UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
CURSO DE BIOMEDICINA

Marina de Lima Marcolin

# EFEITOS DE INTERVENÇÕES PRECOCES E DE DIETA PALATÁVEL SOBRE PARÂMETROS BIOQUÍMICOS E COMPORTAMENTAIS EM RATOS JOVENS

Porto Alegre
2011

Marina de Lima Marcolin

# EFEITOS DE INTERVENÇÕES PRECOCES E DE DIETA PALATÁVEL SOBRE PARÂMETROS BIOQUÍMICOS E COMPORTAMENTAIS EM RATOS JOVENS

Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Bacharel em Biomedicina.

Área de conhecimento: Bioquímica

Orientadora: Profª Drª Carla Dalmaz
Co-orientadora: Doutoranda Rachel Krolow

Porto Alegre
2011

**Agradecimentos**

Aos ratos, sem os quais este trabalho não existiria, meus sinceros agradecimentos e grande respeito.

Ao pessoal do biotério do Departamento de Bioquímica, por toda a ajuda com os animais e com as caixas de isolamento.

À minha orientadora, professora Carla Dalmaz, pela confiança, apoio, incentivo, paciência e ensinamentos.

Aos colegas do Laboratório de Neurobiologia do Estresse, especialmente minha co-orientadora Rachel, por toda a ajuda, força e exemplo; Cristie, pelo exemplo e ensinamentos; Danusa e André, meus braços direitos neste trabalho, grandes companheiros de experimentos.

Ao CNPq, pelo suporte financeiro, imprescindível para que este trabalho se concretizasse.

À UFRGS, por proporcionar um excelente ambiente acadêmico, inspirador e estimulante.

À minha família, por todo apoio e incentivo, por me ensinar a correr atrás dos meus sonhos, por me ajudar a crescer em todos os sentidos.

Aos meus avôs, pela acolhida nos anos inicias da faculdade e por me apresentar esta cidade fantástica que é Porto Alegre.

Aos meus amigos, tanto os de perto quanto os que estão longe, pelas conversas, risadas, desabafos e companheirismo durante todos esses anos.

Aos colegas e professores do Centro Cultural Chinês, por alegrar meu dia com os treinos e me mostrar que o Kung Fu é também uma excelente terapia contra o estresse.

Aos professores, tanto os da faculdade quanto os do colégio, por me apresentarem a Ciência e me mostrarem o que quero ser quando crescer.

Devido a recentes acontecimentos, um especial agradecimento à equipe do Hospital São Lucas, Cascavel-PR, e ao Dr. Marcos Horikawa que, literalmente, salvaram minha vida.

E não menos importante, obrigada aos descobridores da varfarina, dos remédios para asma e inventores das meias de compressão, sem os quais eu não estaria aqui hoje.

**Sumário**

Resumo....6
Abstract....8
Introdução....10
Estresse....10
Sistemas de Resposta ao Estresse....10
Eixo Hipotálamo-Hipófise-Adrenal (HHA)....12
Períodos Críticos do Desenvolvimento....13
Manipulação Neonatal....14
Comportamento Alimentar....15
Manipulação Neonatal e o Comportamento Alimentar....16
Estresse e o Comportamento Alimentar....16
Estresse Oxidativo....17
Objetivo....19
Artigo....20
1. Introduction....21
2. Materials and Methods....23
2.1. Subjects....23
2.2. Neonatal Handling....24
2.3. High Palatable Diet....24
2.4. Food Consumption....24
2.5. Caloric Intake and Caloric Efficiency....25
2.6. Behavioral Tests....25
2.6.1. Open Field....25
2.6.2. Plus Maze....25
2.7. Biochemical Analisys....25
2.7.1. Superoxide Dismutase Activity....26
2.7.2. Glutathione Peroxidase Activity....26
2.7.3. Catalse Activity....26
2.7.4. Total Thiol Content....27
2.7.5. Protein Assay....27
2.7.6. Plasma Levels of Glucose....27
2.8. Statistical Analisys....27

3. Results ..... 27
3.1. Body and Adrenal Glands Weight ..... 27
3.2. Food Consumption ..... 28
3.3. Caloric Intake and Efficiency ..... 28
3.4. Behavioral Tests ..... 28
3.4.1. Open Field ..... 28
3.4.2. Plus Maze ..... 28
3.5. Biochemical Analisys ..... 29
3.5.1. Prefrontal Cortex ..... 29
3.5.2. Hippocampus ..... 29
3.5.3. Plasma ..... 29
4. Discussion ..... 30
References. ..... 32
Conclusão ..... 48
Perspectivas ..... 49
Bibliografia ..... 50

**Resumo**

O estresse é um aspecto presente diariamente em nossas vidas. Desafios reais ou imaginários são interpretados pelo nosso cérebro e respostas adequadas são geradas (sejam elas comportamentais, fisiológicas ou bioquímicas), de modo a restabelecer a homeostase do organismo. As respostas ao estresse são altamente adaptativas e têm a finalidade de promover a sobrevivência do indivíduo. Porém, quando em excesso ou mal-conduzidos, os mesmos sistemas de resposta podem gerar danos e agravar processos fisiopatológicos. Dentre estes sistemas, destaca-se o eixo hipotálamo-hipófise-adrenal (HHA), cuja ativação culmina com a liberação de glicocorticóides (cortisol em humanos, corticosterona em roedores), pelo córtex da glândula adrenal. Estes hormônios afetam vários órgãos, incluindo o sistema nervoso central, regulando sua própria liberação pelo mecanismo de retroalimentação negativa. Além de promoverem as respostas do organismo frente a situações estressoras, os glicocorticóides também afetam a memória, o sistema imune, o apetite e o comportamento alimentar. Alimentos palatáveis, também chamados de "alimentos confortantes", possuem propriedades motivadoras potentes e seu consumo parece estar relacionado com o sistema de reforço-recompensa cerebral, de modo a reduzir a atividade do eixo HHA. Outros fatores também podem alterar o funcionamento do eixo HHA, como intervenções feitas em períodos críticos do desenvolvimento dos filhotes. O eixo HHA tem uma regulação extremamente fina no período pré e pós-natal e distúrbios no padrão normal de secreção de glicocorticóides causados por essas intervenções podem alterar de forma definitiva as respostas do filhote ao estresse. Uma intervenção bastante usada é a manipulação neonatal, que consiste na retirada dos filhotes do ninho por um breve período de tempo. Dessa forma, há um aumento no cuidado maternal e esses filhotes, quando adultos, apresentam modificações endócrinas, bioquímicas e comportamentais que perduram por toda a vida do animal, incluindo menos medo em ambientes novos e maior consumo de alimentos palatáveis.

Uma vez que existe uma forte relação entre estresse, manipulação neonatal e comportamento alimentar, o objetivo deste estudo foi investigar os efeitos desses três fatores sobre parâmetros comportamentais (ansiedade e locomoção), estresse oxidativo em estruturas encefálicas (córtex pré-frontal e hipocampo) e glicose plasmática em ratos Wistar jovens. Ninhadas de ratos Wistar foram manipuladas (10

min/dia) ou não nos dias 1-10 pós-natal. Os machos destes grupos foram subdivididos em quatro subgrupos: dois foram estressados por isolamento na infância (pré-puberdade), e os outros dois, não. Ambos, porém, tiveram acesso a uma dieta palatável ou ração padrão de laboratório concomitante ao estresse durante 7 dias. Os ratos que receberam dieta palatável consumiram mais alimento, mais calorias, ganharam mais peso e tiveram um nível maior de glicose plasmática, porém sua eficiência calórica foi menor do que os grupos que receberam ração padrão. A manipulação diminuiu o efeito da dieta em relação ao ganho de peso e consumo de alimento no primeiro dia de isolamento. A dieta também aumentou o tempo na área central do aparato do Campo Aberto e o tempo nos braços abertos no Labirinto em Cruz Elevado. A atividade das enzimas antioxidantes foi alterada principalmente pela manipulação e dieta nas duas estruturas cerebrais analisadas (córtex pré-frontal e hipocampo), e esses fatores tiveram efeitos opostos na maioria dos casos, com a manipulação aumentando e a dieta diminuindo a atividade das enzimas. O efeito ansiogênico do estresse foi contrabalanceado pelo acesso à dieta palatável e, em menor grau, pela manipulação neonatal. O uso de ambas as condições, no entanto, não parece trazer proteção adicional contra os efeitos do estresse neste período particular de vida, a pré-puberdade.

**Abstract**

Stress is an aspect of our daily lives. Real or perceived challenges are interpreted by our brain and appropriate responses are generated (behavioral, physiological or biochemical) in order to restore homeostasis. The stress responses are highly adaptive and are designed to promote the survival of the individual. However, when excessive or poorly conducted, the same response systems can cause serious damage and aggravate pathophysiological processes. Among these systems, there is the hypothalamic-pituitary-adrenal (HPA), whose activation culminates with the release of glucocorticoids (cortisol in humans, corticosterone in rodents) from adrenal glands' cortex. These hormones affect multiple organs, including the central nervous system, regulating their own release by a negative feedback mechanism. Besides promoting the responses of the organism to stressful situations, glucocorticoids also affect memory, immune system, appetite and eating behavior. Palatable foods, also called "comfort foods", have powerful motivating properties and their consumption appears to be related to the brain reward circuitry, in order to reduce the activity of the HPA axis. Other factors can also alter the functioning of the HPA axis, such as the interventions made during critical periods of development of offspring. The HPA axis regulation is extremely thin in the pre and post-natal period and changes in the normal pattern of secretion of glucocorticoids caused by these interventions may permanently alter the responses of the offspring to stress. An intervention that is widely used is neonatal handling, which consists of removing the pups from the nest for a brief period of time. Thus, there is an increase in maternal care and their pups, when adults, exhibit endocrine, biochemical and behavioral changes that persist throughout the animal's life, including less fear in new environments and greater consumption of palatable foods.

Since there is a strong relationship between stress, neonatal handling and feeding behavior, the aim of this study was to investigate the effects of these three factors on behavioral parameters (anxiety and locomotion), oxidative stress in brain structures (prefrontal cortex and hippocampus) and on plasma glucose of young Wistar male rats. Nests of Wistar rats were handled (10 min/day) or not (control group) on days 1–10 after birth. Males from these groups were subdivided in subgroups: stressed by isolation in childhood (pre-puberty), having access to a high palatable diet or standard lab chow concomitant to stress for 7 days. Rats receiving

high palatable diet consumed more food, more calories, gained more weight and had a greater plasma glucose level, but had lower caloric efficiency than standard chow groups. Handling decreased diet's effect toward body weight gain and food consumption on the first day. Palatable diet also increased time in central area in the open field apparatus and time in open arms in the elevated plus maze. Antioxidant enzymes activities were mainly altered by handling and diet in the two brain structures analyzed, and these factors had opposite effects in most cases, with handling increasing and palatable diet decreasing these enzymes activities. The anxiety-like effect of isolation was counterbalanced by the access to a palatable diet and, in a lesser extent, by neonatal handling. The use of both these conditions, however, does not appear to bring additional protection against the effects of stress in this particular period of life, the pre-puberty.

## Introdução

### Estresse

Quando o corpo é desafiado física ou psicologicamente, o organismo responde com alterações fisiológicas para restabelecer a homeostase, promovendo adaptação e sobrevivência em curto prazo. No entanto, se esses desafios persistirem por longos períodos de tempo, os mesmos sistemas de resposta fisiológica podem causar danos e agravar processos de doença a longo prazo (McEwen, 2000). A relação entre estes aspectos paradoxais desses sistemas de resposta ao estresse é conhecida desde 1936, quando Hans Selye introduziu o conceito de estresse.

Este cientista definiu o estresse como a "Síndrome da Adaptação Geral", ou seja, a resposta adaptativa de um organismo à ação de agentes nocivos – os chamados agentes estressores. A resposta ao estresse seria dividida em três estágios: (1) alarme, onde o agente estressor seria notado; (2) resistência, no qual o organismo estaria combatendo o agente estressor com sucesso; e, por fim, (3) um estado de exaustão, onde o organismo esgotaria sua capacidade de resposta de estresse, daí advindo os seus efeitos deletérios (Selye, 1936, Kopin, 1995).

Assim, a palavra "estresse" tem sido interpretada como o conjunto de respostas do organismo a um estressor. Esse estressor pode ser tanto um desafio ao indivíduo, que potencialmente pode perturbar a homeostase, como também ser apenas uma interpretação inadequada da situação, percebida erroneamente como ameaça, que resulta em uma resposta comportamental e/ou hormonal (McEwen, 2002; Tsigos, 2002).

### Sistemas de Resposta ao Estresse

A resposta ao estresse compreende uma cascata de respostas adaptativas originadas no Sistema Nervoso Central (SNC) e na periferia. Isso causa mudanças temporárias físicas, psicológicas e comportamentais no organismo, afetando, entre outros aspectos, o sistema imune, o metabolismo, o apetite e o comportamento alimentar (Chrousos, 1992).

Há dois sistemas de resposta ao estresse classicamente descritos: (a) o sistema neurovegetativo, com liberação de catecolaminas (adrenalina) pela medula adrenal; e

(b) o eixo hipotálamo-hipófise-adrenal (HHA), com liberação dos glicocorticóides, produzidos no córtex da adrenal sob estímulo hipotalâmico e hipofisário (McEwen, 2002; Tsigos, 2002). A resposta ao estresse leva a alterações comportamentais e metabólicas, num esforço de manter a homeostasia corporal e aumentar as chances de sobrevivência. As alterações geradas vão depender do tipo do estresse, da intensidade, da duração e da idade do animal.

