Universidade Federal de Pernambuco

Centro de Ciências da Saúde

Pós-Graduação em Nutrição

CAROLINA PEIXOTO MAGALHÃES

Programação Nutricional:

Estudo da preferência alimentar e da participação dos receptores serotoninérgicos centrais do tipo 5-HT1B/D no controle da saciedade em ratos.

Recife, 2010

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Tese apresentada ao Programa de Pós-graduação em Nutrição do Centro de Ciências da Saúde da Universidade Federal de Pernambuco para obtenção do título de Doutora em Nutrição.

Orientador: Prof. Dr. Raul Manhães de Castro
Co-orientadora: Profª. Dra. Sandra Lopes de Souza

Recife, 2010

Magalhães, Carolina Peixoto

Programação nutricional: estudo da preferência alimentar e da participação dos receptores serotoninérgicos centrais do tipo 5-HT1B/D no controle da saciedade em ratos / Carolina Peixoto Magalhães . – Recife: O Autor, 2010.

107 folhas ; 30 cm

Orientador: Raul Manhães de Castro

Tese (doutorado) – Universidade Federal de Pernambuco. CCS. Nutrição, 2010.

Inclui bibliografia e anexos.

1. Comportamento alimentar. 2. Desnutrição. 3. Serotonina. 4. Alimento palatável. I. Castro, Raul Manhães. II.Título.

UFPE

616.39 CDD (22.ed.) CCS2011-031

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Tese aprovada em 12 de novembro de 2010

Rhowena Jane Barbosa de Matos, CAV

Ana Elisa Toscano Meneses da Silva, CAV

Wylla Tatiana Ferreira e Silva, CAV

Raul Manhães de Castro, UFPE

Cristiano Mendes da Silva - UNIFESP

Recife, 2010

Ser feliz é reconhecer que vale a pena viver, apesar de todos os desafios, perdas e frustrações. Ser feliz é deixar de ser vítima dos problemas e se tornar autor da própria história. Meus mais profundos agradecimentos a Deus por ter me dado a capacidade de superação e recomeço.

As pessoas que mais amo,

Minha mãe, Calu, minha vida, minha luz, meu sucesso são os seus sucessos, pois somos duas partes de um todo. Te amo demais.

Aos meus filhos, Gabriel e Pedro, hoje ainda pequenos, sem muita noção da importância desse momento para a minha vida e para a deles. Sem vocês, eu não teria tanta coragem.

Aos meus avós, Celso (em memória) e Carmem, pessoas que sempre foram exemplo de luta e sabedoria, meu eterno respeito e admiração. Deixo aqui registrada minha tristeza pela perda tão repentina do meu avô Celso. ***Essa Tese é para você vovô!***

A Geraldo Junior, meu companheiro, meu amigo, meu amor. Você fez parte dessa construção e defesa. Sem você eu não teria conseguido. Muito obrigado por você existir em minha vida.

Te amo.

Agradecimentos

Agradeço especialmente a minha amiga e "irmã" Renata Campina. Aprendemos, sorrimos e choramos. Vivemos essa tese intensamente. Sem sua participação as dificuldades seriam ainda maiores.

À minha grande amiga e companheira Matilde Cesiana, tudo começou na sua casa, quando você me acolheu. Seu coração é enorme e Deus te abençoará sempre.

As minhas companheiras de trabalho, as quais foram muito especiais para a conclusão desse trabalho, são elas: Lucia Pires (participação constante em nossas vidas), Ligia Galindo, Claudia Lagranha, Aline Isabel, Amanda Marcelino e Madge Fechine. Sem a ajuda e participação ativa de cada uma de vocês eu não teria conseguido. Pois descobri que uma tese de doutorado se faz com várias "mãos".

Às minhas fiéis estagiárias, Tássia Karin, Larissa Almeida, Priscilla e Livia Lira, que tanto se doaram e trabalharam, dia e noite, para obtenção de resultados fidedignos, meu muito obrigado.

Aos meus orientadores, Raul Manhães e Sandra Lopes. As discussões, nossos encontros, nossas tentativas, obrigado por terem me feito passar por tudo, foi engrandecedor.

Às minhas companheiras de trabalho Manuela Figueiroa, Lisiane Oliveira, Ana Elisa Toscano e Taciana Rocha. Obrigado pela ajuda e compreensão, e por estarem sempre dispostas a ajudar.

Ao Sr. Paulino e Dr. França, pessoas importantes no aprendizado com as dietas experimentais e com o manuseio e respeito pelos animais de laboratório (respectivamente). Muito obrigado.

Aos meus monitores de anatomia do CAV. Todos, de certa forma, fizeram parte dessa tese. Obrigado a todos vocês por participarem da minha vida e da minha carreira profissional.

Ao técnico André Pukey, por sua ajuda e sincera amizade. Sua participação e compreensão nas horas difíceis foram muito importantes para que eu pudesse finalizar meus trabalhos.

Ao amigo Joe Turner, pela constante presença em nossas vidas. Obrigado por acreditar e confiar em mim.

À Família Sena, vocês entraram em minha vida durante a elaboração dessa tese, acompanharam e torceram por mim. Muito obrigado pelo acolhimento.

A todos aqueles que fazem o Centro Acadêmico de Vitória, local onde encontrei a verdadeira felicidade profissional.

RESUMO

Durante a "janela precoce de plasticidade fisiológica" existem fases de crescimento do sistema nervoso que são sensíveis às agressões ambientais, sendo considerados períodos críticos do desenvolvimento. Esse fenômeno biológico que estabelece a relação entre estímulos no período crítico de desenvolvimento e o estado funcional futuro é denominado de programação. Investigamos os efeitos da desnutrição perinatal sobre o perfil do comportamento alimentar e a participação dos receptores 5-HT1B/D no controle da saciedade e a preferência por alimentos palatáveis em ratos de meia-idade. No primeiro artigo intitulado "The modulatory role of serotonin on feeding behavior" foi realizada uma ampla revisão de literatura sobre o papel desempenhado pelo sistema serotoninérgico sobre o comportamento alimentar. No segundo artigo original intitulado "Perinatal protein restriction induces alteration of control of satiety by 5-HT1B/1D receptor agonist and alterations of alimentary preference in adult rats" investigamos os efeitos da desnutrição perinatal sobre a preferência por alimentos com alto teor de gordura ou sacarose e a ação do agonista 5-HT1B/1D sobre os parâmetros da seqüência comportamental de saciedade em ratos de meia-idade. A desnutrição materna durante a gestação e lactação é capaz de alterar a preferência por alimentos palatáveis e a seqüência comportamental de saciedade. Concluímos que a desnutrição no período crítico do desenvolvimento programa o perfil alimentar de ratos adultos.

Palavras-chave: comportamento alimentar; desnutrição; serotonina; alimento palatável

ABSTRACT

During the "window of early physiological plasticity" there are stages of growth of the nervous system that are sensitive to environmental stressors and are considered critical periods of development. This biological phenomenon that establishes the relationship between stimuli in the critical period of development and the future state is called functional programming. We investigated the effects of perinatal malnutrition on the profile of eating behavior and participation of the receptors 5-HT1B/D in the control of satiety and preference for palatable foods in rats of middle age. In the first article entitled "The modulatory role of serotonin on feeding behavior" was performed a comprehensive review of literature on the role of the serotonergic system on feeding behavior. In the second original article titled "Perinatal protein restriction induces alteration of control of satiety by 5-HT1B receptor and alimentary disorder of preference in adult rats" investigated the effects of perinatal malnutrition on the preference for foods high in fat or sucrose and action agonist 5-HT 1B/1D on the parameters of behavioral satiety sequence in rats of middle age. The maternal malnutrition during pregnancy and lactation can alter the preference for palatable foods and behavioral satiety sequence. We conclude that malnutrition during critical development program the food profile of adult rats.

Keywords: Feeding behavior; malnutrition; serotonin; palatable foods

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1. Apresentação

Nas últimas décadas, vários estudos têm descrito as modificações comportamentais e os mecanismos de controle da ingestão alimentar no período entre o nascimento e o desmame ocorridas após agravos ambientais (Houpt and Epstein, 1973; Zippel, Heidel et al., 2001; Lopes de Souza, Orozco-Solis et al., 2008). Nesse período da vida de humanos e animais, o excesso ou a escassez na oferta de alimentos podem consolidar padrões alimentares que poderão estar envolvidos com determinadas patologias na vida adulta (Barker, 1995). Já que eventos precoces têm efeitos determinantes sobre o padrão saúde-doença na vida adulta cabe investigar as possíveis alterações ocorridas no comportamento alimentar de ratos com idade avançada.

A presente tese intitulada "Programação Nutricional: Estudo da preferência alimentar e da participação dos receptores serotoninérgicos centrais do tipo 5-HT1B/D no controle da saciedade em ratos**"** teve como objetivo estudar a preferência alimentar e a participação do agonista serotoninérgico5-HT1B/1D no controle da saciedade em ratos de meia-idade submetidos à desnutrição perinatal. A fim de abordar aspectos importantes relacionados ao tema, a revisão bibliográfica foi organizada em três capítulos da seguinte forma: 1. A programação por desnutrição e suas consequências; 2. O sistema serotoninérgico e o controle do comportamento alimentar; 3. A programação e a preferência alimentar e 4. A sequência comportamental de saciedade e sua interação com o comportamento alimentar.

Delineados os métodos, os resultados foram apresentados na forma de dois artigos, sendo o primeiro artigo de revisão aceito pela *Nutritional Neuroscience*, e o segundo submetido à revista *Physiology & Behavior*. O primeiro artigo: The modulatory role of serotonin on feeding behavior teve como objetivo situar o papel do sistema serotoninérgico no controle homeostático e hedônico do comportamento alimentar. Para isto procurou-se organizar, sobretudo, do ponto de vista experimental, as evidências da literatura sobre o

assunto. No segundo artigo original: Perinatal protein restriction induces alteration of control of satiety by 5-HT1B/1D receptor agonist and alterations of alimentary preference in adult rats, analisou-se o efeito da desnutrição protéica perinatal sobre a preferência por dieta com alto teor de gordura ou solução de sacarose e a participação do receptor 5-HT1B/1D no comportamento alimentar em ratos de meia-idade.

Hipóteses

- A desnutrição perinatal induz a preferência por alimentos com alto teor de gordura;

- Os animais desnutridos submetidos a doses agudas do agonista serotoninérgico 5-HT1B/1D retornam o ponto de saciedade para os níveis dos animais controle.

Objetivos

Geral:

Estudar a preferência alimentar e a ação do agonista serotoninérgico 5-HT1B/1D no controle da saciedade em ratos de meia-idade submetidos à desnutrição perinatal.

Específicos:

- Organizar as evidências experimentais do papel modulador do sistema serotoninérgico sobre o comportamento alimentar;

- Averiguar se a desnutrição perinatal altera a preferência quanto à ingestão de alimentos ricos em gordura ou solução de sacarose;

- Analisar os efeitos da desnutrição e de um agonista serotoninérgico 5-HT1B/1D sobre os parâmetros da seqüência comportamental de saciedade.

2. Revisão da Literatura

2.1. A PROGRAMAÇÃO POR DESNUTRIÇÃO E SUAS CONSEQUÊNCIAS

No oeste da Holanda, entre 1944 e 1945, ocorreu um período de fome aguda no centro urbano, atingindo a maioria da população a qual foi denominada de *Dutch Hunger Winter*, como resultado de um embargo alemão sobre as ferrovias, impedindo o transporte da região, além de um forte inverno que levou ao congelamento dos rios. Esta recessão provocou uma redução gradativa da ingestão de calorias de 1.800 Kcal/pessoa para níveis abaixo de 1.000 Kcal/pessoa, no decorrer de cinco meses. Esse período terminou abruptamente com a dominação dos países aliados à Holanda e com uma maciça distribuição de alimentos para a população atingida (Burger GCE, 1948). Nesta época, os indivíduos que eram expostos, na vida intra-uterina, a jejum prolongado em decorrência da *Dutch Hunger Winter* apresentavam após o nascimento restrição do crescimento (Ravelli, Stein et al., 1976). Com base nesses achados, pesquisadores procuraram identificar uma janela crítica para o desenvolvimento de características impostas pela nutrição perinatal, que poderiam desencadear seqüelas na vida adulta (McCance and Widdowson, 1974; Pimstone, 1976).

Em 1992 surgiu a Hipótese do Fenótipo Protetor sugerida por Hales e Barker (Hales and Barker, 1992). Segundo esta hipótese, o indivíduo submetido durante o período intra-uterino a uma dieta deficiente em nutrientes, desenvolveria uma resposta adaptativa com o objetivo de maximizar a eficiência quanto ao armazenamento e o uso de energia, para manter-se neste meio e para garantir o crescimento do feto. Esse fenótipo continuaria a beneficiar o indivíduo, se a condição de nutrição deficiente permanecesse durante a vida pós-natal. Na presença de nutrição adequada ou abundante, estas adaptações se tornariam fatores predisponentes ao desenvolvimento de doenças na vida adulta (Hales and Barker, 1992; Hales and Barker, 2001).

Baseado no conceito de programação nutricional fetal, Lucas e colaboradores (Lucas, Baker et al., 1996), observaram que a prole de ratas que foram submetidas à restrição protéica durante a gestação e a lactação apresentou programação no metabolismo dos lipídios. Outros estudos se seguiram e passaram a estudar os efeitos das manipulações nutricionais durante o período perinatal e observaram redução não apenas o crescimento dos filhotes, mas também mudanças na composição corporal (alteração no fluxo sanguíneo, no tamanho e no número de fibras musculares) e no funcionamento de alguns órgãos (pâncreas, rins, fígado), embora tenha levado a preservação de outras estruturas como o cérebro(Desai, Crowther et al., 1996). A deficiência na ingestão protéica em ratas gestantes também gerou aumento da pressão sanguínea dos filhotes (Langley-Evans, 1996) e o surgimento de diabetes tipo II na vida adulta, devido a alterações na sensibilidade dos tecidos a insulina (Okitolonda, Brichard et al., 1987; Grace, Swenne et al., 1990; Ozanne, Smith et al., 1996).

A resposta adaptativa preditiva é outra hipótese que propõe o desenvolvimento de adaptações na vida perinatal baseado em uma predição do ambiente após o nascimento (Gluckman and Hanson, 2004). Quando esta resposta adaptativa preditiva é apropriada, o fenótipo é normal, todavia quando ocorre uma incompatibilidade entre a predição e o ambiente atual, desenvolvem-se as doenças (Gluckman and Hanson, 2004). As principais patologias que se desenvolvem como conseqüências da programação fetal são: a hipertensão arterial, o diabetes mellitus tipo II e a obesidade (Armitage, Taylor et al., 2005; Gluckman and Hanson, 2006). Essas patologias constituem a síndrome metabólica, distúrbio de incidência crescente, sendo responsável pela diminuição na expectativa de vida das populações (Barker, 1995; Desai and Hales, 1997). Desta forma, a nutrição é identificada como o principal fator ambiental capaz de alterar a expressão do genoma fetal podendo levar a conseqüências em longo prazo (Wu, Bazer et al., 2004).

Os mecanismos que levam a uma programação não estão totalmente esclarecidos. Sabe-se, no entanto, que a dieta materna está diretamente relacionada com o desenvolvimento e crescimento fetal. A incompatibilidade entre a demanda de nutrientes oferecida *in utero* e o ambiente pós-natal adicionado aos costumes e estilos adquiridos ao longo da vida como: mudanças nos hábitos alimentares e forma de vida associados com o sedentarismo, podem provocar um forte impacto metabólico e fisiológico no individuo, aumentando o risco da expressão da carga fenotípica adquirida na vida intra-uterina.

2.2. O SISTEMA SEROTONINÉRGICO E O CONTROLE DO COMPORTAMENTO ALIMENTAR

Realizando, a mais de uma década, uma série de experiências visando determinar as conseqüências da desnutrição sobre o funcionamento do sistema nervoso central, com o foco no sistema de neurotransmissão serotoninérgica, nosso grupo, Nutrição, Neuropsicofarmacologia e Imunidade, constatou que animais desnutridos, durante o período perinatal, apresentam após o desmame, um aumento no apetite caracterizado por retardo no aparecimento da saciedade e uma diminuição dos efeitos anorexígenos da serotonina (Lopes de Souza, Orozco-Solis et al., 2008). As repercussões da desnutrição sobre o desenvolvimento estrutural, neuroquímico e integridade funcional de células no sistema nervoso central são bem conhecidas (Morgane, Austin-LaFrance et al., 1993). Estes agravos podem acarretar modificações neuroquímicas (Winick, Rosso et al., 1972), inclusive nos níveis de neurotransmissores, como a serotonina (5-hidroxitriptamina ou 5-HT) (Forbes, Stern et al., 1978; Chen, Peng et al., 1995). Isto se reflete na excitabilidade neural (Guedes, Monteiro et al., 1996; Rocha-de-Melo and Guedes, 1997) e no comportamento dos animais (Manhaes de Castro, Barreto Medeiros et al., 2001; Medeiros, Silva et al., 2001).

A serotonina é um neurotransmissor sintetizado em neurônios dos núcleos da rafe, com projeções para várias regiões do sistema nervoso central (Lidov and Molliver, 1982; Wallace and Lauder, 1983; Azmitia and Gannon, 1986; Meister, 2007). As ações intracelulares da serotonina são mediadas por 7 distintas famílias de receptores (5-HT1 – 5-HT7), contendo 14 diferentes subtipos de receptores (Hoyer, Clarke et al., 1994). Nos mamíferos, os receptores serotoninérgicos regulam uma ampla variedade de processos neurocomportamentais incluindo cognição, estados afetivos, controle motor, integração sensório-motora e o comportamento alimentar (Yoshioka, Matsumoto et al., 1995; Ericsson, Poston et al., 1996; Miyawaki, Meah et al., 1997; Julius, 1998).

Estudos usando antagonistas dos receptores serotoninérgicos sugerem que os subtipos de receptores 5-HT1B e 5-HT2C estão envolvidos na ação inibitória da serotonina sobre a ingestão alimentar (Kennett and Curzon, 1988; Dourish, Clark et al., 1989). Esses receptores reduzem a procura por alimentos em ratos (Dourish, 1995; Yamada, Sugimoto et al., 2003). Os agonistas dos receptores serotoninérgicos e as drogas que aumentam a disponibilidade sináptica de 5-HT, como agentes liberadores de 5-HT ou inibidores de sua recaptação, reduzem o consumo alimentar (Halford, Harrold et al., 2005; Chan, Mancini et al., 2006; Halford, Harrold et al., 2007; Lopes de Souza, Orozco-Solis et al., 2008).

É conhecida a ação da serotonina no controle do comportamento alimentar modulando a fome e a saciedade (Blundell, 1977; Blundell and Hill, 1987; Simansky, 1996; Schuhler, Clark et al., 2005). Porém, a distribuição dos receptores serotoninérgicos em áreas encefálicas relacionadas ao sistema de recompensa alimentar é pouco conhecida. Estruturas encefálicas localizadas nos núcleos da base constituem uma interface essencial entre motivação e ação pela busca do alimento (Jones and Mogenson, 1980). É provável que as vias que envolvem a amigdala, o córtex frontal e o corpo estriado, juntamente com as estruturas do hipotálamo

lateral, executem um papel crítico no controle da motivação do comportamento alimentar (Swanson, 2000). O núcleo pálido ventral exerce importante papel no processamento das informações hedônicas, recebendo fibras do núcleo accumbens e da área tegmental ventral, enquanto projeta-se para os núcleos hipotalâmicos, como hipotálamo lateral, dorsomedial e paraventricular (Groenewegen, Berendse et al., 1993; Kelley, 2004; Kalivas and Volkow, 2005).

