UNIVERSIDADE FEDERAL DE PERNAMBUCO

DESNUTRIÇÃO PREGRESSA E/OU MANIPULAÇÃO FARMACOLÓGICA DO SISTEMA SEROTONINÉRGICO: ESTUDO COMPORTAMENTAL E DA RESPOSTA IMUNE

JAIRZA MARIA BARRETO MEDEIROS

RECIFE

2004

UNIVERSIDADE FEDERAL DE PERNAMBUCO

DESNUTRIÇÃO PREGRESSA E/OU MANIPULAÇÃO FARMACOLÓGICA DO SISTEMA SEROTONINÉRGICO: ESTUDO COMPORTAMENTAL E DA RESPOSTA IMUNE

JAIRZA MARIA BARRETO MEDEIROS

Tese de doutorado submetida a Coordenação do Curso de Pós Graduação em Nutrição do Departamento de Nutrição da Universidade Federal de Pernambuco como requisito para a obtenção do título de Doutor em Nutrição.

RECIFE
2004

UNIVERSIDADE FEDERAL DE PERNAMBUCO

DESNUTRIÇÃO PREGRESSA E/OU MANIPULAÇÃO FARMACOLÓGICA DO SISTEMA SEROTONINÉRGICO: ESTUDO COMPORTAMENTAL E DA RESPOSTA IMUNE

JAIRZA MARIA BARRETO MEDEIROS

ORIENTADORA

Dra. Célia Maria M. B. de Castro

Professor Adjunto do Departamento de Medicina Tropical, da Universidade Federal de Pernambuco.

CO-ORIENTADOR

Dr. Raul Manhães de Castro

Professor Adjunto do Departamento de Nutrição
Centro de Ciências da Saúde
Universidade Federal de Pernambuco

RECIFE
2004

Título: "Desnutrição Pregressa e/ou Manipulação Farmacológica do Sistema Serotoninérgico: Estudo Comportamental e da Resposta Imune.

Nome: **Jairza Maria Barreto Medeiros**

Tese aprovada em: 20/01/2004

Membros da Banca:

Bernira Lara Silveira Andrade da Costa

Carlos Teixeira Brandt

Débora Catarine Nepomuceno de Pontes Pessôa

José Eulálio Cabral Filho

Sílvia Regina Arruda Moraes

FICHA CATALOGRÁFICA

Medeiros, Jairza Maria Barreto
Desnutrição pregressa e/ou manipulação farmacológica do sistema serotoninérgico : estudo comportamental e da resposta imune / Jairza Maria Barreto Medeiros. – Recife : O Autor, 2004.
xiii, 164 folhas : il., fig.

Tese (doutorado) – Universidade Federal de Pernambuco. CCS. Nutrição, 2004.

Inclui bibliografia e anexos.

1. Desnutrição precoce – Ratos – Estudo experimental. 2. Serotonina – Estudo em ratos – Manipulação farmacológica. 3. Ratos – Comportamento agressivo e alimentar – Análise. 4. Imunologia – Ratos – Efeitos da agressividade. I. Título.

613.25:599.3 CDU (2.ed.) UFPE
612.3 CDD (21.ed.) BC2004-037

APRESENTAÇÃO

Este trabalho foi realizado no Laboratório de Fisiologia da Nutrição Naíde Teodósio do Departamento de Nutrição da Universidade Federal de Pernambuco e no Laboratório de Imunopatologia Keizo Asami da Universidade Federal de Pernambuco, sob a orientação da Professora Célia Maria Machado Barbosa de Castro, Doutora em Farmacologia Experimental e Clínica da Universidade Federal do Ceará e co-orientação do Professor Raul Manhães de Castro, Doutor em Farmacologia Experimental e Clínica pela Universidade de Paris 6. Contou com o apoio financeiro da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). No presente trabalho foram estudados os efeitos da desnutrição pregressa e/ou da manipulação farmacológica do sistema serrotoninérgico sobre o consumo alimentar, o comportamento agressivo e a inter-relação entre agressividade intraespecífica e resposta imune. Desta pesquisa, foram originados cinco artigos científicos: O primeiro, intitulado: "Action of selective serotonin reuptake inhibitor on aggressive behavior in adult rat submitted to the neonatal malnutrition" foi publicado na revista: Arq Neuropsiquiatr, 59(3-A):499 - 503, em 2001. Neste estudo, foi demonstrado que ratos adultos submetidos a desnutrição neonatal tornam-se resistentes aos efeitos anti-agressividade do citalopram; O segundo, intitulado: "Early malnourished rats are not affected by anorexia induced by a selective serotonin reuptake inhibitor in the adult life" foi publicado na revista: Nutritional Neuroscience, 5(3):211-214, em 2002. Neste trabalho, foi observado que ratos adultos submetidos a desnutrição precoce não são afetados por anorexia induzida por citalopram; O terceiro, intitulado: "Malnutrition during brain growth spurt alters the effect of fluoxetine on aggressive behavior in adult rats", foi aceito para publicação na revista: Nutritional Neuroscience. Neste manuscrito, foi mostrado que ratos adultos desnutridos precocemente tornam-se hiporresponsivos a ação anti-agressividade da fluoxetina; O quarto, intitulado: "The expression of an intraspecific aggressive reaction before a stressor alters the immune response in rats" foi aceito para publicação na revista: Brazilian Journal of Biology, 65 (3), 2005. No manuscrito foram descritos resultados que levam a hipótese de que a expressão da agressividade intraespecífica ativa o sistema imune e potencializa a resposta humoral antígeno-específica; Por fim, o quinto artigo, intitulado: "Malnutrition during brain growth spurt alters the effect of aggressiveness on the immune response in adult rats", submetido a Physiology and Behavior, demonstra que a desnutrição durante o período de rápido desenvolvimento do cérebro altera a inter-relação entre comportamento agressivo e a resposta imune em ratos adultos. Em conclusão, a desnutrição precoce além de interferir na inter-relação entre agressividade e resposta imune; acarreta efeitos duradouros sobre o funcionamento do sistema serotoninérgico.

Dedicatória

A Deus meu refúgio e fortaleza, por todas as graças concedidas, dedico não só este trabalho, mas todos os momentos da minha vida.

Ao meu esposo Osvaldo Medeiros, cujo amor, apoio e paciência foram indispensáveis para que este trabalho existisse, dedico a realização desse sonho.

Aos meus filhos Mayra e Matheus, pelo amor incondicional em todos os momentos; cuja presença me deu força para enfrentar os desafios. Obrigado por seu amor.

A minha mãe Maria Lícia Batista Barreto, por seu amor infinito, por ter me ensinado a acreditar em Deus e a confiar que com ele todos os sonhos se tornam possíveis.

Ao meu irmão Jairo Osvaldo Batista Barreto, por todo amor e apoio a mim dedicados.

Ao meu pai Nivaldo da Silva Barreto, por seu exemplo de vida, por seus ensinamentos eternos; obrigado por ser a minha estrela-guia....(in memoriam).

Agradecimentos

Aos meus orientadores, Profa. Célia Maria Machado Barbosa de Castro e Prof. Raul Manhães de Castro, com os quais compartilho os méritos desse trabalho. As palavras não são suficientes para agradecer tudo que vocês fizeram por mim!

Ao Prof. José Eulálio Cabral Filho, pela valiosa ajuda na análise estatística, os meus sinceros agradecimentos.

A Profa. Roseanne Porto Dantas Mazza, pela orientação durante o meu período de iniciação científica, deixo aqui minha gratidão.

A todos os estagiários que participaram desse trabalho, especialmente a Emanuella Gomes Feitoza, Kedma Magalhães Lima, Francisco Machado Manhães de Castro, Rosangela Rosendo da Silva e Claudionilo Eugênio Gomes Mudo, amigos e importantes colaboradores.

Às amigas Tereza Cristina Bonfim de Jesus Deiró, Adenilda Queirós Santos, Lúcia Maria Pires Ferreira e a Fátima Alves Diniz pelo apoio.

Aos funcionários Fernanda Almeida, Neci do Nascimento, Roberto Dias, Sônia Pedrosa, Edeones França e Hamilto Salviano, agradeço a atenção e a colaboração.

Ao Laboratório de Fisiologia da Nutrição Naíde Teodósio do Departamento de Nutrição da UFPE e ao Laboratório de Imunopatologia Keizo Asami da UFPE, pelo apoio na realização das pesquisas.

Ao Departamento Ciências da Nutrição da Escola de Nutrição da UFBA, agradeço o apoio.

À CAPES / PICDT / UFBA, pela concessão da bolsa de estudos.

A todos aqueles que direta ou indiretamente contribuíram para realização desse trabalho, os meus sinceros agradecimentos.

RESUMO

Neste estudo, foram investigados os efeitos da desnutrição precoce e/ou do tratamento com inibidor seletivo da recaptação de serotonina (ISRS) sobre o consumo alimentar e o comportamento agressivo em ratos adultos. Outrossim, foram investigadas as repercussões sobre a resposta imune da expressão da agressividade intraespecífica frente a um estressor em ratos adultos submetidos ou não a desnutrição precoce. Para isso, ratos Wistar machos foram divididos em dois grupos de acordo com a dieta materna durante a lactação. O grupo bem-nutrido foi alimentado por mães que receberam uma dieta com 23% de proteína (Labina); o desnutrido por mães que consumiram uma dieta com aproximadamente 8% de proteína (Dieta Básica Regional; DBR). Após o desmame, todos os ratos receberam dieta com 23% de proteína. Aos 90 – 120 dias de idade, cada grupo nutricional foi dividido em subgrupos: grupo CITALOPRAM (AGUDO: uma única injeção i.p. de 20 mg/kg de citalopram; CRÔNICO: injeções i.p. diárias de 10mg/kg ou 20 mg/kg de citalopram por 14 dias); grupo FLUOXETINA (injeções i.p. diárias de 10 mg/kg de fluoxetina por 14 dias); grupo SALINA (AGUDO: uma única injeção i.p. de 0.9% NaCl ; CRÔNICO: injeções i.p. diárias de 0.9% NaCl por 14 dias); grupo CONTROLE (apenas as avaliações imunológicas foram realizadas); grupo CHOQUE NAS PATAS – CP (animais receberam CP individualmente) e grupo RESPOSTA AGRESSIVA - RA (animais receberam CP e apresentaram RA). Durante os 14 dias de tratamento com salina ou citalopram 10 mg/kg, foram avaliados o consumo alimentar e o ganho de peso corporal. Após 60 minutos do tratamento com salina ou citalopram 20 mg/kg e 24h do término do tratamento crônico com salina, citalopram 20 mg/kg ou fluoxetina 10mg/kg, os animais foram submetidos aos testes de agressividade. Para indução da RA os ratos eram submetidos aos pares à sessões de 5 choques nas patas (1,6 mA/2s, de 4 em 4 min). Para as medições imunológicas, amostras de sangue foram coletadas imediatamente, 7 e 15 dias após CP ou RA. Leucócitos e títulos de anticorpos anti-hemácias de carneiro foram analisados. A desnutrição precoce alterou a anorexia induzida por citalopram em ratos adultos. Do mesmo modo, o tratamento com ISRS reduziu as respostas agressivas nos ratos bem-nutridos, mas não nos desnutridos. Além disso, em bem nutridos, o CP reduziu a quantidade de leucócitos. Contudo, a agressividade, foi acompanhada, além da redução do número de leucócitos, por diminuição de linfócitos e aumento de neutrófilos imediatamente após as RA. Ademais, foi observada uma elevação no número de leucócitos associada a um aumento na resposta imune humoral uma semana após as RA em bem nutridos, mas não em ratos desnutridos. Assim, neste estudo a expressão da agressividade intraespecífica frente a um estressor parece modular o sistema imune e potencializar a resposta humoral antígeno específica. Contudo, a desnutrição precoce alterou a inter-relação entre a agressividade e resposta imune; Além disso, a desnutrição durante o período de rápido desenvolvimento do cérebro afeta o funcionamento do sistema serotoninérgico.

ABSTRACT

In this study, the effects of the early malnutrition and/or the treatment with selective serotonin reuptake inhibitor (SSRI) on the food intake and the aggressive behavior in adult rats were investigated. Moreover, the repercussions on the immune response of the expression of the intraspecific aggressiveness before a stressor was investigated in adult rats submitted or not to early malnutrition. Male Wistar rats were allocated into two groups, according to their mothers diet during lactation. The well-nourished group was fed by mothers receiving a 23% protein diet (Purina of Brazil Ltd); the malnourished one by mothers receiving a 8% protein diet approximately ("Regional Basic Diet" - RBD). After weaning, all rats received the 23% diet. On the 90^{th} – 120^{th} day after birth, each nutritional group was divided in subgroups: CITALOPRAM group (ACUTE: a single i.p injection of 20 mg/kg of citalopram; CHRONIC: single daily injection of 10mg/kg or 20 mg/kg of citalopram for 14 days); FLUOXETINE group (single daily injection of 10mg/kg of fluoxetine for 14 days); SALINE group (ACUTE: a single i.p injection of saline (0.9% NaCl); CHRONIC: single daily injection of saline (0.9% NaCl) for 14 days); control group (only the immunological measurements were accomplished); foot-shock (FS) (animals individually received FS) and intraspecific aggressive response (IAR) group (animals received FS and presented IAR). During the 14 days of the chronic treatment with saline or citalopram 10mg/kg, the food intake and body weight gain were evaluated. The animals were submitted to the aggressiveness tests 1-h after the acute treatment or 24h after the chronic treatment with saline, citalopram 20 mg/kg or fluoxetine 10mg/kg. To induce the aggressive response the rats were submited in pairs to sessions of 5 foot shocks (1.6 mA/2s, each 4 min). For immunological measurements, blood samples were collected immediately, 7 and 15 days after FS or IAR. Leukocytes and antibody titer anti-SRBC (sheep red blood cells) were analyzed. The early malnutrition altered the anorexia induced by citalopram in adult rats. In the same way, the treatment with SSRI reduces aggressive response in well-nourished, but not in malnourished ones. Moreover, in well-nourished, the FS reduced the total amount of leukocytes. However, the aggressiveness was accompanied, besides the reduction of the leukocytes number, by lymphocytes decrease and neutrophils increase. Moreover, an elevation in the leukocytes number associated to an increase in the humoral immune response was also observed one week after the IAR in well-nourished, but not in malnourished rats. In this study, the expression of the intraspecific aggressiveness before a stressor seems to modulate the immune system and to potentiate the antigen specific humoral response. However, the early malnutrition altered the interrelation between the aggressive behavior and the immune response. Moreover, the malnutrition during the brain growth spurt affects the functioning of the serotoninergic system.

LISTA DE ABREVIAÇÕES

µA: Micro-ampere.

5 – HIAA: Ácido 5-hidroxindolacético.

5,7-DHT: 5,7–dihidroxitriptamina.

5-HT: Serotonina.

5-HTP: 5-hidroxitriptófano.

8-OH-DPAT: (8-hydroxy-2-(di-n-propylamino) tetralin).

ACTH: Hormônio adrenocorticotrópico.

ANOVA: *Analysis of variance.*

BGS: *Brain grow spurt*

CORT: Corticosteróides.

CP 95,253: (3-(1,2, 3,6 tetrahidro–4–piridinil)-5-propoxipir-rolo(3,2- piridina).

CRH: Hormônio liberador de corticotropina.

DBR: Dieta básica regional.

DMT: 5-methory–N, N– dimethyltryptamine).

DNA: Ácido desoxirribonucleico.

E: Epinefrina.

EDTA: Ácido etileno diamino tetra acético.

ENK: Encefalina.

FS: *Foot-shock.*

GABA: Ácido gama-aminobutírico.

GH: Hormônio do crescimento.

HPA: Hipotálamo-pituitária-adrenal.

I.P: Intraperitonial.

IAR: *Intraspecific aggressive response*

IgA: Imunoglobulina A.

IgG: Imunoglobulina G.

IL-1: Interleucina - 1.

IL-6 : Interleucina - 6.

ISRS: Inibidor seletivo da recaptação de serotonina.

LCE: Líquido cerebro-espinhal.

LT: *Lymphocytes.*

LY 206130: (1-[1-H-indol-4-yloxy]-3-[cyclohexylamino]-2-propanol maleate)

M: *Malnutrition.*

MA: Mileampere.

MAG: *Aggressive response malnourished.*

MAC: *Malnourished acute citalopram*.

MAS: *Malnourished acute saline*.

MC: *Control malnourished.*

MCC: . *Malnourished chronic citalopram.*

MCS: *Malnourished chronic saline.*

MFS: *Foot-shock malnourished.*

MNC: *Malnourished plus citalopram.*

MNS: *Malnourished plus saline.*

MØ: Macrófagos.

NAC: *Nourished acute citalopram*.

NAS: *Nourished acute saline.*

NCC: *Nourished chronic citalopram.*

NCS: *Nourished chronic saline*.

NE: Norepinefrina.

NK: Natural *Killer.*

NPY: Neuropeptídeo Y.

NT: *Neutrophils.*

PCPA: Para-clorofenilanina.

PMA: Phorbol myristate acetate

PSAP: *Point Subtraction Aggression Paradigm.*

RNA: Ácido desoxirribonucléico.

RO 60-0175: (S)-2-(6-chloro-5-fluoro-indol-l-yl)-l-methylethylamine hydrochloride).

S.E.M: Erro padrão da média.

SB 242084: (6-chloro-5-methyl-1-[2(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline).

SD: Desvio padrão.

SN: Sistema Nervoso.

SNA: Sistema Nervoso Autônomo.

SNC: Sistema Nervoso Central.

SP: Substância P.

SRBC: *Sheep red blood cells.*

SSRI: *Selective serotonin reuptake inhibitor.*

TNF: Fator de necrose tumoral.

WBC: *White blood cells.*

WNAG: A*ggressive response well-nourished.*

WNC: *Control well-nourished.*

WNC: *Well-nourished plus citalopram.*

WNFS: *Foot-shock well-nourished.*

WNS: *Well-nourished plus saline.*

SUMÁRIO

INTRODUÇÃO

1 – INTRODUÇÃO

1.1. Desnutrição e Sistema Nervoso

A desnutrição é um dos principais fatores que afeta o desenvolvimento do sistema nervoso (TONKISS *et al*., 2003; PEREZ-TORRERO *et al*., 2003). Todos os nutrientes podem influenciar de alguma maneira o processo de maturação neural, mas a proteína parece ser o componente mais crítico para o desenvolvimento de funções nervosas (MORGANE *et al.,* 2002; CHANG *et al.,* 2003; HERNANDES E ALMEIDA, 2003). Elas fornecem aminoácidos que são essenciais para a constituição de elementos celulares estruturais e funcionais. No processo fundamental de neurotransmissão, vários aminoácidos são precursores de neurotransmissores ou em muitos casos atuam como o próprio neurotransmissor (MORGANE *et al.,* 2002).

No homem, a desnutrição ocorrida nos primeiros 5 anos de vida, pode ter conseqüências devastadoras para o sistema nervoso; durante esse período, o crescimento é rápido e as necessidades de calorias e nutrientes são maiores (BROWN e POLLITT, 1996). Nessa fase da vida, o processo de crescimento e desenvolvimento do encéfalo ocorre com grande intensidade, o que torna sua estrutura mais vulnerável a vários tipos de agressão (MORGANE, *et al.,* 1978). Agressões nutricionais a depender do tipo, da severidade, da duração e do período de ocorrência em relação as etapas de desenvolvimento do cérebro, poderão ocasionar alterações irreversíveis mesmo após recuperação nutricional (MORGANE *et al.,* 1992). Esse período é considerado crítico no desenvolvimento neural e corresponde ao pico de

atividades de eventos específicos como neurogênese, gliogênese, diferenciação celular, migração e sinaptogênese (DOBBING, 1968; MORGANE *et al.,* 1978; MORGANE *et al.,* 1993).

O período crítico de desenvolvimento do encéfalo varia entre as espécies; no homem, inicia-se no período pré-natal (último trimestre de gestação), continuando até os primeiros anos de vida (3 a 4 anos). No rato, corresponde as três primeiras semanas de vida pós-natal (MORGANE *et al.,* 1978).

Em ratos desnutridos, particularmente durante o período neonatal, foi observada redução do conteúdo de DNA no cérebro, no cerebelo e no hipocampo (WINICK *et al*., 1972) e também no conteúdo de RNA no córtex cerebral (CASTILLA *et al*., 1979). Em ratos submetidos à desnutrição pós-natal, foi observado ao desmame, redução do peso, acompanhada de alterações químicas como diminuição da concentração de colesterol, decréscimo cerebelar do conteúdo de DNA, diminuição do conteúdo de proteína da região telencefálica e tronco cerebral e redução da atividade da acetilcolinesterase (SOBOTKA *et al.,* 1974). Outrossim, em ratos desnutridos no início da vida, foi encontrado déficit no número de neurônios no giro denteado (BEDI, 2003). Alterações na forma de neurônios foram também observadas em ratos submetidos a desnutrição durante o período perinatal (RESNICK *et al.,* 1979; BORBA *et al.,* 2000).

A questão é se essas modificações têm conseqüências funcionais. Segundo LEVITSKY (1975), essas alterações podem interferir na capacidade do indivíduo interagir com o seu meio; em outras palavras, elas poderiam

trazer conseqüências comportamentais.

Barreto Medeiros (1998), investigando as conseqüências funcionais da desnutrição precoce sobre o sistema nervoso, em animais adultos, particularmente, sobre a expressão comportamental, observou redução no peso corporal associada a aumento do consumo alimentar e maior sensibilidade dolorosa de animais desnutridos, mesmo após longo período de recuperação nutricional.

Outros estudos têm demonstrado que a desnutrição pode alterar os sistemas de neurotransmissores (MANJARREZ *et al.,* 2003; CHEN *et al.,* 1997), dentre eles o serotoninérgico (CHEN *et al.,* 1992; CHEN *et al.,* 1995). Há relatos na literatura de aumento da atividade serotoninérgica no cérebro de animais desnutridos em desenvolvimento (WIGGNS *et al*., 1984). Assim, um aumento nos níveis de serotonina e ácido 5-hidroxindolacético foi detectado no cérebro de animais desnutridos, desde o nascimento até 300 dias de idade (STERN *et al.,*1975). Outras investigações encontraram um aumento na liberação de serotonina no hipocampo de ratos desnutridos durante o período pré-natal (CHEN *et al.,* 1992; MOLKER *et al.,* 1999; MOLKER *et al.,* 2003). Contudo, CHEN *et al.* (1997), observaram que ratos submetidos à desnutrição protéica pré-natal seguida de reabilitação nutricional não apresentaram alterações nas concentrações de serotonina e norepinefrina. Parece que a reabilitação nutricional pós desmame neutralizou o efeito da agressão nutricional precoce sobre os neurotransmissores.

Os efeitos das agressões nutricionais e farmacológicas sobre os sistemas de neurotransmissão, particularmente o sistema serotoninérgico,

merece atenção especial, pois este sistema elicia ou modula uma ampla variedade de funções do sistema nervoso central (CHOPIN *et al.,* 1994). Entre estas podemos destacar o consumo alimentar e o comportamento agressivo.

1.2. Sistema Serotoninérgico

O sistema serotoninérgico é constituído de neurônios que liberam serotonina (5-HT) e dos receptores específicos para a mesma (MANHÃES DE CASTRO, 1995). Em mamíferos, a maioria dos corpos celulares dos neurônios 5-HT estão localizados nos núcleos bulbares e mesencefálicos da rafe (MAGALHÃES *et al.,* 2000). Estes núcleos enviam projeções que inervam o cérebro, cerebelo, tronco cerebral e a medula espinhal (JACOBS e AZMITIA, 1992). Além do sistema nervoso, a 5-HT ainda pode ser encontrada nas células enterocromafins do trato gastrintestinal, nas plaquetas e em mastócitos (BORNE, 1994). Na figura 1 encontra-se ilustrada a distribuição das vias serotoninérgicas no cérebro.

A serotonina é uma amina derivada do aminoácido essencial triptófano (WURTAMAN, 1982; FERNSTROM, 1991). Sua síntese envolve duas reações: uma de hidroxilação, onde o triptófano se transforma em 5-hidroxitriptófano (5-HTP) pela ação da triptófano hidroxilase e outra de descarboxilação, em que o 5-HTP dá origem à 5-HT, através da ação da enzima 5-hidroxitriptófano descarboxilase (HAMON *et al.,* 1981). A degradação da 5-HT é feita pelas enzimas monoaminoxidase e aldeído desidrogenase, tendo como produto final o ácido 5-hidroxindolacético (5-HIAA) (FERNSTROM, 1983). A síntese e a degradação da serotonina estão ilustradas na figura 2.

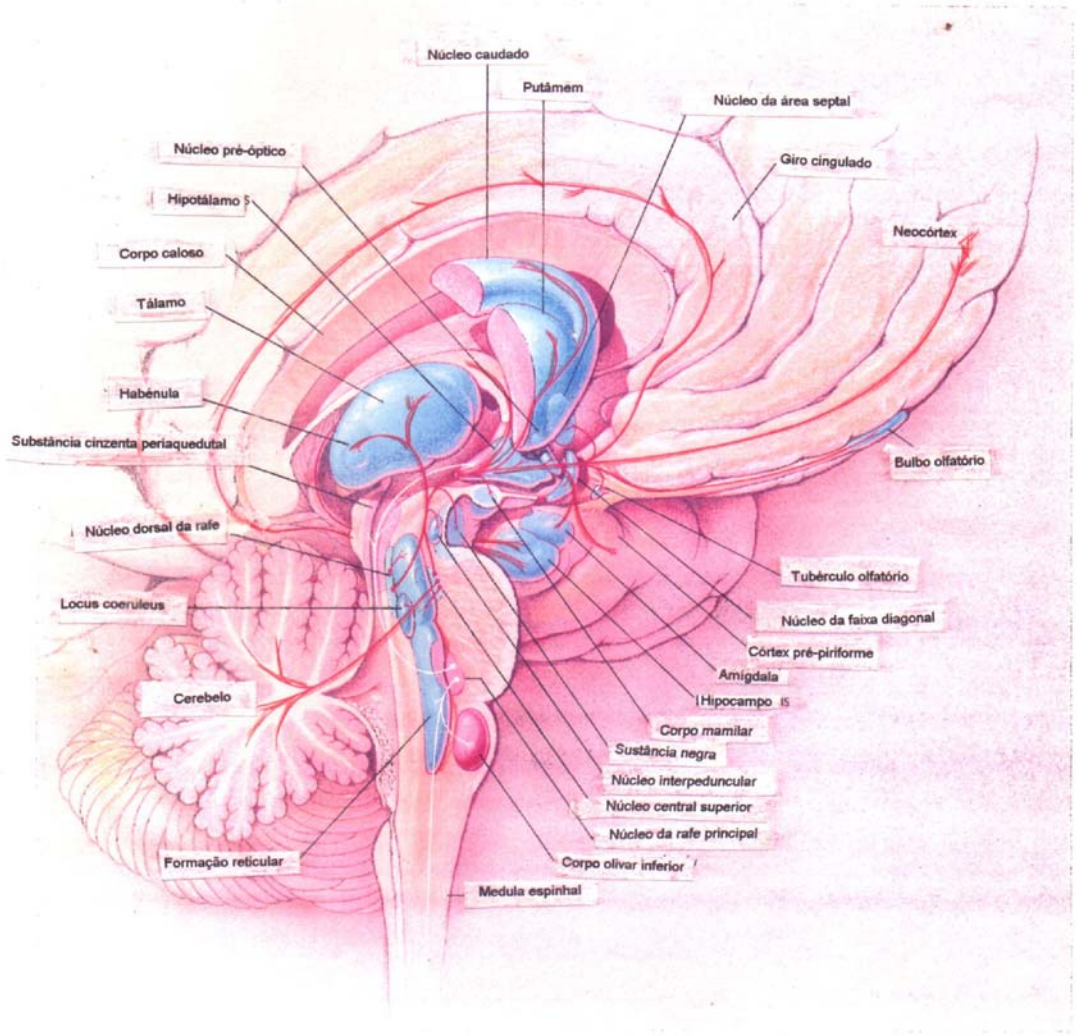


Figura 1 – Distribuição das vais serotoninérgicas no cérebro – Modificado de Snyder, S.H. in: Drugs and the Brain. Scientific American Libery, 1996.

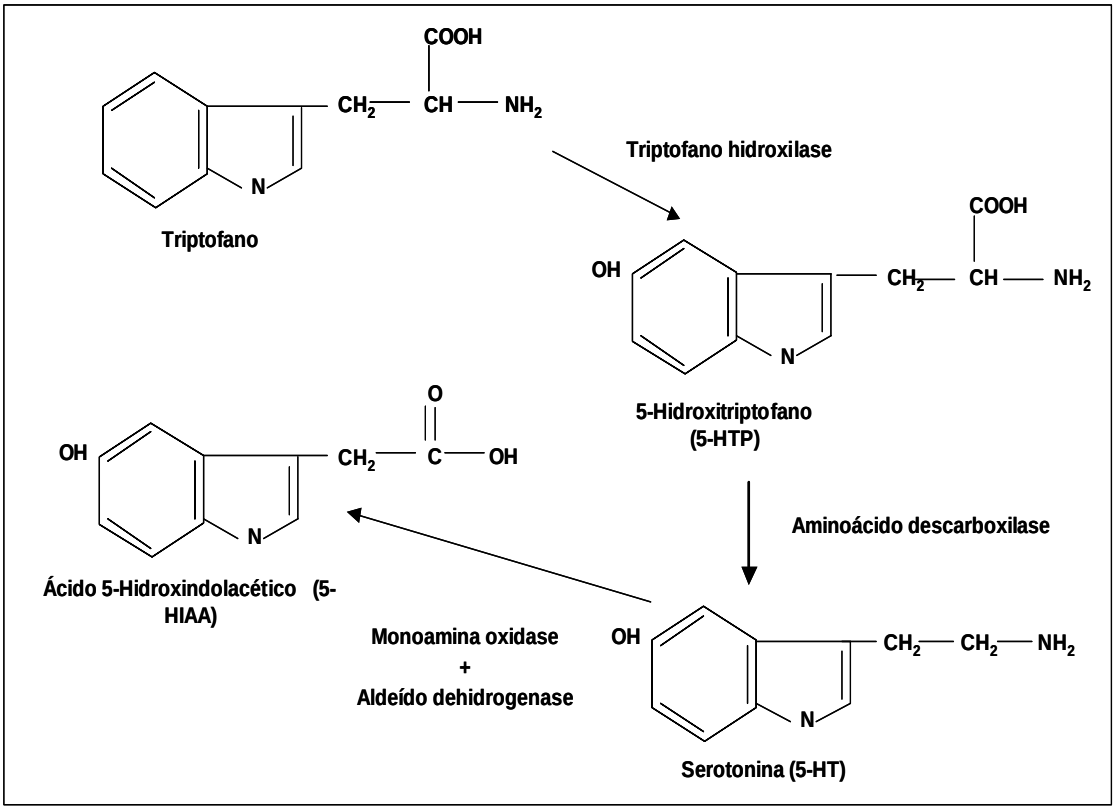


Figura 2 - Síntese e metabolismo da serotonina (SVED, A.F., 1983).

No sistema nervoso central, a 5-HT após a síntese é armazenada em vesículas sinápticas, sendo liberada para a fenda pela ação de impulsos nervosos (BORNE, 1994). Em seguida ela pode atuar em receptores pré-sinápticos, que regulam sua síntese e liberação, ou se ligar a receptores pós-sinápticos, possibilitando a propagação da informação para outros neurônios (BARNES e SHARP, 1999).

Atualmente existem cerca de sete tipos de receptores serotoninérgicos identificados, são eles: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 e 5-HT_7 (BARNES e SHARP, 1999). Vários sub-tipos de receptores também já foram descritos e devidamente classificados (BARNES E SHARP, 1999), como por exemplo, 5-HT_{1A} e 5-HT_{1B} (BOLANOS-JIMENEZ *et al.,* 1993; 1994; MANHÃES DE CASTRO *et al.,* 1996) entre outros.

O aumento na ação da serotonina extracelular sob receptores pós-sinápticos dos neurônios alvos resulta em várias alterações funcionais, refletindo uma ampla distribuição de terminais nervosos serotoninergicos em regiões cerebrais que regulam numerosas funções fisiológicas (FULLER, 1996). Sabe-se que o sistema serotoninérgico participa de funções do sistema nervoso central tais como: sensibilidade a dor, controle do sono, humor, comportamento sexual, consumo alimentar e agressividade (CHOPIN *et al.,* 1994). Além disso, há várias evidências de que a serotonina age como um fator neurotrófico (FABER e HARING, 1999; RADLEY e JACOBS, 2002).

1.2.1. Serotonina e Consumo Alimentar

O papel da serotonina no controle da ingestão alimentar e da saciedade tem sido estudado por muitos pesquisadores (BLUNDEL, 1992; HALFORD e BLUNDEL, 2000; LEE *et al.,* 2002). Na figura 3 encontram-se ilustradas gaiolas individuais utilizadas para o estudo do consumo alimentar em ratos. Segundo Blundel *et al.,* (1995), drogas serotoninérgicas podem reduzir o consumo alimentar e bloquear o ganho de peso corporal de ratos submetidos a dieta rica em gordura (BLUNDEL *et al.,* 1995). Considerável perda de peso foi observada em ratos, após a administração crônica de serotonina (EDWARDS, 1995).

Ademais, alteração funcional do sistema serotoninérgico tem sido associada com distúrbios alimentares (MCBRIDE *et al.,* 1991). Assim, concentrações reduzidas do 5-HIAA no líquido cérebro-espinhal, podem ser encontradas em mulheres com anorexia nervosa e baixo peso corporal (GILBERG, 1983; KAYE *et al.,* 1988), sendo que a normalização de seus níveis é seguida da restauração do peso (KAYE *et al.,*.1988).

Alguns subtipos dos receptores serotoninérgicos, particularmente $5HT_{1A}$, $5HT_{1B}$ e $5HT_{2C}$, tem sido relacionados à ação da serotonina no controle da ingestão alimentar e do peso corporal (DOURISH, 1995; DE VRY e SCHREIBER, 2000; LEE *et al.,* 2002). Assim, pequenas doses de 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin), um agonista do receptor $5\text{-}HT_{1A}$ aumentou a ingesta alimentar em ratos (DOURISH *et al.,* 1985). Além disso, estudo realizado em camundongos demonstrou que a administração de LY206130 (1-[1-H-indol-4-yloxy]-3-[cyclohexylamino]-2-propanol maleate), um

antagonista seletivo do receptor $5HT_{1A}$ potencializou o efeito inibitório da fluoxetina sobre o consumo de leite condensado (LI *et al.,* 1998).

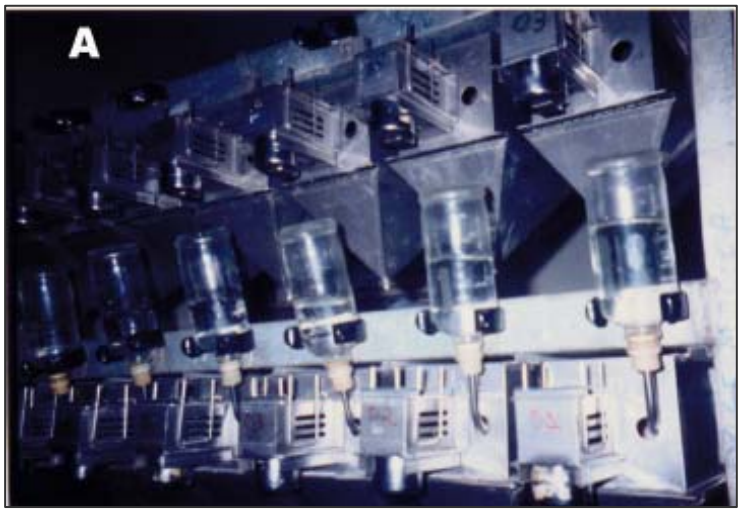


Figura 3– Gaiolas utilizadas para o estudo do consumo alimentar.
(A) – Visão Posterior – Bebedouros e Comedouros.
(B) – Visão Anterior - Gaiolas com coletor de fezes e coletor de urina.

Em ratos, o uso do CP 94,253 (3-(1,2, 3,6 tetrahidro–4–piridinil)-5-propoxipir-rolo(3,2-b)piridina), um agonista altamente seletivo do receptor $5HT_{1B}$, reduziu o consumo alimentar e preservou a seqüência comportamental de saciedade (LEE *et al.,* 2002). Do mesmo modo, Halford e Blundel (1996A) demonstraram que a ativação dos sítios do receptor $5HT_{1B}$ por CP 94,253 é suficiente para reduzir a ingestão alimentar e aumentar a saciedade. Em camundongos, tratamento com RO 60-0175 ((S)-2-(6-chloro-5-fluoro-indol-l-yl)-l-methylethylamine hydrochloride), um agonista do receptor $5HT_{2c,}$ nas doses de 1.0, 3.0 e 10.0 mg\kg de peso corporal produziu redução no consumo alimentar de maneira dose dependente (HEWITT *et al.,* 2002). Este efeito anorexígeno do RO 60-0175 foi atenuado por SB 242084 (6-chloro-5-methyl-1-[2(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline), um antagonista seletivo deste receptor (HEWITT *et al.,* 2002). Segundo Dourish (1995), agonistas seletivos do receptor $5\text{-}HT_{2C}$ apresentam efeito anoréxico e podem ser úteis no tratamento da obesidade.

Alguns fármacos, entre eles os inibidores seletivos de recaptação de serotonina, vêm sendo utilizados na prática clínica no tratamento de transtornos alimentares ou psiquiátricos (TAMMELA *et al.,* 2003; ARMENTEROS e LEWIS, 2003).

1.2.2. Serotonina e Agressividade

A importância da serotonina no controle inibitório da agressão tem sido evidenciada experimentalmente pela manipulação do sistema serotoninérgico em roedores (DATLA *et al.,* 1991; KEELE *et al.,* 2001).

Os níveis cerebrais de serotonina podem ser reduzidos por restrição do triptófano, por inibição da triptófano hidroxilase com para-clorofenilanina, por destruição dos neurônios serotoninérgicos com a neurotoxina, 5,7–dihidroxitriptamina (5,7-DHT) ou por lesão dos núcleos da rafe (EICHELMAN, 1990). Dados experimentais demonstram que a supressão do sistema serotoninérgico aumenta o comportamento agressivo em ratos (APPLEGATE, 1980). Estudo experimental realizado em macacos machos demonstrou aumento da agressividade, relacionado a baixos níveis de serotonina (KYES, 1993).

O triptófano aumenta a síntese de serotonina no cérebro e portanto, deve estimular a liberação de 5-HT e suas funções (VOLVOKA, 1995). Assim, dietas pobres em triptófano facilitam o comportamento muricida em ratos e a suplementação com este aminoácido reduz este comportamento (GIBBONS *et al.,* 1979). Em mulheres submetidas a teste laboratorial de agressividade (PSAP: *Point Subtraction Aggression Paradigm*), a suplementação alimentar com triptófano diminuiu o comportamento agressivo, enquanto a depleção desse aminoácido aumentou a agressividade (MARSH *et al.,* 2002).

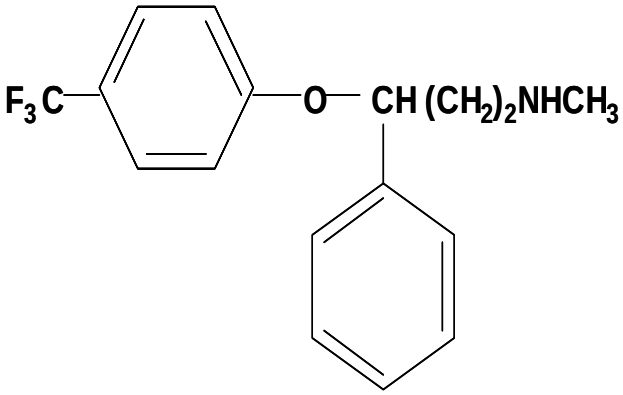
Os níveis do 5-HIAA no LCE foi por muito tempo utilizado como indicativo da atividade serotoninérgica central. Redução dos níveis do 5-HIAA foi observada em pacientes depressivos, com história de violência e tentativa

de suicídio (ASBERG *et al.,* 1976).

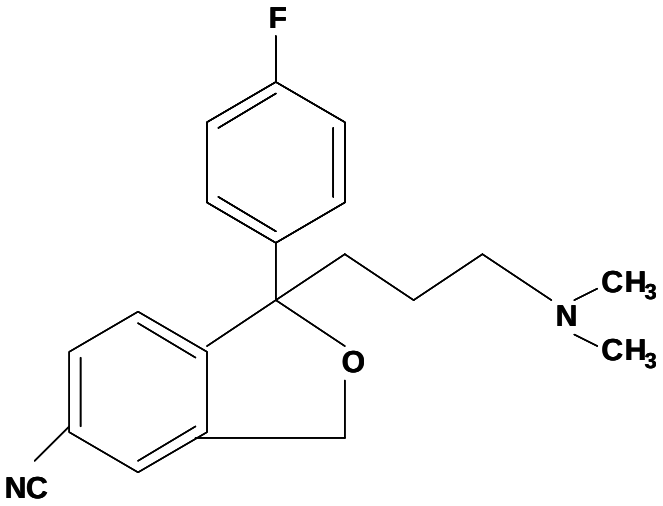
O papel da serotonina no controle do comportamento agressivo tem ainda sido demonstrado através da utilização de instrumentos farmacológicos (MANHÃES DE CASTRO *et al.,* 2001). Há drogas que atuam inibindo a recaptação da serotonina, aumentando a sua disponibilidade sináptica e consequentemente potencializando a ação desse neurotransmissor (HYTTEL, 1994). A fluoxetina e o citalopram, que são inibidores seletivos da recaptação de serotonina (ISRS) (HYTTEL, 1994; SÁNCHEZ e HYTTEL, 1999) são algumas dessas substâncias (Figura 4).

