UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS – NEUROCIÊNCIAS

EFEITOS DO ESTRESSE NEONATAL SOBRE O COMPORTAMENTO ALIMENTAR NA VIDA ADULTA EM RATOS

Dissertação de Mestrado

Patrícia Pelufo Silveira

Porto Alegre, 2004.

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As mãos de meu pai

As tuas mãos têm grossas veias como cordas azuis sobre um fundo de manchas já cor da terra
----- como são belas as tuas mãos
pelo quanto lidaram, acariciaram ou fremiram da nobre cólera dos justos...
Porque há nas tuas mãos, meu velho pai, essa beleza que se chama simplesmente vida.
E, ao entardecer, quando elas repousam nos braços da tua cadeira predileta,
uma luz parece vir de dentro delas...
Virá dessa chama que pouco a pouco, longamente, vieste alimentando na terrível solidão do mundo, como quem
junta uns gravetos e tenta acendê-los contra o vento?
Ah! Como os fizeste arder, fulgir, com o milagre das tuas mãos!
E é, ainda, a vida que transfigura as tuas mãos nodosas...
essa chama de vida ----- que transcende a própria vida ... e que os Anjos, um dia, chamarão de alma.

Mário Quintana

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ABREVIATURAS

ACTH.....Hormônio adrenocorticotrófico

AGRP.....Proteína relacionada ao gene cutia

AVP.....Vasopressina

BDZ.....Benzodiazepínicos

CART.....Peptídeo relacionado à cocaina e à anfetamina

CRH.....Hormônio liberador de corticotrofina

GABA.....Ácido gama-amino butírico

GCs.....Glicocorticóides

HPA.....Hipotálamo-hipófise-adrenal

NPY.....Neuropeptídeo Y

POMC.....Proopiomelanocortina

PVN.....Núcleo paraventricular

RNA.....Àcido ribonucleico

SNC.....Sistema nervoso central

RESUMO

O estresse neonatal leva a alterações comportamentais e neuroquímicas na vida adulta, como menor reatividade ao estresse e aumento da expressão de receptores glicocorticóides no hipocampo. Entretanto, o efeito do estresse neonatal no comportamento alimentar foi pouco estudado. O principal objetivo deste trabalho foi avaliar o comportamento alimentar de ratos submetidos à separação materna no período neonatal, e verificar se havia alteração no estado emocional destes animais que pudesse ser correlacionada com a ingestão de alimentos palatáveis. Ninhadas foram divididas em grupos de ratos intactos, separados da mãe (incubadora a 37°C, 10 min/dia) e com estimulação tátil (estímulo pela mão do experimentador, de forma ântero-posterior no dorso, 10 min/dia), durante os dias 1 a 10 pós-natal, tendo seu comportamento analisado na vida adulta. Ratos que sofreram estresse neonatal (separação materna ou estímulo tátil) apresentam maior consumo de alimentos palatáveis como o doce e o salgado na vida adulta, sem alteração na ingestão de ração padrão ou no consumo de soluções doces e salgadas. Este efeito é persistente até idades mais avançadas. Além disso, estes animais não apresentam alterações em testes comportamentais para verificar ansiedade como o labirinto em cruz elevada, claro-escuro e campo aberto. Da mesma forma, o aumento do consumo de doce não é revertido por diazepam antes do teste. Logo, o estresse neonatal modifica o padrão de preferência alimentar de ratos na vida adulta, e este comportamento não parece ser relacionado a um estado de ansiedade alterado nestes animais. É possível que outros mecanismos de saciedade e recompensa estejam envolvidos.

ABSTRACT

Neonatal stress leads to behavioral and neurochemical alterations in adult life, like decreased stress reactivity and increased expression of glicocorticoid receptor in hippocampus. Few studies have shown the effect of neonatal stress on feeding behavior. The objective of this work is to verify the alterations in feeding behavior of rats submitted to handling in the neonatal period, and if it could be related to emotional status. Nests were selected and separated in intacts, handling (37°C incubateur, 10 min/day) and handling+tactile stimulated rats (10 min/day) Procedures were performed on Days 1–10 after birth. When adults, behavioral tasks were performed. Neonatal stress (hanlding and handling+tactile stimulated rats) had increased ingestion of palatable food like sweet and savory snacks, without alteration in the ingestion of standard lab chow or in the ingestion of sweetened and salty solutions. This effect is persistent throughout oldest ages. Besides that, these animals do not present alterations in behavioral tasks that measure anxiety like plus maze test, light/dark test and open field. The increased sweet food ingestion is not reversed by diazepam administration before the test session. Neonatal stress changes the pattern of food preferences in adult life of rats, and it does not seem to be related to altered anxiety in these animals. It is possible that other mechanisms of satiety and reward may be involved.

1. INTRODUÇÃO

Com a melhoria no atendimento em sala de parto e desenvolvimento técnico e farmacológico nas UTIs neonatais, tem aumentado a sobrevida de recém-nascidos muito doentes ou prematuros (Anthony et al., 2004; Darlow et al., 2003; Harper et al., 2002). Estima-se que a prevalência de recém-nascidos prematuros ou com baixo peso situe-se por volta de 10 a 15%, variando conforme a população estudada (Kilsztajn et al., 2003; Fang et al., 1999; Spencer et al., 1999; Valero et al., 1996; Onah, 2000; Kramer et al., 2002). Este grupo também caracteriza-se por alta morbidade (Hoekstra et al., 2004; Sehgal et al., 2003; Ward et al., 2003) sendo portanto mais suscetível a situações de estresse.

Estudos de seguimento a longo prazo de crianças que sofreram estresse neonatal demonstram que intervenções adversas em períodos precoces do desenvolvimento podem levar a alterações persistentes de sistemas diversos como o nervoso (McQuillen et al., 2004), cardiovascular (Singhal et al., 2004), respiratório (Dezateux et al., 2004) e endócrino – metabólico (Soto et al., 2003). A importância desses efeitos na análise da saúde pública é de fundamental relevância epidemiológica. Entretanto, pelo tempo e pelas dificuldades inatas à realização desse tipo de seguimento em humanos, poucos centros têm conseguido produzir resultados aplicáveis e de qualidade.

Neste contexto, os modelos experimentais em animais surgem como uma alternativa para conhecer o funcionamento dos sistemas, sua morfologia e alterações frente a uso de

fármacos. Desde a década de 50 do século passado o estresse neonatal tem despertado interesse dos pesquisadores e cada vez mais elucidado a importância de um ambiente adequado durante períodos vulneráveis para a promoção do desenvolvimento saudável.

1.1 Estresse

O termo "estresse" tem sido largamente usado de várias formas. Foi introduzido por Seyle no início do século XX como uma adaptação de um conceito existente na Física - estado de tensão sobre um material antes de se partir. Este pesquisador definiu estresse como uma seqüência de reações a uma série de agressões contra a integridade física e psicológica que ameaçavam o estado de equilíbrio do organismo (homeostase). Ultimamente, a palavra "estresse" tem sido interpretada como o conjunto de respostas do organismo a um estressor.

"Estressor" é definido como um desafio ao indivíduo que perturba a homeostase e requer uma resposta fisiológica. Pode também ser apenas uma interpretação errônea da situação, percebida erroneamente como ameaça, que resulta numa resposta comportamental e/ou hormonal (McEwen, 2002; Tsigos et al., 2002).

Há dois sistemas de resposta ao estresse classicamente descritos: (a) o Sistema Vegetativo, com a liberação de adrenalina pela medula adrenal; (b) os glicocorticóides produzidos no córtex da adrenal sob estímulo hipotalâmico e hipofisário (McEwen., 2002; Tsigos et al., 2002). A ativação aguda destes sistemas promove principalmente aumento da disponibilidade de energia e melhora do fluxo sangüíneo para órgãos-alvo, sendo altamente adaptativa (Tsigos et al.., 2002). Entretanto, a exposição crônica a níveis elevados de glicocorticóides pode ser danosa ao organismo (Dallman et al., 2004a; Miller et al., 2002).

O eixo hipotálamo-hipófise-adrenal (HPA) tem uma regulação extremamente fina no período pré e pós-natal imediato, possuindo alta plasticidade (Francis et al., 1999). Distúrbios no padrão normal de secreção de glicocorticóides neste período crítico podem alterar de forma definitiva as respostas do organismo ao estresse (Levine et al., 1967).

1.2 Funcionamento do eixo hipotálamo-hipófise-adrenal

Estímulos externos têm grande impacto sobre o sistema límbico, que se relaciona com o hipotálamo. O controle central do sistema de resposta ao estresse inclui os neurônios parvocelulares do núcleo paraventricular do hipotálamo (PVN). Estas células estão sob influência de vários mecanismos intrínsecos e extrínsecos que regulam a resposta do eixo hipotálamo-hipófise-adrenal ao estresse. Aferências diretas ao PVN são provenientes principalmente da informação sensorial, promovendo respostas do eixo a ameaças reais a homeostasia, e incluem o núcleo do trato solitário, o núcleo da rafe, o órgão subfornicial, o núcleo próprio da *stria terminalis*, o tálamo e regiões hipotalâmicas que circundam o PVN. Aferências indiretas vindas do hipocampo, amígdala, córtex pré-frontal, septo lateral e tálamo ativam os mesmos neurônios parvocelulares na ausência de desafios fisiológicos francos, mas prementes (Herman et al., 2003).

No estado de repouso (basal), o hipotálamo apresenta secreção de hormônio liberador de corticotrifina (CRH) e vasopressina (AVP) de uma maneira pulsátil, com dois ou três picos por hora (Engler et al., 1989). Durante o estresse agudo, a amplitude e a freqüência destes pulsos aumenta, resultando na liberação de adrenocorticotrofina (ACTH) pela hipófise e de cortisol (corticosterona em ratos) pelo córtex da adrenal (Tsigos et al., 1994). Uma série de

situações pode estimular o hipotálamo, como por exemplo: frio, infecção, hemorragia, choque, vibração, estresse emocional e/ou social, contenção, etc. (Miller et al., 2002). Citocinas e outros mediadores de inflamação também são liberados nessas ocasiões e potenciam a ação dos vários componentes do eixo HPA.

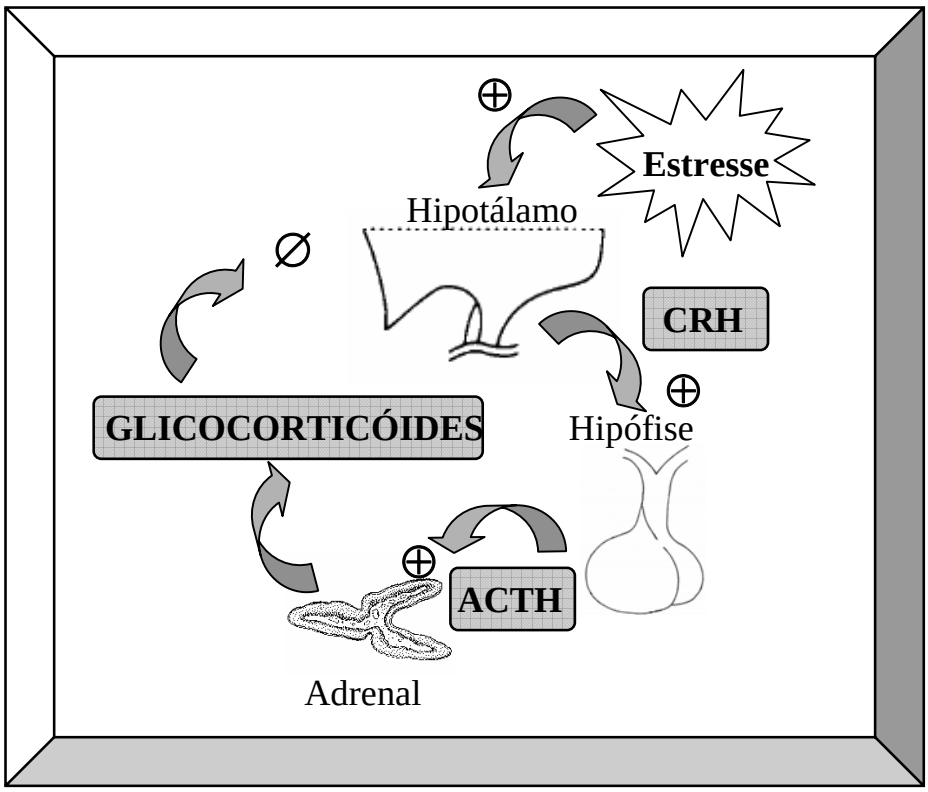

Figura 1: Eixo hipotálamo-hipófise-adrenal (HPA). Estímulos ambientais externos são captados pelo sistema límbico, ativando os sistemas de resposta ao estresse, entre eles o eixo HPA. ⊕ = estimulação; ∅ = inibição.