A desnutrição durante o período perinatal pode programar a estrutura e a função de receptores serotoninérgicos envolvidos no componente hedônico do comportamento alimentar. A programação pode desencadear transtornos alimentares na vida adulta, particularmente relacionada às dietas altamente palatáveis. Assim, o conhecimento da ação da programação sobre áreas hedônicas do comportamento alimentar torna-se relevante, particularmente pela participação de controles cognitivos, olfatórios, gustatórios e emocionais, os quais podem estar envolvidos com o desejo de comer. É provável que os efeitos dos receptores serotoninérgicos envolvam diversas estruturas neuroanatômicas e mecanismos de fome e saciedade. Bem como mecanismos hedônicos que modulem, juntamente com aqueles relacionados à homeostase energética, a busca pelo alimento, a motivação para buscá-lo e a quantidade de alimento que será ingerida.

2.3. A PROGRAMAÇÃO E A PREFERÊNCIA ALIMENTAR

Várias manipulações ambientais durante a gestação e lactação têm resultado em alterações no padrão alimentar adulto (Lopes de Souza, Orozco-Solis et al., 2008; Orozco-Solis, Lopes de Souza et al., 2009). A incidência de estresse durante a lactação aumenta o apetite por alimentos palatáveis (Silveira, Portella et al., 2004), provavelmente em decorrência de uma maior ansiedade levando a um aumento no consumo de alimentos hiperpalatáveis

(Ely, Dapper et al., 1997). Em uma análise do comportamento hiperfágico às dietas hiperpalatáveis, Warwick e Weingarten (1995) observaram a resposta a palatabilidade para os sabores das gorduras e dos carboidratos, e perceberam que os ratos preferiam à dieta rica em gordura à dieta rica em carboidrato, indicando que o sabor palatável da gordura pode ter contribuído para essa resposta hiperfágica (Warwick and Weingarten, 1995). Com base nessas evidências, os animais programados durante o desenvolvimento têm uma preferência para comer alimentos ricos em gordura (Cambraia, Vannucchi et al., 2001; Bellinger, Lilley et al., 2004).

A programação perinatal provoca distúrbios do comportamento alimentar acompanhados de mudanças na expressão hipotalâmica de peptídeos envolvidos na regulação da ingestão de alimentos (Ikenasio-Thorpe, Breier et al., 2007; Delahaye, Breton et al., 2008). O número de neurônios hipotalâmicos que sintetizam peptídeos anorexígenos como o neuropeptídeo Y (NPY), o qual aumenta a ingestão de carboidrato mais do que a ingestão de gordura ou de proteína (Stanley and Leibowitz, 1985; Morley, Levine et al., 1987), a galanina (Tempel, Leibowitz et al., 1988) e a enterostatina (Okada, York et al., 1991) que seletivamente aumentam ou diminuem a ingestão de gordura, estão reduzidos em animais programados (Plagemann, Waas et al., 2000; Ikenasio-Thorpe, Breier et al., 2007). O hipotálamo desempenha um papel fundamental na detecção e integração de sinais nutricionais e hormonais derivados da digestão e do metabolismo de nutrientes (Bernardis and Bellinger, 1996; Williams, Harrold et al., 2000).

A ingestão de alimentos ricos em gordura está associada ao desenvolvimento de superalimentação e da obesidade, em parte pelo seu sabor palatável, alta densidade de energia e os fatores metabólicos (Drewnowski, 1997; French and Robinson, 2003). A natureza atrativa de alimentos com alto teor de gordura é amplamente reconhecida, mas a base para a promoção da ingestão de gorduras em animais desnutridos não foi totalmente explorado.

Estudos têm procurado relacionar a variação da nutrição materna durante o período crítico do desenvolvimento e sua capacidade de alterar os níveis de ingestão de alimentos na prole através da indução de mudanças na expressão, localização e ação de neuropeptídeos específicos responsáveis pela regulação do apetite (Handa, DeJoseph et al., 2000; Edwards, 2001; Edwards and McMillen, 2002; Bloomfield, Oliver et al., 2004).

A quantidade de alimento consumido durante a amamentação pode ter um papel determinante na ingestão de alimentos na vida adulta (Oscai and McGarr, 1978). A ingestão excessiva de alguns nutrientes da dieta está relacionada a um maior risco para determinadas doenças, como hipertensão e diabetes (Krousel-Wood, Muntner et al., 2004). O consumo de ácidos graxos trans e de carboidratos simples está associado às doenças coronarianas (Dyerberg, Eskesen et al., 2004) e as dietas com alto teor glicêmico contribui para um aumento das doenças cardiovasculares (Bell and Sears, 2003). Porém, os mecanismos fisiológicos e moleculares subjacentes que ligam fisiopatologicamente a desnutrição perinatal e a síndrome metabólica permanecem não esclarecidas.

2.4. A SEQUÊNCIA COMPORTAMENTAL DE SACIEDADE E SUA INTERAÇÃO COM O COMPORTAMENTO ALIMENTAR

Nos estudos sobre a neurobiologia do apetite, um trabalho clássico sobre a alimentação de ratos selvagens e de laboratório (Barnett, 1956), salientou o fato de que todas as pesquisas até aquele momento eram produtos finais de alimentação (ou seja, a quantidade de alimento consumido), com pouca ou nenhuma atenção para o comportamento alimentar. Nos últimos 25 anos, a seqüência comportamental de saciedade (SCS) como se tornou conhecida (Montgomery and Willner, 1988), é utilizada para avaliar a especificidade dos comportamentos que modulam o apetite.

Nos roedores, a SCS é caracterizada como uma fase inicial de alimentação, seguida por atividades de limpeza e de locomoção e termina com uma fase final de repouso (Antin J, Gibbs J, Holt J, 1975, Halford JC, Wanninayake SC, 1998). O ato de comer é um dos elementos do comportamento alimentar que está relacionada com a necessidade biológica de ter nutrientes. A ingestão de alimentos é um fator importante para a interpretação da SCS. Pela medição da ingestão alimentar e da duração de alimentação, pode-se calcular a taxa de alimentação ou velocidade de ingestão. A limpeza é a ação que ocorre normalmente depois do período de alimentação (Antin, Gibbs et al., 1975) e a locomoção envolve movimentos ativos dentro da gaiola. A atividade locomotora está relacionado ao comportamento exploratório (Berlyne, Koenig et al., 1966), permitindo ao animal a aquisição de informações que o familiarizem com o ambiente (Berlyne, Koenig et al., 1966). O descanso é caracterizado pela inatividade do animal (Antin, Gibbs et al., 1975; Halford, Wanninayake et al., 1998), é a postura assumida no final da BSS (Antin, Gibbs et al., 1975; Halford, Wanninayake et al., 1998). O aparecimento da postura de repouso na BSS é uma condição causada pela saciedade (Antin, Gibbs et al., 1975).

Por conta da ligação inicial da serotonina e o apetite (Blundell, 1977), não é surpreendente que a metodologia da SCS esteja sendo extensivamente utilizada nas pesquisas sobre a neurotransmissão dessa indolamina e seus vários subtipos de receptores (Lee, Somerville et al., 2004; Thornton-Jones, Vickers et al., 2005; Lievens, Verbaeys et al., 2009). Muitos são os trabalhos nesta área (Clifton, Barnfield et al., 1989; McGuirk, Muscat et al., 1992; Halford, Wanninayake et al., 1998) envolvendo drogas que liberam serotonina (D-fenfluramina) ou inibem a sua recaptação (fluoxetina, sertralina, sibutramina). Consistente com o papel proposto da 5-HT na promoção da saciedade, um dos mais recentes estudos com esses agentes em ratos e camundongos confirmou que os níveis elevados de 5-HT sináptica inibem a ingestão alimentar e aceleram a SCS sem interromper a sua integridade (Rodgers,

Holch et al., 2010). A SCS não é somente produzida pelo aumento no SNC dos níveis de serotonina ou diretamente ativando receptores 5-HT1B e 5-HT2C no hipotálamo. Embora esteja estabelecido que a manipulação farmacológica da serotonina reduza a ingestão alimentar enquanto preservam a SCS, com os receptores 5-HT medeiam esses efeitos?

Blundell (Blundell, 1986) argumentou que a estrutura do comportamento alimentar do animal reflete a operação de variáveis contextuais (Por exemplo, alimentos, drogas, meio ambiente, estado fisiológico) que influenciam a ingestão de alimentos. E o monitoramento do comportamento animal, induzindo alimentação e atividades *nonfeeding* poderia proporcionar um poderoso ensaio bio-comportamental sobre o apetite. Algumas pesquisas foram realizadas para compreender os efeitos das manipulações nutricionais sobre o padrão temporal de alimentação (Ishii, Blundell et al., 2003), a palatabilidade de dietas (Ishii, Blundell et al., 2003), o poder da saciedade de uma determinada dieta ingerida (Bensaid, Tome et al., 2003) e como as dietas hiperproteicas afetam o comportamento alimentar e em particular sobre o consumo alimentar, a taxa de alimentação e a relação entre a ingestão de alimentos e a duração da alimentação (Bensaid, Tome et al., 2003). Estudos da SCS em animais que foram desnutridos na vida perinatal demonstraram uma preservação da estrutura da seqüência comportamental de saciedade e que a hiperfagia observadas em animais desnutridos ocorre por um atraso no aparecimento da saciedade associado a um aumento no tamanho da refeição (Orozco-Solis, de Souza et al., 2009). A prole de matrizes alimentadas com uma dieta com baixo teor de proteína também apresentaram menor latência para comer e passaram mais tempo comendo que controles (Orozco-Solis, de Souza et al., 2009). Os efeitos de manipulações farmacológicas e nutricionais sobre a regulação natural fisiológica da ingestão de alimentos pode ser avaliada através da SCS. A SCS é uma ferramenta experimental sólida para melhorar a nossa compreensão do complexo processo psicofisiológico envolvido na regulação do comportamento alimentar.

3. Métodos

3.1. ANIMAIS

Foram utilizados ratos machos da linhagem *Wistar* com idades entre zero e 400 dias, provenientes da colônia do Biotério do Departamento de Nutrição da Universidade Federal de Pernambuco. As fêmeas nulíparas com peso entre 250 e 300g foram mantidas em ciclo claro/escuro de 12 horas invertido (luz acesa às 18h00), temperatura de 22 ± 1ºC, durante 15 dias para adaptação, com livre acesso à água e a dieta padrão do biotério Labina (Purina® do Brasil S/A). Após o período de adaptação, as fêmeas foram acasaladas na proporção de duas fêmeas para um macho. A gestação foi detectada através da visualização de espermatozóides no esfregaço vaginal. Após essa detecção, as fêmeas foram separadas em gaiolas individuais. Foi oferecida, de acordo com os grupos experimentais, uma dieta com baixo teor de proteína (D8%) e uma dieta normoprotéica (D17%) desde a detecção de espermatozóides no esfregaço vaginal até o 21º dia de lactação. A dieta D17% estava de acordo com recomendações do American Institute of Nutrition-AIN (Reeves, Nielsen et al., 1993) (Tabela 01).

Um dia após o nascimento, todos os filhotes nascido no mesmo dia foram randomizados e separados os machos em 8 filhotes por ninhada. Quando a quantidade de filhotes machos não foi suficiente, a ninhada foi completada com fêmeas. Como critério de inclusão, os animais provenientes de mães que consumiam D8% pesavam entre 5,0 - 6,5g de peso e os animais de mães alimentadas com dieta D17% pesavam entre 6,0 – 7,5g. Segundo a dieta oferecida para as gestantes e lactantes foram obtidos os grupos experimentais: D8% e D17%. Aos 21 dias (P21) de vida ocorreu o desmame e todos os animais, e independente do grupo experimental, passaram a receber dieta a base de caseína (17% de proteína) até completarem 50 dias de vida (P50). Após esse período os animais foram mantidos em gaiolas individuais e receberam dieta padrão de biotério a Labina (normoprotéica com 23,0% de

proteína bruta) até completarem 400 dias (P400). Todos os experimentos foram realizados de acordo com as recomendações do Comitê Brasileiro de Experimentação Animal - COBEA, e aprovado pelo comitê de ética em experimentação animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (processo nº23076.025905/2008-70).

Tabela 1 - Composição Centesimal das dietas experimentais (g/Kg) e do percentual de macronutrientes em relação ao valor energético total.

Macronutrientes	**Dieta Hipoprotéica**	**Dieta Normoprotéica**	**Dieta Hiperpalatável**
g %	100,00	100,0	100,00
Proteínas	8,10	17,30	22,36
Carboidratos	75,10	65,90	48,50
Lipídios	7,00	7,00	15,33
Fibras	5,00	5,00	2,72
***Vitaminas**	1,00	1,00	1,00
†Minerais	3,50	3,50	3,50
Metionina	0,30	0,30	0,30
Proteína de origem animal	-	-	16,66
% Kcal	**362,48**	**363,44**	**421,41**

A dieta hiperpalatável foi elaborada no Departamento de Nutrição da Universidade Federal de Pernambuco, Brasil. *The vitamin mixture contained (milligrams per kilogram of diet): retinol 12, cholecalciferol 0.125, thiamine 40, riboflavin 30, pantothenic acid 140, pyridoxine 20, inositol 300, cyanocobalamin 0.1, menadione 80, nicotinic acid 200, choline 2720, folic acid 10, p-aminobenzoic acid 100, biotin 0.6.†The mineral mixture contained (milligrams per kilogram of diet): $CaHPO_4$ 17 200, KCl 4000, NaCl 4000, MgO 420, $MgSO_4$ 2000, Fe2O2 120, $FeSO_4.7H_2O$ 200, trace elements 400 ($MnSO4.H_2O$ 98, $CuSO4.5H_2O$ 20, $ZnSO4.7H_2O$ 80, $CoSO_4.7H_2O$ 0.16, KI 0.32, sufficient starch to bring to 40 g/kg of diet).

3.2 PESO CORPORAL

O peso corporal dos filhotes foi aferido diariamente do 1º ao 21º dia pós-natal (n=32). Aos 30, 60, 90, 150, 180 e 400 dias de vida peso corporal de cada animal (n= 24) também foi verificado. Após todas as avaliações os animais foram sacrificados para retirada da gordura abdominal e do fígado. Todos os pesos foram aferidos em balança Marte AS 1000C®, classe II, capacidade máxima 1000g, menor divisão 0,01g.

3.3 PREFERÊNCIA ALIMENTAR

Os ratos ao completarem 400 dias de vida (n=18), participaram durante cinco dias consecutivos dos testes de preferência alimentar. As gaiolas onde os animais estavam alocados individualmente incluíam duas garrafas idênticas (Bebedouro de policarbonato com capacidade para 500 ml, rolha de borracha anti-ácida e bico de aço inoxidável, reto), uma das quais continha uma solução de sacarose na concentração de 10% e a outra água filtrada. Concomitantemente, foi oferecido, pelo mesmo período de tempo, dieta padrão (DP) ou dieta hiperpalatável (DH) (Tabela 1). A partir das dietas ofertadas aos animais foram formados os seguintes grupos experimentais: animais controle com acesso a dieta padrão (C/DP), controle com acesso a dieta hiperpalatável (C/HD), desnutrido com acesso a dieta padrão (LP/DP) e desnutrido com acesso a dieta hiperpalatável (LP/HD). O conteúdo dos frascos de água e solução de sacarose foi aferido diariamente, através da medição da quantidade rejeitada. O consumo das dietas foi verificado pesando-se o rejeito e em seguida completando para a quantidade de 80g. Apenas os três últimos dias foram utilizados para análise estatística. Sendo o primeiro e segundo dias considerados como períodos de adaptação dos animais as novas dietas.

As soluções foram fornecidas por 5 dias mas apenas os últimos três dias foram utilizados para avaliação da preferência alimentar. O primeiro e segundo dia foi considero período de adaptação as novas dietas. O conteúdo dos frascos foi aferido diariamente e a quantidade ingerida de cada garrafa foi anotada, o mesmo procedimento foi realizado com a dieta. A dieta foi completada diariamente, após a pesagem para o montante de 80 gramas. A ingestão diária de cada garrafa (ml) foi usada em combinação com o peso corporal para calcular a ingestão de sacarose (ml/kg), ingestão de água (ml/kg) e a ingestão total (ml/kg). O mesmo foi realizado para calcular o consumo de labina (g/kg) e da dieta hiperpalatável (g/kg).

3.4 ESTUDO DA SEQÜÊNCIA COMPORTAMENTAL DE SACIEDADE

O estudo da seqüência comportamental de saciedade ocorreu no 400º dia de vida dos animais (n=28) no horário de 16h00 às 17h00. A análise da SCS foi precedida por privação alimentar de quatro horas e realizada como descrita por Halford (Halford, Wanninayake et al., 1998). Meia hora antes de terminar o período de privação alimentar, uma dose aguda de um agonista do receptor 5-HT 1B/1D (1mg/Kg, p.c.) dissolvido em NaCl 0,9% (Mitsikostas, Papadopoulou-Daifotis et al., 1996) ou solução de salina (0,9%), foram injetadas por via subcutânea, formando os grupos experimentais: controle/salina (C/Sal), controle/ Agonista 5-HT1B/1D (C/5-HT1B/1D), desnutrido/salina (D/Sal) e desnutrido/Agonista 5-HT1B/D (D/5-HT1B/1D). As soluções foram preparadas imediatamente antes do uso. A dose do agonista 5-HT1B/1D foi escolhida para coincidir com aquelas utilizadas em seres humanos (Mitsikostas, Manta et al., 1995). Completada às quatro horas de privação foi oferecido a cada animal 60g de ração labina para realização da SCS. Três experimentadores treinados observaram os comportamentos característicos da seqüência comportamental de saciedade por 1 hora. Ao término desse período, os animais foram pesados e o consumo de ração foi quantificado através da diferença entre a quota oferecida e a quota rejeitada. Os comportamentos foram

categorizados como: comportamento de alimentação (caracterizado por mordida, roedura ou deglutição de ração disposta no comedouro), comportamento de limpeza (caracterizado por lamber o corpo, as patas e a genitália) e comportamento de descanso (caracterizado pela inatividade do animal, por sua posição relaxada, tendo a cabeça curvada sobre o corpo, ou o repouso do animal sobre o fundo da gaiola, estendido de lado ou sobre o ventre). A duração de cada comportamento foi quantificada com cronômetro digital e anotada em protocolo apropriado.

Com os dados obtidos durante a análise da SCS foram calculados outros parâmetros denominados de microestruturais da alimentação: consumo alimentar (g), tempo de duração da alimentação (min), taxa local de alimentação [quantidade de alimento consumido (g)/duração da refeição (min)], ingestão calórica total (Kcal /total), ingestão calórica por peso corporal (Kcal/100g p.c.) e ingestão calórica por minuto (Kcal/min).

3.5 ANÁLISE ESTATÍSTICA

O peso corporal foi analisado usando Two Way Analysis of Variance-ANOVA. Os dados da preferência alimentar e SCS foram analisados por meio de One-Way (ANOVA) seguida pelo teste de *Bonferroni* para comparações múltiplas entre os grupos. O consumo alimentar e os parâmetros microestruturais da alimentação foram analisados usando teste t de *Student*. Os resultados experimentais foram expressos como médias ± EPM. Todos os dados foram analisados utilizando um programa de demonstração SigmaStat 2.03. Antes de utilizar o teste ANOVA, os dados foram submetidos à análise de variância e teste de normalidade, com tolerância de 5%.