A ativação aguda desses sistemas promove respostas clássicas de "luta ou fuga", sendo altamente adaptativas. Nessas circunstâncias, a energia gasta em atividades rotineiras, como ingestão de alimentos, digestão e reprodução, deve ser direcionada a outras funções. Assim, parte da resposta estereotipada ao estresse agudo inclui a supressão do apetite e da ingestão de alimentos (Adam, 2007). Além disso, para lutar ou fugir, o organismo precisa de energia. Como conseqüência, há uma mobilização de estoques energéticos através de glicogenólise, lipólise e catabolismo protéico, aumento da atenção e vigilância, inibição da função gonadal, aumento da taxa de batimentos cardíacos e pressão sangüínea, direcionamento do fluxo sanguíneo para órgãos-alvo (como cérebro, músculo esquelético e coração), para que o organismo supere a situação aversiva e continue vivo (Tsigos, 2002; Majzoub, 2006). Entretanto, quando há uma exposição crônica a situações estressoras ou o controle inadequado das respostas ao estresse, ocorre um aumento nos níveis basais de glicocorticóides circulantes, podendo ser danoso ao organismo (Miller, 2002; Dallman, 2004).

O ambiente social é uma fonte de estresse, tanto para humanos, quanto para os roedores, em especial durante a puberdade. Nesse sentido, muitos estudos em roedores têm mostrado que o isolamento por um longo período de tempo, que abrange a puberdade, tem impacto no comportamento, na "emocionalidade" e na reatividade ao estresse dos adultos (Douglas, 2004; Weiss, 2004; McCormick, 2007). No ambiente natural, esses roedores vivem em grupo e exibem altos níveis de comportamento social, tanto com animais mais novos, quanto mais velhos do que eles (Panksepp, 2007). As interações sociais são gratificantes para os roedores (Panksepp, 2007), enquanto o isolamento social é um evento aversivo e aumenta a atividade do eixo HHA (Douglas, 2004; McCormick, 2007).

**Eixo Hipotálamo-Hipófise-Adrenal (HHA)**

Após a percepção do agente estressor, ocorre a ativação do eixo HHA. Ela inicia com a secreção do hormônio liberador de corticotropina (CRH) do núcleo paraventricular do hipotálamo, que estimula a síntese e liberação do hormônio adrenocorticotrópico (ACTH) da hipófise anterior para a circulação sangüínea. O ACTH, por sua vez, estimula a secreção de glicocorticóides (cortisol em humanos e corticosterona em ratos) pelo córtex da adrenal (Lupien, 2005), como pode ser visualizado na Figura 1 abaixo:

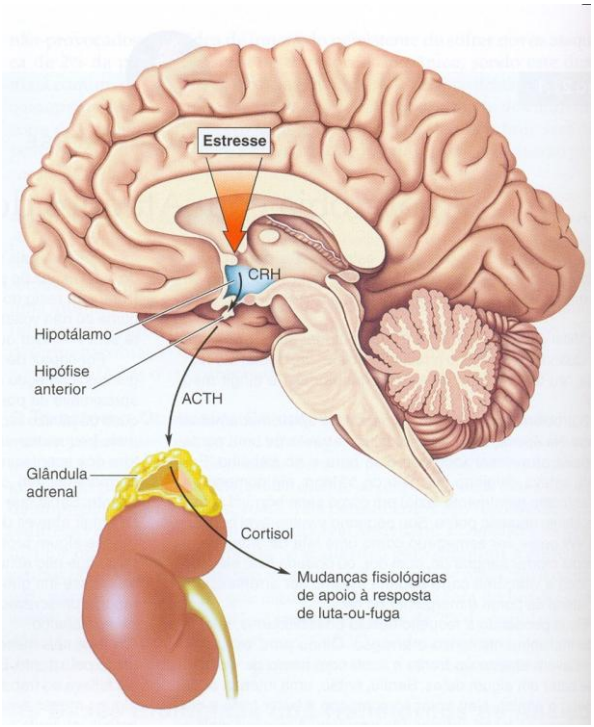



**Figura 1:** Eixo Hipotálamo-Hipófise-Adrenal (HHA). Estímulos ambientais externos são captados pelo sistema límbico, ativando os sistemas de resposta ao estresse, entre eles o eixo HHA.

O eixo HHA é um dos principais sistemas de controle da resposta aos estressores psicológicos e psicossociais nos mamíferos (O'Brien, 1997; Sapolsky, 2000). Sinais neuronais associados ao estressor são traduzidos em uma resposta endócrina no hipotálamo. Como dito anteriormente, aumentos agudos e por tempo limitado nos níveis de glicocorticóides provocam uma resposta adaptativa. Entretanto, o aumento prolongado nos níveis desses hormônios pode ter efeitos negativos no sistema nervoso, além de outros tecidos, sendo associados a dilatação ventricular, atrofia cerebral, redução na capacidade cognitiva (O'Brien, 1997) e

possível neurotoxicidade (Sapolsky, 2000). Esses aumentos causam danos também ao hipocampo, levando a uma *down-regulation* dos receptores de glicocorticóides nessa estrutura e prejudicando sua capacidade de controlar a retroalimentação negativa. Esse ciclo vicioso de eventos é referido como a "cascata dos glicocorticóides" (Sapolsky, 1986).

O eixo HHA tem uma regulação extremamente fina no período pré e pós-natal imediato, possuindo alta plasticidade (Francis, 1999). Distúrbios no padrão normal de secreção de glicocorticóides em períodos críticos do desenvolvimento podem alterar de forma definitiva as respostas do organismo ao estresse (Levine, 1967). Interações genéticas e ambientais regulam os mecanismos neurais envolvidos no desenvolvimento de determinados comportamentos, e experiências sensoriais no início da vida pós-natal podem afetar o desenvolvimento neural e o comportamento de um animal adulto. Estímulos estressantes são algumas das influências ambientais que podem modificar o desenvolvimento neural (Gonzáles, 1990).

**Períodos Críticos do Desenvolvimento**

Existem períodos críticos no desenvolvimento dos filhotes, nos quais diversos sistemas corporais ainda não estão maduros, como o sistema nervoso central. Nesta fase da vida, o principal contato dos filhotes com o mundo exterior é a mãe, e as interações mãe-filhote (quando bem feitas) promovem um ambiente adequado para o desenvolvimento da prole, gerando adultos saudáveis.

Em ratos, as duas primeiras semanas após o nascimento representam o chamado período hiporresponsivo ao estresse (Sapolsky, 1986). Durante essa fase, a resposta do eixo HHA a estímulos nocivos é reduzida (Haltmeyer, 1966; Bartova, 1968), ou seja, há uma exacerbação do mecanismo de retroalimentação negativa dos glicocorticóides na hipófise e diminuição da sensibilidade da adrenal ao hormônio adrenocorticotrópico (ACTH). Conseqüentemente, a estimulação precoce atua sobre o desenvolvimento do sistema nervoso e induz uma variedade de mudanças neuroquímicas e comportamentais no adulto.

Em condições naturais, o desenvolvimento neural em um rato ocorre tipicamente em ambiente escuro e tranqüilo, em que a maior fonte de estimulação provém da mãe e dos seus companheiros de ninho (Caldji, 1998). Entretanto, diversos estudos mostram que a manipulação pós-natal ou outra estimulação do

animal nesse período alteram o comportamento da mãe com seus filhotes, causando mudanças na interação desta com a prole (Caldji, 1998; Francis, 1999; Pryce, 2001; Parent, 2008; Litvin, 2010; Walker, 2010).

**Manipulação Neonatal**

A estimulação neonatal em ratos consiste tipicamente na "manipulação" dos filhotes por alguns minutos no período que abrange as primeiras duas semanas de vida. Dessa forma, a relação mãe-filhote é alterada: as mães de filhotes manipulados lambem mais a sua prole do que mães de filhotes não manipulados (Liu, 1997; Pryce, 2001). Este comportamento da mãe em relação ao filhote promove uma série de respostas comportamentais e fisiológicas na prole, que incluem mudanças na temperatura corporal, na locomoção, na freqüência cardíaca e na reação emocional. A manipulação neonatal também aumenta a expressão de receptores para CRH e $\alpha_2$-adrenorreceptores na amígdala e no locus ceruleus, regiões importantes para a resposta ao medo (Caldji, 1998), assim como de receptores de glicocorticóides no hipocampo e no córtex frontal, envolvidos na regulação da atividade do eixo HHA (Francis, 1996).

Contudo, os níveis basais de corticosterona de animais manipulados e não manipulados não diferem entre si quando adultos, mas as diferenças entre eles parecem ser devido a uma sensibilidade diferencial do sistema nervoso central ao mecanismo de retroalimentação negativa da adrenal (Levine, 1994). A maior concentração de receptores de glicocorticóides no hipocampo de animais manipulados promove um aumento da inibição mediada por esta estrutura cerebral e uma diminuição da excitação mediada pela amígdala na resposta neuroendócrina do eixo HHA nos animais que sofreram estresse neonatal (de Kloet, 1998). Desse modo, na idade adulta, esses animais apresentam uma resposta menos acentuada da secreção de glicocorticóides pela adrenal quando expostos a estímulos estressores (Meaney, 1991; Levine, 1993).

As conseqüências da manipulação neonatal na vida adulta desses filhotes envolvem uma série de alterações comportamentais e endócrinas, incluindo uma diminuição do medo a novos ambientes, com diminuição do comportamento de congelamento e aumento da atividade exploratória (Levine, 1967), além de aumentarem a ingestão de alimentos palatáveis (Silveira, 2004).

## Comportamento Alimentar

O comportamento alimentar envolve complexos mecanismos que incluem aspectos homeostáticos (demanda calórica do organismo), hedônicos e cognitivos. A via homeostática aumenta a motivação para comer em função do balanço energético, sendo ativada quando ocorre a depleção dos estoques de gordura. Substratos energéticos presentes no sangue são importantes reguladores da via homeostática, que também pode ser influenciada pela disponibilidade de nutrientes (Ely, 1997). Por outro lado, a via hedônica, ou regulação baseada na recompensa, pode sobrepor-se à via homeostática durante períodos de relativa abundância de energia, por aumentar o desejo para o consumo de alimentos altamente palatáveis.

Alimentos palatáveis, também chamados "alimentos confortantes", são uma recompensa natural e possuem propriedades motivadoras potentes, sendo utilizados em várias tarefas comportamentais como reforço. A ingestão de soluções de sacarose promove um aumento na liberação de dopamina no núcleo accumbens, córtex pré-frontal e hipotálamo, estruturas relacionadas com o comportamento alimentar (Papaioannou, 2002; Hajnal, 2004; Adam, 2007). Dados da literatura sugerem que é necessária a integridade das vias de dopamina, serotonina e opióides para uma resposta adequada ao estímulo da presença de alimentos (Blundell, 1991). Também foram relatadas relações importantes entre os níveis de corticosterona e a quantidade ingerida de soluções adoçadas, mostrando que os glicocorticóides alteram a preferência e o consumo de alimentos (Dallman, 2003).

A ativação do eixo HHA apresenta grande influência sobre o comportamento alimentar. Em situações de estresse crônico, a alta concentração de glicocorticóides circulantes causa (1) aumento na expressão de RNAm do hormônio liberador de corticotropina (CRH) no núcleo central da amígdala, uma área emocional crítica do cérebro, recrutando a rede de resposta ao estresse crônico; (2) aumento da importância das atividades prazerosas ou compulsivas (ingestão de sacarose, gordura e drogas); na presença de insulina, estresse e glicocorticóides aumentam a ingestão de "alimentos confortantes"; (3) aumento dos depósitos de gordura abdominal, o que permite um aumento na sinalização dos estoques de energia abdominais para inibir a ação de catecolaminas no tronco cerebral e expressão de CRH em neurônios hipotalâmicos que regulam a liberação de ACTH. Todas essas ações visam à redução da atividade do eixo HHA, de forma a reduzir a influência das respostas ao estresse

crônico sobre efeitos comportamentais, autônomos, e neuroendócrinos (Dallman, 2003 e 2005).

**Manipulação Neonatal e o Comportamento Alimentar**

Existem evidências de que a manipulação neonatal altera o comportamento alimentar dos animais na vida adulta. Estudos anteriores do nosso laboratório demonstraram que ratos submetidos à manipulação neonatal apresentaram, na idade adulta, um maior consumo de alimento doce quando este foi oferecido por um curto período de tempo, tanto em aparato de comportamento alimentar quanto na caixa-moradia, sem que houvesse alteração no consumo de ração padrão (Silveira, 2005 e 2006) e este efeito é observado somente após a puberdade (Silveira, 2008). Nestes ratos, não foram encontradas alterações nos níveis basais de corticosterona plasmática com relação aos animais controles (Silveira, 2004). Curiosamente, quando expostos cronicamente a alimentos palatáveis, estes animais parecem ser menos vulneráveis aos efeitos metabólicos adversos de tal sobrecarga de açúcar e gordura (Benetti, 2007).

Em conjunto, estes dados sugerem que tanto a motivação para aproximar-se ou o impacto hedônico da recompensa representado pela comida saborosa podem ser afetados pelo ambiente neonatal. Considerando que o desejo por alimentos palatáveis pode estar associado a distúrbios alimentares, compreender os mecanismos pelos quais estes animais buscam mais avidamente este tipo de alimento pode eventualmente contribuir para a forma de abordagem de distúrbios alimentares.

