UNIVERSIDADE DO EXTREMO SUL CATARINENSE – UNESC

PROGRAMA DE PÓS-GRADUAÇÃO MESTRADO EM CIÊNCIAS DA SAÚDE – PPG-CS

ESTUDO DOS EFEITOS COMPORTAMENTAIS E NEUROQUÍMICOS DO MODELO ANIMAL DE DEPRESSÃO BASEADO NO PARADIGMA DO ESTRESSE CRÔNICO LEVE E VARIADO

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CRICIÚMA
2008

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Ciências da Saúde para obtenção do título de Mestrado em ciências da Saúde.

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CRICIÚMA
2008

AGRADECIMENTOS

Agradeço a todos que de alguma forma me auxiliaram neste processo.

Agradeço à Clarissa por sua capacidade, sua dedicação, inexprimível auxílio e competência.

Agradeço ao Quevedo, meu amigo e orientador, por acreditar em mim e me estimular sempre.

Agradeço ao pessoal do Neurolab pela amizade e constante apoio.

Agradeço à Fernanda, namorada, noiva e mulher, por ser meu foco nos momentos nebulosos.

DEDICATÓRIA

Dedico esta dissertação ao Neurolab e a todos seus integrantes que fizeram e fazem de alguma forma parte de minha história pessoal.

Dedico também à UNESC e seus profissionais que me proporcionaram espaço para crescer juntamente com eles.

ÍNDICE

PARTE I 06

1. RESUMOS 07
1.1 Resumos em Português 07
1.2 Resumos em Inglês (abstract) 08

2.LISTA DE ABREVIATURAS 09

3. INTRODUÇÃO 10
3.1 Teoria Monoaminérgica 12
3.2 Teoria do Estresse – Eixo Hipotálamo-Hipófise-Adrenal 13
3.3 Teoria Molecular Intracelular 16
3.3.1 AMPc 16
3.3.2 Proteínas Kinases (PK) 17
3.3.3 CREB e Transcrição Gênica 17
3.4 BDNF – *Brain-derived neurotrophic factor* 18
3.5 Radicais Livres e Estresse Oxidativo 21
3.6 Modelo Animal de Depressão – Estresse Crônico Leve Variado (ECLV) 22

4 OBJETIVOS DO TRABALHO 24
4.1 – Objetivo Geral 24
4.2 Objetivos Específicos 24

PARTE II 25

1. Artigo "Chronic Mild Stress Paradigm Reduces Sweet Food Intake in Rãs Without Affecting BDNF Protein Levels" 26

2. Artigo "Effects of Chronic Mild Stress Model on The Oxidative Damage Parameters in the Rat Brain" 48

3. Artigo "Effects of Chronic Mild Stress Model on The Oxidative Damage Parameters in the Rat Brain" 70

PATE III 96

1. DISCUSSÃO 97

REFERÊNCIAS 105

PARTE I

1. RESUMOS

1.1 Resumo em Português

O transtorno depressivo maior (TDM) é um dos transtornos mentais mais prevalentes, incapacitantes, gerador de grande sofrimento aos portadores e aos familiares, podendo levar os indivíduos a cometerem suicídio. Cerca de 4.4% do ônus mundial com doenças é empregado no manejo deste transtorno. Apesar disso, as bases biológicas do TDM ainda são pouco conhecidas. O protocolo de estresse crônico leve variado (ECLV) tem sido utilizado como modelo animal de depressão refletindo anedonia em animais. O presente estudo investigou aspectos comportamentais e neuroquímicos de ratos expostos ao protocolo de ECLV. Foram avaliados os seguintes parâmetros: o consumo de comida doce, atividade locomotora, peso da glândula adrenal, peso corporal, níveis da proteína do BDNF no hipocampo, líquor e soro, níveis de oxidação de proteína e lipídeos e atividade das enzimas superóxido dismutase (SOD) e catalase (CAT) em hipocampo, córtex pré-frontal, córtex, cerebelo e estriado. Nossos resultados evidenciaram redução da ingestão de sacarose (anedonia), aumento do peso da glândula adrenal e falta de ganho ponderal nos animais submetidos ao ECLV. Não houve diferenças nos níveis de BDNF em soro, líquor e hipocampo, nem na atividade locomotora em relação ao grupo controle. Foi encontrado aumento da peroxidação protéica (pré-frontal, hipocampo e estriado) e lipídica (cerebelo e estriado). Em relação à atividade das enzimas antioxidantes houve um aumento da atividade da CAT (cerebelo, hipocampo e estriado) e redução da atividade da SOD (pré-frontal, hipocampo e estriado) nos ratos estressados. A geração de ERO em partículas submitocondriais no cérebro de ratos foi demonstrada pelo aumento da geração de superóxido em todas estruturas cerebrais analisadas. Um aumento na geração de TBARS foi observada apenas no córtex. Assim, estes resultados evidenciam que o modelo de ECLV induziu anedonia em ratos Wistar sem alterar os níveis da proteína do BDNF. Entretanto, produtos da degradação de lipídeos e proteínas por radicais livres (superóxido mitocondrial), bem como alterações na atividade das enzimas SOD e CAT, sugerem que o estresse oxidativo contribui em parte no TDM.

Palavras-Chaves: Estresse crônico leve variado. BDNF. SOD. CAT. Transtorno depressivo maior. Estresse oxidativo. ROS.

1.2 ABSTRACT

The major depressive disorder (MDD) is one of the most prevailing, incapacitating mental disorders, generator of great suffering to the ones affected and its family, that could end up in suicide. About 4,4% of the worlds burden with diseases is spent on the treatment of this illness. Despite that, the biological basis of the MDD are still not fully elucidated. The ECLV protocol have been used as an animal model of depression reflecting anedony in them. The present study investigated the behavioral and neurochemical aspects in rats exposed to the ECLV protocol. The following parameters were evaluated: the consuption of sweet food, the motor activity, cerebrospinal fluid and serum, protein and lipids oxidation levels and the activity of SOD and CAT enzymes in hippocampus, pre-frontal cortex, cortex, cerebellum and striatum. Our results show reduction in the sacarosis intake (anedony), an increase on the adrenal gland weight and a lack of ponderal gain on the animal exposeed to the ECLV. There were no differences in the serum, cerebrospinal fluid and hipocampus BDNF, neigther in the motor activity when comparing to the controls. It was found an increase in protein peroxidation (pre-frontal cortex, hipocampus and striatum) as well as in lipid peroxidation (cerebellum and striatum). In regard to the antioxidants enzymes activity there was an increase in CAT activity (cerebellum, hipocampus and striatum) and a reduction in SOD activity (pre-frontal, hipocampus and striatum) on the stressed rats. The generation of ROS in submitochondria particles in the rat brain was demonstrated by the increase of superoxide generation in the all brain structures analyzed. An increased in thiobarbituric acid reactive substances generation was observed only in the cortex. In this manner, these results showed that the ECLV model induced anedony in Wistar rats without altering the levels of BDNF protein. However, free radical (superoxide) products from protein and lipid degradation, as well as SOD and CAT enzymes activity, suggest that the oxidative stress contributes in parts in the MDD pathogenesis.

KEYWORDS: Chronic mild stress. BDNF. SOD. CAT. Major depression. Oxidative stress.

ROS.

2. LISTA DE ABREVIATURAS

5-HT - Serotonina

AC – Adenil Ciclase

ACTH – Hormônio Adrenocorticotrófico

AMPc – Monofosfato de Adenosina Cíclica

ATP – Trifosfato de Adenosina

BDNF – Fator Neurotrófico Derivado de Cérebro

CAT - Catalase

CRE – "*cyclic AMP response element"*

CREB – "*c-response element binding-protein"*

CRH – Hormônio Liberador da Corticotrofina

DAG – Diacilglicerol

ECLV – Estresse Crônico Leve Variado

ECT - Eletroconvulsoterapia

ERO – Espécies Reativas do Oxigênio

HHA – Hipotálamo-Hipófise-Adrenal

LSD – Ácido Lisérgico

OMS – Organização Mundial de Saúde

PK – Proteína Kinase

RG – Receptor de Glicocorticóide

RNAm – Ácido Ribonucléico Mensageiro

SNC – Sistema Nervoso Central

SOD – Superóxido Dismutase

TBARS - Substâncias Reativas Derivadas do Ácido Tiobarbitúrico

TDM – Transtorno Depressivo Maior

TEPT – Transtorno do Estresse Pós-Traumático

TNF – Teste do Nado Forçado

TrkB – Receptor Tirosina Kinase B

3. INTRODUÇÃO

O Transtorno Depressivo Maior (TDM) se caracteriza por episódios isolados ou recorrentes de tristeza e perda de prazer por quase todas as atividades habituais num período superior a duas semanas (American Psychiatric Association, 2000). Os indivíduos também apresentam sintomas adicionais que incluem alterações neurovegetativas sobre o apetite, peso, sono, libido, diminuição da energia e mudança no nível habitual da atividade psicomotora. Sintomas de ordem cognitiva são expressos através do relato de sentimentos injustificados de culpa, ideação ou plano suicida, pensamentos obsessivos sobre morte, dificuldades de concentração e memória prejudicada. Na esfera somática podem estar presente sintomas dolorosos como cefaléias e dores musculares (American Psychiatric Association, 2000; Wise et al., 2006 e Goodwin, 2007). O diagnóstico do TDM só é feito quando os sintomas promovem prejuízo significativo no funcionamento social, profissional ou em outras áreas importantes da vida do indivíduo. Em casos de intensidade mais leve o funcionamento pode parecer normal, mas exige um esforço aumentado e desproporcional (American Psychiatric Association, 2000).

O TDM está entre os transtornos mentais mais prevalentes e pode resultar em incapacidade, morte prematura por suicídio e intenso sofrimento dos pacientes acometidos e seus familiares (Nestler et al., 2002). O TDM é responsável por 4.4% do ônus mundial com doenças, uma contribuição semelhante a das doenças cardiovasculares isquêmicas ou diarréicas (Mann, 2005). As formas graves de depressão acometem cerca de 2-5% da população dos Estados Unidos e aproximadamente 20% sofre de formas mais leves. As mulheres são duas vezes mais acometidas do que homens. A incidência ao logo da vida é de 13% para homens e pode chegar até 25% nas mulheres. (American Psychiatric Association,

2000 e Murray et al., 1997). Um grave prejuízo ocupacional e social atinge aproximadamente 60% das pessoas acometidas (Murray et al,1997).

Segundo estimativas feitas pela Organização Mundial de Saúde (OMS), o TDM era a quarta causa mundial de incapacidade em adultos em 1990 (Murray et al., 1997) e em 2020 o TDM será a segunda maior causa mundial de incapacidade, abaixo somente das doenças isquêmicas do coração (Murray et al. 1997).

Apesar do reconhecimento do impacto devastador gerado pelo TDM no mundo inteiro, muito ainda falta ser elucidado na pesquisa de novos psicofármacos, em um melhor entendimento dos mecanismos genéticos e neurobiológicos subjacentes aos transtornos de humor, caracterizando, então, uma lacuna entre o conhecimento dos transtornos de humor e seu tratamento (Nestler et al., 2002).

A preocupação com o desenvolvimento de novas terapêuticas antidepressivas com mecanismos distintos das atuais, mais eficazes e com ação mais rápida do que duas a seis semanas, conduziu as investigações para a busca do entendimento de outras causas da depressão. A utilização de modelos animais de depressão neste contexto se fez imprescindível para a compreensão e evolução da pesquisa das alterações neuroquímicas dos quadros depressivos como as desencadeadas pelas alterações do estresse crônico, da plasticidade neuronal, das cascatas intracelulares e do estresse oxidativo.

A presente dissertação tem como propósito revisar brevemente algumas teorias neurobiológicas já estabelecidas e apresentar um modelo animal de depressão baseado no estresse que será replicado em nosso laboratório para que possamos responder alguns de nossos questionamentos e contribuir com o avanço do conhecimento da neurobiologia do TDM.

3.1 TEORIA MONOAMINÉRGICA

O tratamento farmacoterápico da depressão revolucionou a forma como ela é atualmente entendida e deu início à era moderna da pesquisa neurobiológica nesta área. No início, os psicofármacos foram descobertos incidentalmente durante o tratamento de pacientes clínicos com melhora nos sintomas de humor. A imipramina (antidepressivo tricíclico) foi descoberta por seu efeito antidepressivo em pacientes que a utilizavam como anti-histamínico e a iproniazida (inibidor da enzima monoaminoxidase) no tratamento de tuberculosos há pouco mais de meio século atrás (Nestler et al., 2002). Essas medicações e outras substâncias como a cocaína, o LSD e a reserpina levaram à hipótese de que a redução das concentrações sinápticas das aminas biogênicas (serotonina, noradrenalina e dopamina) seria o fator causal da depressão (Schildkraut, 1965). Fatores que reduzem a concentração sináptica dos neurotransmissores monoaminérgicos e seu papel no desencadeamento de episódios depressivos vêm sendo estudados. Entre eles estão a hiperatividade da enzima monoaminoxidase, as rotas metabólicas, fusão das vesículas para liberação dos neurotransmissores e a concentração dos precursores dos neurotransmissores (Belmaker et al., 2008).