4. Resultados

4.1 THE MODULATORY ROLE OF SEROTONIN ON FEEDING BEHAVIOR

Review

Modulatory role of serotonin on feeding behavior

Carolina Peixoto Magalhães[1], Manuela Figueiroa Lyra de Freitas[1], Maria Inês Nogueira[2], Renata Cristinny de Farias Campina[3], Luiz Fernando Takase[4], Sandra Lopes de Souza[3], Raul Manhães de Castro[5]

[1]Centro Acadêmico de Vitória/Universidade Federal de Pernambuco, Vitória de Santo Antao, Brasil
[2]Departamento de Anatomia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil
[3]Departamento de Anatomia, Universidade Federal de Pernambuco, Recife, Brasil
[4]Departamento de Morfologia e Patologia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos São Carlos, Brasil
[5]Departamento de Nutrição, Universidade Federal de Pernambuco, Recife, Brasil

The appearance, the odor, and the flavor of foods, all send messages to the encephalic area of the brain. The hypothalamus, in particular, plays a key role in the mechanisms that control the feeding behavior. These signals modulate the expression and the action of anorexigenic or orexigenic substances that influence feeding behavior. The serotonergic system of neurotransmission consists of neurons that produce and liberate serotonin as well as the serotonin-specific receptor. It has been proven that some serotonergic drugs are effective in modulating the mechanisms of control of feeding behavior. Obesity and its associated illnesses have become significant public health problems. Some drugs that manipulate the serotonergic systems have been demonstrated to be effective interventions in the treatment of obesity. The complex interplay between serotonin and its receptors, and the resultant effects on feeding behavior have become of great interest in the scientific community.

Keywords: feeding behavior, serotonergic system, serotonergic receptors

How serotonin modulates feeding behavior?

Feeding behavior is the result of the integration at the level of the central nervous system (CNS), of homeostatic and non-homeostatic signals involved in the maintenance of the energetic balance of the organism. Indeed, several signals produced during the transit of food along the intestine as well as those indicative of the nutritional and energetic status of the body are conveyed to the brain which, in turn, adjusts the ingestion of food and metabolic activity to preserve the energetic homeostasis of the organism. The peripheral homeostatic factors include several peptides released from the gastrointestinal tract (cholecystokinin [CCK], peptide YY, ghrelin), the pancreas (insulin) and adipose tissue (leptin), as well as cell signals derived from the stimulation of intestine mechanoreceptors. In the CNS, all these homeostatic signals are integrated mainly at the level of the brain stem and hypothalamus and involve the action of several neuropeptides (pro-opiomelanocortin [POMC], neuropeptide Y [NPY]) and neurotransmitters (dopamine, noradrenaline, serotonin; Fig. 1). Non-

Correspondence to: Carolina Peixoto Magalhães, Centro Acadêmico de Vitoria/Universidade Federal de Pernambuco, Rua do Alto do Reservatório, S/N – Bela Vista – CEP 55608-680, Vitória de Santo Antão, PE, Brasil
E-mail: peixotocarolina8@hotmail.com
Received 22 April 2010, manuscript accepted 4 August 2010

DOI 10.1179/147683010X12611460764723

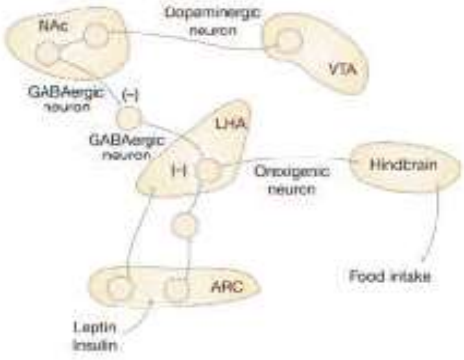

Figure 1 Brain reward circuitry. Perception of pleasure associated with consumption of palatable food involves neuronal activation in the NAc and striatum, which through activation of opiate peptide receptors disinhibits the lateral hypothalamic area and thereby stimulates feeding. Amy, amygdala; GP, globus pallidus; NAc, nucleus accumbens; VTA, ventral tegmental area. Modified from data of Morton *et al*.[136]

homeostatic information (reward, hedonic, cognitive), inter-relate with these homeostatic signals to modulate the aspects of feeding behaviour associated with the search for, or the rejection of, food. These non-homeostatic signals are generated in several encephalic structures like the nucleus accumbens and the ventral tegmental area and use as main messenger endogenous opiates and catecholamines like serotonin and dopamine. The neurotransmitter serotonin plays a key role in the regulation of the different aspects of feeding behavior.[1,2]

Serotonin, or 5-HT, is found in both vertebrate and invertebrate organisms.[3] It is involved in the regulation of energy homeostasis in different animal species (see Tecott[4] for review). In mammals, serotonin is produced by the enterochromaffin cells of the intestine and by the serotoninergic neurons of the raphe nuclei located within the brain stem. The concentration of serotonin in the brain, accounts only for 2% of the total amount of 5-HT in the organism. In spite of this small quantity, serotonin produced in the raphe nuclei plays a key role in the regulation of feeding behavior. Thus, the depletion of 5-HT in rat brain through the intracerebroventricular injection of the neurotoxin 5,7-dihydroxytryptamine or *p*-chlorophenylalanine, an inhibitor of tryptophan hydroxylase, the first and rate-limiting enzyme of serotonin synthesis, leads to hyperphagia and body weight gain.[5,6] In contrast, an increase in the synaptic levels of serotonin as induced by 5-HT uptake inhibitors such as sertraline, fenfluramine and sibutramine, reduces food intake and body weight.[7–10]

Serotonin and the regulation of energy homeostasis

The arcuate nucleus (ARC) of the hypothalamus is the main integrative centre of the metabolic signals produced in the periphery in response to food intake. The ARC is constituted of two populations of neurons. One contains agouti-related protein (AgRP) and neuropeptide Y and the other co-expresses cocaine and amphetamine regulated transcript (CART) and pro-opiomelanocortin. The activation of the former neuronal population by circulating signals enhances food intake whereas the activation of the latter reduces food ingestion.[11,12] These two neuronal groups are directly innervated by serotonergic neurons arising from the dorsal and median raphe nuclei.[13] The activation of the serotonergic neurons leads to the hyperpolarization of NPY/AgRP neurons and to the depolarization of POMC/CART neurons. The combined action of 5-HT in these two groups of neurons produces hypophagia.[1,14] The inhibitory effects of serotonin on NPY/AgRP neurons are mediated by 5-HT2C receptors whereas its stimulatory action on POMC/CART neurons is the result of the specific stimulation of 5-HT1B receptors. The administration of fenfluramine in the nucleus tractus solitary (NTS), also induces the activation of a group of neurons involved in the regulation of food intake.[15–17] For instance, it has been shown that decerebrated rats in which the direct neuronal connexion between the hindbrain and the forebrain has been disrupted exhibit a reduction in food intake in response to the administration of fenfluramine or *m*-chlorophenylpiperazine (mCPP).[18,19] The hypophagia produced by the systemic administration of mCPP is blocked by the injection at the level of the fourth ventricle of a 5-HT2A/2C receptor agonist.[19] Moreover, the analysis of the neuronal activity of the NTS during the phase of feeding using the expression of c-Fos as indicator of cell activity showed that the catecholaminergic neurons of this nucleus are activated in response to the administration of mCPP into the fourth ventricle.[20] Altogether, these observations sustain the idea that the anorexic effects of serotonin are also the result of its direct action in the brain stem.

Several lines of evidence indicate that the anorexic effects of serotonin are associated with its capacity to interact with the melanocortin system which is a fundamental component of centrally regulated energy balance. The melanocortins are a group of small protein hormones derived by post-translational cleavage of the pro-opiomelanocortin gene product. This homeostatic circuit is composed of cells expressing the neuropeptide precursor pro-opiomelanocortin, that after cleavage to α-melanocyte stimulating hormone activates melanocortin-4 receptors (MC4R) expressed on secondary neuron populations located in the paraventricular nucleus and other hypothalamic nuclei. The stimulation of MC4R reduces food intake (see Garfield *et al.*[21] for review), and the genetic inactivation of MC4R induces hyperphagia and increased body weight.[22,23] Interestingly, the hypophagic effect of fenfluramine is suppressed in the agouti mouse.

Interestingly, the yellow agouti mice (A^{vy}), a mutant animal characterized by the constitutive ectopic expression of the agouti gene,[24] exhibit reduced hypophagic effects in response to the administration of fenfluramine and selective MCR4 and MCR3 antagonist.[25] These latter observations indicate that the melanocortin system plays a critical role in the anorexigenic effects of serotonin.

The anorexigenic effects of serotonin are also related to its action on the corticotrophin-releasing neurons located within the paraventricular nucleus of the hypothalamus. These neurons are directly innervated by serotonergic neurons[26] and their stimulation inhibits food intake and reduces body weight. Moreover, the acute administration of fenfluramine,[27] or of selective 5-HT receptor agonists,[26] increases the expression of corticotrophin-releasing hormone (CRH) and reduces food intake. These latter observations are also in line with the idea that corticotrophin-releasing neurons mediate the anorexigenic effects of 5-HT. The micro-injection of serotonin within the paraventricular nucleus reduces food intake by decreasing meal size and slowing the rate of eatin.[28,29] These effects mirror the action of serotonin at the level of the arcuate nucleus.

Also, in terms of action on the homeostatic control, serotonin appears to interact synergistically with an anorexic peripheral agent cholecystokinin. The effect of CCK satiety signaling in the short term seems to depend on the serotonergic system. The CCK is released by the small intestine during feeding and acts to promote satiety and end of feeding.[30] Treatment with CCK-8 increases plasma levels of CCK inhibits food intake;[31] on the other hand, treatment with CCK

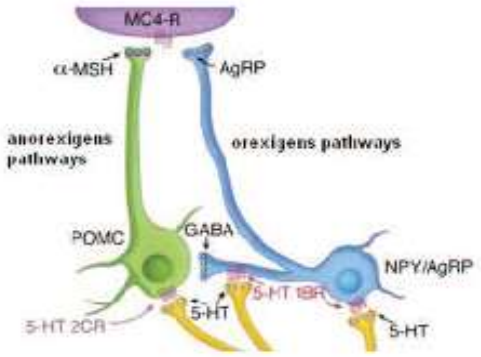

Figure 2 Schematic diagram of proposed 5-HT sites of action' on melanocortin pathways. 5-HT hyperpolarizes and inhibits AgRP neurons and decreases an inhibitory drive onto POMC cells by activation of 5-HT1BRs. 5-HT also activates POMC neurons via activation of 5-HT2CRs. Modified from data of Heisler *et al.*[16]

receptor antagonist reverses this effect and stimulates hunger.[30] The first evidence of the connection mechanisms of satiety controlled by CCK and serotonin was described by Stallone *et al.*[32] who noted that metergoline, a 5-HT antagonist, mitigated the reduction in food intake produced by CCK-8. The CCK appears to stimulate neurons in the dorsal raphe nucleus by acting on the CCK-A receptor.[33] The effect of fenfluramine anorexia was blocked by devazepine, a CCK-A receptor antagonist.[34] Studies have shown that 5-HT3 receptors mediate the enhancing effect of CCK satiety.[35,36] These receptors are found in high density in the NTS, area postrema and dorsal motor nucleus of the vagus.[37,38] These regions represent sites of regulation of food intake in the short term.[39] However, the anorexic effect of fluoxetine, an inhibitor of serotonin re-uptake, was not altered by devazepine[40] indicating that any increased activity of serotonin is mediated by CCK-dependent mechanisms.

Serotonin and non-homeostatic control

Recent studies that investigate the role of serotonin on the motivational aspects of food reward indicate the nucleus accumbens is an important region involved in reward system.[41,42] Serotonergic receptor stimulation in the nucleus accumbens induced various actions on the ingestion of palatable food. Thus, injection of 5-

HT1/7 agonist in the medial portion of the accumbens nucleus promoted reduction in food intake while the 5-HT6 agonist (AMD 386088) promoted an increase.[42] Another study showed that stimulation of 5-HT4 receptor in the nucleus accumbens reduces food intake.[43] Serotonin seems to play a role in the non-homeostatic control of energy balance. This action may depend on the type of receptor stimulated in the nucleus accumbens, and also in other regions. Few studies explore the role of the serotonergic system, stimulating the interest for the development of studies designed to clarify the morphological basis of these mechanisms.

Anatomical structures involved in the control of feeding behavior

The serotonergic system is formed at the beginning of the development of the CNS. The greatest activity in the formation of the serotonergic axonal terminals is in the prosencephalon areas, including the cortex and hippocampus.[44] This occurs in the early prenatal period. Rats have a ratio of one serotonergic neuron for each million neurons. Despite this low ratio, the serotonergic neurons reach all parts of the CNS.[45,46]

The structure, localization, and interaction of the serotonergic system with other systems of neurotransmitters make it a logical participant in certain distinct functions of the nervous system. The anatomical organization of the serotonergic system is similar to that found in other vertebrates.[47] The main source of serotonin in the CNS is in the neurons of the raphe nuclei.[48–51] The serotonergic neurons in raphe nuclei of the CNS trunk encompass the most extensive and complex anatomical/neurochemical system of the CNS in mammals.[52] From the raphe nuclei, the serotonergic neurons send descending projections to the spine and ascending projections to the prosencephalon structures, beyond local reciprocal circuits (Fig. 3).[49,53] The ascending projections reach practically all of the hypothalamic zones.[53] The hypothalamus is one of the structures involved in maintaining homeostasis. It regulates feeding behavior and it controls corporal weight,[54–57] mainly for its connections with neural mechanisms that control appetite.[55–57] The hypothalamic nuclei to dorsomedial (DMH), arcuate, paraventricular (PVH) hypothalamus and lateral hypothalamic area (LH) have a direct relationship with the control of the feeding behavior.[58] Studies have identified in hypothalamus, the place of production, release and area of performance of some molecules that stimulate or inhibit food intake.[59–61] During pre- or postnatal development, the hypothalamus can suffer insult and

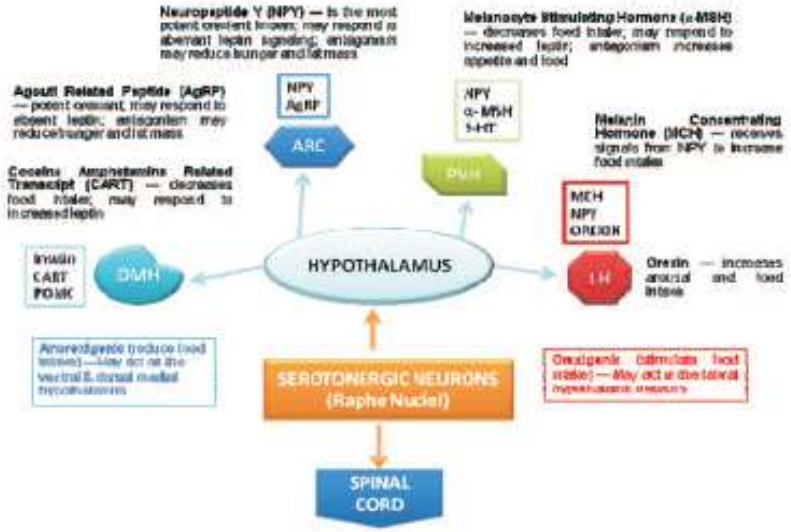

Figure 3 Schematic drawing of the ascending serotonergic that reach the hypothalamic nuclei, pathways involved in the regulation of appetite and satiety. MSH/CART-producing neurons in α-stream and binds to receptors on NPY/AGRP and the hypothalamic arcuate nucleus to orchestrate a series of responses mediated by downstream centers including the paraventricular nucleus (PVN) to control thyroid hormone secretion, feeding behavior, and energy conservation, and by lateral hypothalamic (LH) orexin- and MCH-producing neurons to control arousal responses and feeding behavior

modify its complex involved net in the control of the feeding behavior resulting in structural and functional alterations in adult life.[62,63]

The hypothalamic region is also associated with the regulation of vital functions such as temperature control, biological rhythms, and sexual behavior.[64] The hypothalamus is formed from more than 40 histologically distinct nuclei.[64] The hypothalamic nuclei involved in energy regulation are the following: arcuate, ventromedial, dorsomedial to paraventricular, and the area of the hypothalamolateral region.[65,66] These nuclei form a complex network that regulates energy level. Numerous neuromodulators are also located in these areas. These include the classic neurotransmitters serotonin, catecholamines, and α-aminobutyric acid.[67] Studies in rats indicate an increase in the release of serotonin in the ventromedial nucleus of the hypothalamus related to signs of satiety.[68] The neurons located in the hypothalamic regions also produce chemical messengers that are related to the stimulation or the inhibition of feeding behavior.[69,70] These messengers trigger the expression and secretion of the orexigenic and anorexigenic neuropeptides that regulate food intake and energy expenditure. Most notable among these neuropeptides are the orexigens, NPY and AgRP and the anorexigens, POMC/α-MSH (α-melanocortin-stimulating protein) and CART.[71,72] Thus, there exists an interaction among the central and peripheral signals of satiety. The chemical signals in the neural areas, mentioned above, interact with the peripheral signals insulin, leptin, and ghrelin. Satiety is thus influenced by this interplay of chemical messengers.[73,74]

In the arcuate nucleus of the hypothalamus, an abundant population of neurons exist that co-synthesize NPY and AgRP, both powerful stimulators of food intake.[74–77] Neuropeptide Y and POMC possess, respectively, potent orexigenic and anorexigenic properties and their hypothalamic levels reflect the body's nutritional status with NPY mRNA and NPY release increasing with fasting and decreasing after refeeding.[78–80] Conversely, the expression levels of POMC decrease with fasting and increase with feeding.[80,81] Animals submitted to protein restriction during the perinatal period develop hypophagia in adult life. This is characterized by a delay in the development of satiety and an increase in the size of the meals consumed.[82] In this study, protein restriction promoted an increase in the expression of the orexigenic AgRP, NPY, and a reduction in the hypothalamic levels of the POMC anorexigenic peptide.[82] The protein restriction also caused an increase in abdominal fat and an increase in the levels of the fatty acid triglyceride in the serum of these animals.[82]

The paraventricular nucleus contains multiple neural terminals with neurotransmitters that influence appetite, including NPY, the precursor of the α-MSH, 5-HT, galanin, noradrenalin and an opioid peptide.[83] The paraventricular nucleus is particularly sensitive to the effects of neurotransmitters on feeding and energy expenditure.[83] The ventromedial nucleus affects the satiety mechanism and receives projections from the dorsal raphe nucleus.[84] The dorsomedial nucleus is involved in the beginning and maintenance of food intake.[85] The lateral hypothalamus includes one definite subpopulation of neurons that express orexigenic hormones and melanin-concentrating hormone (MCH), both peptides involved in controlling food intake.[83]

Another nucleus involved in feeding behavior is the nucleus accumbens. It is a cerebral region which is an essential interface between motivation and action.[86] The nucleus accumbens receives a convergence of information from the encephalic regions with the data coming from the amygdala, hippocampus, thalamus, and prefrontal cortex. This information includes emotional learning, memory and cognitive input.[87] With its great network of inputs and neural outputs, the nucleus accumbens functions as a connection between the cortical, hypothalamic circuits, and the part of the brain involved with feeding behavior.[88]

The anatomical organization of these afferent and efferent connections of the nucleus accumbens suggests that it is ideally located to translate the input received into activity leading to eating.[89] The nucleus accumbens integrates received information with emotional and cognitive processes. It also integrates this information with the hypothalamic connections mediating energy expenditure.[88] The nucleus accumbens is also important with respect to the hedonic characteristics of foods that influence the control of appetite.[87]

Another area involved with information relevant to satiety is the nucleus tractus solitarius in the brain. The nucleus tractus solitarius is an area of particular interest because the majority of the gustatory and gastrointestinal neural pathways have their synapses in this region. The information then proceeds from this nucleus to the vagal nerve. It transmits signals related to the gastrointestinal tract, such as the degree of gastric distension, the levels of glucose in the intestinal veins, and the kinds of nutrients in the intestine.[90]

Cholecystokinin plays a role in the ending of a meal. It sends its signals via the NTS using the receiver CCK-

A.[91,92] Some neurons of the NTS are receptive to glucose, some express pro-opiomelanocortin, some are receivers of leptin, and some are receptive to melanocortina-4.[91,92] The nucleus tractus solitarius sends dense projections to the lateral hypothalamus and to the saw parabrachial nucleus. Both of these structures can be particularly important in the function of the orexigenic neurons. They are also involved in the actions of neurons sensitive to glucose levels.[68]