O ACTH aumenta a síntese de glicocorticóides pelo córtex da glândula adrenal. Em situações críticas, os glicocorticóides têm ações de proteção e manutenção da homeostasia: mobilização de estoques energéticos através de gliconeogênese, lipólise e catabolismo protéico,

melhora da função cognitiva, inibição da função gonadal, alteração da homeostasia do cálcio. Os GCs têm importância na própria regulação neuroendócrina, uma vez que atuam em receptores do sistema límbico (especialmente amígdala e hipocampo), do hipotálamo e da hipófise por retroalimentação negativa, encerrando a ativação (de Kloet, 1998). Este hormônio também inibe as citocinas, regulando a atividade do eixo HPA. A inibição causada pelos glicocorticóides limita sua própra ação, prevenindo o organismo de seus efeitos catabólicos, antirreprodutivos e imunossupressivos (Tsigos et al., 2002).

1.3 Estresse neonatal

As primeiras duas semanas de vida de um rato correspondem ao período perinatal humano. Durante essa fase continua a ocorrer o desenvolvimento de vários sistemas, incluindo o sistema nervoso central (SNC). Esses primeiros dias constituem o chamado período hiporresponsivo ao estresse (Sapolsky et al., 1986), uma vez que há uma exacerbação do mecanismo de retroalimentação negativa dos glicocorticóides na hipófise e diminuição da sensibilidade da adrenal ao ACTH (Yoshimura et al., 2003).

Sendo um período crítico de diferenciação, a submissão do rato a um estresse nesses primeiros dias determina alterações neuroquímicas e comportamentais observáveis durante toda a vida. Em essência esses animais apresentam menor medo quando expostos a ambiente novo, maior atividade e exploração (Levine et al., 1967). Estes achados concordam com os resultados neuroendócrinos de persistência da exacerbação da retroalimentação negativa dos glicocorticóides (Ader et al., 1969), redução da expressão de RNA mensageiro para CRH no hipotálamo e diminuição do conteúdo de CRH na eminência média (Plotsky et al., 1992).

Foi demonstrada também maior concentração de receptores glicocorticóides no hipocampo (Meaney et al., 1989), com aumento da inibição mediada pelo hipocampo e diminuição da excitação mediada pela amígdala na resposta neuroendócrina do eixo HPA nos animais que sofreram estresse neonatal (de Kloet et al., 1998). Além disso, há uma marcante diminuição da liberação de noradrenalina no núcleo paraventricular do hipotálamo em resposta a estresse por contenção (Liu et al., 2000). Logo, há uma supressão crônica da resposta de liberação de glicocorticóides frente ao estresse pelo eixo, semelhante ao que ocorre em situações de estresse crônico repetido na vida adulta.

O contato maternal parece ser fundamental para o desenvolvimento de tais alterações em ratos submetidos a estresse neonatal (Cirulli et al., 2003). O fato de retirar os filhotes da ninhada gera na mãe um aumento nos cuidados quando do retorno deles para a caixa-moradia (Branchi et al., 2001; Pryce et al., 2001), e tem-se encontrado correlações entre o comportamento maternal e menor reatividade dos filhotes ao estresse na vida adulta (Liu et al., 1997).

É importante ressaltar aqui que a expressão "estresse neonatal" é muito ampla e abrange uma série de pesquisas com protocolos variados. Evidentemente há larga discordância de resultados se considerarmos o conjunto de estudos sobre intervenções neonatais. Neste estudo estamos enfatizando os efeitos que curtos períodos – até o máximo de uma hora - de separação materna (*handling*) possuem a longo prazo, seguindo o modelo originalmente descrito por Levine e Denemberg na metade do século passado (Levine et al., 1967). Outros estudos, envolvendo longos episódios de separação (*maternal separation* ou *maternal deprivation*) apresentam resultados diversos (Pryce et al., 2001; 2003) e não serão abordados neste trabalho .

1.4 Comportamento alimentar

O hipotálamo foi classicamente relacionado como sendo o centro primário de integração de fatores centrais e periféricos que regulam a homeostase energética. Uma série de peptídeos orexigênicos e anorexígenos que constituem a circuitaria de controle do comportamento alimentar são primariamente produzidos por neurônios hipotalâmicos. Entre estes, o neuropeptídeo Y (NPY) e a proopiomelanocortina (POMC) têm sido largamente estudados por suas ações claramente opostas sobre a ingestão alimentar. O NPY é produzido na região medial do núcleo arqueado do hipotálamo juntamente a outro neuropetídeo chamado Proteína Relacionada ao Gene Cutia (AGRP), ambos francamente orexígenos (Henry, 2003, Sahu, 2004). Por outro lado, células da porção ventro-lateral no núcleo arqueado produzem a POMC e o peptídeo relacionado à cocaína e à anfetamina (CART), ambos anorexígenos (Sahu, 2004).

Entretanto, sinais periféricos também se relacionam com o hipotálamo para regular o apetite (Saper et al., 2002). A leptina, hormônio produzido no tecido adiposo, foi descrita em 1994 como um fator sinalizador do excesso de peso (Zhang et al., 1994), parecendo estar envolvida na saciedade pela ativação do sistema POMC/CART (41). A grelina, por sua vez, é um hormônio produzido no estômago em situações de jejum (Kojima et al. 1999). Acredita-se que ela atue em neurônios hipotalâmicos estimulando a produção de NPY (Dickson, et al., 1997).

Outras substâncias do trato gastrintestinal também atingem o SNC para informar sobre a distensão estomacal e a quantidade de glicose e lipídios no fígado, como a colecistocinina e o peptídeo semelhante ao glucagon-1. Estes hormônios regulam a ingestão alimentar enviando sinais a diversas regiões encefálicas como o núcleo do trato solitário, a amígdala, a área

postrema e o núcleo parabraquial. Acredita-se que estas áreas atuem no comportamento alimentar através de suas conexões com o hipotálamo (Saper et al., 2002).

Não só os mecanismos homeostáticos descritos acima regulam o apetite. Outros fatores como a sensação de recompensa e a palatabilidade também interferem na escolha e na quantidade de alimento ingerida. Estados emocionais como a ansiedade (Inoue et al., 2004) a depressão (Gronli et al., 2004) sabidamente são relacionados a alterações da ingestão de alimento. Diferentes rotas neuroquímicas envolvidas na reação hedônica da comida são afetadas por esses estados emocionais e modulam o apetite, como os opióides (Yeomans et al., 2002), a dopamina (Smith, 2004; Gambarana et al., 2003), a serotonina (Muraki et al., 2004) e o sistema Ácido gama amino butírico - GABA/benzodiazepínicos (Berridge et al., 1995). O estresse, por sua vez, também pode alterar a preferência alimentar, como veremos a seguir.

1.5 Estresse e preferência alimentar

Diferentes tipos de estresse podem alterar o consumo alimentar: contenção (Ely et al., 1997, Pecoraro et al., 2004), ruído (O´Hare et al., 2004), nado forçado (Nagaraja et al., 2003), superpopulação (Nagaraja et al., 2002), choque e estresse emocional (Pijlman et al., 2003). O estresse repetido por contenção na vida adulta aumenta a ingestão de alimentos palatáveis (Ely et al., 1997, Pecoraro et al., 2004). Este efeito parece ocorrer como uma resposta adaptativa ao estresse, atenuando a reatividade do eixo HPA (Pecoraro et al., 2004).

Os glicocorticóides por si são capazes de aumentar a preferência por alimentos palatáveis (Dalman et al., 2003). A remoção dos GCs por adrenalectomia suprime o consumo alimentar em 10-20% e diminui o ganho de peso (Bhatnagar et al., 2000), assim como inibe a

obesidade induzida pelo NPY (Dallman et al., 2004b). Esses efeitos da adrenalectomia são revertidos pela administração de GCs (Freedman et al., 1985). Em humanos, a secreção aumentada de cortisol após estresse correlaciona-se com maior ingestão de alimentos hipercalóricos (Epel et al., 2001).

O tipo de dieta também influi no padrão de secreção hormonal. Dietas forçosamente ricas em gordura aumentam a secreção de GCs tanto basal quanto induzida por estresse (Tannenbaum et al., 1997), talvez funcionando como um tipo de estressor. Ingestão de dieta rica em gordura por 2 a 3 meses reduz a resposta vegetativa ao estresse quando comparada com dieta rica em carboidratos (Buwalda et al., 2001). Por sua vez, dietas hipercalóricas atenuam a resposta do eixo HPA ao estresse (Strack et al., 1997), o que parece ocorrer também em humanos (Epel et al., 2001). O jejum aumenta a secreção de ACTH e corticosterona, reduz a retroalimentação negativa do eixo HPA e a secreção de insulina e leptina (Dallman et al., 1999).

A interação dos GCs com outros sistemas neuroquímicos como o dopaminérgico e o opióide (Samarghandian et al., 2003) parece ser importante na modulação do apetite pelo estresse. O sistema GABA/BDZ também tem seu papel nessa modulação, provavelmente reduzindo a ansiedade, uma vez que o aumento no consumo de doce induzido por estresse crônico por contenção em adultos é revertido pela administração aguda de diazepam (Ely et al., 1997) ou crônica de midazolam (Silveira et al., 2000) antes do teste.

1.6 Programação do eixo HPA

Como visto anteriormente, o organismo é muito hábil em responder a desafios físicos ou psicológicos com padrões de secreção hormonal e com alterações comportamentais. Sob

estresse, o SNC não apenas inicia a secreção rápida de moléculas efetoras como a noradrenalina e os GCs, mas também responde com alterações padronizadas e coordenadas na expressão gênica (McEwen, 1999; Meaney, 2001). Entretanto, embora essa capacidade seja extremamente adaptativa, melhorando a comunicação neuronal e favorecendo a sobrevivência, pode trazer grande impacto sobre a função e integridade neuronais, tanto imediatamente quanto a longo-prazo.

Os mecanismos pelos quais os eventos adversos precoces provocam efeitos a longo prazo permanecem obscuros, mas algumas evidências de alterações organizacionais persistentes nas respostas do SNC ao estresse têm sido demonstradas. Essa propriedade tem sido chamada "programação" (Welberg et al., 2001). Uma vez que há grande integração entre a função do eixo HPA e a expressão comportamental, é possível imaginar que o comportamento (em especial em relação à resposta ao estresse) possa ser programado durante o desenvolvimento (Meaney, 2001).