Além da redução sináptica das monoaminas, foi descoberto que alterações estruturais ou funcionais dos receptores pré e pós-sinápticos para estas substâncias contribuem para o desenvolvimento do quadro depressivo e suas variantes (Belmaker et al., 2008).

Os sistemas monoaminérgicos indubitavelmente desempenham um importante fator na etiopatogenia das complexas facetas e apresentações clínicas do TDM, contudo estudos recentes têm levantado a hipótese de que as alterações das monoaminas possam representar efeitos finais de anomalias intracelulares anteriores (Belmaker et al., 2008).

3.2 TEORIA DO ESTRESSE - EIXO HIPOTÁLAMO-HIPÓFISE-ADRENAL

O estresse ambiental é percebido pelo córtex cerebral e transmitido ao hipotálamo, onde o hormônio liberador da corticotrofina (CRH) é liberado na hipófise anterior. O estímulo do CRH nas células hipofisárias promove a secreção do hormônio adrenocorticotrófico (ACTH) no plasma e este age sobre as células do córtex da glândula adrenal promovendo aumento de seu volume, aumento da produção e da liberação de cortisol na corrente sangüínea. O cortisol por sua vez inibe, por *feedback* negativo, a secreção do CRH e ACTH para manutenção da sua homeostase. O cortisol exerce esta ação inibitória pela sua ligação aos receptores para glicocorticóides (RG) localizados no córtex, hipocampo e hipotálamo (Froger et al., 2004). Esta cadeia de eventos é chamada de eixo hipotálamo-hipófise-adrenal (HHA) (Gillespie et al., 2005).

Anomalias no funcionamento do eixo HHA são bem descritas em alguns pacientes com TDM (Shelton, 2007 e Gillespie et al., 2005). Pacientes deprimidos podem apresentar níveis elevados de cortisol plasmático (Froger et al., 2004 e Burke et al., 2005), do CRH no líquido cefalorraquidiano (Merali et al., 2004 e Froger et al., 2004), aumento do mRNA do CRH e sua proteína em regiões límbicas (Stout et al., 2000 e Merali et al., 2004). Aproximadamente metade dos portadores de TDM grave não reduz os níveis séricos de cortisol após o teste de supressão com dexametasona (Carrol et al., 2007 e Gillespie et al., 2005). Possivelmente pacientes que não revertem a hiper-ativação do eixo HHA possuam uma expressão reduzida dos RG necessários para sinalização por *feedback* negativo (Gillespie et al., 2005 e Froger et al., 2004). A serotonina é o agente responsável pelo início da cascata de sinalização intracelular que culmina com a transcrição dos RG. Vários artigos mostram que

modelos de estresse crônico culminam em uma *downregulation* dos RG tanto no hipocampo quanto no hipotálamo induzindo uma hiper-ativação prolongada do eixo HHA por conseqüente falência da regulação por *feedback* negativo provocada pela ativação desses receptores (Froger et al., 2004; Mizoguchi et al., 2001; Kitraki et al., 1999; Makino et al., 1995 e Johren et al., 1994). A remissão clínica de um episódio depressivo tratado com antidepressivo (aumentando a concentração de serotonina) é acompanhada pela reversão de algumas destas anomalias do eixo HHA (Belmaker et al., 2008).

Eventos adversos precoces, como abuso físico ou sexual, em fases críticas do desenvolvimento infantil podem acarretar alterações permanentes no funcionamento do eixo HHA (Shelton, 2007 e Kaufman et al., 2000). Ratos adultos que foram separados de suas mães quando filhotes apresentam alterações de hiper-ativação do eixo HHA, maior tempo de imobilidade no teste do nado forçado (TNF) e a utilização de antidepressivos reverte esses resultados (Bhansali et al., 2007).

Estudos de neuroimagem e pós-morte em pacientes com depressão crônica, resistente ou recorrente, evidenciam que existe uma redução volumétrica e no número de células na região do hipocampo, córtex órbito-frontal, pré-frontal, núcleo *acumbens* e gânglios da base quando comparados com indivíduos controle (Gonçalves et al., 2006; Sheline et al., 2003; Rajkowska, 2000; Soares et al., 1997 e Elkis et al., 1995). As causas desses achados ainda não estão totalmente elucidadas podendo representar anomalias neurodesenvolvimentais, progressão do transtorno que fundamentalmente envolve perda/atrofia glial e neuronal ou alterações bioquímicas que acompanham os episódios depressivos (Manji et al., 2003; Duman et al., 2000; Gould et al., 2000; Manji et al., 2000; Rajkowska, 2000; Sapolsky, 2000 e Bremner, 1999). Dando suporte à última hipótese, o estresse crônico e a administração de glicocorticóides provocaram atrofia ou até mesmo morte de neurônios hipocampais em ratos e primatas (Bergström et al., 2008, Dranovsky et al., 2006, Duman et al., 2004 e Bremner,

1999). Estudos de ressonância nuclear magnética em pacientes com quadros clínicos constituídos de elevações crônicas dos hormônios do eixo HHA como portadores da síndrome de Cushing e do transtorno do estresse pós-traumático (TEPT) também evidenciaram redução volumétrica hipocampal quando comparado com o grupo controle (Manji et al., 2000 e Brown et al., 1999). Os neurônios do hipocampo apresentam uma grande densidade de receptores glicocorticóides. Estudos de seguimento sugerem que o dano hipocampal estaria associado à exposição direta, elevada e por tempo prolongado de glicocorticóides (Bremner, 1999 e Sapolsky et al., 1990). Outros estudos em animais demonstraram que os glicocorticóides promoviam diminuição das ramificações dendríticas (Wooley et al., 1990), perda neuronal (Uno et al., 1990), alterações na estrutura sináptica (Magarinos et al., 1997), inibição da regeneração neuronal (Gould et al., 1998) e neurogênese no hipocampo (Bremner, 1999). Os glicocorticóides exercem seus efeitos através de uma desestruturação do metabolismo celular (Lawrence et al., 1994) e aumentando a vulnerabilidade dos neurônios aos diversos insultos, incluindo a liberação de aminoácidos excitatórios e o aumento do acúmulo de glutamato extracelular com conseqüente aumento do influxo de cálcio e ativação de cascatas apoptóticas intracelulares (Zhou et al., 2005, Manji et al., 2003, Nestler et al., 2002, Saplosky, 2000 e Saplosky et al., 1985).

Além da ação deletéria dos glicocorticóides sobre os neurônios, outros fatores parecem contribuir para manutenção e instalação destas lesões como a ação lesiva e indutora de apoptose dos radicais livres (Ng et al., 2008) e as alterações na transcrição e liberação das neurotrofinas, as quais são essenciais para manutenção, proliferação e sobrevida neuronal (Castrén et al., 2007). Entre as neurotrofinas o BDNF (*Brain-derived neurotrophic factor*) é uma das mais estudadas atualmente e faz parte de uma das diversas cascatas de eventos bioquímicos que ocorrem no interior dos neurônios.

3.3 TEORIA MOLECULAR INTRACELULAR

A teoria molecular e intracelular vem sendo estudada passando para além da fenda sináptica e observando as alterações subseqüentes à ativação de receptores pós-sinápticos (Hammond et al., 2007). Os dados mais recentes a partir do laboratório de Duman (Kempermann et al., 2003) sugerem a existência de uma cascata celular cuja seqüência seria a seguinte: Ativação do receptor pós-sináptico (por neurotransmissores e/ou BDNF), AMPc, proteínas kinases, CREB (*c-response element binding-protein*), transcrição gênica de RG, BDNF, receptor para BDNF (Shaltiel et al., 2007 e Gonçalves et al., 2006). Esta cascata seria unificadora de mecanismos como a reestruturação dendrítica, aumento da neurogênese hipocampal e aumento da sobrevida das células do SNC (Duman et al., 2006, Duman, 2004 e Duman et al., 2000). Todas estas moléculas foram implicadas na fisiopatologia da depressão e a partir de então viraram alvos para desenvolvimento de possíveis novos psicofármacos com ação antidepressiva (Manji et al., 2003 e Nestler et al., 2002). Vários trabalhos sugerem que o aumento da expressão destes elementos é necessário para a ação terapêutica dos antidepressivos (Duman et al., 2006, Kempermann et al., 2003, Manji et al., 2003 e Nestler et al., 2002). Estes achados acrescentam evidências ao empirismo inicial do uso de antidepressivos (Gonçalves et al., 2006). A seguir apresentamos separadamente os diversos elementos desta cascata.

3.3.1 **AMPc**

A administração crônica de antidepressivos aumenta a expressão do AMPc, o qual dá início à cascata celular acima descrita (Shelton, 2007). Assim, antidepressivos que elevam a concentração sináptica de noradrenalina estimulam a conversão do ATP em AMPc o qual aumenta a sobrevida, mas não a diferenciação de neurônios (Gonçalvez et al., 2006).

3.3.2 PROTEINA KINASES (PK)

A PKA é fosforilada após a ligação da noradrenalina ao seu receptor proteína G acoplada à adenilciclase (AC) que gera o segundo mensageiro AMPc responsável por essa fosforilação (Shelton, 2007). Em contrapartida a PKC é fosforilada como conseqüência da ligação da serotonina em seu receptor acoplado à fosfolipase C que gera o segundo mensageiro diacilglicerol (DAG) fosforilando a PKC (Shelton, 2007). Ambas PK têm em comum a fosforilação do CREB o qual, nessa forma, penetra os poros nucleares e regula a transcrição gênica. Essa seria a rota integrativa entre a ação dos antidepressivos que aumentam a concentração sináptica de serotonina e noradrenalina (Gronli et al., 2006).

Estudos em humanos com TDM evidenciaram níveis reduzidos de PK. Além dessa diminuição, dependendo do subtipo de depressão, melancólica ou atípica, há uma redução maior ou menor (Akin et al., 2005; Pandey et al., 2005 e Akin et al. 2004). A deficiência destas PK ocasionaria uma interrupção da cascata e assim menor fosforilação do CREB e conseqüentemente menor transcrição gênica. Estes achados foram encontrados tanto em sangue periférico quanto em tecido cerebral pós-morte por outros grupos de pesquisa (Gronli et al., 2006; Dwivedi et al., 2004; Dwivedi et al., 2003; Dwivedi et al., 2002; Coull et al., 2000; Pandey et al., 1998 e Pandey et al., 1997).

Apesar do reconhecimento da redução dessas proteínas em pacientes deprimidos, o mecanismo exato para este achado permanece desconhecido. Uma das possíveis explicações seria o aumento da oxidação destas proteínas pela ação de espécies reativas de oxigênio (Adler et al., 1999).

3.3.3 CREB E TRANSCRIÇÃO GÊNICA

Estudos mostram que a expressão do CREB aumenta paralelamente à maturação de novos neurônios (Gonçalves et al., 2006). Após a fosforilação do CREB pela ação das PKA e

PKC, ele penetra no interior do núcleo onde regula a expressão gênica de genes que possuem o *cyclic AMP response element* (CRE) em sua região promotora (Shelton, 2007). Esses genes codificam proteínas chaves que regulam a resposta ao estresse no tecido cerebral como o BDNF (Karege et al., 2004 e Shieh et al., 1998), seus receptores para tirosina kinase (TrkB) (Deogracias et al., 2004) e receptores glicocorticóides (Barrett et al., 1996). Gronli et al. (2006) mostraram que ratos submetidos ao modelo de estresse crônico leve variado (ECLV) apresentavam níveis inferiores aos controles do CREB fosforilado (Gronli et al., 2006).

Essa cascata de eventos evidencia diversos passos subseqüentes à interação das monoaminas com seus receptores pós-sinápticos como a alteração da plasticidade neuronal e as conseqüentes alterações encontradas em hipocampo e outras áreas cerebrais de pacientes com TDM (Blendy, 2006).

3.4 BDNF - *Brain-derived neurotrophic factor*

O BDNF (*Brain-derived neurotrophic factor*) é uma importante proteína membro da família das neurotrofinas e está presente de forma abundante no tecido cerebral e periférico, pois pode ser encontrado em neurônios e plaquetas sendo capaz de cruzar a barreira hemato-encefálica (Duffau et al., 2006 e Karege, 2002). Esta neurotrofina tem ação diversa sobre as células nas distintas regiões cerebrais como crescimento celular, conectividade sináptica, diferenciação e reparo neuronal (Tapia-Arancibia et al., 2004). Além disso, encontra-se envolvido na expressão de diferentes neurotransmissores (Karege, 2002). O BDNF exerce sua ação através da ligação em seu receptor (TrkB) presente na membrana neuronal das sinapses (Castrén et al., 2007).