**Estresse e o Comportamento Alimentar**

O comportamento alimentar pode ser alterado por diferentes fatores, dentre eles o estresse (Ely, 1997). Os hormônios liberados em resposta ao estresse podem afetar o apetite de diferentes formas. A noradrenalina (Halford, 2001) e o hormônio liberador de corticotropina (CRH) têm sido relatados como supressores do apetite frente ao estresse, enquanto o cortisol estimula o apetite durante a recuperação do estresse (Takeda, 2004). Estudos têm mostrado que o aumento na ingestão de alimentos palatáveis, quando há estímulo crônico do eixo HHA e aumento na

liberação de glicocorticóides, seria baseado no sistema de reforço-recompensa encefálico (Adam, 2007).

O aumento nos níveis de glicocorticóides causa mudanças no metabolismo do animal, alterando a produção de insulina, leptina, grelina e neuropeptídeo Y, hormônios envolvidos com o comportamento alimentar. Dessa forma, estes alimentos confortantes conseguiriam reduzir a resposta do animal ao estresse (Pecoraro, 2004). Isso mostra que o eixo HHA não só é um condutor das respostas ao estresse, como também está intimamente relacionado com a regulação endócrina do apetite (Adam, 2007), e esse tipo de ação é considerado uma forma de o organismo repor as energias gastas durante o período em que foi submetido ao estresse.

Alguns estudos em humanos mostraram que indivíduos que eram altamente reativos ao estresse ingeriam mais calorias e que esse comportamento estava associado com o comportamento compulsivo (Freeman, 2004; Epel, 2009). Em concordância com tais estudos, trabalhos usando ratos adultos mostraram que, se esses animais fossem submetidos a um estresse crônico, poderia ocorrer um aumento da ingestão de alimentos palatáveis (Ely, 1997; Pecoraro, 2004; Silveira, 2004). Além disso, a gravidade e a duração da exposição ao estressor são capazes de modificar diferentemente o comportamento alimentar (Krolow, 2010).

Por sua vez, a atividade do eixo HHA também pode ser influenciada pelo tipo de alimento consumido. Uma dieta hipercalórica, rica em alimento doce e gordura, pode levar a uma redução da resposta do eixo ao estresse (Pecoraro, 2004), sugerindo um efeito metabólico periférico da dieta sobre o cérebro (Dallman, 2003). Entretanto, dietas palatáveis podem aumentar os níveis de glicocorticóides basais e induzidos por estresse, possivelmente agindo como um fator estressor *per se* (Tannembaum, 1997; Kamara, 1998).

**Estresse Oxidativo**

O desequilíbrio na homeostase derivado do estresse pode ser sentido pela célula de diversas maneiras. Dentre elas, um importante sistema é o potencial redox. Qualquer sinalização que induza alguma espécie de dano ou estresse a organelas, como afetar o potencial de membrana da mitocôndria ou gerar estresse do retículo endoplasmático rugoso, causa um aumento na produção de espécies reativas de

oxigênio (ERO), além de afetar os sistemas de defesas antioxidantes (Ceccatelli, 2007).

Radicais livres e ERO são constantemente formadas no organismo como parte normal e essencial em processos biológicos (Halliwell, 1994; Murphy, 2011). Porém, quando em excesso, causam dano oxidativo a biomoléculas e estruturas celulares. Se, além do aumento da produção de ERO, há também uma diminuição nas defesas antioxidantes, temos o estado de estresse oxidativo. Assim, as defesas antioxidantes enzimáticas, tais como a superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx), e defesas não-enzimáticas (vitaminas, glutationa) não são capazes de neutralizar espécies reativas de forma eficiente (Halliwell, 2007). Como conseqüência, as proteínas celulares, lipídios e DNA podem ser danificados (Cochrane, 1991).

Como já mencionado, após a exposição a eventos estressores, há aumento dos níveis de glicocorticóides circulantes. Se esse aumento persiste por um longo tempo (por exemplo, estressores crônicos), efeitos deletérios começam a aparecer causando danos ao organismo como um todo, especialmente ao sistema nervoso, e têm sido relacionados com um aumento na geração de ERO (McIntosh, 1996). O cérebro é especialmente vulnerável a danos causados pelos radicais livres por causa de seu alto consumo de oxigênio, abundante teor de lipídios e relativa escassez de enzimas antioxidantes (Olanow, 1992; Halliwell, 2007). Já foi demonstrado que o estresse oxidativo está envolvido na patogênese de muitas doenças (Metodiewa, 2000; Gutteridge, 2000), incluindo aquelas causadas pelo estresse diário (McEwen, 2007).

Não se sabe ao certo se o estresse oxidativo é causa ou conseqüência dos efeitos deletérios decorrentes do estresse psicoemocional, mas ele existe e é encontrado em diversas estruturas e tecidos após eventos estressores. Além disso, os efeitos do estresse oxidativo sobre as células são bem conhecidos, culminando com a morte celular quando não revertido (Epel, 2004 e 2009). Isso pode explicar a degeneração encontrada no SNC após longos períodos de exposição ao estresse (McEwen, 2000, Ceccatelli, 2007).

## Objetivo

Visto que existe uma forte relação entre estresse, manipulação neonatal e comportamento alimentar, o objetivo deste estudo foi investigar os efeitos desses três fatores sobre parâmetros (ansiedade e locomoção), estresse oxidativo em estruturas encefálicas (córtex pré-frontal e hipocampo) e glicose plasmática em ratos Wistar jovens manipulados no período neonatal, estressados por isolamento na infância (pré-puberdade) e com acesso a uma dieta de alta palatabilidade concomitante ao estresse. Nossa hipótese é que a manipulação no período neonatal, por reduzir a resposta ao estresse, poderá prevenir os efeitos da exposição ao estresse no período pré-púbere. Além disso, uma vez que o acesso a uma dieta palatável é capaz de reduzir a resposta ao estresse, também teorizamos que esse acesso poderia contrapor-se aos efeitos do isolamento sobre a ansiedade e o estresse oxidativo em estruturas cerebrais.

**Artigo submetido na revista "Physiology & Behavior"**

# EFFECTS OF EARLY LIFE INTERVENTIONS AND PALATABLE DIET ON ANXIETY AND ON OXIDATIVE STRESS IN YOUNG RATS

Marina de Lima Marcolin [a], André de Noronha Dantas Benitz [a], Danusa Mar Arcego [a,b], Cristie Noschang [a,b], Rachel Krolow [a,b], Carla Dalmaz [a,b]

[a] Laboratório de Neurobiologia do Estresse, Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, RS, Brasil

[b] Programa de Pós-Graduação em Ciências Biológicas – Bioquímica, Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, RS, Brasil

Mailing address: Departamento de Bioquímica, ICBS, UFRGS
Ramiro Barcelos, 2600 (Anexo) Lab: 37.
CEP: 90035-003 Porto Alegre, RS, Brasil
Phone: 55 51- 3308-5570.
Fax: 55 51- 3308-5535.
E-mail: carladalmaz@yahoo.com.br

## Abstract

Early life events can change biochemical, endocrine and behavioral aspects throughout the life of the animal. Since there is a strong relationship between stress, neonatal handling and feeding behavior, the aim of this study was to investigate the effects of these three factors on behavioral parameters (anxiety and locomotion), oxidative stress in brain structures (prefrontal cortex and hippocampus) and on plasma glucose. Nests of Wistar rats were handled (10 min/day) or not (control group) on days 1–10 after birth. Males from these groups were subdivided in subgroups: stressed by isolation in childhood (pre-puberty), having access to a high palatable diet or standard lab chow concomitant to stress for 7 days. Rats receiving high palatable diet consumed more food, more calories, gained more weight and had a greater plasma glucose level, but had lower caloric efficiency than standard chow groups. Handling decreased diet's effect toward body weight gain and food

consumption on the first day. Palatable diet also increased time in central area in the open field apparatus and time in open arms in the elevated plus maze. Antioxidant enzymes activities were mainly altered by handling and diet in the two brain structures analyzed, and these factors had opposite effects in most cases, with handling increasing and palatable diet decreasing these enzymes activities. The anxiety-like effect of isolation was counterbalanced by the access to a palatable diet and, in a lesser extent, by neonatal handling. The use of both these conditions, however, does not appear to bring additional protection against the effects of stress in this particular period of life, the pre-puberty.

**Key-words:** anxiety, high palatable diet, isolation stress, neonatal handling, oxidative stress, pre-puberty

## 1. Introduction

There are critical periods in the development of offspring in which several body systems are not yet mature [1], and the brain is particularly susceptible to environmental influences that occur in early life [2–6]. Interventions in the neonatal period may influence the mother's relationship with the pups, affecting the development of the offspring nervous system and modifying biochemical, endocrine and behavioral aspects throughout the animal's life [7–10].

Experimental models have been developed to study the effects of interventions in early life and its consequences. One such model is the neonatal handling, a brief, repeated and seemingly harmless separation of pups from their mothers. When the pups return to their home cage, there is an increase of maternal care [11,12], which can cause, among other things, a permanent modulation of the hypothalamic-pituitary-adrenal (HPA) of the offspring. The HPA axis is one of the most important neuroendocrine systems that are activated in response to real or perceived challenges of the environment [13]. As adults, handled pups have less fear in new environments, elicited by a decrease in freezing behavior and increased exploration activity [7]. Also, it was observed in these animals an increased intake of palatable foods [14] and a smaller increase in the secretion of glucocorticoids by the adrenal gland in response to stressors, when compared to non-handled animals [15].

During the pre-puberty and adolescence periods, neuronal rearrangements occur and the neural circuitry is structurally remodeled resulting in fine connectivity and functionality of brain regions in adulthood [16,17]. In addition, some studies support the hypothesis that the adolescent brain is particularly vulnerable to stress and the prefrontal cortex would be more susceptible during this period [17]. The social environment is a source of stress, both for humans and for rodents, especially during puberty. Nevertheless, many studies in rodents have shown that isolation for a period of time, covering puberty, have an impact on behavior, emotionality and stress reactivity in adults [18–20]. In their natural environment, these rodents live in groups and exhibit high levels of social behavior, both with younger and older animals [21]. Social interactions are rewarding to rodents [22], while social isolation is an aversive event and increases the activity of the HPA axis [18,20].

The feeding behavior involves complex mechanisms that include caloric demand of the body as well as hedonic and cognitive aspects [23–26]. Moreover, it can be changed by different factors such as nutrient availability and stress [27]. The hormones released in response to stress may affect appetite in different ways. Norepinephrine and corticotropin-releasing hormone (CRH) have been reported as appetite suppressants facing stress [28], whereas cortisol stimulates the appetite during recovery from stress [29]. Studies have shown that increased intake of palatable foods in response to a release of glucocorticoids would be based on a reward-reinforcement system [23]. Thus, these comfort foods could reduce the animal's response to stress [30].

After exposure to stressful events, there are increased levels of circulating glucocorticoids. If this increase persists for a long time (e.g., chronic stressors), deleterious effects begin to appear, damaging the body as a whole, especially the nervous system. These deleterious effects have been reported to increase the generation of reactive oxygen species (ROS) [31]. The brain is especially vulnerable to free radical production and to oxidative damage because of its high oxygen consumption, abundant lipid content and a relative paucity of antioxidant enzymes [32,33]. Although ROS participate in normal physiological processes, when in excess they can cause oxidative damage to biomolecules and cellular structures. If, in addition to the increased production of ROS, there is also a decrease in antioxidant defenses, a state of oxidative stress develops, which is involved in the pathogenesis of many diseases, including those caused by everyday stress [34].

Since there is a strong relationship between stress, neonatal handling and feeding behavior, the aim of this study was to investigate the effects of these three factors on behavioral parameters (anxiety and locomotion) and oxidative stress in brain structures. In addition, we measured a metabolic parameter (plasma glucose) in young Wistar rats neonatally handled and stressed by isolation in childhood (pre-puberty), which had access to a high palatable diet concomitant to stress. Our hypothesis was that neonatal handling and the access to a palatable diet could protect against consequences of stress exposure.

## 2. Materials and Methods

### 2.1. Subjects

All animal proceedings were approved by the Institutional Ethical Committee and followed the recommendations of the International Council for Laboratory Animal Science (ICLAS) and of the Federation of Brazilian Societies for Experimental Biology. All efforts were done to minimize animal suffering as well as to reduce the number of animals.

Fourteen pregnant Wistar rats bred at our own animal facility were randomly selected on gestational day 18, and housed alone in home cages made of Plexiglas (65x25x15 cm) with the floor covered with sawdust and were maintained in a controlled environment: lights on between 07:00 h and 19:00 h, temperature of 22 ± 2°C, cage cleaning twice a week, food and water provided *ad libitum*. The day of birth was considered day 0 and the litters were culled in 8 pups within 24 hours.

The litters were divided in two groups: handled and non-handled. The neonatal handling occurred between days 1-10 after birth, between 11:00 h and 14:00 h, during 10 minutes/day [14]. Once during this period, dirty sawdust was carefully removed from one side of the cage, without disturbing the mother and the nest, and replaced by clean sawdust at that side by the main researcher.

Litters were weaned on postnatal day 21. Only the male offspring were used in this study. Male pups were weighed and distributed into four groups, in such a way that only one animal per litter was used in each group, with 5-8 animals/group. The following groups were used: (1) receiving standard lab chow, and not stressed; (2) receiving a high palatable diet [35], and not stressed; (3) receiving standard lab chow, and stressed by isolation (one animal in a smaller home cage, 27x17x12 cm)

[18]; (4) receiving a high palatable diet, and stressed. These interventions occurred between postnatal days 21-28 and the daily food consumption was measured [36].