O sistema límbico em desenvolvimento (primariamente o hipocampo), hipotálamo e hipófise anterior sintetizam grande quantidade de receptores GCs. A exposição aos GCs neste período altera o desenvolvimento e a função subsequente do sistema límbico e do eixo HPA. Na periferia, o efeito final da programação é secreção alterada dos GCs endógenos por toda a vida. A alta exposição predispõe o indivíduo a doenças neurológicas, metabólicas e cardiovasculares como aterosclerose, imunossupressão, diabetes melitus tipo 2, depressão e déficit cognitivo (Barker , 1996; Phillips et al., 1998; Ward et al., 2004). Evidencia-se mais uma vez a importância do estudo do estresse neonatal para compreensão e prevenção de doenças na vida adulta.

2. OBJETIVOS

2.1 – Investigar os efeitos do estresse neonatal sobre o comportamento alimentar de ratos na vida adulta.

Avaliaremos os efeitos do estresse neonatal sobre o peso corporal e o consumo de alimento doce, salgado, ração padrão, água e soluções doce e salgada no início da vida adulta de ratos. Na idade avançada, avaliaremos novamente o consumo de doce.

2.2 – Investigar se os efeitos do estresse neonatal sobre o comportamento alimentar tem relação com alteração do estado emocional.

Avaliaremos o efeito do estresse neonatal em testes comportamentais de ansiedade como o labirinto em cruz elevado e o teste de transição claro-escuro. Avaliaremos também a locomoção e habituação através da tarefa comportamental de exposição ao campo aberto. Por fim, observaremos o efeito da administração de um fármaco ansiolítico (benzodiazepínico – diazepam) antes do teste de consumo de doce.

3. MÉTODOS E RESULTADOS

OBJETIVO 1 - Investigar os efeitos do estresse neonatal sobre o comportamento alimentar de ratos na vida adulta.

ARTIGO 1

Silveira PP, Portella AK, Clemente Z, Bassani E, Tabajara AS, Gamaro GD, Dantas G, Torres ILS, Lucion AB, Dalmaz C. **Neonatal handling affects feeding behavior of rats.** Physiology & Behavior, 80 (2004) 739-745.

OBJETIVO 2 - Investigar se os efeitos do estresse neonatal sobre o comportamento alimentar tem relação com alteração do estado emocional.

ARTIGO 2

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Neonatal handling alters feeding behavior of adult rats

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Abstract

Stress during the neonatal period leads to a large number of behavioral and biochemical alterations in adult life. The aim of this study is to verify the effects of handling and tactile stimulation during the first 10 days of life on feeding behavior in adult rats. Litters were divided into (1) intact; (2) handled (10 min/day); and (3) handled and tactile stimulated (10 min/day). Procedures were performed on Days 1–10 after birth. When adults, rats were tested for ingestion of sweet and savory snacks. We also measured body weight, ingestion of standard lab chow, and consumption of water and 1% glucose and 1.5% NaCl solutions. Stressed rats (handling and handling+tactile stimulation groups) consumed more sweet (two-way ANOVA, P=.008) or savory snacks (P=.001) than intact ones. This effect was observed in males and females. There were no differences in body weight, ingestion of standard lab chow, water, or in the ingestion of sweetened or salty solutions between groups. The same animals were tested later in life (15 months of age), and the effect was still evident. We suggest that handling during the neonatal period leads to alterations in the CNS of rats, causing an increased ingestion of palatable food in adult life, and this alteration probably persists throughout the whole life.

Keywords: Feeding behavior; Neonatal handling; Tactile stimulation; Palatable food ingestion; Sweet food ingestion

1. Introduction

Several studies have documented the impact of early life events on neuroendocrine and behavioral status in adulthood [1, 2 and 3]. The hypothalamic–pituitary–adrenocortical (HPA) axis is one of the most important neuroendocrine systems activated in response to actual or presumed environmental challenges. In rats, it has been demonstrated that both prenatal and postnatal factors may influence the development of the HPA axis [4, 5, 6, 7 and 8]. Alterations of the HPA axis have been reported to accompany certain psychiatric diseases, such as major depression [9].

Handling during the neonatal period produces, in adult rats, a decrease in emotional-related measurements and an increased activity and exploration in novel situations [10]. The reduced anxiety-related behavior in the adult offspring appears to be related to a greater intensity of maternal behavior performed by the dam induced by the daily short-term separation in the neonatal period [10 and 11].

Neonatally handled rats exhibit, as adults, reduced adrenocorticotrophin and glucocorticoid responses to stress [12]. Furthermore, reduced corticotrophin-releasing hormone (CRH) mRNA expression in hypothalamic tissue and reduced median eminence CRH content have been reported [7]. Maternal behavior and proximity are the most important regulatory factors in determining the infant's adrenocortical activity, and they may suppress HPA axis activity in the infant [13].

It has been shown that 6 h of daily maternal separation during the neonatal period leads to an increased rebound hyperphagia after food restriction in adult rat females [14]. Besides that, rats handled for 15 min early in life demonstrate a greater ingestion of rich carbohydrate food, and this is not reversed by peptides, such as bombesin and amylin [15].

Feeding control is a complex mechanism that includes appetite, motivation, and caloric demands of the organism. It may be altered by different factors, such as biological status, available nutrients, and stress [16]. In rats, a number of agents, including adrenergic agonists, opioids, dinorphin, neuropeptide Y, and galanin, increase food ingestion [17]. Data exist to suggest that the integrity of dopamine, serotonin, and opioid pathways are necessary for an adequate response to the stimulus of the presence of food [17]. An important relation between corticosterone levels and the amount of sweetened solutions drunk have also been reported, showing that glucocorticoids alter food preferences and food intake [18]. Besides that, models of chronic stress have been reported to lead to a wide range of behavioral and physiological disturbances, including altered responses to rewarding stimuli [16 and 19]. In addition, studies in humans have provided evidence of overeating induced by emotional experiences in adults [20].

Previous studies in our laboratory showed that repeated-restraint stress in adult rats leads to a greater ingestion of sweet food [16]. The objective of this study is to verify the effect of handling and tactile stimulation during the neonatal period on palatable food ingestion in adult rats.

2. Material and methods

2.1. Subjects

Eighteen pregnant Wistar rats were randomly selected. They were housed alone in home cages made of Plexiglas (65×25×15 cm) with the floor covered with sawdust and were maintained in a controlled environment (lights on between 0700 and 1900 h, temperature of 22±2 °C). Within 24 h after birth, all litters were culled to eight pups, and they were maintained undisturbed, unless for the stress procedures, which were carried out between 1000 and 1500 h. Eight litters were assigned to the intact group, and ten litters were submitted to the stress procedures.

Weaning occurred on Postnatal Day 22. Rats were housed in groups of four to five per cage and were separated by sex. A total of 138 experimentally adult male and female rats (90 days old; 190–300 g of body weight) were used, and no more than two female and two male pups of the same litter were used per experiment. Rats had free access to food (standard lab rat chow) and water, except during the period when the behavioral tasks were applied. Tasks were performed between 1300 and 1600 h. After being exposed to the behavioral tasks, animals were returned to the vivarium. The same animals were again exposed to the behavioral tasks at age 15 months.

2.2. Neonatal stress model

Nonhandled group—Pups were left undisturbed with the dam until weaning.

Handling—All pups were removed from their home cage, and four of them were placed in a clean cage lined with clean paper towel. This cage was placed into an incubator at 37 °C. After

10 min, the pups were returned to their dams. This procedure was performed during the first 10 days of life, and then the pups were left undisturbed until 22nd day of life.

Handling+tactile stimulation—The other four pups of the litter (see above) had their paws marked with blue ink. They were removed from the nests and placed together (three to four pups) on the examiner's hand. Then, they were gently stroked dorsally from head to tail for 10 min, being replaced into the nest afterwards. This procedure was also performed during the first 10 days of life.

2.3. Behavioral tasks

Rats were tested as adults. The animals were placed in a lightened rectangular box (40×15×20 cm) with side walls and a floor made of wood, and a glass ceiling.

For sweet food ingestion, 10 Froot loops (Kellogg's—pellets of wheat and corn starch and sucrose) were placed in one extremity of the box. Each animal was submitted to five habituation trials of 3 min each, on different days, until the intake reached about four Froot loops per trial. After being habituated under food restriction (80% of habitual ingestion of standard lab chow), the animals were exposed for 3 min to the test session, when the number of ingested pellets was counted. A protocol was established, so that when the animals ate part of the Froot loops (e.g., 1/3 or 1/4), this fraction was considered. This last evaluation was made with the animals fed ad libitum.

For savory snack ingestion, five Cheetos (Elma Cheeps—pellets of corn meal, cheese, and salt) were placed in the same apparatus. Habituation was established in 3 days because rats were

already familiar with the environment, and testing was performed in the same manner as described above.

2.4. Standard lab chow ingestion and water consumption

Rats were placed in single home cages, and the ingestion of food and water was measured for 3 consecutive days. Ingestion was measured by leaving a determined amount of food in the cage and water in the bottle, and checking the remainder the next day. The first day of measurement was not considered in the analysis to exclude the effect of acute social isolation stress.

On another occasion, after the rats had been habituated to the apparatus described above, lab chow was put in the same place where the behavioral tasks were performed. Ingestion was measured for 3 min for 3 consecutive days. This test was performed to verify whether environmental challenge would influence food consumption.

2.5. Consumption of 1% glucose or 1.5% NaCl in drinking water

Rats were placed in single home cages, and the consumption of a solution of 1% glucose in drinking water was measured for 3 consecutive days. On another occasion, the same was tested with a NaCl solution. Animals could choose between ingestion of these liquids or water. Consumption was measured by leaving a determined amount of the solution in the bottle and checking the remaining volume the next day.

2.6. Statistical analysis

Data were expressed as mean±S.E.M. and were analyzed by two-way ANOVA followed by the Student–Newman–Keuls' test, or by repeated-measure ANOVA [21].

3. Results

3.1. Body weight

Body weight was measured at 90 days of life, before the beginning of the behavioral tasks (n=67). Two-way ANOVA showed an effect of sex [$F(1,61)=160.303$, $P<.0001$], where males weighed more than females. There were no statistical differences between groups [$F(2,61)=1.883$, $P=.161$] and no interaction between sex and group [$F(2, 61)=0.617$, $P=.543$] (Data not shown).

3.2. Standard lab chow ingestion and water consumption

There were no statistical differences between nonhandled and treated groups in standard lab chow consumption, either measuring the consumption with rats in single home cages [two-way ANOVA; n=5–8 per group; $F(2,32)=0.972$, $P=.389$] or in the apparatus of the behavioral task [n=9–17 per group; $F(2,64)=0.66$, $P=.523$], and no interaction was observed between sex and group [$F(2,32)=0.634$, $P=.537$]. In single home cages, male rats ate more than females [$F(1,32)=68.252$, $P=.0001$; Fig. 1A]. In the behavioral task apparatus, there were no statistical differences between genders [Data not shown; $F(1,64)=0.88$, $P=.351$], and no interaction was observed between sex and group [$F(2,64)=0.13$, $P=.879$].

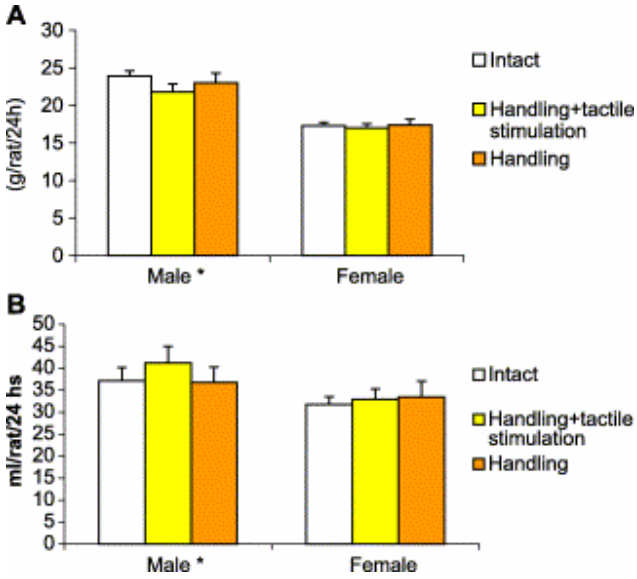

Fig. 1. Food ingestion (A) and water consumption (B) of isolated rats. Data are the means of Days 2 and 3 and are expressed as mean±S.E.M. for grams of food/animal (A), or milliliters of water/animal (B). There is no difference between the groups (two-way ANOVA, P=.389 for food and .69 for water). Males ate and drank more than females (two-way ANOVA, P<.0001 for gender).