Artigos recentes têm sugerido que os antidepressivos e a eletroconvulsoterapia (ECT) poderiam atuar através do aumento na produção das neurotrofinas cerebrais (Castrén et al.,

2007 e Jayatissa et al., 2006). A redução das neurotrofinas poderia estar envolvida na patogênese dos transtornos de humor, pois uma deficiência na produção endógena de neurotrofinas causaria uma interrupção no crescimento, diferenciação e ramificação neuronal com ineficácia da ação antidepressiva (Jayatissa et al., 2006; Gage, 2000 e Gould et al., 2000). O período desde a administração do antidepressivo até a transcrição e tradução da proteína do BDNF, a qual seria essencial para modificação plástica das estruturas neuronais, explicaria o tempo de latência de aproximadamente quatro semanas para a observação da resposta clínica (Altar, 1999).

A relação entre as neurotrofinas e a depressão é muito mais complexa do que primeiramente se pensava. Apesar da sinalização através do BDNF estar claramente envolvida na resposta antidepressiva, a redução dos seus níveis ou de sua sinalização não produziu sintomas depressivos em ratos (Duman et al., 2006 e Saarelainen et al., 2003). Animais com uma diminuição dos receptores TrkB não aumentaram o tempo de imobilidade no teste do nado forçado (Saarelainen et al., 2003), mas desenvolveram comportamento ansioso. De forma contrária, o aumento da sinalização via TrkB reduziu sintomas de ansiedade e comportamentos depressivos em ratos (Koponen et al., 2006 e Koponen et al., 2005). A infusão de BDNF no hipocampo e núcleo da rafe mimetizou efeitos antidepressivos em ratos, enquanto que a infusão na área tegmental ventral produziu um comportamento depressivo (Eisch et al., 2003).

Com relação ao estresse e modelos de depressão, artigos têm demonstrado uma redução na expressão do RNA mensageiro (mRNA) do BDNF após a aplicação do modelo de ECLV em ratos e um aumento com a administração de fármacos antidepressivos (Gonçalves, et al. 2006; Shirayama et al., 2002; Russo-Neustaldt et al., 2001 e Smith et al., 1995). Um artigo extremamente elucidativo revelou o papel da remodelação da cromatina no estresse e transcrição do BDNF (Tsankova et al., 2006). Animais submetidos a um modelo de estresse

apresentavam uma hiper-metilação duradoura de histonas na região promotora do gene do BDNF suprimindo assim sua transcrição. Isso sugere que o estresse crônico induz um estado de repressão da transcrição gênica por período de até um mês após a retirada dos estressores. O uso de antidepressivo não reverte a metilação das histonas, porém gera uma acetilação na mesma proteína o que aumenta a transcrição do mRNA para BDNF (Tsankova et al., 2006).

Em relação à quantidade de proteína total do BDNF existem divergências entre trabalhos quanto a sua variação. Alguns trabalhos mostram redução da proteína total em cérebro de ratos após serem submetidos ao modelo de estresse (Franklin et al., 2006 e Angelucci et al., 2000), enquanto outros mostram ausência de alteração em relação aos controles (Gronli et al., 2006 e Rosenborck et al., 2005). Contudo, estudos de microdiálise mostram que as alterações nas concentrações da proteína do BDNF variam entre áreas distintas do hipocampo e não de forma homogênea no encéfalo (Gronli et al., 2006; Rosenborck et al., 2005; Gronli et al., 2004; Karege et al., 2002 e Conner et al., 1997). Quanto à dosagem do BDNF sérico em humanos, estudos têm demonstrado uma redução na concentração do BDNF em pacientes com transtorno depressivo maior grave em relação aos controles e um aumento nos níveis após o tratamento crônico com antidepressivos (Shimizu et al., 2003). O BDNF é encontrado perifericamente nas plaquetas o que poderia dificultar a interpretação destes achados.

Até então, não há na literatura artigos de correlação entre os níveis séricos, liquóricos e cerebrais de neurotrofinas em modelos animais de depressão e em humanos portadores de transtornos de humor.

3.5 RADICAIS LIVRES E ESTRESSE OXIDATIVO

Atualmente sabe-se que a geração de espécies reativas de oxigênio (ERO) exerce um papel fundamental na fisiopatologia de diversos transtornos neuropsiquiátricos como transtorno de humor bipolar, esquizofrenia, Alzheimer e outros processos demenciais (Frey et al., 2006a; Frey et al., 2006b e Gackowski et al., 2008). As ERO são radicais livres capazes de reagir indiscriminadamente com qualquer tipo de molécula orgânica, extraindo elétrons e gerando novos radicais livres em reações em cadeia altamente citotóxicas e com potencial de oxidar moléculas biológicas incluindo proteínas, lipídeos e DNA (Middleton et al., 2000; Coleman 2001; Yen et al., 2003; Aldred et al., 2004). As lesões causadas pelas ERO podem desencadear um processo de morte celular programada denominado apoptose (Halliwell, 2006). Em pacientes deprimidos observa-se redução volumétrica e no número de células do hipocampo onde a ação dos radicais livres poderia contribuir em parte para este achado (Rajkowska, 2000 e Sapolsky, 2000). Entre as proteínas lesadas pelos radicais livres estariam as PK importantes nas rotas metabólicas intracelulares nos quadros depressivos.

O cérebro é particularmente vulnerável à produção das ERO, porque ele metaboliza 20% do oxigênio corporal total e tem uma capacidade antioxidante limitada (Halliwell, 2006). As enzimas superóxido dismutase (SOD) e catalase (CAT) exercem papel importante na rota de eliminação dos radicais livres gerados no interior das células. A enzima SOD é responsável pela conversão do ânion superóxido em peróxido de hidrogênio, o qual, através da ação da CAT é convertido em oxigênio e água (Halliwell, 2006). Caso a SOD esteja em baixas concentrações há uma tendência ao acúmulo de superópxido e conseqüentemente desencadeia estresse oxidativo. Se a enzima CAT apresentar-se em baixas concentrações o estresse oxidativo se instala pela conversão do peróxido de hidrogênio em hidroxila, altamente reativo

e cititóxico, pois a CAT impede esta reação (Halliwell, 2006). Em situações onde a geração de radicais livres excede a capacidade das defesas antioxidantes, o estresse oxidativo pode levar a degradação da membrana, disfunção celular, dano ao DNA e apoptose (Frey et al. 2006b; Halliwell, 2006 e Gackowski et al., 2007). O tratamento de ratos submetidos ao ECLV com venlafaxina reduz os níveis de peroxidação lipídica e óxido nítrico (ON) no tecido cerebral e em eritrócitos de ratos tratados e aumenta estes parâmetros em controles expostos ao ECLV não tratados, evidenciando presença de estresse oxidativo em ratos com anedonia (Gackowski et al., 2007 e Eren et al., 2007).

3.6 MODELO ANIMAL DE DEPRESSÃO - ESTRESSE CRÔNICO LEVE VARIADO (ECLV)

Os modelos animais de depressão vêm sendo estudados há mais de duas décadas (Mineur et al., 2006; Willner, 2005; Willner et al., 2002; Cabib, 1997; Willner, 1997; Willner et al., 1987 e Katz, 1982). A necessidade para elaboração dos modelos animais de depressão surge da urgência em aprofundar o entendimento das bases biológicas subjacentes aos quadros depressivos e para elaboração de novos psicofármacos (Willner et al., 1987).

Entre eles, encontra-se o modelo do ECLV (Gamaro et al., 2003 e Willner et al., 1987). Este modelo é baseado em um trabalho intenso, com demanda de espaço físico, material e tempo para aplicação. O modelo consiste em expor os animais, seqüencialmente, a uma variedade de estressores distintos e imprevisíveis, por um período de semanas, com intuito de induzir um estado depressivo melancólico caracterizado pela presença de anedonia (Auriacombe et al., 1997 e Willner et al., 1987). Os modelos de depressão animal, baseados no estresse, representam uma aproximação indispensável à patologia em humanos, uma vez que dados clínicos apontam para um importante papel de experiências estressantes (“eventos

de vida adversos") no desenvolvimento, expressão e exacerbação destes transtornos (Kessing, 2007; Gamaro et al., 2003; Cabib, 1997; Willner, 1997 e Willner et al., 1987), bem como devido ao fato de que uma grande proporção dos pacientes portadores de TDM apresentarem disfunção do eixo HHA (Shelton, 2007).

As vantagens do ECLV é que ele possui uma validade aparente, onde quase todos os sintomas da síndrome depressiva são demonstrados no modelo, maior que a maioria dos demais modelos de depressão em animais (Willner, 1997). Estudos mostram que entre as alterações apresentadas estão o distúrbio do sono (Gronli et al., 2004 e Cheeta et al., 1997), apetite (Gronli et al., 2004 e Gamaro et al., 2003), comportamento sexual (libido) (Gronli et al., 2005) e anedonia (Gronli et al., 2004; Gamaro et al., 2003 e Willner, 1997). O modelo do ECLV envolve mais fatores estressantes naturalísticos aproximando proporcionalmente aos enfrentados pelos humanos (Gronli et al., 2006). Animais estressados através deste modelo exibem comportamento anedônico, o qual é inferido pela redução do consumo de sacarose (Willner et al., 1996). Juntamente com esse comportamento, os animais expostos a esse modelo desenvolvem uma variedade de outras seqüelas neurobiológicas e comportamentais, que são revertidas com o uso de antidepressivos em longo prazo (Casorotto et al., 2007 e Jayatissa et al., 2006). Essa reversão das alterações com o uso de antidepressivos confere ao modelo uma boa validade preditiva (Willner, 1997).

Tanto as anormalidades comportamentais produzidas pelo estresse crônico quanto os efeitos paliativos do antidepressivo neste paradigma são difíceis de ser replicado nos diferentes laboratórios por necessitar de equipe eficiente, organizada, com espaço e aparato adequado o que culmina por reduzir sua aplicabilidade geral (Nestler, et al., 2002).

4. OBJETIVOS DO TRABALHO

4.1 OBJETIVO GERAL

Investigar as alterações comportamentais e neuroquímicas de ratos Wistar expostos ao modelo de ECLV.

4.2 OBJETIVOS ESPECÍFICOS

- Testar se o modelo de ECLV realizado no laboratório de neurociências da UNESC induzirá anedonia em ratos Wistar para posterior utilização em pesquisas neurobiológicas.
- Avaliar o efeito no ganho ponderal de ratos expostos ao modelo de ECLV;
- Avaliar indiretamente os efeitos do modelo de ECLV sobre o eixo hipotálamo-hipófise-adrenal de animais experimentais através do aumento do peso da glândula adrenal;
- Avaliar os níveis hipocampais, séricos e liquóricos da proteína do BDNF em ratos submetidos ao modelo de ECLV;
- Avaliar os parâmetros de estresse oxidativo tais como atividade da catalase e da superóxido dismutase, concentração do grupamento carbonil, substâncias reativas ao ácido tiobarbitúrico e superóxido mitocondrial em hipocampo de ratos submetidos ao ECLV;

PARTE II

1. ARTIGO "CHRONIC MILD STRESS PARADIGM REDUCES SWEET FOOD INTAKE IN RATS WITHOUT AFFECTING BDNF PROTEIN LEVELS"

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ABSTRACT

Major depression is a common, serious and recurrent disorder that affects 17-20% of the population of the world. The chronic mild stress (CMS) model has been used as an animal model of depression because induce anhedonia in animals. The present study investigated behavioral, physiological and neurochemical aspects of rats exposed to a CMS procedure. The consumption of sweet food, locomotor activity, body and adrenal gland weight, BDNF protein levels (ELISA-sandwich) evaluated in hippocampus, cerebrospinal fluid and serum were assessed in rats. Our findings demonstrated decrease in sweet food intake, increase of adrenal gland weight and a lack of body weight gain and no changes were observed in BDNF protein levels in serum, cerebrospinal fluid and hippocampus in rats exposed to CMS procedure. Indeed, locomotor activity was not significantly affected. In conclusion, these data reveal that BDNF protein levels were not significantly correlated with the decrease of sweet food consumption observed in CMS exposed animals.

KEY WORDS: anhedonia; depression; BDNF protein levels; chronic mild stress; mood disorder; animal model.

INTRODUCTION

Major depression is a common, serious and recurrent disorder that affects 17-20% of the population of the world and may result in premature death, major social and economic consequences [1]. Major depressive disorder accounts for 4.4% of the total overall global disease burden and a similar prevalence was found in ischemic heart disease [2]. Among persons with major depression, 75-85% has recurrent episodes and 10-30% recovery incompletely and has persistent or residual depressive symptoms throughout the life [2, 3, 4].

The treatment of depression was revolutionized about a half-century ago with the introduction of the antidepressants. This event marked the beginning of the modern era of depression research, which has sought to identify the neurobiological basis of depression [5]. Important advances have been made, but our understanding of the precise molecular and cellular underpinnings of this complex disorder is in its infancy. Therefore, animal models are useful to provide new insights into the neurobiology and pathophysiology of depression.