At postnatal day 28, the food was removed from the cages at the beginning of light cycle. Two hours later, two behavioral tests were performed consecutively: Open Field [37] and Plus Maze [38]. Both tests were recorded and analyzed by computer programs.

After 8 hours of fasting, the animals were weighted again and killed by decapitation. Trunk blood was collected and the brain was removed, dissected (prefrontal cortex and hippocampus) and frozen at -70ºC for further analysis. Also, adrenal glands were dissected and weighed.

### 2.2. Neonatal handling

Pups of handled group were gently removed from the nest and placed in an incubator at 32ºC. The cages with the mothers remained in the same room and, after 10 minutes, pups were returned to their home cages. The researcher changed gloves for the manipulation of each litter to avoid the spread of any kind of odor from nest to nest. Pups of non-handled groups were kept with their mothers without interventions until weaning.

### 2.3. High palatable diet

The high palatable diet used in this study is enriched with simple carbohydrates, and it is made with condensed milk, sucrose, vitamins and salts mix, powder standard lab chow, purified soy protein, soy oil, water, methionine and lysine. The nutritional content of this diet is similar to that of a standard lab chow (including 22% protein and 4-6% fat), however most carbohydrates in the palatable diet were sucrose (from condensed milk and from sucrose); in contrast, the standard lab chow had carbohydrates mainly from starch. The palatable diet was made at postnatal day 20 and the pellets were daily switched.

### 2.4. Food consumption

Previously weighed amounts of standard lab chow and high palatable diet were offered and the remaining amount was measured each day to evaluate the consumption. The food consumption was measured per cage and, in the control

cages, the amount of food consumed was divided by the number of animals per cage to determine mean consumption per animal.

### 2.5. Caloric intake and caloric efficiency

To verify the amount of kilocalories consumed per animal, we multiplied the amount of food ingested by the caloric content per gram of chow or diet. The standard lab chow has a caloric content of 3.24 kcal/g, whereas the high palatable diet has 4.5 kcal/g (being 38% more caloric than the standard chow). And to verify gain weight by energy intake, we calculated the caloric efficiency dividing the weight gain in milligrams by the total amount of kilocalories consumed in the period.

### 2.6. Behavioral tests

#### 2.6.1. Open Field

The open field consisted of an open wooden arena (60x40 cm) with 12 equally divided squares measuring 15x13,3 cm. Fifty centimeter high walls bordered the field. The frontal wall was made of glass, which allows the animal's observation by the researcher. The behavioral test was conducted in an observational room using red light illumination. The animals were observed for 5 min and the locomotion activity (number of line crossings), rearing (standing upright on the hind legs) and time of grooming were analyzed. After these 5 minutes, the animals were placed in the Plus Maze apparatus.

#### 2.6.2. Plus Maze

The elevated plus maze apparatus was made of wood and consisted of two opposed open arms (48,5x10 cm), two opposed enclosed arms with no roof (48,5x9,5x49 cm), and an open square (13x10 cm) in the center. The maze was elevated 50 cm above the floor. The behavioral test was conducted in the same observational room using red light illumination. The animal was placed in the center of the plus maze, facing one of the open arms, and remained in the apparatus for 5 min. The number of entries and the time spent in the open or enclosed arms, frequency of head dipping, rearings and time grooming were analyzed.

### 2.7. Biochemical analysis

For determination of antioxidant enzymes activities in prefrontal cortex and hippocampus, these brain structures were homogenized in 10 volume (w:v) ice-cold 50 mM potassium phosphate buffer (pH 7.4), containing 1 mM EDTA. The homogenate was centrifuged at 960 x g for 10 min at 4ºC and the supernatant was used. For determination of plasma glucose and lipid profile, the trunk blood was collected into tubes with EDTA and centrifuged at 960 x g at 4ºC for 10 minutes. The plasma was separated and frozen at -70ºC for further analysis.

### 2.7.1. Superoxide dismutase activity

SOD activity was determined using a RANSOD kit (Randox Labs., USA). This method employs xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a formazan dye that is assayed spectrophotometrically at 492 nm at 37ºC. The inhibition in production of the chromogen is proportional to the activity of SOD present in the sample [39]. SOD activity was expressed as U per mg of protein, one unit of SOD being defined as the amount that causes 50% inhibition of the rate of reduction of INT under the conditions of the assay.

### 2.7.2. Glutathione peroxidase activity

GPx activity was determined according to Wendel [40], with modifications. The reaction was carried out at 37ºC in a solution containing 20 mM potassium phosphate buffer (pH 7.7), 1.1 mM EDTA, 0.44 mM sodium azide, 0.5 mM NADPH, 2 mM glutathione and 0.4 U glutathione reductase. The activity of GPx was measured taking tertbutylhydroperoxide as the substrate at 340 nm. The contribution of spontaneous NADPH oxidation was always subtracted from the overall reaction ratio. GPx activity was expressed as nmol NADPH oxidized per minute per mg protein.

### 2.7.3. Catalase activity

CAT is an enzyme that degrades hydrogen peroxide ($H_2O_2$), and its activity assessment is based upon establishing the rate of $H_2O_2$ degradation spectrophotometrically at 240 nm at 25ºC [41]. CAT activity was calculated in terms of µmol of $H_2O_2$ consumed per minute per mg of protein, using a molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$.

### 2.7.4. Total thiol content

Total thiol content was determined spectrophotometrically based in the reduction of 5,5 '-dithiobis-2-nitrobenzoic acid (DTNB) by thiol groups, which become oxidized (disulfide), yielding a yellow compound (TNB) whose absorption is measured at 412 nm [42].

### 2.7.5. Protein assay

The total protein concentrations were determined by the Lowry method with bovine serum albumin as the standard [43], and it was used to normalize previous analysis.

### 2.7.6. Plasma levels of glucose

Plasma glucose was measured using commercial kit from Wiener, Rosario, Argentina [36].

## 2.8. Statistical analysis

For food consumption, statistical analysis was performed by repeated measures ANOVA (time was the within subjects factor; between subjects factors were handling, stress and diet). For the other analysis, a three-way ANOVA was performed, with handling, diet and stress as factors. Data were expressed as mean ± standard error of mean and significance was given by $p<0.05$.

# 3. Results

## 3.1. Body and adrenal glands weight

The body weight of all animals increased with time [$F(1,46)=1609.23$, $p<0.005$] (data not shown). At day 21, no difference was found between groups, but at day 28, groups receiving palatable diet had higher body weights, compared to groups receiving chow [$F(1,46)=10.05$, $p<0.005$]. Weight gain was also increased by palatable diet [$F(1,46)=26.19$, $p<0.005$] and there was an interaction between handling and diet [$F(1,46)=4.47$, $p<0.05$], since handling by itself increased weight gain (Figure 1). There was no difference regarding adrenal glands weight (data not shown).

### 3.2. Food consumption

Over the seven days, the consumption of all groups increased with time [$F(1,20)=25.93$, $p<0.001$] (data not shown), and groups receiving high palatable diet had higher consumption than groups receiving standard lab chow [$F(1,26)=17.79$, $p<0.0005$] (Figure 2). During the first day of exposure to the palatable diet, when analyzing only isolated animals, we observed the effect of handling during the neonatal period [$F(1,22)=5.03$, $p<0.05$] in addition to diet's effect [$F(1,22)=6.7$, $p<0.05$], since handling increased consumption when compared to non-handled animals (Figure 3).

### 3.3. Caloric intake and caloric efficiency

Caloric intake was increased by exposure to the palatable diet [$F(1,46)=277.4$, $p<0.001$] (Figure 4). However, despite the increase in weight gain and in caloric intake, caloric efficiency was decreased by diet [$F(1,46)=32.9$, $p<0.001$] (Figure 5).

### 3.4. Behavioral tests

#### 3.4.1. Open Field

Time in the central squares of the Open Field was increased by exposure to the palatable diet [$F(1,46)=6.568$, $p<0.05$] (Figure 6), but no difference was found in total crossings, crossings in the peripheral area, time for the first crossing, frequency of rearings and time of grooming (Table 1).

#### 3.4.2. Plus Maze

Time in the open arms of the Plus Maze was decreased by stress [$F(1,38)=5.224$, $p<0.05$], increased by palatable diet [$F(1,38)=6.589$, $p<0.05$] and there was an interaction between handling and stress [$F(1,38)=5.816$, $p<0.05$], with handling reducing the effect of stress (Figure 7). Stress also decreased frequency of head dipping [$F(1,46)=12.362$, $p<0.001$]. Time of grooming was increased by stress [$F(1,46)=9.089$, $p<0.005$] and decreased by handling [$F(1,46)=5.001$, $p<0.05$]. No difference was found in frequency of rearings (Table 2).

### 3.5. Biochemical measures

#### 3.5.1. Prefrontal Cortex

An interaction was found between handling and diet on SOD activity [$F(1,36)=8.382$, $p<0.01$] and on SOD/GPx ratio [$F(1,34)=11.637$, $p<0.005$], since handling only increased them in chow groups, not groups receiving palatable diet. CAT activity was increased by diet [$F(1,43)=4.481$, $p<0.05$] and there was an interaction between stress and diet on GPx activity, since stress only increased it in chow groups [$F(1,37)=4.627$, $p<0.05$]. Total thiol content was decreased by handling [$F(1,31)=5.004$, $p<0.05$] and increased by diet [$F(1,31)=8.995$, $p<0.001$]. No difference was found in SOD/CAT ratio (Table 3).

#### 3.5.2. Hippocampus

SOD activity was increased by handling [$F(1,39)=8.366$, $p<0.01$] and decreased by diet [$F(1,39)=8.673$, $p<0.01$]. There was an interaction between handling and diet [$F(1,36)=9.143$, $p<0.005$] and another between the three variables [$F(1,36)=7.496$, $p<0.01$] on CAT activity. Diet decreased GPx activity [$F(1,37)=9.251$, $p<0.005$] and SOD/CAT ratio [$F(1,34)=4.93$, $p<0.05$], and in both there were interactions between handling and diet [$F(1,37)=6.432$, $p<0.05$ and $F(1,34)=4.27$, $p<0.05$, respectively], with handling accentuating diet's effect. SOD/GPx ratio was increased by handling [$F(1,34)=9.33$, $p<0.05$] and no difference was found on total thiol content (Table 4).

#### 3.5.3. Plasma glucose

Glucose level was increased by diet [$F(1,37)=12.81$, $p< 0.001$] (Figure 8).

## 4. Discussion

Juvenile animals receiving a high palatable diet showed increased caloric intake, and gained more weight at the end of the week. However, their caloric efficiency was lower than groups receiving standard lab chow. These results suggest that, even consuming more calories, groups receiving this diet did not gain as much weight as expected. Therefore, this excess of calories was possibly used in other processes, such as increased basal metabolism. Another study of our group [36]

using adult animals receiving chocolate (rich in fat and sugar) did not observed reduced caloric efficiency, and this difference could be related to the age of the animals (in the present study we used animals in the pre-puberty phase). The type of palatable food used may also be important, as well as the fact that the stress in that study was chronic (50 days), and it is known that chronically stressed animals do not show the same behavior and do not experience the consequences that animals exposed to acute or sub acute stress do [27,44–46]. Furthermore, we observed that when animals are isolated, they decrease consumption in the first day, which increases over time. This decreased consumption may reflect the stress of being isolated. Also in the first day of isolation, besides the effect of palatable diet (increasing consumption), a handling effect appears, since handled animals showed a different pattern of consumption, suggesting that these animals are less susceptible to stress consequences. This observation agrees with reports from the literature showing reduced stress response in handled adults [13,15]. It is important to consider that the present results were observed before puberty.

Both elevated plus-maze and open field task have been used to assess neurobehavioral profiles of animals under the influence of anxiogenic/anxiolytic agents [47,48]. In the present study, the open field task was used to assess locomotion capacity and anxiety-like behavior. The results suggest that the access to a high palatable diet during the pre-puberty period had an anxiolytic effect (since it increased time in central squares of the open field and time in the open arms of the plus maze apparatus), while stress (isolation) was anxiogenic. Therefore, such diet seems to have properties of "comfort foods", as postulated in the literature [30,49]. These results agree with the hypothesis that a reward-based eating would decrease the response to stress when animals have access to a palatable diet [23,30]. Also, the high palatable diet had no influence over crossings and rearings in open field, which is consistent with Souza [35]. Therefore, it is possible that exposure to social stress during the pre-puberty period may lead to increased consumption of food rich in carbohydrates and fat, contributing to the increasing epidemic of obesity which has been observed also in children.

It is well established that neonatally handled rats exhibit in adulthood decreased stress response [7,10,50] facing both acute [51] and chronic stress situations [50,52]. When adults, handled rats show less fear in novel environments, a greater exploratory behavior and lower anxiety [44,53–55]. Those results were not

observed in juveniles, since handling by itself had no effect on anxiety or locomotion. On the other hand, it partially prevented stress-induced anxiogenic effects in the plus maze apparatus, thus adding further support to the suggested protective effect of handling on some adverse environmental effects.