A Fluoxetina é uma amina secundaria, que é metabolizada a norfluoxetina, considerada um inibidor da recaptação da serotonina mais seletivo e mais potente (BAUMANN, 1996; SANCHEZ e HYTTEL, 1999). Segundo Wong (1993), a fluoxetina tem um potencial para inibir a recaptação da serotonina duas vezes maior que sua eficácia em bloquear a recaptação de dopamina e noradrenalina.

O citalopram é uma amina terciária, que é metabolizada a N-dimetilcitalopram e N-didmethylcitalopram, que são ISRS menos potentes (BAUMANN, 1996). Em ratos, tratamento com citalopram (10 mg/kg i.p. duas vezes ao dia durante 14 dias), não resultou em modificações adaptativas dos sítios de captação de serotonina e não teve efeito sobre o autorreceptor 5-HT1B, e os receptores 5-HT3 e 5-HT4 (GOBBI *et al.,* 1997). Outrossim, trabalho experimental demonstrou um aumento na concentração de serotonina no cortex frontal de ratos, após tratamento crônico com citalopram (20 mg/kg/dia, i.p., por 14 dias), 10 e 12 h após a última dose (ARBORELIUS *et al.,* 1996).



Fluoxetina



Citalopram

Figura 4 - Estrutura química de ISRS (BAUMANN, P. e ROCHAT, B., 1995)

Há evidências indicando que ISRS diminui o comportamento agressivo em várias espécies e modelos de agressão (DATLA *et al.,* 1991; SÁNCHEZ e HYTTEL, 1994; SPERRY *et al.,* 2003). Em ratos, a agressividade foi reduzida após administração central de fluoxetina (DATLA *et al.,* 1991). Em camundongos, tratamento agudo com fluoxetina reduziu a agressividade induzida por isolamento (SÁNCHEZ e HYTTEL, 1994). Do mesmo modo, em pardais o tratamento agudo com fluoxetina reduziu o comportamento agressivo (SPERRY *et al.,* 2003). Outrossim, em ratos tratamento neonatal com citalopram (20 mg/Kg, sc, do 2° ao 21° de vida) diminuiu a agressividade intraespecífica na vida adulta (MANHÃES DE CASTRO *et al.,* 1998). O efeito inibidor do citalopram, sobre a agressividade induzida por isolamento, foi também demonstrado em camundongos pré tratados com uma dose subefetiva do precursor da serotonina (SÁNCHEZ e HYTTEL, 1994; SÁNCHEZ e MEIRE, 1997).

Dentre os modelos experimentais utilizados para o estudo da agressividade, destaca-se o comportamento agressivo induzido por choque elétrico nas patas (EICHELMAN, 1990). Segundo, EICHELMAN e THOA (1973) este padrão de comportamento foi descrito pela primeira vez por O'kelly e Steckle em 1939 e desde então, vem sendo utilizado por muitos pesquisadores (DATLA *et al.,* 1991; SILVA, 1997; MANHÃES DE CASTRO *et al.,* 2001). Este modelo é caracterizado pela administração repetida de pulsos elétricos nas barras do piso de uma gaiola contendo dois ratos (VOLVOKA, 1995; Figura 5). Quando o choque nas patas é administrado os ratos assumem postura de luta (Figura 6).

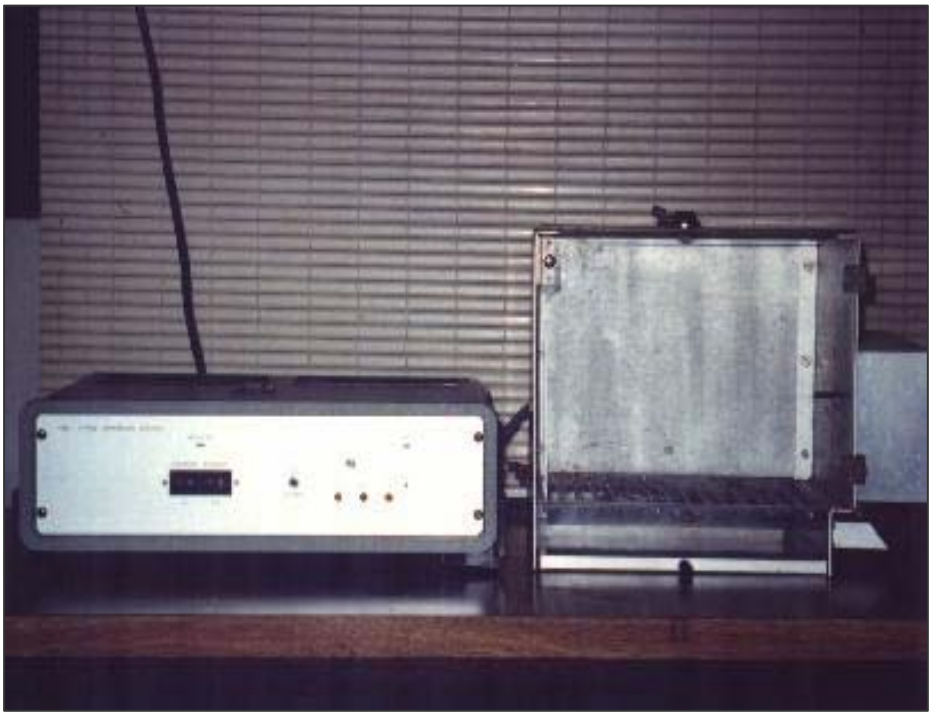


Figura 5 – Aparelho utilizado para administração de choque elétrico nas patas.

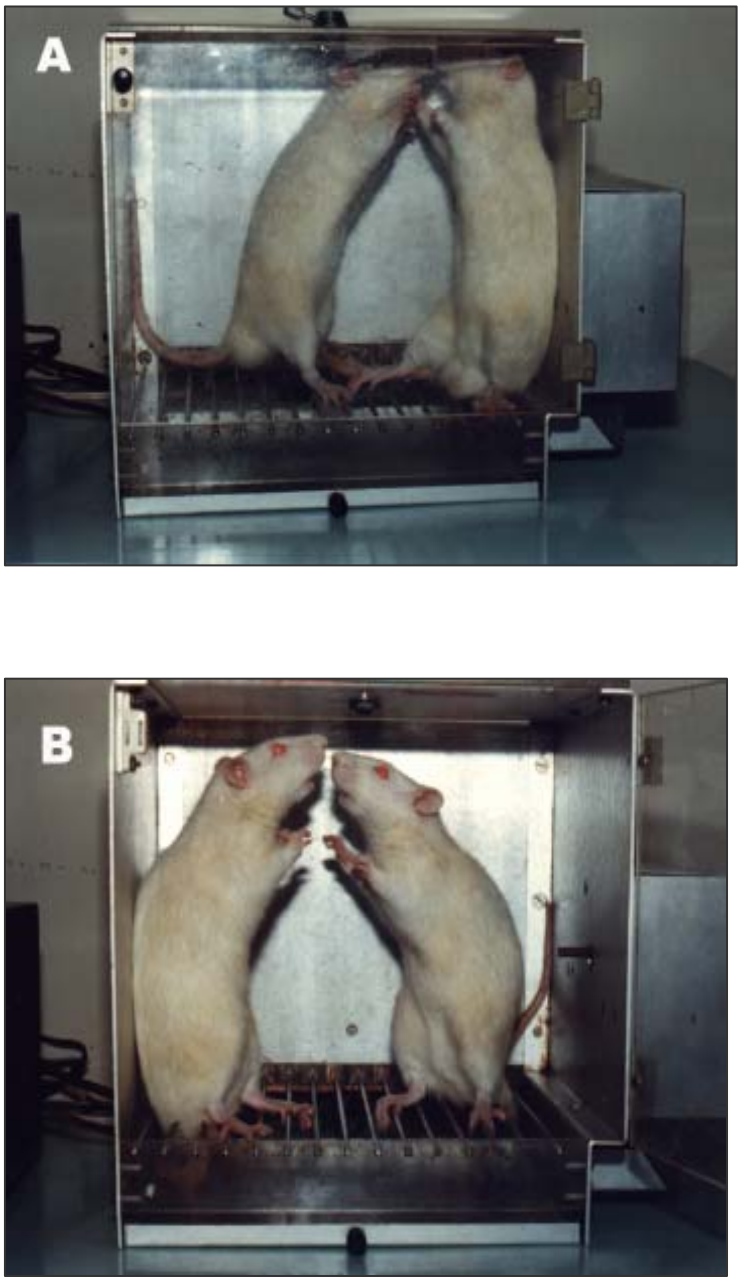


Figura 6 (A e B) – Resposta ao choque nas patas consideradas como agressivas.

1.3. Agressividade e Sistema Imune

Nos últimos anos, muitos pesquisadores têm se dedicado a estudar como as reações emocionais podem interferir na resposta imune (STEFANSKI e ENGLER, 1998; DRÉAU *et al.,* 1999; GROOT *et al.,* 2002). Na maioria dos casos, a atenção tem sido focalizada principalmente sobre a influencia do estresse sobre a função imune (DE CASTRO *et al.,* 2000). São poucos os estudos sobre a inter-relação entre agressividade e resposta imune (COHEN *et al.,* 1997; DEVOINO *et al.,* 2003A).

Em primatas, níveis mais altos de agressividade foram associados com menor suscetibilidade a infeção respiratória (COHEN *et al.,* 1997). Já em camundongos geneticamente agressivos foi encontrada redução na atividade das células Natural *Killer* (NK) naqueles menos agressivos (PETITTO *et al.,* 1993). Sabe-se que as alterações imunológicas podem variar a depender da postura assumida pelos animais durante um confronto (STEFANSKI e ENGLER, 1999). Assim, Devoino *et al.,* (2003A) Observaram que a ocorrência de comportamento agressivo em ratos submissos produziu imunoestimulação. Isto significa que frente a uma situação estressante, as respostas dos sistemas fisiológicos podem ser diferentes.

Há duas principais vias pelas quais o sistema nervoso central pode influenciar a resposta imune periférica (Figura 7). Uma é o sistema nervoso autônomo (SNA), onde as catecolaminas (epinefrina e norepinefrina) liberadas dos terminais nervosos controla a atividade de órgãos imunes primários e secundários (FRIEDMAN e IRWIN, 1997). E a outra é o eixo hipotálamo-pituitário-adrenal (HPA) (MAIER e WATKINS, 1998). O principal iniciador dos

efeitos imunorregulatórios deste eixo é o hormônio liberador de corticotrofina (CRH) (LAWRENCE e KIM, 2000). O hipotálamo libera o CRH em resposta a uma variedade de estímulos(MAIER e WATKINS, 1998). O CRH induz as células da pituitária a produzir e liberar no sangue o ACTH (VIZI, 1998). Este último por sua vez, estimula a síntese e liberação de glicocorticóide (cortisol no homem e corticosterona no rato) pela glândula supra renal (MAIER e WATKINS, 1998; LAWRENCE e KIM, 2000). Catecolaminas e glicocorticoides são potentes mediadores de muitos aspectos da imunidade, incluindo migração e proliferação de linfócitos (FELSNER *et al.,* 1992; BENSCHOP *et al.,* 1993; SHAKHAR e BEM-ELIYAHU, 1998).

A estimulação do eixo hipotálamo-pituitário-adrenal parece constituir um dos elementos fundamentais da resposta adaptativa de um organismo na maioria das situações ameaçadoras a que pode ser submetido (CASTELNAU e LÔO, 1993). O eixo HPA e também outros elementos partícipes da resposta ao estresse, como o sistema nervoso simpático (SGOIFO *et al.,* 1996; LAWRENCE e KIM, 2000) são componentes estreitamente associados à expressão da resposta agressiva.

Há várias evidências na literatura mostrando que células imunes podem liberar hormônios e neuropeptídeos (OTTAVIANI e FRANCESCHI, 1996; BLALOCK *et al.,* 1985). Outrossim, receptores para substâncias como, adrenalina, serotonina, acetilcolina, histamina, endorfina, adrenocorticotropina e neuropeptideos que estão implicados nas reações de estresse e agressividade, tem sido identificados nas membranas de distintas populações de células imunes (BLALOCK *et al.,* 1985; DANTZER, 1991). Essas evidências explicam pelo menos em parte como reações emocionais e de

estresse podem alterar a resposta imune.

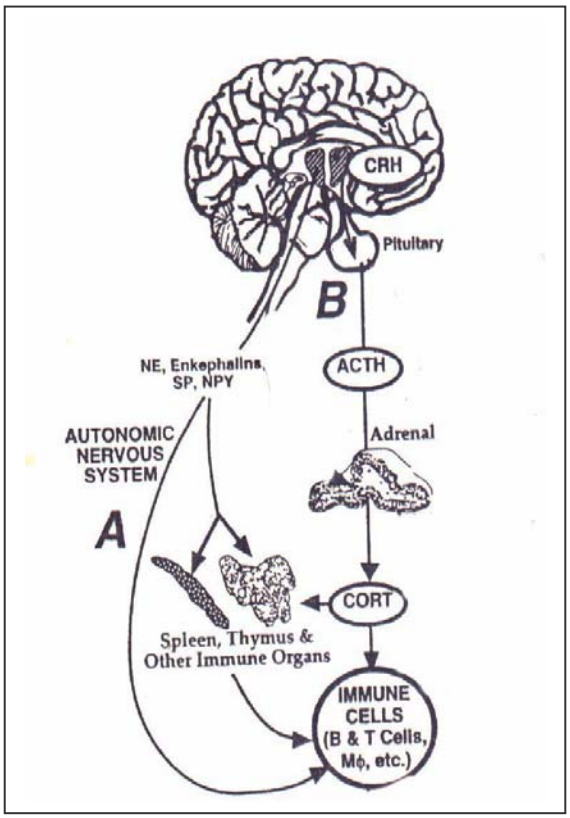


Figura 7 – Esquema representando as duas principais vias de conexão entre o cérebro e o sistema imune: CRH = hormônio liberador de corticotropina; ACTH = hormônio adrenocorticotropico; CORT = corticosteróides; NE = norepinefrina; ENK = encefalina; SP = substância P; NPY = neuropeptídeo Y; MØ = macrófagos (MODIFICADO de MEIER e WATKINS, 1998).

1.4 Desnutrição e Sistema Imunológico.

Este tema será apresentado na forma de artigo de revisão intitulado: " **HOW DOES THE MALNUTRITION ALTER THE IMUNE SYSTEM?**" Publicado na revista: *An. Fac. Med. Univ. Fed. Pernamb*., Recife, v.48 (1), 2003.

O sistema imunológico atua em um ambiente multissistêmico podendo desse modo, sofrer a influência de diversos fatores inerentes ao indivíduo. Entre esses fatores o estado nutricional é um dos mais importantes. Assim, estudar a influência da desnutrição sobre o sistema imune tem sido objeto de muitas pesquisas. Este artigo, tras uma revisão de como a desnutrição pode alterar o sistema imune. Nesta revisão é interessante notar, que são escassos os estudos sobre as repercussões tardias da desnutrição neonatal, seguida por recuperação nutricional.

HOW DOES THE MALNUTRITION ALTER THE IMMUNE SYSTEM?

Jairza Maria Barreto-Medeiros[1]
Célia Maria Machado Barbosa De-Castro[2]
Adriana Maria da Silva Teles[1]
Eduardo José Neponuceno Montenegro[1]
Raul Manhaes-de-Castro[3]

Barreto-Medeiros JM, De-Castro CMBB, Teles AMS, Montenegro EJN, Manhães-de-Castro R.How does the malnutrition alter the immune system? An. Fac. Med. Univ. Fed. Pernamb. Recife, 48(1), p.69-71, 2003.

ABSTRACT: The nutritional state is one of the principal factors that can affect the functioning of the immune system and consequently to alter the capacity of the organism to win the aggressions of the milieu. That is due to the fact that the cells of the immune system as the cells of any other system depend on nutrients for its appropriate function. The present work makes a revision of as the malnutrition it can alter the immune system.

KEYWORDS: Malnutrition; Immune system.

INTRODUCTION

Happening in the first years of the man's life, the malnutrition (M) can have serious consequences for the organism, because that period is characterized by intense growth process and systemic development[1]. During that specific period, the growth is fast and the needs of calories and proteins are larger[1]. Like this, in that phase of the life, nutritional aggressions can cause alterations in functioning of organs and systems (among them, the immune system), which play important role for maintenance of the homeostasis and integrity of the body. The effects of the M on the immune system will be discussed in this work.

In the last 30 years, studies have been confirming that deficiency of nutrients harms the immune response and takes to frequent infections that result in mortality, especially in children[9]. The complex interactions among nutrition, infection and immunity are not still well understood. In that context, it is difficult to determine the specific role of each one of the involved factors. The Infections induce to great metabolic alterations that result in nitrogen negative balance. On the other hand, the M of any origin can result in alteration of the immune response[8].

Smythe and colls observed in children undernourished atrophy in the tonsils and reduction in the rate of transformation of lymphocytes[20]. Reduction of the size and weight of the thymus during the M are also observed by other researchers[23]. A decrease in the weight of the spleen was observed in rats submitted to the diet with low percentage in proteins[20]. Chandra (1997) refers that, in the protein-energy M, there is also a significant loss of the lymphoid cells in the spleen, in the lymphnods and in the thymus-dependent paracortical areas[8].

In the protein-energy M there is a significant damage of several aspects of the immune response, including: decrease of lymphocytes, smaller titles of antibodies in response to vaccines, alteration of the phagocytic function, decrease of the activity of the complement and of the cytokine production[7,9,17].

Most of the studies demonstrate that the M seems to have a particularly vast effect on the immunity mediated by cells[22,24]. Consequently, undernourished individuals are more susceptible to infection for obligatory intracellular pathogens. In mice, submitted to the moderate restriction of calories and proteins, a decrease was observed in the

[1]Doutorando(a) em Nutrição - UFPE.
[2]Profa. Adjunta do Depto de Medicina Tropical - UFPE
[3]Prof. Adjunto de Fisiologia da Nutrição do Depto de Nutrição - UFPE

Correspondência: Célia M. M. Barbosa de Castro. Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Recife, CEP 50670-901 PE, Brasil, Fax: 55 81 32718485 E-mail: ccastro@lika.ufpe.br

immunity mediated by cells[10]. According to Keusch (1981), a significant damage in the immunity mediated by the cells T, it is also observed in the severe M[16]. In the same way, Chandra (1997) tells that in the M there is a reduction in the proportion of T-helper lymphocytes and in the number of T-suppressor cells[8].

The macrophages play an important part in the defense of the host [11]. However, researches have been demonstrating that in the M several functions are harmed in these cells[21,2]. Reynolds et al. (1992) observed that the protein deficiency harms the activation of the macrophages[21]. This damage can commit the immune effector mechanisms, dependent of the macrophage activation, including rejection of intracellular pathogens. Besides, the macrophages number in the exudato peritoneal is reduced in undernourished animals[21]. In undernourished patients, it has also been observed a retard and a decrease in the mobilization of the monocytes[26].In rats, submitted to the protein privation, they were also found quantitative reductions, bankruptcy of the maturation and decrease of the proliferate capacity of the cells of Kupffer[14].

The macrophage is the largest source of liberation of IL-1, a cytokine that acts increasing the proliferation of the T lymphocytes. Reynalds and collaborators (1992) observed that, in rats, the protein deficiency harms the production and, consequently, the activity of IL-1 in response to endotoxins[21]. Besides, the production of TNF for activated macrophages, it is also harmed in the M process[3].

The migration and the cellular adhesion are two important stages in the phagocytic process. However, in mice submitted to the protein deficiency, it was observed decrease in the fibronectin expression and reduction in the adhesion of macrophages[2]. It was verified in experimental research that dietary restriction for 7 days reduces the expression of molecules of adhesion of polymorphonuclear neutrophils in the cavity peritoneal, in rat[15]. This implicates in damage of the response mediated by those phagocytes.

The complement system is another component of the immune system that can be altered in the M. The concentration and activity of most of the components of the complement are reduced in the nutritional depletion. Studies in children showed a reduction of the activity and of the components of the complement[16]. Besides the alterations mentioned previously, the cutaneous response of late hypersensibility is quite depressed in the M[6]. Nohr and colls (1985), investigating in mice, the effects of short period of nutritional privation on the immunity, observed that the test of late cutaneous hypersensibility and the responses to specific antigens were depressed[19]. In that study the nutritional recovery was enough to recuperate the corporal weight, but it didn't correct the appraised parameters, of the immune response.

Chandra (1991) refers that, for most of the authors, the humoral immunity is not affected directly by the protein-energy M, particularly when the antigen is not thymus-dependent[5]. According to Stiehm (1980), the B lymphocytes subpopulations, the serum IgA and IgG levels and immunoglobulin synthesis and metabolism are usually normal or increased in the M[27]. Nahani and collaborators (1996) investigating the relationship between nutritional state and immune response in children, they observed that the immunoglobulins G and D were normal in the moderate and severe M, however IgA was just elevated in the serious M[18].

Salimata and colls (1983) investigating in mice, the effects of a deficient alimentary ingestion, during the suckling period, on the immune response, observed that the animals maintained with an insufficient feeding, they presented a the depressed humoral immunity; the same not being observed with relationship to the animals nutritionally recovered[23]. As referred them researchers, the protein-energy M for short period, same induced in the critical period of development of the immune system, relatively, it produces smaller effect.

Besides the effects of the M mentioned previously, several studies have been demonstrating that the M can alter the nervous system and the endocrine system[1,12]. In the same way, undernourished rats in the critical period of development of the encephalon were not affected for anorexia induced by SSRI in the adult life[1]. Likewise, in a recent article, we observed that M during the suckling period causes reduction in the corticosterone levels in adult rats[12]. As the interaction among the physiologic systems (particularly Nervous System, Endocrine System and Immune System) can interfere in the immune response[12,13] we can conclude that the immune alterations provoked by the deficit nutritional, they cannot just be consequence of the lack of nutrients for the immune cells, but also of neuro-endocrine disturbances that happen in the M.

Barreto-Medeiros JM, De-Castro CMBB, Teles AMS, Montenegro EJN, Manhães-de-Castro R. Como a desnutrição altera o sistema imune? An. Fac. Med. Univ. Fed. Pernamb. Recife, 48(1), p.69-71, 2003.

RESUMO: O estado nutricional é um dos principais fatores que pode afetar o funcionamento do sistema imune e consequentemente alterar a capacidade do organismo de vencer as agressões do ambiente. Isso se deve ao fato de que as células do sistema imunológico como as células de qualquer outro sistema, dependem de nutrientes para o seu funcionamento apropriado. O presente trabalho faz uma revisão de como a desnutrição pode alterar o sistema imunológico.

UNITERMOS: Desnutrição; Sistema imune.

REFERENCES

1. Barreto Medeiros JM, Cabral Filho JE, De Souza SL, Freitas Silva SR, Mendes da Silva C, Deiró TCBJ et al. Early malnourished rats are not affected by anorexia induced by a selective serotonin reuptake inhibitor in adult life. Nutr Neurosci 2002; 5:211-214.
2. Borelli P, Souza IP, Borojevic R, Dagli ML Z, kang HC. Protein Malnutrition: Some Aspects of the in vitro adhesion of peritoneal mouse macrophages. Ann Nutr Metab 1998; 42: 367-373.
3. Bhaskaram P, Sivakumar B. Interleukin-1 in malnutrition. Arch Dis Child 1986; 61:182-5.
4. Brown JL, Pollitt E. Malnutrition, poverty and intellectual development. Sci Am 1996; 38-43.
5. Chandra RK. 1990 McCollum aware lecture: nutrition and immunity: lessons from the past and new insights into the future. Am. J. Clin. Nutr 1991; 53:1087.
6. Chandra RK. Protein - energy malnutrition and immunological responses. J. Nutr 1992; 122: 597-600.
7. Chandra RK, Kumari S. Nutrition and immunity: An overview. J Nutr 1994; 124: 1433-1435.
8. Chandra RK. Nutrition and immune system: na introdução. Am J Clin Nutr 1997, 66: 460 - 463.
9. Chandra RK. Nutrition and immunology: from the clinic to cellular biology and back again. Proc Nutr Soc 1999; 58 681-3.
10. Conge GA, Gouache P, Lagrange PH, Lemonnier D. Effect of moderate protein-calorie deprivation on cell-mediated immunity in mice. Ann Nutr Metab 1983; 27:286-95.
11. De-Castro CMB, Manhães de Castro R, Medeiros AF, Queirós Santos A, Silva WTF, Lima Filho JL. Effect of stress on the production of O2 in alveolar macrophages. J Neuroimmunol 2000; 108: 68-72.
12. De-Castro CMB, Manhães de Castro R, Queirós Santos A, Barreto-Medeiros JM, Silva WTF, Teles MAS et al. Neonatal Malnutrition Reduces Superoxide Production of Alveolar Macrophages in Adult Life. Free Radic Res, 2003. "in press".
13. De Simoni MG. Two-way communication pathways between the brain and the immune system. Neurosci Res Commun 1997; 21: 163-172.
14. Honda Y, Takahashi K, Naito M, Fujiyama S. The role of macrophage colony-stimulating factor in the differentiation and proliferation of Kupffer cells in the liver of protein-deprived mice. Lab Invest 1995; 72:696.
15. Ikeda S, Saito H, Fukatsu K, Inoue T, Han I, Furukawa S, Matsuda T, Hidemura A. Dietary restriction impairs neutrophil exudation by reducing CD11b/CD18 expression and chemokine production. Arch Surg 2001; 136 :297-304.
16. Keusch GT. Host defense mechanisms in protein energy malnutrition. Adv Exp Med Biol 1981; 135:183-209.
17. Lesourd MB. Nutrition and immunity in the elderly: modification of immune rsponses with nutritional treatments. Am J Clin Nutr 1997; 66: 478-484.
18. Nahani J, Nik-aAeen A, Rafii M, Mohagheghpour N. Effect of malnutrition on several parameters of the immune system of children. Nutr Metab 1976; 20:302-6.
19. Nohr CW, Tchervenkov JI, Meakins JL, Christou NV. Malnutrition and humoral immunity: Short-term acute nutritional deprivation. Surgery 1985, 14-16.
20. Pessoa DCNC. Efeitos da desnutrição pela Dieta Básica Regional (DBR), em três gerações de ratos, sobre a reprodução, crescimento corporal e desenvolvimento de órgãos. Tese (Doutorado em Nutrição) - Universidade Federal de Pernambuco, Recife 1997, p 118.
21. Revillard JP, Cozon G. Experimental models and mechanisms of immune deficiencies of nutritional origin. Food Addit Contam 1990; 1:82-6.
22. Rikimaru T, Taniguchi K, Yartey JE, Kennedy DO, Nkrumah FK. Humoral and cell-mediated immunity in malnourished children in Ghana. Eur J Clin Nutr 1998, 52:344-50.
23. Salimata W, Lemonnier D, Bleiberg F, Delorme J. Early Nutritional Experiments: Effects on the Humoral and Cellular Immune Responses in Mice. J Nutr 1983; 113: 1131-1139.
24. Scrimshaw NSE, Sangiovanni JP. Synergism of nutrition, infection, and immunity: na overview. Am J Clin Nutr 1997, 66: 464-477.
25. Sirisinha S, Edelman R, Suskind RM. Complement and C3-proactivator levels in children with protein calorie malnutrition and effect of dietary treatment. Lancet 1973; 1:1016-9.
26. Smythe PM, Brereton-Stiles GG, Grace HJ. Thymo-lymphatic deficiency .and depression of cell-mediated immunity in protein-calorie malnutrition. Lancet 1971; 11:939-43.
27. Stiehm ER. Humoral immunity in malnutrition. Fed Proc 1980; 39: 3093-7.

JUSTIFICATIVA

2 - JUSTIFICATIVA

O estudo das respostas agressivas em animais, desnutridos durante o período crítico de desenvolvimento do encéfalo e submetidos a manipulação farmacológica do sistema serotoninérgico na vida adulta é de grande importância, para se verificar possíveis alterações permanentes que o sistema serotoninérgico possa sofrer no processo de desnutrição.

Outro aspecto importante é analisar a resposta imune em animais normais ou desnutridos após comportamento agressivo. Que repercussão tem a agressividade na resposta imune? E qual o papel da desnutrição nesse fenômeno?

Alguns fármacos, entre eles os inibidores de recaptura de serotonina, vêm sendo utilizados na prática clínica no tratamento de transtornos alimentares ou psiquiátricos. Assim, investigar os efeitos dessas drogas sobre os comportamentos alimentar e agressivo é relevante, particularmente no indivíduo desnutrido no início da vida.

OBJETIVOS

3 - OBJETIVOS

3.1 - Geral

Investigar os efeitos da desnutrição pregressa e/ou da manipulação farmacológica do sistema serotoninérgico sobre o consumo alimentar, o comportamento agressivo e a inter-relação entre agressividade intraespecífica e resposta imune em ratos adultos.

3.2 - Específicos

- Estudar os efeitos da desnutrição durante o período de rápido desenvolvimento do cérebro e do tratamento com citalopram sobre o comportamento agressivo;

- Analisar os efeitos da desnutrição durante o aleitamento sobre o consumo alimentar em animais tratados ou não com citalopram;

- Avaliar os efeitos da fluoxetina sobre a agressividade intraespecífica em animais submetidos à desnutrição durante o período de rápido desenvolvimento do cérebro;

- Investigar as conseqüências sobre a resposta imune da expressão da agressividade intraespecífica frente a um estressor;

- Analisar os efeitos da desnutrição durante o período de aleitamento sobre o padrão leucocitário e a resposta imune humoral em animais submetidos à agressividade intraespecífica.

HIPÓTESES

4 – HIPÓTESES

- Na dependência de sua ação sobre a recaptura da 5-HT, o tratamento farmacológico crônico reduz as respostas agressivas intraespecíficas.

- Tratamento crônico com citalopram diminui o consumo alimentar.

- Agressividade intraespecífica diminui a resposta imune.

- Desnutrição precoce reduz o efeito do tratamento com inibidor seletivo de recaptação de serotonina sobre o comportamento agressivo.

- Desnutrição durante o período de aleitamento reduz a anorexia induzida por citalopram.

- A desnutrição pregressa, mesmo após longo periodo de recuperação nutricional, prejudica a inter-relação entre o comportamento agressivo e a resposta imune.

APRESENTAÇÃO DOS ARTIGOS

5 – APRESENTAÇÃO DOS ARTIGOS

No presente trabalho foram avaliados em ratos adultos os efeitos da desnutrição precoce e/ou de inibidor seletivo de recaptação de serotonina sobre o consumo alimentar e agressividade intraespecífica. Outrossim, foram estudadas as inter-relações entre desnutrição pregressa, agressividade e parâmetros da resposta imune. Desta pesquisa, foram originados cinco artigos científicos. Destes, dois foram publicados, dois aceitos e um está submetido. Todos os artigos serão apresentados aqui em ordem cronológica e em suas versões originais.

5.1. Primeiro artigo, intitulado: **"ACTION OF SELECTIVE SEROTONIN REUPTAKE INHIBITOR ON AGGRESSIVE BEHAVIOR IN ADULT RAT SUBMITTED TO THE NEONATAL MALNUTRITION".** Publicado como artigo original na revista: *Arq Neuropsiquiatr*, 59(3-A):499 – 503, 2001.

Neste artigo, foram estudados os efeitos da desnutrição durante o aleitamento sobre a agressividade intraespecífica em ratos adultos tratados ou não com inibidor seletivo de recaptação de serotonina. Neste estudo foi observado que a desnutrição durante o período de desenvolvimento rápido do cérebro tornou os ratos adultos resistentes aos efeitos anti-agressividade do citalopram.

Arq Neuropsiquiatr 2001;59(3-A):499-503

ACTION OF SELECTIVE SEROTONIN REUPTAKE INHIBITOR ON AGGRESSIVE BEHAVIOR IN ADULT RAT SUBMITTED TO THE NEONATAL MALNUTRITION

Jairza Maria Barreto Medeiros[1], Cristiano Mendes da Silva[2], Everton Botelho Sougey[3], José Audisio Costa[4], Célia Maria M. Barbosa de Castro[5], Raul Manhães de Castro[6]

ABSTRACT- The effect of the malnutrition during suckling on the aggressiveness was investigated in adult rats treated or not with citalopram, a selective serotonin reuptake inhibitor (SSRI). The animals were divided into two groups according to the diet used: nourished group– the rats received the control diet with 23% protein during the life; and malnourished group– the rats had its mothers submitted to diet with 7.8% protein during suckling. At 120 days of age, each group was sub-divided according to the treatment: acute – consisting a single i.p. injection of saline solution or 20-mg/Kg citalopram; chronic – consisting the single injections (1 per day during 14 days) of saline or 20 mg/Kg citalopram. The acute or chronic treatment with SSRI reduces aggressive response in nourished rats, but not in malnourished ones. Thus, the malnutrition during the critical period of brain development seems to induce durable alterations in the function of the serotoninergic neurotransmission

KEY WORDS: aggressive behavior, malnutrition, serotonin.

Ação de inibidor seletivo da recaptação de serotonina sobre comportamento agressivo em rato adulto submetido à desnutrição neonatal

RESUMO - O efeito da desnutrição durante a lactação sobre a agressividade foi investigado em ratos adultos tratados ou não com citalopram, um inibidor seletivo da recaptação de serotonina (ISRS). Os animais foram divididos em dois grupos de acordo com a dieta: grupo nutrido– ratos que receberam toda a vida dieta controle (23% de proteína); e grupo desnutrido– ratos que tiveram suas mães submetidas a dieta com 7,8% de proteína na lactação. Aos 120 dias de idade, cada grupo foi sub-dividido conforme o tratamento: agudo – consistindo de injeção única i.p. de solução salina ou 20mg/Kg de citalopram; crônico - consistindo de injeções únicas (1 por dia durante 14 dias) de salina ou 20mg/Kg de citalopram. O tratamento agudo ou crônico com ISRS reduziu a resposta agressiva nos ratos nutridos, mas não nos desnutridos. Assim, a desnutrição durante o período crítico de desenvolvimento do cérebro parece acarretar alterações duradouras na função da neurotransmissão serotoninérgica.

PALAVRAS-CHAVE: comportamento agressivo, desnutrição, serotonina.

The role of serotonin in the control of the aggressive behavior has been demonstrated through the use of pharmacological instruments[1]. There are drugs that act through the selective serotonin (5-HT) reuptake inhibition; these substances increase the availability of 5-HT in the synapse and, consequently, the action of this monoamine[2]. Among these substances are the citalopram, one of the most selective serotonin reuptake inhibitors (SSRI)[3]. The growth of the central nervous system (CNS) and its developmental processes (gliogenesis, neuronal differentiation, migration, synaptogenesis, etc) occur with great intensity during the suckling period in the rat[4]. The brain is more vulnerable to several types of the aggressions in that phase[4]. Thus, nutritional insults can cause irrevers-

Estudo realizado no Departamento de Nutrição, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brasil; [1]Doutoranda em Nutrição da UFPE, Professora assistente do Departamento de Ciências da Nutrição, Universidade Federal da Bahia; [2]Mestrando em Nutrição da UFPE; [3]Doutor em Psiquiatria da Universidade Estadual de Campinas, Professor Adjunto do Departamento de Neuropsiquiatria, UFPE; [4]Doutor em Fisiologia da Universidade de São Paulo (USP - Ribeirão Preto), Professor Adjunto do Departamento de Nutrição, UFPE; [5]Doutora em Farmacologia da Universidade Federal do Ceará, Professora Adjunta do Departamento de Medicina Tropical, UFPE; [6]Doutor em Farmacologia Experimental e Clínica da Universidade de Paris VI, Professor Adjunto do Departamento de Nutrição, UFPE. CAPES, CNPq, UFPE and UFBA supported this investigation.

Received 12 December 2000, received in final form 2 March 2001. Accepted 12 March 2001.

Dr. Raul Manhães de Castro - Departamento de Nutrição, UFPE, Cidade Universitária - 50670-901 Recife PE - Brasil. FAX 81 271 8473. E-mail: rcastro@nutricao.ufpe.br

ible alterations[5]. This period is called "brain growth spurt"[6]. In the man, it begins in the prenatal period (last quarter of gestation) continuing until the first years of life[4].

The malnutrition during the neonatal period in rats results in major neurochemical alterations including those in the neurotransmitter systems[7]. The effects of nutritional insults on the neurotransmitter systems, in particular the serotoninergic one, deserves special attention. This system participates in a wide variety of the functions of the CNS[8]. There are several experimental evidences about the effects of malnutrition on the serotoninergic system[9-11]. However, there are few works on the effects of manipulations of this system in undernourished subjects, particularly concerning the behavioral expression[12,13].

Thus, the present work investigated the effects of the malnutrition during the critical period of brain development and the effects of the treatment with SSRI, on the aggressive behavior in adult rats.

METHOD

Male Wistar rats maintained at room (23 ± 1° C) and on a light (6:00 a.m. to 6:00 p.m.) –dark (6:00 p.m. to 6:00 a.m.) cycle was used. The male offspring of rats were kept with 6 animals. The animals were divided according to the diet employee during suckling: nourished group – the rats received the control diet with 23% of protein (Purina of Brazil Ltd.); and malnourished group– the rats had its mothers submitted to diet with 7.8% protein ("Regional Basic Diet" - RBD) during suckling. The composition of RDB is shown in Table 1[14]. After weaning (24 days after birth), all rats received the control diet *ad libitum* until the day of the experiment. Body weights were determined on the 1st, 24th, 60th and 120th day. On the 120th day after birth, each group was subdivided according to the paradigm used for drug treatment: acute – consisting a single i.p. injection (1ml/kg) of saline (0,9% NaCl solution) or citalopram (20 mg/Kg, Lundbeck); chronic – consisting the single injections (1ml/kg; 1 per day during 14 days) of saline or 20 mg/Kg citalopram. The citalopram was dissolved in saline. It was formed 8 experimental groups, each one containing 20 animals. This way, the groups of adult rats were constituted, into nourished or malnourished ones in the suckling period, acute or chronically treated or not with citalopram. During the 14 days of the chronic treatment the animals were housed individually in cage. The animals were submitted to the aggressiveness tests 1-h after the acute treatment or 24h after the chronic treatment. The aggressiveness tests were accomplished

Table 1. Centesimal composition of "Regional Basic Diet" (RBD).

Ingredients	Centesimal composition						
	g%	Proteins	Carbohydrates	Fats	Ash	Fibers	Kcal %
Beans [a]	18.34	3.99	10.66	0.24	0.57	1.09	60.76
Manioc flour	64.81	0.84	48.59	0.12	0.43	5.64	198.80
Poor fat-dried and salted	3.74	2.74	-	0.06	0.06	-	11.50
Dried and salted meat fat	0.35	-	-	0.35	-	-	3.15
Sweet potato [a]	12.76	0.30	9.99	0.03	0.20	0.48	41.43
	100	7.87	69.24	0.80	1.26	7.21	315.6

a. cooked and dried.

Table 2. Body weight of rats nourished or malnourished during suckling.

Experimental groups	Weight (g)			
	1st day	24th day	60th day	120th day
Nourished groups	7.53 ± 0.13	44.35 ± 1.55	214.9 ± 6.76	322.75 ± 9.77
Malnourished groups	7.29 ± 0.12	23.75 ± 1.07*	173.3 ± 8.71*	275.25 ± 5.34*

Rats nourished received the control diet with 23% protein during the life; and rats malnourished had its mothers submitted to diet with 7.8% protein during suckling. The animals were weighed on the 1st, 24th, 60th and 120th day. The data are reported as mean ± SD. * $p < 0.01$ compared to the nourished group at the same age (two-tailed Student t-test).

in an acoustic isolated room, by using a box (20 x 20 x 20 cm) with the floor consisting of parallel metallic bars (inter-bar distance: 1.3cm), connected to an electric scrambled current source. The test consisted of placing a pair of rats of the same group (matched by weight) in the box, where they received a session of stimuli to induce aggressive responses. Each stimulus (an electric foot-shock) was represented by a 1.6 mA - 2 s current pulse. Each session lasted 20 min and was composed by 5 stimuli separated by a 4-min interval. During the first 3 min of this interval, the duration of the aggressive response was quantified by using a digital chronometer. So the total time for observation of aggressive behavior was 900 s. The annotations and the verification of the equipment were preceded in the last minute of each interval. The aggressive response was defined as the presentation of, at least, one of the two following behaviors: a) the animals stayed lifted up on the hind paws, facing one to the other, in a threatening attitude but without direct contact, or b) they maintained evident physical contact (besides being scratched, exhibition of the teeth and emission of characteristic vocalization). The data were compared by the two-tailed Student t-test (body weight) or by the Mann-Whitney U-test (aggressiveness) with the level of significance set at $P \leq 0.05$.