The chronic mild stress (CMS) model has been shown to induce lower consumption of sucrose (sweet food) postulated to reflect anhedonia (the loss of interest or pleasure) in animals, one of the two core symptoms required for diagnosis of a major depressive episode in humans [6, 7, 8]. The exposure of rats to CMS also induces changes in hypothalamic-pituitary-adrenal axis, body weight and adrenal glands [9], all these symptoms are consistent with human depression. Abnormalities of the hypothalamic-pituitary-adrenal axis in depressed patients are well described [10]. CMS leads to increased activity of the HPA axis, including adrenal hypertrophy and corticosterone hypersecretion [11].

The CMS model is based on intensive labor, space demanding and long stress procedure which consists of exposing animals sequentially to a variety of mild and unpredictable "stressors" (e.g. isolation, water and food deprivation, restraint, forced swimming, flashing light exposure) for a period of 4-6 weeks. The protocol is regarded as being close to model the human situation, consisting more of daily hassles than traumatic events [12, 13].

It is worth mentioning that the validity of the CMS model has been questioned, since the decrease in sucrose consumption is not consistently observed following the stress procedure among distinct laboratories [14, 15, 16, 17]. Due to this fact, many attempts to develop and/or replicate the original model have allowed that the stress-induced decrease in sucrose consumption varies within experiments, among laboratories, and within animal strains used [18, 5].

The neurotrophin brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and the most widespread growth factor in the brain. BDNF has diverse functions in the adult brain as a regulator of neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity [19, 20, 21]. Several studies support the hypothesis of BDNF involvement in depression and suggest that depressive disorder induce a marked decrease in plasma BDNF levels [for review see 22, 23]. Moreover, literature findings report that BDNF expression is also modified by stress [for review see, 24]. In a recent review article, was described changes on hippocampal BDNF mRNA levels, but not in BDNF protein levels, in stressed rats [25].

In this context, the present study investigated the effects of chronic mild stress paradigm on behavioral, physiological and neurochemical parameters in rats under our experimental conditions. The measurement of sweet food intake was used as an index of anhedonia-related behavior. Body and adrenal gland weight were evaluated in rats aiming to

assess the physiological responses during a CMS situation. Indeed, BDNF protein levels were assessed in hippocampus, cerebrospinal fluid and serum of rats subjected to the CMS paradigm in order to further correlate behavioral and physiological observations with neurochemical findings.

EXPERIMENTAL PROCEDURE

Animals

Male Wistar rats (3-4 months old, weighting 220-310 g) were obtained from our breeding colony (UNESC). The animals were housed 5 to cage with food and water available *ad libitum* and they were maintained on a 12-h light/ dark cycle (lights on at 7:00 am). All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

Chronic mild stress

Chronic mild stress (CMS) protocol was utilized conform the literature [26]. Rats were divided in two groups: controls and stressed. Controls were kept undisturbed in their home cages during the 40 days of experiment, receiving only ordinary care with daily supports of food and water. By contrast, the stressed group was subjected to a 40-day chronic mild stress paradigm. Individual stressors and length of time applied each day are listed in Table 1. The following stressors were used: (i) 24 h of food deprivation; (ii) 24 h of water deprivation; (iii) 1-3 h of restraint, as described later, (iv) 1,5-2 h of restraint at 4° C; (v) forced swimming

during 10 or 15 min, as described later; (vi) flashing light during 120-210 min; (vii) isolation (2-3 days). Stress was applied at distinct periods everyday, in order to minimize its predictability.

Restraint was carried out by placing the animal in a 25 cm x 7 cm plastic tube and adjusting it with plaster tape on the outside, so that the animal was unable to move. A 1-cm hole at the far end of the tube was present for animal breathing. Forced swimming was carried out by placing the animal in a glass tank measuring 50 cm (height) x 47 cm (diameter) with 30 cm of water at 23 ± 2° C. Exposure to flashing light was made by placing the animal in a 60 cm x 60 cm x 25 cm plywood made box divided in 16 cells of 15 cm x 15 cm x 25 cm with a frontal glass wall. A 40 W lamp, flashing at frequency of 60 flashes/min, was used.

Consumption of sweet food

After 40 days of treatment, consumption of sweet food was measured in 30 rats (15 controls and 15 stressed). Animals were placed in a lightened rectangular box (40 cm × 15 cm × 20 cm) with a ceiling, floor, side walls made of wood and divided into 12 equal rectangles by black lines. Ten “Froot Loops” (Kellogg’s® pellets of wheat and corn starch and sucrose) were placed in one extremity of the box. Animals were submitted to five 3-min trials, once a day, in order to become familiarized. After being habituated, animals were exposed to two test sessions, 3 min each, when the number of ingested pellets and the spontaneous locomotor activity (crossings of black lines and rearings) were registered [26, 27].

Briefly, the consumption of 1/3 or 1/4 of the “Froot Loops” pellet by an animal was considered as one pellet. Five trial of sweet food consumption were used to assess the anhedonia-like behavior in rats exposed to the CMS paradigm. Three trials (three consecutive days) of sweet food intake were made with the animals 22-h food deprived which were used

as a motivating stimulus; however it may also be an acute stressor. Due to this fact, in the last two trials, these tests were made with animals fed ad libitum [26].

Body and adrenal gland weight

Body weight was measured at the beginning (1st day) and at the last day (40th day) of the chronic mild stress protocol. At the seventh day after consumption of sweet food, rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) given intraperitoneally. After death, the adrenal gland was removed through laparotomy, and weighted in an analytical balance. Adrenal gland weight was used in this study as an indirect parameter of hypothalamic-pituitary-adrenal axis activation [8, 26].

Neurochemical analysis

Blood (serum) and cerebrospinal fluid were collected by cardiac puncture and transcutaneous cisternal puncture, respectively, whereas after rats' death, hippocampus were immediately isolated. Serum, cerebrospinal fluid and hippocampus were stored at - 80º C for posterior analyses of BDNF protein levels. BDNF levels in were measured by anti-BDNF sandwich-ELISA, according to the manufacturer instructions (Chemicon, USA). Briefly, hippocampus was homogenized in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride and 1 mM EGTA. The blood was immediately centrifuged at 3000×*g* for 5 min, and serum was kept frozen at −80 ◦C until assayed. The cerebrospinal fluid was immediately centrifuged at 3000×*g* for 5 min, and supernatant was kept frozen at −80 ◦C until assayed. Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluent. The plates were then washed four times with sample diluent, and a monoclonal anti-BNDF rabbit antibody diluted to 1:1000 in sample diluent was added

to each well and incubated for 3 h at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1:1000) was added to each well and incubated at room temperature for 1 h. After addition of streptavidin-enzyme, substrate and stop solution, the amount of BDNF was determined by absorbance in 450 nm. A standard curve was produced and it ranged from 7.8 to 500 pg/ml of BNDF. This curve was obtained from a direct relationship between Optical Density and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 15.0 was utilized for statistical analyses. All data are expressed as mean ± standard error of the mean of *n* animals, and have been statistically analyzed with the Student's t-test for unpaired data. *P* values less than 0.05 were considered statistically significant.

RESULTS

As depicted in Figure 1A, CMS group decreased sweet food intake when compared with control group (t=8.208; df=28; p=0.001). In the open-field test (Figure 1B), 40-days of chronic unpredictable stressful protocol reduced, but not in a significant manner, the number of crossings (t=1,805; df=27; p=0.082) and rearings (t=1,945; df=27; p=0.062) compared to non-stressed rats.

The effects of the CMS protocol in body and adrenal gland weight were illustrated in Figure 2A and 2B, respectively. After the 40-day of CMS protocol, rats displayed lack of body weight gain in comparison with control group (t=4.442; df=28; p=0.001). By contrast,

CMS procedure induced an increase of rat adrenal gland weight compared with non-stressed rats (t=3.119; df=28; p=0.004).

Figure 3 showed that CMS procedure did not modify the availability of BDNF protein levels in the rat hippocampus (t=1.407; df=18; p=0.177), and cerebrospinal fluid (t=1.457; df=13,925; p=0.167) compared to control. In the periphery, the level of BDNF protein in serum of rats exposed to the CMS paradigm was increased, but not in a significant way, compared to control group (t=1.968; df=12; p=0.073).

DISCUSSION

The present study demonstrated that: (1) CMS rat displayed reduced sweet food intake, without significant changes in locomotor activity; (2) CMS rats showed lack of body weight gain, and increased adrenal gland weight compared to non-stressed rats; (3) concentrations of BDNF protein levels were not altered in hippocampus, and cerebrospinal fluid. However, a non significant increase in the BDNF protein levels in serum was observed in rats exposed to CMS paradigm compared to control.

The CMS paradigm, which was originally described [6], is a model of depression obtained by using chronic unpredictable mild stressors [for a review see: 12]. In the CMS model, both consumption of and preference for sucrose intake as well as decreased intracranial self stimulation behavior have served as markers of generalized decrease in sensitivity to reward, which behavior is quite related to anhedonia [6, 7, 27-30]. In accordance with the literature, present data confirm that rats exposed to CMS procedure consume less sweet food compared to non-stressed rats.

Literature data support the fact that stressed rats had a lack of body weight gain as well as behavioral alterations [26, 31]. Emotional changes, such as exposure to stressful situations can influence feeding behavior, and it has been demonstrated that chronic exposure to stressors may alter body weight of rats [32]. In accordance with the literature, our findings also demonstrated lack of body weight gain in CMS rats compared to non-stressed rats.

Our findings also observed an increase in adrenal gland weight in those rats exposed to chronic mild unpredictable stress. Distinct authors have already suggested an increase of the rat adrenal weight after 14 [9] or 28 days [33] of CMS paradigm. These changes in adrenal gland could be due to the increase of adrenocorticotropin circulating hormone which is released in high concentrations during stressful situations by anterior pituitary gland [for a review see: 34].

As stressful life events have a substantial causal association with depression and anxiety, stress paradigms have long been used to model these diseases. Notably, it has been shown that stress can lead to neuronal atrophy and loss in several brain regions, including the hippocampus. The hippocampus plays an important role in the regulation of stress responses and it expresses high levels of BDNF protein and mRNA in the normal adult rat [35]. Numerous studies have also documented that stress decreases the expression of BDNF mRNA in the hippocampus [36, 37, 38, 39]. Alterations in BDNF synthesis have been suggested to be on the basis of the pathophysiology of depressive states [for a review see: 24]. Here, we showed that rats exposed to the CMS paradigm did not display any alterations in BDNF protein levels in the rat hippocampus.

In humans, BDNF levels are decreased in serum of unmedicated patients suffering from depression [40]. Indeed, low levels of serum BDNF is associated with vulnerability to develop mood disorders in healthy subjects [41]. Notably, in the serum of rats exposed to CMS procedure we observed a trend to increase the BDNF protein levels compared to control.

Therefore, this lack of alteration (i.e., reduction of increase) in the serum BDNF protein of chronic mild stressed rats could be due to (1) the animal's difference in developing anhedonia behavior, and (2) a neuroprotective mechanism that could be activated in animals which were exposed to stressful situations. To support this view, was reported an upregulation of BDNF mRNA in those CMS-exposed rats, which were resistant to the development of anhedonia [39]. As far as we are aware, this is the first time that BDNF protein levels were assessed in serum and cerebrospinal fluid of rats subjected to a CMS paradigm. However, we evaluated BDNF protein levels and did not BDNF mRNA as evaluated in other studies. Thus, unlike unmedicated depressive patients, the CMS procedure did not elicit any alteration in rat serum or cerebrospinal fluid BDNF levels.

Despite the anhedonic-related behavior and body weight alterations displayed by rats subjected to CMS paradigm, our findings demonstrated that the exposure to this chronic mild unpredictable stressful situation did not alter BDNF protein levels in serum, cerebrospinal fluid and hippocampus. These findings are contrasting with human observations that showed a correlation between depressive disorders and a decreased level of serum BDNF [40, 41,42]. In fact, our findings reveal that BDNF protein levels were not significantly correlated with the decrease of rats sucrose intake observed in those animals subjected to the CMS paradigm. In a recent review article, changes on BDNF mRNA levels in hippocampus was described, but did not reported studies that sowed decreased in hippocampus BDNF protein levels in stressed rats [25]. Gronli et al. showed that BDNF protein is decreased in specific regions of the hippocampus (dentate gyrus), but not in the hole structure as one [13].

In conclusion, this study demonstrates that decreased sweet food intake, which reflects an anhedonia-like behavior, was observed in our rats exposed to a CMS paradigm. Despite the behavioral alterations, BDNF protein levels in hippocampus, cerebrospinal fluid and serum were not changed in comparison with control. These observations support in part the view

that the CMS paradigm is an animal model of depression which mimics alterations observed in depressive patients, suggesting that stress change only mRNA BDNF levels in analyzed structures.

Acknowledgements: This research was supported by grants from CNPq (ECG, FK and JQ), FAPESC (ECG and JQ), Instituto Cérebro e Mente (FK, ECG and JQ) and UNESC (ECG and JQ). FK and JQ are CNPq Research Fellows. SSV is holder of a CAPES studentship, and CMC is holder of a CNPq studentship.