These connections form a series of complex pathways by which the amount of food intake is regulated.[93] This includes signals that are integrated in the hypothalamic centers. For example, stimulation of the vagal nerve produces activity in the NTS similar to the electric stimulation of the paraventricular nucleus hypothalamus.[93]

Feeding behavior and serotonergic receptors

In the most recent studies of feeding behavior, the main focus has been on the types and subtypes of serotonergic receptors that regulate feeding behavior.[20,68,94,95] Serotonin exerts its influence by means of interactions with a variety of receptors.[96] On the basis of structural and operational characteristics, the 5-HT receptors have been divided into seven distinct classes, 5HT1 through 5HT7. Additionally, 16 subtypes of this receptor have been identified.[97,98] The receptors 5-HT1A (pre-synaptic),[99] 5-HT1B,[100] 5HT2A[101] and 5-HT2C,[102,103] have been investigated for their roles in serotonergic actions on food intake. Their interactions with other neurotransmission systems are not yet well understood.[103,104]

There is evidence that the receptor 5-HT1B plays a key role in the central modulation of food intake.[105] The receptor 5-HT1B predominantly seems to be pre-synaptic in its localization.[106] This receptor is widely expressed in all the regions of the CNS, including gyrus of cingulo, entorrinal cortex, hippocampus, striatum, sub-thalamus nucleus, raphe nuclei, Purkinje cells in the cerebellum and spinal cord.[106] Raised concentrations of this receiver have been found in areas important in the control of food intake, such as in the paraventricular and ventromedial hypothalamic nuclei.[100] These nuclei receive dense neural networks from serotonergic projections of the raphe nuclei.[107]

The levels of 5-HT in the hypothalamus play important roles in the selection of macronutrients, the size of the meal, and the body weight.[68,107] Low doses of agonists of 5-HT affect feeding when injected into the hypothalamus.[68] The 5-HT1B and 5-HT2C receptor subtypes are important mediators of serotonin-related hypophagia but it is not completely known how they interact to support this serotonergic function. They have been recently shown to, respectively, inhibit NPY/Agouti-related peptide neurons and stimulate pro-opiomelanocortin neurons in the arcuate nucleus (Fig. 2).[14] 5-HT1B has been shown to be hyper-responsive to agonist stimulation in 5-HT2C knockout mice, demonstrating that these serotonergic receptors are able to accomplish compensatory adaptations.[108]

The receptor 5HT1B regulates mechanisms that affect the process by which satiety is achieved.[109] The administration of the selective agonist 5-HT1B (CP-94,253), reduces ingestion and modifies the behavior relative to achieving satiety.[108,110,111] The changes noted are more complex than a simple reduction in the amount of pellets and sucrose solution consumed by the experimental animal.[112]

Administration of various mixtures of agonists for the 5-HT receptor has been useful in the analysis of feeding behavior. A hypophagic effect was observed with the administration of the agonist 5-HT2A/2C, DOI, and 5-HT1B/2C, *m*-CPP and 1-(*m*-trifluoromethylphenyl)-piperazine (TFMPP).[113,114] These promote hypophagia by causing a more rapid achievement of satiety.[110] Pre-treatment with DOI in the paraventricular nucleus of hypothalamus inhibits the food intake stimulated by the NPY.[115] The *m*-CPP, the CP-94,253 and the BW 723C86 induce hypophagia after injection of relatively low doses.[116] On the other hand, injection of similar doses of *m*-CPP or CP-94,253 in the fourth-intracerebro-ventricular (4th-icv) also induced hypophagia.[19] These findings suggest 5-HT1B/2C in the brain is also involved with the hypophagic effect of the agonist of the receptor. When injected in the parabrachial nucleus of the brain, CP-94,253 was 50 times more powerful in the reduction of food intake when compared with local injection into the hypothalamus.[117] It is important to identify the parabrachial nucleus 5-HT1B and 5-HT2C prior to treatment.[117] The receptors located in the brain are necessary for the hypophagic effect of *m*-CPP and the CP-94,253.[18,19]

The receptor 5-HT2C is detected exclusively in the central nervous system and plays a role in various physiological and behavioral processes. In human brain slices, *in situ* hybridization reveals significant mRNA expression in both the hypothalamic paraventricular and ventromedial nuclei, and labeling in the amygdala is also observed.[118] Increased anxiety,[25] hyperlocomotion,[119] and adult-onset hyperphagia and obesity[120] have been reported with receptor 5-HT2C null mice, confirming the importance of receptor 5-HT2C in the regulation of

emotion, locomotion, and appetite and metabolic rate control. The receptor 5-HT2C is post-synaptic for the entire CNS.[121–123] Its involvement in the regulation of appetite initially was suggested by the anorexegenic effect of the non-specific 5-HT2C and meta-chlorophenylpiperazine. The receptor 5-HT2C seems to modulate feeding behavior by promoting reduction in the size of the meal. This effect can be verified by administration of its agonist Ro 60-0175.[124] It was also observed that the corporal weight of rats was reduced after continuous administration of Ro 60-0175 for 14 days. This was attributed to a reduction in food intake.[102] Elements that promote reduction in food intake, such as leptin and fenfluramine, also seem to act through the 5-HT2C receptor.[125–128] Studies have suggested the agonists of the 5-HT2C receptor are important instruments in the treatment of obesity.[103] These agonists do not simply reduce food intake in rats or mice on a short-term basis. These agents can also reduce the body weight of the animal when administered on a longer-term basis to treat obesity.[103] Study demonstrates lorcaserin to be a potent and selective 5-HT2C agonist, with an 18- and 104-fold functional selectivity over 5-HT2A and 5-HT2B receptors, respectively.[129] Lorcaserin shows rapid oral absorption and efficacy to decrease food intake and body weight gain in the rat. Administration of lorcaserin to the rat for 28 days resulted in transiently reduced food intake and sustained reductions in body weight gain compared with vehicle.[129] These data show lorcaserin to have potential as a safe and efficacious treatment for obesity.[129]

Pharmacological agents that increase the activity of 5-HT in the CNS cause inhibition of food intake and promote loss of weight. Studies using selective and non-selective antagonistic agents using serotonergic receptors suggest the subtypes of the receptor 5-HT1B and 5-HT2C mediate the inhibiting action of 5-HT in feeding behavior.[130,131]

Conclusions

The mechanisms that control feeding behavior are strongly influenced by neurotransmitters, especially serotonin. Pharmacological and genetic studies have consistently demonstrated the fundamentality of the serotonergic system in the regulation of appetite and feeding behavior. Moreover, they have highlighted the involvement of specific serotonin receptor subtypes in mediating these effects. The participation of 5-HT2C and 5-HT1B receptors are widely accepted notion in the anorectic response in several paradigms.[132–135] Involvement of serotonergic transmission demonstrated by treatment with serotonin releasers has also confirmed that systems modulators influence feeding behavior.[133] The neurotransmitter serotonin, its receptor 5-HT1B subtypes, and its 5-HT2C sub-types are of critical importance in the mechanisms that control feeding behavior. Knowledge of the actions of these agents, their receptors, and their susceptibility to inhibition is critical to understanding feeding behavior. Further characterization of the actions of 5-HT1B and 5-HT2C receptor subtypes in controlling feeding behavior is necessary. Pharmacological studies using more selective drugs are needed. It is most probable that multiple neurotransmitters, anatomical structures, and other mechanisms influence hunger and satiety. The pleasurable aspects of eating as well as the basic need for energy homeostasis also play a role. Together they determine hunger, satiety, and feeding behavior. It is apparent that the serotonergic system offers therapeutic potential in the unremitting battle against obesity.

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4.2 PERINATAL PROTEIN RESTRICTION INDUCES ALTERATION OF CONTROL OF SATIETY BY 5-HT1B/1D RECEPTOR AGONIST AND ALTERATIONS OF ALIMENTARY PREFERENCE IN ADULT RATS

[1]Carolina Peixoto Magalhães; [2]Renata Cristinny de Farias Campina; [3]Larissa Cavalcanti do Amaral Almeida; [4]Priscilla Alves Santos; [5]Amanda Alves Marcelino; [1]Lisiane dos Santos Oliveira; [6]Sandra Lopes de Souza; [7]Raul Manhães de Castro

[1]Professora de Anatomia do Centro Acadêmico de Vitória – CAV/UFPE – Brazil

[2]Doutoranda da Pós-graduação em Neuropsiquiatria da Universidade Federal de Pernambuco– Brazil;

[3]Graduanda em fisioterapia pela Universidade Federal de Pernambuco– Brazil

[4]Graduanda em Nutrição pela Universidade Federal de Pernambuco– Brazil

[5]Mestranda da Pós-graduação em Nutrição pela Universidade Federal de Pernambuco– Brazil

[6]Professora de Anatomia da Universidade Federal de Pernambuco – Brazil

[7]Professor do Departamento de Nutrição da Universidade Federal de Pernambuco – Brazil

Corresponding author:

Carolina Peixoto Magalhães

Centro Acadêmico de Vitória – CAV/UFPE

Rua Alto do Reservatório s/n – Bela Vista – Vitória de Santo Antão – PE

CEP: 55608-680

Fone: 55-81-9245-9274

e-mail: peixotocarolted@hotmail.com

1. Introdution

The developing brain is vulnerable to environmental insults, and this seems to be true in all mammalian species [1]. According to the hypothesis of a critical period of development, formulated by Dobbing [2], perinatal malnutrition causing irreversible effects on the developing rat brain. Among the neurotransmitter systems that may be affected by early nutritional deficit is the serotonergic system [3]. This system becomes important neurotransmission by serotonin (5-hydroxytryptamine or 5-HT) in brain development and modulation of axonal outgrowth, neural differentiation and synaptogenesis [4-6]. The functions of serotonin in the central nervous system are numerous, and involve control of appetite, sleep, memory and learning, temperature regulation, mood and endocrine regulation [7-9].

The discovery of multiple serotonin receptors in the central nervous system led to the improvement of anorectic drugs selectively acting as agonists at various serotonin receptor subtypes. We identified at least five subtypes of receptors involved in feeding behavior, such as 5-HT1A, 5-HT1B, 5-HT2, 5-H1D [10-12] and 5-HT3 [13]. Studies using serotonin receptor antagonists show that the subtypes 5-HT1B and 5-HT2C receptors are involved in the inhibitory action of serotonin on food intake [14-15]. The ability of serotonin in regulating satiety and macronutrient selection provides the basis for the pharmacological treatment of obesity and eating disorders [16].

Undernourished in early life show deficits in satiety and exhibit a strong preference for fatty foods [17-18]. Another factor that influences the power consumption and adiposity is the quality and quantity of available food. Studies with laboratory animals indicate that the hyperphagia and obesity can be induced by cafeteria diet, high in fat and sugar (eg, biscuits, milk, cheese) [19]. The preference for an excessive intake of fat and high sugar foods is often attributed to its palatability, ie, the hedonic response to taste [20-21]. The fat content of foods has an important impact on the palatability, energy density and metabolic actions post-feeding behavior [22]. Rats generally prefer to eat more foods high in fat compared to foods low in fat [23-24].

The feeding behavior of animals is an adaptive response, arising from the demands of the internal environment and is modulated by limitations imposed by the external environment [25]. Satiety is an event subsequent to food intake that inhibits hunger and keeps this effect during a certain period of time [26]. Animals deprived of food for a prior period exhibit a characteristic sequence of behavioral changes [27]. In the rat, the cessation of feeding is followed by a short period of exploratory behavior and cleaning, which is replaced by rest and / or relaxation [27]. This sequence of activities is called the behavioral satiety sequence (BSS) [28]. This characteristic behavior of the mouse allows us to use the behavioral satiety sequence as a tool to test the action of drugs or other manipulations may act on the onset of satiety [29-30].

In the present study investigated the effects of perinatal malnutrition on the preference of the animals on the intake of foods rich in fat or sucrose solution and the effects of pharmacological manipulation with 5-HT1B/1D receptor agonist on the parameters of behavioral satiety sequence.

2. Materials and Methods

2.1 Animals

We used male Wistar rats aged between zero and 400 days of the colony from the animal facilities of the Department of Nutrition, Federal University of Pernambuco. The nulliparous females weighing between 250 and 300g were kept in light/dark cycle of 12 hours reversed (lights on at 18.00), 22 ± 1 º C for 15 days for adaptation, with free access to water and standard diet Labina (Purina ®, Brazil S / A). After the adjustment period, females were mated at a ratio of two females to one male. Pregnancy was detected by visualization of sperm in vaginal smear. Being considered the 1st day of pregnancy. At this time, the dams were separated and were offered with low-protein diet (LP) and a normal protein diet (C) to compose the experimental groups. Birds were fed the 1st pregnancy until day 21 of lactation. The normal protein diet was in accordance with recommendations of the American Institute of Nutrition, AIN [31] (Table 01).

One day after birth, male offspring of the same experimental group were weighed, randomized and separated to form a litter with 8 puppies. When the amount of male offspring was not enough, the litter was supplemented with females. As inclusion criteria, the pups from mothers who consumed LP weighed between 5.0 to 6.5 g and fed normal protein diet weighed between 6.0 to 7.5 g. On day 21 (P21) of life weaning occurred and all animals, regardless of the experimental group began receiving the normal protein diet until they complete 50 days of life (P50). After this period the animals were kept in individual cages and were fed a standard animal house (normal protein with 23.0% crude protein) until they complete 400 days (P400). All experiments were performed in accordance with the recommendations of the Brazilian Committee of Animal Experiments - COBEA and approved by the ethics committee on animal experimentation of the Center for Biological Sciences, Federal University of Pernambuco (Case No. 23076.025905/2008-70).

Table 1 - Chemical composition of experimental diets (g/kg) and the percentage of macronutrients in relation to total energy value.

Ingredients	low-protein 8%	control 17%	palatable diet
g %	100,00	100,0	100,00
Casein	8,10	17,30	22,36
Sucrose	75,10	65,90	48,50
Fat	7,00	7,00	15,33
Cellulose	5,00	5,00	2,72
* Vitamin mix	1,00	1,00	1,00
† Mineral mixture	3,50	3,50	3,50
Metionin	0,30	0,30	0,30
Animl protein	-	-	16,66
% Kcal	**362,48**	**363,44**	**421,41**

The high fat diet was prepared at the Department of Nutrition, Federal University of Pernambuco, Brazil.* The vitamin mixture contained (milligrams per kilogram of diet): retinol 12, cholecalciferol 0.125, thiamine 40, riboflavin 30, pantothenic acid 140, pyridoxine 20, inositol 300, cyanocobalamin 0.1, menadione 80, nicotinic acid 200, choline 2720, folic acid 10, p-aminobenzoic acid 100, biotin 0.6.

† The mineral mixture contained (milligrams per kilogram of diet): CaHPO4 17 200, KCl 4000, NaCl 4000, MgO 420, MgSO4 2000, Fe2O2 120, FeSO4$7H2O 200, trace elements 400 ($MnSO4.H_2O$ 98, $CuSO4.5H_2O$ 20, $ZnSO4.7H_2O$ 80, $CoSO_4.7H_2O$ 0.16, KI 0.32, sufficient starch to bring to 40 g/kg of diet).

2.2 Body weight

The weight of each pup was recorded daily from 1 to 21 days postnatal (n = 32). After weaning, the body weight of each animal (n = 24) was measured at 30, 60, 90, 150, 180 and 400 days. All weights were measured in balance Mars ® AS 1000C, Grade II, 1000g capacity, lower division 0.01 g.

2.3 Food Preference

The rats were housed in individual cages. Each cage contained two identical bottles (polycarbonate Trough for 500 ml, rubber stopper and spout anti-acid stainless steel, straight). It was offered for 5 consecutive days, a bottle with sucrose solution at a concentration of 10% and the other filtered water bottle, both in volume of 500ml. Concomitantly, each animal was offered a portion of 80g per day of standard diet (SD) or a palatable diet (PD) (Table 1). Thus, all animals were simultaneously exposed to water, sucrose solution and one of the above diets.

From the diets offered to animals were formed the following experimental groups: control animals with access to standard diet (C/SD), control with access to palatable diet (C/PD), malnourished with access to standard diet (LP/SD) and malnourished with access to palatable diet (LP/PD). The contents of the bottles of water and sucrose solution were checked daily by measuring the amount rejected. The consumption of diets was verified by weighing the waste and then supplementing for the amount of 80g. Only the last three days were used for statistical analysis. As the first and second days considered as periods of animals to adapt to new diets.

2.4 Study of the Behavioral Satiety Sequence

When the animals were 400 days of age was conducted to study the behavioral satiety sequence. The analysis of the BSS was conducted for one hour as described by [32] and preceded by a four-hour food deprivation. Half an hour before the end of the period of food deprivation, an acute

dose 5-HT1B/1D receptor agonist (1 mg/kg, bw) dissolved in 0.9% NaCl [33] or saline solution (0.9%) were injected subcutaneously. From the injection of the solutions were graduates of the experimental groups: C/saline, C/5-HT1B/1D, LP/saline and LP/5-HT1B/1D. The solutions were prepared immediately before use. The dose 5-HT1B/1D receptor agonist was chosen to match those used in humans [33].

Completed the four-hour food deprivation was offered to the animals of groups 60g Labina to conduct the analysis of BSS by three trained experimenters. The observations of behaviors characteristic of behavioral satiety sequence were performed for 1 hour. After this period, the animals were weighed and food intake was quantified by the difference between the quota and the quota offered rejected.

The behavior observed in the BSS were classified as Eating (characterized by biting, gnawing or swallowing food prepared at the feeder), Grooming (characterized by licking the body, legs and genitalia) and Resting (characterized by inactivity of the animal, by its relaxed position with his head bent over the body, or the animal is resting on the bottom of the cage, lying on its side or on the belly). The duration of each behavior was quantified with digital timer and recorded in the appropriate protocol.

With the data obtained during analysis of the BSS were calculated other parameters called microstructure of feeding: food intake (g), Meal duration (min), Amount of food consumed (g)/meal duration (min), Total energy intake (Kcal total), Caloric intake per body weight (kcal/100g) and Caloric intake per minute (kcal/min).

2.6 Statistical Analysis

The experimental results were expressed as mean ± SEM. All data were analyzed using a demonstration program SigmaStat 2.03. The microstructural parameters of feeding were analyzed using the Student t test. Body weight and food intake were analyzed using Two-Way Analysis of Variance ANOVA and data were analyzed by BSS through a one-way (ANOVA) followed by

Bonferroni test for multiple comparisons between groups. Before using the ANOVA, data were subjected to analysis of variance and normality test, with 5% tolerance.

3. Results

3.1 Effects of perinatal malnutrition on body weight of rats

During the nursing phase, we observed a decrease in body weight of malnourished animals from the 3rd day of life (9.74 ± 1.29 g C, n = 32 vs 6.95 ± 0.64 g LP, n = 32, Fig 1), this reduction lasts until the animals reached 21 days. After this period, body weight was measured during three subsequent moments. In 30 (85.77 ± 10.99 g C, n = 20 LP vs 48.86 ± 5.44 g, n = 24, Fig 1), 60 (250.63 ± 21.50 g C, n = 15 vs 215 LP, 44 ± 17.38 g, n = 15, Fig 1) and 400 ° (496.22 ± 41.36g C, n = 16 vs. 456.19 ± 27.14 g LP, n = 18, Fig 1) day. We observe that the malnourished animals at all periods evaluated post-lactation weighed less than control rats, indicating that even with a process of nutritional rehabilitation there was no recovery of body weight deficits produced by early protein malnutrition (Figure 1).