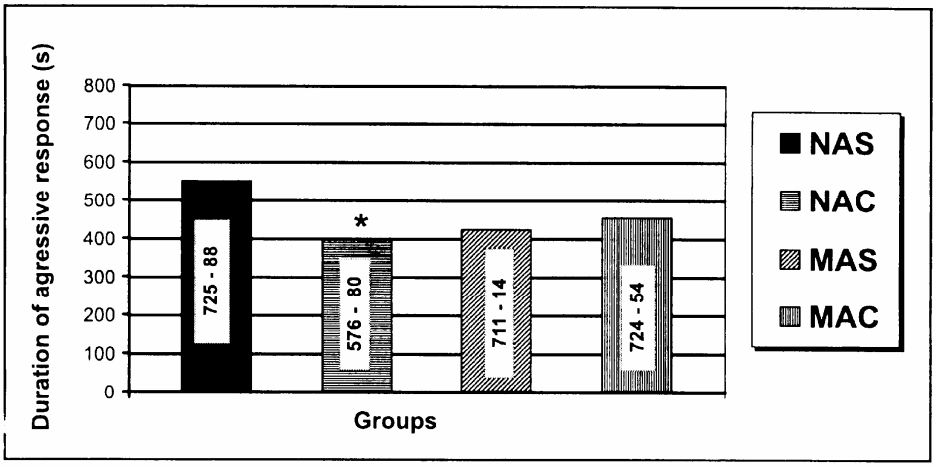


*Fig 1. Aggressive responses of rats nourished or malnourished during sucking period, submitted or not to acute treatment with citalopram. The groups nourished acute saline (NAS), malnourished acute saline (MAS) nourished acute citalopram (NAC), malnourished acute citalopram (MAC) were obtained. For each group (20 rats/group), the data are reported as median of time of aggression in seconds, with a range reported inside the columns. *P<0.05 compared to saline-treated nourished animals (Mann – Whitney two-tailed U-test).*

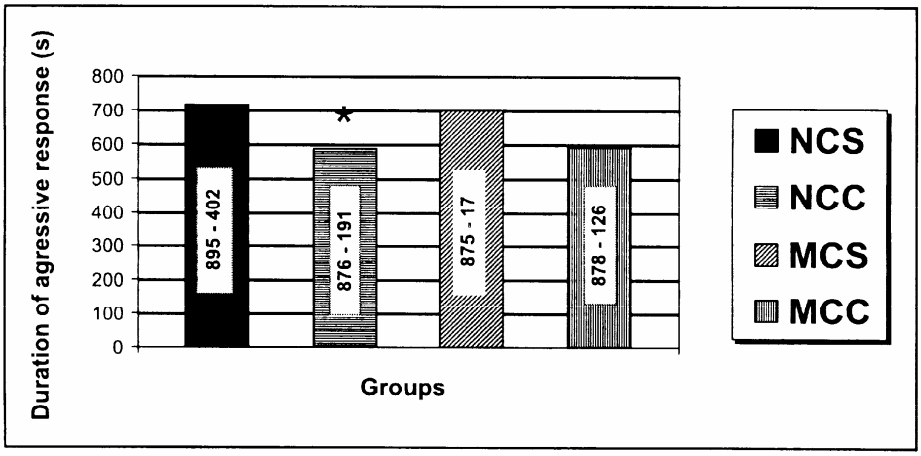


*Fig 2. Aggressive responses of rats nourished or malnourished during sucking period, submitted or not to chronic treatment with citalopram. The groups nourished chronic saline (NCS), malnourished chronic saline (MCS) nourished chronic citalopram (NCC), malnourished chronic citalopram (MCC) were obtained. For each group (20 rats/group), the time of aggression this represented in the columns (median), inside the same ones they are the values maximum and minimum. * P<0,05 compared to saline treated nourished animals (Mann – Whitney two-tailed U-test).*

RESULTS

Compared to the nourished (Table 2) , malnourished rats showed a reduction in body weight on the 24th, 60th and 120th day. The acute or chronic treatment with citalopram reduced the aggressive responses in nourished group but not in malnourished one (Figs 1 and 2). The high values of the time of aggressiveness in the animals submitted to the chronic treatment can be due to the largest time of isolation in the cages.

DISCUSSION

The present study showed that malnutrition during the critical period of brain development impaired the weight evolution of the rats. This effect can be a consequence of the protein deficiency that was imposed the mothers during the suckling period. In this phase, the protein deficiency causes alterations in the quality of the maternal milk[15,16] that induces damage of the growth of the body in several species of mammals[4]. The treatment with citalopram reduced the intraspecific aggressive response in the nourished rats. This anti-aggressiveness effect of the selective serotonin reuptake inhibitor (SSRI) may be a consequence of an increase in the serotoninergic transmission. The SSRI increase the availability of 5-HT in the synapse and, consequently, the action of this monoamine[2]. However, the malnutrition during brain growth spurt blocked the effect of the SSRI on aggressive behavior in adult rats.

The reduction of aggressiveness after treatment with SSRI found in the present work agrees with previous studies accomplished in humans[17] and in animals[2,18]. The serotonin has an important role on the emotional processes[19]. Serotoninergic projections innervate cerebral areas that participate in the control of the aggressive behavior[19]. The reduction of the serotoninergic activity seems to increase the aggressiveness[20]. In contrast, the SSRI treatment increasing the synaptic availability of the 5-HT diminishes the aggressive behavior[8,21]. The reduction of the aggressiveness after the treatment with SSRI may be the consequence of the action of the serotonin on the postsynaptic receptors[20,22,23].

In the present study, the neonatal malnutrition interfered in the effect of the SSRI on the intraspecific aggressiveness in the adult rats. This alteration could be related to the nutritional insult during the neonatal phase. The malnutrition during the neonatal period results in major neurochemical consequences, including those in the neurotransmitter systems[7]. In rats, the first serotoninergic neurons appear between the 12° and the 14° day of gestation[24], but the final density and definitive location of terminals, it is established during the postnatal maturation of the central nervous system[25]. Thus, the malnutrition imposed early in life could cause alterations in the serotonin neurotransmission system, reflecting on its functional responses to drugs. Some drugs which act in the central nervous system has its effects diminished in the malnourished animals[12,13,26]. This suggests a sequel of malnutrition on the serotoninergic system.

In conclusion, the malnutrition during the critical period of brain development renders adult rats resistant to the effect of the SSRI on the aggressive response. The nutritional insult appears to have an enduring effect upon the functioning of the serotoninergic system. Though, it is not still clear which components of the serotoninergic system are altered in a persistent way by the nutritional aggression.

Acknowledgements - The authors thank Lúcia Maria Pires Ferreira for her superb and timely assistance.

REFERENCES

1. Manhães-de-Castro R, Barreto Medeiros JM, Deiró TCBJ, Mendes da Silva C, Freitas Silva SR, Souza SL. Neonatal treatment with selective serotonin reuptake inhibitor reduces intraspecific aggression in rats. Neurobiologia 1998;61:61-69.
2. Sanchez C, Meier E. Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Psychopharmacology 1997;129:197-205.
3. Hyttel J. Effect of a specific 5-HT uptake inhibitor, citalopram (Lu 10-171), on 3H-5-HT uptake in rat brain synaptosomes in vitro. Psychopharmacology 1978;60:13-18.
4. Morgane PJ, Miller M, Kemper T, et al. The effects of protein malnutrition on the developing central nervous system in the rat. Neurosci Biobehav Rev 1978;2:137-230.
5. Morgane PJ, Austin-la France RJ, Bronzino J, Tonkiss J, Galler JR. Malnutrition and developing central nervous system. In Isaacson RL, Jensen KF (eds). The vulnerable brain and environmental risks. New York: Plenum Press 1992;1:2-42.
6. Dobbing J. Vulnerable periods in developing brain. In Davison AN, Dobbing J (eds). Applied neurochemistry. Oxford: Blackwell 1968:287-316.
7. Winick M, Brasel JA, Rosso P. Nutrition and cell growth. In Winick M (ed). Nutrition and development. London: John Wiley & Sons 1972:49-98.
8. Chopin P, Moret C, Briley M. Neuropharmacology of 5-hydroxytryptamine$_{1B-1D}$ receptor ligands. Pharmacol Therap 1994;62:385-405.
9. Stern WC, Miller M, Forbes WB, Morgane PJ, Resnick O. Ontogeny of the levels of biogenic amines in various parts of the brain and in peripheral tissue in normal and protein malnourished rats. Exper Neurol 1975;49:314-326.
10. Wiggins RC, Fuller G, Enna SJ. Undernutrition and the development of brain neurotransmitter systems. Undernutr Brain Develop 1984;35:2085-2094.
11. Chen JC, Tonkiss J, Galler J, Volicer L. Prenatal protein malnutrition in rats enhances serotonin release from hippocampus. J Nutr 1992;122: 2138-2143.
12. Hall RD, Leahi JP, Robertson WM. Hyposensitivity to serotoninergic stimulation in protein malnourished rats. Physiol Behav 1983;31:187-195.
13. Almeida SS, Tonkiss J, Galler JR. Malnutrition and reactivity to drugs acting in the central nervous system. Neurosci Biobehav Rev 1996;20:389-402.
14. Teodosio NR, Lago ES, Romani SAM, Guedes RCA. A regional basic diet from Northeast Brazil as a dietary model of experimental malnutrition. Arch Latinoamer Nutr 1990;40:533-547.

15. Marin MC, Thomas ME, Serres C, Mercuri O. Protein-energy malnutrition during gestation and lactation in rats affects growth rate brain development and essential fatty acid metabolism. J Nutr 1995; 125: 1071-1024.

16. Sturman JA, Devine E, Resnick O, Morgane PJ. Maternal protein malnutrition in the rat: effect on protein and two enzymes in the milk. Nutr Res 1986;6:437-442.

17. Coccaro EF, Kavoussi RJ. Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. Arch Gen Psychiatry 1997;54: 1081-1088.

18. Sanchez C, Hyttel J. Isolation-induced aggression in mice: effects of 5-hydroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT_{1A} receptors. Eur J Pharmacol 1994;264:241-247.

19. Olivier B, Mos J, Oorschot RV, Hen R. Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 1995;28(suppl 2):80-90.

20. Kyes GB. Aggression and brain serotoninergic responsivity: response to slides in male macaques. Physiol Behav 1993;2:205-208.

21. Fuller RW. Pharmacology of central serotonin neurons. Ann Rev Pharmacol Toxicol 1980;20:111-127.

22. Applegate CD. 5,7-Dihydroxytryptamine-induced mouse killing and behavioral reversal with ventricular administration of serotonin in rats. Behav Neural Biol 1980;30:178-190.

23. Eichelman BS. Neurochemical and psychopharmacologic aspects of aggressive behavior. Ann Rev Med 1990;41:149-158.

24. Lauder JM, Bloom FE. Ontogeny of monoamine neurons in locus coeruleous, raphe nuclei and substantia nigra of the rats I, Cell differentiation. J Comp Neurol 1974;155:469-481.

25. Lidov HGW, Molliver ME. An immunohistochemical study of serotonin neuron development in the rat: ascending pathways and terminal fields. Brain Res Bull 1982;8:389-416.

26. Rotta FT, Mello CF, Rocha JBT, Souza DOG. Early undernutrition blocks the effect of naltrexone on rat exploratory behavior. Braz J Med Biol Res 1988;2:305-312.

5.2. Segundo artigo, intitulado: **“EARLY MALNOURISHED RATS ARE NOT AFFECTED BY ANOREXIA INDUCED BY A SELECTIVE SEROTONIN REUPTAKE INHIBITOR IN THE ADULT LIFE”** Publicado como artigo original na revista: *Nutritional Neuroscience*, 5(3):211–214, 2002.

Em seqüência ao estudo apresentado no primeiro artigo, referente a influencia da desnutrição sobre o sistema serotoninérgico, o presente trabalho teve como principal objetivo investigar os efeitos da desnutrição durante o aleitamento sobre o consumo alimentar em ratos adultos tratados ou não com citalopram. Neste estudo foi observado que a desnutrição durante o período de desenvolvimento rápido do cérebro alterou a anorexia induzida por citalopram em ratos adultos.

Nutritional Neuroscience, 2002 Vol. 5 (3), pp. 211–214

Taylor & Francis
healthsciences

Early Malnourished Rats are not Affected by Anorexia Induced by a Selective Serotonin Reuptake Inhibitor in Adult Life

J.M. BARRETO MEDEIROS[a], J.E. CABRAL FILHO[b], S.L. DE SOUZA[a], S.R. FREITAS SILVA[a], C. MENDES DA SILVA[c], T.C.B.J. DEIRÓ[a], J.M. MONTEIRO[a], R.C.A. GUEDES[c], C.M.M.B. DE CASTRO[d] and R. MANHÃES DE CASTRO[b,*]

[a]*Departamento Ciências da Nutrição, Universidade Federal da Bahia, Salvador, BA, Brazil;* [b]*Instituto Materno Infantil de Pernambuco, Recife, PE, Brazil;* [c]*Departamento de Nutrição, Universidade Federal de Pernambuco, Cidade Universitária, 50670-901, Recife, PE, Brazil;* [d]*Laboratório de Imunologia Keizo Asami, Universidade Federal de Pernambuco, Cidade Universitária, Recife, PE, Brazil*

(Received 11 October 2001; Revised 8 November 2001; In final form 7 January 2002)

The effect of early postnatal malnutrition upon food intake and its modulation by the selective serotonin reuptake inhibitor (SSRI) citalopram, was investigated in adult rats. Sixty four Wistar rats were allocated to two groups, according to their mother's diet during lactation. Mothers receiving a 23% protein diet fed the well-nourished group; mothers receiving 8% protein diet fed the malnourished. After weaning, all rats received the 23% protein diet *ad libitum*. On the 120th day after birth, each nutritional group was divided in two subgroups (each one, $n = 16$) which received a single daily injection of citalopram (10 mg/kg) or saline (0.9% NaCl) for 14 days. Chronic treatment with citalopram decreased both the food intake and weight gain in the well-nourished rats, but not in the malnourished ones. These data are consistent with findings concerning the nutritional manipulation of the nervous system during its higher vulnerable phase, suggesting that early malnutrition alters the effect of treatment of SSRI in adult rats, and that malnutrition during the critical period of brain development affects the serotoninergic system.

Keywords: Feeding behavior; Food intake; Malnutrition; Selective serotonin reuptake inhibitor

INTRODUCTION

The brain growth spurt (BGS) is an early phase of mammal development in which the brain growth occurs at a high velocity (Dobbing, 1968). This period varies among animal species throughout the gestation and suckling periods. In humans, it begins in the prenatal period (last quarter of gestation) continuing until the first years of life; in rats it corresponds to the first three weeks of postnatal life (Dobbing, 1968). It has been shown that nutritional insults in this phase can cause deleterious long-lasting effects on the brain and neural functions (Winick *et al.*, 1972; Morgane *et al.*, 1978; 1993). Depending on the severity of the nutritional deficit, these effects may not be able to be reversed even by further dietary treatment or environmental stimulation (Morgane *et al.*, 1978; 1992).

Some of the consequences of protein malnutrition are major neurochemical alterations including those in neurotransmitter systems (Wiggins *et al.*, 1984; Chen *et al.*, 1992; 1995). Rats submitted early in life to low protein diets reveal altered brain levels of noradrenaline, dopamine, and serotonin (Resnick *et al.*, 1979; Wiggins *et al.*, 1984; Chen *et al.*, 1995). The effects of malnutrition on the serotoninergic system has deserved special attention, since this system plays a role in several functions, such as, pain sensitivity, sleep control, mood, sexual behavior, aggression and feeding control (Chopin *et al.*, 1994). Moreover, there is mounting evidence that serotonin applied to embryonic rat tissue acts as a neurotrophic factor, this action including growth stimulation and improved survival of the serotoninergic neurons themselves (Palén *et al.*, 1979; Liu and Lauder, 1992). On the other hand, PCPA-induced

*Corresponding author. Tel.: +81 271-8471. Fax: +81-271-8473. E-mail: rcastro@nutricao.ufpe.br

ISSN 1028-415X print/ISSN 1476-8305 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1028415O290029197

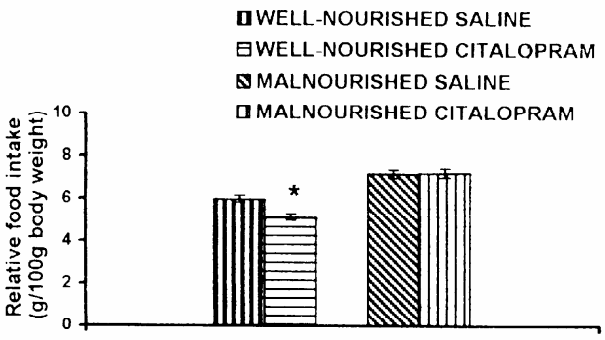


FIGURE 1 Relative food intake of citalopram or saline-treated adult rats, fed by mothers receiving a 23% or a 8% protein diet during lactation. Data represent mean (+ SEM) of 16 animals. Comparisons between saline and citalopram in the same nutritional group were made by two-tailed Students *t*-test ($*p < 0.001$).

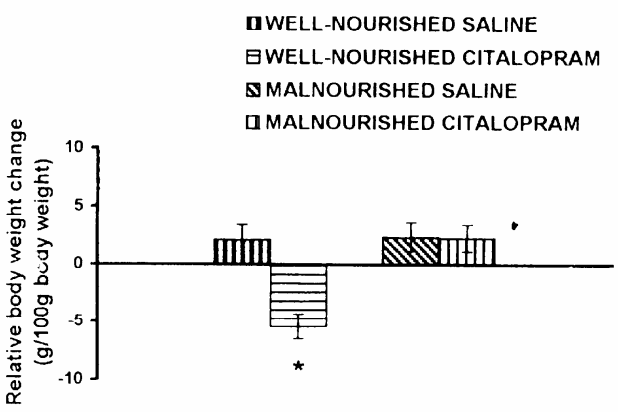


FIGURE 2 Relative body weight change of citalopram or saline-treated adult rats, fed by mothers receiving a 23% or a 8% protein diet during lactation. Data represent mean (+ SEM) of 16 animals. Comparisons between saline and citalopram in the same nutritional group were made by two-tailed Students *t*-test ($*p < 0.001$).

serotonin depletion in rats aged 10–20 days resulted in diminished serotonin synaptic density in adult life (Mazer *et al.*, 1997). As a consequence, the possibility exists that protein energy malnutrition imposed during the initial phase of life (so providing an increased brain serotonin concentration) could induce long-lasting biobehavioral effects which depend on neural serotoninergic activity. It is not surprising that prenatal protein malnutrition enhances brain levels of serotonin and reduces the REM sleep of adult rats as shown by Datta *et al.* (2000). However, although serotoninergic drugs have been largely used in the treatment of psychiatric or feeding disorders, there are few studies which have been done concerning the influence of pharmacological manipulations of the serotoninergic system in malnourished individuals.

The present study was undertaken to test the hypothesis that protein malnutrition during BGS induces alterations on the feeding behavior of adult rats treated with citalopram, one of the most selective serotonin reuptake inhibitors (SSRI) (Hyttel, 1978).

MATERIALS AND METHODS

Sixty four male Wistar rats maintained at a room temperature of 23 ± 1°C and on a light–dark cycle (light 6:00 a.m.–6:00 p.m.) were used. During suckling, the offsprings were kept in groups of six pups, randomly assigned to each mother. They were distributed in two nutritional groups according to the mother diet during lactation: the well-nourished group was fed by mothers receiving a 23% protein diet (purina chow) and the malnourished group, by mothers fed a 8% protein diet. After weaning (on the 25th day of age) all rats received the 23% protein diet. On the 120th day after birth, the well-nourished animals (body weight: 350 ± 42 g) and the malnourished ones (275 ± 30 g) were divided according to the four following treatment: (a) well-nourished plus saline (WNS); (b) well-nourished plus citalopram (WNC); (c) malnourished plus saline (MNS); (d) malnourished plus citalopram (MNC). Single injections of citalopram (10 mg/kg, i.p., 1 ml/100 mg, dissolved in saline) or saline (0.9% NaCl) were administered during 14 days (120th–134th day of life). During the treatment period, the animals were housed in individual cages ($15 \times 15 \times 25\,cm^3$) each one supplied with a meal dispenser, a water dispenser and a collector for feces and urine. The body weight and the offered food amount were daily registered on an electronic balance (accuracy 0.1 g) between 12:00 a.m. and 2:00 p.m.. The daily food intake of each animal was calculated by the difference between the amount (g) of meal offered and that rejected during the 24-h period. The relative changes (food intake or body weight gain/100 g body weight) of these variables in the treatment period were calculated. The difference between saline and citalopram treatment in each nutritional group was analyzed by two-way Student's *t*-test with $p < 0.05$. The experimental protocol for this work was approved by the Ethical Committee for animal research of the Federal University of Pernambuco, Recife, Pernambuco, Brazil.

RESULTS

As can be seen in Fig. 1, during the 14 days of the pharmacological treatment period, the WNC group reduced its relative food intake when compared to WNS ($t = 3.778$, $p =< 0.001$), but the MNC did not differ significantly from the MNS group ($t = -0.137$, $p = 0.892$). Concerning the relative body weight change (Fig. 2), the WNC group revealed a weight

loss equivalent to 5.4% as compared to the WNS one ($t = 4.490$, $p < 0.001$). Both the MNC and MNS groups showed a relative weight gain of 2.4%, the difference between them not being significant ($t = 0.058$, $p = 0.954$).

DISCUSSION

The inhibitory role of serotonin on feeding control (Toornvliet *et al.*, 1996; Halford and Blundel, 1996; Halford *et al.*, 1997) is supported by the present results, since well-nourished animals injected with citalopram presented both a reduced food intake and a lower body weight gain, when compared to saline-injected animals of the same nutritional group. The supposed mechanism of this effect is the increased brain serotonin release following synaptic blocking of the amine induced by citalopram, as shown by others (Hyttel, 1978; Gobbi *et al.*, 1997). In fact, the 14-day treatment period used here has been considered by others as being sufficient to induce significant changes in the serotoninergic system (Arborelius *et al.*, 1996; Barreto Medeiros *et al.*, 2001; Manhães-de-Castro, 2001).

The citalopram-induced anorexic effect observed in this study corroborates findings with other serotoninergic agents (Lucki *et al.*, 1988; Halford and Blundel, 1996; Lucas *et al.*, 1998; Li *et al.*, 1998). Hyttel (1994) indicates that the repeated citalopram administration maintains a selective and potent inhibition of serotonin reuptake. It is well known that serotonin, tryptophan and some serotoninergic agonists produce fasting and substantial anorexia (Blundel, 1992). Therefore, it is likely that the hypophagic effect of citalopram, as observed in this work, results from its action on the serotoninergic system.

The lack of response to the citalopram anorexic action observed in malnourished animals is a major subject of this study and deserves attention. Diminished bio-behavioral responses of malnourished individuals have been known for a long time. (Levstsky and Barnes, 1972). These authors showed that rats submitted to early malnutrition present a reduced sensorial interaction with the environment. Rotta *et al.* (1988) showed that early malnutrition blocks the effect of opioid-antagonists (naltrexone) on rat exploratory behavior. In addition, neural hypo-responsiveness of undernourished animals to pharmacological manipulations of neurotransmitter systems was elsewhere observed (Guedes *et al.*, 1992). These authors have shown that, in contrast to well-nourished control rats, the rats submitted during suckling to protein deficiency (8% protein diet) failed to change the velocity of cortical spreading depression, when challenged by a GABA agonist (diazepam) or GABA-antagonists (picrotoxin and bicuculline).

Furthermore, as long ago as the eighties, Hall *et al.* (1983) showed that early protein malnourished rats stimulated by the serotonin agonist *N*, *N*-dimethyl-tryptamine presented a lower performance than well-nourished animals in several behavioral tests, such as the serotoninergic syndrome, the rota-rod and the treadmill. These responses might be associated to serotonin neuronal processes and to alterations of brain serotonin due to malnutrition. In rat embryos, the first serotoninergic neurons appear between the 12th and the 14th gestation day (Lauder and Bloom, 1974), and the final density and definitive location of terminals establish during postnatal maturation of the central nervous system (Lidov and Molliver, 1982). This density and location might be improved by the increased brain serotonin deriving from malnutrition, because of the role of this amine on serotoninergic ontogenesis itself (Whitaker-Azmitia, 1991; Liu and Lauder, 1992). It is worth noting that $5HT_{1a}$ receptors mediate this ontogenetic activity (Yan *et al.*, 1997). Moreover, a 30% reduction of Bmax values of 3H—paroxetine binding was observed in the frontal cortex of young rats which had undergone chronic food restriction (Huether *et al.*, 1997). So it is likely that changes in serotoninergic receptors are involved in brain neurotransmitter responses to malnutrition.

From the above results, it can be hypothesized that the non-responsiveness of the malnourished to citalopram anorexic effect here observed is a consequence of a down-regulation of serotonin receptors. In brain tissue, a down-regulation of serotonin receptors by increased serotonin availability is well established (Chaput *et al.*, 1986; De Montigny and Blier, 1991). In the present study, the down-regulation would be induced by the long-lasting increased levels of brain serotonin following the poor protein diet imposed on the mothers during lactation. This view is supported by Hall *et al.* (1983) findings. They had the point of view that low behavioral responsiveness of malnourished rats to a serotonin agonist might reflect a hypo-sensitivity of serotonin receptors induced by malnutrition.

In conclusion, it has been demonstrated a SSRI hypo-responsiveness of rats submitted to protein restriction during the BGS and then nutritionally recovered for a long period. This suggests that early malnutrition affects serotoninergic receptors in a permanent way, so altering the adult feeding behavior.

Acknowledgements

The authors thank Lúcia Maria Pires Ferreira for her superb and timely assistance. This investigation was supported by CAPES, CNPq, UFPE and UFBA.

References

Arborelius, L., Nomikos, G.G., Salmi, P., Hertel, P., Grillner, P., Hook, B., B, ., Hacksell, U. and Svensson, T.H. (1996) "The 5-HT receptor antagonist (s)-UH-301 the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic with citalopram", *Naunyn-Schmiedeberg's Archives of Pharmacology* **353**, 630–640.

Barreto Medeiros, J.M., Mendes da Silva, C., Sougey, E.B., Costa, J.A., De Castro, C.M.M.B. and Manhães de Castro, R. (2001) "Action of selective serotonin reuptake inhibitor on aggressive behavior in adult rats submitted to the neonatal malnutrition", *Arquivos de Neuro-psiquiatria* **59**(3-A), 499–503.

Blundel, J.E. (1992) "Serotonin and the biology of feeding", *American Journal of Clinical Nutrition* **55**, 155S–159S.

Chaput, Y., De Montigny, C. and Blier, P. (1986) "Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain", *Naunyn-Schmiedeberg's Archives of Pharmacology* **333**, 342–348.

Chen, J.C., Tonkiss, J., Galler, J. and Volicer, L. (1992) "Prenatal protein malnutrition in rats enhances serotonin release from hippocampus", *Journal of Nutrition* **122**, 2138–2143.

Chen, J.C., Tonkiss, J., Galler, J.R. and Volicer, L. (1995) "Effect of prenatal malnutrition on release of monoamines from hippocampal slices", *Life Sciences* **57**, 1467–1475.

Chopin, P., Moret, C. and Briley, M. (1994) "Neuropharmacology of 5-hydroxytryptamine$_{1B/1D}$ receptor ligands", *Pharmacology and Therapeutics* **62**, 385–405.

Datta, S., Patterson, E.H., Vincitore, M., Tonkiss, J., Morgane, P.J. and Galler, J.R. (2000) "Prenatal protein malnourished rats show changes in sleep changes in sleep/wake behavior as adults", *Journal of Sleep Research* **9**, 71–79.

De Montigny, C. and Blier, P. (1991) "Desensitization of terminal 5-HT autoreceptors by 5-HT reuptake blockers", *Archives General Psychiatry* **48**, 483–484.

Dobbing, J. (1968) "Vulnerable periods in developing brain", In: Davison, A.N. and Dobbing, J., eds, Applied Neurochemistry (Blackwell, Oxford), pp 287–316.

Gobbi, M., Crespi, D., Foddi, M.C., Fracasso, C., Mancini, I., Parotti, L. and Mennini, T. (1997) "Effects of chronic treatment with fluoxetine and citalopram on 5-HT uptake, 5-HT1B autoreceptors, 5-HT3 and 5-HT4 receptors in rats", *Naunyn-Schmiedeberg's Archives of Pharmacology* **356**, 22–28.

Guedes, R.C.A., Cabral-Filho, J.E. and Teodósio, N.R. (1992) "GABAergic mechanisms involved in cortical sptreading depression in normal and malnourished rats", *Experimental Brain Research series* **23**, 17–26.

Halford, J.C.G. and Blundel, J.E. (1996) "The 5-HT$_{1b}$ receptor agonist Cp-94,253 reduces food intake ad preserves the behavioural satiety sequence", *Physiology and Behavior* **60**, 933–939.

Halford, J.C.G., Lawton, L.C.L. and Blundel, J.E. (1997) "The 5-HT$_1$ receptor agonist MK-212 reduces food intake and increases resting but prevents the behavioural satiety sequence", *Pharmacology, Biochemistry and Behaviour* **56**, 41–46.

Hall, R.D., Leahy, J.P. and Robertson, W.M. (1983) "Hyposensitivity to serotonergic stimulation in protein malnourished rats", *Physiology and Behavior* **3**, 187–195.

Huether, G., Zhou, D., Schmidt, S., Wiltfang, J. and Rüther, E. (1997) "Long-term food restriction down-regulates the density of serotonin transporters in the rat frontal cortex", *Society Biological Psychiatry* **41**, 1174–1180.

Hyttel, J. (1978) "Effect of a specific 5-HT uptake inhibitor, citalopram (Lu 10-171), on 3H-5-HT uptake in rat brain synaptosomes *in vitro*", *Psychopharmacology* **60**, 13–18.

Hyttel, J. (1994) "Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs)", *International Clinical Psychopharmacology* **9**(Suppl. 1), 19–26.

Lauder, J.M. and Bloom, F.E. (1974) "Ontogeny of monoamine neurons in locus coeruleous, raphe nuclei and substantia nigra of the rats 1, cell differentiation", *Journal of Comparative Neurology* **155**, 469–481.

Levstsky, D.A. and Barnes, R.H. (1972) "Nutritonal and environmental interactions in the behavioral development of the rat: long-term effects", *Science* **176**, 68–71.

Li, D.L., Simmons, R.M.A. and Iyengars, S. (1998) "5-HT$_{1A}$ receptor antagonists enhance the functional activity of fluoxetine in a mouse model of feeding", *Brain Research* **781**, 121–128.

Lidov, H.G.W. and Molliver, M.E. (1982) "A immunohistochemical study of serotonin neuron development in the rat: ascending pathways and terminal fields", *Brain Research Bullettim* **8**, 389–416.

Liu, J. and Lauder, J.M. (1992) "Serotonin promotes region-specific glial influences on cultured serotonin and dopamine neurons", *Glia* **5**, 306–317.

Lucas, J.J., Yamamoto, A., Scearce-levie, K., Saudou, F. and Hen, R. (1998) "Absence of fenfluramine-induced anorexia and reduced c-Fos induction in the hypothalamus and central amygdaloid complex of serotonin 1B receptor Knock-out mice", *The Journal of Neuroscience* **18**, 5537–5544.

Lucki, I., Kreider, M.S. and Simansky, K.J. (1988) "Reduction of feeding behavior by the serotonin uptake inhibitor sertraline", *Psychopharmacology* **96**, 289–295.

Manhães-de-Castro, R., Barreto Medeiros, J.M., Mendes da Silva, C., Ferreira, L.M.P., Guedes, R.C.A., Cabral Filho, J.E. and Costa, J.A. (2001) "Reduction of intraspecific aggression in adult rats by neonatal treatment with a selective serotonin reuptake inhibitor", *Brazilian Journal of Medical and Biological Research* **34**, 121–124.

Mazer, C., Muneyyirci, J., Taheny, K., Raio, N., Borella, A. and Whitaker-Azimitia, P. (1997) "Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rats: a possible model of neurodevelopmental disorders with cognitive deficits", *Brain Research* **760**, 68–73.

Morgane, P.J., Miller, M., Kemper, T., Stern, W., Forbes, W., Hall, R., Bronzino, J., Kissane, J., Hawrylewick, E. and Resnick, O. (1978) "The effects of protein malnutrition on the developing central nervous system in the rat", *Neuroscience and Biobehavioral Reviews* **2**, 137–230.

Morgane, P.J., Austin-la France, R.J., Bronzino, J., Tonkiss, J. and Galler, J.R. (1992) "Malnutrition and developing central nervous system", In: Isaacson, R.L. and Jensen, K.F., eds, The Vulnerable Brain and Environmental Risks (Plenum Press, New York) **Vol. 1**, pp 2–42.

Morgane, P.J., Austin-la France, R.J., Bronzino, J., Tonkiss, J., Diaz-Cintra, S., Cintra, L., Kemper, T. and Galler, J.R. (1993) "Prenatal malnutrition and development of the brain", *Neuroscience and Biobehavioral Reviews* **17**, 91–128.

Palén, K., Thorneby, L. and Emanuelsson, H. (1979) "Effects of serotonin antagonists on chick embryogenesis", *Wilhelm Roux's Archives of Developmental Biology* **187**, 89–103.

Resnick, O., Miller, M., Fobs, W., Hall, R., Kenper, T., Bronzino, J. and Morgane, J.P. (1979) "Developmental malnutrition protein: influences on the central nervous system of the rat", *Neuroscience and Biobehavioral Reviews* **4**, 232–246.

Rotta, F.T., Mello, C.F., Rocha, J.B.T. and Souza, D.O.G. (1988) "Early undernutrition blocks the effect of naltrexone on rat exploratory behavior", *Brazilian Journal of Medical and Biological Research* **21**, 305–312.

Toornvliet, A.C., Pijl, H., Hopman, E., Wever, B.M.E. and Meinders, A.E. (1996) "Serotoninergic drug-induced weight loss in carbohydrate craving obese patients", *International Journal of Obesity* **20**, 917–920.

Whitaker-Azmitia, P.M. (1991) "Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology", *Pharmacological Reviews* **43**, 553–561.

Wiggins, R.C., Fuller, G. and Enna, S.J. (1984) "Undernutrition and the development of brain neurotransmitter systems", *Undernutrition and Brain Development* **35**, 2085–2094.

Winick, M., Brasel, J.A. and Rosso, P. (1972) "Nutrition and cell growth", In: Winick, M., ed, Nutrition and Development (Wiley, New York), pp 49–98.

Yan, W., Wilson, C.C. and Haring, J.H. (1997) "5-HT1a receptors mediate the neurotrophic effect of serotonin on developing dentate gramile cells", *Developmental Brain Researech* **98**, 185–190.

5.3. Terceiro artigo, intitulado: **“MALNUTRITION DURING BRAIN GROWTH SPURT ALTERS THE EFFECT OF FLUOXETINE ON AGGRESSIVE BEHAVIOR IN ADULT RATS”.** Aceito para publicação na revista: *Nutritional Neuroscience*, 2003.

Ainda, dando continuidade aos estudos referentes a influência da desnutrição sobre o sistema serotoninérgico, o presente trabalho teve como principal objetivo investigar os efeitos da fluoxetina (outro ISRS) sobre a resposta agressiva em ratos adultos submetidos à desnutrição durante o período de rápido desenvolvimento do cérebro. Neste estudo, nós observamos uma hiporresponsividade a fluoxetina em ratos adultos submetidos à desnutrição precoce.

Running Title: Malnutrition, serotonin and aggressive behavior

Malnutrition during Brain Growth Spurt Alters the Effect of Fluoxetine on Aggressive Behavior in Adult Rats.

J. M. BARRETO-MEDEIROS[a], E. G. FEITOZA[a], K. MAGALHÃES[a], J.E. CABRAL-FILHO[b], F.M. MANHÃES-DE-CASTRO[a] , C. M. M. B. DE-CASTRO[c] , R. MANHÃES-DE-CASTRO[a] *

[a]Departamento de Nutrição, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil; [b]Instituto Materno Infantil de Pernambuco, Recife, PE, Brazil; [c]Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Recife, PE, Brazil.

KEY WORDS: aggressive behavior, malnutrition, selective serotonin reuptake inhibitor.

*Corresponding author: Departamento de Nutrição, Universidade Federal de Pernambuco, Cidade Universitária, 50670-901,Recife, PE, Brasil. Tel.: (081) 271.8471. Fax: (081) 271.8473. E-mail: rcastro@nutricao.ufpe.br.

Number of pages: 13
Number of figures: 01

The effect of the malnutrition during suckling period on the aggressive behavior was investigated in adult rats treated or not with fluoxetine, a selective serotonin reuptake inhibitor. Sixty-four Wistar male rats were allocated in two groups, according to their mother's diet during lactation. The well-nourished group was fed by mothers receiving a 23% protein diet; the malnourished one by mothers receiving a 8% protein diet. After weaning, all rats received the 23% protein diet. On the 90th day after birth, each nutritional group was divided in two subgroups, which received respectively a single daily injection of fluoxetine (10 mg/kg) or saline (0.9% NaCl) for 14 days. The treatment with fluoxetine reduced the aggressive response in well-nourished but not in malnourished rats. These findings suggest that the serotoninergic system has suffered consequences of the malnutrition during the critical period of brain development, persisting even after a long period of nutritional recovery.

The growth of the central nervous system and its developmental processes (gliogenesis, neuronal differentiation, migration, synaptogenesis, etc) occur with great intensity during the suckling period in the rat (Morgane *et al.,* 1993). This period is called “brain growth spurt” (Dobbing, 1968) and in it the brain is highly vulnerable to several kinds of challenges. Nutritional insults (Morgane *et al.,* 1992) or pharmacological manipulations early in life (Manhães de Castro *et al.,* 1993) can cause irreversible alterations.

The effects of malnutrition on neurotransmitters systems, in particular the serotoninergic one, has deserved special attention, since this system plays a role in several functions, such as pain sensitivity, sleep control, mood, sexual behavior, feeding control and aggressiveness (Chopin *et al.,* 1994).

The role of serotonin in the control of aggressive behavior has been demonstrated through the use of pharmacological instruments (Manhães de Castro *et al.,* 2001). On the other hand, there are drugs that act as selective serotonin reuptake inhibitors increase serotonin availability in synapses and, consequently, the action of this neurotransmitter (Sánchez and Meier, 1997). Fluoxetine, a potent serotonin reuptake inhibitor (Hyttel, 1994; Sánchez and Hyttel, 1999), is one of those substances.

Mounting evidences indicate that fluoxetine decreases aggressive behavior in various animal species and models of aggression (Datla *et al.,* 1991; Sánchez and Hyttel, 1994; Sperry *et a*., 2003). However, to our knowledge, there are no studies on the effects of fluoxetine in the aggressive behavior of adult rats malnourished early in life. The present work aimed to investigate the effect of fluoxetine on aggressiveness of adult rats submitted to malnutrition during the brain growth spurt.

Sixty-four Wistar male rats maintained at a room temperature of 23 ± 1ºC and on a light–dark cycle (light 6:00 a.m. - 6:00 p.m.) were used. During the suckling period,

the offsprings were housed in polyethylene cages in litters of 6 pups, randomly distributed to each mother. They were then allocated in two nutritional groups according to the mother diet during lactation: a well-nourished group (n=32) fed by mothers receiving a 23% protein diet (Purina chow) and a malnourished group (n=32) fed by mothers receiving a 8% protein diet. Following weaning (on the 24th day of age), all rats were fed with the 23% protein diet *ad libitum*. Body weights were determined on the 1st, 24th and 90th day. On the 90th day after birth, the well-nourished animals and the malnourished ones were divided according to the four following treatments (n=16, each one): well-nourished plus saline; well-nourished plus fluoxetine; malnourished plus saline; malnourished plus fluoxetine. Single injections of fluoxetine (10 mg/kg, i.p., 1 ml/100mg, dissolved in saline) or saline (0.9% NaCl) were administered during 14 days. Twenty-four hours following this treatment period the animals were submitted to aggressiveness tests. The tests were accomplished in an acoustic isolated room, by using a box (20 x 20 x 20 cm) with one the walls transparent and the floor formed by parallel metallic bars (inter-bars distance: 1.3cm), connected to an electric scrambled current source. The test consisted of placing a pair of rats of the same treatment group (matched by weight) in the box, where they received a session of stimuli to induce fight responses. Each stimulus (an electric foot-shock) was produced by a 1,6 mA - 2 s current pulse. Each session lasted 20 min being composed by 5 stimuli separated by a 4 min interval. During the first 3 min of this interval, the duration of the aggressive responses was measured by using a digital chronometer. So the total time for observation of aggressive behavior was 900s. In the last minute of each interval, annotations and verification of the equipment were proceeded. The aggressive response was defined as the presentation of, at least, one of the two following behaviors: a) the animals stayed lifted up on the hind paws, facing

one to the other, in a threatening posture but without direct contact, or b) the animals maintained evident physical contact (besides being scratched, exhibition of the teeth and emission of characteristic vocalization). For statistical comparisons, data were previously tested for normality (Kolmogorov-Smirnov test) and variance homogeneity (Levene median test). Body weights passed ($p>0.05$) in both criteria, but aggressiveness, not (variance homogeneity, $p<0.05$). So body weights (between nutritional groups) were compared by Student t-test, and aggressiveness by Kruskal-Wallis, one way analysis of variance followed by Dunn's test for multiple comparisons. The null hypothesis was rejected when $p \leq 0.05$.

Compared to well-nourished animals, the malnourished ones suffered a reduction of the body weight ($p<0.05$) both in suckling and in adult periods (table I). Concerning the aggressive behavior (figure 1), observed after pharmacological manipulation, the fluoxetine groups showed a reduction of aggressiveness as compared to the well-nourished saline group (Dunn test, $p<0.05$). However, there was no significant difference between malnourished saline and malnourished fluoxetine animals.

The results of the present study showed that malnutrition during the critical period of brain development induces a persistent body weight deficit in rats. The data corroborate previous experimental evidence showing lower body weight of animals submitted to malnutrition during the suckling period (Sobokta *et al.*, 1974). Therefore, body weight reduction caused by the maternal low protein diet attests to the efficiency of the employed malnutrition model.

The inhibitory effect of fluoxetine on aggressive behavior observed by others (Datla *et al.*, 1991; Fuller, 1996) is supported by this work, since the well-nourished animals fluoxetine-treated reduced its aggressiveness as compared to saline treated ones. This effect could be explained by an increased extracellular availability of serotonin in the

brain induced by fluoxetine (Baumann and Rochat, 1995). This is a plausible hypothesis since an anti--aggressiveness action of serotonin in humans beings (Coccaro and Kavoussi, 1997) and in animals (Sánchez and Hyttel, 1994; Sperry, *et al.,* 2003) has been demonstrated. In contrast, an increased aggressiveness has been observed after diminished brain serotonin (Kyes, 1993).