REFERENCES

1. Kessler RC, McGonagle KA, Zhao S et al (1994) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch. Gen. Psychiatry 51:8-19.
2. Mann, JJ (2005) The medical management of depression. N. Engl. J. Med. 353:1819-34.
3. Mueller TI, Leon AC, Keller MB et al (1999) Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. Am. J. Psychiatry 156:1000-1006
4. Keller MB, Lavori PW, Rice J et al (1986) The persistent risk of chronicity in recurrent episodes of nonbipolar major depressive disorder: a prospective follow-up. Am. J. Psychiatry 143:24-28.
5. Nestler EJ, Gould E, Manji H et al (2002) Preclinical model: Status of basic research in depression. Biol. Psychiatry 52:503-528.
6. Willner P, Towell A, Sampson D et al (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93:358-364.
7. Willner P (1997) Validity, reability and utility of the chronic mild stress model of depression: a 10-years review and evaluation. Psychopharmacology 134:319-329.
8. Katz RJ, Roth KA, Carroll BJ (1981) Animal models and human depressive disorders. Neurosci. Biobehav. Rev. 5:231–246.
9. Harro J, Tonissaar M, Eller M et al (2001) Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. Brain Res. 899: 227–239.

10. Gillespie CF, Nemeroff CB (2005) Hypercortisolemia and depression. Psychosom. Med. 67:26-28.

11. Vollmayr B, Henn FA (2003) Stress models of depression. Clin. Neurosci. Res. 3:245-251.

12. Wilner P (2005) Chronic mild stress (CMS) revisited: consistency and behavioral-neurobiological concordance in the effects of CMS. Neuropsychobiology 52:90-110.

13. Gronli J, Bramham C, Murison R et al (2006) Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper. Pharmacol. Biochem. Behav. 85:842-849.

14. Forbes NF, Stewart CA, Matthews K et al (1996) Chronic mild stress and sucrose consumption: validity as a model of depression. Physiol. Behav. 60:1481–4.

15. Harris RB, Zhou J, Youngblood BD et al (1997) Failure to chage exploration or saccharin preference in rats exposed to chronic mild stress. Physiol. Behav. 63:91-100.

16. Hatcher JP, Bell DJ, Reed TJ et al (1997) Chronic mild stress-induced reductions in saccharin intake depend upon feeding status. J. Psychopharmacol. 11:331-8.

17. Matthews K, Forbes N, Reid IC (1995) Sucrose consumption as an hedonic measure following chronic unpredictablemild stress. Physiol. Behav. 57:241–8.

18. Gronli J, Murison R, Bjorvatn B et al (2004) Chronic mild stress affects sucrose intake and sleep in rats. Behav. Brain. Res.150:139-147.

19. Hashimoto K, Shimizu E, Iyo M (2004) Critical role of brain-derived neurotrophic factor in mood disorders. Brain Res. Rev. 45:104–14.

20. Lewin GR, Barde YA (1996) Physiology of the neurotrophins. Annu. Rev. Neurosci.19:289–317.

21. Blum R, Konnerth A (2005) Neurotrophin-mediated rapid signaling in the central nervous system: mechanisms and functions. Physiology (Bethesda) 20:70–8.

22. Castren E, Voikar V, Rantamaki T (2007) Role of neurotrophic factors in depression. Curr. Opin. Pharmacol. 7:18–21.

23. Lee BH, Kim H, Park SH et al (2007) Decreased plasma BDNF level in depressive patients. J. Affect. Disord. 101:239-44.

24. Tapia-Arancibia L, Rage F, Givalois L et al (2004) Physiology of BDNF: focus on hypothalamic function. Front. Neuroendocrinol. 25:77–107.

25. Martinowich K, Manji H, Lu B (2007). New insights into BDNF function in depression and anxiety. Nat. Neurosci. 10:1089-93.

26. Gamaro GD, Manoli LP, Torres IL et al (2003) Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. Neurochem. Int. 42:107-14.

27. Vianna MR, Alonso M, Viola H et al (2000) Role of hippocampal signaling pathways in long-term memory formation of a nonassociative learning task in the rat. Learn. Mem. 7:333-40.

28. Haidkind R, Eller M, Harro M et al (2003) Effects of partial locus coeruleus denervation and chronic mild stress on behaviour and monoamine neurochemistry in the rat. Eur. Neuropsychopharmacol. 13:19–28.

29. Baker SL, Kentner AC, Konkle At et al (2006) Behavioral and physiological effects of chronic mild stress in female rats. Physiol. Behav. 87: 314–22.

30. Jayatissa MN, Bisgaard C, Tingstrom A et al (2006) Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. Neuropsychopharmacology 31:2395–404.

31. Bekris S, Antoniou K, Daskas S, et al (2005) Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behav. Brain. Res. 161:45-59.

32. Dess NK, Raizer J, Chapmen CD et al (1988) Stressors in the learned helplessness paradigm: effects on body weight and conditioned taste aversion in rats. Physio. Behav. 44:483–490

33. Konarska M, Stewart RE, McCarty R (1990) Predictability of chronic intermittent stress: effects on sympathetic-adrenal medullary responses of laboratory rats. Behav. Neural. Biol. 53, 231–243.

34. O'Connor TM, O'Halloran DJ, Shanahan F (2000) The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. Q.J.M. 93:323-33.

35. Conner JM, Lauterborn JC, Yan Q et al (1997) Distribution of brainderived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J. Neurosci. 17:2295–313.

36. Smith MA, Makino S, Kvetnansky R et al (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J. Neurosci. 15:1768–1777.

37. Duman RS & Monteggia LM (2006) A neurotrophic model for stress-related mood disorders. Biol. Psychiatry 59:1116–1127.

38. Russo-Neustadt A, Ha T, Ramirez R et al (2001) Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav. Brain. Res. 120:87–95.

39. Shirayama Y, Chen AC, Nakagawa S et al (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J. Neurosci. 22:3251–61.

40. Bergström A, Jayatissa MN, Mørk A et al (2008) Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study. Brain. Res. 1196:41-52.

41. Karege F, Perret G, Bondolfi G et al (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry. Res. 109:143-8.

42. Lang UE, Hellweg R, Gallinat J (2004) BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. Neuropsychopharmacology 29:795-8.

LEGENDS OF FIGURES

Figure 1 – Effect of CMS paradigm on consumption of sweet food (1A) and on number of crossings and rearings of rats subjected to the open-field test (1B). Bars represent means ± S.E.M. of 15 rats. * $p<0.05$ vs. control according to Student's t-test

Figure 2 - Effect of CMS paradigm on rat body (2A) and adrenal gland (2B) weight in the 1st and 40th day of experiment. Bars represent means ± S.E.M. of 15 rats. * $p<0.05$ vs. control according to Student's t-test.

Figure 3 – Effect of CMS paradigm on BDNF protein levels assessed in the hippocampus, cerebrospinal fluid and serum of rats. Bars represent means ± S.E.M. of 15 rats.

FIGURES

Figure 1A

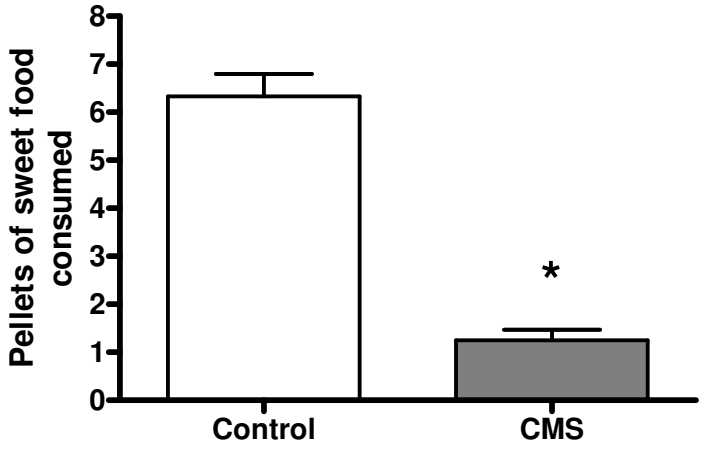


Figure 1B

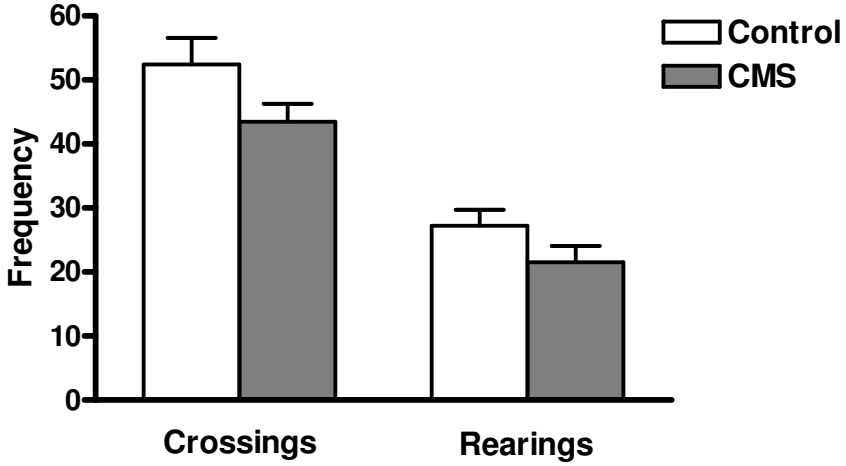


Figure 2A

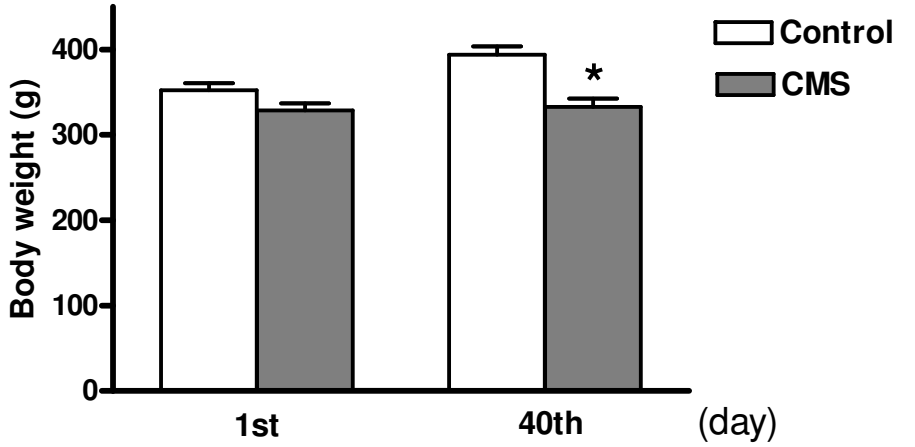


Figure 2B

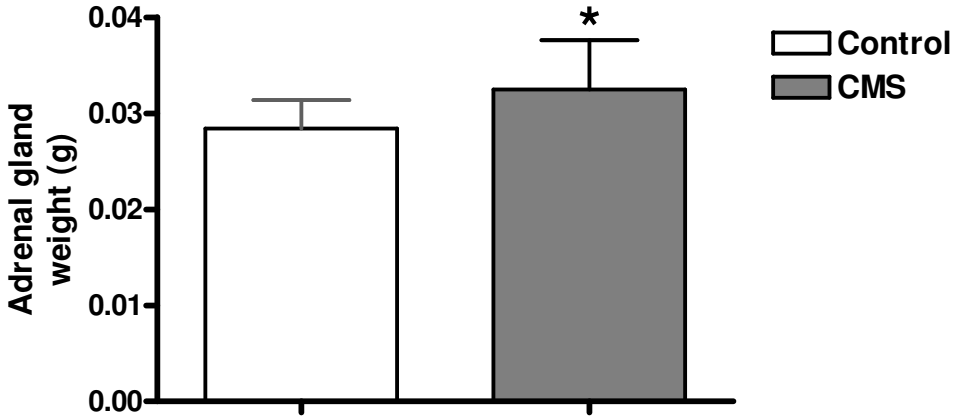
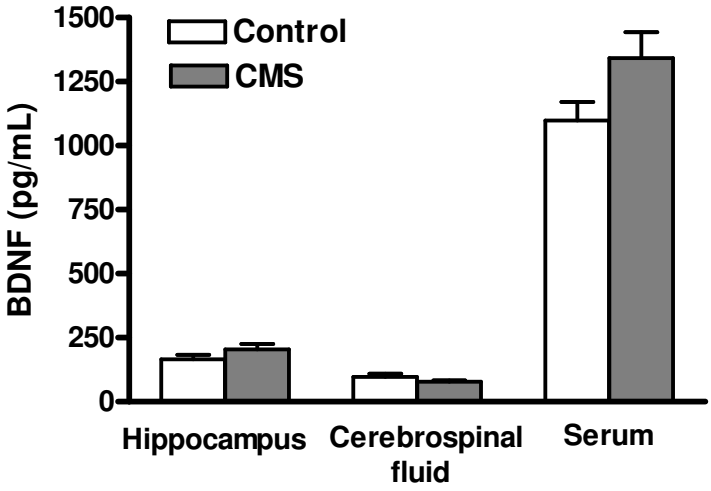


Figure 3


1500
1250
1000
750
500
250
0
BDNF (pg/mL)
Control
CMS
Hippocampus
Cerebrospinal fluid
Serum

TABLES

Table 1 - Schedule of stressor agent used during the chronic treatment.