Several evidence point to a relation between anxiety-like behavior and oxidative stress [56–59], and some studies have suggested that psychosocial stress may lead to alteration in some cellular processes, which may cause oxidative stress [60–62]. The brain is especially vulnerable to free radical production and to oxidative damage because of its high oxygen consumption, abundant lipid content and a relative paucity of antioxidant enzymes [32,33,63]. Excessive sugar intake, leading to an elevated level of plasmatic glucose, has been considered the main source of free radicals production in glucose intolerant situations [35]. An overload of glucose can block the flux of electrons transport chain, generating elevated levels of reactive nitrogen and oxygen species [64,65]. In this study, antioxidant enzyme activities were mainly influenced by handling and diet, with opposed effects; in most cases, handling increased and diet decreased activities. The isolation stress only affected cortical GPx and hippocampal CAT. A previous study of our group found no difference between handled and non-handled animals on the antioxidant enzyme activities of handled animals [66], but the analyses were made in adult animals, instead of in young ones, as is the case of this study. The present results suggest that neonatal interventions affect antioxidant enzyme activities and the access to a palatable diet tends to reduce this effect. However, since no measurements of damage to macromolecules and production of free radicals were made, it is not possible to conclude if there is a state of oxidative stress, or if it is involved in behavioral changes found in this study.

Since food rich in sugar may cause deleterious effects, including changes on metabolic parameters, we also evaluated plasma glucose. Palatable diet increased plasma glucose, and this increase is considered a risk factor for some conditions, such as metabolic syndrome [67]. Another study of our group showed that the metabolic profile exhibited by handled adult animals suggests a particular metabolic response concerning energy storage and expenditure when exposed long term to a highly palatable diet [68], and handling has been suggested to protect these animals from risks resulting from increases in the offer of nutrients. On the other hand, our results suggest that, during infancy, handled animals do not present a profile that

would protect them from exposure to increased offer of highly energetic substrates in the form of palatable food.

In conclusion, both handling during the neonatal period and access to a high palatable diet were able to reduce the effects of stress on anxiety-like behavior generated by isolation. The use of both these conditions, however, does not appear to bring additional protection against the effects of stress in this particular period of life, the pre-puberty. This study also points to the importance of the previous life history of the animal when studying behavioral and physiological disturbances. Understanding how these factors (interventions and the type of diet during development) affect brain and behavior can help to elucidate the pathophysiological mechanisms related to eating disorders.

**Acknowledgments:** CNPq, PRONEX-FAPERGS.

**References**

[1] Knudsen EI. Sensitive periods in the development of the brain and behavior. J Cogn Neurosci. 2004; 16(8):1412–25.

[2] Singer W. Development and plasticity of cortical processing architectures. Science. 1995; 270(5237):758–64.

[3] Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. Science. 1996; 274(5290):1133–8.

[4] Grossman AW, Churchill JD, McKinney BC, Kodish IM, Otte SL, Greenough WT. Experience effects on brain development: possible contributions to psychopathology. J Child Psychol Psychiatry. 2003; 44(1):33–63.

[5] Hensch TK. Critical period mechanisms in developing visual cortex. Curr Top Dev Biol. 2005; 69:215–37.

[6] Friederici AD. The neural basis of language development and its impairment. Neuron. 2006; 52(6):941–52.

[7] Levine S, Haltmeyer GC, Karas GG, Denenberg VH. Physiological and behavioral effects of infantile stimulation. Physiol Behav. 1967; 2(1):55–9.

[8] Levine S. Infantile experience and resistance to physiological stress. Science. 1957; 126(3270):405.

[9] Meaney MJ, Diorio J, Francis D, Widdowson J, LaPlante P, Caldji C, et al. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. Dev Neurosci. 1996; 18(1-2):49–72.

[10] Padoin MJ, Cadore LP, Gomes CM, Barros HM, Lucion AB. Long-lasting effects of neonatal stimulation on the behavior of rats. Behav Neurosci. 2001; 115(6):1332–40.

[11] Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science. 1997; 277(5332):1659–62.

[12] Pryce CR, Bettschen D, Feldon J. Comparison of the effects of early handling and early deprivation on maternal care in the rat. Dev Psychobiol. 2001; 38(4):239–51.

[13] Levine S. The ontogeny of the hypothalamic-pituitary-adrenal axis: the influence of maternal factors. Ann N Y Acad Sci. 1994; 746:275–88.

[14] Silveira PP, Portella AK, Clemente Z, Bassani E, Tabajara AS, Gamaro GD, et al. Neonatal handling alters feeding behavior of adult rats. Physiol Behav. 2004; 80(5):739–45.

[15] Meaney MJ, Mitchell JB, Aitken DH, Bhatnagar S, Bodnoff SR, Iny LJ, et al. The effects of neonatal handling on the developmental of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. Psychoneuroendocrinology. 1991; 16(1-3):85–103.

[16] Andersen, SL. Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev. 2003; 27(1-2):3–18.

[17] Buwalda B, Geerdink M, Vidal J, Koolhaas JM. Social behavior and social stress in adolescence: a focus on animal models. Neurosci Biobehav Rev. 2011; 35(8):1713–21.

[18] Douglas L, Varlinskaya E, Spear LP. Rewarding properties of social interactions in adolescent and adult male and female rates: impact of social versus isolate housing of subjects and partners. Dev Psychobiol. 2004; 45(3):153–62.

[19] Weiss IC, Pryce CR, Jongen-Rêlo AL, Nanz-Bahr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res. 2004; 152(2):279–95.

[20] McCormick CM, Mathews IZ. HPA functions in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. Pharmacol Biochem Behav. 2007; 86(2):220–33.

[21] Panksepp JB, Jochman KA, Kim JU, Koy JJ, Wilson ED, Chen Q, et al. Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. PLoS One. 2007; 2(4):e351.

[22] Panksepp JB, Lahvis GP. Social reward among juvenile mice. Genes Brain Behav. 2007; 6(7):661–71.

[23] Adam TC, Epel ES. Stress, eating and the reward system. Physiol Behav. 2007; 91(4):449–58.

[24] Finlayson, G, Bryant JE, Blundell JE, King, NA. Acute compensatory eating following exercise is associated with implicit hedonic wanting for food. Physiol Behav. 2009; 97(1):62–7.

[25] Krause M, German PW, Taha SA, Fields HL. A pause in nucleus accumbens neuron firing is required to initiate and maintain feeding. J Neurosci. 2010; 30(13):4746–56.

[26] Joseph RJ, Alonso-Alonso M, Bond DS, Pascual-Leone A, Blackburn GL. The neurocognitive connection between physical activity and eating behaviour. Obes Rev. 2011, 12(10):800–12.

[27] Ely DR, Dapper V, Marasca J, Corrêa JB, Gamaro GD, Xavier MH, et al. Effect of restraint stress on feeding behavior of rats. Physiol Behav. 1997; 61(3): 395–8.

[28] Halford JC. Pharmacology of appetite suppression: implication for the treatment of obesity. Curr Drug Targets. 2001; 2(4):353–70.

[29] Takeda E, Terao J, Nakaya Y, Miyamoto K, Baba Y, Chuman H, et al. Stress control and human nutrition. J Med Invest. 2004; 51(3-4):139–45.

[30] Pecoraro NC, Reyes F, Gomez F, Bhargava A, Dallman MF. Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. Endocrinology. 2004; 145(8):3754–62.

[31] McIntosh LJ, Sapolsky RM. Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin-induce toxicity in neuronal culture. Exp Neurol. 1996; 141(2):201–6.

[32] Olanow CW. An introduction to the free radical hypothesis in Parkinson's disease. Ann Neurol. 1992; 32 Suppl:S2–9.

[33] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th ed. Oxford University Press, Oxford; 2007.

[34] Gutteridge JMC, Halliwell B. Free radicals and antioxidants in the year 2000. A historical look to the future. Ann N Y Acad Scis. 2000; 899:136–47.

[35] Souza CG, Moreira JD, Siqueira IR, Pereira AG, Rieger DK, Souza DO, et al. High palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. Life Sci. 2007; 81(3):198–203.

[36] Fachin A, Krolow R, Noschang, CG, Pettenuzzo LF, Bertinetti L, Billodre MN, et al. Stress effects on rats chronically receiving a highly palatable diet are sex-specific. Appetite. 2008; 51(3):592–8.

[37] Silveira, PP, Portella AK, Clemente Z, Gamaro G.D, Dalmaz C. The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior. Int J Dev Neurosci. 2005; 23(1):93–9.

[38] Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav. 1986; 24(3):525–9.

[39] Delmas-Beauvieux MC, Peuchant E, Dumon MF, Receveur MC, Le Bras M, Clerc M. Relationship between red blood cell antioxidant enzymatic system status and lipoperoxidation during the acute phase of malaria. Clin Biochem. 1995; 28(2):163–9.

[40] Wendel A. Glutathione peroxidase. Methods Enzymol. 1981; 77:325–33.

[41] Aebi H. Catalase *in vitro*. Methods in Enzymol. 1984; 105:121–6.

[42] Aksenov MY, Markesbery WR. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett. 2001; 302(2-3):141–5.

[43] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265–75.

[44] D'Aquila PS, Brain P, Willner P. Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. Physiol Behav. 1994; 56(5):861–7.

[45] MacNeil G, Sela Y, McIntosh J, Zacharko RM. Anxiogenic behavior in the light-dark paradigm following intraventricular administration of

cholecystokinin-8S, restraint stress, or uncontrollable foot shock in the CD-1 mouse. Pharmacol Biochem Behav. 1997; 58(3):737–46.

[46] Morilak DA, Cecchi M, Khoshbouei H. Interactions of norepinephrine and galanin in the central amygdala and lateral bed nucleus of the stria terminalis modulate the behavioral response to acute stress. Life Sci. 2003; 73(6):715–26.

[47] Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 2003; 463(1-3):3–33.

[48] Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev. 2005; 29(8):1193–205.

[49] Dallman MF, Pecoraro NC, Akana SF, la Fleur SE, Gomez F, Houshyar H, et al. Chronic stress and obesity: a new view of "comfort food". PNAS. 2003; 100(20):11696–701.

[50] Panagiotaropoulos T, Papaioannou A, Pondiki S, Prokopiou A, Stylianopoulou F, Gerozissis K. Effect of neonatal handling and sex on basal and chronic stress- induced corticosterone and leptin secretion. Neuroendocrinology. 2004; 79(2):109–18.

[51] Meaney MJ, Aitken DH, Viau V, Sharma S, Sarrieau A. Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. Neuroendocrinology. 1989; 50(5):597– 604.

[52] Ladd CO, Thrivikraman KV, Huot RL, Plotsky PM. Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates. Psychoneuroendocrinology. 2005; 30(6):520–33.

[53] Ferré P, Núñez JF, García E, Tobeña A, Escorihuela RM, Fernández-Teruel A. Postnatal handling reduces anxiety as measured by emotionality rating and hyponeophagia tests in female rats. Pharmacol Biochem Behav. 1995; 51(2-3):199–203.

[54] McIntosh J, Anisman H, Merali Z. Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects. Dev Brain Res. 1999; 113(1-2):97–106.

[55] Severino GS, Fossati IAM, Padoin MJ, Gomes CM, Trevizan L, Sanvitto GL, et al. Effects of neonatal handling on the behavior and prolactin stress response in male and female rats at various ages and estrous cycle phases of females. Physiol Behav. 2004; 81(3):489–98.

[56] Rammal H, Bouayed J, Younos C, Soulimani R. Evidence that oxidative stress is linked to anxiety-related behaviour in mice. Brain Behav Immun. 2008; 22(8):1156–9.

[57] Hovatta I, Juhila J, Donner J. Oxidative stress in anxiety and comorbid disorders. Neurosci Res. 2010; 68(4): 61–75.

[58] Salim S, Asghar M, Chugh G, Taneja M, Xia Z, Saha K. Oxidative stress: A potential recipe for anxiety, hypertension and insulin resistance. Brain Res. 2010; 1359:178–85.

[59] Salim S, Asghar M, Taneja M, Hovatta I, Chugh G, Vollert C, et al. Potential contribution of oxidative stress and inflammation to anxiety and hypertension. Brain Res. 2011; 1404:63–71.

[60] Mällo T, Matrov D, Köiv K, Harro J. Effect of chronic stress on behavior and cerebral oxidative metabolism in rats with high or low positive affect. Neuroscience. 2009; 164(3):963–74.

[61] Bouayed J, Rammal H, Soulimani R. Oxidative stress and anxiety: relationship and cellular pathways. Oxid Med Cell Longev. 2009; 2(2):63–7.

[62] Vollert C, Zagaar M, Hovatta I, Taneja M, Vu A, Dao A, et al. Exercise prevents sleep deprivation-associated anxiety-like behavior in rats: Potential role of oxidative stress mechanisms. Behav Brain Res. 2011; 224(2):233–40.

[63] Metodiewa D, Kóska C. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders. An overview. Neurotox Res. 2000; 1(3):197–233.

[64] Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000; 404(6779):787–90.

[65] Brownlee, M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005; 54(6):1615–25.

[66] Noschang GC, Krolow R, Fontella FU, Arcego DM, Diehl LA, Weis SN, et al. Neonatal handling impairs spatial memory and leads to altered nitric oxide

production and DNA breaks in a sex specific manner. Neurochem Res. 2010; 35(7):1083–91.

[67] Kyrou I, Chrousos GP, Tsigos C. Stress, visceral obesity, and metabolic complications. Ann N Y Acad Sci. 2006; 1083:77–110.

[68] Benetti CS, Silveira PP, Portella AK, Diehl LA, Nunes E, de Oliveira VS, et al. Could preference for palatable foods in neonatally handled rats alter metabolic patterns in adult life? Pediatr Res. 2007; 62(4):405–11.