Although fluoxetine seems to enhance not only serotonin availability but that of dopamine and norepinephrine as well (Hyttel, 1994; Wong *et al.,* 1995), the participation of these catecholamines in the reduction of aggressive behavior can be ruled out. In fact, evidences indicate that dopamine and norepinephrine facilitate and do not inhibit the aggressiveness (Eichelman, 1990; Datla *et al.,* 1991; Pruus *et al.,* 2000).

Since fluoxetine has also an anxiolytic effect (Sánchez and Meier 1997), the possibility exists that hipoaggressiveness here observed would be associated to a reduced anxiety of the animals. This influence however could be little because the anxiolytic effect of fluoxetine is weak (De Vry *et al.,* 1993)

The reduced response to the fluoxetine of malnourished animals as compared to those malnourished that did not receive the drug is a major subject of this study and is noteworthy. In rats, the first serotoninergic neurons appear between the 12° and the 14° gestation day (Lauder and Bloom, 1974) but the final density and definitive location of terminals are established during the postnatal maturation of the central nervous system (Lidov and Molliver, 1982). Therefore, the malnutrition imposed early in life could cause alterations in the serotonin neurotransmission system, reflecting on its functional responses to drugs. Medeiros *et al*. (2001) showed that adult rats malnourished during suckling are hypo-responsive to the inhibitory effect of citalopram -another selective serotonin reuptake inhibitor- on the intraspecific aggressive behavior. Barreto-

Medeiros *et al.* (2002) also observed that early malnourished rats are not affected in adult life by anorexia induced by citalopram.

We cannot rule out the possibility of an anti-aggressive response of malnourished rats to other fluoxetine doses. However the similar responsiveness between malnourished saline and malnourished fluoxetine groups, here observed, allows us suppose that the anti-aggressiveness effect of fluoxetine does not occur, at least, by chronic administration of a 10mg dose.

Furthermore, as long ago as the eighties, Hall *et al.* (1983) showed that early protein malnourished rats stimulated by the serotonin agonist N, N-dimethyltryptamine presented a lower performance than well-nourished animals in several behavioral tests, such as the serotoninergic syndrome, the rota-rod and the treadmill. This lower responsiveness was supposed by these authors to be associated to serotonin neuronal processes and to alterations of brain serotonin due to malnutrition (Hall *et al.*, 1983).

On the basis of this work we can suggest that, the hypo-responsiveness to fluoxetine of rats submitted to protein restriction during the brain growth spurt and then nutritionally recovered, is due to that nutritional deficit. Therefore the nutritional insult early in life could to have a long-lasting effect upon the functioning of the serotoninergic system.

Acknowledgements

The authors thank Lúcia Maria Pires Ferreira for her superb and timely assistance. Jairza Maria Barreto Medeiros is the recipient of a fellowship from the CAPES/PICDT/UFBA.

References

Barreto Medeiros, J.M., Cabral Filho, J.E., De Souza, S.L., Freitas Silva, S.R., Mendes da Silva, C., Deiró, T.C.B.J., Monteiro, J.M., Guedes, R.C.A., De Castro, C.M.M.B. and Manhães de Castro, R. (2002) “Early malnourished rats are not

affected by anorexia induced by a selective serotonin reuptake inhibitor in adult life", *Nutritional Neuroscience* **5**, 211-214.

Bauman, P. and Rochat, B. (1995) "Comparative pharmacokinetics of selective serotonin reuptake inhibitors: a look behind the mirror", *International Clinical Psychopharmacology* **10**,15-21.

Chopin, P., Moret, C. and Briley, M. (1994) "Neuropharmacology of 5-hydroxytryptamine$_{1B/1D}$ receptor ligands", *Pharmacology and Therapeutics* **62,**385-405.

Coccaro, E.F. and Kavoussi, R.J. (1997) "Fluoxetine and impulsive aggressive behavior in personality-disordered subjects" *Archives General Psychiatry* **54,**1081-1088.

Datla, K.P., Mitra, S.K., Bhattacharya, S.K. (1991) "Serotonergic modulation of footshock induced aggression in paired rats" Indian Journal of Experimental Biology **29**, 631-635.

De Vry, J., Benz, U., Schreiber, R., Traber, J. (1993) "Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs", *European Journal of Pharmacology* **249**, 331-339.

Dobbing, J.(1968) "Vulnerable periods in developing brain". In: Davison, A.N. and Dobbing, J., eds, *Applied Neurochemistry* (Blackwell, Oxford), pp 287-316.

Eichelman, B.S. (1990) "Neurochemical and psychopharmacologic aspects of aggressive behavior", *Annual Review of Medicine* **41**, 149-158.

Fuller, R.W. (1996) "Fluoxetine effects on serotonin function and aggressive behavior", *Annals of the New York Academy of Sciences* **20,** 90-97.

Hall, R.D., Leahi, J.P. and Robertson, W.M. (1983) "Hyposensitivity to serotoninergic stimulation in protein malnourished rats", *Physiology and Behavior* **31**, 187-195.

Hyttel, J. (1994) "Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs)". *International Clinical Psychopharmacology* **9** (suppl. 1), 19-26.

Kyes, G.B. (1993) "Aggression and brain serotoninergic responsivity: response to slides in male macaques", *Physiology and Behavior* **2**, 205-208.

Lauder, J.M. and Bloom, F.E. (1974) "Ontogeny of monoamine neurons in locus coeruleous, raphe nuclei and substantia nigra of the rats 1, Cell differentiation", *Journal of Comparative Neurology* **155,** 469-481.

Lidov, H.G.W. and Molliver, M.E. (1982) "A immunohistochemical study of serotonin neuron development in the rat: ascending pathways and terminal fields", *Brain Research Bulletin* **8**, 389-416.

Manhäes-de-Castro, R., Barreto Medeiros, J. M., Mendes da Silva, C., Ferreira, L.M.P., Guedes, R.C. A., Cabral Filho, J.E. and Costa, J.A. (2001) "Reduction of

intraespecific aggression in adult rats by neonatal treatment with a seletive serotonin reuptake inhibitor", Brazilian *Journal of Medical and Biological Research* **34**, 121-124.

Manhäes-de-Castro, R., Cabral Filho, J.E., Costa, J. A., Costa, F.B.R., Galindo, M.A.C. and Hecksher, C.A. (1993)" Neonatal treatment with naloxone causes permanent hyperalgesia in rats", Brazilian *Journal of Medical and Biological Research* **26**, 747-751. .

Medeiros, J.M., Mendes da Silva, C., Sougey, E.B., Costa, J. A., De Castro, C.M.M.B. and Manhães de Castro, R. (2001) "Action of selective serotonin reuptake inhibitor on aggressive behavior in adult rats submitted to the neonatal malnutrition", *Arquivos de Neuro-psiquiatria* **59** (3-A), 499-503.

Morgane, P.J., Austin-la France, R.J., Bronzino, J., Tonkiss, J. and Galler, J. R. (1992) "Malnutrition and developing central nervous system", In: Isaacson, R.L. and Jensen, K.F., eds, *The vulnerable brain and environmental risks*. (Plenum Press, New York) **Vol. 1**, pp 2-42.

Morgane, P.J., Austin-la France, R.J., Bronzino, J., Tonkiss, J., Diaz-Cintra, S., Cintra, L., Kemper, T. and Galler, J. R. (1993) "Prenatal malnutrition and development of the brain", *Neuroscience and Biobehavioral Reviews* **17**, 91-128.

Pruus, K., Rudissaar, R., Skrebuhhova-Malmros, T., Allikmets, L., Matto, V. (2000) "Development of apomorphine-induced aggressive behavior: comparison of adult male and female Wistar rats*" Methods and Findings in Experimental and Clinical Pharmacology* **22,** 47-50.

Sánchez, C. and Hyttel, J. (1994) "Isolation-induced aggression in mice: effects of 5-hydroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT_{1A} receptors", *European Journal of Pharmacology* **264**, 241-247.

Sánchez, C. and Meier, E. (1997) "Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression", *Psychopharmacology* **129**:197-205.

Sánchez,C. and Hyttel, J. (1999) "Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding, *Cellular and Molecular Neurobiology* **19:** 467-489.

Sobokta, T. J., Cook, M. P. and Brodie, R. E (1974) "Neonatal malnutrition: neurochemical, hormonal and behavioral manifestations", *Brain Research* **26**:443-457.

Sperry, T.S., Thompson, C.K. and Wingfield, J.C. (2003) "Effects of acute treatment with 8-OH-DPAT and fluoxetine on aggressive behaviour in male song sparrows (Melospiza melodia morphna)", *Journal of Neuroendocrinology* **15**, 150-160.

Wong, D.T., Bymaster, F.P., Reid, L.R., Mayle, D.A., Krushinsk,i J.H., Robertson, D.W. (1993) "Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain", *Neuropsychopharmacology* **8**, 337-44.

TABLE I. Body weight of well-nourished or malnourished rats during suckling.

	Body Weight (g)		
Experimental Groups	1st day	24th day	90th day
Well-nourished	7.5 ±0.15	49.0 ±0.85	314.2 ±9.83
Malnourished	7.3 ±0.11	23.8 ±1.07*	265.3 ±7.84*

Rats were fed by mothers receiving a 23% (Well-nourished group) or a 8% (malnourished group) protein diet during lactation. The animals were weighed on the 1st, 24th, and 90th day. The data are reported as mean (± SEM) of 16 animals. Comparisons between well-nourished and malnourished groups at the same age, were made by two-tailed Student t-test (* $p<0.05$).

FIGURE 1

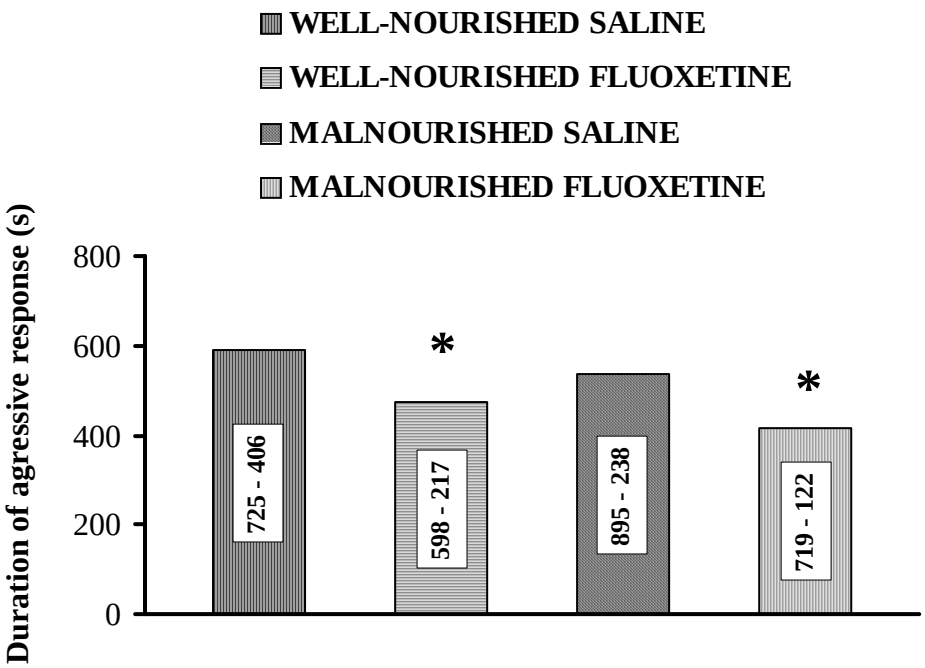

WELL-NOURISHED SALINE
WELL-NOURISHED FLUOXETINE
MALNOURISHED SALINE
MALNOURISHED FLUOXETINE
Duration of agressive response (s)
800
600
400
200
0
725 - 406
598 - 217
895 - 238
719 - 122
*
*

Figure Legends

Figure 1. Aggressive responses of fluoxetine or saline-treated adult rats, fed by mothers receiving a 23% or a 8% protein diet during lactation. Columns represent the medians of 16 animals; maximum and minimum values are inside columns. Comparisons among groups (Kruskal-Wallis test, $p=0.01$) and multiple comparisons between groups (Dunn's test,*$p<0.05$): well-nourished fluoxetine < well-nourished saline and malnourished fluoxetine < well-nourished saline.

5.4. Quarto artigo, intitulado: **“THE EXPRESSION OF AN INTRASPECIFIC AGGRESSIVE REACTION BEFORE A STRESSOR ALTERS THE IMMUNE RESPONSE IN RATS”**. Artigo original aceito para publicação na revista: *Brazilian Journal of Biology*, 65 (3), 2005.

Nesse artigo, foi avaliado em ratos adultos se a expressão ou não da agressividade intraespecífica frente a um estressor pode alterar a resposta imune. Nesta pesquisa, foi observado que a expressão da agressividade intraespecífica parece ativar a resposta imune e potencializar a resposta humoral antígeno específica.

THE EXPRESSION OF AN INTRASPECIFIC AGGRESSIVE REACTION BEFORE A STRESSOR ALTERS THE IMMUNE RESPONSE IN RATS.

Jairza Maria Barreto-Medeiros[1], Emmanuela Gomes Feitoza[1], Kedma Magalhaes[1], Rosângela R da Silva[2], Francisco Machado Manhaes-de-Castro[1], Raul Manhaes-de-Castro[1], Célia Maria Machado Barbosa De-Castro[2*]

[1]Departamento de Nutrição, Universidade Federal de Pernambuco, Cidade Universitária, 50670-901, Recife, PE, Brazil; [2]Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Cidade Universitária, 50670-901, Recife, PE, Brazil.

Key words: intraspecific aggressiveness, leukocytes, humoral immune response.

Palavras-Chaves: agressividade intraespecífica, leucócitos, resposta imune humoral.

Running Title: intraspecific aggressive behavior and immune response

[*]Correspondence to: Dra. Célia Maria Machado Barbosa de Castro. Laboratório de Imunopatologia Keizo Asami, UFPE, Recife, CEP 50670-901 PE, Brasil, Fax: 55 81 32718485
E-mail: ccastro@lika.ufpe.br

Number of figures: 04

ABSTRACT

The repercussion on the immune response of the expression of the intraspecific aggressiveness before a stressor was investigated in rats. To the 90 days of life, the animals were divided in three groups: control group (only the immunological measurements were accomplished); foot-shock (FS) (animals individually received FS) and intraspecific aggressive response (IAR) group (animals received FS and presented IAR). For immunological measurements, blood samples were collected immediately, 7 and 15 days after FS or IAR. FS reduced the total amount of leukocytes. However, the aggressiveness was accompanied, besides the reduction of the leukocytes number, by lymphocytes decrease and neutrophils increase. Moreover, an elevation in the leukocytes number associated to an increase in the humoral immune response was also observed one week after the IAR. In this study, the expression of the intraspecific aggressiveness before a stressor seems to activate the immune system and to potentiate the antigen specific humoral response.

RESUMO

A expressão de uma reação agressiva intraespecífica frente a um estressor altera a resposta imune em ratos.

A repercussão sobre a resposta imune da expressão da agressividade intraespecífica frente a um estressor foi investigada em ratos. Aos 90 dias de vida, os animais foram divididos em três grupos: grupo controle (foram realizadas apenas as mensurações imunológicas); choque nas patas (FS) (os animais receberam FS individualmente) e grupo resposta agressiva intraespecífica (IAR) (os animais receberam FS e apresentaram IAR). Para as medições imunológicas, amostras de sangue foram coletadas imediatamente, 7 e 15 dias após FS ou IAR. O FS reduziu a quantidade total de leucócitos. Contudo, a agressividade, foi acompanhada, além da redução do número de leucócitos, por diminuição de linfócitos e aumento de neutrófilos. Ademais, foi observada também uma elevação no número de leucócitos associada a um aumento na resposta imune humoral uma semana após as IAR. Neste estudo, a expressão da agressividade intraespecífica frente a um estressor parece ativar o sistema imune e potencializar a resposta humoral antígeno específica.

INTRODUCTION

In several species, the aggressiveness is important for the survival, because the aggressive behavior guarantees access to food, reproduction, protection of the pups, struggle against the predators and defense of territory (Volvaka, 1995). On the other hand, the aggressiveness can take to traumas, wounds and also to higher susceptibility to the exposition to new diseases (Granger et al., 2000).

The hypothalamus-pituitary-adrenal axis (HPA) constitutes one of the fundamental elements of the mammalian adaptive response in most of the threatening situations (Castelnau & Lôo, 1993). The HPA axis and also other participating elements of the response to stress, as the sympathetic nervous system (Sgoifo et al., 1996; Lawrence & Kim, 2000), are components straightly associated to the expression of aggressive response. In animals, the intraspecific aggressiveness can be induced by means of painful electrical foot-shocks (Eichelman, 1990; Manhães de Castro et al., 2001). This kind of electric stimulus is a typical neurogenic stressor (Sawchenko, 2000), which activates the HPA axis as well as the sympathetic-adrenal system.

In the nervous system (NS), the receptors for some chemical messengers have their functional parameters altered by stress situations (Manhães de Castro et al., 1996). Moreover, these chemical messengers are implied in the genesis of stress, of anxiety (Dantzer, 1991; Toth, 2003) and of aggressiveness (Manhães of Castro et al., 2001; Medeiros et al., 2001). Stressors can provoke alterations on the immune response, characterizing a possible neuroimmune modulation and also indicating the straight relationship among the immune, nervous and endocrine systems (Dardenne & Savino, 1996; Lawrence & Kim, 2000). The relationship among these physiological systems is reinforced by evidences showing that immune cells can interact with hormones, neuropeptides and neurotransmitters and they can also produce them

(Blalock et al, 1985; Ottaviani & Franceschi, 1996). However, according to the situations, the behavioral and immunological alterations can vary before a stressor (Stefanski & Engler, 1999; Devoino et al., 2003), denoting, therefore, differences in the responses of the physiological systems.

There are several evidences demonstrating that stress and emotional reactions affect the immune system (Stefanski & Engler, 1998; De Castro et al., 2000; Gasparotto et al., 2002). However, studies about interrelations between the aggressive behavior and the immune function in mammals are scarce; above all, controlling the interference of the stress factor. The present work investigates the repercussion on the immune response of the intraspecific aggressiveness expression or not before a stressor in rats.

MATERIALS AND METHODS

Animals and experimental groups

All the manipulations were performed in male Wistar rats. The animals were kept under controlled conditions (*ad libitum* access to water and food, 12h light-dark cycle and 23±1ºC room temperature) during all the study period. In the 90th day after the birth, the animals were distributed in three groups: one control group (n=16, in which only the immunological measurements were accomplished), one foot-shock group (n=14, composed by animals that individually received foot-shocks – FS) and one FS group + intraspecific aggressive response - IAR (n=16; composed by animals disposed in boxes in pairs that received foot-shocks and presented aggressive response).

Stress induced by foot-shocks

Rats were submitted to the stress induced by foot-shocks in an isolated room, using a box, 20 x 20 x 20cm, with the floor consisting of parallel metallic bars (interbar distance: 1,3cm), connected to an electric scrambled current source. The test consisted of placing one rat in the box, where it received a session of electric stimuli. Each stimulus (an electrical foot-shock) was represented by a 1,6mA – 2sec current pulse. Each session lasted 20min and was composed by 5 stimuli separated by a 4min interval. During the first 3min of this interval, the behavioral response was analyzed. The annotations and the verification of the equipment were done in the last minute of each interval.

Intraspecific aggressive response study

The aggressiveness test consisted in placing a pair of rats of the aggressive response group (matched by weight) in the box, where they received a session of electric stimulus under the same conditions above referred. The aggressive response was defined as the presentation of, at least, one of the two following behaviors: a) the animals stayed lifted up on the hind paws, facing one to the other, in a threatening attitude but without direct contact, or b) they maintained evident physical contact (besides scratches, exhibition of the teeth and emission of characteristic vocalization).

Blood sampling

Blood samples were collected immediately, 7 and 15 days after submitting the rats to the stress induced by foot-shocks or after the aggressiveness test. Blood was collected by the tail clip method as previously described by Dhabhar et al 1996. The

samples were used for leukocyte (about 20 µl, 3% EDTA) and antibody titer analysis.

White blood cells (WBC) and leukocyte subsets

Total WBC number was determined in a hemocytometer. The percentage of lymphocyte and neutrophils was determined with a microscope (May-Grün-Wald/Giemsa staining).

Immunization

The animals were immunized immediately after the stress induced by foot-shocks or after the aggressive behavior. Sheep red blood cells (SRBC) were prepared by washing citrated sheep blood three times in sterile saline. Animals were immunized intraperitoneally with 10^8cells/ml in a volume of approximately 0,5ml.

Determination of antibody titer

Blood samples were collected before (negative control of antibody titer anti-SRBC) and 7 e 15 days after immunization. Samples were subsequently centrifuged at 3000rpm for 5min and the supernatant collected. Serum complement was then inactivated at 56ºC for 30min and stored at –20ºC. Twofold serial dilutions of inactivated serum, saline, and a 1% SRBC solution were then made in microwells glass. The highest dilution at which aggregation of SRBCs was still evident was considered to be the antibody titer.

Statistical analysis

For statistical comparisons, data were previously tested for normality

(Kolmogorov-Smirnov test) and variance homogeneity (Levene median test). It has been verified that most of the data didn't correspond to one of these criteria at least. So the results were compared by Kruskal-Wallis, one way analysis of variance followed by Dunn's test for multiple comparisons. Statistical significance was defined as $p \leq 0.05$.

RESULTS

Effect of stress induced by foot-shocks and of the intraspecific aggressive response on WBC.

Compared to the control group, the animals that received foot shocks presented an immediate reduction in the total leukocytes counting. However, there was no difference 7 and 15 days after the foot-shocks. In the same way, the aggressive response group presented an immediate reduction in the total leukocytes counting. However, 7 days after the aggressive response, an increase was observed in the total number of those cells (Fig.1).

Effect of stress induced by foot-shocks and of the intraspecific aggressive response on leukocyte subsets

There was no difference in the lymphocytes and neutrophils percentages between control and foot-hocks groups. However, the aggressiveness reduced the percentage of lymphocytes and increased the percentage of neutrophils immediately after the expression of the aggressive behavior. Moreover, 7 and 15 days after the induction of the aggressive response, no difference was observed in the lymphocytes and neutrophils percentages (Figs.2 and 3).

Effect of stress induced by foot-shocks and of the intraspecific aggressive response on humoral immunity.

Compared to the control, there was no alteration in the humoral immune response of the animals submitted to the foot-shocks. However, the intraspecific aggressiveness increased the titers of anti-SRBC antibodies 7 days after the immunization. Besides, 15 days after the immunization, no difference was found in the humoral immune response between the aggressive response and control groups (Fig. 4).

PLEASE INSERT RELATED FIGURES HERE

DISCUSSION

Studies about interrelations between the aggressive response and the immune function in mammals are scarce; above all, controlling the interference of the stress factor. Thus, in this study, the possibility that the expression or not of intraspecific aggressiveness before a stressor alters the immune response in adult rats was evaluated. The results demonstrated that the stress induced by the foot-shocks markedly reduced the amount of leukocytes in the blood current. However, the alterations in the leukocyte number were reverted a week after ceasing the stress. Our data corroborate experimental evidences indicating a reduction of the total number of leukocytes in animals submitted to acute stress (Dhabhar et al., 1994; Dhabhar et al., 1995). It seems that stressing experiences suppress the ability of the immune system to respond to strange agents (Dhabhar & Mcewen, 1996).

The electrical foot-shock is a classic stress model (Sawchenko, 2000), very

known for provoking increases in biosynthetic and secretory activity of chemical messengers in the HPA axis (Kant et al., 1984) and in the sympathetic-adrenal system (McCarty & Kopin, 1978). Thus, it is very probable that substances as adrenaline and glucocorticoids mediate the effects of stress on the leukocytary pattern observed in the present work. Corroborating this hypothesis, some studies have been demonstrating that organs and cells of the immune system express receptors for these chemical messengers and are, therefore, regulated by them (Blalock et al, 1985; Dantzer, 1991). Glucocorticoids are physiologic agents that have the potential to inactivate macrophages as well as other cells of the immune system (Celada & Nathan, 1994). In a recent article, it was observed that under stress situations, there is a decrease in the superoxid production by alveolar macrophages activated with PMA associated to an increase of the serum corticosterone levels (De Castro et al., 2000).

Concerning the humoral immune response, in this study, the foot-shocks didn't alter the titers of anti-SRBC antibodies. In contrast, a previous study demonstrated that rats submitted immediately and 24 hours after the immunization to a session of electrical foot-shocks (16 shocks, with 1,6mA, 5s long and 4min interval) presented an increase in the humoral immune response (Wood et al., 1993). It is worth to stand out that, in the present study, the stress session consisted of 5 shocks in the paws, with 1,6mA, 2s long and 4min interval. Moreover, the animals were immunized immediately after the stress session and the humoral response was evaluated 7 and 15 days after the immunization. Thus, it is possible that methodological differences as the number and the duration of the electric stimuli, as well as the period of application of the stressor in relation to the administration of the antigen, are responsible for the discrepancy among the different studies.

In this study, the foot-shock followed by the expression of the aggressive

response is also accompanied of lymphocyte decrease and neutrophils increase, besides the characteristic reduction of the number of leukocytes due to stress. Moreover, an elevation in the leukocyte number associated to an increase in the humoral immune response was observed a week after the aggressive interactions. The HPA axis and also other elements of the response to stress are straightly related to the expression of aggressive behavior (Sgoifo et al., 1996; Lawrence & Kim, 2000). Thus, we can suppose that, in the present work, the immunological alterations induced after the expression of the aggressive behavior are mediated by neuro-endocrine components specifically associated to this behavioral response. Thus, a specific neuro-endocrine profile (synthesis and liberation of several chemical messengers) associated to the activation of the aggressiveness neural circuits, distinct from that of stress, would affect in a particular way the different immunological components. This hypothesis is plausible and it also corroborates the evidences that immune cells can interact with hormones, neuropeptides and neurotransmitters (Blalock et al, 1985; Ottaviani & Franceschi, 1996).

The number and the proportion of leukocytes in the blood give an important representation of the distribution state of leukocytes in the organism and of the immune system state of activation (Dhabhar et al., 1995). Thus, in the present work, the alterations observed in the leukocytary pattern, specifically related to the presence of aggressiveness, distinct from those of the stress, can result of the differential activation of the immune system faced with the new behavioral component. That differential activation of the immune system can be an adaptive response of the organism to assure a more effective immune defense.

In this study, it is interesting to notice that, 7 days after the aggressive behavior, there was an increase in the titers of anti-SRBC antibodies. The largest production of

antibodies found in the aggressive group reinforces the hypothesis that intraspecific aggressiveness can activate the immune system, increasing its capacity to react against strange antigens. Corroborating this hypothesis, Cohen et al. (1997) observed that primates with higher levels of aggressive behavior presented smaller susceptibility to respiratory infection. In the same way, Devoino et al. (2003) observed that the occurrence of aggressive behavior in rats previously submissive produced immune stimulation.

Another important aspect to consider is that aggressiveness is also modulated by neurotransmitters systems. Among them, the dopaminergic system stands out (Poshivalov, 1986; Miczek et al., 1994). Moreover, there are several evidences of the involvement of the dopamine in the modulation of the immune system (Devoino et al. 1994;1997). Thus, it is possible that the immunological alterations observed in the rats that expressed aggressiveness have also been mediated by dopaminergic mechanisms. However, more studies are necessary to elucidate this subject.

In conclusion, the expression of the intraspecific aggressiveness before a stressor seems to activate the immune system and to potentiate the antigen-specific humoral response. This suggests that the aggressive behavior can increase the capacity of the organism to respond to noxious agents, and thus, it favors the survival of the species.

Acknowledgements: We thank Lúcia Maria Pires Ferreira for their superb assistance. Jairza Maria Barreto Medeiros is the recipient of a fellowship from the CAPES/PICDT/UFBA. This work was supported by CAPES, CNPQ and UFPE.

REFERENCES

BLALOCK, J. E., HARBOUR, M. C, MENAMIN D. & SMITH E. M., 1985, Peptides and hormones shared by the neuroendocrine and immunologic systems. J Immunol., 135:858-861.

CASTELNAU, E. T. & LÔO, H. L'ENCÉPHALE, 1993, Revue de Psychiatrie Clinique Biologique et Thérapeutique, 1:137-208.

CELADA, A. & NATHAN, C, 1994, Macrophage activation revisited. Immunol Today., 15:100-102.

COHEN, S., LINE, S., MANUCK, S. B., RABIN, B. S., HEISE, E.R. & KAPLAN, J. R., 1997, Chronic social stress, social status, and susceptibility to upper respiratory infections in nonhuman primates. Psychosom Med, 59:213–21

DANTZER, R., 1991, Des émotions à l'immunité et vice versa. Neuro Psy., 6:511-518.

DARDENNE, M. & SAVINO, W., 1996, Interdependence of endocrine and immune systems. Adv Neuroimmunol., 6:297-307.

DE CASTRO, C. M. M. B., MANHÃES DE CASTRO, R, MEDEIROS, A. F., QUEIRÓS SANTOS, A., FERREIRA E SILVA, W.T. & FILHO, J. L. L., 2000, Effect of stress on the production de O_2^- In alveolar macrophages. J Neuroimmunol., 108:68-72.

DEVOINO, L., ALPERINA, E. & PAVINA, T., 2003, Immunological consequences of the reversal of social status in C57BL/6J mice. Brain Behav Immun., 17:28–34.

DEVOINO, L., ALPERINA, E., GALKINA, O. & ILYUTCHENOK, R., 1997, Involvement of brain dopaminergic structures in neuroimmunomodulation. Int J Neurosci., 93:213–228.

DEVOINO, L., IDOVA, G., ALPERINA, E.M. & CHEIDO, M., 1994, Brain

neuromediator systems in the immune response control: Pharmacological analysis of pre- and postsynaptic mechanisms. Brain Res., 633:257–274.

DHABHAR, F. S. & MCEWEN, B. S., 1996, Stress-induced enhancement of antigen-specific cell-mediated immunity. J. Immunol., 156:2608-2615

DHABHAR, F. S., MILLER, A. H., MCEWEN, B. S. & SPENCER, R. L., 1995, Effects of stress on immune cell distribution - dynamics and hormonal mechanisms. J. Immunol., 154:5511-5527.

DHABHAR, F. S., MILLER, A. H., STEIN, M, MCEWEN, B. S. & SPENCER, R. L., 1994, Diurnal and stress-induced changes in distribuition of peripheral blood leukocyte subpopulations. Brain Behav Immunn., 8:66.

DHABHAR, F. S., MILLER, A. H., STEIN, M., MCEWEN, B. S. & SPENCER, R. L., 1996, Stress-Induced Changes in Blood LeuKocyte Distribution- Role of Adrenal Steroid Hormones. J. Immunol., 157:1638-1644

EICHELMAN, B. S., 1990, Neurochemical and psychopharmacologic aspects of aggressive behavior. Annu Rev Med.,41:149–158.

GASPAROTTO, O. C., IGNACIO, Z. M., LIN, K. & GONCALVES, S., 2002, The effect of different psychological profiles and timings of stress exposure on humoral immune response. Physiol Behav., 76:321-326

GRANGER, D. A., BOOTH, A. & JOHNSON, D. R., 2000, Human Aggression and Enumerative Measures of Immunity. Psychosom Med., 62:583–590.

KANT, G. J., MOUGEY, E. H., PENNINGTON, L. L. & MEYERHOFF, J. L., 1984, Graded footshock stress elevates pituitary cyclic amp and plasma ß-endorphin, ß-LPH, corticosterone and prolactin. Life Sci., 33:2657-2663.

LAWRENCE, A. D. & KIM, D., 2000, Central/peripheral nervous system and immune responses. Toxicology, 142:189–201.

MANHÃES DE CASTRO, R., BOLÃNOS-JIMÉNEZ, F., SEGUIN, L., SARHAN, H., DRIEU, K. & FILLION, G., 1996, Sub-chronic cold stress reduces 5-HT1A receptor responsiveness in the old but not in the young rat. Neurosci Lett., 203:21-24.

MANHÃES-DE-CASTRO, R., BARRETO MEDEIROS, J. M., MENDES-DA-SILVA, C., FERREIRA, L. M. P., GUEDES, R. C. A. & CABRAL-FILHO, J., E., COSTA, J. A., 2001, Reduction of intraspecific aggression in adult rats by neonatal treatment with a selective serotonin reuptake inhibitor. Braz J Med Biol Res., 34:121-124.

MCCARTY, R. & KOPIN, I. J., 1978, Sympatho-adrenal medulary activity and behavior during exposure to footshook stress: a comparison of seven rat strains. Physiol Behav., 21:567-572.

MEDEIROS, J.M., MENDES DA SILVA, C., SOUGEY, E. B., COSTA, J.A., DE CASTRO, C. M. M. B. & MANHÃES DE CASTRO, R., 2001, Action of selective serotonin reuptake inhibitor on aggressive behavior in adult rats submitted to the neonatal malnutrition. Arq Neuropsiquiatr., 59:499-503.

OTTAVIANI, E. & FRANCESCHI, C., 1996, The neuroimmunology of stress from invertebrates to man. Prog Neurobiol., 48:421-440.

SAWCHENKO, P. E., LI, H. Y & ERICSSON, A., 2000, Circuits and mechanisms governing hypothalamic responses to stress: a tale of paradigms. Brain Res., 122:61–78.

SGOIFO, A., BOER, S. F., HALLER, J., & KOOLHAS, J. M., 1996, Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. Physiol Behav., 60:1403-1407.

STEFANSKI, V. & ENGLER, H., 1998, Effects of Acute and Chronic Social stress on Blood Cellular Immunity in Rats. Physiol Behav., 64:733–741.

STEFANSKI, V. & ENGLER, H., 1999 Social Stress, dominance and blood cellular immunity. J Neuroimmunol., 94:144-152.

TOTH, M., 2003, 5-HT(1A) receptor knockout mouse as a genetic model of anxiety. Eur J Pharmacol., 463:177-184.

VOLVOKA, J., 1995, Neurobiology of Violence. Ed. American Psychiatric Press, Washington, 21 – 76.

WOOD, P. G., KAROL, M. H., KUSNECOV, A. W. & RABIN, B. S., 1993, Enhancement of antigen-specific humoral and cell-mediated immunity by electric footshock in rats. Brain, Behavior and Immunity., 7:121-134.

Figure 1

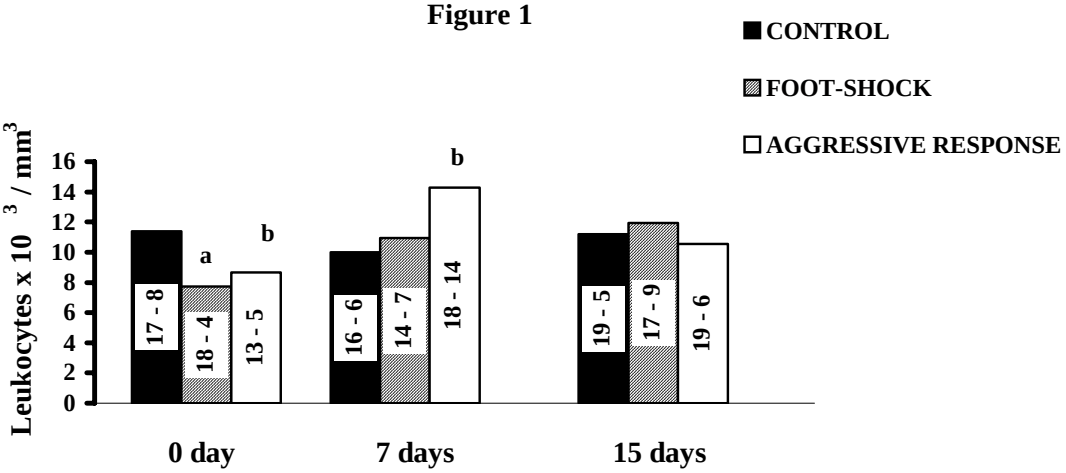

CONTROL
FOOT-SHOCK
AGGRESSIVE RESPONSE
Leukocytes x 10 3 / mm3
16
14
12
10
8
6
4
2
0
a
b
b
17 - 8
18 - 4
13 - 5
16 - 6
14 - 7
18 - 14
19 - 5
17 - 9
19 - 6
0 day
7 days
15 days

Figure 2

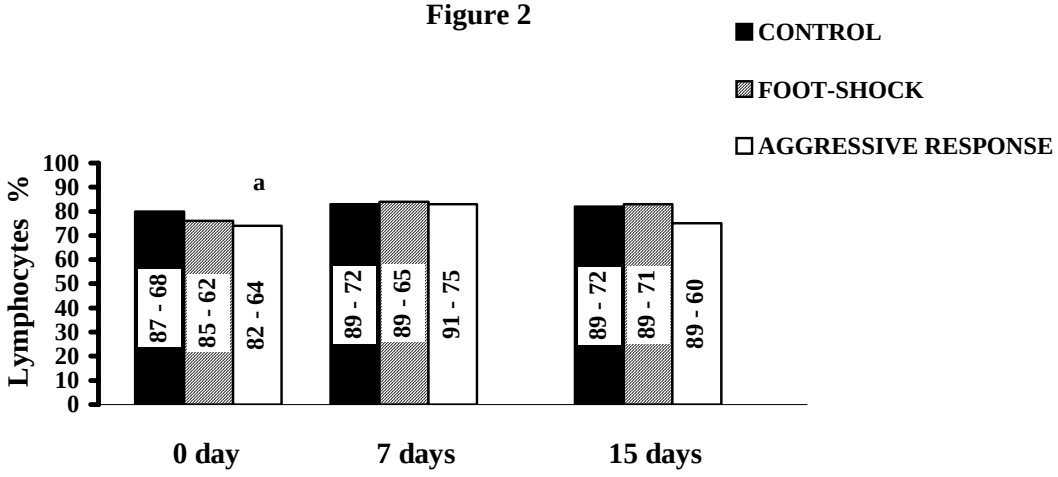

CONTROL
FOOT-SHOCK
AGGRESSIVE RESPONSE
Lymphocytes %
100
90
80
70
60
50
40
30
20
10
0
a
87 - 68
85 - 62
82 - 64
89 - 72
89 - 65
91 - 75
89 - 72
89 - 71
89 - 60
0 day
7 days
15 days

Figure 3

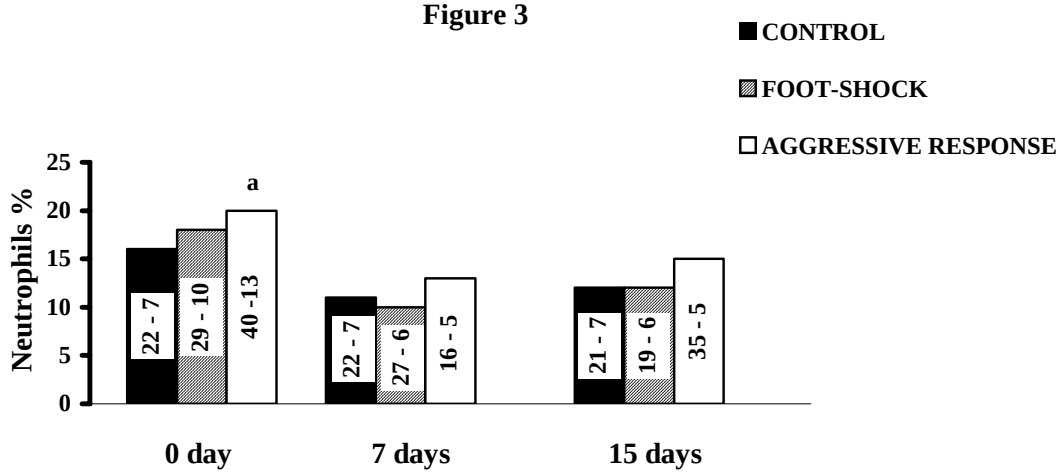

CONTROL
FOOT-SHOCK
AGGRESSIVE RESPONSE
Neutrophils %
25
20
15
10
5
0
a
22 - 7
29 - 10
40 -13
22 - 7
27 - 6
16 - 5
21 - 7
19 - 6
35 - 5
0 day
7 days
15 days

Figure 4

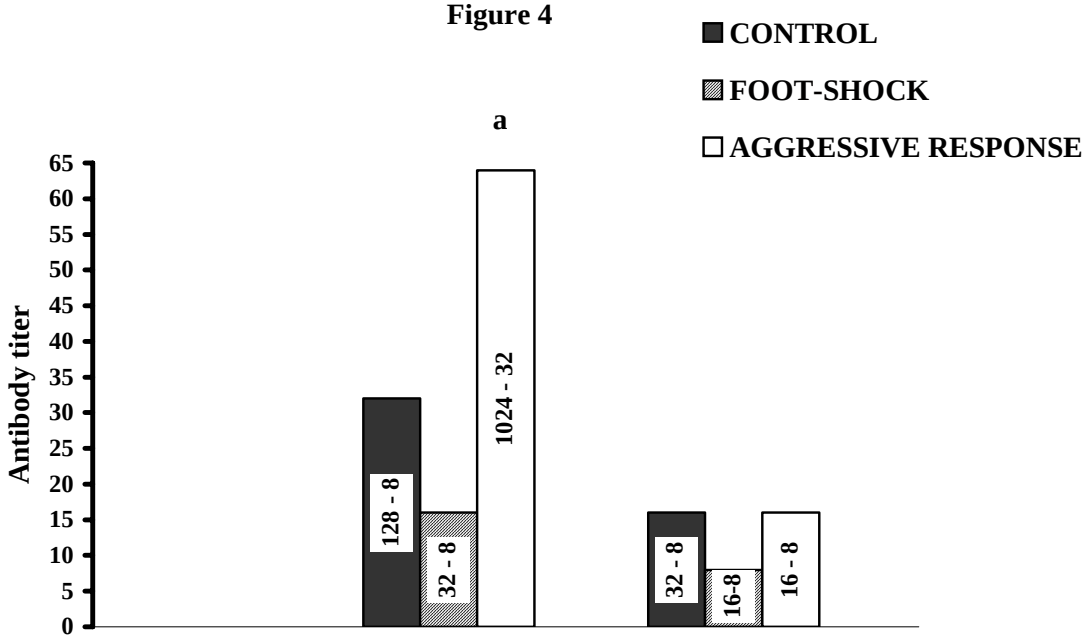

CONTROL
FOOT-SHOCK
AGGRESSIVE RESPONSE
a
Antibody titer
65
60
55
50
45
40
35
30
25
20
15
10
5
0
128 - 8
32 - 8
1024 - 32
32 - 8
16-8
16 - 8

FIGURE LEGENDS

Fig.1. Leukocytes were counted immediately, 7 and 15 days after submitting the rats to the stress induced by foot-shocks or after the aggressive behavior, as described in Methods. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between [a] Control x Foot shock; [b] Control x Aggressive Response ware made by Dunn's test ($p < 0.05$).