Day of treatment	*Stressor used*	*Duration*
Day 1	Water deprivation	24 h
Day 2	Food deprivation	24 h
Day 3	Isolation	24 h
Day 4	Isolation	24 h
Day 5	Isolation	24 h
Day 6	Flashing light	3 h
Day 7	Food deprivation	24 h
Day 8	Forced swimming	10 min
Day 9	Restraint	1 h
Day 10	Water deprivation	24 h
Day 11	No stressor applied	-
Day 12	No stressor applied	-
Day 13	Restraint + cold	2 h
Day 14	Flashing light	2.5 h
Day 15	Food deprivation	24 h
Day 16	Forced swimming	15 min
Day 17	Isolation	24 h
Day 18	Isolation	24 h
Day 19	Isolation	24 h
Day 20	Water deprivation	24 h
Day 21	Food deprivation	24 h

Day 22	Flashing light	3 h
Day 23	Restraint	2 h
Day 24	Isolation	24 h
Day 25	Isolation	24 h
Day 26	Restraint + cold	1.5 h
Day 27	Forced swimming	10 min
Day 28	Flashing light	3.5 h
Day 29	No stressor applied	-
Day 30	Food deprivation	24 h
Day 31	Restraint	3 h
Day 32	Flashing light	2 h
Day 33	Water deprivation	24 h
Day 34	Restraint + cold	2 h
Day 35	Forced swimming	15 min
Day 36	Isolation	24 h
Day 37	Isolation	24 h
Day 38	No stressor applied	-
Day 39	Flashing light	3 h
Day 40	Forced swimming	10 min

2. ARTIGO "EFFECTS OF CHRONIC MILD STRESS MODEL ON THE OXIDATIVE DAMAGE PARAMETERS IN THE RAT BRAIN"

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ABSTRACT

Major depression is characterized for symptoms at the psychological, behavioral and physiological levels. The chronic mild stress (CMS) model has been used as an animal model of depression. This study investigated aspects of rats exposed to CMS. The consumption of sweet food, locomotor activity, body weight, lipid and protein oxidation levels and on superoxide dismutase and catalase activities in the rat hippocampus, prefrontal cortex and cortex were assessed in rats. Our findings demonstrated decreased of sweet food intake but locomotor activity was not affected, a lack of body weight gain, increased protein (prefrontal, hippocampus, striatum and cortex) and lipidic peroxidation (cerebellum and striatum), increased catalase (cerebellum, hippocampus, striatum, cortex) and decreased superoxide dismutase activity (prefrontal, hippocampus, striatum and cortex) in stressed rats. In conclusion, this results support the idea that stress produces oxidants and imbalance between superoxide dismutase and catalase activities contributing, at least in part, to stress-related diseases, such as depression.

KEY-WORDS: Major depression, Chronic Mild Stress, Reactive oxygen species, Antioxidants activity .

INTRODUCTION

Major Depression is a serious and recurrent disorder often manifested with symptoms at the psychological, behavioral and physiological levels affecting 17-20% of the population of the world and may result in premature death, major social and economic consequences (1). This disorder accounts for 4.4% of the total overall global disease burden, a contribution similar to that ischemic heart disease or diarrheal diseases (2). Among persons with major depression, 75-85% have recurrent episodes (3, 4) and 10-30% recovery incompletely and have persistent, residual depressive symptoms (2).

The brain is particularly vulnerable to reactive oxygen species (ROS) production because it metabolizes 20% of total body oxygen and has a limited amount of antioxidant capacity. In situations where the generation of free radicals exceeds the capacity of antioxidant defense, oxidative stress may lead to membrane degradation, cellular dysfunction and apoptosis. Oxidative stress can result from increased production of ROS, decreased antioxidant defense or failure to repair oxidative damage. ROS are free radicals or reactive anions/molecules containing oxygen atoms such as hydroxyl radical, superoxide and peroxynitrite. ROS can cause cell damage by enzyme inactivation, lipid peroxidation and DNA modification (review see: 5). Oxidative stress is well known to contribute to neuronal degeneration in the central nervous system (CNS) in the process of aging as well as in neurodegenerative diseases such as amyotrophic lateral sclerosis (6), Alzheimer's dementia (7) and Parkinson's disease (8). Recent studies have consistently reported increase ROS in plasma on patients with major depression, especially with melancholia associated (9). Recent study showed evidences of oxidative stress in Major Depression as reflected in increased oxidative stress from frontal regions of patients compared to those of matched controls (10).

Numerous attempts have been made in order to set up animal models of depression or at least of some disease aspects (11, 12). The chronic mild stress (CMS) model has been shown to induce lower consumption of sucrose (sweet food) postulated to reflect anhedonia (the loss of interest or pleasure) in animals, one of the two core symptoms required for diagnosis of a major depressive episode in humans (13, 14, 15). The exposure of rats to CMS also induces changes in hypothalamic-pituitary-adrenal axis, weight body and adrenal glands all consistent with human depression (16).

Because of these findings, we designed the present study to investigate the effects of CMS paradigm on lipid and protein oxidation levels (markers of oxidative stress) and on superoxide dismutase (SOD) and catalase (CAT) activities (the major antioxidant enzymes) in rat brain.

MATHERIALS AND METHODS

Animals

Male Wistar rats (3-4 months, 220-310 g) were obtained from our breeding colony (UNESC). The animals were housed 5 to a cage with food and water available *ad libitum* and were maintained on a 12-h light/ dark cycle (lights on at 7:00 am). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

Experimental Procedure

Chronic mild stress (CMS) protocol was adapted from the previous procedure (17). The animals were divided in two groups: control and stressed. Control were kept undisturbed in their home cages during the 40 days of treatment receiving only ordinary daily care with daily supports of food and water. The 40-days chronic mild stress paradigm was used for the animals in the stressed group. Individual stressors and length of time applied each day are listed in Table 1. The following stressors were used: (i) 24h of food deprivation; (ii) 24h of water deprivation; (iii) 1-3h of restraint, as described later, (iv) 1,5-2h of restraint at 4°C; (v) forced swimming during 10 or 15 min, as described later; (vi) flashing light during 120-210min; (vii) isolation (2-3 days). Stress was applied at different times everyday, in order to minimize its predictability.

Restraint was carried out by placing the animal in a 25cm x 7cm plastic tube and adjusting it with plaster tape on the outside, so that the animal was unable to move. There was a 1cm hole at the far end for breathing. Forced swimming was carried out by placing the animal in a glass tank measuring 50cm x 47cm 40cm with 30cm of water at 23±2°C. Exposure to flashing light was made by placing the animal in a 60cm x 60cm x 25cm plywood made box divided in 16 cells of 15cm x 15cm x25cm with a frontal glass wall. A 40w lamp, flashing at frequency of 60 flashes/min, was used.

Apparatus

After 40 days of treatment, consumption of sweet food was measured in 30 animals (15 controls and 15 stressed) to verified anhedonia. The animals were placed in a lightened rectangular box (40 cm × 15 cm × 20 cm) with a glass ceiling, floor, side walls made of wood and divided into 12 equal rectangles by black lines. Ten Froot loops (Kellogg's® pellets of

wheat and corn starch and sucrose) were placed in one extremity of the box. Animals were submitted to five 3 min trials, one per day, in order to become familiarized with this food. After being habituated, the animals were exposed to two test sessions, 3 min each, when the number of ingested pellets and motor activity (crossings of the black lines and rearings were counted) (Gamaro et al, 2003) was measured. A protocol was established so that when the animal ate part of the Froot loops (e.g. 1/3 or 1/4) this fraction was considered. These two evaluations were made with the animals submitted to fasting (during a period of 22 h prior to the behavioral task) or with animals fed ad libitum. These evaluations were made since food deprivation, which is used in many behavior tasks as a motivating stimulus, may also be an acute stressor (17)

Body weight was measured at the beginning and after of stress protocol. After consumption of sweet food, the animals were killed by decapitation, hippocampus, prefrontal and cortex were immediately isolated and stored at -80ºC for posterior analyses for oxidative stress in submitochondrial particles.

Oxidative stress parameters

The oxidative damage was measured through the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, as previously described (18). The samples were mixed with 1 mL of trichloroacetic acid (TCA) 10% and 1 mL of thiobarbituric acid (TBARS) 0.67% and were then heated in a boiling water bath for 15 minutes. TBARS were determined by the absorbance at 535 nm. Oxidative damage to proteins was measured by the quantification of carbonyl groups based on the reaction with dinitrophenylhidrazine (DNPH), as previously described (19). Proteins were precipitated by the addition of 20%

trichloroacetic acid and were redissolved in DNPH; the absorbance was read at 370 nm. To determine CAT activity, the brain tissue was sonicated in 50 mmoL/L phosphate buffer (pH 7.0), and the resulting suspension was centrifuged at 3000 *g* for 10 minutes. The supernatant was used for enzyme assay. CAT activity was measured by the rate of decrease in hydrogen peroxide absorbance at 240 nm (20). SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation, as previously described (21). All biochemical measures were normalized to the protein content, with bovine albumin as standard (22).

Statistical analysis

Results were presented as mean ± SEM. Differences among experimental groups were determined by student t test. P values <0.05 were considered to indicate statistical significance.

RESULTS

Figure 1 illustrated the effects of the CMS in sucrose intake, locomotor activity and body weight. As depicted in **Figure 1A**, CMS Group decreased sucrose intake when compared with Control Group (t=3.978; df=21.979 P=0,001). Interesting, in **figure 1B**, the open-field test, the CMS Group did not modify the number of crossing (t=0.235; df=28; P=0,0816) and rearing (t=0,113; df=28; P=0,911) compared to control rats. **Figure 1C** illustrated the effects of CMS in body weight. After the 40-day of chronic mild stress

protocol, rats displayed lack of body weight gain when compared with control group (t=5.899; df=28; P=0.0001)

The **Figure 2** showed the effects of the CMS in oxidative variables in rat brain. **Figure 2A** demonstrate that CMS increased protein peroxidation – Carbonyl in Prefrontal (t=9.812; df=6; p=0.0001), Hippocampus (t=12.329; df=4.191; p=0.0001), Striatum (t=7.997; df=3.207; p=0.003) and Cortex (t=10.015; df=4.265; p=0.0001) in comparison with control group. The **Figure 2B** showed increase lipidic peroxidation – TBARS in Cerebellum (t=4.277; df=7.902; p=0.003) and Striatum (t=5.123; df=7; p=0.001) in comparison with control group. **Figure 2C** displayed increase catalase activity in Cerebellum (t=3.220; df=6; p=0.018), Hippocampus (t=3.220; df=6; p=0.018), Striatum (t=2.694; df=8; p=0.027) and Cortex (t=2.623; df=6; p=0.039) in comparison with control group. As depicted in **Figure 2D** CMS group deceased superoxide dismutase activity in Prefrontal (t=13.541; df=6; p=0.0001), Hippocampus (t=7.383; df=6; p=0.0001), Striatum (t=3.327; df=3.046; p=0.044) and Cortex (t=3.241; df=3.023; p=0.047) in comparison with control group

DISCUSSION

The present study demonstrated that: (1) CMS rats displayed reduced sweet food intake, without significant changes in locomotor activity; (2) CMS rats showed decreased body weight compared to non-stressed rats; (3) increased protein peroxidation (prefrontal, hippocampus, striatum and cortex), lipidic peroxidation (cerebellum and striatum), (4) increase catalase activity (cerebellum, hippocampus, striatum, cortex) and decreased

superoxide dismutase activity (prefrontal, hippocampus, striatum and cortex in stressed rats compared to control.

The CMS paradigm (13) is an animal model of depression obtained by using chronic unpredictable mild stressors (for a review see: 23). In the CMS model, both consumption of and preference for sucrose intake as well as decreased intracranial self stimulation behavior have served as markers of generalized decrease in sensitivity to reward, which behavior is quite related to anhedonia (13, 17; 24). In accordance with the literature, present data confirm that rats exposed to CMS procedure consume less sweet food compared to non-stressed rats. Literature data support the fact that stressed rats had a severe loss of body weight as well as behavioral alterations (17; 24). Emotional changes, such as exposure to stress situations can influence feeding behavior, and several studies have demonstrated that chronic exposure to stressors may alter body weight of rats (25). In accordance with the literature, our findings also demonstrated lack of body weight gain in stressed rats compared to body weight gain in non-stressed rats.

In this context, oxidative stress in rat brain structures may play a role in the pathogenesis of depression (26). A study using an animal model of repeated restraint stress showed that this model induced an increase in TBARS levels in hippocampus (27). In one study demonstrated that animal model of immobilization stress caused significant increases in lipid peroxidation in the cerebral cortex, cerebellum and hippocampus compared to the unstressed controls; significant increases in levels of protein oxidation were also found in the cortex, hypothalamus and striatum; oxidative nuclear DNA damage increased after stress in all brain regions, although only the cerebral cortex showed a statistical significant increase (28). In humans was demonstrated elevated ROS in plasma of patients with major depression, especially in those with melancholic type (9).