There were no statistical differences between groups on water consumption [n=5–8 per group; F(2,32)=0.375, P=.690]. Males drank more water than females [F(1,32)=4.829, P=.035; Fig. 1B]. There was no interaction between sex and group [F(2,32)=0.287, P=.752].

3.3. Consumption of 1% glucose and 1.5% NaCl in drinking water

As displayed in Fig. 2, there were no statistical differences between groups [two-way ANOVA for glucose, F(2,30)=0.984, P=.386; and for NaCl, F(2,30)=0.485, P=.621; n=6–7 per group]. There was no effect of sex [for glucose, F(1,30)=2.854, P>.05; for NaCl, F(1,30)=0.028, P>.05] and no interaction between sex and group for glucose [F(2,30)=0.646, P>.05]. An interaction was observed for NaCl [F(2,30)=4.262, P=.023].

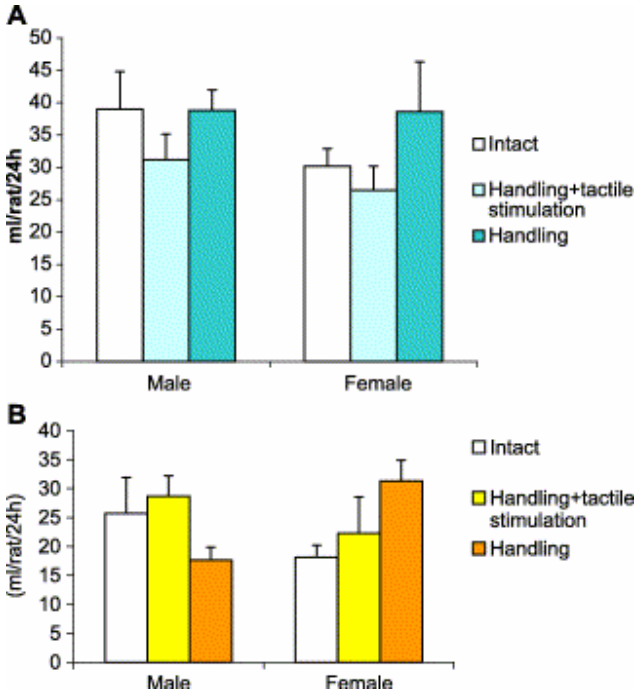

Fig. 2. Consumption of (A) 1% glucose in drinking water for males and females and (B) of 1.5% NaCl in drinking water. Data are the means of Days 2 and 3 and are expressed as mean±S.E.M. (milliliters of solution/animal). There is no difference between the groups (two-way ANOVA, P=.386 for 1% glucose and P=.687 for 1.5% NaCl solutions). There was no effect of time (repeated-measure ANOVA, P=.109). An interaction was observed for NaCl [F(2,30)=4.262, P=.023].

3.4. Sweet food ingestion measurement

3.4.1. At 3 months of age (n=11–27 per group)

During habituation, there was an effect of time showing that rats ate more as the days passed by [repeated-measure ANOVA, F(4,380)=77.59, P<.0001]. There were also effects of group [F(2,95)=11.42, P<.001] and of sex [F(1,95)=3.99, P<.05], with interactions between time and group [F(8,380)=2.73, P<.01], and time and sex [F(4,380)=3.32, P<.05]. For Day 2 onwards,

both the handling group and the tactile stimulation group ate significantly more than intact animals (Fig. 3).

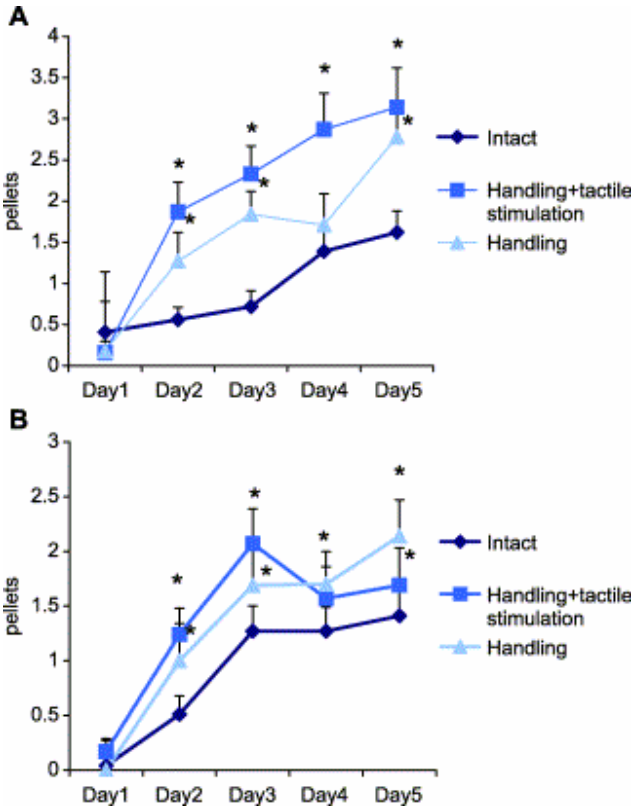

Fig. 3. Habituation to sweet food—Days 1 to 5 in (A) males and (B) females. Repeated-measure ANOVA showed an effect of time (P<.001), and effect of sex (P<.05), and of group (P<.001). *Significantly different from intact rats (Student–Newman–Keuls test, P<.05).

In the test session, both the handled group and the group submitted to handling+tactile stimulation ate more than the intact group [two way ANOVA, $F(2,95)=4.903$, $P=.009$]. There was no effect of gender in the experiment [$F(1,95)=1.790$, $P>.05$; Fig. 4] and no interaction between sex and group [$F(2,95)=1.067$, $P>.05$].

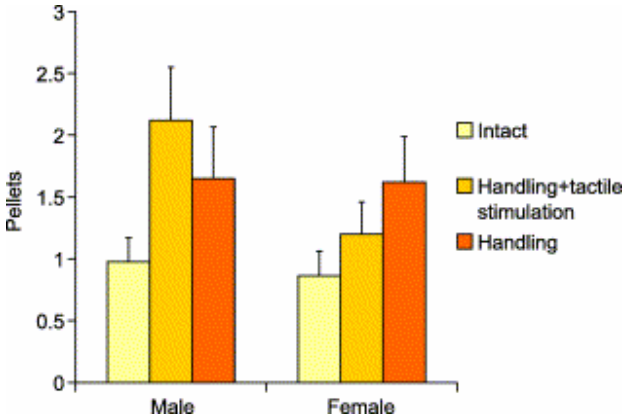

Fig. 4. Sweet food ingestion during the test session. A two-way ANOVA showed an effect of group ($P<.01$).

3.4.2. At 15 months of age (n=5–7 per group)

This test was performed without renewed habituation to the apparatus, that is, the animals were exposed once to sweet food without habituation and without food restriction. Both the handled group and the group submitted to handling+tactile stimulation ate more than the intact group [two way ANOVA, $F(2,29)=7.964$, $P=.002$ for group effect]. There was no effect of gender [$F(1,29)=3.397$, $P>.05$; Fig. 5], but a significant interaction between sex and group was observed in this experiment [$F(2,29)=6.057$, $P=.006$].

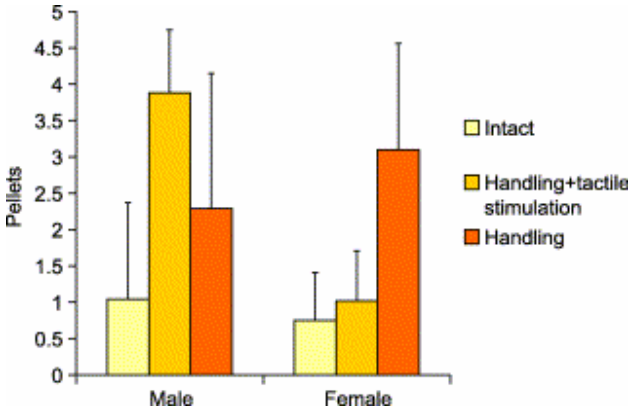

Fig. 5. Sweet food ingestion at 15 months of age. A two-way ANOVA showed an effect of group (P<.005) and a significant interaction between sex and group (P=.006).

3.5. Savory snacks ingestion

During habituation, there were no differences between groups [repeated-measure ANOVA, F(2,58)=0.72, P>.05], no difference between sexes [F(1,58)=1.56, P>.05], and no interaction between sex and group [F(2,58)=0.90, P>.05]. However, an effect of time was noted, where all the rats ate more as the days passed by [repeated-measure ANOVA, F(2,116)=41.23, P<.001]. In the test session, with animals fed ad libitum, there were differences between groups, when handled and handling+tactile stimulation groups ate more than the nonhandled group [two-way ANOVA, F(2,58)=4.556, P=.015]. There was no effect of gender [F(1,58)=2.629, P>.05] and no interaction between sex and group [F(2,58)=0.348, P>.05; Fig. 6].

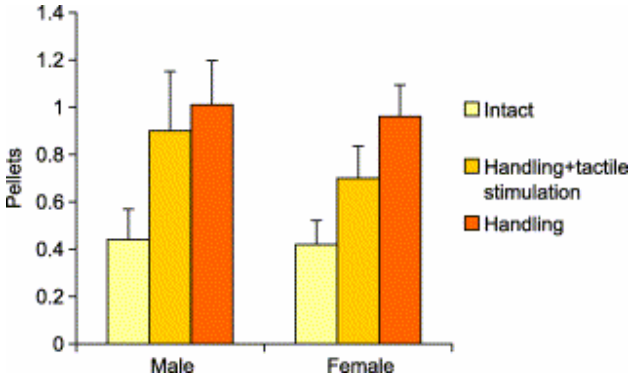

Fig. 6. Savory snacks ingestion during the test session. A two-way ANOVA showed an effect of group (P<.001).

4. Discussion

We observed an increased appetite for sweet and savory palatable food in response to neonatal stress. In the case of sweet food, appetite was independent of the hunger condition, that is, the effect was already evident during habituation when rats were under food restriction but was particularly present when the animals were fed ad libitum. This is also a long-lasting effect in as much as it persisted until the animals reached 15 months of age. It is important to point out that at 15 months of age, there was an interaction between sex and group concerning the consumption of sweet pellets. While both males and females that had been submitted to handling presented higher intake, it was just the males from the handling+tactile stimulation group that presented an effect, and the females ate an amount of pellets similar to the control group.

There was no alteration in habitual ration intake between groups. Under the conditions described, there was no significant difference in weight gain between the groups in the adult rats. Weight was not measured during development. Other studies [14] have reported that

neonatal stress decreases the body weight of rats when measured at 3 weeks of age, without alteration in standard lab chow ingestion. It is possible that the animals gained weight to reach the weight of control rats in adulthood. The period of handling, the different strains, and the gender of experimental animals used may explain the different responses to stress [15, 22, 23 and 24]. Thus, using Wistar rats in a model of handling and handling+tactile stimulation for short periods during the first 10 days of life, we verified no effect either on body weight or on standard lab chow intake when adults. However, increased appetite for sweet and savory palatable food was observed.