Fig.2. Lymphocytes were analyzed immediately, 7 and 15 days after submitting the rats to the stress induced by foot-shocks or after the aggressive behavior, as described in Methods. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between [a] Control x Aggressive Response ware made by Dunn's test ($p < 0.05$).

Fig.3. Neutrophils were analyzed immediately, 7 and 15 days after submitting the rats to the stress induced by foot-shocks or after the aggressive behavior, as described in Methods. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between [a] Control x Aggressive Response ware made by Dunn's test ($p < 0.05$).

Fig.4. The antibody titers were analyzed 7 e 15 days after immunization, as described in Methods. The animals were immunized immediately after the stress induced by foot-shocks or after the aggressive behavior. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between [a] Control x Aggressive Response ware made by Dunn's test ($p < 0.05$).

5.5. Quinto artigo, intitulado: **“EARLY MALNUTRITION ALTERS THE EFFECT OF AGGRESSIVENESS ON THE IMMUNE RESPONSE IN RATS”.** Artigo original submetido a publicação na revista: *Physiology and Behavior*, 2003.

Dando prosseguimento ao estudo apresentado no quarto artigo, sobre a inter-relação entre agressividade e resposta imune, o presente manuscrito teve como objetivo principal, observar os efeitos do comportamento agressivo intraespecífico sobre a resposta imune de ratos adultos submetidos a desnutrição precoce. Neste trabalho, foi encontrado que a desnutrição durante o período de rápido desenvolvimento do cérebro alterou a inter-relação entre comportamento agressivo e resposta imune em ratos adultos.

Early malnutrition alters the effect of aggressiveness on the immune response in rats

Jairza Maria Barreto-Medeiros[1], Kedma de Magalhaes Lima[1] , Emanuella Gomes Feitoza[1], Francisco Machado Manhaes-de-Castro[1], José Eulálio Cabral-Filho[2], Raul Manhaes-de-Castro[1], Célia Maria Machado Barbosa De-Castro[3*]

[1]Departamento de Nutrição, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil; [2]Instituto Materno Infantil de Pernambuco, Recife, PE, Brazil [3]Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Recife, PE, Brazil.

Running Title: malnutrition, aggressiveness, immunity

[*]Correspondence to: Dra. Célia Maria Machado Barbosa de Castro. Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Recife, CEP 50670-901 PE, Brasil, Fax: 55 81 32718485 E-mail: ccastro@lika.ufpe.br

Abstract

The effect of the malnutrition during suckling period on the imune response was investigated in adult rats submitted the intraspecific aggressiveness. Eighty-four Wistar male rats were allocated in two groups, according to their mother's diet during lactation. The well-nourished group was fed by mothers receiving a 23% protein diet; the malnourished one by mothers receiving a 8% protein diet. After weaning, all rats received the 23% protein diet. On the 90th day after birth, each nutritional group was divided in tree subgroups: control group (only the immunological measurements were accomplished); foot-shock (FS) (animals individually received FS) and intraspecific aggressive response (IAR) group (animals received FS and presented IAR). For immunological measurements, blood samples were collected immediately, 7 and 15 days after FS or IAR. In well-nourished rats the FS reduced the total amount of leukocytes. However, the aggressiveness was accompanied, besides the reduction of the leukocyte number, by lymphocytes decrease and neutrophils increase. Moreover, an elevation in the leukocyte number associated to an increase in the humoral immune response was also observed one week after the IAR in well-nourished but not in malnourished rats. In this study, the early malnutrition altered the interrelation between the aggressive behavior and the immune response.

Keywords: malnutrition, intraspecific aggressiveness, leukocyte and humoral immune response.

1. Introduction

The relationship between aggressive behavior and immune function is still little understood; it is well known, however, that particularly the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis are associated to the expression of aggressiveness (1,2,3). Catecholamines and glucocorticoids are powerful mediators of several immunologic responses (4,5). The responsiveness of both HPA axis and sympathetic-adrenal system seems to be altered by the perinatal malnutrition (6,7,8) as well. In a recent study, we observed that adult rats that were malnourished early in life are not affected by citalopram-induced anorexia (9). We have also found a hypo-responsiveness to the inhibitory effect of citalopram upon intraspecific aggressive behavior (10). Mounting evidences indicate that nutritional insults, during the period of fast brain development, can cause deleterious long-lasting effects on the brain and neural functions. (11,12,13). Depending on the severity of nutritional deficiency, these effects may not be reversible even by further dietary treatment or environmental stimulation (14,15).

The present study was accomplished to test the hypothesis that malnutrition during the period of fast brain development the so called brain growth spurt alters the relationship between the aggressive behavior and the humoral immune response, even after a long period of nutritional recovery.

2. Materials and methods

2.1 Animals and experimental groups

Male Wistar rats maintained at a room temperature of 23 ± 1ºC and in a light–dark cycle (light 6:00 a.m. - 6:00 p.m.) were used. During the suckling period, the

offsprings were housed in polyethilene cages in littler of 6 pups, randomly assigned to each mother. They were distributed in two nutritional groups according to the mother diet during lactation: a well-nourished group was fed by mothers receiving a 23% protein diet (purina chow) and a malnourished group, by mothers fed a 8% protein diet. After weaning (on the 25th day of age) all rats received the 23% protein diet. At the 90th day after birth each group was subdivided in three subgroups, as follows: foot-shock group (composed by animals that individually received one session foot-shock), aggressive response group (composed by animals disposed in pairs in the boxes receiving foot-shock session as well) and a control receiving no foot-shock. Immunological measurements were accomplished in each animal immediately and at 7 and 15 days following these treatments. Determination of the corticosterone levels in the serum of 15 well-nourished and 15 malnourished adult rats was assayed.

2.2. Determination of the corticosterone levels in the serum.

Samples of blood were collected in chilled tubes. The samples were centrifuged at 1000-x g for 5 min, and the supernatants were removed and stored at –20 ^{o}C until assayed for corticosterone. The serum levels of corticosterone, after their extraction with ethyl acetate, were quantified using the corticosterone kit, 1235 AutoDELFIA^{TM} automatic immunoassay system (EG&G WALLAC).

2.3. Stress induced by foot-shocks

Rats were submitted to stress induced by foot-shocks in an isolated room, using a box, 20 x 20 x 20 cm, with the floor consisting of parallel metallic bars (interbar distance: 1,3 cm), connected to an electric scrambled current source. The test consisted of placing one rat in the box, where it received a session of electric stimuli. Each

stimulus (an electrical foot-shock) was represented by a 1,6 mA – 2 sec current pulse. Each session lasted 20 min and was composed by 5 stimuli separated by a 4 min interval. During the first 3 min of this interval, the aggressive response was analyzed. The annotations and the verification of the equipment were done in the last minute of each interval.

2.4. Intraspecific aggressive response study

The aggressiveness test consisted in placing a pair of rats of the same group (matched by weight) in the box, where they received a session of electric stimulus under the same conditions of the anterior section. The aggressive response was defined as the presentation of, at least, one of the two following behaviors: a) the animals stayed lifted up on the hind paws, facing one the other, in a threatening attitude but without direct contact, or b) they maintained evident physical contact (besides scratches, exhibition of the teeth and emission of characteristic vocalization).

2.5. Blood sampling

Blood samples were collected immediately, 7 and 15 days after submitting the rats to the stress induced by foot-shocks or after the aggressiveness test. Blood was collected by the tail clip method as previously described (16). Samples were used for leukocyte (about 20 µl, EDTA a 3%) and antibody titer analysis.

2.6. *White blood cells (WBC) and leukocyte subsets*

Total WBC counts were determined in a hemocytometer. The percentage of lymphocyte and neutrophils was determined with a microscope (May-Grün-wald/Giemsa staining).

2.7. Immunization

The animals were immunized immediately after the stress induced by foot-shocks or after the aggressive behavior. Sheep red blood cells (SRBC) were prepared by washing citrated sheep blood three times in sterile saline. Animals were immunized intraperitoneally with 10^8 cells/ml in a volume of approximately 0.5 ml.

2.7. Determination of Antibody titer

Blood samples were collected before (negative control of antibody titer anti-SRBC) and 7 e 15 days after immunization. Samples were subsequently centrifuged at 3000 rpm for 5 min and the supernatant collected. Serum complement was then inactivated at 56ºC for 30 min and stored at -20^0C. Twofold serial dilutions of inactivated serum, saline, and a 1% SRBC solution were then mixed in miccrowells glass. The highest dilution at which aggregation of SRBCs was still evident was considered to be the antibody titer.

2.8. Statistical analysis

Corticosterone levels between nutritional groups were compared by the two-tailed Student t-test. Leukocyte and antibody titer were transformed in log and analyzed by one-way ANOVA followed by Tukey test for multiple comparisons. The null hypothesis was rejected when $p \leq 0,05$.

3. Results

3.1. Corticosterone level in the serum

The corticosterone levels in the serum of the malnourished animals were lower ($p<0.001$) when compared to that of the well-nourished animals (Figure 1).

3.2. Effect of stress induced by foot shocks and of the intraspecific aggressive response on WBC in rats well nourished or malnourished during suckling period

In the well-nourished group, the animals that received foot shocks presented an immediate reduction in the total leukocytes counting compared to the control. In the same way, the aggressive response well-nourished group presented an immediate reduction in the total leukocytes counting. However, no difference was observed in the total leukocytes counting among the malnourished animals. Moreover, also there was no significant difference 7 and 15 days after the foot-shocks in both well-nourished and malnourished groups. However, 7 days after the aggressive response, an increase was observed in the total number of those cells between the well-nourished animals but not in malnourished (Table 1).

3.3. Effect of stress induced by foot shocks and of the intraspecific aggressive response on leukocyte subsets in rats well nourished or malnourished during suckling period

There was no difference in the lymphocytes and neutrophils percentages between control and foot-shocks in both well-nourished and malnourished groups. However, the aggressiveness reduced the percentage of lymphocytes and increased the percentage of neutrophils immediately after the expression of the aggressive behavior in well-nourished animals, but not in malnourished ones. Besides, 7 and 15 days after the induction of the aggressive response, there was no significant difference in both well-nourished and malnourished groups when compared to the respective controls (Table 2).

3.4. Effect of stress induced by foot-shocks and of the intraspecific aggressive response on humoral immunity in rats well nourished or malnourished during suckling period

Compared to the control, there was no alteration in the humoral immune response of the either well-nourished or malnourished animals submitted to the foot-shocks. However, the intraspecific aggressiveness increased the titers of anti-SRBC antibodies 7 days after the immunization in the well-nourished group but not in malnourished one. Moreover, 15 days after the immunization the aggressive response didn't change the humoral immune response in both well-nourished and malnourished groups when compared to the respective controls (Table 3).

PLEASE INSERT RELATED FIGURES HERE

4. Discussion

In this study, the expression of the intraspecific aggressive response was accompanied by the reduction of the leukocytes counting, the lymphocytes decrease and the neutrophils increase. Besides, an elevation in the leukocytes counting associated to an increase in the humoral immune response one week after the aggressive interactions was observed. The HPA axis and also other elements of the response to stress are related to the expression of aggressive behavior (17,18). However, in this study, it was observed that differently from the aggressiveness, the stress induced by the foot-shocks just reduced the amount of leukocytes in the blood current. That reduction was reverted one week after ceasing the stress. Thus, we can suppose that, in the present study, the immunological alterations induced after the expression of the aggressive behavior is mediated by neuro-endocrine components specifically associated to this behavioral response. A specific neuro-endocrine profile (synthesis and liberation of several chemical messengers), associated to expression of the aggressive behavior, distinct from that of stress, may have affected the

immunological components in a particular way. This hypothesis is plausible and it also corroborates evidences that immune cells can interact with hormones, neuropeptides and neurotransmitters (19,20,21).

In the present study, the malnutrition seems to have interfered in the interrelation between the aggressive behavior and the immunological parameters studied. The mechanism responsible for that alteration is still not clear. It is known that the perinatal malnutrition alters the activity of the HPA axis before a stressor (22,23). In rats, it was observed that maternal malnutrition interferes in the fetal development of the HPA axis, by producing alterations in the adrenal and in the pituitary (24).

Moreover, in this study, it was interesting to notice that the early malnutrition reduced the serum corticosterone levels in adult life. It is known that glucocorticoids can assure that specific leukocytes be present in the right place in the right time to respond to incentives begun by stress inductor agents (25). Thus, it is very probable that the similar immune response between malnourished control and malnourished submitted to intraspecific aggressiveness, here observed, be consequence of neuro-endocrine alterations provoked by the malnutrition during the critical period of development of the brain.

Another important aspect to consider is that the perinatal period is marked by the rapid expansion of the leukocytes population and formation of lymphoid organs (26,27). Important events for the immunocompetence are initiated still in the embryo and they continue during the first week of life (28,29,30). In this period, there are some functionally active T cells and just in the fifth and in the sixth weeks of life the subpopulations of T cells are totally immunocompetent (31). Thus, in this work it is possible that the nutritional deficiency during the neonatal period has also affected in a long lasting way the immune system, making it hyporesponsive to the expression of

the aggressive response.

In conclusion, the malnutrition during the brain growth spurt altered the interrelation between the aggressive behavior and the immune response. The nutritional insult seems to have a long-lasting effect upon the functioning of neuro-endocrine components specifically associated to the expression of aggressive response and mediators of the humoral immune response.

Acknowledgements

The authors thank Lúcia Maria Pires Ferreira for her superb and timely assistance. Jairza Maria Barreto Medeiros is the recipient of a fellowship from the CAPES/PICDT/UFBA. This investigation was supported by CAPES, CNPQ and UFPE.

References

1. Haller, J. Alpha-2 Adrenoceptor Blockade and the Response to Intruder Aggression in Long-Evans Rats. Physiol Behav. 58:101-106; 1995a.
2. Haller, J.Catecholamine Stimulation and the Response to Behavioral Challenge in Wistar Rats. Pharmacol. Biochem. Behav. 514:789-794;1995b.
3. Veenema, H. A.; Meijer, O.C.; Kloet, E.R.; Koolhaas, J.M.; Bohus B.G. Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. Hormones. J. Immunol. 43: 197–204; 2003.
4. Felsner, P.; Hofer, D.; Rinner, I.; Porta, S.; Korsatko, W.; Schauenstein, K. Adrenergic suppression of peripheral blood T cell reactivity in the rat is due to activation of peripheral alpha 2-receptors. J. Neuroimmunol. 57(1-2):27-34; 1995.
5. De Castro, C.M.M.B.; Manhaes de Castro, R.; Medeiros, A.F.; Queirós Santos, A.;

Ferreira e Silva, W.T.; Filho, J.L.L. Effect of stress on the production de O_2^- In alveolar macrophages. J. Neuroimmunol. 108:68-72; 2000.

6. Kehoe, P., Mallinson, K., Bronzino, J., McCormick, C.M. Effects of prenatal protein malnutrition and neonatal stress on CNS responsiveness. Dev. Brain Res.132:23–31; 2001.
7. Lesage, J.; Blondeau, B.; Grino, M.; Bre´Ant, B. Maternal Undernutrition during Late Gestation Induces Fetal Overexposure to Glucocorticoids and Intrauterine Growth Retardation, and Disturbs the Hypothalamo-Pituitary Adrenal Axis in the Newborn Rat. Endocrinology. 142:1692 – 1702; 2001.
8. Jezova, D.; Skultetyova, I.; Makatsori, A.; Moncek, F.; Duncko, R. Hypothalamo-pituitary-adrenocortical axis function and hedonic behavior in adult male and female rats prenatally stressed by maternal food restriction. Stress. 5:177-83; 2002.
9. Barreto Medeiros, J.M.; Cabral Filho, J.E.; De Souza, S.L.; Freitas Silva, S.R.; Mendes da Silva, C.; Deiró, T.C.B.J.; Monteiro, J.M.; Guedes, R.C.A.; De Castro, C.M.M.B; Manhães de Castro, R. Early malnourished rats are not affected by anorexia induced by a selective serotonin reuptake inhibitor in adult life. Nutr. Neurosci. 5:211-214; 2002.
10. Medeiros, J.M.; Mendes da Silva, C.; Sougey, E.B.; Costa, J. A.; De Castro; C.M.M.B.; Manhães de Castro, R. Action of selective serotonin reuptake inhibitor on aggressive behavior in adult rats submitted to the neonatal malnutrition. Arq Neuropsiquiatr. 59(3-A):499-503; 2001.
11. Morgane, P.J.; Austin-la France, R.J.; Bronzino, J.; Tonkiss, J. Diaz-Cintra, S.; Cintra, L.; Kemper, T.; Galler, J. R. Prenatal malnutrition and development of the brain. Neurosci Biobehav Rev. 17: 91-128; 1993.
12. Datta, S.; Patterson, E.H.; Vincitore, M.; Tonkiss, J.; Morgane, P.J.; Galler, J.R.

Prenatal protein malnourished rats show changes in sleep changes in sleep/wake behavior as adults. J. Sleep Res. 9: 71-9; 2000.

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

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

15. Morgane, P.J.; Austin-la France, R.J.; Bronzino, J.; Tonkiss, J.; Galler, J.R. Malnutrition and developing central nervous system. In Isaacson R.L., Jensen K.F. (eds). The vulnerable brain and environmental risks. New York Plenum Press 1:2-42; 1992.

16. Dhabhar, F.S; Miller, A.H.; Stein, M.; Mcewen, B.S.; Spencer, R.L Stress-Induced Changes in Blood LeuKocyte Distribution- Role of Adrenal Steroid Hormones. J. Immunol.157:1638-1644; 1996.

17. Sgoifo, A.; Boer, S.F.; Haller, J.; Koolhas, J.M. Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. Physiol. Behav. 60:1403-1407; 1996.

18. Lawrence, A.D.; Kim, D. Central/peripheral nervous system and immune responses. Toxicology. 142:189–201; 2000.

19. Blalock, J.E.; Harbour, M.C.; Menamin, D.; Smith, E.M. Peptides and hormones shared by the neuroendocrine and immunologic systems. J. Immunol. 135:858-861; 1985.

20. Young, M.R.; Matthews, J.P. Serotonin regulation of T-cell subpopulations and of macrophage accessory function. Immunology. 84:148-152; 1995.

21.Ottaviani, E.; Franceschi, C. The neuroimmunology of stress from invertebrates to man. Prog Neurobiol. 48:421-440; 1996.

22.Lesage, J.; Dufourny, L.; Laborie, C.; Bernet, F.; Blondeau, B.; Avril, I.; Breant, B.; Dupouy, J.P. Perinatal malnutrition programs sympathoadrenal and hypothalamic-pituitary-adrenal axis responsiveness to restraint stress in adult male rats. J Neuroendocrinol. 14:135-43; 2002.

23.Leonhardt, M.; Lesage, J.; Dufourny, L.; Dickes-Coopman, A.; Montel, V.; Dupouy, J.P. Perinatal maternal food restriction induces alterations in hypothalamo-pituitary-adrenal axis activity and in plasma corticosterone-binding globulin capacity of weaning rat pups. Neuroendocrinology. 75:45-54; 2002.

24.Hawkins, P.; Steyn, C.; McGarrigle, H.H.; Calder, N.A.; Saito, T.; Stratford, L.L.; Noakes, D.E; Hansona, M A. Cardiovascular and hypothalamic-pituitary-adrenal axis development in late gestation fetal sheep and young lambs following modest maternal nutrient restriction in early gestation. Reprod. Fertil. Dev. 12:443-56; 2000.

25.Dhabhar, F.S.; Miller, A.H.; Mcewen, B.S.; Spencer, R.L Effects of stress on immune cell distribution - dynamics and hormonal mechanisms. J. Immunol.154:5511-5527;.1995.

26.Mosier, D.E.; Johnson, B.M. Ontpgeny of mouse lymphocyte function II. Development of yhe ability to produced antibody is modulated by T lymphocytes. J. Exp. Med. 141:216-226; 1975.

27.Szewczuk, M.R.; Sherr, D.H.; Siskind, G.W. Ontogeny of B lymphocyte function. VI. Ontogeny of thymus cell capacity to facilitate the functional maturation of B lymphocytes. Eur. J. Immunol. 8:370-3; 1978

28.Papiernik, M.; Ezine, S. Postnatal development of T cells. III. Thymus

independency of T-cell-dependent antigen response in the neonatal spleen. Immunology. 46:209-14; 1982.

29. Kale, S.A.; Rao, S.GHaemopoietic stem cells during development of mouse embryo. Indian J Exp Biol. 30:371-376; 1992.

30. Gobel, T. W. F. The T-dependent immune system. In: Poultry Immunology. T. F. Davidson, T. R. Morris, and L. N. Payne, ed. Poultry Science Symposium Series. Vol.24. Carfax Publishing, Oxfordshire, U. K.;.1996.

31. Mosier, D.E.; Cohen, P.I. Ontogeny of mouse T lymphocyte function. Fed. Proc. 34:137-140; 1975.

Figure 1

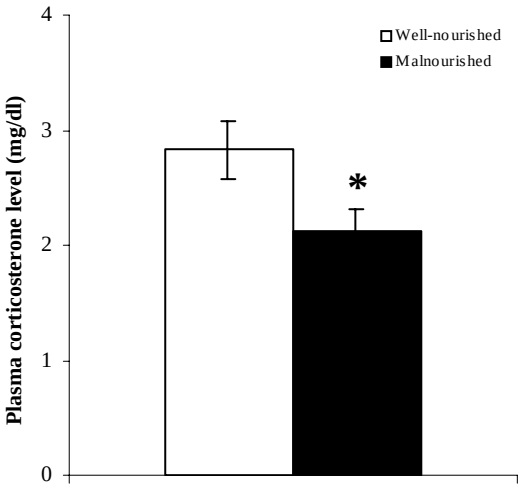

Plasma corticosterone level (mg/dl)
4
3
2
1
0
Well-nourished
Malnourished
*

Table 1

Groups (n=14)	Time (days after induction)					
	0		7		15	
WNC	12280	± 737	10425	± 846	10768	± 726
WNAG	8623	± 685[a]	13841	± 698[a]	11140	± 841
WNFS	9052	±1285[b]	10995	± 593	12093	± 551
MC	10321	± 666	10561	± 561	10054	± 757
MAG	11016	±1009	10307	± 443	10357	± 995
MFS	10243	± 571	12527	± 827	10996	±1251

Table 2

Groups(n=14)	Time (days after induction)					
	0		7		15	
	LT (%)	NT(%)	LT(%)	NT(%)	LT(%)	NT(%)
WNC	81 ±1,21	15 ±1,21	82 ±1,34	13 ±1,22	82 ±1,53	12 ±1,42
WNAG	72 ±1,29*	25 ±2,06*	83 ±0,86	12 ±0,62	74 ±2,59	15 ±1,54
WNFS	75 ±1,93	18 ±1,72	80 ±2,36	13 ±1,99	81 ±1,48	12 ±1,21
MC	81 ±2,35	16 ±2,22	80 ±1,52	15 ±1,43	79 ±1,50	17 ±1,90
MAG	79 ±2,36	15 ±1,88	82 ±1,37	14 ±1,37	79 ±2,65	15 ±2,18
MFS	81 ±1,33	16 ±1,51	77 ±1,49	17 ±1,07	74 ±1,72	21 ±1,33

Table 3

Groups(n=14)	Time (days after induction	
	7	15
WNC	46 ±10,69	16 ±2,52
WNAG	114 ±34,65*	22 ±2,40
WNFS	20 ±02,18	10 ±1,00
MC	26 ±03,77	15 ±1,65
MAG	62 ±12,19	33 ±5,86
MFS	33 ±17,32	29 ±9,17

FIGURE LEGENDS

Figure 1. Corticosterone was measured by radioimmunoassay in the plasma and [1,2,6,7-^{3}H] corticosterone as radioligand described in Materials and methods. Data represent the mean ± S.E.M. from fifteen animals. Comparisons between well-nourished and malnourished group at the same age were made by two-tailed Student t-test (* $p<0,001$).

Table 1. Leukocytes were analyzed immediately (0), seven (7) and fifteen (15) days after submitting the rats to the stress induced by foot-shocks or after the aggressive behavior. Data represent the mean ± S.E.M. for each group: control well-nourished (WNC); aggressive response well-nourished (WNAG); foot-shock well-nourished (WNFS); control malnourished (MC); aggressive response malnourished (MAG); foot-shock malnourished (MFS). Comparisons among groups (ANOVA, $p<0,05$) and multiple comparisons between groups (Tukey, test,*$p<0,05$): [a]WNFS x WNC and [b]WNAG x WNC.

Table 2. Lymphocytes (LT) and neutrophils (NT) were analyzed immediately (0), seven (7) and fifteen (15) days after submitting the rats to the stress induced by foot-shocks or after the aggressive behavior. Data represent the mean ± S.E.M. for each group: control well-nourished (WNC); aggressive response well-nourished (WNAG); foot-shock well-nourished (WNFS); control malnourished (MC); aggressive response malnourished (MAG); foot-shock malnourished (MFS). Comparisons among groups (ANOVA, $p<0,05$) and multiple comparisons between groups (Tukey test,*$p<0,05$):

*WNAG x WNC.

Table 3. The antibody titers were analyzed seven (7) e fifteen (15) days after immunization. The animals were immunized immediately after the stress induced by foot-shocks or after the aggressive behavior. Data represent the mean ± S.E.M. for each group: control well-nourished (WNC); aggressive response well-nourished (WNAG); foot-shock well-nourished (WNFS); control malnourished (MC); aggressive response malnourished (MAG); foot-shock malnourished (MFS). Comparisons among groups (ANOVA, $p<0,05$) and multiple comparisons between groups (Tukey test,*$p<0,05$): *WNAG x WNC.

DISCUSSÃO GERAL

6 – DISCUSSÃO:

O presente estudo mostrou que tratamento com inibidor seletivo de recaptação de serotonina reduz a agressividade intraespecífica e o consumo alimentar em ratos adultos. Demonstrou ainda, que a expressão da agressividade intraespecífica é acompanhada por alteração na quantidade de células imunes e por aumento nos títulos de anticorpos específicos anti-hemácias de carneiro. Além disso, alterações somáticas e funcionais foram evidenciadas e parecem estar associadas à desnutrição imposta no período crítico de desenvolvimento do cérebro. Assim, déficit no peso corporal, redução da resposta a inibidor seletivo de recaptação de serotonina e hiporresponsividade à ação da agressividade intraespecífica sobre a resposta imune foram observados em ratos desnutridos, mesmo após longo período de recuperação nutricional.

A redução da agressividade após o tratamento com inibidor de recaptação de serotonina encontrada no presente trabalho, pode ser uma conseqüência de um aumento na neurotransmissão serotoninérgica. Os ISRS aumentam a disponibilidade sináptica de serotonina e consequentemente potencializam a ação desta monoamina (HYTTEL, 1978; HYTTEL, 1994). Os resultados parciais deste trabalho foram publicados (MEDEIROS *et al.,* 2001) e corroboram os dados de outros pesquisadores (SANCHEZ e HYTTEL, 1994; COCCARO e KAVOUSSI, 1997; REIST *et al.,* 2003).

O papel da serotonina no controle do comportamento agressivo está relacionado a distribuição das vias serotoninérgicas, especialmente as projeções que inervam áreas cerebrais envolvidas nos processos emocionais

(OLIVER *et al.,* 1995). A redução da atividade serotoninergica parece aumentar a agressividade (ANNEMOON *et al.,* 2000; KEELE *et al.,* 2001). Em contraste, o aumento da disponibilidade sináptica de serotonina parece reduzir esse comportamento (MARSH *et al.,* 2002). Assim, redução dos níveis de serotonina foi associada com aumento de comportamento agressivo induzido por isolamento em ratos (KEELE *et al.,* 2001). Baixos níveis de 5-HIAA foram encontrados em primatas com agressividade intensa (MEHLMAN *et al.,* 1994). Outrossim, em mulheres, a suplementação alimentar com triptófano reduziu a resposta agressiva (MARSH *et al.,* 2002).

O efeito inibitório da fluoxetina e do citalopram sobre o comportamento agressivo é suportado pelo presente estudo, uma vez que animais nutridos tratados com esses ISRS apresentaram redução do comportamento agressivo, quando comparados aos animais tratados com salina do mesmo grupo nutricional. Estes resultados concordam com estudos anteriores realizados em humanos (COCCARO e KAVOUSSI, 1997; REIST *et al.,* 2003; ARMENTEROS e LEWIS, 2003) e em animais de várias espécies, (SANCHEZ e HYTTEL, 1994; SPERRY *et al.,* 2003; PERREAULT *et al.,* 2003). Assim, em indivíduos portadores de desordens de personalidade com história de comportamento agressivo impulsivo foi observada redução da agressividade após tratamento crônico com fluoxetina (COCCARO e KAVOUSSI, 1997). Do mesmo modo, tratamento crônico com citalopram foi efetivo em reduzir o comportamento agressivo impulsivo em homens com desordens de personalidade (REIST *et al.,* 2003). Em crianças e adolescentes, tratamento crônico com citalopram também reduziu o comportamento agressivo impulsivo (ARMENTEROS e LEWIS, 2003). Além

disso, diminuição da agressão induzida pelo isolamento foi observada em camundongos, após o tratamento agudo com sertralina, fluoxetina e fluvoxamina (SANCHEZ e HYTTEL, 1994). Em pardais, tratamento agudo com fluoxetina diminuiu o comportamento agressivo (SPERRY *et al*., 2003). Outrossim, tratamento agudo e crônico com fluoxetina reduziu a agressividade em peixes (PERREAULT *et al.,* 2003). Em suas pesquisas, Sperry *et al*., (2003) e Perreault *et al.,* (2003) utilizaram o modelo intruso-residente para indução do comportamento agressivo.

Neste estudo, foi também demonstrado que animais bem nutridos tratados com citalopram (10mg/kg/dia) por 14 dias apresentam redução do consumo alimentar e conseqüente perda de peso corporal quando comparados com os animais tratados com salina do mesmo grupo nutricional (dados publicados; BARRETO-MEDEIROS *et al.,* 2002). Este efeito anoréxico pode ser uma conseqüência do aumento da serotonina extra celular seguido ao bloqueio sináptico da recaptação dessa amina, induzido pelo tratamento farmacológico. Corroborando essa hipótese, estudos têm mostrado que duas semanas de tratamento com inibidor seletivo de recaptação de serotonina são suficientes para provocar alterações significantes no sistema serotoninergico (ARBORELIUS *et al.,* 1996; PERREAULT *et al*., 2003).

O efeito hipofágico do citalopram observado no presente trabalho, corrobora achados de alteração na ingesta alimentar induzida por outros agentes serotoninergicos (HALFORD e BLUNDEL, 1996B; LUCAS *et al.,* 1998; Li *et al.,* 1998). É bem conhecido que serotonina, drogas que estimulam sua liberação ou bloqueiam sua recaptação, triptófano e alguns agonistas de receptores serotoninérgicos produzem rápida e substancial anorexia

(BLUNDEL, 1992; HALFORD e BLUNDEL, 2000; LEE *et al*., 2002). Em ratos, tratamento com fluoxetina (20 e 40 mg/Kg de peso corporal) reduziu a ingesta de leite condensado 1 hora após sua administração (LI *et al.,* 1998). Do mesmo modo, ratos tratados com sertralina (10mg/Kg), outro inibidor seletivo de recaptação de serotonina, apresentaram redução do consumo alimentar (LUCKI *et al.,* 1988). Portanto, é bem provável que o efeito hipofágico do citalopram resulte de sua ação sobre o sistema serotoninérgico. Além disso, Hyttel (1994) afirma que a administração repetida de citalopram mantém uma seletiva e potente inibição da recaptação de serotonina. Assim, um aumento constante da disponibilidade sináptica da serotonina e consequente potencialização do efeito inibitório dessa amina sobre o comportamento alimentar é uma hipótese muito plausível.

Poucos são os estudos sobre os efeitos de manipulações do sistema serotoninérgico em indivíduos desnutridos, particularmente sobre a expressão comportamental. Neste estudo, foram investigados os efeitos da desnutrição pregressa sobre o consumo alimentar e o comportamento agressivo em ratos adultos tratados com inibidor seletivo de recaptação de serotonina. Os resultados obtidos mostraram que ratos adultos submetidos à desnutrição neonatal tornam-se resistentes aos efeitos hipofágico e anti-agressividade de ISRS. Além disso, foi observado que os animais desnutridos durante o aleitamento pelo uso da Dieta Básica Regional (DBR) apresentam redução de peso corporal até a idade adulta.

O déficit de peso corporal apresentado pelos animais desnutridos, atesta a eficiência do modelo de desnutrição empregado. A DBR possui apenas 7.87% de proteínas, sendo 96% de proteínas de origem vegetal,

provenientes de feijão e de raízes e apenas 4%, de carne salgada e seca (TEODÓSIO *et al.,* 1990). É, portanto, dieta pobre em proteínas em termos quantitativos e qualitativos. O baixo valor biológico das proteínas da DBR, ou seja seu teor deficitário em alguns aminoácidos essenciais (TEODÓSIO *et al.,* 1990), parece ser importante na gênese de seus efeitos deletérios.

Nossos dados corroboram evidências experimentais indicando redução ponderal de animais submetidos à desnutrição precoce (BORBA *et al.,* 2000; MANJARREZ *et al.,* 2003). Assim, Borba *et al.,* (2000) observaram menor peso corporal em ratos jovens (30 a 40 dias de vida) submetidos à dieta hipoproteica (7,8% de proteína) durante o período neonatal. Do mesmo modo, Manjarrez *et al.,* (2003) também constataram que redução (50%) na cota alimentar materna durante o período gestacional afeta o ganho de peso corporal dos filhotes. É importante ressaltar que as repercussões da desnutrição precoce sobre o ganho de peso corporal parece ter sido duradouro. A oferta, portanto, de dieta nutricionalmente adequada, a partir do desmame, não foi eficiente na recuperação das eventuais alterações originadas no período crítico do desenvolvimento do SN.

Esse inadequado ganho de peso corporal pode ser uma conseqüência da deficiência protéica imposta as ratas mães durante o período de aleitamento. Corroborando essa hipótese, alguns estudos encontraram alteração na qualidade do leite de ratas desnutridas (PINE *et al.,* 1994; MARÍN *et al.,* 1995). Assim, Pine *et al.,* (1994) demonstraram que dieta hipoproteica (9% de proteína) durante a lactação diminui o conteúdo de proteína e de lactose do leite. Do mesmo modo, Marín *et al.,* (1995) observaram redução na concentração de ácidos graxos saturados no leite de ratas submetidas à

restrição (5, 10 e 15%) de proteínas durante a gestação e lactação. Parece que, a capacidade materna de manter a lactação depende dos nutrientes da dieta, do grau de reserva corporal materno e da capacidade de mobilização dos tecidos, pelas fêmeas (PESSOA, 1997).

A hiporresponsividade dos animais desnutridos a fluoxetina e ao citalopram aqui observada, pode estar relacionada aos efeitos da agressão nutricional sobre o sistema serotoninergico. Em ratos, os primeiros neurônios serotoninérgico aparecem entre o 12º e o 14º dia de gestação (LAUDER e BLOOM, 1974), mas a densidade final e localização definitiva dos terminais serotoninérgicos é estabelecida durante a maturação pós-natal do sistema nervoso central (LIDOV e MOLLIVER, 1982). Ademais, há várias evidências de que a desnutrição durante a fase perinatal resulta em conseqüências neuroquímicas, incluindo os sistemas de neurotransmissores (BLATT *et al.,*1994; MANJARREZ *et al.,* 2003). Neste sentido, Blatt *et al.,* (1994) observaram que ratos desnutridos no período pré-natal possuem um decréscimo das fibras serotoninérgicas do giro denteado, da CA1 da formação hipocampal e redução dos sítios de captação de serotonina na CA3 e CA1. Manjarrez *et al.,* (2003) encontraram elevação no conteúdo de serotonina no cortex e no tronco cerebral de ratos submetidos à desnutrição também no período pré-natal. Assim, no presente trabalho, a desnutrição precoce pode ter causado alterações duradouras, que refletiram sobre as respostas funcionais do sistema serotoninérgico às drogas, especialmente aquelas que agem especialmente neste sistema de neurotransmissão.

Corroborando, esta hipótese, Hall *et al.,* (1983) verificaram que ratos desnutridos precocemente e estimulados por um agonista

serotoninergico (DMT: 5-methory–N, N– dimethyltryptamine) apresentaram menor desempenho que os animais nutridos em vários testes comportamentais, tais como *rota-rod* e *treadmill* (HALL *et al.,* 1983). Segundo Almeida *et al.,* (1996) ratos submetidos à desnutrição no início da vida são também hiporreativos aos efeitos anxiolíticos de drogas que agem sobre os receptores centrais 5-HT1A (ALMEIDA *et al.,* 1996). Assim, outros estudos objetivando evidenciar as possíveis alterações que o sistema serotoninérgico sofreu mediante a agressão nutricional, durante o período de rápido desenvolvimento do encéfalo, são necessários para esclarecer os mecanismos responsáveis pela alteração da responsividade ao tratamento com inibidor seletivo de recaptação de serotonina sobre a agressividade intraespecífica e o consumo alimentar, observados no presente trabalho.

Há grande número de evidências demonstrando que estresse e reações emocionais afetam o sistema imune (STEFANSKI e ENGLER, 1998; DE CASTRO *et al*., 2000; GASPAROTTO *et al*., 2002). Contudo, são poucos os estudos sobre a inter-relação entre o comportamento agressivo e função imune em mamíferos; sobretudo, controlando a interferência do fator estresse. Assim, o presente trabalho investigou se a expressão da agressividade intraespecífica frente a um estressor altera a resposta imune em ratos adultos. Os resultados demonstraram que a agressividade intraespecífica reduziu a contagem total de leucócitos, diminuiu o percentual de linfócitos e aumentou o percentual de neutrófilos no sangue de ratos adultos. Ademais, uma elevação na contagem total de leucócitos associada a um aumento nos títulos de anticorpos anti-hemácias de carneiro foram observados uma semana após as interações agressivas.

Até agora, pouco se conhece sobre os mecanismos responsáveis pelas alterações em variáreis imunológicas induzidas pelo comportamento agressivo. Entretanto, é bem conhecido que o eixo HPA e também outros elementos da resposta ao estresse como o sistema nervoso simpático estão estreitamente relacionados à expressão deste comportamento (SGOIFO *et al.,* 1996; LAWRENCE e KIM, 2000; CAROBREZ *et al.,* 2002). É importante destacar que neste estudo, diferente da agressividade foi observado que o estresse induzido pelo choque nas patas reduziu apenas a quantidade de leucócitos na corrente sangüínea. E as alterações no número de leucócitos foram revertidas uma semana após cessar o estresse. Assim, podemos supor que, no presente trabalho, as alterações imunológicas induzidas após expressão do comportamento agressivo são mediadas por componentes neuro-endócrinos associados especificamente a esta resposta comportamental. Um perfil neuro-endócrino (síntese e liberação de vários mensageiros químicos) específico associado à agressividade, distinto daquele do estresse, pode ter afetado de modo característico os distintos componentes imunológicos. Esta hipótese é plausível e corrobora também evidências de que células imunes podem interagir com hormônios, neuropeptídeos e neurotransmissores (BLALOCK *et al.,* 1985; OTTAVIANI e FRANCESCHI, 1996). Ademais, em um estudo recente foi observado que a presença de citocinas no hipotálamo, particularmente a interleucina–1 (IL-1), potencializou o comportamento agressivo em gatos e que pré-tratamento com um antagonista seletivo do receptor 5-HT2 bloqueou este efeito (HASSANAIN *et al.,* 2003). Estes pesquisadores sugerem que o efeito facilitatório da IL-1 sobre o comportamento emocional em gatos pode ser mediado por este tipo de

receptor serotoninérgico (HASSANAIN *et al.,* 2003).

O número e a proporção de leucócitos no sangue fornece uma importante representação do estado de distribuição de leucócitos no organismo e aparente estado de ativação do sistema imune (DHABHAR *et al.,* 1995). Assim, no presente trabalho as alterações no padrão leucocitário observadas após as interações agressivas podem ser resultado da ativação do sistema imune frente a um novo componente comportamental. Segundo SPRENT e TOUGH (1994), a circulação contínua das células imune do sangue para os diversos compartimentos imunes e o retorno para a circulação é essencial para a manutenção de uma defesa imune efetiva.