We demonstrate in this study an increased protein oxidation in prefrontal, hippocampus, striatum and cortex and, increased lipidic peroxidation in cerebellum and striatum. Other studies have reported numerous oxidative disturbances parameters in patients with major depression, including oxidative damage in erythrocytic membranes (suggested by the depletion of omega-3 fatty acids) (29); elevated lipid peroxidation products and elevated superoxide anion (O_2^-) generation (30) and oxidative DNA damage (31). Therefore, our findings of an increased lipid and protein oxidation are consistent with previous results suggesting oxidative stress as crucially involved in the pathophysiology of depression.

In this context, previous studies have demonstrated that alterations on the redox state can lead to an imbalance between SOD and CAT activities and to oxidative stress (32, 33). A recent study demonstrated that patients with major depression, especially with melancholia, presented elevated antioxidative enzyme activities – catalase and superoxide dismutase in the plasma (9). Catalase metabolizes the excess of H2O2 (hydrogen peroxide) producing O_2 + H_2O, thereby decreasing the intracellular redox status. The brain is particularly prone to oxidative damage due to its relative high content of peroxidizable fatty acids and limited antioxidant capacity (5). In situations which superoxide dismutase levels are increased without a concomitant catalase increase, the intermediate product hydrogen peroxide may accumulate and generate hydroxyl radicals, which may lead to lipid and protein oxidation (damage). We demonstrated increase catalase activity in cerebellum, hippocampus, striatum, cortex and decreased superoxide dismutase activity in prefrontal, hippocampus, striatum and cortex. However, several enzymes generate hydrogen peroxide, including xanthine, urate, coproporplyrinogen III, glucose, lysyl, monoamine and d-amino acid oxidases, as well as superoxide dismutase (34).

In conclusion, the present findings support the idea that stress produces oxidants and imbalance between SOD and CAT activities it is possible that the oxidative damage in stress could contribute, at least in part, to stress-related diseases, such as depression.

REFERENCES

1. Kessler RC, McGonagle KA, Zhao S et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. ***Arch. Gen. Psychiatry 1994; 51(1):8-19.***

2. Mann, JJ. The medical management of depression. ***N Engl J Med*** **2005; 353(17):1819-34.**

3. Mueller TI, Leon AC, Keller MB et al. Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. ***Am J Psychiatry*** **1999; 156(17):1000-1006**

4. Keller MB, Lavori PW, Rice J et al. The persistent risk of chronicity in recurrent episodes of nonbipolar major depressive disorder: a prospective follow-up. ***Am J Psychiatry*** **1986; 143(1):24-28.**

5. Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. ***Proc Soc Exp Biol Med*** **1999;222(3):236-45.**

6. Rosen DR, Siddique T, Patterson D et al. Mutations in Cu/Zn-superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. ***Nature*** **1993; 362(6435):59–62.**

7. Durany N, Münch G, Michel TM et al. Investigations on oxidative stress and therapeutical implications in dementia. ***Eur Arch Psychiatry Clin Neurosci.*** **1999; 249(Suppl), 68–73.**

8. Abraham S, Soundararajan CC, Vivekanandhan S et al. Erythrocyte antioxidant enzymes in Parkinson's disease. ***Indian J Med Res*** **2005; 121(2): 111–115.**

9. Bilici M, Efe H, Köroğlu MA et al. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. ***J Affect Disord*** **2001; 64(1):43-51**

10. Michel TM, Frangou S, Thiemeyer D et al. Evidence for oxidative stress in the frontal cortex in patients with recurrent depressive disorder--a postmortem study. ***Psychiatry Res*** **2007; 151(1-2):145-50.**

11. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. ***Trends Pharmacol Sci*** **2002; 23(5):238–45.**

12. Holmes PV. Rodent models of depression: reexamining validity without anthropomorphic inference. ***Crit Rev Neurobiol*** **2003;15(2):143–74.**

13. Willner P, Towell A, Sampson D et al. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. ***Psychopharmacology*** **1987; 93(3):358-364.**

14. Willner P. Validity, reability and utility of the chronic mild stress model of depression: a 10-years review and evaluation. ***Psychopharmacology*** **1997; 134(4):319-329.**

15. Katz RJ, Roth KA, Carroll BJ. Animal models and human depressive disorders. ***Neurosci Biobehav Rev*** **1981; 5(2):231–246.**

16. Harro J, Tonissaar M, Eller M et al (2001) Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. ***Brain Res*** **899(1-2): 227–239.**

17. Gamaro GD, Manoli LP, Torres IL et al. Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures**.** ***Neurochem Int*** **2003; 42(2):107-14.**

18. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. ***Methods Enzymol*** **1990; 186(2):407-21.**

18. Levine RL, Williams JA, Stadtman ER, et al. Carbonyl assays for determination of oxidatively modified proteins. ***Methods Enzymol*** **1994;233:346-57.**

20. Aebi H. Catalase in vitro. ***Methods Enzymol*** **1984;105:121-6.**

21. Bannister JV, Calabrese L. Assays for superoxide dismutase. ***Methods Biochem Anal*** **1987;32:279-312.**

22. Lowry OH, Rosebrough NJ, Farr AL et al. Protein measurement with the Folin phenol reagent. ***J Biol Chem*** **1951;193:265-75.**

23. Wilner P. Chronic mild stress (CMS) revisited: consistency and behavioral neurobiological concordance in the effects of CMS. ***Neuropsychobiology*** **2005; 52(2):90-110.**

24. Bekris S, Antoniou K, Daskas S et al. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. ***Behav Brain Res*** **2005; 161(1):45-59.**

25. Dess NK, Raizer J, Chapmen CD et al. Stressors in the learned helplessness paradigm: effects on body weight and conditioned taste aversion in rats. ***Physiol Behav.*** **1988; 44(4-5):483–490.**

26. Eren I, Naziroglu M, Demirdas A. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. ***Neurochem Res*** **2007; 32(7): 1188–1195.**

27. Fontella FU, Siqueira IR, Vasconcellos AP et al. Repeated restraint stress induces oxidative damage in rat hippocampus**.** ***Neurochem Res*** **2005 30(1):105-11.**

28. Liu J, Wang X, Shigenaga MK et al. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. ***FASEB J*** **1996; 10(13):1532-8.**

29. Peet M, Murphy B, Shay J et al. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients**.** ***Biol Psychiatry*** **1998; 43(5): 315–319.**

30. Sarandol A, Kirli S, Akkaya C et al Oxidativeantioxidative systems and their relation with serum S100 B levels in patients with schizophrenia: Effects of short term antipsychotic treatment. ***Prog Neuropsychopharmacol Biol Psychiatry*** **2007; 1(6):1164–1169.**

31. Forlenza MJ, Miller GE. Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. ***Psychosom Med*** **2006; 68(1): 1–7.**

32. Klamt F, Dal-Pizzol F, Conte da Frota ML et al. Imbalance of antioxidant defense in mice lacking cellular prion protein. ***Free Radic Biol Med*** **2001; 30(10):1137–1144 259.**

33. Andrades M, Ritter C, Moreira JC et al. Oxidative parameters differences during non-lethal and lethal sepsis development. ***J Surg Res*** **2005; 125(1):68–72.**

34. Halliwell B & Gutteridge J. *Free Radicals in Biology and Medicine*. 4nd ed. ***Oxford: Biosciences; 2007*****.**

TABLE

Table 1 - Schedule of stressor agent used during the chronic treatment.

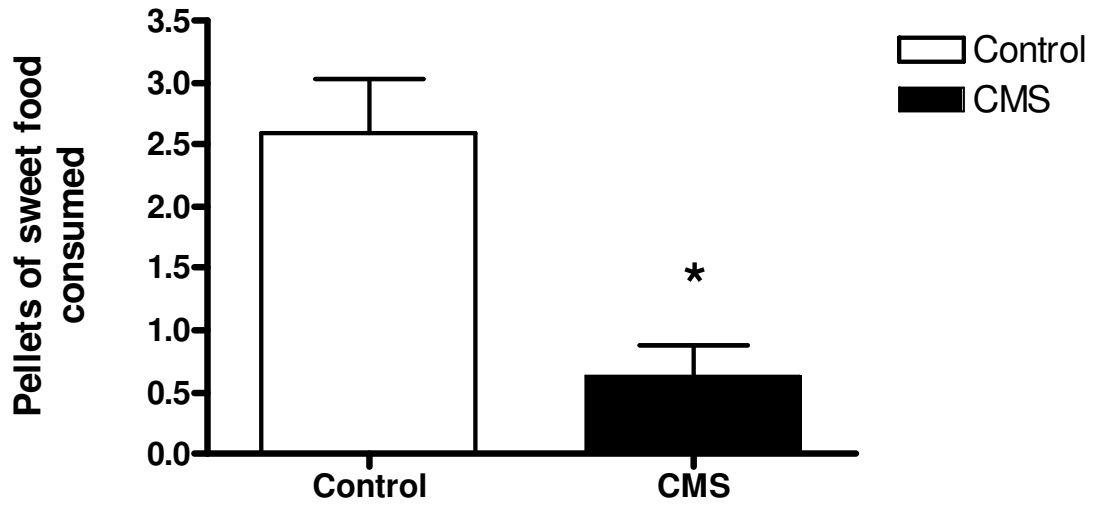
Day of treatment	***Stressor used***	***Duration***
Day 1	Water deprivation	24 h
Day 2	Food deprivation	24 h
Day 3	Isolation	24 h
Day 4	Isolation	24 h
Day 5	Isolation	24 h
Day 6	Flashing light	3 h
Day 7	Food deprivation	24 h
Day 8	Forced swimming	10 min
Day 9	Restraint	1 h
Day 10	Water deprivation	24 h
Day 11	No stressor applied	-
Day 12	No stressor applied	-
Day 13	Restraint + cold	2 h
Day 14	Flashing light	2.5 h
Day 15	Food deprivation	24 h
Day 16	Forced swimming	15 min
Day 17	Isolation	24 h
Day 18	Isolation	24 h
Day 19	Isolation	24 h
Day 20	Water deprivation	24 h
Day 21	Food deprivation	24 h

Day 22	Flashing light	3 h
Day 23	Restraint	2 h
Day 24	Isolation	24 h
Day 25	Isolation	24 h
Day 26	Restraint + cold	1.5 h
Day 27	Forced swimming	10 min
Day 28	Flashing light	3.5 h
Day 29	No stressor applied	-
Day 30	Food deprivation	24 h
Day 31	Restraint	3 h
Day 32	Flashing light	2 h
Day 33	Water deprivation	24 h
Day 34	Restraint + cold	2 h
Day 35	Forced swimming	15 min
Day 36	Isolation	24 h
Day 37	Isolation	24 h
Day 38	No stressor applied	-
Day 39	Flashing light	3 h
Day 40	Forced swimming	10 min

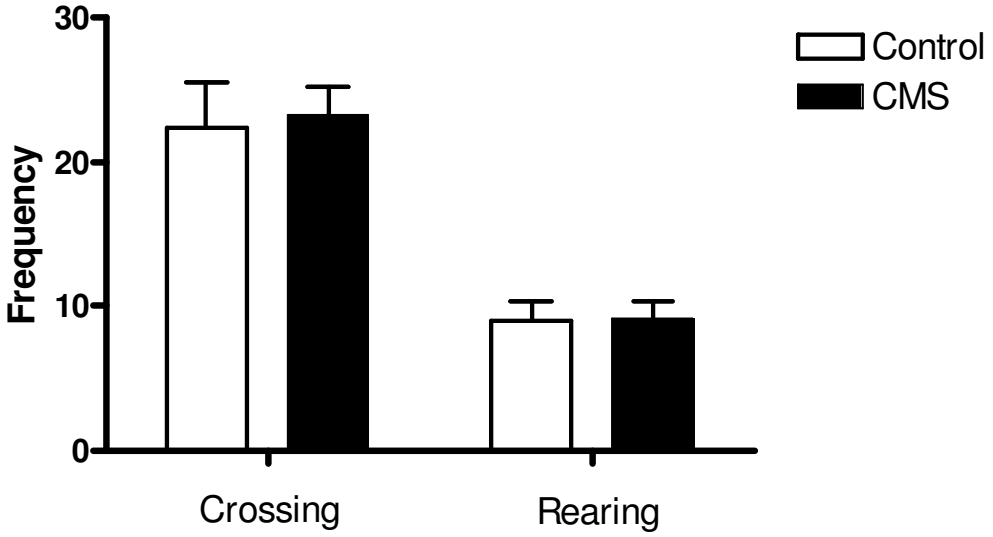
FIGURES

FIGURE 1

A)



B)


30
20
10
0
Frequency
Control
CMS
Crossing
Rearing

C)