In as much as both groups of stressed rats (i.e., submitted to handling and submitted to handling+tactile stimulation) demonstrated a comparable alteration in palatable food ingestion, and the common characteristic between them was a brief handling, we conclude that the effect observed in this experiment was induced by handling itself, and no effect of tactile stimulation was observed in this parameter. We cannot discard, however, the possibility that the increased stimulation upon reunion with the dam may cause the observed effects on feeding, rather than the separation (handling) per se.

Other studies have found an increased intake of 3% NaCl solution in adult rats separated from their dams for 24 h in the neonatal period or injected with furosemide on the 12th day of life [25]. In our experiment, a short period of handling caused no difference in the consumption of solutions, such as 1% glucose or 1.5% NaCl, but increased sweet and savory palatable food ingestion. It is possible that the procedural differences between these studies and ours, particularly different maternal separation periods, have led to this conflicting results. Concerning the intake of sweet fluids, a recent study [26] found no differences in the intake of

a sucrose solution between neonatal handling during 15 min and the control group—similar to the results of the present study.

It is interesting that handling and tactile stimulation affect the intake of sweet and savory snacks but not the intake of sweet and salty drinking solutions. Previous papers usually only monitored one of these two types of consumption. This difference suggests that it is not the sweet or salty taste itself but that other characteristics of the food may be important, such as texture. In addition, the environment may exert influence in this result in as much as solid foods were presented in a behavioral apparatus different from the home cages of the animals, and it is known that rats submitted to neonatal stress act differently in new sets [3, 15, 22 and 27].

In the experiments to investigate consumption of both solids and liquids, male rats consumed more than females due to their greater body weight. If data are expressed in relation to body weight, the differences between males and females are insignificant (data not shown).

The central mechanisms involved in stress-induced overeating are very complex. Many agents, such as β-adrenoceptor agonists, beta-endorphin, glucocorticoids, dynorphin, neuropeptide Y, and galanine, stimulate food intake [20 and 28]. The effects of pharmacological and behavioral treatments on the hedonic response to feeding are another important dimension of eating behavior. Serotonin, dopamine, and opioid peptides play a role in the response to food stimuli. Neonatal stress could influence any of the above mechanisms, stimulating the appetite for palatable food.

For a long time, dopamine was thought to be the mediator of reward and positive reinforcement. However, more recent studies have found that dopaminegric neurons respond to environmental stimuli (like food) that attract the animal's attention [29]. Sweet stimuli increase quantity and metabolism of dopamine in the nucleus accumbens [30]. Neonatal stress may cause an alteration in the interpretation of stimuli, leading rats to perceive sweet or savory food as more significant stimuli when they are presented to them. In addition, maternal separation increases acquisition of cocaine self-administration in adult rats [31]; it may be that handling results in a greater sensitivity to the reinforcing effects of palatable food. It is interesting that a recent study has shown increased turnover of dopamine in several brain regions in animals submitted to neonatal stress [32].

Other systems, such as the opioid system, may be involved in the development of the effects observed in the present study. Several studies have indicated that endogenous opioids are released in response to a variety of social stimuli; two of these (milk transfer and somatosensory contact) are of particular relevance to infants [33]. Other studies suggest the presence of opioid routes between the central nucleus of the amygdala, paraventricular nucleus and nucleus of the solitary tract, and all these nuclei are involved in feeding behavior [34 and 35]. Therefore, alterations in the opioid system response to palatable stimuli may also be involved in these effects.

Neonatal handling may also lead to altered maternal behavior towards pups, and this effect has been shown to decrease the ACTH and corticosterone response to acute stress and to increase hippocampal glucocorticoid receptor mRNA expression, enhance glucocorticoid feedback sensitivity, and decrease levels of CRH mRNA [7 and 8]. Glucocorticoids and CRH are

important modulators of feeding behavior [18 and 36]; therefore, alterations in the HPA axis may also be involved in the effects observed in feeding behavior. In addition, corticosteroids are known to increase serotonin receptor numbers in the hippocampus [37], and neonatal handling increases serotonin metabolism only in brain regions where glucocorticoid receptor expression is altered [38]. Serotonin release is also involved in mood control and eating behavior [39, 40 and 41]. Thus, the effect on palatable food consumption induced by neonatal handling described in the present study may also be related to an alteration in the serotonergic system.

In summary, we showed that neonatal handling leads to an increased appetite for both sweet and savory palatable food, without alteration to body weight, consumption of standard lab chow, water, and sweet or salty solutions. This effect is persistent throughout old age. Neonatal handling is known to lead to various alterations in CNS systems involved in reward, pleasure, feeding, and responses to stress [7, 32, 38, 42, 43 and 44], but the specific mechanisms responsible for the effects observed in the present study are not known. Other studies are needed to verify which alterations induced by neonatal handling in the nervous system cause this effect upon feeding behavior.

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The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior

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Abstract

Brief periods of handling during the neonatal period have been shown to have profound and long-lasting physiological consequences. Previous studies performed in our laboratory have demonstrated that handling the pups during the neonatal period leads to increased sweet food ingestion in adult life. The objective of this study is to verify if this effect could be explained by the enhanced anxiety levels in these animals. Litters were divided in: (1) intact; (2) handled (10 min in an incubater/day) and (3) handled + tactile stimulation (10 min/day). Procedures were performed on days 1–10 after birth. When adults, rats were tested in the elevated plus maze apparatus, light dark exploration test and open field test. They were also tested for sweet food ingestion, being injected with 2 mg/kg diazepam or vehicle 60 min before the test. Handling and handling + tactile stimulation do not alter performance in the plus maze test, but handled rats presented more crossings in the light/dark exploration test and open field (two-way ANOVA). Females also spent more % time in the open arms in the plus maze and more time in the lit compartment in the light/dark test, presenting more crossings in both tests. Both treated rats (handled and handled + tactile stimulation groups) consumed more sweet food than intact ones (two-way ANOVA). When diazepam was injected prior to the measurement of sweet food ingestion, there was no effect of the drug. We suggest that handling during the neonatal period leads to plastic alterations in the central nervous system of these animals,

causing an increased ingestion of palatable food in adult life, and this alteration does not express an anxiety-like behavior.

Keywords: Feeding behavior; Neonatal handling; Tactile stimulation; Anxiety; Diazepam; Plus maze test

Many studies suggest that stress early in life can promote long-term changes in multiple neurotransmitter systems and brain structures (Francis and Meaney, 1999 and Fleming et al., 1999; Meaney and Aitken, 1985). Handling in the neonatal period produces a decrease in emotionality-related measurements and an increased activity and exploration in novel situations in adult rats (Levine et al., 1967). We have studied the feeding behavior of adult rats submitted to neonatal stress, and have shown that neonatal handling leads to an increased appetite for palatable food, both sweet and savory snacks, without alteration in body weight, consumption of standard lab chow, water and sucrose or NaCl solutions (Silveira et al., 2004). This effect is persistent throughout older ages (Silveira et al., 2004). Handling during the neonatal period is known to lead to various alterations in CNS systems involved in reward, pleasure, feeding and responses to stress, but the specific mechanisms responsible for the effects observed on feeding behavior are not known.

Neonatal handling in rats has been used as a model of the possible effects of early adverse events in adult life. It has been linked to the presentation of some pathologies, such as major depression, bulimia/anorexia and anxiety (Kalinichev et al., 2002; Wigger and Neumann, 1998). In addition, 15 min handling during the neonatal period has been shown to induce increased levels of mRNA for the γ2 subunit of the GABAA receptor complex, which confers high affinity to benzodiazepine binding in several brain structures (Caldji et al., 2000). Changes

in postsynaptic GABA receptor function during adult life were also observed after two episodes of handling during neonatal life (Hsu et al., 2003).

Food consumption when rats are exposed to a known type of food in an environment different from their home cage may be affected by the anxiety state of the animal (Merali et al., 2003). Therefore, previous results showing increased consumption of sweet and savory snacks by animals handled during the neonatal period could be influenced by anxiety. Additionally, previous studies from our laboratory showed that repeated restraint stress in adult rats leads to a greater ingestion of sweet food (Ely et al., 1997), which is reversed by acute diazepam administration before the test session, in a dose that does not affect feeding by itself (Ely et al., 1997), and by chronic administration of midazolam (Silveira et al., 2000). This increased consumption of sweet food is believed to be due to increased anxiety levels in these animals. In this context, it is possible that similar mechanisms are induced by neonatal handling or adult stress exposure, increasing sweet food ingestion. Therefore, our hypothesis is that the increased sweet food consumption observed in animals handled during the neonatal period may be due to anxiety and, if so, will be attenuated using an anxiolytic drug (diazepam). In order to do so, we chose a dose that, while presenting an anxiolytic effect, had no effect on feeding by itself in the conditions of the experiment (Ely et al., 1997). We also submitted these rats to behavioral tests that might reveal some effects of neonatal handling: the elevated plus maze and the light–dark exploration tests, used to indicate anxiety-related behavior (Ho et al., 2002, Holmes et al., 2001 and Fernandez et al., 2004), and open field exposure, used to evaluate habituation and motor activity (Carlini et al., 2002).

1. Experimental procedures

1.1. Subjects

Pregnant Wistar rats were randomly selected. They were housed alone in home cages made of Plexiglas (65 cm × 25 cm × 15 cm) with the floor covered with sawdust and were maintained in a controlled environment (lights on between 07:00 and 19:00 h, temperature of 22 ± 2 °C). Within 24 h after birth, all litters were culled to eight pups and they were maintained undisturbed, unless for the handling procedures, that were carried out between 10:00 and 15:00 h.

Weaning occurred on postnatal day 22. No more than two male and two female pups were used per litter. Rats were housed in groups of four to five per cage, and separated by sex. A total of ninety-four experimentally adult male and female rats (90-days-old at the beginning of behavioral tests; 190–300 g body weight) were used. Rats had free access to food (standard lab rat chow) and water, except during the period when the behavioral tasks were applied. Behavioral tasks were performed between 13:00 and 16:00 h, and each animal was used in two or three behavioral tests. After being exposed to the behavioral tasks, animals were returned to the vivarium.

1.2. Neonatal handling

1.2.1. Non-handled group

Pups were left undisturbed with the dam until weaning. Cleaning of the cages was done each 5 days, changing part of the sawdust without touching the animals.

1.2.2. Handling

Pups were removed from their home cage and placed in a clean cage lined with clean paper towel. This cage was placed in an incubator at 37 °C. After 10 min, pups were returned to their dams. This procedure was performed during the first 10 days of life, and then pups were left undisturbed until the twenty-second day of life.

1.2.3. Handling + tactile stimulation

Pups were removed from the nests and placed together (3–4 pups) on the examiner's hand. Then they were gently stroked dorsally from head to tail for 10 min, being replaced in the nest afterwards. This procedure was also performed during the first 10 days of life.

1.3. Plus maze test

The elevated plus maze apparatus was made of wood and consisted of two opposed open arms (50 cm × 10 cm), two opposed enclosed arms with no roof (50 cm × 10 cm × 40 cm), and an open square (10 cm × 10 cm) in the center. The maze was elevated 50 cm above the floor. The behavioral test was conducted in the 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 were analyzed.

1.4. Light/dark exploration test

The light/dark apparatus consisted of an open-topped wooden arena (70 cm × 10 cm), half painted black and half white. Twenty-seven centimeter high walls bordered the field, and the two compartments were freely communicated at the center. The white compartment was illuminated by bright, direct white light, and the dark compartment received no light at all. The

experiment was conducted with room lights off. Crossing from one side to another was considered when the rat left only the distal third of the body in the original compartment. Time spent in the lit compartment and entries made to the lit compartment were recorded during 15 min.