3.2 Effects of perinatal malnutrition on food preference of rats

We evaluated the preference of the animals eating the sucrose solution at 10% or palatable diet. With respect to sucrose intake, control rats with access to standard diet ingest more sucrose than controls rats access to the PD (C/PD 21.45 ± 3.88 g, n = 10 vs C/PD 15.39 ± 1, 79g, n = 9, Fig 2A). In the comparison between the malnourished animal, we observed that malnourished with access to palatable diet ingest a smaller amount of sucrose solution, compared to the malnourished/PD (LP/PD 11.03 ± 3.08 g, n = 9 vs. LP/PD 27.45 ± 2.44 g, n = 10, Fig 2 A).

In the comparison between groups showed that the malnourished animals ingest more sucrose than controls, when both have access standard diet (LP/SD 27.45 ± 2.44 g, n = 10 vs SD/SD 21.45 ± 3, 88g, n = 10, Fig 2 A). When the animals were malnourished and controls ingesting palatable diet,

there was a decrease in sucrose intake by malnourished group compared with controls (SD/PD 15.39 ± 1.79 g, n = 9 vs LP/PD 11.03 ± 3.08 g, n = 9, Fig 2 A).

Regarding the preference standard or palatable diets was observed in intra-group comparison, which controls ingest a larger amount of palatable diet, which controls access to standard diet (SD/SD 3.23 ± 0.58, N = 8 vs. SD/PD 4.14 ± 0.36, N = 8, Fig 2 B). In the comparison between the preference of malnourished animals by standard or palatable diets, showed that malnourished prefer eating the palatable diet, compared to a standard diet (LP/SD 3.95 ± 0.70, N = 8 vs. LP/PD 4.70 ± 0.32, N = 8, Fig 2 B).

3.3 Effects of perinatal malnutrition on the behavioral satiety sequence

The behavioral satiety sequence was analyzed in four experimental groups. In Figure 3, it was observed that animals from the four groups studied were one of BSS typical behavior characterized by constant progression initiated by Eating followed by an active behavior of grooming before they reach the stage of resting. There was no disruption of the behavioral sequence in any of the groups.

Comparing the groups C/saline and C/5-HT1B/1D no changes of transition feeding-resting with maintenance of grooming (Figure 4). There was no significant difference between the total time of eating, grooming and resting between the C /saline and C/5-HT1B/1D (Table 3). There was no difference in the microstructural parameters of power among the groups (Table 6).

Assessing the effects of malnutrition on the BSS, it was observed that there was a delay in the transition between the eating-resting in the LP/saline, compared to C/saline (Figure 5). In the comparison between groups, there was an increase in the total time of eating ($p < 0.001$, Table 4), reduction in resting time of the LP/saline compared to C/saline ($p < 0.001$, Table 4). In the microstructural parameters of power between the groups, there was an increase in eating time and increased amount of food eaten, with no change in intake rate by the LP/saline (table 6).

Evaluating the effects of 5-HT1B/1D receptor agonist in malnourished animals, we observed a reduction in the transition between the eating-resting compared to LP/5-HT1B/1D and LP/saline (Figure 6). There was a reduction in the total time of eating and grooming group LP/5-HT1B/1D with increased rest time compared with group LP/saline ($p<0.01$, Table 5). As for the microstructural parameters of eating, reduction in eating time, with no change in food intake. However, there was an increase in intake rate by LP/5-HT1B/1D group (table 6).

Table 3 - Total time (min) spent in the behavioral satiety sequence in control animals that received a dose of saline solution or 5-HT1B/1D receptor agonist receptor

Grupos Experimentais	Alimentação	Limpeza	Descanso
Control Saline	81,47±6,64	51,15±8,24	130,89±13,76
Control 5-HT1B/1D	87,06±6,94	44,64±8,60	134,07±15,08

Data are expressed as mean ± SEM (n = 14). Two Way (ANOVA), *Bonferroni* post-test. * Indicates difference intra-group ($p <0.01$). E # indicates difference between groups ($P <0.001$).

Table 4 - Total time (min) spent in the behavioral satiety sequence in control animals that received doses of saline.

Grupos Experimentais	Alimentação	Limpeza	Descanso
Control Saline	81,47±6,64	51,15±8,24	130,89±13,76
LP Salina	112,27±5,93#	55,01±7,37	85,94±12,74#

Data are expressed as mean ± SEM (n = 14). Two Way (ANOVA), *Bonferroni* post-test. # indicates difference between groups ($P <0.001$).

Table 5 - Total time (min) spent in the behavioral satiety sequence in malnourished animals that received a dose of saline solution or 5-HT1B/1D receptor agonist.

Grupos Experimentais	Alimentação	Limpeza	Descanso
LP Saline	112,27±5,93	55,01±7,37	85,94±12,74
LP 5-HT1B/1D	78,05±6,93*	41,86±8,60*	133,69±14,38*

Data are expressed as mean ± SEM (n = 14). Two Way (ANOVA), *Bonferroni* post-test. * Indicates difference intra-group ($p < 0.01$).

Table 6 - Parameters microstructure of feeding during the BSS controls and malnourished animals that received acute dose of saline solution or 5-HT 1B/D receptor agonist to 400 days of life.

	Duração da alimentação (min)	Consumo Relativo (g)	Taxa alimentação (min)	Energia total (Kcal)	Kcal/100g p.c.	Kcal/min
Control/ Saline	17,17±0,94	1,13±0,10	0,070±0,0071	3,96±0,08	0,80±0,07	0,25±0,024
Control/ 5-HT1B/1D	18,00±1,12	1,36±0,14	0,071±0,0077	4,22±0,34	0,86±0,08	0,27±0,028
LP/saline	21,73±1,73$^{\#}$	1,48±0,09$^{\#}$	0,064±0,0031	5,08±0,33	1,12±0,75	0,23±0,010
LP 5-HT1B/1D	16,08±1,21*	1,29±0,09	0,079±0,0050*	4,82±0,34	1,06±0,08	0,26±0,015

Data are expressed as mean ± SEM (n = 14). Using Student's t-test. * indicates intra-group difference ($p < 0.05$). $^{\#}$ difference between groups ($p < 0.05$).

4. DISCUSSION

Concerning the offspring of females fed a diet low in protein, our findings corroborate the studies of Langley-Evans [34] and Lopez-de-Souza [35] demonstrating that the pups are born with similar weight and reduction in body weight of malnourished children is identified only from the third day of lactation. This growth retardation persisted until the body malnourished animals completing 400 days, despite the standard diet with food from the time of weaning. Cheral et al, [36] also demonstrated in our study, protein malnutrition during gestation and lactation prevents the pups to gain weight in adulthood causing retarded growth and physical maturation. C. Petry J, et al. [37]

showed an increase at 90 days of life of serum catecholamines in rats malnourished during gestation and lactation. These data support the hypothesis that the plasma catecholamine levels lead to a decrease in body fat mass in animals that suffered early protein restriction.

Our study evaluated the preference of malnourished animals by a diet high in fat or high in simple carbohydrate (sucrose 10%). We realized that the control and malnourished animals prefer high-fat diet that may partly reflect the greater acceptability of fat compared with the standard diet. However, there was a tendency to eat more of the malnourished diet rich in fat than the controls, but this finding was not significant. Our data agree with Sclafani and Warwick [22, 24], rats prefer to eat foods high in fat compared to standard diet. The fat content of foods has a major impact on palatability, thermogenesis, energy density and metabolic actions post-feeding behavior [24, 38-39]. The preference for high fat diet observed in our study may be related to post-ingestive action of diets rich in fat that not only promote excess, but also influence the food preferences through a process of nutrient-conditioned flavor-caloric value.

Saccharin is a sweetener traditionally considered highly palatable not only induces hyperphagia, but also serves as an effective reinforcement of operant behavior [40]. The intake of sugars is influenced by two types of brain systems: those associated with the regulation of feeding and energy homeostasis and those associated with reward [41-42]. In our study, there was an increase in consumption of sucrose solution by malnourished animals when they were eating a diet with low fat content compared to controls. In addition to the systems of homeostatic control of appetite, the processes that govern the taste and odor, and therefore the palatability of food can also be an object programming [43]. A study that confirms our own, demonstrated that rats with access to a solution of sugar (sucrose or glucose), in addition to standard diet and water, typically consume 60% of total calories as sugar and increasing the energy consumption of about 20 % [19]. This increase may have occurred due to changes in factors related to the reward system, which can be changed according to the perinatal malnutrition.

According to David Benton [44], there is a "seesaw" between sugar and fat, where the ingestion of a diet low in fat causes an increase in intake of a diet high in sugar and vice versa. In our study, this preference was identified when we offer malnourished and control groups, diets with high fat diet and another group with low fat, along with a sucrose solution. All animals, regardless of the experimental group who were eating a diet low in fat, drank a higher amount of sucrose solution. We observed that rats have a preference for the solution which contains more calories and this reflects the nature of the rewards of post-ingestive effect of the nutrient [45]. Thus, the rats invariably prefer to attach the flavor with more calories [46-48].

When we offer control and malnourished animals a diet high fat, while a sucrose solution identified a reduction in sucrose intake by malnourished animals. Sclafani A. [49] showed that post-ingestive action of nutrients can, in turn, have powerful effects on the preference and food intake. The rats generally prefer to eat foods high in fat compared to foods low in fat [23-24], probably due to the prolonged effect of satiety caused by fat. Malnutrition perinatal caused malnourished animals' preference for fat compared to sucrose. Thus, the reduction in sucrose intake found in our study may have occurred due to increased satiety caused by the power of fat compared to sucrose.

This study also evaluated the behavioral satiety sequence and the microstructural parameters of the power to determine whether early protein restriction alters the effect 5-HT1B/1D receptor agonist regarding the initiation and maintenance of power until reaching satiety in animal half age. The 5-HT1B/1D receptor agonist when applied acutely to control bodies does not cause changes in behavior of the total time of feeding, grooming and the rest of the BSS. These findings contrast the studies of Blundell & Halford [50], who observed by using a selective of 5-HT 1B receptor agonist, the CP-94, 253, a reduction in the total time feeding and an increased frequency of rest. These discrepant results may have occurred because of the selectivity of agonist and the dose used in our experiments. With respect to the microstructural parameters of feeding, our results corroborate the studies of Lee, MD, et al. [51], through which a dose-response study, noted that just after the dose of 2.5 mg / kg, the agonist CP-94, 253, there is a reduction in food intake.

In evaluating the effect of malnutrition on the parameters of the BSS, we found that perinatal malnutrition causes in middle-aged animals, a delayed onset of satiety, with increased food intake. This result corroborates the studies Orosco-Solis et al. [52] also observed that hyperphagia in offspring of animals with 35 days of life that were undernourished in perinatal life. Malnutrition causes a reduction in the number of neurons with NPY [53], suggesting that changes in dietary behavior associated with perinatal malnutrition occur because of an anatomical disruption in hypothalamic nuclei regulating food intake [53-54]. Orozco-solis [52] also observed in animal studies planned, changes in levels of hypothalamic orexigenic and anorexigenic peptides.

In the malnourished bodies 5-HT1B/1D receptor agonist resulted in a return point of satiety to the levels of controls. Malnourished animals reduce feeding time, but does not change the amount of food ingested. There is evidence that serotonin is involved in modulating the sensation of hunger / satiety [55-56]. Our results corroborate the study of our group that demonstrated through the use of the agonist CP-94,253 a 40% reduction in food intake in rats and controls under the same conditions and dose of this agonist decreased it by only 10% on food intake in malnourished rats [18]. Based on this line of evidence, we argue that malnutrition causes alterations in 5-HT1B receptors, leading to reduction in the hypophagic effect of serotonin. In malnourished animals middle-aged an increase in intake rate. Probably, so malnourished they could eat the same amount of food in a shorter time, it was necessary to increase the speed of digestion.

It is likely that the hypophagic effects of serotonergic drugs are dependent on the dose used, the selectivity of agonists and differential involvement of their receptors [57]. However, the precise role of serotonin receptors is not well understood, particularly its effect on organisms programmed by perinatal malnutrition.

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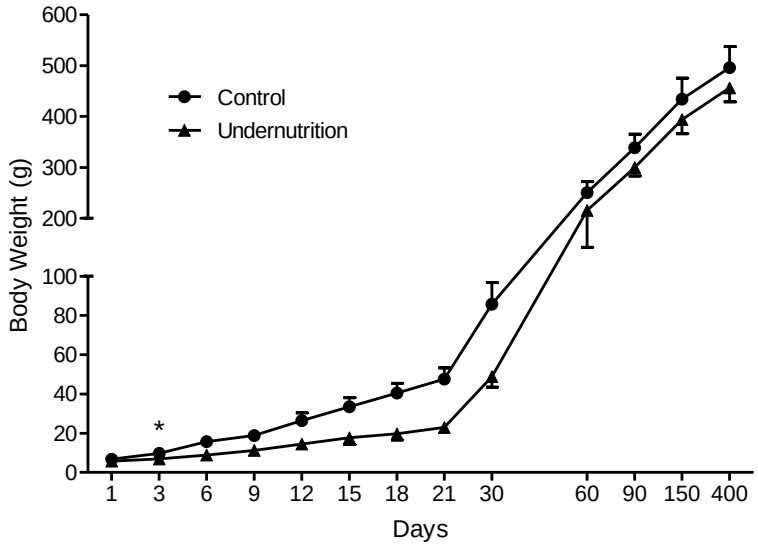

Figure 1 - Effect of nutritional manipulation during lactation (n = 16) and during adulthood on body weight (n = 12). Data are expressed as mean ± SEM. For body weight was performed two-way repeated measures (ANOVA). * indicates the beginning of the difference between groups, P <0.05.

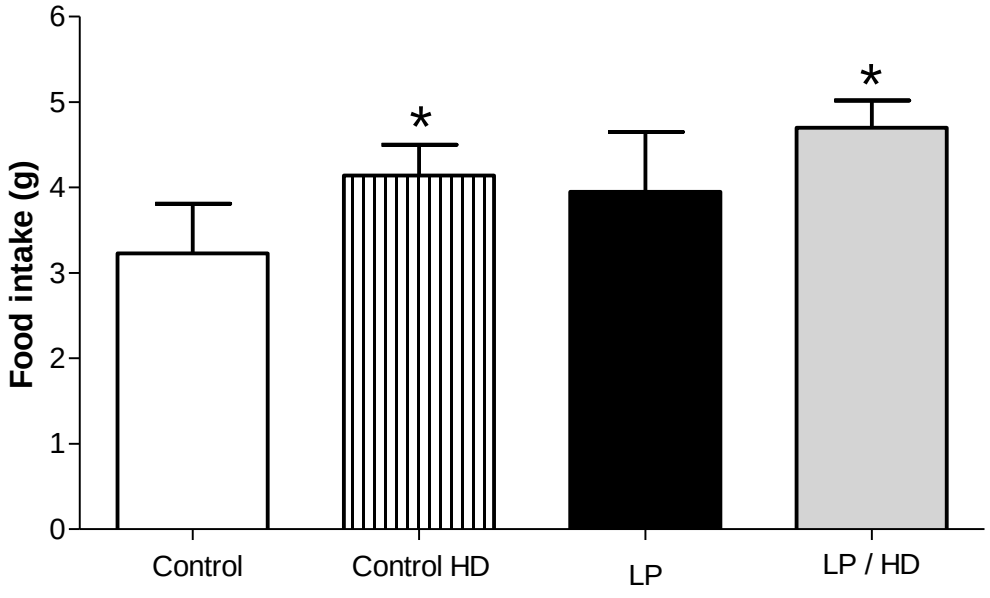

Figure 2 – Ingestion palatable diet in the range of three consecutive days of control (n = 9) and LP (n = 9) at 400 days. Data are expressed as mean±SEM. One-way ANOVA. * Indicates difference intra-group and # difference between groups; P <0.05.

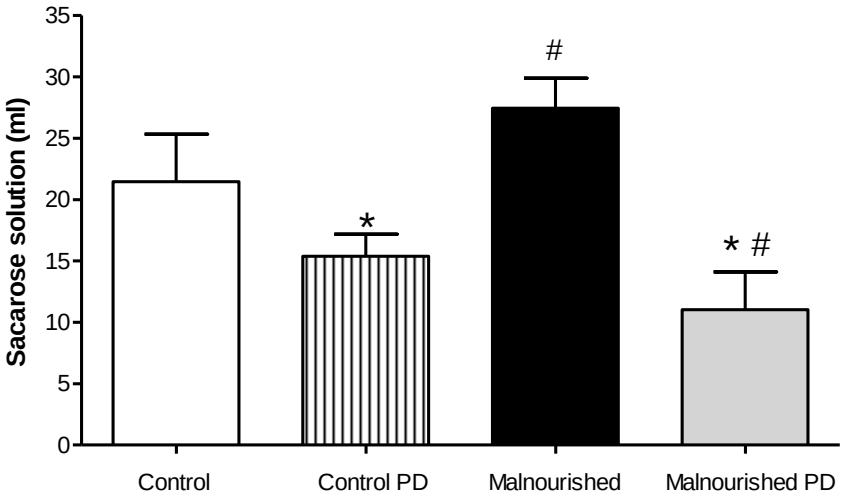

Figure 3– Ingestion of sucrose solution in the range of three consecutive days of control (n = 9) and LP (n = 9) at 400 days. Data are expressed as mean±SEM. One-way ANOVA. * Indicates difference intra-group and # difference between groups; P <0.05.

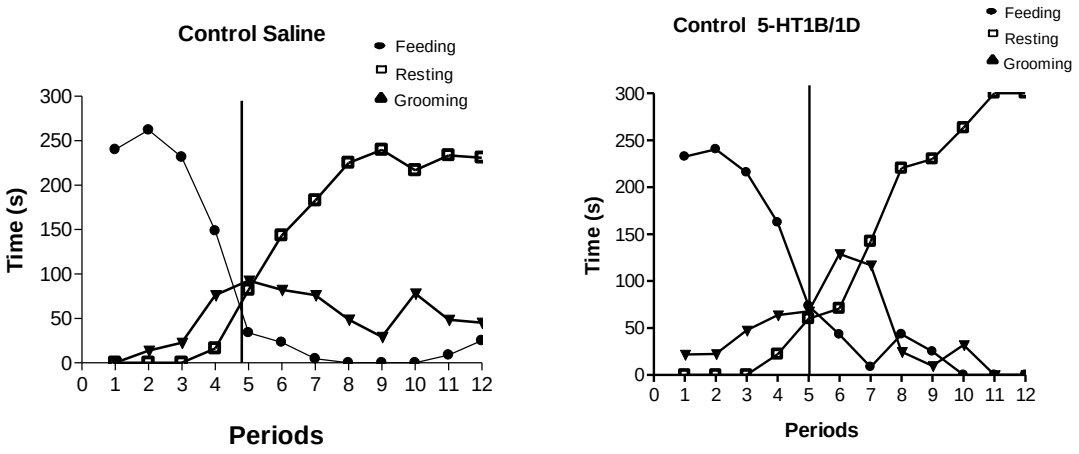

Figure 4 - Behavioral satiety sequence in control rats (n = 14) with 400 days of age treated acutely with saline (0.9% NaCl) or 5-HT1B/1D receptor agonist (1mg / kg) solution. The data from each behavioral category was expressed as a proportion of the total number of observations in the period. Two-way ANOVA followed by *Bonferroni t-test*, ($p < 0.05$).