Ressaltamos, que as alterações no padrão leucocitário observadas imediatamente após as interações agressivas foram restritas a curto período de tempo, pois uma semana após o confronto nós encontramos aumento no número total de leucócitos e nos títulos de anticorpos anti-hemácias de carneiro. Nossos dados concordam em parte com os resultados encontrados por GASPAROTTO *et al.,* (2002). Estes investigadores observaram que camundongos submissos expostos a conflito social por duas semanas apresentam redução nos títulos de anticorpos anti-hemácias de carneiro; entretanto aqueles animais dominantes submetidos a confronto social por três semanas apresentaram aumento na imunidade humoral (GASPAROTTO *et al*., 2002). Ademais, Devoino *et al.,* (1993) encontraram aumento da resposta imune humoral cinco dias após imunização com hemácias de carneiro em camundongos agressivos comparados aos controles. Do mesmo modo, Devoino *et al.,* (2003A) observaram que a ocorrência de comportamento agressivo em ratos anteriormente submissos produziu estimulação da

resposta imune cinco dias após imunização com hemácias de carneiro.

A maior produção de anticorpos encontrada no grupo agressivo reforça a hipótese que agressividade intraespecífica parece ativar o sistema imune, aumentando sua capacidade de reagir contra antígenos estranhos. Corroborando esta hipótese, Petitto *et al.,* (1993) encontraram uma menor atividade de células NK associada a uma maior suscetibilidade de desenvolvimento de tumor em camundongos menos agressivos comparados àqueles mais agressivos (PETITTO *et al.,* 1993). Outrossim, Cohen *et al.,* (1997) observaram que primatas com níveis mais altos de comportamento agressivo apresentavam menor suscetibilidade a infecção respiratória. Do mesmo modo, estudo realizado em indivíduos com desordem de personalidade verificou que homens que exibiam moderado comportamento agressivo apresentavam maior número de células linfóides circulantes (GRANGER *et al.,* 2000)

Outro aspecto importante a se considerar é que agressividade é modulada também por sistemas de neurotransmissores (EICHELMAN, 1990; VAN ERP e MICZEK, 2000). Ademais, há um grande número de evidências do envolvimento desses mensageiros químicos na modulação da resposta imune (DEVOINO *et al.,* 1997; MOSSNER e LESCH, 1998; KUBERA *et al.,* 2000). Assim, é tentador especular que substâncias como dopamina e serotonina possam ter mediado de forma, direta ou não, as alterações imunológicas observadas nos ratos que expressaram agressividade. Entretanto, no presente estudo não é possível identificar qual desses neurotransmissores é mediador dos resultados encontrado. Outros estudos são necessários para esclarecer essa questão.

Neste estudo, o choque nas patas não alterou os títulos de anticorpos anti-hemácias de carneiro. Em contraste, estudo anterior demonstrou que ratos submetidos imediatamente e 24 horas após a imunização a uma sessão de choque elétrico nas patas (16 choques, com 1.6 mA, 5s de duração e 4 min de intervalo) apresentaram aumento na resposta imune humoral (WOOD *et al.,* 1993). Entretanto, uma acentuada redução nos títulos de anticorpos anti-hemácias de carneiro foi observada em camundongos submetidos a choque elétrico (360 choques, com 150 µA, 2s de duração e 9s de intervalo) nas patas 72 horas após imunização (ZALCMAN e ANISMAN, 1993). Nesse mesmo estudo, Zalcman e Anisman (1993) não encontraram diferença na resposta imune humoral dos camundongos submetidos aos choques nas patas 24 horas e 48 horas antes ou imediatamente e 24 horas após a inoculação do antígeno (ZALCMAN e ANISMAN, 1993). Vale ressaltar que no presente estudo, a sessão de estresse consistiu de 5 choques nas patas, com 1.6 mA, 2s de duração e 4 min de intervalo. Ademais, os animais foram imediatamente imunizados após a sessão de estresse e a resposta humoral foi avaliada 7 e 15 dias depois da imunização. Assim, é possível que diferenças metodológicas como o número e a duração dos estímulos elétricos, bem como o período de aplicação do estressor em relação à administração do antígeno, sejam responsáveis pela discrepância entre os diferentes estudos.

A falta de efeitos da agressividade intraespecífica sobre a resposta imune observada em animais adultos submetidos à desnutrição no período neonatal é uma das principais questões neste estudo e merece atenção. Não estão claros os mecanismos responsáveis pela diminuição da resposta do

sistema imune em animais desnutridos frente à agressividade. Entretanto, alguns aspectos merecem ser considerados.

Em ratos o período perinatal pode ser considerado crítico para o desenvolvimento do sistema imunológico (GOBEL *et al.,* 1996). Eventos importantes para a imunocompetência são iniciados ainda no embrião e continuam durante a primeira semana de vida (PAPIERNIK e EZINE, 1982; KALE *et al.,* 1992). Neste período há uma rápida expansão da população de leucócitos e formação de órgãos linfóides (MOSIER e JOHNSON, 1975; SZEWEZUK *et al.,* 1978). Outrossim, em camundongos foi observado que no período embrionário há algumas células T funcionalmente ativas e apenas na quinta e na sexta semanas de vida as subpopulações de células T estão totalmente imunocompetentes (MOSIER e COHEN, 1975). Assim, neste trabalho é possível que a deficiência nutricional durante o período neonatal tenha também afetado de modo duradouro o sistema inume, tornando-o hiporresponsivo a expressão da resposta agressiva.

Ademais, estudos recentes tem demonstrado que desnutrição precoce pode alterar a responsividade dos sistemas nervoso e endócrino frente a uma situação estressante (KEHOE *et al.,* 2001; LESAGE *et al.,* 2002; SEBAAI *et al.,* 2002). Assim, KEHOE *et al.,* (2001) investigando os efeitos da desnutrição proteica pré-natal e do estresse neonatal sobre a responsividade do SNC, observaram que os animais desnutridos não apresentaram alteração dos níveis de corticosterona quando submetidos ao estresse de isolamento. Entretanto, Lesage *et al.,* (2002) verificaram que animais desnutridos submetidos a estresse de contenção apresentavam maior concentração plasmática de corticosterona livre e menor nível de adrenalina. Outrossim, em

ratos foi observado que desnutrição materna interfere no desenvolvimento fetal do eixo HPA, por produzir alteração na adrenal e na pituitária (HAWKINS *et al.,* 2000).

Lesage *et al.,* (2001) demostraram que redução alimentar materna durante o final da gestação induz a retardo no crescimento intra-uterino e superexposição do feto a corticosterona, o qual interfere no desenvolvimento do eixo HPA. Neste último estudo os filhotes desnutridos apresentaram redução do peso da adrenal, diminuição da expressão de receptores para mineralocorticoides e glicocorticoides no hipocampo e redução do ACTH no plasma. Do mesmo modo, Sebaai *et al.,* (2002) observaram que ratos submetidos a desnutrição perinatal apresntaram na vida adulta, redução de peso corporal associada a aumento dos receptores para mineralocorticóides nas CA2 e CA3 do hipocampo e decréscimo nos receptores para glicocorticoides nas áreas CA1, CA3 e no giro denteado.

No presente estudo, foi interessante notar que a desnutrição precoce reduziu os níveis séricos de corticosterona na vida adulta. Sabe-se que glicocorticoides podem assegurar que leucócitos específicos estejam presentes no local certo e na hora certa para responder a estímulos iniciados por agentes indutores de estresse (DHABHAR *et al.,* 1995). Assim, é bem provável que a similar resposta imune entre desnutrido controle e desnutrido submetido a agressividade intraespecífica, aqui observada, seja conseqüência de alterações neuro-endócrinas provocadas pela desnutrição durante o período crítico de desenvolvimento do cérebro.

Outro aspecto importante a se considerar, é que a desnutrição precoce interfere no desenvolvimento do sistema nervoso, particularmente dos sistemas de neurotransmissores (CHEN *et al.,* 1997; MORGANE *et al.,* 2002). Ademais, como foi referido anteriormente estes mensageiros químicos são mediadores de muitos aspectos da resposta imune (SEMPERE *et al.,* 2003; Devoino *et al.,* 2003B). Assim, não podemos excluir a possibilidade de que a falta de inter-relação entre agressividade e resposta imune observada nos animais desnutridos seja conseqüência também dos efeitos duradouros da desnutrição sobre os sistemas de neurotransmissores. Vale lembrar, que no presente estudo os ratos submetidos à desnutrição no período neonatal tornaram-se na vida adulta hiporresponssivos aos efeitos anti-agressividade ou anorexigêno de inibidores seletivos da recaptação da serotonina.

Em conclusão, o comportamento agressivo parece aumentar a capacidade do organismo para responder a agentes nocivos. Ademais, o insulto nutricional precoce parece acarretar efeitos duradouros sobre o funcionamento do sistema serotoninergico em ratos adultos. Do mesmo modo, a desnutrição pregressa, mesmo após longo período de recuperação nutricional, interfere na inter-relação entre o comportamento agressivo e resposta imune. Esses achados tornam-se de grande relevância, pois permitem compreender aspectos relacionados à saúde que por questões éticas são inviáveis de serem estudados na espécie humana.

CONCLUSÕES

7 – CONCLUSÕES

Os resultados do presente estudo nos permitem concluir que:

- Ratos adultos submetidos à desnutrição durante o período de rápido desenvolvimento do cérebro tornam-se hiporresponsivos aos efeitos de ISRS sobre a agressividade intraespecífica.

- Ratos adultos submetidos à desnutrição pregressa não são afetados pela anorexia induzida por citalopram.

- A expressão da resposta agressiva parece modular a resposta imune potencializando a resposta humoral para um antígeno específico.

- Desnutrição durante o período de rápido desenvolvimento do cérebro interfere na inter-relação entre comportamento agressivo e resposta imune em ratos adultos.

- Desnutrição imposta durante o período de rápido desenvolvimento do cérebro afeta os sistemas fisiológicos causando seqüelas persistentes.

PERSPECTIVAS

8 – PERSPECTIVAS

- Estudar a relação entre agressividade e resposta imune em ratos adultos submetidos à desnutrição em diferentes fases de desenvolvimento.

- Investigar o efeito do tratamento crônico com diferentes doses de fluoxetina sobre a agressividade intraespecífica em ratos adultos submetidos ou não a desnutrição durante o período de aleitamento.

- Avaliar individualmente o padrão comportamental dos animais submetidos à agressividade intraespecífica, para correlacionar posturas dominantes e submissas com alterações imunológicas.

- Analisar os níveis de corticosterona em ratos adultos normais ou desnutridos, submetidos à agressividade intraespecífica.

REFERÊNCIAS BIBLIOGRÁFICAS

9 – REFERÊNCIAS BIBLIOGRÁFICAS

ALMEIDA, S.S.; TONKISS, J.; GALLER, J.R. Malnutrition and reactivity to drugs acting in the central nervous system. **Neuroscience and Biobehavioral**, 20(3): 389-402, 1996.

ANNEMOON, M.; VAN ERP M.; KLAUS A. M. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. **The Journal of Neuroscience**, 20(24): 9320-9325, 2000.

APPLEGATE, C.D. 5,7-Dihydroxytryptamine-induced mouse killing and behavioral reversal with ventricular administration of serotonin in rats. **Behavioral and Neural Biology**, 30: 178-190, 1980.

ARBORELIUS, L.; NOMIKOS, G.G.; SALMI, P.; HERTEL, P.; GRILLNER, P.; HOOK, B. B.; HACKSELL, U.; SVENSSON, T.H. The 5-HT receptor antagonist (s)-UH-301 the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic with citalopram. **Naunyn-Schmiedeberg's Archives of Pharmacology,** 353: 630-640, 1996.

ARMENTEROS, J.L.; LEWIS, J.E. Citalopram treatment for impulsive aggression in children and adolescents: an open pilot study. **Journal of the American Academy of Child and Adolescent Psychiatry,** 41(5): 522-9, 2002.

ASBERG, M.; TRASKMAN, L.; THOREN, P. 5-HIAA in the cerebrospinal fluid: a biochemical suicide predictor? **Archives of General Psychiatry**, 33: 1193–1197, 1976.

BARNES, N.M.; SHARP, T. A review of central 5-HT receptors and their function. **Neuropharmacology**, 38: 1083-1152, 1999.

BARRETO MEDEIROS JM. Agressão intraespecífica e consumo alimentar em ratos adultos: Efeitos da desnutrição pregressa e/ou de inibidor seletivo de recaptação de serotonina, Recife, 1995. 90p. **Tese** (Mestrado), Universidade Federal de Pernambuco, 1998.

BARRETO MEDEIROS, J.M.; CABRAL FILHO, J.E.; DE SOUZA, S.L.; FREITAS SILVA, S.R.; MENDES DA SILVA, C.; DEIRÓ, T.C.B.J.; MONTEIRO, J.M.; GUEDES, R.C.A.; DE CASTRO, C.M.M.B.; MANHÃES DE CASTRO, R. Early malnourished rats are not affected by anorexia induced by a selective serotonin reuptake inhibitor in adult life. **Nutritional Neuroscience,** 5: 211-214, 2002.

BAUMAN, P.; ROCHAT, B. Comparative pharmacokinetics of selective serotonin reuptake inhibitors: a look behind the mirror. **International Clinical Psychopharmacology,** 10: 15-21,1995.

BAUMANN, P. Pharmacology and pharmacokinetics of citalopram and other SSRIs. **International Clinical Psychopharmacology**, 1: 5-11, 1996.

BEDI, K.S. Nutritional effects on neuron numbers. **Nutritional Neuroscience**, 6(3):.141-52, 2003.

BENSCHOP, R.J.; OOSTVEEN, F.G.; HEIJNEN, C.J.; BALLIEUX, R.E. Beta 2-adrenergic stimulation causes detachment of natural killer cells from cultured endothelium. **European Journal of Immunology,** 23(12): 3242-7, 1993.

BHASKARAM, P.; SIVAKUMAR, B. Interleukin-1 in malnutrition. **Archives of Disease in Childhood**, 61: 182-5, 1986.

BLALOCK, J.E.; HARBOUR, M.C.; MENAMIN, D.; SMITH, E.M. Peptides and hormones shared by the neuroendocrine and immunologic systems. **Journal of Immunology,** 135: 858-861, 1985.

BLATT, G. J.; CHEN, J.; ROSENE, D.L.; VOLICER, L.; GALLER, J.R. Prenatal protein malnutrition effects on the serotonergic system in the hippocampal formation: An immunocytochemical, ligand binding, and neurochemical study. **Brain Research Bulletin,** 34: 507-518, 1994.

BLUNDEL, J.E. Serotonin and the biology of feeding. **American Journal of Clinical Nutrition,** 55: 155S-159S, 1992.

BLUNDELL, J.E.; LAWTON, C.L.; HALFORD, J.C.G. Serotonim, eating behavior, and fat intake. **Obesity Research**, 4: 471-476, 1995.

BOLAÑOS, F.J.; MANHÃES DE CASTRO, R.; FILLION, G. Antagonis by citalopram and tianeptine of presynaptic 5HT $_{1B}$ heteroreceptors inhibiting acetylcholine release. **European Journal of Pharmachology**, 242: 1-6, 1993.

BOLAÑOS, F.J.; MANHÃES DE CASTRO, R.; FILLION, G. Effect of chronic antidepressant treatment on $5HT_{1B}$ presynaptic heteroreceptor inhibiting acetylcholine release. **Neuropharmacology**, 31: 77-81, 1994.

BORBA, J.M.C; ARAUJO, M.S.; PICANÇO-DINIZ, C.W.; MANHAES DE CASTRO, R.; GUEDES, R.C.A. Permanent and transitory morphometric changes of NADPH-diaphorase-containing neurons in the rat visual cortex after early

malnutrition. **Brain Research Bulletin,** 53(2) 193-201, 2000.

BORELLI, P.; SOUZA, I.P.; BOROJEVIC, R.; DAGLI, M.L.Z.; KANG, H.C. Protein Malnutrition: Some Aspects of the in vitro adhesion of peritoneal mouse macrophages. **Annals of Nutrition and Metabolism**, 42: 367-373, 1998.

BORNE, R.F. Serotonin: the neurotransmitter for the '90s. **Drugs Topics,** 10:108, 1994.

BROWN, J.L.; POLLITT, E. Malnutrition, poverty and intellectual development. **Scientific American**, 38-43, 1996.

CAROBREZ, S.G.; GASPAROTTO, O.C.; BUWALDA, B.; BOHUS, B. Long-term consequences of social stress on corticosterone and IL-1beta levels in endotoxin-challenged rats. **Physiology and Behavior**, 76(1): 99-105, 2002.

CASTELNAU, E. T.; LÔO, H. L'encéphale. **Revue de Psychiatrie Clinique Biologique et Thérapeutique**, 1: 137-208, 1993.

CASTILLA, L.; CRAVIOTO, A.; CRAVIOTO, J. Efectos a corto plazo de la interacción estimulación-desnutrición proteico calórica sobre el desarrollo bioquímico del sistema nervioso central. **Gaceta Medica de Mexico**. 155: 225-233, 1979.

CHANDRA, R.K. 1990 McCollum aware lecture: nutrition and immunity: lessons from the past and new insights into the future. **American Journal of Clinical Nutrition**, 53: 1087, 1991.

CHANDRA, R.K. Nutrition and immune system: An introdução. **American Journal of Clinical Nutrition**, 66: 460 – 463, 1997.

CHANDRA, R.K. Nutrition and immunology: from the clinic to cellular biology and back again. **Proceedings of the Nutrition Society**, 58: 681-3, 1999.

CHANDRA, R.K. Protein – energy malnutrition and immunological responses. **Journal of Nutrition**, 122: 597 – 600, 1992.

CHANDRA, R.K.; KUMARI, S. Nutition and immunity: An overview. **Journal of Nutrition,** 124: 1433-1435, 1994.

CHANG, Y.M.; GALLER, J.R.; LUEBKE, J.I. Prenatal protein malnutrition results in increased frequency of miniature inhibitory postsynaptic currents in rat CA3 interneurons. **Nutritional Neuroscience**, 64 (4): 263-7, 2003.

CHAPUT, Y.; DE MONTIGNY, C.; BLIER, P. Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain**. Naunyn-Schmiedeberg's Archives of Pharmacology,** 333: 342 – 348, 1986.

CHEN, J.C.; TONKISS, J.; GALLER, J.; VOLICER, L. Prenatal protein malnutrition in rats enhances serotonin release from hippocampus. **Journal of Nutrition,** 122: 2138–2143, 1992.

CHEN, J.C.; TONKISS, J.; GALLER, J.R.; VOLICER, L. Effect of prenatal malnutrition on release of monoamines from hippocampal slices. **Life Sciences** 57: 1467-1475, 1995.

CHEN, J.C.; TURIAK, G.; GALLER, J.; VOLICER, L. Postnatal changes of brain monoamine levels i n prenatally malnourished and control rats. **International Journal of Developmental Neuroscience**, 15 (2): 257-263,

1997.

CHOPIN, P.; MORET, C.; BRILEY, M. Neuropharmacology of 5-hydroxytryptamine$_{1B/1D}$ receptor ligands. **Pharmacology and Therapeutics,** 62: 385-405, 1994.

COCCARO, E.F.; KAVOUSSI, R.J. Fluoxetine and impulsive aggressive behavior in personality-disordered subjects **Archives General Psychiatry,** 54: 1081-1088, 1997.

COHEN, S.; LINE, S.; MANUCK, S. B.; RABIN, B. S.; HEISE, E.R.; KAPLAN, J. R. Chronic social stress, social status, and susceptibility to upper respiratory infections in nonhuman primates. **Psychosomatic Medicine,** 59: 213–21, 1997.

CONGE, G.A.; GOUACHE, P.; LAGRANGE, P.H.; LEMONNIER, D. Effect of moderate protein-calorie deprivation on cell-mediated immunity in mice. **Annals of Nutrition and Metabolism**, 27: 286-95, 1983.

DANTZER, R. Des émotions à l'immunité et vice versa. **Neuropsy**., 6:511-518, 1991.

DARDENNE, M.; SAVINO, W. Interdependence of endocrine and immune systems. **Advances in Neuroimmunology,** 6: 297-307,1996.

DATLA, K.P.; MITRA, S.K.; BHATTACHARYA, S.K. Serotonergic modulation of footshock induced aggression in paired rats. **Indian Journal of Experimental Biology,** 29: 631-635, 1991.

DATTA, S.; PATTERSON, E.H.; VINCITORE, M.; TONKISS, J.; MORGANE, P.J.;

GALLER, J.R. Prenatal protein malnourished rats show changes in sleep changes in sleep/wake behavior as adults. **Journal of Sleep Research,** 9: 71-9, 2000.

DE CASTRO, C.M.M.B.; MANHÃES DE CASTRO, R.; MEDEIROS, A.F.; QUEIRÓS SANTOS, A.; FERREIRA E SILVA, W.T.; FILHO, J. L. L. Effect of stress on the production de O_2^- In alveolar macrophages. **Journal of Neuroimmunology**, 108: 68-72, 2000.

DE MONTIGNY C.; BLIER P. Desensitization of terminal 5-ht autoreceptors by 5-HT reuptake blockers. **Archives General Psychiatry,** 48: 483 – 484, 1991.

DE SIMONI, M.G. Two-way communication pathways between the brain and the immune system. **Neuroscience Research and Communications**, 21(3): 163-172, 1997.

DE VRY, J.; BENZ, U.; SCHREIBER, R.; TRABER, J. Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs. **European Journal of Pharmacology,** 249: 331-339, 1993.

DE VRY, J.; SCHREIBER, R. Effects of selected serotonin 5-HT(1) and 5-HT(2) receptor agonists on feeding behavior: possible mechanisms of action. **Neuroscience and Biobehavioral Reviews**. 24(3): 341-53, 2000.

DE-CASTRO, C.M.B.; MANHÃES DE CASTRO, R.; QUEIRÓS SANTOS, A.; BARRETO-MEDEIROS, J.M.; SILVA, W.T.F.; TELES, M.A.S.; CABRAL-FILHO, J.E. Neonatal Malnutrition Reduces Superoxide Production of Alveolar Macrophages in Adult Life. **Free Radical Research**,2003. “in press”.

DEVOINO, L.; ALPERINA, E.; GALKINA, O.; ILYUTCHENOK, R. Involvement

of brain dopaminergic structures in neuroimmunomodulation. **International Journal of Neuroscience**, 93: 213–228, 1997.

DEVOINO, L.; ALPERINA, E.; KUDRYAVTSEVA, N.; POPOVA, N. Immune responses in male mice with aggressive and submissive behavior patterns: Strain differences. **Brain Behavior and Immunity,** 7, 91–96, 1993.

DEVOINO, L.; ALPERINA, E.; PAVINA, T. Immunological consequences of the reversal of social status in C57BL/6J mice. **Brain Behavior and Immunity**, 17: 28–34, 2003A.

DEVOINO, L.; CHEIDO, M.; ALPERINA, E.; IDOVA, G. Evidence for a role of dopaminergic mechanisms in the immunostimulating effect of micro -opioid receptor agonist DAGO. **International Journal of Neuroscience,** 113(10): 1381-1394, 2003B.

DEVOINO, L.; IDOVA, G.; ALPERINA, E.M.; CHEIDO, M. Brain neuromediator systems in the immune response control: Pharmacological analysis of pre- and postsynaptic mechanisms. **Brain Research**, 633: 257–274, 1994.

DHABHAR, F. S.; MILLER, A. H.; STEIN, M.; MCEWEN, B. S.; SPENCER, R. L. Diurnal and stress-induced changes in distribuition of peripheral blood leukocyte subpopulations. **Brain Behavior and Immunity,** 8: 66, 1994.

DHABHAR, F.S.; MILLER, A.H.; MCEWEN, B.S.; SPENCER, R.L. Effects of stress on immune cell distribution - dynamics and hormonal mechanisms**.** **Journal of Immunology,** 154: 5511-5527, 1995.

DHABHAR, F.S.; MILLER, A.H.; STEIN, M.; MCEWEN, B.S.; SPENCER, R.L.

Stress - induced changes in blood leukocyte distribution- role of adrenal steroid hormones. **Journal of Immunology,** 157: 1638-1644,1996.

DOBBING J. Vulnerable periods in developing brain. In: Davison, A.N.; Dobbing, J. (Ed). Applied Neurochemistry, Oxford Blackwell, p. 287-316, 1968.

DOURISH CT. Multiple serotonin receptors: opportunities for new treatments for obesity? **Obesity Research,** 4: 449-462, 1995.

DOURISH, C.T.; HUTSON, P.H.; CURZON, G. Low doses of the putative serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) elicit feeding. **Psychopharmacology**, 86: 197-204, 1985.

DRÉAU, D.; SONNENFELD, G.; FOWLER, N.; MORTON, D.S.; LYTE, M. Effects of Social Conflict on Immune Responses and *E. coli* Growth Within Closed Chambers in Mice. **Physiology and Behavior**, 67 (1) 133–140, 1999.

EDWARDS, S. Chronic systemic administration of 5-HT produces weight loss in mature adult males rats. **Physiology and Behavior**, 58 (3): 629–631, 1995.

EICHELMAN, B.S. Neurochemical and psychopharmacologic aspects of aggressive behavior. **Annual Review of Medicine,** 41: 149-158,1990.

EICHELMAN, B.S.J.; THOA, B.T. The aggressive monoamines. **Biological Psychiatry,** 6(2): 143 – 164, 1972.

FABER, K.M.; HARING, J.H. Synaptogenesis in the postnatal rat fascia dentata is influenced by 5-HT1a receptor activation. **Brain Research. Developmental Brain Research,** 114(2): 245-52, 1999.

FELSNER, P.; HOFER, D.; RINNER, I.; PORTA, S.; KORSATKO, W.; SCHAUENSTEIN, K. Adrenergic suppression of peripheral blood T cell reactivity in the rat is due to activation of peripheral alpha 2-receptors. **Journal of Neuroimmunology**, 57(1-2): 27-34,1995.

FERNSTROM, J. D. Role of precursor availability in control of monoamine biosynthesis in brain. **Physiological Reviews,** 63(2): 484-546, 1983.

FERNSTROM, J.D. Effects of the diet and other metabolic phenomena on brain tryptophan uptake and serotonin synthesis. **Advances in Experimental Medicine and Biology,** 294: 369-76, 1991.

FRIEDMAN, E.M.; IRWIN, M.R. Modulation of immune cell function by the autonomic nervous system. **Pharmacology and Therapeutics,** 74(1): 27-38. 1997.

FUKUDA, M.T.H.; FRANÇOLIN-SILVA, A.L.; ALMEIDA, S.S. Early postnatal protein malnutrition affects learning and memory in the distal but not in the proximal cue version of the Morris water maze. **Behavioural Brain Research**, 133: 271-277, 2002.

FULLER, R.W. Pharmacology of central serotonin neurons. **Annual Review of Pharmacology and Toxicolology,** 20: 111 - 127, 1980.

FULLER, R.W. Fluoxetine effects on serotonin function and aggressive behavior. **Annals of the New York Academy of Sciences,** 20: 90-97, 1996.

GASPAROTTO, O. C.; IGNACIO, Z. M.; LIN, K.; GONCALVES, S. The effect of different psychological profiles and timings of stress exposure on humoral

immune response. **Physiology and Behavior**, 76: 321-326, 2002.

GIBBONS, J.L.; BARR, G.A.; BRIDGER, W.H. Manipulations of dietary tryptophan: effects on mouse killing behavior serotonin in the rat. **Brain Research**, 169: 139-153, 1979.

GILBERG, C. Low dopamine and serotonin levels in anorexia nervosa. **American Journal of Psychiatry**, 140: 948-949, 1983.

GOBBI, M.; CRESPI, D.; FODDI, M.C.; FRACASSO, C.; MANCINI, L.; PAROTTI, L.; MENNINI, T. Effects of chronic treatment with fluoxetine and citalopram on 5-HT uptake, 5-HT1B autoreceptors, 5-HT3 and 5-HT4 receptors in rats. **Naunyn-Schmiedeberg's Archives of Pharmacology**, 356: 22-28, 1997.

GOBEL, T. W. F. The T-dependent immune system. In: Poultry Immunology. T. F. Davidson, T. R. Morris, and L. N. Payne, ed. Poultry Science Symposium Series. Vol.24. Carfax Publishing, Oxfordshire, U. K., 1996.

GRANGER, D. A.; BOOTH, A.; JOHNSON, D. R. Human Aggression and Enumerative Measures of Immunity. **Psychosomatic Medicine**, 62: 583–590, 2000.

GROOT, J.; BOERSMA, W.J.; SCHOLTEN, J.W.; KOOLHAAS, J.M. Social stress in male mice impairs long-term antiviral immunity selectively in wounded subjects. **Physiology and Behavior**, 75(3): 277-85, 2002.

GUEDES, R.C.A.; CABRAL-FILHO, J.E.; TEODÓSIO, N.R. GABAergic mechanisms involved in cortical sptreading depression in normal and

malnourished rats. **Experimental Brain Research series,** 23: 17-26, 1992.

HALFORD, J.C.; BLUNDELL, J.E. Pharmacology of appetite suppression. **Progress in Drug Research**, 54: 25-58, 2000.

HALFORD, J.C.G.; BLUNDEL, J.E. The 5-HT_{1b} receptor agonist CP-94,253 reduces food intake ad preserves the behavioural satiety sequence. **Physiology and Behavior**, 60: 933-939, 1996A

HALFORD, J.C.G.; BLUNDELL, J.E. Metergoline antagonize fluxotine-induced suppression of food intake but not changes in the behavioural satiety sequence*.* **Pharmacology, Biochemistry and Behaviour,** 54: 745-75, 1996B

HALFORD, J.C.G.; LAWTON, L.C.L.; BLUNDEL, J.E. The 5-HT_2 receptor agonist MK-212 reduces food intake and increases resting but prevents the behavioural satiety sequence*.* **Pharmacology, Biochemistry and Behaviour,** 56: 41-46. 1997.

HALL, R.D.; LEAHI, J.P.; ROBERTSON, W.M. Hyposensitivity to serotoninergic stimulation in protein malnourished rats. **Physiology and Behavior,** 31: 187-195, 1983.

HALLER, J. Alpha-2 Adrenoceptor Blockade and the Response to Intruder Aggression in Long-Evans Rats. **Physiology and Behavior,** 58: 101-106, 1995A.

HALLER, J.Catecholamine Stimulation and the Response to Behavioral Challenge in Wistar Rats. **Pharmacology, Biochemistry and Behavior**. 514: 789-794, 1995B.

HAMON, M.; BOURGOIN, S.; ARTAUD, F.; EL MESTIKAWY, S. The respective roles of tryptophan uptake and tryptophan hydroxylase in the regulation of serotonin synthesis in the central nervous system. **Journal of Physiology,** 77(2-3): 269-79, 1981.

HASSANAIN, M.; ZALCMAN, S.; BHATT, S.; SIEGEL, A. Interleukin-1 beta in the hypothalamus potentiates feline defensive rage: role of serotonin-2 receptors. **Neuroscience,** 120(1): 227-33, 2003.

HAWKINS, P.; STEYN, C.; MCGARRIGLE, H.H.; CALDER, N.A.; SAITO, T.; STRATFORD, L.L.; NOAKES, D.E.; HANSONA, M A. Cardiovascular and hypothalamic-pituitary-adrenal axis development in late gestation fetal sheep and young lambs following modest maternal nutrient restriction in early gestation. **Reproduction, Fertility, and Development,**12: 443-56, 2000.

HERNANDES, A.S.; ALMEIDA, S.S.Postnatal protein malnutrition affects inhibitory avoidance and risk assessment behaviors in two models of anxiety in rats. **Nutritional Neuroscience**, 6(4):213-9, 2003.

HEWITT, K.N.; LEE, M.D.; DOURISH, C.T.; CLIFTON, P.G. Serotonin 2C receptor agonists and the behavioural satiety sequence in mice. **Pharmacology, Biochemistry and Behavior,** 71(4): 691-700, 2002.

HONDA, Y.; TAKAHASHI, K.; NAITO, M.; FUJIYAMA, S. The role of macrophage colony-stimulating factor in the differentiation and proliferation of Kupffer cells in the liver of protein-deprived mice. **Laboratory Investigation,** 72: 696, 1995.

HUETHER, G.; ZHOU, D.; SCHMIDT, S.; WILTFANG, J.; RÜTHER, E. Long-

term food restriction Down-regulates the density of serotonin transporters in the rat frontal cortex. **Society Biological Psychiatry,** 41: 1174-11180, 1997.

HYTTEL, J. Effect of a specific 5-Ht uptake inhibitor, citalopram (Lu 10-171), on 3H-5-Ht uptake in rat brain synaptosomes in vitro. **Psychopharmacology,** 60: 13-18, 1978.

HYTTEL, J. Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). **International Clinical Psychopharmacology,** 9(suppl. 1): 19-26, 1994.

IKEDA, S.; SAITO, H.; FUKATSU, K.; INOUE, T.; HAN, I.; FURUKAWA, S.; MATSUDA, T.; HIDEMURA, A. Dietary restriction impairs neutrophil exudation by reducing CD11b/CD18 expression and chemokine production. **Archives of Surgery,** 136 :297-304, 2001.

JACOBS, B.L.; AZMITIA, E.C. Structure and function of the brain serotonin system. **Physiological Reviews,** 72(1): 165-229, 1992.

JEZOVA, D.; SKULTETYOVA, I.; MAKATSORI, A.; MONCEK, F.; DUNCKO, R. Hypothalamo-pituitary-adrenocortical axis function and hedonic behavior in adult male and female rats prenatally stressed by maternal food restriction. **Stress,** 5: 177-83, 2002.

KALE, A.S.; RAO, S.G. Haemopoietic stem cells during development of mouse embryo. **Indian Journal of Experimental Biology**, 30: 371-376, 1992.

KANT, G. J.; MOUGEY, E. H.; PENNINGTON, L. L.; MEYERHOFF, J. L.; Graded footshock stress elevates pituitary cyclic amp and plasma ß-endorphin,

ß-LPH, corticosterone and prolactin. **Life Sciences,** 33: 2657-2663, 1984.

KAYE, W.H.; GWIRTSMAN, H.E.; GEORGE, D.T.; JIMERSON, D.C.; EBERT, M.H. CSF-5-HIAA concentrations in anorexia nervosa: reduced values in underweight subjects normalize after weight gain. **Biological Psychiatry,** 23: 102-105, 1988.

KEELE, N.B. Phenytoin inhibits isolation-induced aggression specifically in rats with low serotonin. **Neuroreport,**12(6): 1107-12, 2001.

KEHOE, P.; MALLINSON, K.; BRONZINO, J.; MCCORMICK, C.M. Effects of prenatal protein malnutrition and neonatal stress on CNS responsiveness. **Developmental Brain Research,** 132: 23–31, 2001.

KEUSCH, G.T. Host defense mechanisms in protein energy malnutrition. **Advances in Experimental Medicine and Biology,** 135: 183-209, 1981.

KUBERA, M.; SIMBIRTSEV, A.; MATHISON, R.; MAES, M. Effects of repeated fluoxetine and citalopram administration on cytokine release in C57BL/6 mice. **Psychiatry Research,** 96(3): 255-66, 2000.

KYES, G.B. Aggression and brain serotoninergic responsivity: response to slides in male macaques. **Physiology and Behavior,** 2: 205-208, 1993.

LAUDER, J.M.; BLOOM, F.E. Ontogeny of monoamine neurons in locus coeruleous, raphe nuclei and substantia nigra of the rats 1, Cell differentiation, **Journal of Comparative Neurology,** 155: 469-481,1974.

LAWRENCE, A.D.; KIM, D. Central/peripheral nervous system and immune responses. **Toxicology,** 142: 189–201, 2000.

LEE, M.D.; KENNETT, G.A.; DOURISH, C.T.; CLIFTON, P.G. 5-HT1B receptors modulate components of satiety in the rat: behavioural and pharmacological analyses of the selective serotonin1B agonist CP-94,253. **Psychopharmacology,** 164(1): 49-60, 2002.

LEONHARDT, M.; LESAGE, J; DUFOURNY, L; DICKES-COOPMAN, A.; MONTEL, V.; DUPOUY, J.P. Perinatal maternal food restriction induces alterations in hypothalamo-pituitary-adrenal axis activity and in plasma corticosterone-binding globulin capacity of weaning rat pups. **Neuroendocrinology,** 75: 45-54, 2002.

LESAGE, J.; BLONDEAU, B.; GRINO, M.; BRE´ANT, B. Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. **Endocrinology,** 142: 1692–1702, 2001.

LESAGE, J.; DUFOURNY, L.; LABORIE, C.; BERNET, F.; BLONDEAU, B.; AVRIL, I.; BREANT, B.; DUPOUY, J.P. Perinatal malnutrition programs sympathoadrenal and hypothalamic-pituitary-adrenal axis responsiveness to restraint stress in adult male rats. **Journal of Neuroendocrinology,** 14: 135-43, 2002.

LESOURD, M.B. Nutrition and immunity in the elderly: modification of immune rsponses with nutritional treatments. **American Journal of Clinical Nutrition,** 66: 478-484, 1997.

LEVSTSKY, D.A.; BARNES, R.H. Nutritonal and environmental interactions in

the behavioral developmen of the rat: Long-term effects. **Science,** 176: 68-71,1972.

LI, D.L.; SIMMONS, R.M.A.; IYENGARS, S. 5-HT_{1A} receptor antagonists enhance the functional activity of fluoxetine in a mouse model of feeding. **Brain Research,** 781: 121-128, 1998.

LIDOV, H.G.W.; MOLLIVER, M.E. A immunohistochemical study of serotonin neuron development in the rat: ascending pathways and terminal fields. **Brain Research Bulletin,** 8:389-416, 1982.

LIU, J.; LAUDER, J.M. Serotonin promotes region-specific glial influences on cultered serotonin and dopamine neurons. **Glia,** 5: 306–317, 1992.

LUCAS, J.J.; YAMAMOTO, A.; SCEARCE-LEVIE, K.; SAUDOU, F.; HEN, R. Absence of fenfluramine-induced anorexia and reduced c-Fos induction in the hypothalamus and central amygdaloid complex of serotonin 1B receptor Knock-out mice. **The Journal of Neuroscience,** 18: 5537-5544, 1998.

LUCKI, I.; KREIDER, M.S.; SIMANSKY, K.J. Reduction of feeding behavior by the serotonin uptake inhibitor sertraline. **Psychopharmacology,** 96: 289-295, 1988.

MAGALHÃES, C.P.; MANHAES DE CASTRO, R.; DE CASTRO, C.M.M.; CABRAL-FILHO, J.E.; SOUGEY, E.B. Sbstratos anatômicos do sistema serotoninérgico**. Anais da Faculdade de Medicina da Universidade Federal de Pernambuco,** 45(2): 149-152, 2000.

MAIER, S.F.; WATKINS LR. Cytokines for phychologists: implications of

bidirectional immune-to-brain communication for understanding behavior, mood and cognition. **Psychological Review,** 105(1):83-107, 1998.

MANHÃES DE CASTRO, R. Etude de la Participation des Recepteures Centraux de la Serotonine du type 5-HT_{1b} dans les reactions cerebrales au stress et dans le mecanisme d'action des antidepresseurs. Paris, 1995, **Tese** (Doutorado) – Universite Pierre et Marie Curie, Paris, França, 1995.

MANHÃES DE CASTRO, R.; BOLÃNOS-JIMÉNEZ, F.; SEGUIN, L.; SARHAN, H.; DRIEU, K.; FILLION, G. Sub-chronic cold stress reduces 5-HT1A receptor responsiveness in the old but not in the young rat. **Neuroscience Letters,** 203: 21-24,1996.

MANHÄES-DE-CASTRO, R.; BARRETO MEDEIROS, J. M.; MENDES DA SILVA, C.; FERREIRA, L.M.P.; GUEDES, R.C.A.; CABRAL FILHO, J.E.; COSTA, J.A. Reduction of intraespecific aggression in adult rats by neonatal treatment with a seletive serotonin reuptake inhibitor. **Brazilian Journal of Medical and Biological Research,** 34: 121-124, 2001.

MANHÄES-DE-CASTRO, R.; BARRETO MEDEIROS, J.M.; DEIRÓ T.C.B.J.; MENDES DA SILVA, C.; FREITAS SILVA, S.R.; SOUZA, S.L. Neonatal treatment with selective serotonin reuptake inhibitor reduces intraespecífic aggression in rats. **Neurobiologia,** 61(2): 61-9, 1998.

MANHÄES-DE-CASTRO, R.; CABRAL FILHO, J.E.; COSTA, J. A.; COSTA, F.B.R.; GALINDO, M.A.C.; HECKSHER, C.A. Neonatal treatment with naloxone causes permanent hyperalgesia in rats. **Brazilian Journal of Medical and Biological Research,** 26: 747-751, 1993.

MANJARREZ, G.; MANUEL, L.; MERCADO, R.; HERNANDEZ, J. Serotonergic receptors in the brain of in utero undernourished rats. **International Journal of Developmental Neuroscience**, 21: 283–289, 2003.

MARÍN, M.C.; THOMÁS, M.E.; SERRES, C.; MERCURI, O. Protein-energy malnutrition during gestation and lactation in rats affects growth rate brain development and essential fatty acid metabolism. **Journal of Nutrition**, 125: 1017-1024, 1995.

MARSH, D.M.; DOUGHERTY, D.M.; MOELLER, F.G.; SWANN, A.C.; SPIGA, R. Laboratory-measured aggressive behavior of women: acute tryptophan depletion and augmentation. **Neuropsychopharmacology,** 26(5): 660-71.2002.

MAZER, C.; MUNEYYIRCI, J.; TAHENY, K.; RAIO, N.; BORELLA, A.; WHITAKER-AZIMITIA, P. Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rats: a possible model of neurodevelopmental disorders with cognitive deficits. **Brain Research,** 760:68-73, 1997.

McBRIDE, P.A.; ANDERSON, G.M.; KHAIT, V.D.; SUNDAY, S.R.; HALMI, K.A. Serotonergic responsevity in eating desordens. **Psychopharmacology Bulletin,** 27 (3): 365-372, 1991.