1.5. Open field test

The open field consisted of an open wooden arena (53.5 cm × 35 cm) with 12 equally divided squares measuring 12.5 cm2 by means of white adhesive tape. Forty-five centimeter high walls bordered the field. The animals were observed directly and continuously for 5 min. The following behavioral components were measured: locomotion (the number of line crossings) and rearing (standing upright on the hind legs). These measurements were made on two consecutive days (training and test sessions).

1.6. Effects of diazepam on sweet food ingestion

The animals were placed in a lightened rectangular box (40 cm × 15 cm × 20 cm) with floor and side walls made of wood and a glass ceiling. Ten froot loops (Kellogg's®; pellets of wheat and corn starch and sucrose) were placed in one extremity of the box. Each animal was submitted to five habituation trials of 3 min each, on different days, when the intake reached about three froot loops per trial. The animals were habituated under food restriction (80% of habitual ingestion of standard lab chow).

The evaluation of sweet food intake in control, handled and handled + stimulated rats was made with the animals fed ad libitum. An injection of diazepam (2 mg/kg) or vehicle (40% propylene glycol, 10% ethanol and 10% sodium benzoate/benzoic acid, pH 7.4) was

administered intraperitonially 60 min prior to this behavioral task. The volume injected was 1 ml/kg. The animals were then exposed to the same apparatus for 3 min (test session) when the number of ingested pellets was counted. A protocol was established so that when the animals ate part of the froot loops (e.g., 1/3 or 1/4), this fraction was considered.

1.7. Statistical analysis

Data were expressed as mean ± standard error of the mean, and analyzed by multivariate ANOVA, followed by the Student–Newman–Keuls' test, when indicated (Downe and Heath, 1970).

2. Results

The effect of diazepam administration on sweet food consumption in these animals is shown in Fig. 1. When rats that were handled or submitted to handling + tactile stimulation during the neonatal period were tested in adult life, we observed an effect of the group [$F(2, 32) = 5.43$; $P < 0.009$], since rats submitted to handling or handling + tactile stimulation ate more sweet food compared to controls. No effect of drug administration was observed [$F(1, 32) = 0.91$; $P > 0.1$] or sex [$F(1, 32) = 0.186$; $P > 0.1$]. Since both males and females behaved in the same way, the results for the pooled animals are displayed in Fig. 1.

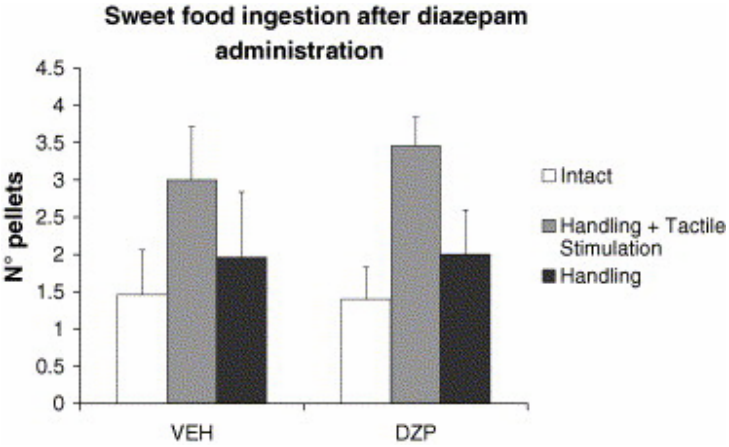

Fig. 1. Number of sweet food pellets consumed during the 3-min test session in animals submitted to neonatal handling or handling + tactile stimulation (10 min/day during the first 10 days of life), or intact animals, measured in the adult life. Sixty minutes before the test, the animals received i.p. 2 mg/kg diazepam or vehicle. Data are expressed as mean ± S.E.M. N = 4–8 animals per group. O, a two-way ANOVA showed a significant effect of neonatal treatment ($P < 0.01$) and no effect of the drug ($P = 0.765$). There was no interaction between the variables ($P = 0.886$).

Rats were exposed to the plus maze apparatus when adults. Two-way ANOVA showed no differences in the time spent in the open arms between groups [$F(2, 67) = 0.344$; $P > 0.1$], and no differences between male and female rats in this parameter [$F(1, 67) = 2.955$; $P = 0.09$], as is displayed in Fig. 2A. There were also no differences in the number of entries in the open arms between groups [$F(2, 67) = 2.41$; $P = 0.1$] but there was an effect of sex [$F(1, 67) = 8.24$; $P = 0.005$], as can be observed in Fig. 2B. The percentage of time spent in the open arms was higher in females [$F(1, 67) = 4.56$; $P = 0.036$], and there was no interaction between sex and group [$F(2, 67) = 1.509$; $P > 0.1$].

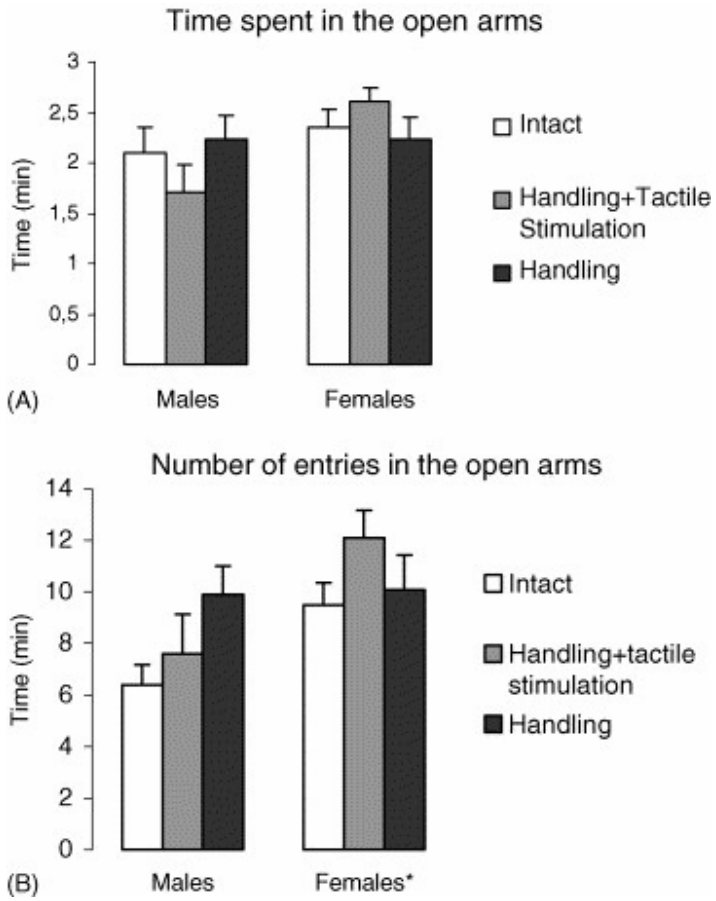

Fig. 2. Performance on the elevated plus maze of the intact, handled and exposed to handling + tactile stimulation male and female rats. (A) Mean ± S.E.M. of the time spent in the open arms during the 5-min exposure to the elevated plus maze. (B) Mean ± S.E.M. of the number of entries into the open arms. N = 9–15 animals per group. (*) Female rats presented more entries in the open arms, and there was no effect of neonatal treatment (two-way ANOVA).

Since there were no differences between groups submitted to handling and to handling + tactile stimulation in the behavioral tests used, we performed the light/dark exploration and open field tests using just the handled group to compare with non-handled rats. In the light/dark exploration test, females spent more time in the lit compartment in relation to males

[F(1, 71) = 12.06; P = 0.001], and they also made more crossings [F(1, 71) = 7.58; P = 0.007]. Handled rats made more crossings compared to non-handled ones [F(1, 71) = 3.79; P = 0.05], with no difference in the time spent in the lit side [F(1, 71) = 0.57; P = 0.81]. There was no interaction between group and sex in relation to the time spent in the lit side [F(1, 71) = 1.43; P = 0.235] and to the number of crossings [F(1, 71) = 1.56; P = 0.215]. Fig. 3 displays these results.

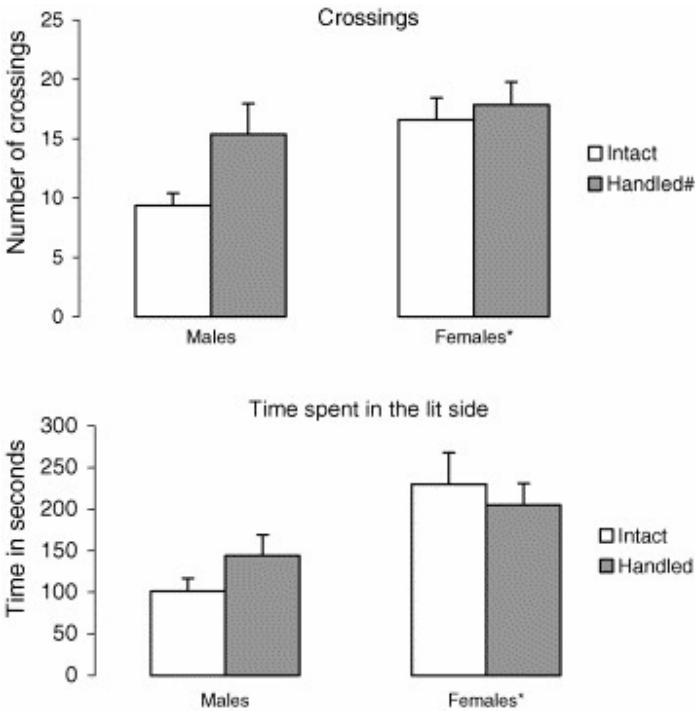

Fig. 3. Light/dark exploration test of the intact and handled male and female rats. (A) Mean ± S.E.M. of the number of crossings. Female rats presented more crossings (*), as did handled rats (#). (B) Mean ± S.E.M. of the time spent in the lit side, where females spent more time (*). N = 15–25 animals per group (two-way ANOVA).

In the open field test, when analyzing the number of crossings, a repeated measures ANOVA revealed an effect of group [$F(1, 45) = 7.87$; $P = 0.007$], since handled animals presented more crossings, especially in the second day (see interaction below), and an effect of sex [$F(1, 45) = 23.61$; $P = 0.001$], since female rats exhibited more crossings. There was a significant effect of session, which is due to habituation to the new environment [$F(1, 45) = 19.32$; $P = 0.001$] and a significant interaction between session and group [$F(1, 45) = 6.18$; $P = 0.017$]. With regard to the number of rearings, a repeated measures ANOVA revealed no effect of group [$F(1, 45) = 0.101$; $P > 0.1$], and a significant effect of sex [$F(1, 45) = 20.41$; $P = 0.001$], since female rats exhibited more rearings. There was a significant effect of session, which is due to habituation to the new environment [$F(1, 45) = 9.28$; $P = 0.004$], and no significant interactions (Fig. 4).