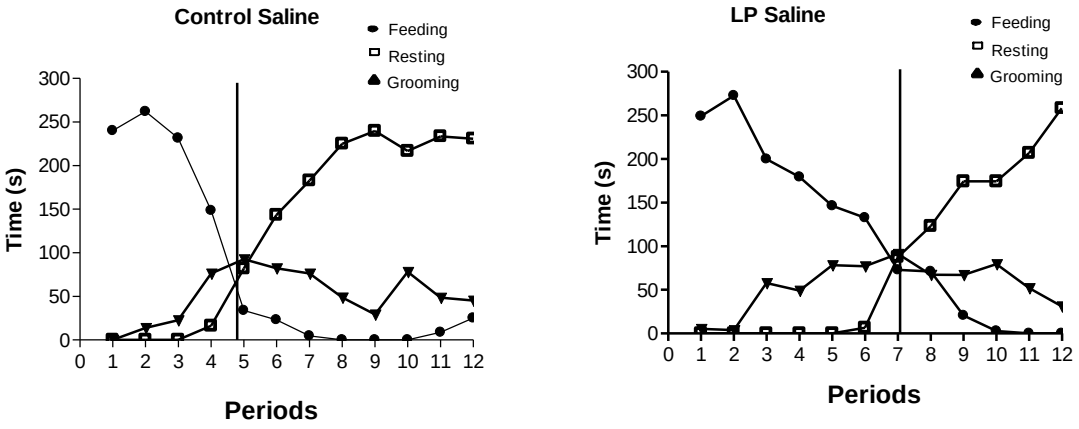

Figure 5 - Behavioral satiety sequence in control (n = 14) and LP rats (n = 14) with 400 days of age treated acutely with saline (0.9% NaCl) solution. The data from each behavioral category was expressed as a proportion of the total number of observations in the period. Two-way ANOVA followed by *Bonferroni t-test*, ($p < 0.05$).

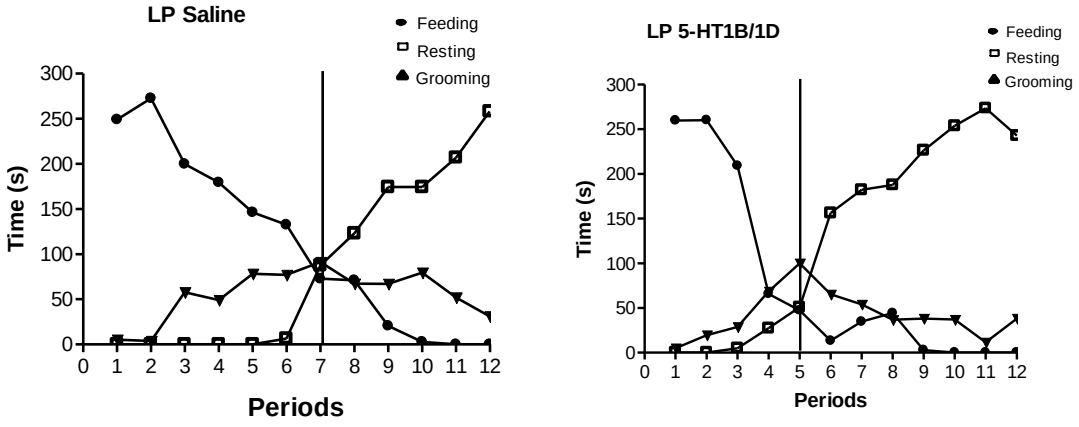

Figure 6 - Behavioral satiety sequence LP rats (n = 14) with 400 days of age treated acutely with saline (0.9% NaCl) or 5-HT1B/1D receptor agonist (1mg / kg) solution. The data from each behavioral category was expressed as a proportion of the total number of observations in the period. Two-way ANOVA followed by *Bonferroni t-test*, ($p < 0.05$).

5. Considerações Finais

Concluímos que a desnutrição perinatal, em animais de meia-idade, aumenta a preferência por alimentos com alto valor energético, altera a seqüência comportamental de saciedade, prolongando o ponto de saciedade e aumentando a ingestão, provavelmente em decorrência de uma desorganização anatômica dos núcleos hipotalâmicos responsáveis pela regulação da ingestão alimentar (Plagemann, Harder et al., 2000). Os animais desnutridos que receberam a dose do agonista serotoninérgico 5-HT1B/1D, retornam o ponto de saciedade para os níveis dos animais controles, diminuindo o tempo de duração da alimentação, porém aumentando a velocidade de ingestão. Esse aumento da velocidade de ingestão alimentar ocorre, provavelmente, para que os desnutridos consigam comer a mesma quantidade de alimento em um período de tempo mais curto. O uso de agonistas que agem sobre o sistema serotoninérgico auxilia na investigação sobre o papel da serotonina no comportamento alimentar e a função de cada um dos receptores serotoninérgicos a cerca de sua ação sobre a duração, tamanho da refeição e início da saciedade. Agentes que atuam como agonistas de receptores 5-HT1B são importantes para definir o papel deste subtipo de receptor da serotonina no comportamento alimentar. Roedores não são obviamente seres humanos e as observações referentes ao apetite e alterações de comportamento não são diretamente aplicáveis aos seres humanos.

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Anexos

ANEXO A: Aprovação do Comitê de Ética

Universidade Federal de Pernambuco
Centro de Ciências Biológicas
Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
fones: (55 81) 2126 8340 | 2126 8351
fax: (55 81) 2126 8350
www.ccb.ufpe.br

Recife, 12 de janeiro de 2009

Ofício nº 96/09

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: **Prof.º Raul Manhaes de Castro**
Departamento de Nutrição- CCS
Universidade Federal de Pernambuco
Processo nº 23076. 025905/ 2008 - 70

Os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) avaliaram seu projeto de pesquisa intitulado " ***Programação serotoninérgica do controle hedônico do comportamento alimentar: regiões encefálicas de regulação e receptores serotoninérgicos***".

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

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

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

Atenciosamente,

Observação:
Origem dos animais: Biotério do Departamento de Nutrição; Animal; Rato; Wistar; Sexo: Machos e fêmeas; Idade: Adultos e de 1^0 ao 120^0 dia de vida pós - natal; Número de animais previsto no protocolo: 72 animais.

Profa. Maria Teresa Jansen
Presidente do CEEA

CCB: Integrar para desenvolver

ANEXO B**:** Carta de aceite do artigo “The modulatory role of serotonin on feeding behavior”

Nutritional Neuroscience - new proofs

30/9/2010 Para Carolina Magalhaes

De: **BRUCE HADDOCK** (b.haddock1@btinternet.com)

Enviada:quinta-feira, 30 de setembro de 2010 14:01:10

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Dear Carolina

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Best wishes

Gill

ANEXO C: Documentação de encaminhamento do artigo “Perinatal protein restriction induces alteration of control of satiety by 5-HT1B receptor and disorder of alimentary preference in adult rats”

Dear carolina,

Your submission entitled "Perinatal protein restriction induces alteration of control of satiety by 5-HT1B receptor and disorder of alimentary preference in adult rats" has been received by Physiology & Behavior

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ANEXO D: Documentação de encaminhamento do artigo “Can perinatal protein restriction induce an emotional pattern specific in middle-aged rats**?**

Ms. Ref. No.: PHB-D-10-00497
Title: Can perinatal protein restriction induce an emotional pattern specific in middle-aged rats?

Physiology & Behavior

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ANEXO E: Artigo “Can perinatal protein restriction induce an emotional pattern specific in middle-aged rats?

Can perinatal protein restriction induce an emotional pattern specific in middle-aged rats?

[1]Carolina Peixoto Magalhães; [2]Renata Cristinny de Farias Campina; [3]Tassia Karin Ferreira Borba; [4]Livia de Almeida Lira; [5]Raquel da Silva Aragão; [6]Sandra Lopes de Souza; [7]Raul Manhães de Castro

[1]Professora de Anatomia do Centro Acadêmico de Vitória – CAV/UFPE – Brazil;

[2]Doutoranda da Pós-graduação em Neuropsiquiatria da Universidade Federal de Pernambuco– Brazil;

[3]Graduada em Ciências Biológicas pela Universidade Federal de Pernambuco– Brazil;

[4]Mestranda da Pós-graduação em Neuropsiquiatria da Universidade Federal de Pernambuco– Brazil;

[5]Doutoranda da Pós-graduação em Nutrição da Universidade Federal de Pernambuco– Brazil ;

[6]Professora de Anatomia da Universidade Federal de Pernambuco – Brazil

[7]Professor do Departamento de Nutrição da Universidade Federal de Pernambuco – Brazil

Corresponding author:

Carolina Peixoto Magalhães

Rua Alto do Reservatório s/n – Bela Vista – Vitória de Santo Antão – PE

CEP: 55608-680

Fone: 55-81-9245-9274

e-mail: peixotocarolted@hotmail.com

Abstract

During critical periods, an adequate supply of nutrients is essential for maintaining the growth and functional development of all systems. We studied the effects of perinatal malnutrition in young and middle-aged rat models testing three behaviors related to anxiety, depression and locomotor activity in rats. Half of the animals were randomly assigned to a low-protein diet (D8%), while the others received a normal protein diet (D17%). The body weight of the pups was measured daily from 1 to 21 days postnatal, and periodic measurements were conducted until day 400. Behavior was studied using the elevated plus maze (EPM), forced swimming test (FST) and open field test (OFT). At 60 days of life, in the EPM malnourished animals showed an increased frequency in open arms, stayed longer in the open arms and demonstrated an increase in head diving behavior. In behavioral assessments in the OFT, there was an increase in the distance traveled, average speed and number of stops. It was observed that middle-aged animals spent less time in open arms, with a higher frequency of self-cleaning behavior compared to controls. The FST demonstrated a reduction in escape attempts, with a consequent increase in immobility behavior and decrease in distance traveled and the average power compared to controls. The malnourished animals show a general decline, with greater weakness and vulnerability as they age, and it may be assumed that the effects of perinatal malnutrition may worsen and exert greater effects in animals with advanced age.

Keywords: Malnutrition; Programming; Forced swimming test; Elevated plus maze

1. Introduction

Epidemiological studies confirming the association between the intrauterine environment and late onset of adverse cardiovascular and metabolic diseases led to the concept of programming (Lucas, 1991). Changes occurring during the critical period of development of physiological systems can program the metabolism in a lasting or even permanent manner, allowing the individual to adapt and survive in the face of environmental changes (Hales and Barker, 1992). This phenomenon is known as metabolic programming (Barker, 1995; de Moura and Passos, 2005). This type of programming is associated with the development of chronic health problems such as obesity, diabetes and

cardiovascular disease (Armitage, Taylor et al., 2005; Cottrell and Ozanne, 2008) in addition to psychopathology such as anxiety and depression (Darnaudery and Maccari, 2008).

Especially during critical periods of development, an adequate supply of nutrients is essential for maintaining the growth and functional development of all organ systems (Hales and Barker, 1992; Orozco-Solis, Lopes de Souza et al., 2009). Malnutrition is one of the non-genetic factors that can affect the developing nervous system (Levitsky and Barnes, 1972; Morgane, Austin-LaFrance et al., 1993). Malnutrition may lead to neurochemical changes (Winick, Rosso et al., 1972), including affecting the levels of neurotransmitters (Leahy, Stern et al., 1978; Foote, Bloom et al., 1983; Chen, Turiak et al., 1995; Mokler, Galler et al., 2003), with effects on excitability (Guedes, Monteiro et al., 1996; Rocha-de-Melo and Guedes, 1997) and behavior (Manhaes de Castro, Barreto Medeiros et al., 2001; Silva, Bernardi et al., 2001; Lopes de Souza, Orozco-Solis et al., 2008).

For experimental studies on malnutrition, the Wistar rat is a good animal of choice because it is relatively easy to change the nutritional status of pups by changing the maternal diet. Undernutrition during pregnancy and / or lactation can alter the behavior of rats in various models of anxiety (Almeida SS, 1994; Almeida, Tonkiss et al., 1996; Pereira-da-Silva, Cabral-Filho et al., 2009), particularly in the elevated plus maze, a test used to measure behavioral changes are interpreted as low levels of anxiety and / or high impulsivity. The test is based on the natural aversion of mice to high and open areas as well as on their natural and spontaneous propensity to explore a new environment. The elevated plus maze is undoubtedly one of the most commonly used devices for ethological animal models in contemporary research on anxiety (Estanislau, Ramos et al.; Rodgers RJ, 1993; Leite-Almeida, Almeida-Torres et al., 2009).

Reports on malnutrition associated with permanent changes in the nervous system include behavioral changes, such as emotion, motivation and anxiety (Levitsky and Strupp, 1995). As the co-morbidity between anxiety and depression is a notable problem in disorders of human behavior, a possible relationship between the behaviors observed in tests related to these psychopathologies is of great importance. The forced swimming test, originally described by Porsolt and colleagues (Porsolt,

Le Pichon et al., 1977), is the most commonly used laboratory test to assess the potential clinical activity of antidepressant drugs (Weiss, McFarland et al., 1998; Cryan, Page et al., 2002; Cryan, Valentino et al., 2005). The test is based on the observation that mice eventually become immobile when placed in a water tank, discontinuing active avoidance behavior, such as climbing or swimming (Cryan, Page et al., 2002). The original forced swimming test offered by Porsolt (Porsolt, Le Pichon et al., 1977; Porsolt, Anton et al., 1978) was a model of depression with features similar to the model of learned helplessness. The affective state of rodents after an initial exposure to forced swimming test was labeled as "behavioral despair (no hope)". Knowledge of the behavior in the forced swimming test may improve the knowledge about behaviors related to depression (Cryan, Valentino et al., 2005). In addition, in this study, the open field test was used, along with other behavioral tests, in order to understand the effects of malnutrition on anxiety and depression and their relationship to locomotion. Generally, in these studies behaviors related to anxiety and depression are found to affect locomotor activity.

Due to the lack of comparative studies on the consequences of programming on the emotional behavior between early and advanced ages, we aimed to delineate the behavioral profile of experimental animals that have been nutritionally programmed. For this, we studied the effects of perinatal malnutrition in rats young and middle-aged animal models using three behaviors related to anxiety, depression and locomotor activity.

2. Materials and Methods

2.1. Animals

We used Wistar rats aged between zero and 400 days from the animal facilities of the Department of Nutrition, Federal University of Pernambuco. Nulliparous females weighing between 250 and 300g were kept for a 15 day adaptation period in a light / dark cycle of 12 hours reversed (light on at 6:00 pm and off at 6:00 am), 22 ± 1 ^{0}C, with free access to water and standard diet (Purina ®, Brazil S / A). After the adjustment period, females were mated at a ratio of two females to one

male. The presumptive diagnosis of pregnancy was made through observation of sperm in vaginal smear, after which the females were placed in individual cages for progression of gestation. Half of the animals were randomly assigned to a diet low protein (D8%), while the others received a normal protein diet (D17%) for the period between the detection of sperm in vaginal smears until day 21 of lactation. The diet D17% was in line with recommendations of the American Institute of Nutrition, AIN (Table 1).

Table 1 - Composition of experimental diets (g / kg) and the percentage of macronutrients in relation to total energy value.

Ingredients	low-protein 8%	control 17%
g %	100,00	100,0
Casein	8,10	17,30
Sucrose	75,10	65,90
Fat	7,00	7,00
Cellulose	5,00	5,00
* Vitamin mix	1,00	1,00
† Mineral mixture	3,50	3,50
Metionin	0,30	0,30
% Kcal	**362,48**	**363,44**

*The vitamin mixture contained (milligrams per kilogram of diet): retinol 12, cholecalciferol 0.125, thiamine 40, riboflavin 30, pantothenic acid 140, pyridoxine 20, inositol 300, cyanocobalamin 0.1, menadione 80, nicotinic acid 200, choline 2720, folic acid 10, p-aminobenzoic acid 100, biotin 0.6. †The mineral mixture contained (milligrams per kilogram of diet): CaHPO4 17 200, KCl 4000, NaCl 4000, MgO 420, MgSO4 2000, Fe2O2 120, FeSO4$7H2O 200, trace elements 400 ($MnSO4.H_2O$ 98, $CuSO4.5H_2O$ 20, $ZnSO4.7H_2O$ 80, $CoSO_4.7H_2O$ 0.16, KI 0.32, sufficient starch to bring to 40 g/kg of diet).

One day after birth, all male pups born on the same day were randomly assigned to groups of eight pups. When the number of male offspring was not sufficient, the group was supplemented with females. The criteria for inclusion in malnourished or control group were: all malnourished pups weighed between 5.0-6.5g and all pups for control group weighed between 6.0-7.5g. The experimental

cohorts consisted of a total of 32 pups from mothers fed on the D8% diet and 32 pups from mothers fed on the D17% diet. On day 21 (P21) of life all animals were weaned and began receiving casein-based diet (17%) until 50 days of life. After this period, they received standard diet (Purina ®) (normal diet with 23.0% protein) until they completed 400 days (P400). All experiments were conducted in accordance with the recommendations of the Brazilian Committee of Animal Experiments - BCAE and approved by the ethics committee on animal experimentation of the Center for Biological Sciences, Federal University of Pernambuco (No. 23076.025905/2008-70).

2.2 Body weight

The body weight (g) of pups was measured daily from 1 to 21 days postnatal. After weaning, the animals were kept in collective boxes with two animals each until the beginning of behavioral tests, when they were separated into individual boxes. In order to monitor the growth rate, the body weight of each animal was measured at 30, 60, 90, 150, 180 and 400 days of life. All body weights were measured using a Marts ® balance: AS 1000C, Class II, 1000g capacity, lower division 0.01 g.

2.3 Elevated plus maze

The elevated plus maze was made of wood and consisted of two open arms (50cm X 10cm) and two opposing closed arms (50cm high), which crossed the open arms. In the center of the intersection of the arms there was a central area (10cm X 10cm), which connected with all arms, both opened and closed. The maze was elevated 50 cm from the ground, as described by Pellow et al. (Pellow, Chopin et al., 1985). The side walls of the open arms were 1 cm tall made of wood, to prevent the animals' falling. The maze was placed in an experimentation room (2.0mX 3.0m), which provides communication with the vivarium where the animals were maintained with their light cycle reversed. The room was illuminated with a 40W red fluorescent light, a highly sensitive camera (Sharp model VL-L63B) was mounted in the ceiling at a distance of 2.50 meter, above the maze. The camera was connected to a VCR (Toshiba X61M ®) and a computer located in the next room, which was equipped with a program to capture and store movies. The videotapes were analyzed using the

program X-Plo-Rat ® 2005, version 1.1.0, developed at the Laboratory of Behavioral Exploratory - Ribeirão Preto - SP, Brazil. The behavioral tests in the elevated plus maze were made in the morning between 9:00 and 11:00 am, with animals in the D17% and D8% groups aged between 60 and 400 days of life. To begin testing, each animal was brought from the vivarium and placed in the experimentation room. Each animal was removed from its cage and placed in the central part of the elevated plus maze, with its head toward one of the enclosed arms. Each test lasted 5 minutes. After testing each animal, the elevated plus maze was cleaned with a 10% alcohol solution. The activities performed by the animals and the behaviors that were evaluated at the elevated plus maze are described respectively in tables 2 and 3.

Table 2 – Description of activities in the elevated plus maze

Activities	Description
Frequency of entries in open arms	An entry in the open arm was defined as the mouse having all four paws inside the arm.
Time spent in open arms	Total time spent by the animal while remaining open arm.
Duration in the center of the elevated plus maze	Total time spent by the animal in the center of the maze.

Table 3 – Description of behavior in the elevated plus maze

Ethological analysis	Behavior	Description
Lifting	When the animal got up on his hind legs.	Included as a classical measure of exploration in a new environment (Almeida SS, 1994; Almeida, Tonkiss et al., 1996).
Diving head	When the animal examined by looking beneath the outstretched arms toward the floor.	Described as risk assessment behaviors (Rodgers RJ, 1993; Almeida SS, 1994).
Stretching	When the animal was a lengthening of the head and shoulders followed by retraction to original position.	

Attempt to enter the open arm	When the animal tried to enter the open arm, placing both front legs, and then returned to the starting position.	Confidence measures to determine the levels of fear / anxiety in experimental animals (Pellow, Chopin et al., 1985; Cruz, Frei et al., 1994).
Self- cleaning	When the animal licked or scratched body parts.	