MCCARTY, R.; KOPIN, I. J., Sympatho-adrenal medulary activity and behavior during exposure to footshook stress: a comparison of seven rat strains. **Physiology and Behavior,** 21: 567-572, 1978.

MEDEIROS, J.M.; MENDES DA SILVA, C.; SOUGEY, E.B.; COSTA, J. A.; DE

CASTRO, C.M.M.B.; MANHÃES DE CASTRO, R. Action of selective serotonin reuptake inhibitor on aggressive behavior in adult rats submitted to the neonatal malnutrition. **Arquivos de Neuro-psiquiatria,** 59(3-A): 499-503, 2001.

MEHLMAN, P.T.; HIGLEY, J.D.; FAUCHER, I.; LILLY, A.A.; TAUB, M.; VICKERS, J.;SUOMI, S.J.; LINNOILA, M. Low CSF 5-HIAA concentrations, severe aggression and impaired impulse control in nonhuman primates. **American Journal of Psychiatry,** 151: 1485– 1491,1994.

MAIER, S. F.; WATKINS, L. R. Cytokines for Psychologists: Implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. **Psychological review,** 105(1):83-107, 1998.

MOKLER, D.J.; BRONZINO, J.D.; GALLER, J.R.; MORGANE, P.J. The effects of median raphe electrical stimulation on serotonin release in the dorsal hippocampal formation of prenatally protein malnourished rats. **Brain Research,** 838(1-2): 95-103, 1999.

MOKLER, D.J.; GALLER, J.R.; MORGANE, P.J. Modulation of 5-HT release in the hippocampus of 30-day-old rats exposed in utero to protein malnutrition. **Brain Research. Developmental Brain Research,** 14:142(2): 203-8, 2003.

MORGANE, P.J.; AUSTIN-LA FRANCE, R.J.; BRONZINO, J., TONKISS, J.; GALLER, J. R. Malnutrition and developing c~~entral ne~~rvous system", In: Isaacson, R.L. and Jensen, K.F., eds, The vulnerable brain and environmental risks. (Plenum Press, New York) Vol. 1, pp 2-42, 1992.

MORGANE, P.J.; AUSTIN-LA FRANCE, R.J.; BRONZINO, J.; TONKISS, J.;

DIAZ-CINTRA, S.; CINTRA, L.; KEMPER, T.; GALLER, J. R. Prenatal malnutrition and development of the brain. **Neuroscience and Biobehavioral Reviews,** 17: 91-128, 1993.

MORGANE, P.J.; MILLER, M.; KEMPER, T.; STERN, W.; FORBES, W.; HALL, R.; BRONZINO, J.; KISSANE, J.; HAWRYLEWICK AND RESNICK, O. The effects of protein malnutrition on the developing central nervous system in the rat. **Neuroscience and Biobehavioral Reviews,** 2: 137-230, 1978.

MORGANE, P.J.; MOKLER, D.J.; GALLER, J.R. Effects of prenatal protein malnutrition on the hippocampal formation. **Neuroscience and Biobehavioral Reviews**, 26: 471-83, 2002.

MOSIER, D.E.; COHEN, P.I. Ontogeny of mouse T lymphocyte function. **Fed. Proc.,** 34: 137-140, 1975.

MOSIER, D.E.; JOHNSON, B.M. Ontegeny of mouse lymphocyte function II. Development of yhe ability to produced antibody is modulated by T lymphocytes. **Journal of Experimetnal Medicine,** 141: 216-226, 1975.

MOSSNER, R.; LESCH, K.P. Role of serotonin in the immune system and in neuroimmune interactions. **Brain Behavior and Immunity,** 12(4): 249-71, 1998.

NAHANI, J.; NIK-AAEEN, A.; RAFII, M.; MOHAGHEGHPOUR, N. Effect of malnutrition on several parameters of the immune system of children. **Annals of Nutrition and Metabolism,** 20: 302-6, 1976.

NOHR, C.W.; TCHERVENKOV, J.I.; MEAKINS, J.L.; CHRISTOU, N.V.

Malnutrition and humoral immunity: Short-term acute nutritional deprivation. **Surgery**, 14-16, 1985.

OLIVIER, B.; MOS, J.; OORSCHOT, R. VAN; HEN, R. Serotonin receptors and animal models of aggressive behavior. **Pharmacopsychiatry,** 28 (2): 80-90, 1995.

OTTAVIANI, E.; FRANCESCHI, C. The neuroimmunology of stress from invertebrates to man. **Progress in Neurobiology,** 48: 421-440, 1996.

PALÉN, K.; THORNEBY, L.; EMANUELSSON, H. Effects of serotonin antagonists on chick embryogenesis**. Wilhelm Roux's Archives of Developmental Biology**, 187: 89 – 103, 1979.

PAPIERNIK, M.; EZINE, S. Postnatal development of T cells. III. Thymus independency of T-cell-dependent antigen response in the neonatal spleen. **Immunology,** 46: 209-14, 1982.

PEREZ-TORRERO, E.; TORREROB, C.; COLLADO, P.; SALAS, M. A paradigm of undernourishing and neonatal rehabilitation in the newborn rat. **Nutritional Neuroscience,** 6(2): 113-115, 2003.

PERREAULT, A.N.H; SEMSAR, K.; GODWIN, J. Fluoxetine treatment decreases territorial aggression in a coral reef fish. **Physiology and Behavior,** 79: 719– 724, 2003.

PESSOA, D.C.N.C. Efeitos da desnutrição pela Dieta Básica Regional (DBR), em três gerações de ratos, sobre a reprodução, crescimento corporal e desenvolvimento de órgãos. **Tese** (Doutorado em Nutrição) - Universidade

Federal de Pernambuco, Recife, p.118, 1997.

PETITTO, J.M.; LYSLE, D.T.; GARIEPY, J.L.; CLUBB, P.H.; CAIRNS, R.B.; LEWIS, M.H.Genetic differences in social behavior: relation to natural killer cell function and susceptibility to tumor development. **Neuropsychopharmacology**, 8(1): 35-43, 1993.

PINE, A.P.; JESSOP, N.S.; OLDHAM, J.D. Maternal protein reserves and their influence on lactational performance in rats. 3. The effects of dietary protein restriction and stage of lactation on milk composition. **British Journal of Nutrition,** 72(6): 815-30. 1994.

PRUUS, K.; RUDISSAAR, R.; SKREBUHHOVA-MALMROS, T.; ALLIKMETS, L.; MATTO, V. Development of apomorphine-induced aggressive behavior: comparison of adult male and female Wistar rats**. Methods and Findings in Experimental and Clinical Pharmacology,** 22: 47-50, 2000.

RADLEY, J.J.; JACOBS, B.L. 5-HT1A receptor antagonist administration decreases cell proliferation in the dentate gyrus. **Brain Research,** 955(1-2): 264-7, 2002.

REIST, C.; NAKAMURA, K.; SAGART, E.; SOKOLSKI, K.N.; FUJIMOTO, K.A. Impulsive aggressive behavior: open-label treatment with citalopram. **Journal of Clinical Psychiatry,** 64(1): 81-5, 2003.

RESNICK, O.; MILLER, M.; FOBS, W.; HALL, R.; KENPER, T.; BRONZINO, J.; MORGANE, J.P. Developmental malnutrition protein: influences on the central nervous system of the rat. **Neuroscience and Biobehavioral Reviews,** 4: 232-246, 1979.

REVILLARD, J.P.; COZON G. Experimental models and mechanisms of immune deficiencies of nutritional origin. **Food Additives and Contaminants,** 1:82-6, 1990.

RIKIMARU, T.; TANIGUCHI, K.; YARTEY, J.E.; KENNEDY, D.O.; NKRUMAH, F.K. Humoral and cell-mediated immunity in malnourished children in Ghana. **European Journal of Clinical Nutrition,** 52(5): 344-50, 1998.

ROTTA, F.T.; MELLO, C.F.; ROCHA, J.B.T.; SOUZA, D.O.G. Early undernutrition blocks the effect of naltrexone on rat exploratory behavior. **Brazilian Journal of Medical and Biological Research,** 21: 305 – 312, 1988.

SALIMATA, W.; LEMONNIER, D.; BLEIBERG, F.; DELORME, J. Early nutritional experiments: effects on the humoral and cellular immune responses in mice. **Journal of Nutrition**, 113: 1131-1139, 1983.

SÁNCHEZ, C.; HYTTEL, J. Isolation-induced aggression in mice: effects of 5-hydroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT_{1A} receptors, **European Journal of Pharmacology,** 264: 241-247, 1994.

SÁNCHEZ, C.; MEIER, E. Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. **Psychopharmacology,** 129: 197-205, 1997.

SÁNCHEZ,C.; HYTTEL, J. Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. **Cellular and Molecular Neurobiology,** 19: 467-489, 1999.

SAWCHENKO, P. E.; LI, H.Y.; ERICSSON, A. Circuits and mechanisms

governing hypothalamic responses to stress: a tale of paradigms. **Brain Research,** 122: 61–78, 2000.

SCRIMSHAW, N.S.E.; SANGIOVANNI, J.P. Synergism of nutrition, infection, and immunity: na overview. **American Journal of Clinical Nutrition,** 66: 464-477, 1997.

SEBAAI, N.; LESAGE, J.; VIEAU, D.; ALAOUI, A. DUPOUY, J.P.; DELOOF, S. Altered control of the hypothalamo-pituitary-adrenal axis in adult male rats exposed perinatally to food deprivation and/or dehydration. **Neuroendocrinology,** 76(4) :243-53. 2002.

SEMPERE, T.; CEDENO, N.; URBINA, M.; LIMA, L. 8-[3H]-hydroxy-2-(di-n-propylamino)tetralin binding sites in blood lymphocytes of rats and the modulation by mitogens and immobilization. **Journal of Neuroimmunology,** 138(1-2): 8-16. 2003.

SGOIFO, A.; BOER, S.F.; HALLER, J.; KOOLHAS, J.M. Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. **Physiology and Behavior,** 60: 1403-1407, 1996.

SHAKHAR, G.; BEN-ELIYAHU, S. In vivo beta-adrenergic stimulation suppresses natural killer activity and compromises resistance to tumor metastasis in rats. **Journal of Immunology,** 160(7): 3251-8, 1998.

SILVA, C.I. Resposta agressiva de ratos hipoglicêmicos ou tratados com d-fenfluramina após privação de sono paradoxal. Recife, 1997. 61p. **Tese** (Mestrado). Universidade Federal de Pernambuco, 1997.

SIRISINHA, S.; EDELMAN, R.; SUSKIND, R.M. Complement and C3-proactivator levels in children with protein calorie malnutrition and effect of dietary treatment. **Lancet**, 1: 1016-9, 1973.

SMYTHE, .PM.; BRERETON-STILES, G.G.; GRACE, H.J. Thymo-lymphatic deficiency .and depression of cell-mediated immunity in protein-calorie malnutrition. **Lancet,** 11: 939-43, 1971.

SOBOKTA, T. J.; COOK, M. P.; BRODIE, R. E Neonatal malnutrition: neurochemical, hormonal and behavioral manifestations. **Brain Research,** 26: 443-457, 1974.

SPERRY, T.S.; THOMPSON, C.K.; WINGFIELD, J.C. Effects of acute treatment with 8-OH-DPAT and fluoxetine on aggressive behaviour in male song sparrows (Melospiza melodia morphna). **Journal of Neuroendocrinology,** 15: 150-160, 2003.

SPRENT, J.; TOUGH, D.F. Lymphocyte life-span and memory. **Science,** 265(5177): 1395-400, 1994.

STEFANSKI, V.; ENGLER, H. Social Stress, dominance and blood cellular immunity. **Journal of Neuroimmunology**, 94:144-152, 1999

STEFANSKI, V.; ENGLER, H.Effects of Acute and Chronic Social stress on Blood Cellular Immunity in Rats. **Physiology and Behavior,** 64: 733–741, 1998.

STERN, W.C.; MILLER, M.; FORBES, W.B.; MORGANE, P.J.; RESNICK, O. Ontogeny of the levels of biogenic amines in various parts of the brain and in

peripheral tissue in normal and protein malnourished rats. **Experimental Neurology,** 49: 314-326, 1975.

STIEHM, E.R. Humoral immunity in malnutrition. **Fed Proc,** 39: 3093-7, 1980.

STURMAM, J.A.; DEVINE, E.; RESNICK, O.; MORGANE, P.J. Maternal protein malnutrition in the rat: effect on protein and two enzymes in the milk. **Nutrition Reseaech,** 6: 437-442, 1996.

SVED, A. F. Precursor control of the function of monoaminérgic Neurons. IN: Wurtman, R. J. e Wurtman, J. J. Nutrition and the brain. Reven Press, 1983.

SNYDER, S.H. Drugs and the Brain. Scientific American Libery, 1996.

SZEWCZUK, M.R.; SHERR, D.H.; SISKIND, G.W. Ontogeny of B lymphocyte function. VI. Ontogeny of thymus cell capacity to facilitate the functional maturation of B lymphocytes. **European Journal of Immunology,** 8: 370-3, 1978.

TAMMELA, L.I.; RISSANEN, A.; KUIKKA, J.T.; KARHUNEN, L.J.; BERGSTROM, K.A.; REPO-TIIHONEN, E.; NAUKKARINEN, H.; VANNINEN, E.; TIIHONEN, J.; UUSITUPA, M. Treatment improves serotonin transporter binding and reduces binge eating. **Psychopharmacology,** 170(1): 89 – 93, 2003.

TEODÓSIO, N.R.; LAGO,E.S.; ROMANI, S.A.M.; GUEDES, R.C.A. A regional basic diet from Northeast Brazil as a dietary model of experimental malnutrition. **Archivos Latinoamericanos de Nutricion,** 40: 533 – 547, 1990.

TONKISS, J.; BONNIE, K.E.; HUDSON, J.L.; SHULTZ, P.L.; DURAN, P.;

GALLER J.R. Ultrasonic call characteristics of rat pups are altered following prenatal malnutrition. **Developmental Psychobiology,** 43 (2): 90-101,2003.

TOORNVLIET, A.C.; PIJL, H.; HOPMAN, E.; WEVER, B.M.E.; MEINDERS, A.E. Serotoninergic drug-induced weight loss in carbohydrate craving obese patients*.* **International Journal of Obesity,** 20: 917-920, 1996.

TOTH, M. 5-HT(1A) receptor knockout mouse as a genetic model of anxiety. **European Journal of Pharmacology,** 463: 177-184. 2003.

VAN ERP, A.M.; MICZEK, K.A. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. **Journal of Neuroscience,** 20(24): 9320-5, 2000.

VEENEMA, H. A.; MEIJER, O.C.; KLOET, E.R.; KOOLHAAS, J.M.; BOHUS B.G. Differences in basal and stress-induced HPA regulation of wild house mice selected for high and low aggression. **Hormones and Behavior,** 43: 197–204, 2003.

VIZI, E.S. Receptor-mediated local fine-tuning by noradrenergic innervation of neuroendocrine and immune systems. Stress of life: from molecules to man. **Annals of New York Acadademy Sciences,** 30: 388 – 396, 1998.

VOLVOKA, J. Neurobiology of Violence. Ed. American Psychiatric Press, Washington, 21 – 76, 1995.

WHITAKER-AZMITIA, P.M. Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology. **Pharmacological Reviews***,* 43: 553-561,1991.

WIGGINS, R.C.; FULLER, G.; ENNA, S.J. Undernutrition and the development of brain neurotransmitter systems. **Undernutrition and Brain Development,** 35: 2085-2094, 1984.

WINICK, M.; BRASEL, J. A.; ROSSO, P. Nutrition and cell growth. In: Winick, M, Nutrition and development (John Wiley & Sons), pp. 49-98,1972.

WONG, D.T.; BYMASTER, F.P.; REID, L.R.; MAYLE, D.A.; KRUSHINSK, I.J.H.; ROBERTSON, D.W. Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain, **Neuropsychopharmacology,** 8: 337-44, 1993.

WOOD, P. G.; KAROL, M. H.; KUSNECOV, A. W.; RABIN, B. S. Enhancement of antigen-specific humoral and cell-mediated immunity by electric footshock in rats. **Brain, Behavior and Immunity,** 7: 121-134, 1993.

WURTMAN, R.J. Nutrients that modify brain function. **Scientific American.** 246(4): 50-59, 1982.

YAN, W.; WILSON, C.C.; HARING, J.H. 5-HT1a receptores mediate the neurotrophic effect of serotonin on developing dentate gramile cells. **Developmental Brain Researech,** 98: 185-190, 1997.

YOUNG, M.R.; MATTHEWS, J.P. Serotonin regulation of T-cell subpopulations and of macrophage accessory function. **Immunology,** 84: 148-152, 1995.

ZALCMAN, S.; ANISMAN, H. Acute and chronic stressor effects on the antibody response to sheep red blood cells. **Pharmacology, Biochemistry and Behavior**, 46(2): 445-52, 1993.

OUTRAS PUBLICAÇÕES

Tratamento neonatal com inibidor seletivo de recaptação da serotonina: evolução nutricional e efeito tardio sobre a depressão experimental

Neonatal treatment with SSRI: nutritional evolution and late effect on the experimental depression

Cristiano Mendes da Silva[1]
Jairza Maria Barreto Medeiros[2]
Daniela Eugênia Costa Antunes[3]
Allan Delano Urbano Cunha[4]
Valdenilson Ribeiro Ribas[4]
Maria Flávia Simões de França[4]
Raul Manhães de Castro[5]

Resumo

A serotonina participa da fisiopatologia da depressão em humanos e em modelos experimentais. Essa amina influencia o crescimento e o desenvolvimento do tecido nervoso, incluindo também os neurônios serotoninérgicos. No presente trabalho, foram investigadas as repercussões do tratamento neonatal com fluoxetina, um inibidor seletivo de recaptação da serotonina, sobre o comportamento de ratos adultos submetidos ao teste do nado forçado (NF). Para induzir o comportamento depressivo, os animais foram conduzidos a 15 minutos de NF em um tanque. Após 24h, cada animal foi avaliado durante 5 minutos de NF, sendo secado em seguida em câmara de aquecimento (32°C) por 15 minutos. Durante o NF, a latência da tentativa de fuga (LTF) e o tempo de imobilidade comportamental (TI) foram avaliados. Quando comparados ao grupo-controle (ratos tratados durante o mesmo período com volume equivalente de solução salina), os animais tratados com fluoxetina apresentaram redução do comportamento depressivo. Os resultados indicam que a administração crônica de fluoxetina no início da vida reduz o comportamento depressivo em ratos adultos e sugere que isso pode ter sido conseqüência da alteração neonatal da atividade serotoninérgica.

Unitermos: depressão; serotonina; inibidor de recaptação da 5-HT; neurogênese

[1] Professor-adjunto do Departamento de Nutrição da UFPE. Doutor em Farmacologia Experimental e Clínica da Universidade de Paris VI
[2] Doutoranda em Nutrição da UFPE
[3] Mestrando em Nutrição da UFPE
[4] Bolsista de Iniciação Científica CNPq PIBIC
[5] Estagiário do Laboratório de Fisiologia da Nutrição Naíde Teodósio, Departamento de Nutrição, UFPE

The serotonin plays a role at the pathophysiology of the depression in humans and experimental models. This amine influences also growth and development of the nervous tissue including also serotoninergic neurons. In present work was investigated the repercussions of treatment in early of the life with fluoxetine, a selective serotonin reuptake inhibitor, on behavior from adults rats during forced swimming test (FST). Depressive behavior was induce in all animals, they were submitted by 15 min to the FST. 24 h later was measured the behavioral despair (BD) and the time of immobility (TI) by 5 min in the FST. Immediately after they were removed of water and placed to dry in a heated enclosure (32°C/15min). When compared to the control group (rats treated during the same period with equivalent volumes of saline solution), the fluoxetine group showed decrease of the depressive behavior. The results indicate which the repeated administration from fluoxetine early in life reduces the depressive behavior in adulthood and suggests that the increased brain serotoninergic activity could exert a role in this effect.

Uniterms: depression; serotonin; selective serotonin reuptake inhibitor; neurogenesis

Introdução

Estudos em animais e humanos demonstram o papel da serotonina (5-hidroxitriptamina, 5-HT) na depressão[9]. Essa relação entre 5-HT e depressão tem sido demonstrada por meio da utilização de instrumentos farmacológicos[7]. Evidências experimentais, envolvendo a participação de receptores serotoninérgicos na fisiopatologia da depressão e nos mecanismos de ação de drogas antidepressivas, têm sido observadas em estudos bioquímicos, eletrofisiológicos e comportamentais[14].

Uma outra função da 5-HT está relacionada à sua participação na embriogênese e no crescimento cerebral[17,23], sugerindo um possível efeito neurotrófico[26] ou um sinal para o desenvolvimento de neurônios embriônicos[4,6]. As alterações oriundas da ontogênese do sistema nervoso (SN) (gliogênese, diferenciação neuronal, migração, sinaptogênese, etc.) ocorrem com muita rapidez[24]. No rato, inicia-se durante a lactação[13]. No homem a ontogênese do SN tem início na fase pré-natal (último trimestre de gestação), continuando até os primeiros anos de vida[11]. O crescimento e o desenvolvimento do SN ocorrem durante a gestação e a lactação, constituindo um período crítico para o desenvolvimento encefálico[3]. Esse período apresenta vulnerabilidade a agressões nutricionais e farmacológicas, que poderão conduzir a drásticas alterações morfológicas, funcionais e comportamentais[7,8,10]. Essas alterações poderão se tornar irreversíveis, dependendo da magnitude da agressão[8]. Assim, é provável que as modificações neurais induzidas pelo tratamento crônico com um inibidor seletivo da recaptação de 5-HT (ISRS), no início da vida, persistam até a vida adulta. As prováveis alterações provocadas pela administração neonatal de ISRSs parecem produzir efeitos a longo prazo sobre comportamentos relacionados com a função serotoninérgica[10]. Entre esses comportamentos, está a modulação do comportamento alimentar e emocional[1]. A possibilidade de manipulação farmacológica controlada do sistema serotoninérgico no período neonatal constitui uma ferramenta importante para o estudo ontogenético do SN e de suas funções. No presente trabalho, foi investigado o efeito tardio do tratamento neonatal com um ISRS sobre o comportamento de ratos adultos submetidos ao teste do nado forçado.

Material e método

Animais e tratamentos

Foram utilizados ratos da linhagem Wistar, provenientes da Colônia do Departamento de Nutrição da Universidade Federal de Pernambuco. Todos os animais foram mantidos no biotério sob condições de controle do ambiente (temperatura entre 23° ± 2°C; ciclo claro-escuro 12/12 horas, água e comida *ad libitum*). Os animais foram separados aleatoriamente 24 horas após o nascimento em ninhadas de 6 filhotes por mãe, formando dois grupos. Um grupo recebendo fluoxetina (Lundbeck, 10 mg/kg, sc, dissolvido em salina: grupo Fluox, n = 26 ratos). O outro grupo (controle, n = 26 ratos) recebeu um volume equivalente de solução salina (NaCl 0,9%, 1 ml/kg, sc). Ambos os tratamentos foram induzidos do 1° ao 21° dia do pós-natal (período de lactação). Os pesos corporais foram determinados do 1° ao 21° e aos 60 dias de vida.

Avaliação comportamental

Os animais com idade entre 60 e 63 dias, pesando em torno de 175 g e 240 g, foram avaliados quanto à depressão experimental, segundo o teste do nado forçado (NF), método previamente descrito por Porsolt *et al.*[18], embora com algumas modificações.

Esse procedimento consiste em expor o animal a uma situação de estresse, em que o rato é forçado a nadar. Após um período inicial de vigorosa atividade natatória em direção à borda (denominada latência da tentativa de fuga), eventualmente diminui a intensidade dos movimentos, produzindo apenas os movimentos necessários para manter sua cabeça fora da água. Essa característica foi classificada como imobilidade comportamental, indicando um possível estado de desespero do animal ao perceber que não tem como escapar.

Os ratos foram colocados individualmente em um tanque (42 cm de altura e 104,5 cm de diâmetro) cujo nível da água não permitia ao animal apoiar-se no assoalho nem subir pela borda. A temperatura da água foi mantida em 25°C. Durante 15 minutos os animais foram submetidos a natação forçada, período pré-teste. Após os 15 minutos de NF cada animal foi conduzido a uma câmara de aquecimento (CA, 32°C) durante 15 minutos e, em seguida, retornaram para suas gaiolas. Vinte e quatro horas após o pré-teste todos os animais foram submetidos a uma nova sessão de NF durante 5 minutos, período de teste. Neste mo-

mento, foi realizada a avaliação comportamental. Em seguida foram novamente conduzidos para a CA. Os parâmetros comportamentais aqui avaliados (latência da tentativa de fuga e imobilidade comportamental) foram quantificados em segundos (s) com auxílio de cronômetros digitais.

Análise estatística

A evolução ponderal, representada pelo ganho de peso corporal (expresso como x ± ep), foi analisada pelo *Student's "t" test*. Os resultados referentes a análise comportamental (expressos como mediana e percentis 25 75) foram avaliados pelo *Mann Whitney U test*. O nível de significância para todos os testes estatísticos utilizados foi de $p < 0,05$.

Resultados

Os resultados apresentados são comparados aos dados do grupo-fluoxetina, onde o grupo-fluoxetina apresentou uma redução no ganho de peso corporal ($p < 0,05$) a partir do 9º dia de vida, continuando até o desmame em torno do 21º dia (Figura 1). Aos 60 dias de idade não houve diferença estatisticamente significativa entre os pesos corporais dos grupos em estudo (Tabela I).

Tabela I – Peso corporal de ratos tratados durante a lactação com fluoxetina ou salina

Ratos receberam fluoxetina (10 mg/kg, sc; grupo fluoxetina) ou salina (0,9% de NaCl, 1 ml/kg, sc; grupo controle) do 1º ao 21º dia de vida. Os animais foram pesados no 1º, 21º e aos 60 dias de idade. Os dados estão discriminados como média e erro padrão (x ± ep).

Grupos	Peso corporal (g)		
	1º dia de vida	21º dia de vida	60º dia de vida
Controle	7,49 ± 0,72 (26)	46,35 ± 7,73 (26)	213,67 ± 19,97 (13)
Fluoxetina 10 mg	7,61 ± 0,71 (26)	36,33 ± 8,07* (26)	208,62 ± 27,31 (13)

* $p < 0,05$ comparado ao grupo controle para a mesma idade, o *Student's "t" test (two tailed)* foi aplicado.

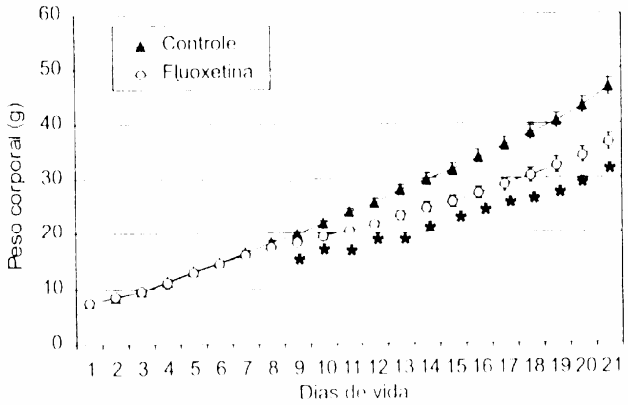


Figura 1 – Efeito do tratamento neonatal com fluoxetina sobre o desenvolvimento ponderal de ratos

* $p < 0,05$ comparado ao grupo controle (*Student's "t" test, two tailed*)

Efeito da administração de fluoxetina durante o aleitamento sobre o crescimento ponderal. Ratos receberam fluoxetina (10 mg/kg, sc; grupo fluoxetina) ou salina (1 ml/kg de NaCl 0,9 sc; grupo controle), diariamente, do 1º ao 21º dia de idade. Todos os animais foram pesados diariamente. Os dados estão representados como x ± ep do peso corporal em gramas, para os diferentes grupos: controle (n = 26) e fluoxetina (n = 26).

Em relação ao estudo do comportamento depressivo, os parâmetros foram avaliados durante o teste do NF: a latência da tentativa de fuga (LTF) e o tempo de imobilidade comportamental (TI), ambos alterados no grupo-fluoxetina. A LTF do grupo fluoxetina foi maior ($p < 0,01$) enquanto o TI foi menor ($p < 0,01$) quando comparado ao grupo-controle (Figuras 2 e 3), sugerindo que os ratos tratados com fluoxetina apresentaram um comportamento menos depressivo que os controles.

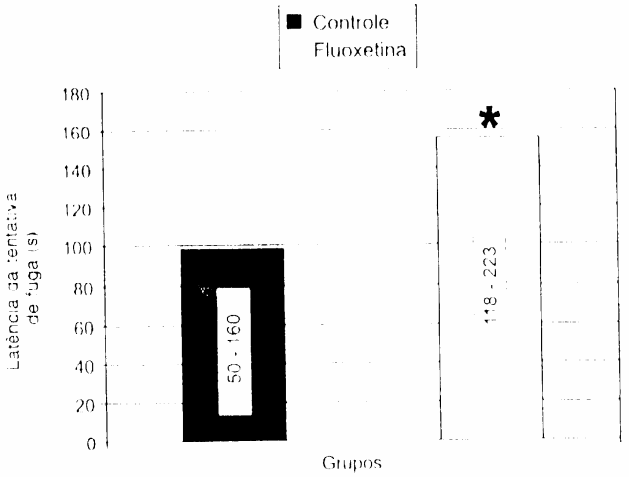


Figura 2 – Efeito do tratamento neonatal com fluoxetina sobre a latência da tentativa de fuga

* $p < 0,01$ comparado ao grupo salina (*Mann Whitney U test*)

Latência da tentativa de fuga (LTF) de ratos tratados com fluoxetina (10 mg/kg, sc; grupo fluoxetina) ou salina (1 ml/kg, sc de NaCl 0,9%; grupo salina), diariamente do 1º ao 21º dia de idade. Todos os animais foram submetidos à avaliação da depressão experimental aos 60 dias de idade. Os dados estão representados como medianas (colunas) e valores expressos em percentis 25 75 (no interior das colunas) do tempo de LTF em segundos, para os diferentes grupos: fluoxetina (n = 26) e controle (n = 26).

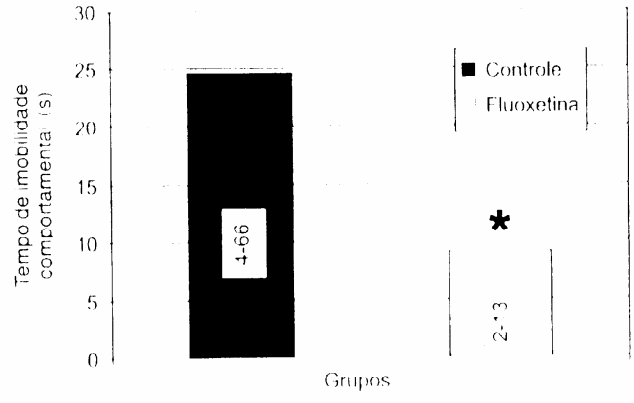


Figura 3 – Efeito do tratamento neonatal com fluoxetina sobre o tempo de imobilidade comportamental

* $p < 0,05$ comparado ao grupo salina (*Mann Whitney U test*)

Tempo de imobilidade comportamental (TI) de ratos tratados com fluoxetina (10 mg/kg, sc; grupo fluoxetina) ou salina (1 ml/kg, sc de

NaCl 0,9%; grupo salina) diariamente do 1º ao 21º dia de idade. Todos os animais foram submetidos à avaliação da depressão experimental aos 60 dias de idade. Os dados estão representados como medianas (colunas) e valores expressos em percentis 25-75 (no interior das colunas) do TI em segundos, para os diferentes grupos: fluoxetina (n = 26) e controle (n = 26).

Discussão

O presente estudo demonstrou que a administração crônica de fluoxetina durante o período crítico de desenvolvimento cerebral prejudicou a evolução do peso corporal e reduziu o comportamento depressivo na idade adulta em ratos. Esses efeitos podem estar correlacionados com alterações do sistema serotoninérgico ocorridas durante o desenvolvimento como sugerido por Palén *et al.*[17]. Os autores observaram que a administração de drogas que atuam sobre a síntese e a liberação de serotonina ou sobre a ativação de receptores serotoninérgicos, durante a embriogênese, poderia resultar em distúrbios do crescimento e desenvolvimento em diversos tecidos, incluindo o SN. Esses achados foram confirmados por meio da inibição da síntese de 5-HT no sistema nervoso central (SNC) embrionário, após administração materna de paraclorofenilalanina [pCPA], segundo Lauder *et al.*[5]. Neste estudo foi demonstrado um retardo na diferenciação dos neurônios embrionários em embriões tratados com pCPA[5]. A diminuição dos níveis de 5-HT no período neonatal também resultou em redução da densidade de espículas dendríticas no SNC em desenvolvimento[26]. Essas alterações morfológicas foram de caráter específico e permanente[26], possivelmente se refletindo em danos sobre a sinaptogênese. Em contraste, neurônios embrionários do mesencéfalo de ratos cultivados com 5-HT apresentaram um aumento na densidade e sobrevida dos neurônios serotoninérgicos[6]. Embora ainda não esteja totalmente esclarecido se essas alterações persistem até a vida adulta e proporcionam danos funcionais, nossos dados concordam com essa possibilidade.

Em relação à redução do ganho de peso nos ratos tratados com fluoxetina, nossos resultados podem estar associados ao papel exercido pela serotonina sobre o controle do comportamento alimentar, inibindo a ingestão de alimentos[21]. A fluoxetina é um antidepressivo que inibe seletivamente a recaptação neuronal da 5-HT[15], reduzindo a fome e a ingestão alimentar em humanos[11] e produzindo hipofagia em ratos[25].

A manipulação farmacológica do sistema serotoninérgico durante o desenvolvimento poderia ter causado uma diminuição pós-tratamento, persistente até a idade adulta, do comportamento depressivo induzido pelo teste do NF. É bem conhecida a relação entre depressão e alteração da função do sistema de neurotransmissão da serotonina[22]. Nesse contexto, estudos realizados em nosso laboratório já demonstraram alterações do comportamento emocional em ratos adultos submetidos a tratamento neonatal com ISRS[10]. A 5-HT está envolvida na neurobiologia da depressão, assim como nos mecanismos de ação de agentes antidepressivos[22]. Essa relação entre 5-HT e depressão é reforçada ainda mais ao se avaliar a influência dos vários subtipos de receptores serotoninergicos[7]. A presença de múltiplos tipos de receptores serotoninérgicos corrobora a hipótese de que drogas com ação seletiva, em alguns desses receptores cerebrais, podem ter propriedades psicotrópicas específicas em transtornos emocionais[7].

A diminuição nas concentrações de 5-HT cerebral pode precipitar a recorrência da depressão em pacientes deprimidos[12], enquanto a manipulação de receptores serotoninérgicos por meio de fármacos parece apresentar propriedades antidepressivas em alguns modelos animais[19]. O NF é um teste comportamental que tem sido utilizado para avaliar a eficácia clínica de vários tratamentos antidepressivos[16]. A redução do comportamento depressivo, durante o NF, nos ratos tratados com fluoxetina, parece estar relacionada com a função do sistema serotoninérgico[3,16]. A inibição do processo de recaptação da 5-HT pela fluoxetina resulta em aumento de sua disponibilidade sináptica, possivelmente acentuando ou facilitando sua ação[20]. A diminuição do comportamento depressivo no teste do NF, observada no presente trabalho, parece estar associada a mecanismos neuroadaptativos desenvolvidos no período neonatal. Esses processos persistiriam até a vida adulta.

Referências

1. Chopin P, Moret C, Briley M. Neuropharmacology of 5-hydroxytryptamine$_{1B/1D}$ receptor ligands. Pharmacology & Therapeutics 1994; 62: 385-405.
2. Detke MJ, Lucki I. Detection of serotoninergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. Behav Brain Res 1996; 73(1-2): 43-6.
3. Dobbing J. Vulnerable periods in developing brain. In: Davison AN, Dobbing J (ed.). Applied Neurochemistry. Oxford, Blackwell, 287-316, 1968.
4. Lauder JM, Wallace JA, Krebs H. Roles of serotonin in neuroembriogenesis. Adv Exp Med Biol 1981; 133: 477-506.
5. Lauder JM, Towle AC, Patrick P, Henderson P, Krebs H. Decreased serotonin content of embryonic raphe neurons following maternal administration of p-chlorophenylalanine: a quantitative immunocytochemical study. Dev Brain Res 1985; 20: 107-14.
6. Liu J, Lauder JM. Serotonin promotes region-specific glial influences on cultures serotonin and dopamine neurons. Glia 1992; 5(4): 306-17.
7. Mac Sweeney CP, Lesourd M, Gandon JM. Antidepressant-like effects of alnespirone (S 20499) in the learned helplessness test in rats. Eur J Pharmacol 1998; 345: 133-7.
8. Manhães de Castro R, Cabral Filho JE, Costa JA, Costa FBR, Galindo MAC, Hecksher CA. Neonatal treatment with naloxone causes permanent hyperalgesia in rats. Braz J Med Biol Res 1993; 26: 747-51.
9. Manhães de Castro R, Peregrino A, Sourgey E, Barreto Medeiros JM, Deiró TCBJ. Depression: repercussion in serotoninergic system. Neurobiologia 61(2): 45-55, 1998.
10. Manhães de Castro R, Barreto Medeiros JM, Deiró TCBJ, Mendes da Silva C, Freitas Silva SR, Souza SL. Neonatal treatment with selective serotonin reuptake inhibitor reduces intraspecific aggression in rats. Neurobiologia 1998; 61(2): 61-9.
11. McGuirk J, Silverstone T. The effect of the 5-HT re-uptake inhibitor fluoxetine on food intake and body weight in healthy male subjects. J Obesity 1990; 14: 361-72.

12. Meltzer HY. Role of serotonin in depression. Ann N Y Acad Sci 1990; 600: 486-95.
13. Morgane PJ, Miller M, Kemper T, Stern W, Forbes W, Hall R, Bronzino J, Kissane J, Hawrylewick E, Resnick O. The effects of protein malnutrition on the developing central nervous system in the rat. Neur Bio Rev 1978; 2: 137-230.
14. Newman ME, Lerer B, Shapira B. 5-HT_{1A} receptors-mediated effects of antidepressants. Prog Neuro-psychopharmacol Biol Psychiatry 1993; 17: 1-19.
15. OLIVIER B, MOS J, Van Der Heyden JAM, Hartog J. Serotonergic modulation of social interactions in isolated male mice. Psychopharmacology 1989b; 97: 154-6.
16. Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I. Serotoninergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology 1999; 142 (2): 162-7.
17. Palén K, Thörneby L, Emanuelsson H. Effects of serotonin antagonists on chick embriogenesis. Wilhelm Roux's Arch Dev Biol 1979; 187: 89-103.
18. Porsolt RD, LE Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977; 266: 730-732.
19. Robinson DS, Alms DR, Shrotriya RC, Messina ME, Wickramaratna P. Serotoninergic anxiolytics and treatment of depression. Psychopathology 1989; 22: 27-36 Suppl. 1.
20. Sánchez C, Hyttel J. Isolation-induced aggression in mice: effects of 5-hidroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT_{1A} receptors. Eur J Pharmacol 1994; 264(3): 241-7.
21. Simansky KJ. Serotonergic control of the organization of feeding and satiety. Behav Brain Res 1996; 73(1-2): 37-42.
22. Stahl S. 5-HT_{1A} receptors and pharmacotherapy. Psychopharmacol Bull 1994; 30: 39-43.
23. Whitaker-Azmitia PM. Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology. Pharmacol Rev 1991; 43(2): 553-61.
24. Winick M. Malnutrition and brain development. The Journal of Pediatrics 1969; 74(5).
25. Wong DT, Reid LR, Threlkeld PG. Suppression of food intake in rats by fluoxetine: comparison of enantiomers and effects of serotonin antagonists. Pharmacol Biochem Behav 1988; 31, 475.
26. Yan W, Wilson CC, Haring JH. 5-HT1a receptors mediate the neurotrophic effect of serotonin on developing dentate gramile cells. Dev Brain Res 1997; 98: 185-90.

Brazilian Journal of Medical and Biological Research (2001) 34: 121-124
ISSN 0100-879X Short Communication

Reduction of intraspecific aggression in adult rats by neonatal treatment with a selective serotonin reuptake inhibitor

R. Manhães de Castro,
J.M. Barreto Medeiros,
C. Mendes da Silva,
L.M.P. Ferreira,
R.C.A. Guedes,
J.E. Cabral Filho
and J.A. Costa

Departamento de Nutrição, Centro de Ciências da Saúde, Universidade Federal de Pernambuco, Recife, PE, Brasil

Abstract

Most studies suggest that serotonin exerts an inhibitory control on the aggression process. According to experimental evidence, this amine also influences growth and development of the nervous tissue including serotoninergic neurons. Thus, the possibility exists that increased serotonin availability in young animals facilitates a long-lasting effect on aggressive responses. The present study aimed to investigate the aggressive behavior of adult rats (90-120 days) treated from the 1st to the 19th postnatal day with citalopram (CIT), a selective serotonin reuptake inhibitor (20 mg/kg, *sc*, every 3 days). Aggressive behavior was induced by placing a pair of rats (matched by weight) in a box (20 x 20 x 20 cm), and submitting them to a 20-min session of electric footshocks (five 1.6-mA - 2-s current pulses, separated by a 4-min intershock interval). When compared to the control group (rats treated for the same period with equivalent volumes of saline solution), the CIT group presented a 41.4% reduction in the duration of aggressive response. The results indicate that the repeated administration of CIT early in life reduces the aggressive behavior in adulthood and suggest that the increased brain serotoninergic activity could play a role in this effect.