**Table 1.** Effect of neonatal handling, isolation and palatable diet on behavior in the Open Field

| Groups | | | Total crossings | Crossings in peripheral area | Time for first crossing | Frequency of rearings | Time of grooming |
|---|---|---|---|---|---|---|---|
| **Non-handling** | **Standard chow** | **Non-stress** | 112.71 ± 11.43 | 103.57 ± 10.80 | 3.08 ± 0.68 | 42.00 ± 6.85 | 10.15 ± 1.56 |
| | | **Stress** | 97.67 ± 6.87 | 89.00 ± 6.22 | 6.35 ± 2.40 | 44.17 ± 4.20 | 6.66 ± 0.96 |
| | **Palatable diet** | **Non-stress** | 113.14 ± 6.32 | 101.71 ± 5.52 | 5.70 ± 1.57 | 45.14 ± 4.37 | 7.12 ± 1.91 |
| | | **Stress** | 117.60 ± 6.38 | 106.40 ± 6.99 | 6.02 ± 0.88 | 48.80 ± 7.27 | 6.45 ± 2.52 |
| **Handling** | **Standard chow** | **Non-stress** | 96.71 ± 9.93 | 87.14 ± 9.23 | 8.80 ± 1.49 | 53.43 ± 4.85 | 7.01 ± 2.71 |
| | | **Stress** | 119.50 ± 5.52 | 109.00 ± 5.68 | 5.56 ± 0.86 | 54.88 ± 2.97 | 6.68 ± 2.07 |
| | **Palatable diet** | **Non-stress** | 112.71 ± 7.69 | 100.57 ± 6.35 | 6.91 ± 1.77 | 50.57 ± 4.43 | 4.80 ± 1.51 |
| | | **Stress** | 114.71 ± 5.39 | 102.29 ± 4.96 | 8.37 ± 2.63 | 49.57 ± 5.34 | 6.35 ± 1.64 |

Data expressed as mean ± S.E.M. N= 5-8/group. Three-way ANOVA showed no difference was found in total crossings, crossings in the peripheral squares, time for the first crossing, frequency of rearings and time of grooming ($p>0.05$).

**Table 2.** Effect of neonatal handling, isolation and palatable diet on behavior in the Plus Maze

| Groups | | | Frequency of head dipping | Frequency of rearings | Time of grooming |
|---|---|---|---|---|---|
| **Non-handling** | **Standard chow** | **Non-stress** | 9.71 ± 3.50 | 15.57 ± 2.96 | 19.61 ± 5.62 |
| | | **Stress** | 5.67 ± 2.59 | 15.50 ± 3.63 | 19.25 ± 6.62 |
| | **Palatable diet** | **Non-stress** | 18.00 ± 4.33 | 14.14 ± 1.40 | 8.59 ± 3.49 |
| | | **Stress** | 4.00 ± 1.64 | 18.00 ± 3.29 | 33.12 ± 11.05 |
| **Handling** | **Standard chow** | **Non-stress** | 14.29 ± 2.66 | 17.14 ± 2.58 | 5.53 ± 2.75 |
| | | **Stress** | 8.63 ± 2.00 | 22.88 ± 2.46 | 10.61 ± 4.63 |
| | **Palatable diet** | **Non-stress** | 18.14 ± 4.13 | 18.57 ± 3.20 | 9.63 ± 2.26 |
| | | **Stress** | 10.43 ± 2.43 | 16.86 ± 1.67 | 12.07 ± 2.83 |

Data expressed as mean ± S.E.M. N= 5-8/group. Three-way ANOVA showed a significant effect of stress on frequency of head dipping ($p<0.001$), on time of grooming ($p<0.005$) and effect of handling on time of grooming ($p<0.05$). No difference was found in frequency of rearings ($p>0.05$).

**Table 3.** Effect of neonatal handling, isolation and palatable diet on biochemical measures in prefrontal cortex

| Groups | | | SOD | CAT | GPx | SOD/CAT | SOD/GPx | Thiol |
|---|---|---|---|---|---|---|---|---|
| Non-handling | Standard chow | Non-stress | 7.35 ± 1.69 | 2.78 ± 0.22 | 22.24 ± 1.26 | 2.54 ± 0.46 | 0.32 ± 0.06 | 60.05 ± 2.38 |
| | | Stress | 7.93 ± 1.56 | 3.65 ± 0.63 | 25.35 ± 1.56 | 2.57 ± 0.66 | 0.31 ± 0.05 | 64.33 ± 3.53 |
| | Palatable diet | Non-stress | 9.66 ± 3.28 | 4.61 ± 0.51 | 22.36 ± 3.26 | 2.04 ± 0.56 | 0.36 ± 0.09 | 68.87 ± 8.45 |
| | | Stress | 8.35 ± 2.38 | 3.88 ± 0.54 | 17.39 ± 3.37 | 2.82 ± 1.26 | 0.46 ± 0.08 | 66.27 ± 10.12 |
| Handling | Standard chow | Non-stress | 11.52 ± 0.74 | 3.70 ± 0.19 | 21.69 ± 2.59 | 3.14 ± 0.22 | 0.59 ± 0.09 | 58.56 ± 3.50 |
| | | Stress | 12.79 ± 2.53 | 3.46 ± 0.40 | 23.92 ± 0.88 | 3.76 ± 0.68 | 0.53 ± 0.09 | 60.12 ± 3.25 |
| | Palatable diet | Non-stress | 8.54 ± 1.31 | 4.07 ± 0.42 | 22.78 ± 1.53 | 2.33 ± 0.56 | 0.35 ± 0.05 | 65.58 ± 2.87 |
| | | Stress | 7.92 ± 1.43 | 3.45 ± 0.23 | 22.32 ± 1.32 | 2.26 ± 0.34 | 0.34 ± 0.05 | 58.01 ± 3.31 |

Data expressed as mean ± S.E.M of SOD (U/mg protein), CAT (μmol $H_2O_2$ consumed/ min/mg protein) and GPx (nmol NADPH oxidized/min/mg protein) activity and total thiol content (nmol TNB/mg de protein). N= 5-8/group. Three-way ANOVA showed an interaction between handling and diet on SOD activity ($p<0.01$) and on SOD/GPx ratio ($p<0.005$), diet effect on CAT activity ($p<0.05$), interaction between stress and diet on GPx activity ($p<0.05$), handling and diet effect on total thiol content ($p<0.05$ and $p<0.001$, respectively) and no difference on SOD/CAT ratio ($p>0.05$).

**Table 4.** Effect of neonatal handling, isolation and palatable diet on biochemical measures in hippocampus

| Groups | | | SOD | CAT | GPx | SOD/CAT | SOD/GPx | Thiol |
|---|---|---|---|---|---|---|---|---|
| Non-handling | Standard chow | Non-stress | 5.64 ± 0.73 | 3.80 ± 0.43 | 17.39 ± 0.68 | 2.05 ± 0.22 | 0.42 ± 0.03 | 52.69 ± 2.98 |
| | | Stress | 5.32 ± 0.84 | 4.48 ± 1.29 | 16.64 ± 0.85 | 2.17 ± 0.42 | 0.44 ± 0.04 | 52.57 ± 4.81 |
| | Palatable diet | Non-stress | 4.60 ± 0.90 | 3.51 ± 0.78 | 15.53 ± 1.03 | 2.07 ± 0.48 | 0.36 ± 0.02 | 52.83 ± 4.17 |
| | | Stress | 4.86 ± 0.86 | 4.39 ± 0.69 | 16.57 ± 1.13 | 1.73 ± 0.52 | 0.38 ± 0.03 | 52.32 ± 3.61 |
| Handling | Standard chow | Non-stress | 11.03 ± 1.15 | 4.50 ± 1.08 | 19.67 ± 1.69 | 2.81 ± 0.35 | 0.57 ± 0.05 | 52.63 ± 4.56 |
| | | Stress | 6.52 ± 0.69 | 3.23 ± 0.32 | 17.06 ± 1.00 | 2.78 ± 0.38 | 0.49 ± 0.05 | 53.25 ± 4.96 |
| | Palatable diet | Non-stress | 5.61 ± 1.19 | 5.15 ± 0.72 | 15.24 ± 0.60 | 1.49 ± 0.18 | 0.46 ± 0.06 | 52.18 ± 2.48 |
| | | Stress | 5.18 ± 1.27 | 4.65 ± 1.42 | 13.61 ± 1.18 | 1.78 ± 0.32 | 0.47 ± 0.08 | 50.83 ± 3.53 |

Data expressed as mean ± S.E.M of SOD (U/mg protein), CAT (μmol $H_2O_2$ consumed/ min/mg protein) and GPx (nmol NADPH oxidized/min/mg protein) activity and total thiol content (nmol TNB/mg de protein). N= 5-8/group. Three-way ANOVA showed a significant effect of handling and diet on SOD activity (both $p<0.01$), an interaction between handling and diet and another one between the three variables on CAT activity ($p<0.005$ and $p<0.01$, respectively), diet effect and an interaction between handling and diet on GPx activity ($p<0.005$ and $p<0.05$ respectively) and on SOD/CAT ratio (both $p<0.05$), handling effect on SOD/GPx ratio ($p<0.05$) and no difference on thiol content ($p>0.05$).

**Legends to figures**

**Figure 1**. Body weight gain of handled (H) and stressed (Iso) animals receiving standard chow or palatable diet. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of diet ($p<0$) and an interaction between handling and diet ($p<0.05$).

**Figure 2.** Average consumption during seven days of handled (H) and stressed (Iso) animals receiving standard chow or palatable diet. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of diet ($p<0.0005$).

**Figure 3.** First day consumption of standard chow or palatable diet of handled (H) and stressed (Iso) animals. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of diet ($p<0.05$). Considering only stressed animals, there was also an effect of handling ($p<0.05$).

**Figure 4.** Caloric intake of handled (H) and stressed (Iso) animals receiving standard chow or palatable diet. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of diet ($p<0.001$).

**Figure 5.** Caloric efficiency of handled (H) and stressed (Iso) animals receiving standard chow or palatable diet. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of diet ($p<0.001$).

**Figure 6.**Time in central squares in the open field apparatus of handled (H) and stressed (Iso) animals receiving standard chow or palatable diet. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of diet ($p<0.05$).

**Figure 7.** Time in open arms in the plus maze apparatus of handled (H) and stressed (Iso) animals receiving standard chow or palatable diet. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of stress ($p<0.05$), diet ($p<0.05$) and an interaction between handling and stress ($p<0.05$).

**Figure 8.** Plasma glucose of handled (H) and stressed (Iso) animals receiving standard chow or palatable diet. Data expressed as mean ± S.E.M. Three-way ANOVA showed a significant effect of diet ($p$<0.001)