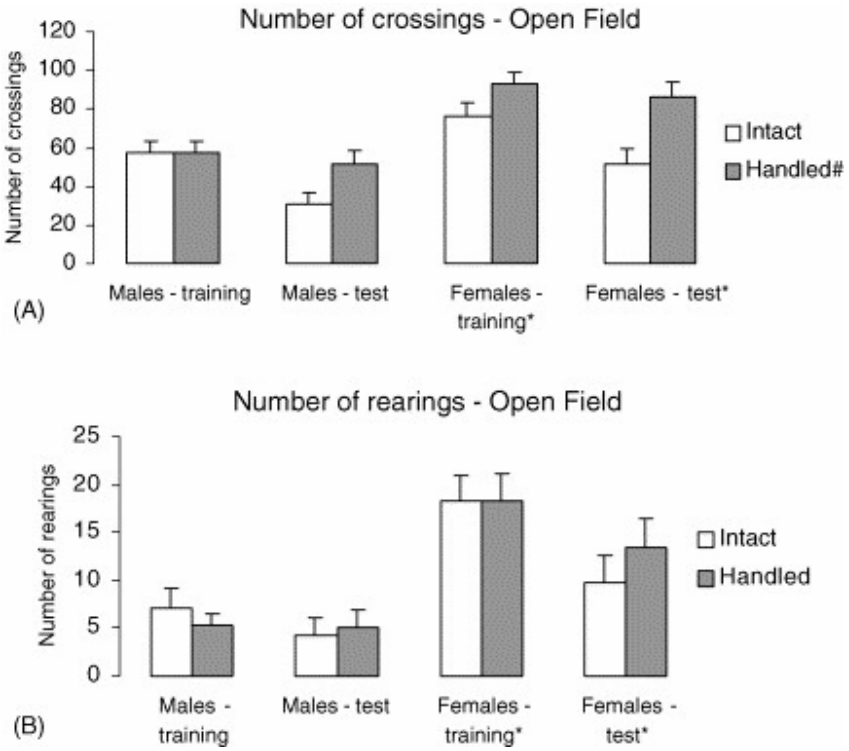

Fig. 4. Performance of the intact and handled male and female rats in the open field test. (A) Mean ± S.E.M. of crossings during the 5 min of exposure to the open field. Female rats presented more crossings (*), as do handled rats (#). (B) Mean ± S.E.M. of the number of rearing during the 5-min exposure to the open field. Female rats presented more rearings (*). N = 11–15 animals per group (two-way ANOVA).

3. Discussion

In this study, we observed that adult rats submitted to neonatal stress presented an increased appetite for palatable sweet food, according to previous reports (Silveira et al., 2004). As observed above, food consumption when rats are exposed to a known type of food in an environment different from their home cage may be affected by the anxiety state of the animal (Merali et al., 2003), and previous studies from our laboratory showed that repeated restraint stress in adult rats leads to a greater ingestion of sweet food (Ely et al., 1997), which is reversed by acute diazepam administration before the test. In the present study, however, the increased appetite for sweet food was not reversed by diazepam administration. This result suggests that the increased consumption of sweet food is not due to increased anxiety levels in these animals. Therefore, the mechanisms induced by neonatal handling or adult stress exposure, increasing sweet food ingestion, are probably different.

We also found that behavioral measures of anxiety were not altered in these animals, since there were no differences between groups in the plus maze or the light/dark tests when considering time spent in the open arms or in the lit compartment. There is some controversy in the literature about neonatal stress and anxiety-behavior measurements. It was found that handling for 15 min, or maternal separation for 180 min, equally decrease anxiety assessed by

the plus maze test, being different in male and female rats (the effect is more evident in females; McIntosh et al., 1999). Another study found less exploration of the open arms of the plus maze and greater startle amplitudes in rats with 180 min of maternal separation (Kalinichev et al., 2002). A reduction in the number of entries and in the amount of time spent in the open arms, and an enhanced ACTH response to the elevated plus maze exposure was also found (Wigger and Neumann, 1998); this last finding was observed only in males. Twenty-four hours of maternal separation on different days of the neonatal period (4, 9 or 18 days of life) does not affect anxiety measured using the plus maze test (Lehmann et al., 1999). It is possible that the procedural differences between these studies, particularly the different maternal separation periods, have led to these conflicting results.

In agreement with other reports in the literature (Marcondes et al., 2001, Imhof et al., 1993 and Ramos et al., 1997), female rats presented reduced anxiety, as expressed by the higher percentage of time spent in the open arms of the plus maze, as well as increased time spent in the lit compartment, in the light/dark test. This increased percentage of time spent in the open arms seems to be correlated with higher estradiol levels (Marcondes et al., 2001). It is interesting that some reports have shown that significant sex differences in the plus maze performance are observed within the range of 60 and 120 days (Imhof et al., 1993), which is the age range that we used in the present study. Additionally, females presented higher ambulation in the open field test, which also agrees with the literature (Ramos et al., 1997; Lehmann et al., 1999).

Most of the ontogenetic changes in brain GABAA receptors in rats occur in the neonatal period, earlier than 20 days of age (Laurie et al., 1992). An alteration in this system, particularly

in central BZ sensitivity (Caldji et al., 2000), could induce a state of altered anxiety levels. In the present study, however, no evidence of increased anxiety was observed.

Benzodiazepines (BZ) are a pharmacological group of drugs that inhibit anxiety symptoms. They can reverse behavioral effects of stress, such as stress-induced analgesia (Willer and Ernst, 1986). On the other hand, diazepam can affect food ingestion (Cooper, 1983a and Cooper, 1983b). Studies have reported an increase of solid food (hyperphagia) and fluid (hyperdipsia) intakes with acute use of BZD in satiated animals or in previously deprived ones (Britton et al., 1981; Cooper, 1983a and Cooper, 1983b). In the present study, the increase of ingestion induced by BZD was not observed, and the control groups treated with diazepam (2 mg/kg) or vehicle had similar intakes of froot loops. We chose this dose in order to investigate whether the increased sweet food consumption observed in animals that were handled during the neonatal period might be due to anxiety and, if so, would be attenuated using an anxiolytic drug. For example, diazepam reverses the increased sweet food ingestion induced by chronic stress in adult rats when given acutely (Ely et al., 1997), as does midazolam when administered chronically (Silveira et al., 2000). In the present study, even though the behavioral alteration (increased consumption) is observed, diazepam was unable to reverse the enhanced sweet food ingestion induced by handling during the neonatal period. Therefore, the mechanisms of increased sweet food ingestion in these two models (chronic stress in adult rats and neonatal handling) involve different mechanisms.

Many agents stimulate food intake such as β-adrenoceptors agonists, beta-endorphin, glucocorticoids, dynorphin, neuropeptide Y and galanine (Yates, 1992 and Tataranni et al., 1996). Serotonine, dopamine and opioid peptides play a role in the response to food stimuli.

Additionally, pharmacological and behavioral treatments may affect the hedonic response to feeding, and neonatal stress could influence any of the above systems, stimulating the appetite for palatable food.

Another finding from this study was the interaction observed between group and session in the exposure to the open field. While control animals showed a reduction in the number of crossings in the second session, when compared to the first one, which is interpreted as habituation to this new environment (Schildein et al., 2002 and Vianna et al., 2001), animals that were handled during the neonatal period presented no habituation, continuing to present a high number of crossings. This lack of habituation could be related to memory (Schildein et al., 2002, Jost et al., 2002 and Vianna et al., 2001) or to a higher ambulatory activity, as reported in the literature (Meaney et al., 1991). This later possibility would agree with the increased number of crossings presented by this group in the light/dark exploration test. The higher ambulatory activity presented by neonatally-handled animals could be due to increased dopaminergic activity (Papaioannou et al., 2002). Effects on memory could not be excluded, but reports concerning memory effects of precocious experiences have shown that, in the laboratory rat and mouse, neonatal handling enhances learning in different tasks in adulthood (Meaney et al., 1988, Tang, 2001, Beane et al., 2002, Bredy et al., 2004 and Tang and Reeb, 2004).

In summary, we find that neonatal handling leads to an increased appetite for palatable food, and this effect is probably not related to altered levels of anxiety in these animals. More studies considering other systems related to feeding behavior are important for the understanding of

this phenomenon.. These studies may be important for the comprehension of the mechanisms of some feeding disorders.

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4. DISCUSSÃO

O objetivo deste trabalho foi verificar se um estímulo estressor no início da vida poderia alterar o padrão de comportamento alimentar de ratos adultos, em especial em relação ao consumo de alimentos palatáveis. Além disso, pretendíamos observar se as alterações do padrão de ingestão alimentar nesses animais tinham relação com o estado emocional, particularmente com a ansiedade. Vimos que o estresse neonatal leva a um aumento do consumo de alimentos palatáveis em animais adultos que sofreram estresse neonatal. Resultados compatíveis foram encontrados em ratos Sprague-Dawley que sofreram 15 minutos de separação materna nas primeiras 3 semanas de vida (McIntosh et al., 1999). Períodos mais longos de separação da mãe também parecem afetar o comportamento alimentar, aumentando a hiperfagia de rebote após um período de restrição alimentar, em especial em fêmeas (Iwasaki et al., 2000).

Conforme discutido no ARTIGO 1, o maior consumo de doce evidenciado nos animais estressados é independente da fome, uma vez que no dia anterior ao teste os ratos haviam recebido ração padrão à vontade. Além disso, este aumento não é acompanhado por maior consumo de ração padrão. Logo, parece que o componente hedônico do controle do apetite tem importância na alteração do comportamento alimentar de ratos que sofreram estresse neonatal.

Sabe-se que vários sistemas relacionados ao prazer, recompensa e saciedade são afetados por intervenções no período neonatal. Há relato, por exemplo, de aumento do

metabolismo dopaminérgico no hipotálamo de ratos que foram expostos ao estresse neonatal (Papaioannou et al., 2002), o que pode significar que a recompensa associada ao alimento doce nestes animais seja maior em relação a ratos intactos. Alguns estudos têm demonstrado que os neurônios dopaminérgicos respondem a estímulos ambientais que atraem a atenção do animal, tendo portanto relevância no aprendizado (Schultz et al., 1993). O estresse neonatal poderia estar alterando a percepção de estímulos ambientais, levando os animais a interpretar a presença de alimento palatável num ambiente diferente da caixa moradia como um estímulo mais significativo do que normalmente é percebido por ratos intactos. A maior secreção de dopamina nesta situação de apresentação do alimento (Bassareo et al., 1999) talvez leve ao maior consumo, uma vez que a atividade do sistema dopaminérgico em algumas áreas específicas como o núcleo accumbens e o córtex pré-frontal acompanha o ato de comer (Gambarana et al., 2003; Kalsbeeket al., 1988).

A serotonina modula a liberação de dopamina pelo núcleo *accumbens* durante o consumo de alimentos (Helm et al., 2003). Em ratos estressados no período neonatal, há aumento de serotonina no hipocampo, hipotálamo e estriado (Papaioannou et al., 2002). É interessante notar que a maior disponibilidade de serotonina, como durante o uso de inibidores de sua recaptação, direciona a preferência alimentar para determinados componentes da dieta (Konkle et al., 2003), podendo levar inclusive à perda de peso. Como neste estudo vimos que o estresse neonatal altera a preferência alimentar sem interferir no consumo de ração padrão e sem aumentar o peso corporal, é possível que este neurotransmissor tenha um papel no efeito observado.

A atividade serotoninérgica em neurônios hipotalâmicos também parece ser afetada pela disponibilidade de alimento no período neonatal (Zippel et al., 2001). Como sabemos, curtos períodos de separação materna no início da vida levam a um maior cuidado maternal, e

possivelmente a um maior tempo de amamentação da ninhada pela mãe (Liu et al., 1997). Pode ser pensado que a disponibilidade de alimento diferenciada para estes animais influencie o desenvolvimento da rede regulatória hipotalâmica, programando permanentemente o consumo alimentar.

Há evidências de maior atividade serotoninérgica em regiões cerebrais onde há maior expressão de receptores glicocorticóides causada pelo estresse neonatal (Smythe et al., 1994), ou seja, hipocampo e córtex frontal, durante os primeiros dias de vida. Entretanto, estas alterações não persistem até a vida adulta. É possível que o sistema serotoninérgico tenha importância como mediador da hiporresponsividade do eixo HPA que ratos que sofreram estresse neonatal apresentam quando adultos, e que a modificação do padrão de secreção de GCs seja responsável pela preferência alimentar diferenciada. A serotonina teria, portanto, uma ação indireta sobre os efeitos que observamos na vida adulta. Por outro lado, como vimos, o padrão de secreção de glicocorticóides por si só é capaz de aumentar a preferência por alimentos palatáveis (Dallman et al., 2003).