2.4 Forced swimming test

Swimming sessions were conducted with rats at 60 and 400 days of life, placing them individually in an aluminum tank (50 cm high x 50 cm diameter) containing sufficient water warmed to a temperature of 23^0C, ± 2^0C so the rat could not touch the bottom of the tank. The first session (pretest) was conducted by forcing the rats to swim for 15 minutes. At the end of the session the animal was removed from the tank, dried with a towel and placed in a warmed cage (23^0C, ± 2^0C) for 15 minutes. Subsequently, the animal was returned to its cage. Twenty-four hours later, all animals underwent an ethological forced swimming test swim for five minutes, which was recorded by the video camera for later analysis by a trained observer (Table 4). Depression was measured in terms of total duration of immobility behavior and latency.

Table 4 – Description of behavior in the forced swimming test

Ethological analysis	Description
Attempted Escape	When the animal had four legs in use, either swimming or trying to climb the side wall of the tank.
Immobility behavior	When the animals floated without struggling, making only movements necessary to keep their heads above water.
Diving	When the animal could not keep his head above water and let the muzzle submerge.
Latency	Time elapsed between placing the animal in the tank and the first immobility observed.

2.5 Open field

A system for monitoring the movements of small animals in an open field setting, called the Sequence Capture Video Program, was developed in cooperation with the Department of Physics and collaboration of the Undergraduate Program in Biomedical Engineering (both at the Federal University of Pernambuco, Brazil). The system consisted of a circular open field surrounded by walls 30 cm high, painted black. The floor of the field was covered with soft surface ethyl vinyl acetate (EVA), also black, aiming to facilitate the movement of the animal while providing a greater visual contrast when recorded by a digital camera. Directly above the center of the field a digital camera (VTR ® 6638 - CCTV System) was fixed to the ceiling of the room to record the animal's movements. The distance from the camera to the center of the base of the field was of 2.40 m. The camera was equipped with an infrared sensor and LED lighting, provided a resolution of 420 lines and speed between 1/60 and 1/100 s, and was able to record images with minimal lighting (down to 0.1 lux). The camera was coupled to the computer via a capture card.

The digital image was transferred to a computer using software possessing a capture rate of 30 frames per second, with the video 240 pixels high by 320 pixels long. The videos were saved in AVI format, and were further divided into tables for analysis. Paint ® software was used to isolate the image of the animal, masking it from objects in the surrounding field. To analyze the captured images, we developed software based on MATLAB ® which is capable of performing the processing of frames taken from each video. Through an interface, the evaluator entered data on each animal and information that was used to analyze the frames. Due to the difference in color between the animal and the open field, the program was able to use the intensity of the pixels to establish the complete image of the animal. The image analysis software determined the midpoint of the image, which was then used to represent position of the animal. This in turn allowed the determination of the xy coordinates of the animal in the frame. Utilizing the animal's position in each frame, it was possible to reconstruct the animal's trajectory and by adding the information from the mass of the animal and time between each frame, it was possible to establish the parameters described in Table 5 (Aragão RS, 2010).

Table 5 – Description of behavior in the open field

Analysis	Description
Distance (m)	The sum of all displacements performed by the animal. It was considered that the animal was moving when it shifted its position by 50% of its length in at most three frames. The software featured built-in converter-meter pixels, where the conversion scale was added by the researcher.
Average speed (m/s)	The ratio of total displacement to the time the animal was in motion: ΔS / ΔT, where ΔS corresponds to the total displacement in meters, and ΔT the total analysis time less downtime in seconds.
Average power (mW)	Power produced during the period of displacement. This was considered to be the capacity of a body in motion to dissipate kinetic energy in relation to travel time: $mV^2 / 2\Delta T$, where m is the mass of the animal in grams, V the average speed in m/s, and ΔT the total analysis time less downtime in seconds.
Time of stops (s)	Total time that the animal remained standing in the open field.
Number of stops	Total number of stops made in the open field during the experiment.

The animals were evaluated at day 60 and day 400. Evaluations were performed during the dark phase of the reversed cycle (between 09:00 am and 11:00am) to utilize the greatest period of activity of the animal. Each animal was placed individually in the center of the field and filmed for five minutes while it moved freely. After each animal, the field was cleaned with a hypochlorite solution (10%), and the EVA flooring was changed to eliminate odors that could affect the following animal's behavior.

2.6 Statistical analysis

Student's t-test was used, when appropriate, to compare groups. All data were expressed as mean±SEM. When indicated test was two-way repeated measures (ANOVA) followed by post-hoc Holm-Sidak method.

3. Results

3.1 Effect of malnutrition on body weight

Perinatal malnutrition caused a reduction in body weight beginning by the 3rd day of lactation and lasting through weaning at 21 days (Fig. 1). Body weight continued to be checked at the 30th, 60th, 90th, 150th, 180th and 400th days of life. We observe that the malnourished animals at all periods evaluated post-weaning weighed less than control animals (Fig. 1).

3.2 Behavioral assessments

3.2.1 Elevated plus maze

At 60 days the animals were evaluated in the elevated plus maze. As illustrated in Figure 2, the malnourished animals showed an increased frequency in open arms (D17%= 5±0.79; D8%= 8± 0.92), stayed longer in the open arms (D17%= 46.12± 6.41; D8%= 75.13 ± 10.65), and an increase in diving head behavior (D17%= 10.20±1.35; D8%=15.3±1.38) compared to controls of the same age. The other ethological parameters evaluated, such as lifting, stretching, trying to enter the open arm and self-cleaning did not show significant

When assessed in the elevated plus maze, it was observed that middle-aged animals in the perinatal malnourished group spent less time in open arms (D17%= 30.78 ± 8.34; D8%= 9±2.82), with a higher frequency of self-cleaning behavior compared to controls (fig.3).

3.2.2 Forced swimming test

In the forced swimming test, ethological analysis of attempted escape behavior and immobility time of animals at 60 days showed no significant differences between the malnourished group and controls of the same age. The frequency of diving (D17%= 21.41±3.51; D8%=44.0 ± 5.48) and latency to immobility (D17%= 88.90 ± 8.15; D8%= 69.30 ± 4.71) differed significantly between malnourished animals at 60 days compared with controls (Fig. 4).

At 400 days of age, the swimming test demonstrated a reduction by the malnourished animals in escape attempts (D17%= 266.26 ± 4.23; D8%= 252.15 ± 5.13), with a consequent increase in immobility behavior (D17%= 33.73 ± 4.23; D8%= 47.84 ± 5.13). In addition, a significant increase in

the frequency of diving (D17%=30.46 ± 3.50; D8%= 46.73 ± 4.50) and a decrease in latency of immobility (time) (D17%= 81.26 ± 3.72; D8%= 63.20 ± 4.32) was observed in the malnourished animals at 400 days compared to controls of the same age (Fig. 5).

3.2.3 Open Field

In behavioral assessments in the open field, there was an increase in distance traveled, average speed and number of stops of the malnourished animals at 60 days of life, with a decrease in the duration of stop time for these animals (Table 2). At 400 days of life, we observed a decrease in distance traveled and the average power of malnourished compared to controls of the same age. Other than an increase in the duration of the stops of these animals (Table 6), we did not observe significant behavior changes.

Table 6 - Results of t-test applied in the measurement of activities in the open field with controls and malnourished animals at 60 and 400 days

Activities	Control 60 days	Low-protein 60 days	Control 400 days	Low-protein 400 days
Distance (m/s)	18,60±1,99	23,70±1,16[a]	20,09±1,26	15,33±1,03[b]
Average power (mW)	2,23±0,30	2,21±0,22	4,57±0,44	2,52±0,23[b]
Average speed (m/s)	0,060±0,0057	0,079±0,0039[b]	0,067±0,004	0,051±0,003[b]
Time of stops (s)	216,86±7,96	191,90±5,23[b]	226,66±4,83	241,85±3,46[b]
Number of stops	68,39±4,25	84,23±2,78[b]	69,08±4,55	66,83±4,20

Data expressed as mean ± SEM (n = 12 for each group). Analysis between groups with [a]P <0.05, [b]P <0.01

4. Discussion

Various prior studies have focused on the effects of malnutrition on gestation or gestation and suckling (Anguita, Sigulem et al., 1993; Burns, Desai et al., 1997; Bertin, Gangnerau et al., 1999). However, lactation appears to be the crucial period in mammals for the establishment of programming

(Ramos, Teixeira et al., 2000; Moura, Franco de Sa et al., 2002; Passos, Ramos et al., 2002). Lopes de Souza et al. (Lopes de Souza, Orozco-Solis et al., 2008) observed a reduction in body weight of programmed animals on the third day after birth. This change was also observed in our studies. The delay in body growth seen during lactation continued until 400 days of life. During lactation, malnutrition appears to be more severe with respect to weight gain. This information is consistent with other studies that indicated the importance of critical periods for growth and development of the organism (Pucciarelli and Goya, 1983; Kagotani, Hashimoto et al., 1989; Reichling and German, 2000; Grove and Smith, 2003).

4.1 Elevated plus maze

In young animals, malnutrition reduced the level of anxiety and / or caused more impulsivity in the elevated plus maze test on the cross, as indicated by a high frequency of entries and a longer stay in the open arms and a higher incidence of diving head behavior (used by animal to assess the risk). These data are consistent with some authors who have found perinatal malnutrition to predict levels of anxiety using models such as light-dark transition (Brioni, Cordoba et al., 1989; Santucci, Daud et al., 1994), the elevated plus maze (Almeida SS, 1994; Almeida, Tonkiss et al., 1996) and elevated T maze (Hernandes and Almeida, 2003). These findings may be related to changes in neural and / or neurochemical systems (Almeida SS, 1994; Almeida, Tonkiss et al., 1996). In the open field, we noticed an increase in distance traveled, average speed and number of stops in the open field test. The animals move about more and more quickly, stopping more often, but spending less time in each stop. This is consistent with a reduction in anxiety, characterized by further exploration in the open field and reduction in time that the animal stopped. Perinatal malnutrition increases exploratory behavior (Sobotka, Cook et al., 1974; Almeida, Tonkiss et al., 1996; Duran, Cintra et al., 2005) and emotional lability.

In contrast to this observation in young animals, programmed animals in middle-age had higher levels of anxiety, with a reduction in impulsivity, characterized by a reduction in time spent in open arms and an increase in grooming behavior. Behavioral responses related to anxiogenic situations

are connected to the limbic structures like hippocampus and amygdala (Millan, 2003; Bremner, Vythilingam et al., 2004). In the open field test we found that perinatal malnutrition decreases the distance traveled and increases the time the animals remain stationary, which may be related to higher levels of anxiety. Stanislaus, et al., (Estanislau, Ramos et al.) Observed an inverse relationship between the behaviors used as indices of anxiety and behavioral despair in the forced swimming test. These results disagree with our study, probably because of the age of the animals. Other studies with animals bred selectively found an association between low open arm exploration in the elevated plus maze and high immobility in the forced swimming test (Overstreet, Rezvani et al., 1992; Prasad, Imamura et al., 1997; Keck, Welt et al., 2001; Hinojosa, Spricigo et al., 2006). However, most studies of *outbred* animals were not able to find a relationship (Hilakivi and Lister, 1990; Andreatini and Bacellar, 1999).

Malnutrition during gestation and childhood leads to deficits and distortions of brain structure and function (Bedi, Birzgalis et al., 1982; Dobbing, 1988; Bedi, 1992; Bedi, 1994). These deficits may be due to a failure of many "normal" neurons and glial cells to be generated during the period of neurogenesis and gliogenesis and / or may be due to actual loss of cells due to the non-programmed cell death as a result of the lack of sufficient nutrients (Dobbing, 1988; Jacobson, Jacobson et al., 1991). Malnutrition causes structural changes (Granados-Rojas, Aguilar et al., 2004; Lister, Blatt et al., 2005) and neurochemical changes (Del Angel-Meza, Ramirez-Cortes et al., 2002; Mokler, Galler et al., 2003) of the hippocampus, reducing its mossy fiber system (Granados-Rojas, Aguilar et al., 2004) and the number of neurons in layers of principal cells of the hippocampal formation (Lister, Blatt et al., 2005). Malnutrition causes changes in both the serotonergic and GABAergic system in hippocampal formation (Mokler, Galler et al., 2003). Food restrictions (Brunell and Spear, 2006; Doremus, Varlinskaya et al., 2006) resulted in behavioral changes related to anxiety and depression with changes in the serotonergic system.

4.2 Forced swimming test

Malnutrition in young animals causes changes in ethological parameters of animal behavior. There was a decrease in latency and an increase in the frequency of dives, indicating a predisposition to behaviors related to depression. According Morgane et al (Morgane, 1978), the period of gestation and lactation in rats is a phase of continued development of the brain, therefore making this period the most vulnerable to attacks that can lead to permanent functional and morphological changes, including on behavioral expression (Manhães de Castro R.; Cabral Filho, 1993; Blundell and King, 1998; Manhaes de Castro, Barreto Medeiros et al., 2001). One of the parameters used to characterize depressive activity is the time of latency. Studies have used this parameter to detect the efficacy of fluoxetine, an antidepressant, in the forced swimming test (Contreras, Rodriguez-Landa et al., 2001; Carlezon, Pliakas et al., 2002; Padovan and Guimaraes, 2004). The time of latency also proved a useful parameter to study the antidepressant effects of caloric restriction in transgenic mice (Koponen, Rantamaki et al., 2005; Lutter, Krishnan et al., 2008). The forced swimming test has been used to study the action of antidepressant drugs (Beique, de Montigny et al., 2000; Martinez-Mota, Estrada-Camarena et al., 2002). Serotonergic drugs such as selective inhibitors of serotonin reuptake, in general, cause a reduction in immobility behavior, with a consequent increase in the time devoted to attempted escape (Detke, Rickels et al., 1995; Lopez-Rubalcava and Lucki, 2000) and these behaviors may be related to serotonergic function (Carlsson, Svensson et al., 1985; Uphouse, Salamanca et al., 1991). One should take into consideration that this neurotransmitter system is implicated in several important roles in emotional disorders (Clenet, De Vos et al., 2001; Middlemiss, Price et al., 2002; Blier and Ward, 2003).

We observed that perinatal undernutrition causes a decrease in latency and in attempts to escape in middle-aged animals, with consequent increased time of immobility during the forced swimming test. The posture of immobility in the forced swimming test is interpreted as failure in behavior directed at an attempt to escape (state of despair) or the development of a passive behavior of the animal (Porsolt, Le Pichon et al., 1977; Porsolt, Anton et al., 1978; Kitada, Miyauchi et al., 1981; Cryan, Page et al., 2002). Other authors (Borsini and Meli, 1988) agree that immobility in the forced

swimming test may be a consequence of an adaptive response to a stressful situation, instead of behavioral despair. Willner, P. (Willner, 1990) suggests that immobility is not due to generalized hypoactivity, but reluctance to maintain effort. For him, the increase in immobility was not necessarily accompanied by a reduction of locomotor activity. However, in our experiments perinatal malnutrition causes in middle-aged animals hypoactivity in the open field and increased immobility in the forced swimming test. The divergence of data probably occurs due to age at assessment of the animals and type of perinatal treatment. Perinatal malnutrition is associated with permanent changes in the nervous system including those responsible for emotion, motivation and anxiety (Levitsky and Strupp, 1995). Studies have reported that with increasing age, the animals showed loss related to measures of learning and memory (water maze), motor coordination (running, walking on a bridge, wire suspension), and the level of spontaneous locomotion (Forster, Dubey et al., 1996). The motor and cognitive impairments related to age are associated with a number of morphological and functional changes that involve different parts of the brain that are normally associated with these functions (Joseph, 1988; Gage, Dunnett et al., 1989). The hippocampus, striatum and cerebral cortex structures are vulnerable to a variety of insults (Walsh, Kelly et al., 1994) that result in deficiencies in cognition, memory or motor function.

We conclude that the animals show a general decline, with greater weakness and vulnerability as they age, and it may be assumed that the effects of perinatal malnutrition may worsen and exert greater effects in animals with advanced age. In this study, perinatal malnutrition has increased the vulnerability of animals to behaviors related to anxiety and depression. This effect manifests as a reduced emotional response in young animals, with a consequent increase in exploratory behavior, followed by a reversal of these behaviors in middle-aged animals, which are more prone than normal animals to behaviors that characterize depressive states. Thus, an analysis of behavioral contexts may be necessary to understand fully anxiety and depression in animal experiments. A direct link has been established between perinatal malnutrition and development of metabolic syndrome, as well as between neonatal stress and the incidence of anxiety and depression in adulthood (Heim and

Nemeroff, 1999; Gross and Hen, 2004). Also note that the emotional behavior of animals is not just a time / state "positive" or "negative", but has multiple dimensions, including anxiety, exploration, locomotion, risk assessment, warning and general ability to fight (Salome, Viltart et al., 2002).

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Figure 1. Effect of perinatal nutritional manipulation during the lactation period (n = 32) and adult (n = 12) on body weight. Data are expressed as mean ± SEM. For body weight was performed two-way repeated measures (ANOVA) * denotes the initial moment of difference and lasts until the end of the experiment ($p < 0.05$).

Figure 2. Effects of perinatal undernutrition on the activities in the elevated plus maze in animals with 60 days of life (n = 9). Mean ± SEM of (A) frequency in the open arms, (B) time in the open arms and (C) diving head behavior. * Indicates the difference between the groups was significant ($p < 0.05$).

Figure 3. Effects of perinatal undernutrition on the activities in the elevated plus maze animals at 400 days (n = 14). Mean ± SEM of (A) time in open arms and (B) self-cleaning. * Indicates the difference between the groups was statistically significant ($p < 0.05$).

Figure 4. Effects of perinatal undernutrition on the activities of forced swimming of animals with 60 days of life (n = 12). Mean ± SEM frequency (A) dip and (B) latency time. * Indicates the difference between the groups was statistically significant ($p < 0.05$).

Figure 5. Effects of perinatal undernutrition on the activities of forced swimming of animals at 400 days (n = 15). Mean ± SEM of (A) attempt to escape, (B) immobility behavior, (C) frequency of diving and (D) latency time. * Indicates the difference between the groups is statistically significant ($p < 0.05$).