**Figure 1**

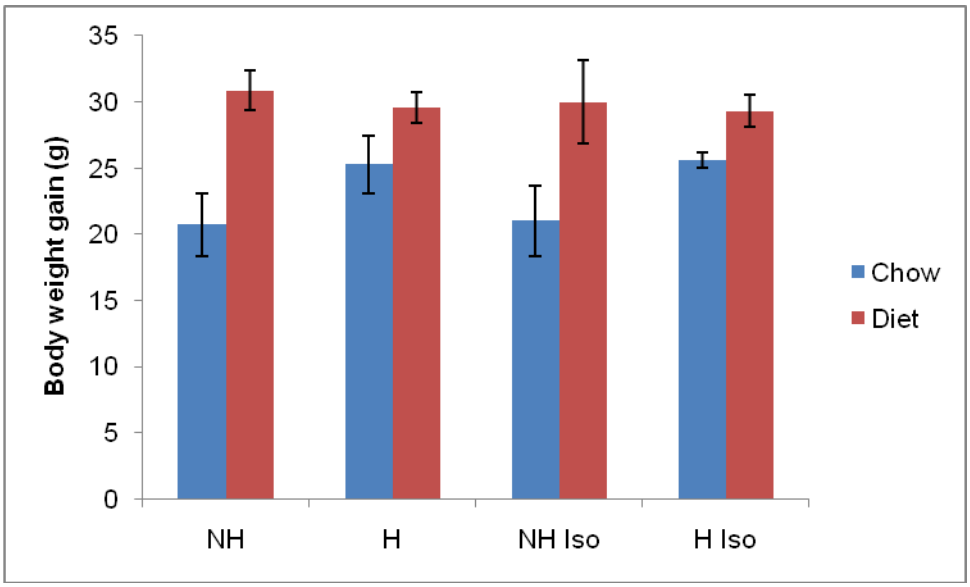



**Figure 2**

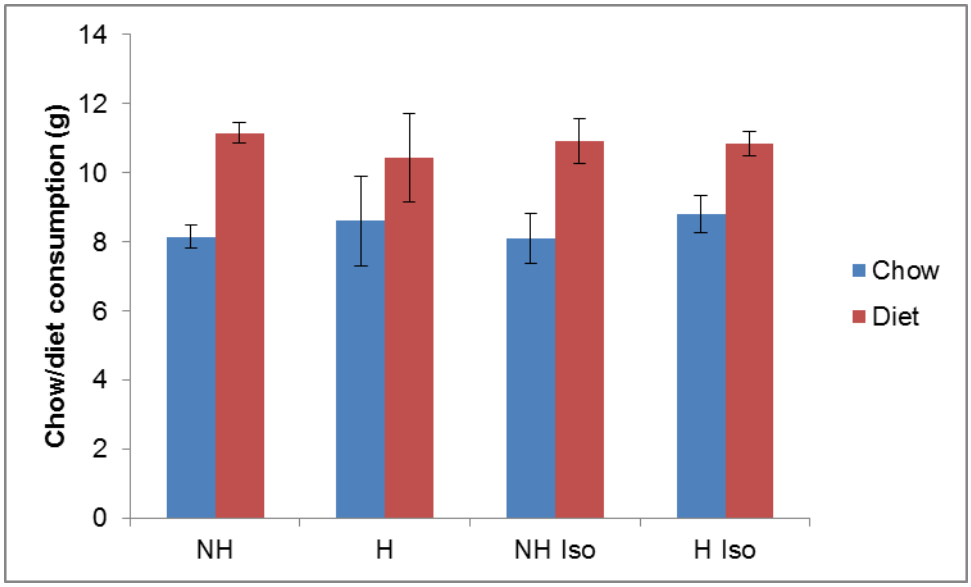


**Figure 3**

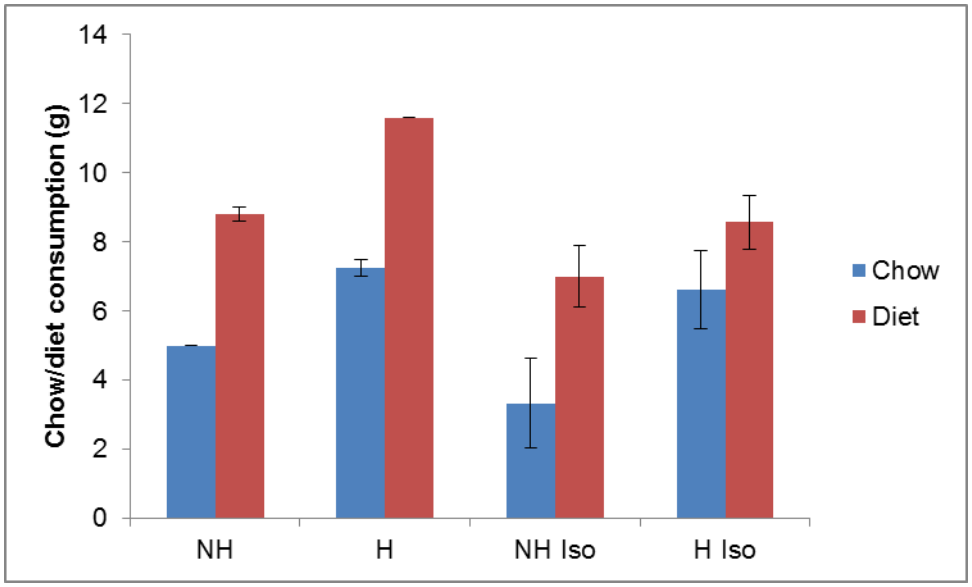



**Figure 4**

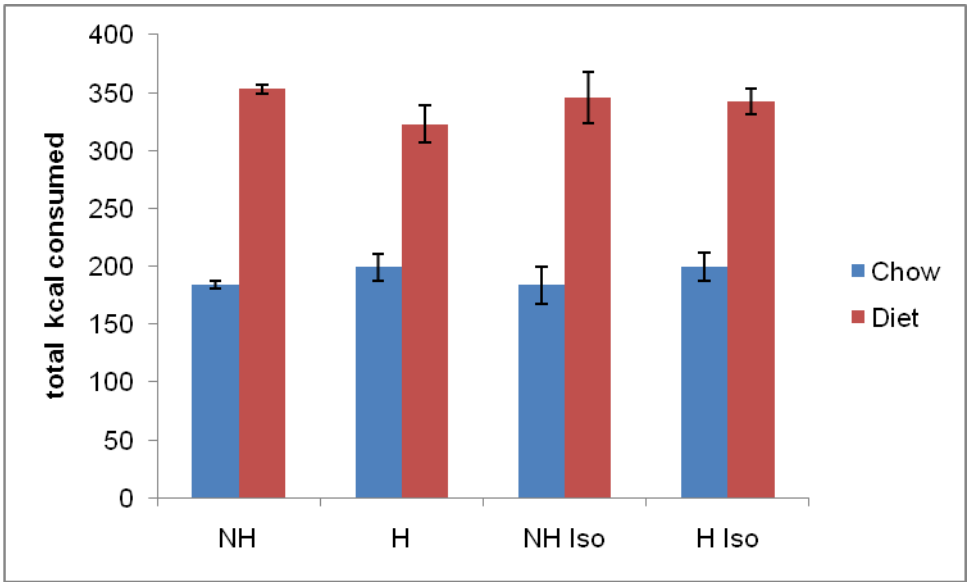


**Figure 5**

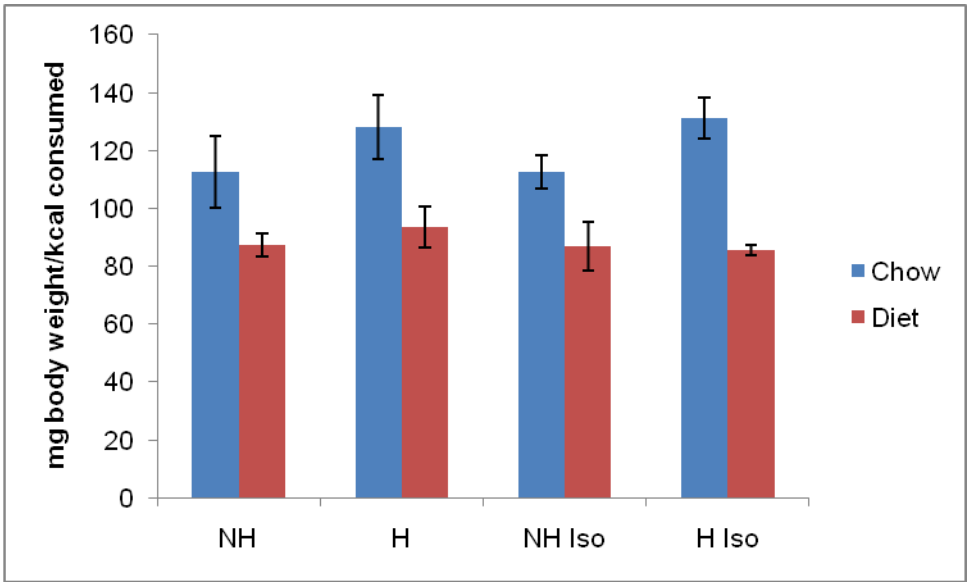



**Figure 6**

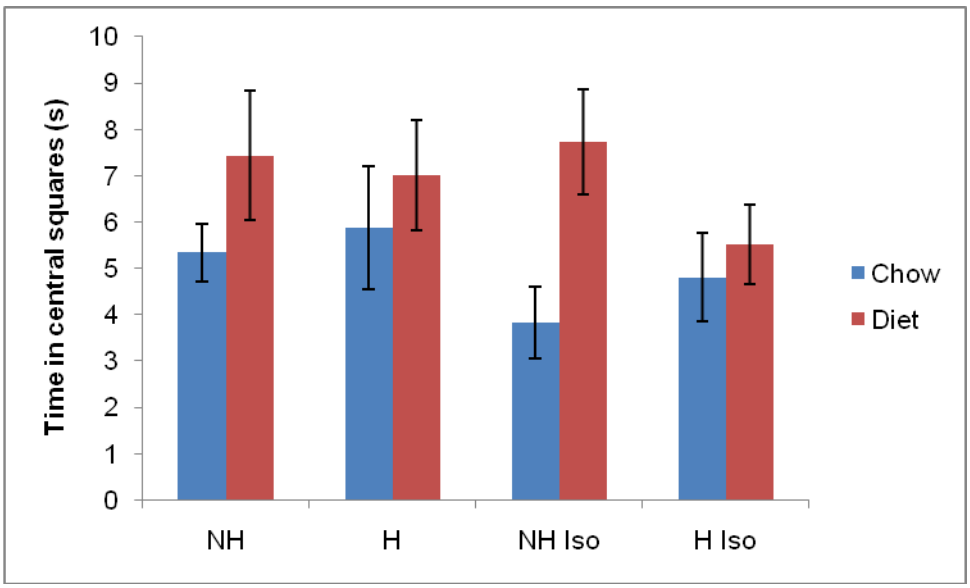


**Figure 7**

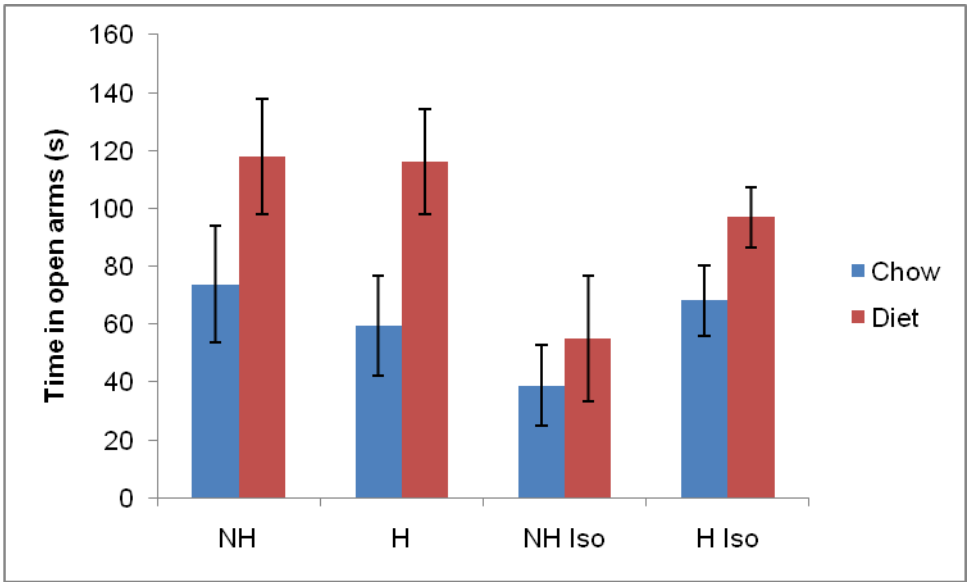
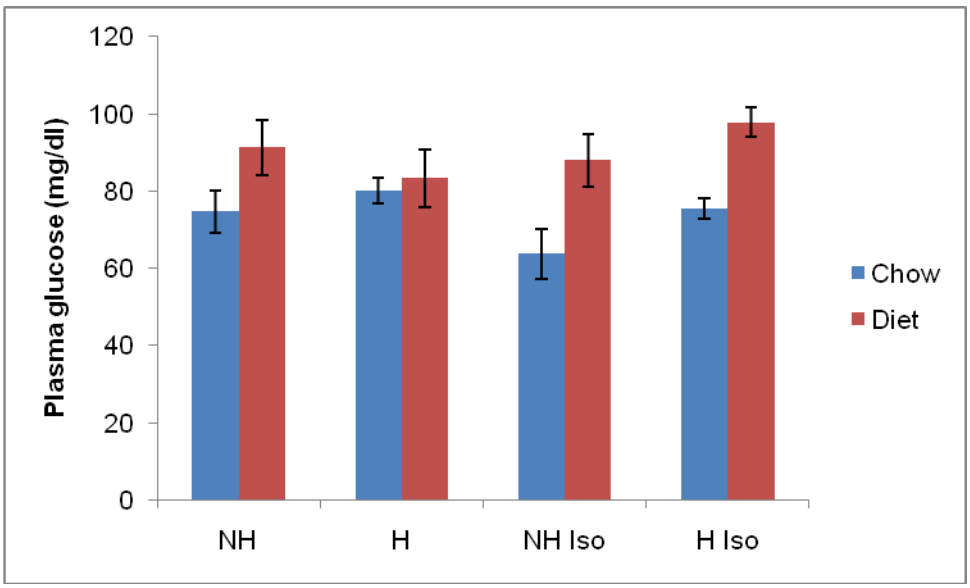



**Figure 8**



## Conclusão

Mesmo consumindo mais calorias e ganhando mais peso, os grupos que receberam dieta palatável não ganharam tanto peso quanto esperado, pois tiveram uma menor eficiência calórica. Possivelmente, esse excesso de calorias foi usado em outros processos, como aumento do metabolismo basal. Como houve um aumento na glicemia causado pela dieta, nossos resultados sugerem que, durante a infância, esses animais não apresentam um perfil metabólico peculiar que poderia protegê-los da exposição a uma grande oferta de substratos altamente energéticos na forma de alimentos saborosos, como parece haver em animais adultos. No primeiro dia de isolamento, os animais que foram manipulados apresentaram um padrão diferente de consumo, sugerindo que esses animais são menos suscetíveis aos efeitos do estresse, como apontam dados da literatura.

Os resultados deste trabalho sugerem que o acesso a uma dieta palatável durante o período pré-púbere teve um efeito ansiolítico (uma vez que aumentou o tempo nos quadrados centrais do Campo Aberto e o tempo nos braços abertos do Labirinto em Cruz Elevado), enquanto que o estresse por isolamento teve efeito ansiogênico. Dessa forma, a dieta utilizada parece apresentar propriedades de "alimento confortante" como postulado na literatura. Estes resultados estão de acordo com a hipótese de que uma alimentação baseada no sistema de reforço-recompensa diminuiria a resposta ao estresse quando os animais têm acesso a uma dieta palatável. Além disso, a dieta palatável não alterou a locomoção (cruzamentos e levantamentos no Campo Aberto), de acordo com dados da literatura. Assim, é possível que a exposição ao estresse social no período pré-púbere posso levar a um aumento no consume de alimentos ricos em carboidratos e gordura, contribuindo para o aumento na epidemia de obesidade que se observa também em crianças. A manipulação durante o período neonatal não teve influência sobre a atividade exploratória, ao contrário do que se observa em animais adultos, mas preveniu parcialmente os efeitos ansiogênicos do isolamento, reforçando dados da literatura a respeito do efeito protetor da manipulação sobre eventos ambientais adversos.