A insulina também tem sido descrita como reguladora do comportamento alimentar. Inicialmente apenas seu papel homeostático de supressão do apetite após a alimentação foi evidenciado, mas progressivamente sua ação central foi sendo descoberta (Malabu et al., 1993). Atualmente, tem-se dado atenção ao possível efeito da insulina como reguladora do aspecto hedônico da alimentação (Figlewicz, 2003a), em especial quando correlaciona-se com o nível de glicocorticóides circulante (Strack et al., 1995).

Neurônios dopaminérgicos da área tegmental ventral possuem receptores para insulina (Figlewicz, 2003b), e este hormônio parece diminuir a transmissão dopaminérgica por estimular a recaptação de dopamina (Patterson et al., 1998). A tradução comportamental destes achados neuroquímicos evidencia-se no teste de preferência condicionada ao lugar. Neste

experimento, testa-se a habilidade de um alimento palatável (disponível durante as sessões de treino) de condicionar a preferência dos ratos a permanecerem no local que eles associaram à presença do alimento (num dia de teste, sem a presença do alimento). O condicionamento e a expressão desse comportamento demonstra integridade das vias dopaminérgicas (Papp, 1989), e o protocolo exige que os animais permaneçam em restrição alimentar (portanto, hipoinsulinêmicos) para que a preferência se desenvolva (Swerdlow et al., 1983). Além disso, a injeção de insulina inibe a formação de preferência nesse teste (Figlewicz, 2004). Logo, a insulina regula a recompensa associada à alimentação interferindo na transmissão dopaminérgica e pode ser um componente importante dos efeitos vistos neste trabalho.

A leptina age sobre o comportamento alimentar de maneira similar à insulina nos aspectos descritos acima (Figlewicz, 2004). Este hormônio é importante na regulação do metabolismo e funções neuroendócrinas, influenciando a atividade do eixo HPA (Heiman et al., 1997). A leptina parece estar envolvida na etiologia do período hiporresponsivo ao estresse em ratos (Salzmann et al., 2004). É curioso perceber que animais que sofreram estresse neonatal exibem um padrão de secreção de GCs na vida adulta semelhante a níveis encontrados no período hiporresponsivo ao estresse, e que a secreção de leptina nesses ratos é diferenciada: episódios curtos e repetidos de separação da mãe levam a menor nível de leptina na vida adulta (Panagiotaropoulos et al., 2004). Sendo a leptina um sinalizador dos estoques energéticos do organismo, um menor nível circulante pode levar ao aumento de consumo alimentar específico para dietas hipercalóricas e, portanto, palatáveis.

Pouco se sabe sobre o papel da leptina nas alterações do comportamento encontradas em animais adultos que sofreram estresse neonatal. Um estudo interessante (Oates et al., 2000) demonstrou que este hormônio injetado no período neonatal aumenta o gasto energético e diminui o peso dos filhotes, assim como reduz a liberação de GCs frente a um estressor.

Poderia se pensar que a manipulação diária dos filhotes levasse a uma alteração dos níveis de leptina da mãe, e que o hormônio fosse transferido ao filhote pelo leite materno (Casabiell et al., 1997), induzindo assim as alterações do eixo HPA persistentes já descritas. Entretanto, surpreendentemente, não há modificações significativas do comportamento maternal nas ninhadas que recebem leptina, apesar dos filhotes exibirem o padrão típico de menor responsividade ao estresse. É possível que a injeção diária (independente de ser salina ou leptina) funcione como um estressor, impedindo que se visualize as diferenças entre os grupos.

A leptina também é responsável pela modulação de outros sistemas que controlam o comportamento alimentar. Por exemplo, a leptina causa diminuição da liberação do NPY no hipotálamo (Thorsell et al., 2002). Os GCs em nível fisiológico são capazes de antagonizar parcialmente a ação da leptina na perda de peso e diminuição do apetite, mas não são capazes de reverter a inibição do NPY causada pela leptina (Solano et al., 1999). É interessante ressaltar que apesar de consumirem maior quantidade de determinados componentes da dieta, animais que sofreram estresse neonatal não consomem mais ração padrão nem são mais pesados (não apresentam maior peso corporal) em relação aos ratos controles. A secreção de GCs diferenciada de ratos que sofreram estresse neonatal pode ter uma ação dual influenciando mais de um sistema de controle do comportamento alimentar, ou um mesmo sistema de forma diferente em dois níveis de regulação diversos. Como exemplo, vê-se que longos períodos de deprivação materna diminuem o nível de NPY no hipocampo (Husum et al., 2002), e a interação entre os sistemas NPY/GCs afeta o apetite: NPY administrado no terceiro ventrículo ou no PVN estimula tanto o consumo alimentar quando o eixo HPA (Hanson et al., 1995).

Outros hormônios interagem com o eixo HPA influenciando o comportamento alimentar. O estresse aumenta a expressão gênica de grelina e este hormônio parece modular as respostas ao estresse, em especial em relação à ansiedade (Asakawa et

al., 2001). Além disso, a grelina diminui a liberação de serotonina em sinaptossomas de hipotálamo (Brunetti et al., 2002)., e parece assim estar estimulando o apetite.

Sistemas neuroquímicos relacionados com prazer também podem estar envolvidos no maior consumo de alimento palatável. O sistema opióide é afetado por episódios de separação materna, que levam a aumento da dinorfina A e B no hipotálamo, amígdala (Ploj, 2003a, 1999) e estriado (Ploj, 1999). Também foi observada maior densidade de receptores delta na amígdala basolateral destes animais (Ploj, 2003b). Tendo maior densidade de receptores, é possível que o tônus opióide seja maior em ratos que sofreram estresse neonatal, levando a maior sensação de prazer na presença de um estímulo agradável.

É curioso que os animais consumam mais alimento palatável mas não tenham maior ingestão de soluções doces e salgadas em relação aos controles. Isso sugere que não apenas a palatabilidade do alimento seja importante, mas outros aspectos como a atitude de comer e a textura do alimento podem ser características que influenciam neste achado (Naim et al., 1986).

Em ratos adultos, o estresse repetido por contenção também causa aumento no consumo de doce, sendo que o diazepam reverte o efeito do estresse, fazendo com que o consumo retorne a níveis comparáveis aos de animais controle (Ely et al., 1997). Através dos resultados encontrados no ARTIGO 2, vimos que o aumento de consumo de doce observado em ratos que sofreram estresse neonatal não é revertido com o uso de um medicamento ansiolítico como o diazepam.

Em doses elevadas, o diazepam isoladamente estimula o comportamento alimentar, tanto na busca quanto na ingestão do alimento (Foltin, 2001). Pensava-se que o uso de ansiolíticos influenciava o consumo alimentar apenas por reduzir a ansiedade e o medo dos animais. Porém mais tarde viu-se que, com o uso crônico desses fármacos, adquiria-se tolerância ao efeito sedativo mas não ao aumento do apetite. Logo, a ativação do sistema BDZ

parece produzir um poderoso aumento da resposta hedônica da palatabilidade (Berridge, 1995). Se as teorias desenhadas acima sobre envolvimento de componentes hedônicos do comportamento alimentar influenciando o maior consumo de doce causado pelo estresse neonatal, o uso de BDZs poderia inclusive servir como grande potencializador desse efeito, levando animais estressados a ingerirem muito mais doce quando sob ação desses fármacos. Entretanto, não encontramos resultados significativos: o diazepam não foi capaz de reverter o efeito do estresse neonatal, como acontece em animais estressados na vida adulta, mas também não funcionou como reforçador do componente hedônico do doce pois não aumentou o consumo deste alimento pelos animais.

A preferência por doce está relacionada à menor ansiedade em ratos adultos (Desousa et al., 1998). Vários estudos sugerem que a expressão comportamental da ansiedade pode ser prevista através da análise do sistema BZDs: ratos com maior *binding* para receptores BDZ são menos ansiosos (Harro et al., 1990). A separação da mãe em espisódios curtos no período neonatal aumenta o *binding* para receptores BDZ periféricos (Weizman et al., 1999), e leva a aumento dos níveis de receptores $GABA_A$ no *locus coeruleus* e no núcleo do trato solitário, assim como maior afinidade dos benzodiazepínicos ao receptor GABA nestas regiões e na amígdala (Caldji et al., 2000). Estes achados concordam com a descrição de menor medo e menor reatividade ao estresse em animais adultos submetidos ao estresse neonatal. Entretanto, neste estudo, não foi possível estabelecer diferenças significativas nas medidas comportamentais de ansiedade entre animais estressados e intactos usando testes como o labirinto em cruz elevado e o teste de transição claro/escuro. Um resultado consistente em nosso trabalho foi o de maior atividade dos ratos estressados nestes testes, o que pode significar maior exploração e menor medo, e possivelmente ser interpretado como menor ansiedade. Diferenças nos protocolos de estresse neonatal, especialmente em relação ao tempo de manipulação diária e à duração da

intervenção neonatal podem ser responsáveis pelo conflito entre o resultado deste trabalho e dados da literatura.

A injeção intracerebroventricular de CRH produz respostas comportamentais, fisiológicas e imunológicas semelhantes àquelas induzidas pelo estresse, independente da ação da hipófise e da adrenal (Momose et al., 1999). Entre estas respostas encontram-se a inibição do apetite e o aumento de ansiedade. Curtos períodos de manipulação neonatal são sabidamente causadores de alterações persistentes do eixo HPA, como redução do CRH hipotalâmico e da eminência média (Plotsky et al., 1992). A análise comportamental concorda com estes dados, como já citamos: o estresse neonatal leva a menos medo e menos ansiedade em ambientes novos na vida adulta (Levine et al., 1997). No estudo atual, entretanto, apesar de evidenciarmos efeitos sobre o apetite, não encontramos efeito da manipulação neonatal em testes comportamentais de ansiedade.

Por fim, vemos que o comportamento alimentar tem diferentes formas de regulação centrais e periféricas, como visto acima, e o estresse pode influenciar estes mecanismos reguladores em vários pontos. Mesmo os GCs por si têm ação sobre o consumo de alimentos, e como animais expostos a intervenções neonatais apresentam resposta ao estresse diferenciada e secreção de GCs típica, é possível que essa propriedade também influencie a ingestão alimentar diretamente ou através da modulação de sistemas-chave que regulam o apetite. Logo, este trabalho abre perspectivas para novos estudos a fim de compreendermos os mecanismos relacionados aos efeitos do estresse neonatal sobre o consumo de alimento na vida adulta.

5. CONCLUSÕES

O estresse neonatal aumenta o consumo de alimentos palatáveis (doce e salgado) na vida adulta. Esse efeito não é acompanhado de alterações do consumo de ração padrão, água ou soluções palatáveis doces e salgadas. Além disso, a preferência por doce é evidente mesmo em épocas mais tardias da vida.

O maior consumo de doce em ratos submetidos ao estresse neonatal não é revertido por injeção de diazepam logo antes do teste. Da mesma forma, estes animais não apresentam comportamento compatível com ansiedade em testes como o labirinto em cruz elevado e o teste de transição claro/escuro.

É possível que o estresse neonatal esteja agindo por mecanismos de programação para alterar a preferência alimentar dos animais na vida adulta. Uma vez que existe a preferência alimentar estabelecida, supõe-se que, numa dieta de livre acesso a qualquer alimento, a seleção de determinados componentes possa contribuir para o desenvolvimento de doenças relacionadas ao consumo alimentar, como a aterosclerose. O impacto deste achado é muito significativo, ou seja, mesmo em períodos muito precoces do desenvolvimento, mínimas intervenções agressivas têm poder para gerar efeitos danosos que repercutem a longo prazo.

A busca pela compreensão dos mecanismos e sistemas neuroquímicos pelos quais o estresse neonatal interfere no consumo alimentar pode contribuir para melhorar o padrão de saúde-doença através de ações preventivas ou terapêuticas específicas.

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