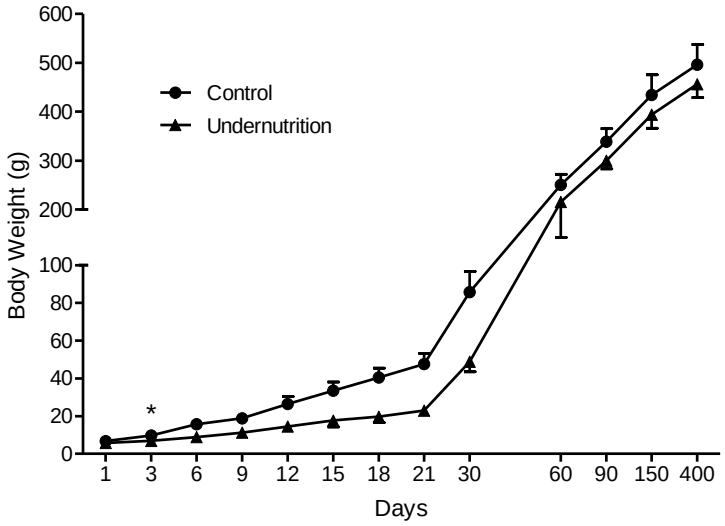

Figure 1

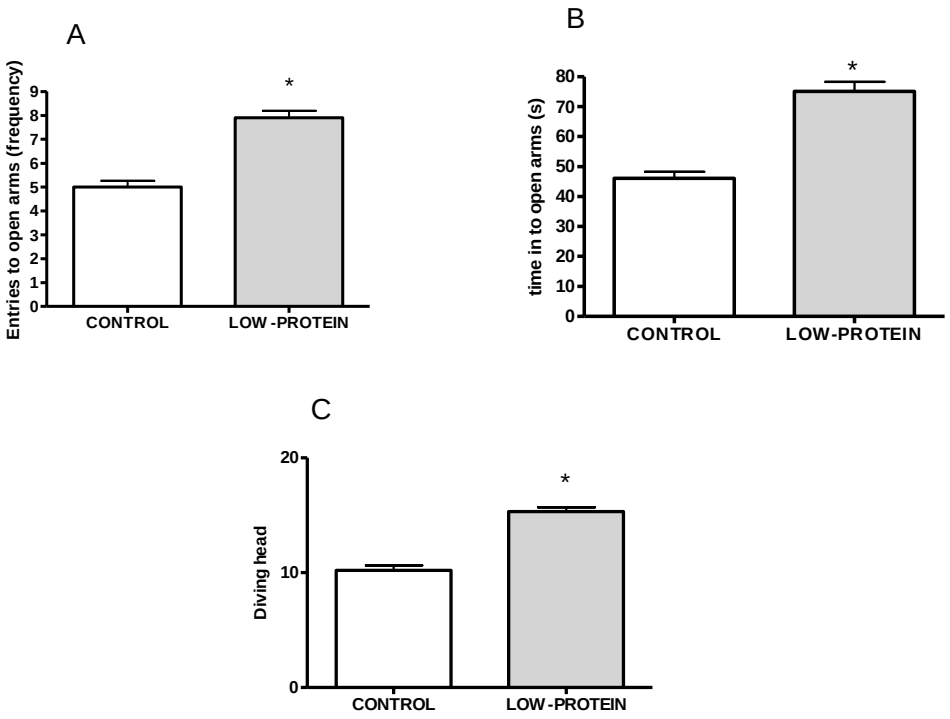
A
Entries to open arms (frequency)
9
8
7
6
5
4
3
2
1
0
*
CONTROL
LOW -PROTEIN
B
time in to open arms (s)
80
70
60
50
40
30
20
10
0
*
CONTROL
LOW-PROTEIN
C
Diving head
20
10
0
*
CONTROL
LOW -PROTEIN

Figure 2

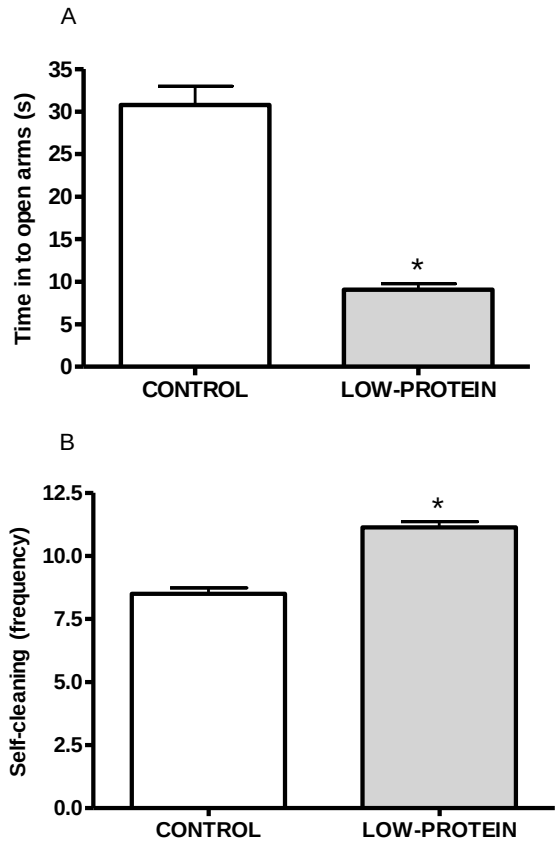

Figure 3

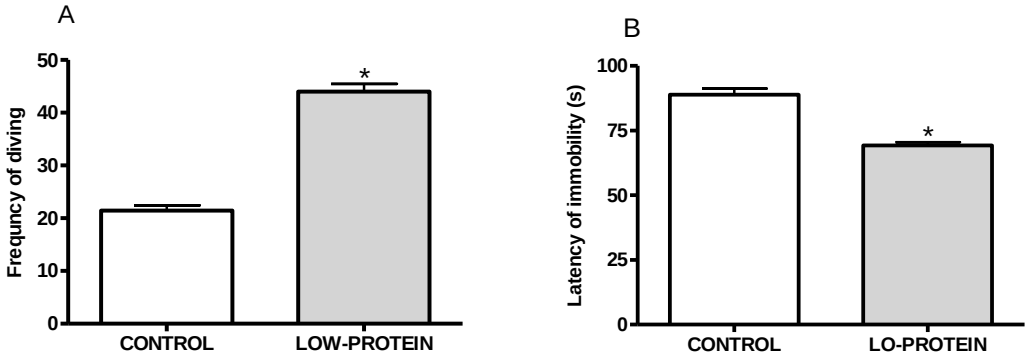

Figure 4

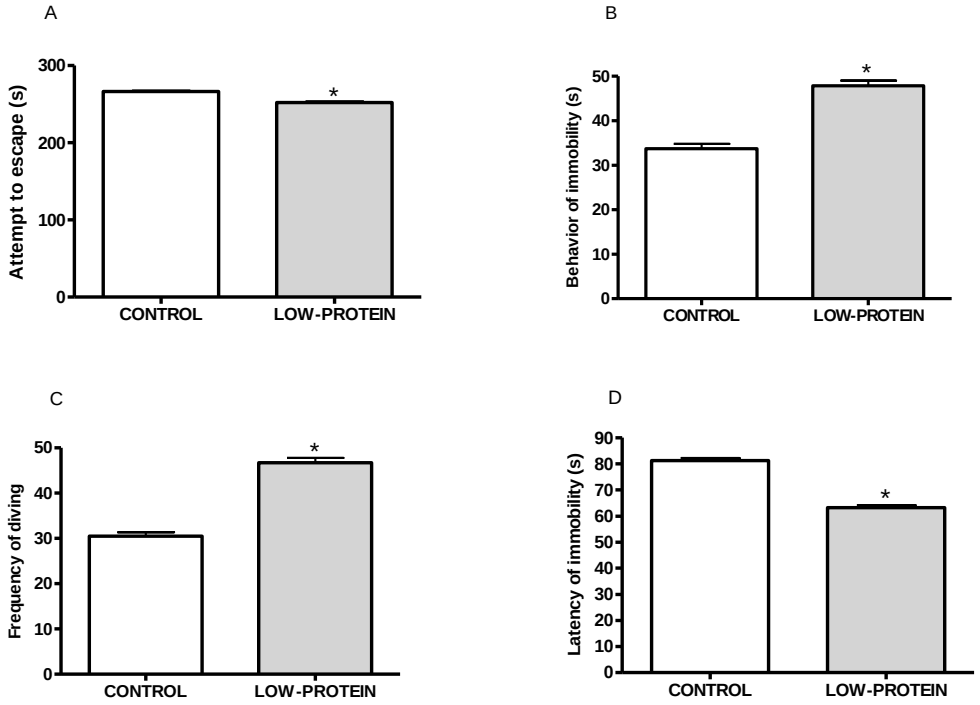
A
Attempt to escape (s)
300
200
100
0
*
CONTROL
LOW-PROTEIN
B
Behavior of immobility (s)
50
40
30
20
10
0
*
CONTROL
LOW-PROTEIN
C
Frequency of diving
50
40
30
20
10
0
*
CONTROL
LOW-PROTEIN
D
Latency of immobility (s)
90
80
70
60
50
40
30
20
10
0
*
CONTROL
LOW-PROTEIN

Figure 5

ANEXO F: Resumos de trabalhos apresentados no Simpósio sobre o Cérebro/ 2010

PROGRAMAÇÃO PERINATAL POR DESNUTRIÇÃO: CONSEQUÊNCIAS PRECOCES E TARDIAS SOBRE A ANSIEDADE EM RATOS.

Barbosa, I. F. S[1]; Silva, R. M. da[1]; Campina, R. C F.[2]; Borba, T. K. F.[3]; Lira, L. A.[4]; Souza, S. L.[5]; Manhães-de-Castro, R.[5]; Magalhães, C. P.[6]

1.Graduandas em Licenciatura em Ciências Biológicas do Centro Acadêmico de Vitória
2. Doutoranda da Pós-graduação de Neuropsiquiatria/UFPE
3. Graduada em Ciências Biológicas/UFPE
4.Mestranda da Pós-graduação de Neuropsiquiatria/UFPE
5.Docente da UFPE
6. Docente de Anatomia do CAV

Centro Acadêmico de Vitória, Rua Alto do Reservatório, s/n Bela Vista, Vitória de Santo Antão.

E-mail: isabelly-barbosa@bol.com.br

O período perinatal é de fundamental importância para o desenvolvimento de todos os órgãos e sistemas, nesta fase as mudanças no meio ambiente influenciarão a programação do metabolismo fetal de maneira duradoura e até permanente, para a adaptação do organismo às situações diversas, como a falta de alimentos, esse fenômeno é conhecido como programação metabólica. Este tipo de programação está associado com o desenvolvimento de vários problemas crônicos de saúde, tais como obesidade, diabetes e doenças cardiovasculares além de psicopatologias, tais como ansiedade e depressão. No presente estudo, utilizou-se o teste do labirinto elevado em cruz (LEC) descrito por Pellow, et al. para avaliar níveis de ansiedade, em ratos jovens e de meia-idade, desnutridos (dieta hipoprotéica 8% caseína) e controles (dieta normoprotéica 17% caseína). Como resultado obteve-se que a desnutrição, na idade de 60 dias, diminui o nível de ansiedade e aumenta a impulsividade, como indicado pela alta freqüência de entradas (D17% 5±0,79; D8% 8±0,92) e permanência nos braços abertos (D17% 46,12±6,41; D8% 75,13±10,65) e maior incidência do mergulho de cabeça (medida de avaliação de risco). No grupo desnutrido de meia-idade, houve aumento na ansiedade, com diminuição da impulsividade, indicado na baixa permanência nos braços abertos (D17% 30,78±8,34; D8% 9±2,82) e maior taxa de tempo gasto em auto-limpeza (D17% 8,5±0,94; D8% 11,13±0,86) comparados ao controle. Esses resultados mostram as conseqüências da desnutrição em diferentes períodos de vida. Conclui-se que a desnutrição no início do desenvolvimento pode ser considerada como fator debilitador do organismo com conseqüentes implicações em doenças físicas e emocionais.

Palavras-chave: período perinatal, desnutrição, labirinto elevado em cruz, ansiedade.

CONSEQUÊNCIAS DA DESNUTRIÇÃO PERINATAL SOBRE O COMPORTAMENTO DEPRESSIVO EM RATOS JOVENS E DE MEIA-IDADE

Silva, R. M. da[1], Barbosa, I. F. S.[1], Campina, R. C F.[2]; Santos, P. A.[3]; Almeida, L. C. A.[3]; Souza, S. L.[4]; Magalhães, C. P.[5]; Manhães-de-Castro, R.[4];

1. Graduandas de Ciências Biológicas do Centro Acadêmico de Vitória (CAV);
2. Doutoranda da Pós-graduação em Neuropsiquiatria/UFPE
3. Graduandas da Universidade Federal de Pernambuco
4. Docentes da Universidade Federal de Pernambuco
5. Docente do CAV/UFPE

Centro Acadêmico de Vitória, Rua Alto do Reservatório, s/n Bela Vista, Vitória de Santo Antão-PE.

E-mail: renata_maria19@hotmail.com

No início da vida dos mamíferos há fases onde ocorrem intensas modificações funcionais e estruturais que são chamadas de períodos críticos. Nesses períodos ocorrem à formação de todos os órgãos e sistemas fisiológicos dos indivíduos. Estudos vêm demonstrando que fatores epigenéticos ao incidirem em períodos críticos da vida podem repercutir sobre o desenvolvimento de órgãos e tecidos provocando alterações estruturais e fisiológicas permanentes, ocasionando inúmeras doenças e problemas psicopatológicos, como a ansiedade e a depressão. Para a realização deste estudo, foram utilizados ratos Wistar com idades de 60 (n=14) e 400 dias (n=14), divididos em dois grupos: desnutrido (dieta hipoprotéica 8%) e controle (dieta normoprotéica 17%). Realizamos o teste do nado forçado para avaliar os comportamentos relacionados à depressão. O teste é baseado na observação de que roedores eventualmente desenvolvem imobilidade quando são colocados em tanques de água, depois que param com o comportamento de fuga ativa, como a escalada ou a natação. O conhecimento sobre a estrutura comportamental no teste do nado forçado pode melhorar o conhecimento a cerca dos comportamentos relacionados à depressão. Observamos que os animais desnutridos, com 60 dias, apresentam diminuição no tempo de latência (D17% 88,90±8,15; D8% 69,30±4,71) e aumento na freqüência de mergulhos (D17% 21,41±3,51; D8% 44,0±5,48). Nos animais desnutridos, com 400 dias, houve uma diminuição no tempo de latência (D17% 81,26±3,72; D8% 63,20±4,32) e na tentativa de escapar (D17% 266,26±4,23; D8% 252,15±5,13) e aumento do tempo de imobilidade (D17% 33,73±4,23; D8% 47,84±5,13) com significante aumento na freqüência de mergulho (D17% 30,46±3,50; D8% 46,73±4,50). Concluímos que a desnutrição no período perinatal, pode ter efeitos ao longo da vida sobre o comportamento emocional, relacionado à depressão.

Palavras-chave: Desnutrição, Sistema Nervoso Central, Depressão, Teste do nado forçado

RESTRIÇÃO PROTÉICA PERINATAL ALTERA A PREFERÊNCIA ALIMENTAR DE RATOS

Tassia Karin Ferreira Borba; Renata Cristiny de Farias Campina; Livia de Almeida Lira; Carolina Peixoto Magalhães; Sandra Lopes de Souza; Raul Manhães de Castro

O estado nutricional precoce altera posteriormente o comportamento alimentar, o balanço energético e o peso corporal. O encéfalo em desenvolvimento é vulnerável aos insultos ambientais. As repercussões da desnutrição sobre o desenvolvimento estrutural, neuroquímico e integridade funcional de células nervosas no sistema nervoso central são bem conhecidas. Investigamos os efeitos da desnutrição perinatal sobre o peso corporal, o consumo alimentar, a motivação dos animais quanto à preferência por alimentos ricos em gordura ou sacarose. Foram utilizados ratos machos da linhagem *Wistar* com idades entre zero e 400 dias. Foi oferecida uma dieta com baixo teor de proteína (D8%) e uma dieta normoprotéica (D17%) durante a gestação e lactação. Para o teste de preferência foi utilizada uma dieta hiperpalatável e solução de sacarose a 10%. Durante o aleitamento, observamos diminuição no peso corporal dos desnutridos a partir do 3º dia de vida (D17% 9,74±1,29; D8% 6,95±0,64). O aumento no consumo alimentar foi detectado a partir do 7º dia de lactação (D17%= 35,01±1,98; D8%=27,52±2,22). Aos 30 (D17%= 14,40 ±2,78; D8%= 7,80 ± 2,82) e 400 dias (D17%= 30,90 ±4,56; D8%=26,96 ± 3,81), os animais desnutridos consumem menos que os controles. Com relação à preferência, houve uma menor ingestão de solução de sacarose entre os animais controles/DH (15,39±1,79) e controle/labina (21,45±3,88) e entre o grupo desnutrido/DH (11,03±3,08) e desnutrido/labina (27,45±2,44). Comparando os grupos controle/labina (21,45±3,88) e desnutrido/labina (27,45±2,44) observamos um aumento na ingestão de sacarose pelo grupo desnutrido/labina. Na avaliação da preferência por dieta rica em gordura, observamos um aumento da ingestão pelo grupo controle/DH (4,14±0,36) comparado ao controle/labina (3,23±0,58). Houve um aumento na ingestão de DH pelo grupo desnutrido/DH (4,70±0,32), comparado ao grupo desnutrido/labina (3,95±0,70). Concluímos que a desnutrição precoce altera as respostas metabólicas e diferentes respostas motivacionais relacionadas ao sistema de recompensa alimentar.

Palavras-chave: comportamento alimentar; recompensa alimentar; hipercalórica

ANEXO G: Resumos de trabalhos apresentados na FeSB/ 2010

DESNUTRIÇÃO PERINATAL INDUZ ALTERAÇÕES NO CONTROLE DA SACIEDADE PELO RECEPTOR 5-HT1B

Larissa Cavalcanti do Amaral Almeida; Renata Cristinny de Farias Campina; Priscilla Alves Santos; Lisiane dos Santos Oliveira; Carolina Peixoto Magalhães; Sandra Lopes de Souza; Raul Manhães de Castro

O estado nutricional precoce fornece pistas para desenvolvimento fetal preparando os filhotes para o ambiente após o nascimento. O encéfalo em desenvolvimento é vulnerável aos insultos ambientais. As funções da serotonina no sistema nervoso central são numerosas, e parecem envolver os controles do apetite, sono, aprendizado e regulação endócrina. A capacidade da serotonina em regular a saciedade e seleção de macronutrientes fornece a base para o tratamento farmacológico da obesidade e transtornos alimentares. Foram utilizados ratos machos da linhagem *Wistar* com 400 dias. Foi oferecida, de acordo com os grupos experimentais, uma dieta com baixo teor de proteína (D8%) e uma dieta normoprotéica (D17%) durante a gestação e lactação. Investigamos os efeitos da desnutrição perinatal com manipulação farmacológica utilizando agonista 5-HT_{1B} sobre os parâmetros da seqüência comportamental de saciedade (SCS). A análise da SCS foi precedida por privação alimentar de quatro horas. Meia hora antes de terminar o período de privação, uma dose aguda de sumatriptan (1mg/Kg, p.c.) ou solução de salina (0,9%) foi injetada via subcutânea. Foram calculados parâmetros denominados de microestruturais da alimentação. Na análise dos grupos desnutrido/salina e desnutrido/sumatriptan observou-se diferença entre os tempos de alimentação (D8%/sal=112,27±5,93; D8%/sumatriptan=78,05±6,93) e descanso (D8%/sal=85,94±12,74; D8%/sumatriptan=133,69±14,38). Na análise entre os grupos controle/sumatriptan e desnutrido/sumatriptan, houve diferença significativa no tempo total de alimentação (D17%/sumatriptan=87,06±6,94; D8%/sumatriptan=78,05±6,93), limpeza (D17%/sumatriptan=44,64±8,60;D8%/sumatriptan=41,86±8,60) e descanso (D17%/sumatriptan=134,07±15,08; D8%/sumatriptan=133,69±14,38). Na avaliação microestrutural da alimentação observou-se que os animais desnutridos/salina passaram mais tempo comendo, com um maior consumo relativo, um maior ganho energético relativo e total, comparados aos controle/salina. Os animais desnutrido/sumatriptan passaram menos tempo comendo com taxa alimentar maior, comparados aos desnutridos/salina. Sugerimos que a restrição protéica durante o desenvolvimento atenua a ação inibitória da 5-HT sobre a ingestão alimentar através de uma diminuição da sensibilidade dos receptores 5-HT_{1B}.

Palavras-chave: agonista 5-HT; desnutrição; programação

ANEXO H: Certificado de Honra ao Mérito

FeSBE 2010

25 a 28 de agosto de 2010
Águas de Lindóia – São Paulo

CERTIFICADO DE HONRA AO MÉRITO

A FeSBE tem a grande satisfação de conferir este certificado de Honra ao Mérito pela brilhante apresentação do trabalho

26.077 - DESNUTRIÇÃO FETAL PROGRAMA A SEQUÊNCIA COMPORTAMENTAL DE SACIEDADE EM RATOS de autoria de Almeida, L. C. A., Lira, L. A., Magalhães, C. P., Lopes de Souza, S., Manhaes-de-Castro,R. - Depto de Nutrição/Universidade Federal de Pernambuco, UFPE

na XXV Reunião Anual da Federação de Sociedades de Biologia Experimental – FeSBE, realizada na cidade de Águas de Lindóia – SP, de 25 a 28 de agosto de 2010.

Comissão Organizadora