Em relação aos parâmetros de estresse oxidativo, os resultados sugerem que as intervenções neonatais aumentam a atividade de enzimas antioxidantes e que o acesso a uma dieta palatável tende a reduzir este efeito. Porém, como ainda não foram feitas dosagens de dano a macromoléculas e produção de radicais livres, não é

possível dizer que há um quadro de estresse oxidativo e que este esteja envolvido com as alterações comportamentais encontradas neste estudo.

Como o efeito da exposição a uma dieta palatável é influenciado pela manipulação, este estudo também aponta para a importância da história de vida anterior do animal quando se estuda alterações comportamentais e fisiológicas. Ainda existem poucos trabalhos envolvendo manipulação neonatal e alimentos palatáveis, e os resultados encontrados variam devido aos diferentes tipos de dietas utilizadas, período de vida em que o animal é exposto e a duração da exposição ao alimento. Com o aumento constante de indivíduos obesos, estressados e com grande acesso a alimentos calóricos e palatáveis, é importante entender as relações entre o consumo, a compulsão e o estresse. Este padrão alimentar alterado pode ter origem em períodos mais precoces na vida do indivíduo, pois perturbações no início da vida podem causar alterações persistentes em sistemas importantes no controle do apetite e de resposta a situações estressoras. O eixo HHA parece ser o elemento-chave que interliga esses fatores (manipulação, estresse e dieta), e o entendimento de como eles afetam a atividade deste eixo pode ajudar a elucidar os mecanismos fisiopatológicos que dão origem e estão presentes em distúrbios alimentares.

**Perspectivas**

- Dosar o conteúdo de radicais livres pela técnica de DCF;
- Dosar nitritos, para ver se há produção de espécies reativas de nitrogênio;
- Verificar dano ao DNA celular no hipocampo e no córtex pré-frontal;
- Verificar atividade dos complexos da cadeia respiratória;
- Dosar as concentrações plasmáticas de insulina, leptina e grelina, hormônios relacionados ao comportamento alimentar;
- Verificar possíveis alterações neuroquímicas nas outras estruturas cerebrais retiradas dos animais (bulbo olfatório, hipotálamo e estriado);
- Verificar se o aumento na ingestão de dieta palatável é realmente devido a uma preferência dos animais por essa dieta; para isso, pretendemos deixá-los crescerem até os 60 dias com acesso tanto à ração padrão quanto à dieta palatável;
- Verificar sistemas dopaminérgicos, colinérgicos e serotoninérgicos;

- Procurar elucidar os mecanismos da diminuição da eficiência calórica.

**Bibliografia**


Adam TC, Epel ES. Stress, eating and the reward system. Physiol Behav. 2007; 91(4): 449–58.

Bartova, A. Functioning of the hypothalamo-pituitary-adrenal system during postnatal development in rats. Gen Comp Endocrinol. 1968; 10(2): 235–9.

Benetti CS, Silveira PP, Portella AK, Diehl LA, Nunes E, de Oliveira VS, Dalmaz C, Goldani MZ. Could preference for palatable foods in neonatally handled rats alter metabolic patterns in adult life? Pediatr Res. 2007; 62(4): 405–11.

Blundell J. Pharmacological approaches to appetite suppression. Trends Pharmacol Sci. 1991; 12(4): 147–57.

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. PNAS. 1998; 95(9): 5335–40.

Ceccatelli S, Tamm C, Zhang Q, Chen M. Mechanisms and modulation of neural cell damage induced by oxidative stress. Physiol Behav. 2007; 92(1-2): 87–92.

Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. JAMA. 1992; 267(9): 1244–52.

Cochrane, CG. Mechanisms of oxidant injury of cells. Mol Aspects Med. 1991; 12(2): 137–47.

Dallman MF, Akana SF, Laugero KD, Gomez F, Manalo S, Bell ME, Bhatnagar S. A spoonful of sugar: feedback signals of energy stores and corticosterone. Physiol Behav. 2003; 79(1): 3–12.

Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, Bell ME, Bhatnagar S, Laugero KD, Manalo S. Chronic stress and obesity: a new view of "comfort food". PNAS. 2003; 100(20): 11696–701.

Dallman MF, Akana SF, Strack AM, Scribner KS, Pecoraro NC, la Fleur SE, Houshyar H, Gomez F. Chronic stress-induced effects of corticosterone on brain: direct and indirect. Ann N Y Acad Sci. 2004. 1018: 141–50.

Dallman MF, Pecoraro NC, la Fleur SE. Chronic stress and comfort foods: self-medication and abdominal obesity. Brain Behav Immun. 2005; 19(4):275–80.

de Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. Endocr Rev. 1998; 19(3): 269–301.

Douglas L, Varlinskaya E, Spear LP. Rewarding properties of social interactions in adolescent and adult male and female rates: impact of social versus isolate housing of subjects and partners. Dev Psychobiol. 2004; 45(3): 153–62.

Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. PNAS. 2004; 101(49): 17312–5.

Epel ES. Psychological and metabolic stress: a recipe for accelerated cellular aging? Hormones. 2009; 8(1): 7–22.

Ely DR, Dapper V, Marasca J, Corrêa JB, Gamaro GD, Xavier MH, Michalowski MB, Catelli D, Rosat R, Ferreira MB, Dalmaz C. Effect of restraint stress on feeding behavior of rats. Physiol Behav. 1997; 61(3): 395–8.

Francis D, Diorio J, LaPlante P, Weaver S, Seckl JR, Meaney MJ. The role of early environmental events in regulating neuroendocrine development. Moms, pups, stress, and glucocorticoid receptors. Ann N Y Acad Sci. 1996; 794:136–52.

Francis DD, Meaney MJ. Maternal care and the development of stress responses. Curr Opin Neurobiol. 1999; 9(1): 128–34.

Freeman LM, Gil KM. Daily stress, coping and dietary restraint in binge eating. Int J Eat Disord. 2004; 36(2): 204–12.

González AS, Rodríguez Echandía EL, Cabrera R, Fóscolo MR, Fracchia LN. Neonatal chronic stress induces subsensitivity to chronic stress in adult rats. I. Effects on forced swim behavior and endocrine responses. Physiol Behav. 1990; 47(4): 735–41.

Gutteridge JMC, Halliwell B. Free radicals and antioxidants in the year 2000. A historical look to the future. Ann N Y Acad Sci. 2000; 899: 136–47.

Hajnal A, Smith GP, Norgren R. Oral sucrose stimulation increases accumbens dopamine in the rat. Am J Physiol Regul Integr Comp Physiol. 2004; 286(1):R31–7.

Halford JC. Pharmacology of appetite suppression: implication for the treatment of obesity. Curr Drug Targets. 2001; 2(4): 353–70.

Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. Environ Health Perspect. 1994; 102: Suppl 10:5–12.

Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th ed. Oxford University Press, Oxford; 2007.

Haltmeyer GC, Denenberg VH, Thatcher J, Zarrow MX. Response of the adrenal cortex of the neonatal rat after subjection to stress. Nature. 1966; 212(5068):1371–3.

Kamara K, Eskay R, Castonguay T. High-fat diets and stress responsivity. Physiol Behav. 1998; 64(1): 1–6.

Kopin IJ. Definitions of stress and sympathetic neuronal responses. Ann N Y Acad Sci. 1995; 771: 19–30.

Krolow R, Noschang CG, Arcego DM, Andreazza AC, Peres W, Gonçalves CA, Dalmaz C. Consumption of a palatable diet by chronically stressed rats prevents effects on anxiety-like behavior but increases oxidative stress in a sex-specific manner. Appetite. 2010; 55: 108–116.

Levine S. Infantile experience and resistance to physiological stress. Science. 1957; 126(3270): 405.

Levine S, Haltmeyer GC, Karas GG, Denenberg VH. Physiological and behavioral effects of infantile stimulation. Physiol Behav. 1967; 2(1): 55–9

Levine S. The psychoendocrinology of stress. Ann N Y Acad Sci. 1993; 697: 61–9.

Levine S. The ontogeny of the hypothalamic-pituitary-adrenal axis: the influence of maternal factors. Ann N Y Acad Sci. 1994; 746: 275–88.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science. 1997; 277 (5332), 1659–62.

Litvin Y, Tovote P, Pentkowski NS, Zeyda T, King LB, Vasconcellos AJ, Dunlap C, Spiess J, Blanchard DC, Blanchard RJ. Maternal separation modulates short-term behavioral and physiological indices of the stress response. Hormones and Behavior. 2010; 58(2): 241–9.

Lupien SJ, Fiocco A, Wan N, Maheu F, Lord C, Schramek T, Tu MT. Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology. 2005; 30(3):225–42.

Majzoub JA. Corticotropin-releasing hormone physiology. Eur J Endocr. 2006; 155: S71–6.

McCormick CM, Mathews IZ. HPA functions in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors.

Pharmacol Biochem Behav. 2007; 86(2): 220–33.

McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. Brain Research. 2000; 886 (1-2): 172–189.

McEwen BS. Allostasis, allostatic load, and the aging nervous system: role of excitatory amino acids and excitotoxicity. Neurochem Res. 2000; 25(9-10): 1219–31.

McEwen BS. Sex, stress and the hippocampus allostasis, allostatic load and the aging process. Neurobiology Aging. 2002; 23(5): 921–39.

McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiological Reviews. 2007; 87: 873–904.

McIntosh LJ, Sapolsky RM. Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin-induce toxicity in neuronal culture. Exp Neurol. 1996; 141(2): 201–6.

Meaney MJ, Mitchell JB, Aikten DH Bhatnagar S, Bodnoff SR, Iny LJ, Sarrieau A. The effects of neonatal handling on the developmental of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. Psychoneuroendocrinology. 1991; 16(1-3): 85–103.

Metodiewa D, Kóska C. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders. An overview. Neurotox Res. 2000; 1(3): 197–233.

Miller DB, O'Callaghan JP. Neuroendocrine aspects of the response to stress. Metabolism. 2002; 51(6 Suppl 1):5–10.

Murphy MP, Holmgren A, Larsson NG, Halliwell B, Chang CJ, Kalyanaraman B, Rhee SG, Thornalley PJ, Partridge L, Gems D, Nyström T, Belousov V, Schumacker PT, Winterbourn CC. Unraveling the biological roles of reactive oxygen species. Cell Metab. 2011; 13(4): 361–6.

O'Brien JT. The "glucocorticoid cascade" hypothesis in man. Br J Psychiatry. 1997; 170: 199–201.

Olanow CW. An introduction to the free radical hypothesis in Parkinson's disease. Ann Neurol. 1992; 32 Suppl: S2–9.

Panksepp JB, Jochman KA, Kim JU, Koy JJ, Wilson ED, Chen Q, Wilson CR, et al. Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. PLoS One. 2007; 2(4): e351.

Panksepp JB, Lahvis GP. Social reward among juvenile mice. Genes Brain Behav. 2007, 6(7): 661–71.

Papaioannou A, Dafni U, Alikaridis F, Bolaris S, Stylianopoulou F. Effects of neonatal handling on basal and stress-induced monoamine levels in the male and female rat brain. Neuroscience. 2002; 114(1): 195–206.

Parent CI, Meaney MJ. The influence of natural variations in maternal care on play fighting in the rat. Developmental Psychobiology. 2008; 50(8): 767–76.

Pecoraro NC, Reyes F, Gomez F, Bhargava A, Dallman MF. Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. Endocrinology. 2004; 145(8): 3754–62.

Pryce CR, Bettschen D, Feldon J. Comparison of the effects of early handling and early deprivation on maternal care in the rat. Dev Psychobiol. 2001; 38(4): 239–51.

Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr Rev. 1986; 7(3): 284–301.

Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res. 1986; 396(1): 64–76.

Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry. 2000; 57(10): 925–35.

Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive suppressive stimulatory and preparative actions. Endocr Rev. 2000; 21(1): 55–89.

Selye H. A Syndrome produced by diverse nocious agents. Nature. 1936; 138: 32.

Silveira PP, Portella AK, Clemente Z, Bassani E, Tabajara AS, Gamaro GD, Dantas G, Torres ILS, Lucion AB, Dalmaz C. Neonatal handling alters feeding behavior of adult rats. Physiol Behav. 2004; 80(5):739–45.

Silveira PP, Portella AK, Clemente Z, Gamaro G.D, Dalmaz C. The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior. Int J Dev Neurosci. 2005; 23(1): 93–9.

Silveira PP, da Silva Benetti C, Ayres C, Pederiva FQ, Portella AK, Lucion AB, Dalmaz C. Satiety assessment in neonatally handled rats. Behav Brain Res. 2006; 173(2): 205–10.

Silveira PP, Portella AK, Crema L, Correa M, Nieto FB, Diehl L, Lucion AB,

Dalmaz C. Both infantile stimulation and exposure to sweet food lead to an increased sweet food ingestion in adult life. Physiol Behav. 2008; 93(4-5): 877–82.

Takeda E, Terao J, Nakaya Y, Miyamoto K, Baba Y, Chuman H, et al. Stress control and human nutrition. J Med Invest. 2004; 51(3-4): 139–45.

Tannenbaum BM, Brindley DN, Tannenbaum GS, Dallman MF, McArthur MD, Meaney MJ. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. Am J Physiol. 1997; 273(6): 1168–77.

Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res. 2002; 53(4): 865–71.

Walker CD. Maternal touch and feed as critical regulators of behavioral and stress responses in the offspring. Developmental Psychobiology. 2010; 52(7): 638–50.

Weiss IC, Pryce CR, Jongen-Rêlo AL, Nanz-Bahr NI, Feldon J. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res. 2004; 152(2): 279–95.