Correspondence
R. Manhães de Castro
Departamento de Nutrição, CCS
UFPE, Cidade Universitária
50670-901 Recife, PE
Brasil
Fax: +55-81-271-8473
E-mail: rcastro@nutricao.ufpe.br

Research supported by CNPq and PROINCNPq.

Received December 28, 1999
Accepted November 8, 2000

Key words
- Aggression
- Serotonin
- Neonatal treatment
- Development

Serotonin has been shown to have multiple functions as a neurotransmitter by exerting modulatory effects on neural excitability (1). There is mounting evidence of its participation in pain sensitivity, body thermoregulation, sleep, feeding behavior and mood (2). Brain areas known to be involved in emotional processes, such as hippocampus, hypothalamus, amygdala and cerebral cortex, are innervated by serotoninergic pathways ascending from mesencephalic nuclei (3,4). Studies on animals and humans have pointed out that the serotoninergic system may play a role in several aggressive behaviors (4,5). An inverse relationship seems to exist between central serotoninergic function and aggressiveness since reducing central serotonin levels facilitates aggression and this increased aggression is impaired by elevation of serotonin beyond normal levels (2,5). Also, the use of animal models of aggressiveness suggests that serotonin receptors are implicated in aggressive responses (4,6).

Furthermore, experimental evidence indicates that serotonin can influence embryogenesis and growth (7,8) presumably by acting as a developmental signal (9) or a neurotrophic factor (10). A role of serotonin in regulating the development of serotonin neurons themselves has also been demonstrated (7). Moreover, it is well known that very fast growth and development of the nervous system occurs during pregnancy and suckling and pharmacological or nutritional manipulations can induce drastic morphological and functional changes in this process. These changes can become irreversible depending on previous treatment and/or environmental factors (11,12). Thus, it is likely that the neural modifications induced by early serotonin treatment persist until adult life. As a consequence, the possibility exists that the use of serotonin agonists in the initial phase of life could have long-lasting effects on behaviors related to serotoninergic function. Since, to our knowledge, there are no data concerning this point, the investigation of the possible long-term effects of early manipulation of the serotoninergic system is highly desirable. The objective of the present study was to test the hypothesis that the administration of a selective serotonin reuptake inhibitor - citalopram (CIT) - to suckling rats induces changes in aggressiveness during adulthood.

Newborn male Wistar rats from the colony of the Department of Nutrition, Federal University of Pernambuco (Brazil), were assigned randomly to two groups (6 pups per litter) 24 h after birth. One group (CIT group, 16 rats) received citalopram (20 mg/kg, *sc*, dissolved in saline, 1 ml/kg; Lundbeck, Copenhagen, Denmark), and the other (control group, 24 rats) received an equivalent volume of saline (0.9% NaCl). The treatments were applied every 3 days from the 1st to the 19th postnatal day (suckling period). Body weights were determined on the 1st, 21st (weaning) and 90th-120th day. The animals were maintained at a room temperature of 23 ± 1°C, on a light-dark cycle of 12/12 h (lights on at 7:00 a.m.), with free access to water and food. When they reached 90-120 days of age they were submitted to aggressiveness tests. The tests were performed in an acoustically isolated room using a box (20 x 20 x 20 cm) with the floor consisting of parallel metal bars (interbar distance: 1.3 cm) connected to a scrambled electric current source. The test consisted of placing a pair of rats from the same group (matched by weight) in the box, where they received a session of stimuli to induce aggressive responses.

Each stimulus (an electric footshock) consisted of a 1.6-mA - 2-s current pulse. Each session lasted 20 min and included 5 stimuli separated by a 4-min interval. During the first 3 min of this interval, the duration of the aggressive response was measured with a digital chronometer. The total time of aggressive behavior observation was 900 s. In the last minute of each interval, the data were recorded and the equipment was checked. The aggressive response was defined as the presentation of at least one of the two following behaviors: a) the animals stood up on the hind paws facing each other in a threatening attitude but without direct contact, or b) they maintained evident physical contact (scratching, tooth baring and emission of characteristic vocalization). Data were compared by the two-tailed Student *t*-test (body weight) or by the Mann-Whitney U-test (aggressiveness) with the level of significance set at $P \leq 0.05$.

Compared to the controls (Table 1), CIT rats showed a reduction in body weight both on the 21st (46.8%, $P<0.01$) and 90th-120th day (14.5%, $P<0.01$). A significant difference in aggressive response was also observed (Table 2), with CIT rats presenting a reduced duration of aggressiveness (41.4% lower than control, $P<0.02$).

The present study showed that chronic administration of CIT during the critical period of brain development impaired the weight evolution and reduced the aggres-

siveness of the rats. These effects may be related to an alteration of the serotoninergic system occurring during development, as suggested by Palén et al. (8). In a study on chicken embryos, these authors observed that drugs acting on serotonin synthesis and release, or on the activation of serotoninergic receptors may cause disturbances of growth and development in several tissues, including the nervous system, during embryogenesis. Some findings suggest improved neuronal development of serotoninergic neurons of embryogenic nervous tissue treated with serotonin (7). Cultured serotonin neurons of embryogenic rat mesencephalon show an increased density and survival of serotoninergic neurons (9). Although it is unknown whether this higher density of serotonin neurons persists until adult life, our data agree with this possibility. In fact the weight reduction found in the CIT group could be associated with the role of serotonin in feeding control by inhibiting food intake (13).

The reduced aggression duration during adulthood may be also related to the pharmacological manipulation of the serotoninergic system during development, since a close relationship seems to exist between aggressiveness and the function of this neurotransmitter system (2,4). In this context, it is noteworthy that changes in emotional behavior have been already shown in both young and adult rats previously submitted to chronic nutritional injury (14), a condition which is known to alter brain serotonin concentration (15).

The reduction of aggressiveness presented by the CIT group could be related to the serotoninergic action both at pre- and postsynaptic sites. Several studies have indicated such inhibitory role through the activation of serotoninergic receptors (4).

In rats, the elevation of serotonin and 5-hydroxyindoleacetic acid levels in the amygdala, diencephalon and brain stem produced a reduction in the latency to muricidal behavior (16).

In the present study a nonspecific effect mediated by serotonin (such as the antinociceptive one) cannot be ruled out because of the high CIT dose used. However, the data support those obtained by others (17), who found a reduction of aggressive behavior in psychotic and borderline human patients treated with CIT. This anti-aggressiveness effect of CIT may be also related to its action on the serotoninergic system. Increased serotonin concentration in the frontal cortex of adult rats has been observed after chronic treatment with high doses of CIT, the same as used here (20 mg kg^{-1} day^{-1}, *ip*, for 14 days) (18). This point deserves attention since

Table 1 - Body weight of rats treated with citalopram or saline during suckling.

Rats received citalopram (20 mg/kg, *sc*; CIT group) or saline (0.9% NaCl, 1 ml/kg, *sc*; control group) every 3 days from the 1st to the 19th day of age. The animals were weighed on the 1st, 21st and 90th-120th day of age. The data are reported as mean ± SD. *P<0.01 compared to the control group at the same age (two-tailed Student *t*-test).

Experimental groups	Weight (g)		
	1st day	21st day	90th-120th day
Control group (N = 24)	7.2 ± 1.0	48.1 ± 7.3	334.1 ± 42.4
CIT group (N = 16)	7.0 ± 0.9	25.6 ± 5.2*	285.6 ± 31.0*

Table 2 - Aggressiveness of adult rats treated with citalopram or saline during suckling.

Rats received citalopram (20 mg/kg, *sc*; CIT group) or saline (0.9% NaCl, 1 ml/kg, *sc*; control group) every 3 days from the 1st to the 19th day of life. Later on, at 90-120 days of age, pairs of animals from the same group were submitted to a footshock session to elicit an aggressive response. The aggressive response was evaluated on the basis of time of aggression (900-s observation). Results are reported as median (Md) and 25 and 75 percentiles (PE_{25-75}). *P<0.02 compared to the control group (two-tailed Mann-Whitney U-test).

Experimental groups	Duration of aggressiveness (s)	
	Md	PE_{25-75}
Control group (N = 24)	679.5	51.1-82.9
CIT group (N = 16)	398.0*	6.9-72.8

CIT inhibits the reuptake of serotonin, consequently increasing its synaptic availability and enhancing its inhibitory effect on aggressive behavior. On the other hand, the repeated administration of selective serotonin reuptake inhibitors can produce down-regulation both of the post- and presynaptic 5-HT_{1B} receptors, in this case improving the serotonin release (19). According to Saudou et al. (20), the 5-HT_{1B} receptors seem to be very important in the control of aggressive behavior, since mutant mice lacking these receptors presented exacerbated aggressiveness. The fact that CIT administrated during suckling affected aggressive behavior much later in life suggests that 1) permanent morphological and/or functional alterations were produced during the period of fast brain development, and 2) these alterations interfere with the behavioral responses of adult rats mediated by the serotoninergic system.

Thus, the reduction of aggressiveness observed in the present study could be associated with neuroadaptive mechanisms developed during the neonatal period which last into adult life.

Further investigations demonstrating possible alterations of the serotoninergic system as a consequence of pharmacological manipulation early in life are necessary to elucidate the mechanisms responsible for the behavioral changes observed in this study.

References

1. Aghajanian GK & Marek GJ (1997). Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology*, 36: 589-599.
2. Chopin P, Moret C & Briley M (1994). Neuropharmacology of 5-hydroxytryptamine $_{1B}$ receptor ligands. *Pharmacology and Therapeutics*, 62: 385-405.
3. Takeuchi Y (1988). Distribution of serotonin neurons in the mammalian brain. In: Osborne NN & Hamon M (Editors). *Neuronal Serotonin*. John Wiley & Sons Ltd, New York, 25-56.
4. Olivier B, Mos J, Van Oorschot R & Hen R (1995). Serotonin receptors and animal models of aggressive behavior. *Pharmacopsychiatry*, 28 (Suppl): 80-90.
5. Kyes RC, Botchin MB, Kaplan JR, Manuck SB & Mann JJ (1995). Aggression and brain serotoninergic responsiveness: response to slides in male macaques. *Physiology and Behavior*, 2: 205-208.
6. Sanchez C & Hyttel J (1994). Isolation-induced aggression in mice: effects of 5-hydroxytryptamine uptake inhibitors and involvement of postsynaptic 5-HT_{1A} receptors. *European Journal of Pharmacology*, 264: 241-247.
7. Whitaker-Azmitia PM (1991). Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology. *Pharmacological Reviews*, 43: 553-561.
8. Palen K, Thorneby L & Emanuelsson [illegible] (1979). Effects of serotonin antagonists on chick embryogenesis. *Wilhelm Roux's Archives of Developmental Biology*, 187: 89-103.
9. Liu J & Lauder JM (1992). Serotonin promotes region-specific glial influences on cultured serotonin and dopamine neurons. *Glia*, 5: 306-317.
10. Yan W, Wilson CC & Haring JH (1997). 5-HT1a receptors mediate the neurotrophic effect of serotonin on developing dentate granule cells. *Developmental Brain Research*, 98: 185-190.
11. de-Castro RM, Cabral-Filho JE, Costa JA, Costa FBR, Gallindo MAC & Hecksher CA (1993). Neonatal treatment with naloxone causes permanent hyperalgesia in rats. *Brazilian Journal of Medical and Biological Research*, 26: 747-751.
12. Noback CR & Eisenman LM (1981). Some effects of protein calorie undernutrition on the developing central nervous system of the rat. *Anatomical Record*, 201: 67-73.
13. Simansky KJ (1996). Serotonergic control of the organization of feeding and satiety. *Behavioural Brain Research*, 73: 37-42.
14. Teodosio MR, Cabral Filho JE, Guedes RCA, Costa JA, Costa FRB & Silva AT (1979). Learned and emotional behavior in chronically malnourished rats. *Acta Physiologica Latinoamericana*, 29: 255-262.
15. Stern WC, Miller M, Forbes WB, Morgane PJ & Resnick O (1975). Ontogeny of the levels of biogenic amines in various parts of the brain and in peripheral tissue in normal and protein malnourished rats. *Experimental Neurology*, 49: 314-326.
16. Sakata T, Fuchimoto H, Kodama J & Fukushima M (1975). Changes of brain serotonin and muricide behavior following chronic administration of theophylline in rats. *Physiology and Behavior*, 15: 449-453.
17. Syvalahti EKG, Kallioniemi H & Lehto H (1994). Citalopram in patients with unsatisfactory response to neuroleptics: an open follow-up study. *Methods and Findings in Experimental and Clinical Pharmacology*, 16: 49-55.
18. Arborelius L, Nomikos GG, Hertel P, Salmi P, Grillner P, Hook BB, Hacksell U & Svensson TH (1996). The 5-HT1A receptor antagonist (S)-UH-301 augments the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic treatment with citalopram. *Naunyn Schmiedeberg's Archives of Pharmacology*, 353: 630-640.
19. Briley M & Moret C (1993). Neurobiological mechanisms involved in antidepressant therapies. *Clinical Neuropharmacology*, 16: 387-400.
20. Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC & Hen R (1994). Enhanced aggressive behavior in mice lacking 5-HT_{1B} receptor. *Science*, 265: 1875-1878.

Arq Neuropsiquiatr 2002;60(4):928-931

NEONATAL TREATMENT WITH FLUOXETINE REDUCES DEPRESSIVE BEHAVIOR INDUCED BY FORCED SWIM IN ADULT RATS

Cristiano Mendes-da-Silva[3], Sandra Lopes de Souza[3], Jairza Maria Barreto-Medeiros[2], Sebastião Rogério de Freitas-Silva[4], Daniela Eugênia Costa Antunes[5], Allan Delano Urbano Cunha[5], Valdenilson Ribeiro Ribas[6], Maria Flávia Simões de França[6], Maria Inês Nogueira[7], Raul Manhães-de-Castro[1]

ABSTRACT - Serotonin plays a role at the pathophysiology of depression in humans and in experimental models. The present study investigated the depressive behavior and the weigh evolution in adult rats (60 days) treated from the 1st to the 21st postnatal day with fluoxetine, a selective serotonin reuptake inhibitor (10 mg/kg, sc, daily). The depressive behavior was induced by the forced swim test (FST). The animals were submitted to two sessions of FST: 1st session for 15 min and the 2nd session 24h later, for 5 min. During the 2nd session the Latency of the Attempt of Escape (LAE) and Behavioral Immobility (BI) were appraised. The Fluoxetine group when compared to the Control group, showed an increase in LAE and a decrease in BI. The neonatal administration of fluoxetine reduced the depressive behavior in adult rats, possibly by increase in the brain serotonergic activity. This alteration can be associated to process of neuroadaptation.

KEY WORDS: depression, serotonin, selective serotonin reuptake inhibitor, neurogenesis

Tratamento neonatal com fluoxetina reduz o comportameto depressivo induzido pelo nado forçado em ratos adultos

RESUMO - Estudos em humanos e em modelos experimentais demonstram que a serotonina (5-HT) participa da fisiopatologia da depressão. O presente estudo investigou o comportamento depressivo e a evolução ponderal de ratos adultos jovens (60 dias) tratados do 1º ao 21º dia pos-natal com fluoxetina, um inibidor seletivo de recaptação da serotonina, (10 mg/kg, sc, diariamente). A depressão experimental foi induzida através do teste de nado forçado (NF). Os animais foram submetidos a duas sessões de NF, a primeira por 15 min e a segunda apos 24 h, por 5 min. Durante os 5 min de NF a latência da tentativa de fuga (LTF) e o tempo de imobilidade (TI) foram avaliados. O grupo tratado com fluoxetina apresentou aumento da LTF e redução do TI comparado ao controle. A administração neonatal de fluoxetina reduziu o comportamento depressivo em ratos adultos, possivelmente em função do aumento da atividade serotoninérgica cerebral. Esta alteração poderá estar relacionada a processos neuroadaptativos.

PALAVRAS-CHAVE: depressão, serotonina, inibidor de recaptação da 5-HT, neurogênese.

Studies in animals and humans have demonstrated the role of serotonin (5-hidroxytryptamine, 5-HT) in psychiatric depressions[1], through the use of pharmacological tools[2]. Experimental evidences of serotonin receptors involvement in the pathophysiology of depression and in the action mechanisms of antidepressant drugs, come from various biochemical, electrophysiological and behavioral approaches[3]. Animal models have largely contributed to the understanding of the 5HT receptors and depressive behavior relations[4]. Adult rats treated with antidepressants such as the selective serotonin reuptake inhibitor (SSRIs) presented behavioral changes in the forced swim test (FST), a recognized experimental model for depression studies[5].

The SSRIs increase the synaptic availability of 5-HT accentuating or facilitating its action[6,7]. According to some researches the chronic administration of

Estudo realizado no Departamento de Nutrição, Universidade Federal de Pernambuco (UFPE), Recife PE, Brasil e no Departamento de Anatomia, Universidade de São Paulo (USP), São Paulo SP, Brasil: [1]Doutor em Farmacologia Experimental e Clínica da Universidade de Paris VI, Professor Adjunto do Departamento de Nutrição, UFPE; [2]Doutoranda em Nutrição da UFPE, Professora Auxiliar do Departamento de Ciências da Nutrição, Universidade Federal da Bahia; [3]Doutorando em Anatomia da USP; [4]Mestrando em Nutrição da UFPE; [5]Bolsista de Iniciação Científica CNPq-PIBIC; [6]Estagiário do Laboratório de Fisiologia da Nutrição Naide Teodosio, Departamento de Nutrição, UFPE; [7]Doutor em Ciências da Universidade Estadual de São Paulo, Professor Assistente Doutor Departamento de Anatomia, Instituto de Ciências Biomédicas -USP. CAPES and UFPE supported this investigation.

Received 7 February 2002, received in final form 14 June 2002. Accepted 17 June 2002.

Dr. Raul Manhães de Castro – Departamento de Nutrição, UFPE - Cidade Universitária - 50670-901 Recife PE - Brasil. FAX: 81 271.8473. E-mail: rcastro@nutricao.ufpe.br.

SSRIs during the neonatal stage (days 1 to 21, suckling period) induces several behavioral changes in adult life[8,9]. Other studies demonstrated that chronic use of the SSRIs increases the expression of brain-derived neurotrophic factor (BDNF), one target gene of antidepressant treatment, in rat limbic structures, most notably the hippocampus[10]. In addition, the BDNF plays an important role in development, synapse remodeling[11] and it has trophic effects on serotonergic neurons in the central nervous system[12].

Furthermore, experimental works indicates that itself 5-HT can influence the embryogenesis and the growth[13,14] presumably by acting as a developmental sign[15] or as a neurotrophic factor[16,17]. Moreover, it is well known that very fast growth and development of the nervous system occur during pregnancy and suckling, and that pharmacological or nutritional manipulations at this phase, can induce drastic morphological and functional changes in these processes[15,18,19]. Drastic consequences have been also observed in behavior[9]. These alterations can become irreversible depending on the magnitude of the aggression[19]. Thus, there is a possibility that the use of SSRIs, in the initial phase of the life, could present long-lasting effects on behaviors related to the serotonergic function[9], such as the emotional behaviors[20]. Therefore, since there are no data concerning this point, the investigation of the possible long-lasting effects caused by early manipulations of the serotonergic system is highly desirable. This study proposed to test the hypothesis that the administration of a selective serotonin reuptake inhibitor - fluoxetine - to suckling rats, promotes changes in depressive behavior induced by forced swim in adult rats.

METHOD

Animals and treatments

Wistar rats were maintained at a room temperature of 23 ± 1 °C, on a light-dark cycle of 12:12 hours (light on at 7:00 a.m.), with free access to water and food. The animals were assigned randomly to two groups (6 pups per litter) 24 h after birth. One group (Fluoxetine group, 26 rats) received fluoxetine (10 mg/kg, *sc*, dissolved in saline solution, 1 ml/kg), and the other (Control group, 26 rats) received an equivalent volume of saline (NaCl 0.9%). The treatments were applied every day from the 1st to the 21st postnatal day (suckling period). Body weights were determined at 1st to the 21st (weaning) and 60th day.

Behavioral evaluation

The animals aged around 60 days, weighing 220-240g, were evaluated with regard to depressive behavior induced by forced swim (Forced Swim Test), modified method of Porsolt et al.[21]. This procedure consists of exposing an animal to a situation of inescapable stress, in which the rat is forced to swim. After an initial period of vigorous swimming activity in the direction to the tank border (denominated Latency of the Attempt of Escape), the animal reduces the intensity of the movements, just producing the necessary movements to maintain its head out of the water. This answer was classified as behavioral immobility, indicating a possible state of despair of the animal when it realizes that there is no escape.

The rats were placed individually in a tank (height, 42 cm; diameter, 104.5 cm), whose level of water do not allow the animal to lean on the floor, nor arise by the border. The temperature of the water was maintained in 25 °C. The animals were submitted to the forced swimming during 15 minutes (Pre-test). After the 15 min of forced swim each animal was led to dry in the Camera of Heating (CH; 32 °C /15min), an then returned to their cages. Twenty-four hours after the Pre-test, all the appraised animals were put back inside of the tank. At this time, the individual behavioral evaluation was accomplished and quantified during 5 minutes of swim (Test); soon after they were again led for CH. The behavioral parameters as Latency of the Attempt of Escape (LAE) and Behavioral Immobility (BI) were quantified in seconds (s) with aid of digital chronometers.

Statistical analysis

The corporal weight evolution (expresses mean ± SEM) was analyzed by Student's "t" test. The behavioral parameters (expressed as median and percentiles 25-75) were appraised for Mann-Whitney two-tailed test. The significance level adopted for all the used statistical tests was $p<0.05$ (Statgraphics Statistical Graphics System v.6.0, Manugistics, Inc. and Statistical Graphics).

RESULTS

Compared to the Control group, the Fluoxetine group presented a reduction in the corporal weight gain ($p < 0.05$) starting from the 9 th day of life and continuing to 21st day (Fig 1). At the 60th day of age none difference was observed among the corporal weights of the groups (Table 1).

The behavioral parameters were appraised during the FST. LAE of the Fluoxetine group was significantly larger ($p < 0.01$) while BI was smaller ($p < 0.01$) when compared with the Control group (Tables 2 and 3).

DISCUSSION

The present study demonstrated that chronic administration of fluoxetine, during the critical period of the nervous system development, besides harming the evolution of the corporal weight, in adult rats, also reduced the depressive behaviors induced by FST. These effects can be correlated with the reported developmental alterations of the serotonergic system, as suggested by Palén et al.[13]. These authors

Table 1. Body weight comparisons between the Control and Treated Groups at three different days.

Experimental groups	Weight (g)		
	1st day	21st day	60th day
Control group	7.49 ± 0.73 (26)	47.45 ± 7.73 (26)	214.67± 19.97 (13)
Fluoxetine group	7.60 ± 0.71 (26)	35.32 ± 8.07* (26)	209.62 ± 27.31 (13)

The treated group received fluoxetine (10mg/kg, sc; Fluoxetine group). The control group received saline (0.9% NaCl, 1ml/kg, sc) from the 1st to the 21st day of age. The weight of the 1st, 21st and 60th day of age are compared and reported as mean + SEM. * $p < 0.05$ (unpaired two-tailed Student *t* test). Number of animals (n) are presented below the weight columns.

Table 2. Latency of the attempt of escape of rats treated with fluoxetine or saline during the suckling period.

Experimental groups	Latency of the attempt of escape (s)	
	Md	PE_{25-75}
Control group (n=26)	154.5	117-222
Fluoxetine group (n=26)	97.5	49-161

Latency of the attempt of escape (LAE) of rats treated with fluoxetine or saline during the suckling period. All the animals were submitted to experimental depression at the 60 day of age. For each group (26 rats/group), the LAE is represented as median (Md) and 25 and 75 percentiles (PE_{25-75}). * $p < 0,01$ compared to the Control group. (Mann-Whitney two-tailed U-test).

Table 3. Behavioral Immobility (BI) of rats treated with fluoxetine or saline during the suckling period.

Experimental groups	Behavioral Immobility (s)	
	Md	PE_{25-75}
Control group (n=26)	9	2-13
Fluoxetine group (n=26)	24.5	4-66

Behavioral Immobility (BI) of rats treated with fluoxetine or saline during the suckling period. All the animals were submitted to the experimental depression at the 60 day of age. For each group (26 rats/group), the BI is represented as median (Md) and 25 and 75 percentiles (PE_{25-75}). * $P < 0.01$ compared to the Control group. (Mann-Whitney two-tailed U-test).

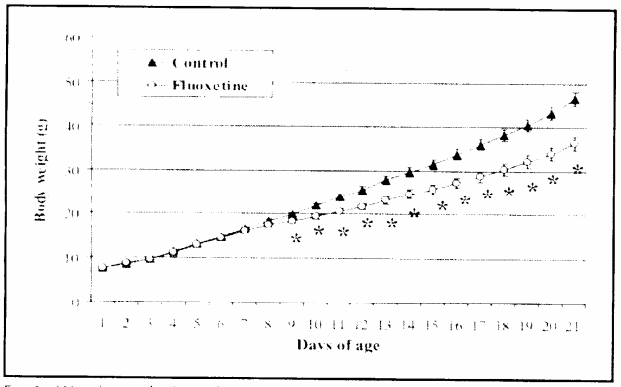


Fig 1. Weight evolution of rats treated with fluoxetine or saline during the suckling period. Rats received fluoxetine (10mg/kg, sc; Fluoxetine group, n=26) or saline (0.9% NaCl, 1ml/kg, sc; Control group, n=26) from the 1st to the 21st day of age. The animals were weighted daily. The data are reported as mean ± SEM. * $P < 0.05$ compared to the Control group at the same age (unpaired two-tailed Student t test).

observed that the administration of drugs acting on the synthesis and serotonin liberation, or on the activation of the serotonergic receptors, during the embriogenesis, could result in disturbances of the growth and development of several tissues, including the nervous one. These data were confirmed by Lauder et al.[22], that demonstrated a delay in the neuronal differentiation, through inhibition in the embryonic synthetic pathway of 5-HT, after maternal administration of p-chlorophenylalanine (pCPA). Decrease on the levels of 5-HT in the neonatal period, also induced reduction of the dendritic spines density in the developing nervous system. This constitute morphologic alterations of specific and permanent character, possibly resulting in damages on the synapses establishment[15]. In contrast, cultured embryonic serotonergic neurons of the mesencephalon of rats presented an increase of its density and survival[19].

Other hypotesis can be relationship to the BDNF, we believe which chronic administration of SSRIs in the early life may to affect this neurotrophin, consequently leading the possible process of the neuroadaptation. Accordingly with recent study a infusion of BDNF into the of hippocampus produced antidepressant effect in the FST[23]. In spite of that, it is not totally been clarified that these alterations may persist until the adult life promoting functional damages, our data corroborate this possibility.

Our observations about the reduced corporal weight gain of fluoxetine treated rats can be attributed to the inhibitory action of serotonin, controlling the food ingestion[24]. Although, the stress provided by chronic treatment with fluoxetine can induce decreased body weight, possibly by affect the hipothalamic-pituitary-adrenal axis resulting disturbances in the control of corticoid function, this fact was not considered in this study. Since, saline controls animals also were treated chronically. Besides, the chronic fluoxetine treatment normalize the corticosterone secretion in depressed pacients or experimental models[25]. Fluoxetine is an antidepressant drug that selectively inhibits the neuronal 5-HT uptake, conse-

quently increasing its synaptic availability[26], and so reduces the hunger and the alimentary ingestion in humans[27] and produces hipofagia in rats[28].

The pharmacological manipulation of the serotonergic system during the development, might have caused the post-treatment decrease of the depressant behaviors induced by the FST, that persisted until the adult age, confirming the well known relationship between depression and functional alteration of the serotonergic system[29]. In this context, the studies accomplished at our laboratory already demonstrated alterations of the aggressive behavior, in adult rats submitted to neonatal treatment with selective serotonin reuptake inhibitor[9]. The 5-HT is involved in the neurobiology of depression, as well as in the action mechanisms of antidepressant agents[30]. The presence of multiple types of serotonergic receptors corroborates the hypothesis that drugs with selective action in some of them, can have specific properties in emotional disorders[2].

The decrease in the concentrations of brain serotonin can precipitate the recurrence of the depression in depressed patients[31] while the manipulation of serotonergic receptors by pharmacological tools has evidenced antidepressant properties in some animal models[32]. The FST has been used to evaluate the effectiveness of several antidepressant treatments[5]. It was realized after evaluation of the several activity of the animals (dates not published), yet no alteration was observed. The reduction of depressant behavior in the FST, in the Fluoxetine group, seems to be related to the function of the serotonergic system[5]. The inhibition of the 5-HT uptake process by fluoxetine results possibly in its increased availability in the synaptic cleft, accentuating or facilitating its action[6]. The decreased depressant behavior evaluated in adult life after neonatal treatment, in the FST observed in the present study, seems to be associated with neuroadaptive mechanisms developed at the time of treatment, that persists until adult life.

Acknowledgements - CAPES and UFPE that supported this investigation.

REFERENCES

1. Manhaes-de-Castro R, Peregrino A, Sougey E, Barreto-Medeiros JM, Deiro TCBF. Depression: repercussion in serotoninergic system. Neurobiologia 1998;61:45-55.
2. Mac Sweeney CP, Fesourd M, Gandon JM. Antidepressant-like effects of almespirone (S 20499) in the learned helplessness test in rats. Eur J Pharmacol 1998;345:133-137.
3. Newman ME, Lerer B, Shapira B. 5-[illegible] receptors-mediated effects of antidepressants. Prog Neuropsychopharmacol Biol Psychiatry 1993;17:1-19.
4. O'Neill MF, Conway MW. Role of 5-[illegible] and 5-HT1B receptors in the mediation of behavior in the forced swim test in mice. Neuropsychopharmacology 2001;24:391-399.
5. Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I. Serotoninergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology (Berl) 1999;142:162-167.
6. Sanchez C, Hyttel L. Isolation-induced aggression in mice: effects of 5-hydroxytryptamine uptake inhibitors and involvement of post-synaptic 5-HT$_{1A}$ receptors. Eur J Pharmacol 1994;264:241-247.
7. Hiemke C, Hartter S. Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacol Ther 2000;85:11-28.
8. Hansen HH, Sánchez C, Meier E. Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in adult rats: a putative animal model of depression? J Pharmacol Exp Ther 1997;283:1333-1341.
9. Manhaes-de-Castro R, Barreto-Medeiros JM, Mendes-da-Silva C, et al. Reduction of intraspecific aggression in adult rats by neonatal treatment with a selective serotonin reuptake inhibitor. Braz J Med Biol Res 2001;34:121-124.
10. Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 1996;16:2365-2372.
11. Lipska BK, Khaing ZZ, Weickert CS, Weinberger DR. BDNF mRNA expression in rat hippocampus and prefrontal cortex: effects of neonatal ventral hippocampal damage and antipsychotic drugs. Eur J Neurosci 2001;14:135-144.
12. Lyons WE, Mamounas LA, Ricaurte GA, et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc Natl Acad Sci USA 1999;96:15239-15244.
13. Palen K, Thorneby L, Emanuelsson H. Effects of serotonin antagonists on chick embriogenesis. Wilhelm Roux's Arch Develop Biol 1979;187:89-103.
14. Whitaker-Azmitia PM. Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology. Pharmacol Rev 1991;43:553-561.
15. Yan W, Wilson CC, Haring JH. 5-HT1a receptors mediate the neurotrophic effect of serotonin on developing dentate granule cells. Brain Res Dev Brain Res 1997;98:185-190.
16. Lauder JM, Wallace JA, Krebs H. Roles of serotonin in neuroembriogenesis. Adv Exp Med Biol 1981;133:477-506.
17. Liu J, Lauder JM. Serotonin promotes region-specific glial influences on cultures serotonin and dopamine neurons. Glia 1992;5:306-317.
18. Dobbing J. Vulnerable periods in developing brain. In: Davison, AN, Dobbing J (Eds.). Applied Neurochemistry. Oxford: Blackwell 1968;287-316.
19. Manhaes-de-Castro R, Cabral Filho JE, Costa JA, Costa FBR, Galindo MAC, Hecksher CA. Neonatal treatment with naloxone causes permanent hyperalgesia in rats. Braz J Med Biol Res 1993;26:747-751.
20. Chopin P, Moret C, Briley M. Neuropharmacology of 5-hydroxytryptamine$_{(1B-1D)}$ receptor ligands. Pharmacol Ther 1994;62:385-405.
21. Porsolt RD, Le Pichon M, Jaltre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730-732.
22. Lauder JM, Towle AC, Patrick P, Henderson, P, Krebs H. Decreased serotonin content of embryonic raphe neurons following maternal administration of p-chlorophenylalanine: a quantitative immunocytochemical study. Brain Res 1985;352:107-114.
23. Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002;22:3251-3261.
24. Simansky KJ. Serotonergic control of the organization of feeding and satiety. Behav Brain Res 1996;73:37-42.
25. Duncan GE, Knapp DJ, Carson SAW, Breese GR. Differential effects of chronic antidepressant treatment on swim stress- and fluoxetine-induced secretion of corticosterone and progesterone. J Pharmacol Exp Ther 1998;285:579-587.
26. Olivier B, Mos J, van der Heyden JA M, Hartog J. Serotonergic modulation of social interactions in isolated male mice. Psychopharmacology (Berl) 1989;97:154-156.
27. Mcgurk J, Silverstone T. The effect of the 5-HT re-uptake inhibitor fluoxetine on food intake and body weight in healthy male subjects. Int J Obes 1990;14:361-372.
28. Wong DT, Reid LR, Threlkeld PG. Suppression of food intake in rats by fluoxetine: comparison of enantiomers and effects of serotonin antagonists. Pharmacol Biochem Behav 1988;31:475-479.
29. Stahl S. 5-HT$_{1A}$ receptors and pharmacotherapy. Psychopharmacol Bull 1994; 30:39-43.
30. Meltzer HY. Role of serotonin in depression. Ann N Y Acad Sci 1990; 600:486-499.
31. Robinson DS, Alms DR, Shrotriya RC, Messina MF, Wickramaratna P. Serotonergic anxiolytics and treatment of depression. Psychopathology 1989;22(Suppl 1):27-36.
32. Detke MJ, Lucki I. Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. Behav Brain Res 1996;73:43-46.

AGRESSIVIDADE INTRAESPECÍFICA ALTERA PADRÃO LEUCOCITÁRIO EM RATOS ADULTOS

DE CASTRO, C.M.M.B.; BARRETO MEDEIROS, J.M.; LIMA, K.M.; FEITOZA, L.G.; MUDO, C.L.G.; SILVA, R.R.; MAGALHÃES, V.; MANHÃES DE CASTRO, R.

Dep. de Nutrição, Lab. de Imunopatologia Keizo Asami, UFPE-Recife

Objetivos: Não há dados na literatura sobre comportamento agressivo e padrão leucocitário (PL), todavia a ativação do sistema imune (SI) pode ser avaliada através deste parâmetro. Avaliamos a contagem total (CTL) e diferencial de leucócitos (CDL) em ratos submetidos ou não à agressividade intraespecífica (AI). **Métodos:** Foram utilizados ratos Wistar machos, entre 90 e 120 dias, nos quais induziu-se (grupo agressivo; n = 14) ou não (grupo controle; n = 14) resposta agressiva intraespecífica (RAI). A indução da RAI era obtida através de sessão (20 min) de choques nas patas (1,6mA/2s, 4 em 4 min, 5 vezes). Imediatamente após, de cada animal, foi coletada amostra de sangue para realização da CTL e CDL. Para análise estatística, foi utilizado o teste t de Student para a CTL e a CDL (valores em médias e EP). **Resultados:** Na CTL: o grupo agressivo (8755 ± 677) apresentou valores menores que o grupo controle (12735 ± 830). Na CDL (%), houve diferença entre os grupos apenas nos linfócitos (L) e neutrófilos (N): grupo agressivo: L (69500 ± 2,4); N (24857 ± 2,5) x grupo controle: L (76143 ± 1,8); N (18643 ± 1,6). **Conclusões:** No presente estudo, os resultados parciais mostraram que a AI alterou a CTL e a CDL. Assim, a influência imediata da agressividade sobre o PL parece ativar o SI, modificando as proporções entre os distintos tipos celulares. Contudo, outros estudos sobre aspectos da imunidade são ainda necessários.

Apoio: CNPq, Propesq, Proin-Capes

308

ANEXOS

UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
COMISSÃO DE ÉTICA EM EXPERIMENTAÇÃO ANIMAL

Recife, 24 de outubro de 2003

Ofício nº 202/2003
Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
A Ilma Célia Maria Machado Barbosa de Castro
Departamento de Medicina Tropical, CCS/UFPE

Após o recebimento de sua carta de encaminhamento solicitando a análise do procedimento com animais do projeto de pesquisa intitulado **"Desnutrição e manipulação famacológica do sistema serotoninérgico: estudo dos parâmetros do comportamento agressivo, da resposta imune e de suas interrelações"**, processo Nº 23076.007692/2003-90, os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) analisaram os aspectos relativos aos protocolos experimentais adotados.

Concluímos que, na versão atual, os procedimentos descritos para manejo e cuidado dos animais em experimentação 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.

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, ressaltamos ainda que o sacrifício dos animais experimentais, realizado no presente trabalho, justifica-se pelo fato de não existirem recursos alternativos para a realização do procedimento científico. Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais descritos no projeto analisado.

Atenciosamente,

Profa. Miriam Camargo Guarnieri
Presidente da Comissão de Ética em Experimentação Animal

Profa. Belmira Lara da S. Andrade da Costa
Vice-Presidente da Comissão de Ética em Experimentação Animal

UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
COMISSÃO DE ÉTICA EM EXPERIMENTAÇÃO ANIMAL

Ofício nº 199/2003 Recife, 24 de outubro de 2003

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
A Ilma Célia Maria Machado Barbosa de Castro
Departamento de Medicina Tropical, CCS/UFPE

Após o recebimento de sua carta de encaminhamento solicitando a análise do procedimento com animais do estudo experimental intitulado **"Pode a expressão ou não da agressividade intraespecífica frente a um estressor alterar a responsta imune?"**, processo Nº 23076.007691/2003-45, os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) analisaram os aspectos relativos aos protocolos experimentais adotados.

Concluímos que os procedimentos descritos para manejo e cuidado dos animais em experimentação 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.

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, ressaltamos ainda que o sacrifício dos animais experimentais, realizado no presente trabalho, justifica-se pelo fato de não existirem recursos alternativos para a realização do procedimento científico. Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais descritos no projeto analisado.

Atenciosamente,

Profa. Miriam Camargo Guarnieri
Presidente da Comissão de Ética em Experimentação Animal

Profa. Belmira Lara da S. Andrade da Costa
Vice-Presidente da Comissão de Ética em Experimentação Animal

Célia M. M. B. de Castro

De: "Instituto Internacional de Ecologia" <iie@iie.com.br>
Para: <ccastro@lika.ufpe.br>
Enviada em: Segunda-feira, 13 de Outubro de 2003 12:49
Assunto: Aceitação RBB-102/03

Prezado(a) Dr(a) Celia Maria M.B. de Castro

Pela presente, vimos informar-lhe que seu manuscrito intitulado "THE EXPRESSION OF AN INTRASPECIFIC AGGRESSIVE REACTION BEFORE A STRESSOR ALTERS THE IMMUNE RESPONSE IN RATS", foi aceito em 09/10/2003 no Brazilian Journal of Biology.

Data prevista para publicacao agosto/2005 no volume 65 numero 3.
Atenciosamente

Profa. Dra. Takako Matsumura Tundisi
Editora Chefe
Brazilian Journal of Biology

-----Mensagem original-----
De: cprasa [mailto:cprasa@lsuhsc.edu]
Enviada em: sexta-feira, 12 de dezembro de 2003 15:24
Para: rcastro@nutricao.ufpe.br
Assunto: MS#NN 29903

Dr. R. Manhaes DeCastro
Departmento de Nutrição
Centro de Ciências Da Saúde
Universidade Federal de Pernambuco
50670-901 Recife/PE, BRASIL
Telephone: +55-81-271-8471
FAX: +55-81-271-8473
E-mail: rcastro@nutricao.ufpe.br

DATE RECEIVED: June 16, 2003, FIRST REVIEW COMPLETED: August 6, 2003,
MANUSCRIPT ACCEPTED: December 12, 2003.

TITLE:

Malnutrition during Brain Growth Spurt Alters the Effect of Selective Serotonin Reuptake Inibitor on Aggressive Behavior in Adult Rats

AUTHOR(S):

J.M. Barreto-Medeiros, E.G. Feitoza, K. Magalhaes, J.E. Cabral Filho, F.M. Manhaes-De-Castro, C.M. M.B. De-Castro, R. Manhaes-De-Castro.

I am pleased to inform you that the above manuscript has been accepted for publication.

We will complete final editorial processing and forward your manuscript to our typesetter for scheduled publication in the next available issue of the journal. Minor changes, abbreviations, etc. will be made by our copy editor and you will be able to check them at the time you receive the proofs.

Sincerely,

Chandan Prasad, PhD
Editor-in-Chief
Nutritional Neuroscience

INBOX: Confirmation new paper No. jmbm.AT.ufba.br/2003121... (8 de 37)

Data: Sat, 13 Dec 2003 04:38:08 GMT
De: physiolbehav@biol.rug.nl
Para: jmbm@ufba.br
Assunto: Confirmation new paper No. jmbm.AT.ufba.br/20031213/1 submitted to PHB

This is an automated email
13-Dec-2003

Dear Dr barreto medeiros,

Thank you for submitting the paper entitled:

'Early malnutrition alters the effect of aggressiveness on the immune response in rats'

to Physiology & Behavior

The e-submission number for your paper is
PHB_jmbm.AT.ufba.br_20031213/1

Your paper will be evaluated as quickly as possible;
and we will be in touch again in due course.

physiolbehav@biol.rug.nl (outside NA. However depending on subject NA authors can also submit to Jaap Koolhaas)