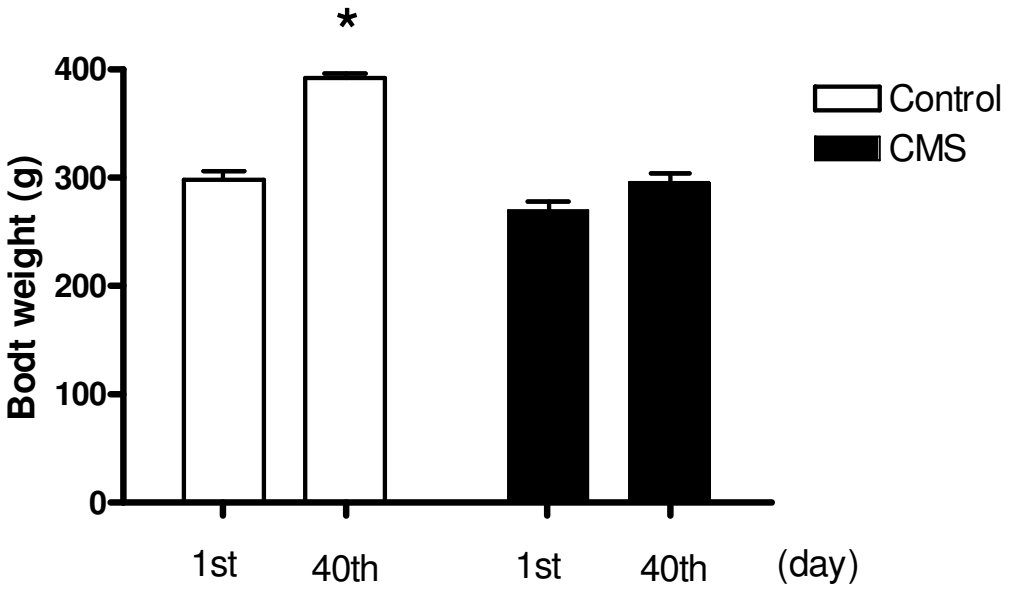
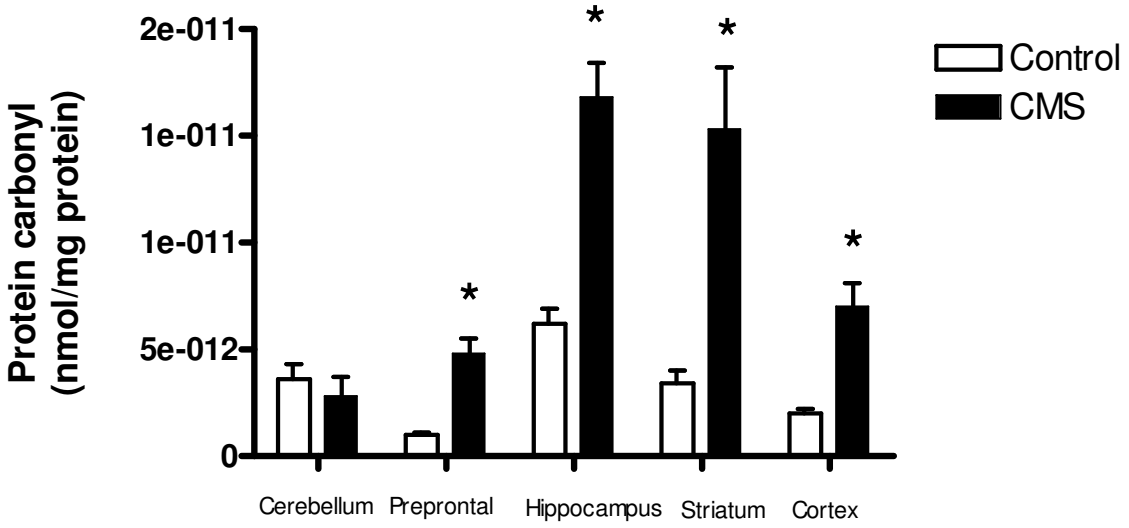
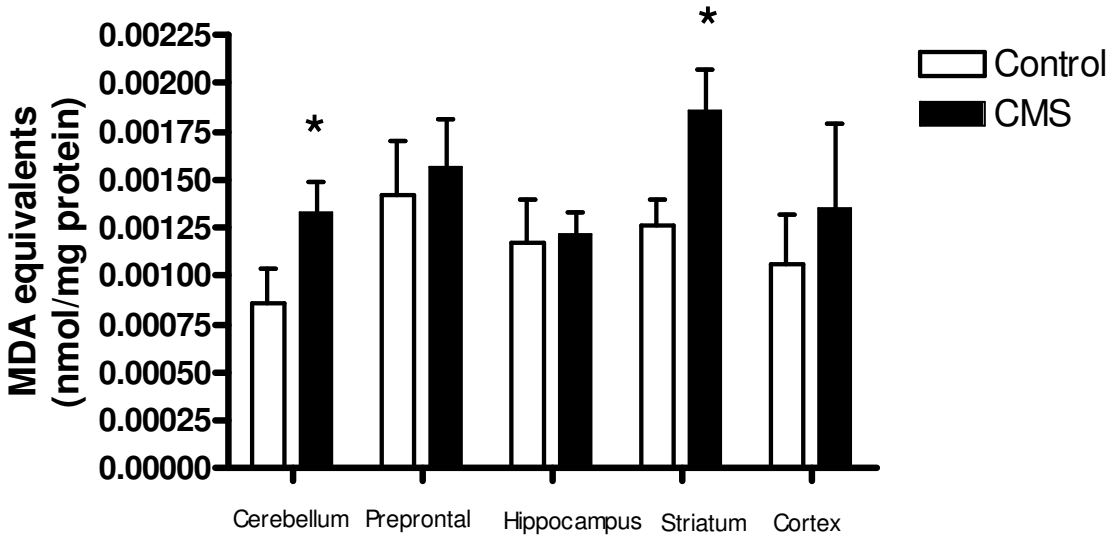

*
400
300
200
100
0
Bodt weight (g)
Control
CMS
1st
40th
1st
40th
(day)

FIGURE 2

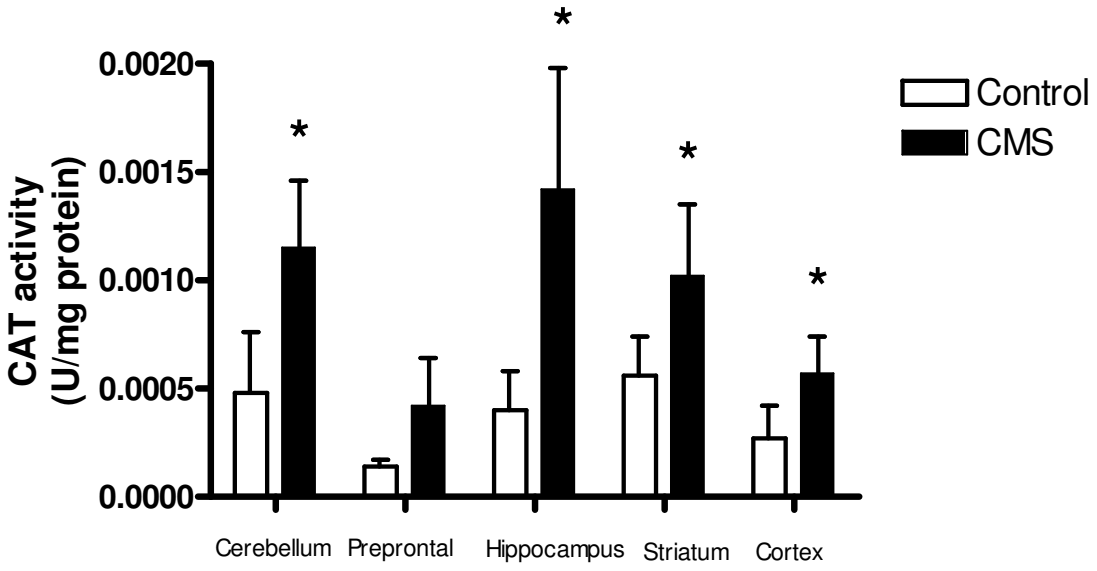
A)


Protein carbonyl (nmol/mg protein)
2e-011
1e-011
1e-011
5e-012
0
Control
CMS
Cerebellum
Preprontal
Hippocampus
Striatum
Cortex

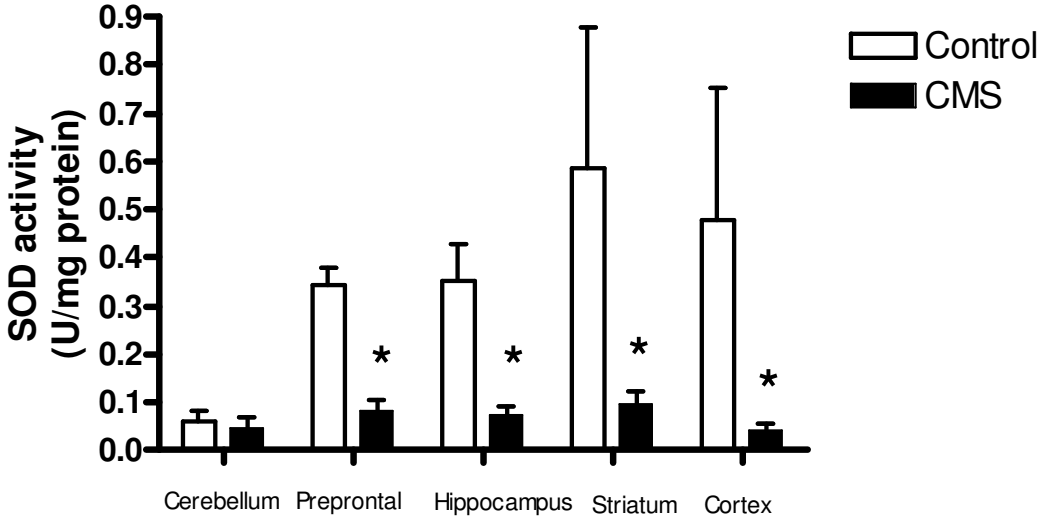
B)


MDA equivalents (nmol/mg protein)
0.00225
0.00200
0.00175
0.00150
0.00125
0.00100
0.00075
0.00050
0.00025
0.00000
Control
CMS
Cerebellum
Preprontal
Hippocampus
Striatum
Cortex

C)


CAT activity (U/mg protein)
0.0020
0.0015
0.0010
0.0005
0.0000
Control
CMS
Cerebellum
Preprontal
Hippocampus
Striatum
Cortex

D)


SOD activity (U/mg protein)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
CMS
Cerebellum
Preprontal
Hippocampus
Striatum
Cortex

LEGENDS OF FIGURES

Figure 1 – Effect of CMS paradigm on consumption of sweet food (1A), number of crossings and rearings of rats subjected to the open-field test (1B) and rat body weight in the 1st and 40th day of experiment (1C). Bars represent means ± S.E.M. of 15 rats. * $p<0.05$ vs. control according to Student's t-test

Figure 2 - Effect of CMS paradigm on protein (2A) and lipidic (2B) peroxidation, catalase (2C) and superoxide dismutases (2D) activity in rat brain. Bars represent means ± S.E.M. of 15 rats. * $p<0.05$ vs. control according to Student's t-test.

3. ARTIGO "INCREASED OXIDATIVE STRESS IN SUBMITOCHONDRIAL PARTICLES INTO THE BRAIN OF RATS SUBMITTED TO THE CHRONIC MILD STRESS PARADIGM"

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ABSTRACT

Major depression is a common, serious and recurrent disorder, characterized for symptoms at the psychological, behavioral and physiological levels. Recent studies have suggested that reactive oxygen species (ROS) may play a role in the pathophysiology of bipolar disorder. The chronic mild stress (CMS) model has been used as an animal model of depression, since it induces some symptoms (anhedonia) of a major depressive episode in humans. The present study investigated behavioral, physiological and neurochemical aspects of rats exposed for 40 days to CMS. To this aim, consumption of sweet food, locomotor activity and body weight were assessed in stressed and control rats. Additionally, the generation of ROS was evaluated using a spectrophotometric assay in submitochondrial particles in the rat hippocampus, prefrontal cortex and cortex. Our findings demonstrated decreased sweet food intake in rats subjected to CMS procedure compared to control. Indeed, a lack of body weight gain was observed in stressed rats. Locomotor activity was not affected in stressed rats. The generation of ROS in submitochondria particles in the rat brain was demonstrated by the increase of superoxide generation in the all brain structures analyzed. An increased in thiobarbituric acid reactive substances generation was observed only in the cortex. In conclusion, these observations support the view that the CMS paradigm is an animal model of depression which mimics alterations observed in depressive patients and it is a useful model to test the hypothesis of altered brain energy metabolism associated to neuropsychiatric disorders.

KEY-WORDS: Major depression, Chronic Mild Stress, Reactive oxygen species, submitochondrial particles.

INTRODUCTION

Major depression is a serious disorder often manifested with symptoms at the psychological, behavioral and physiological levels. It is a serious and recurrent disorder that affects 17-20% of the population of the world and may result in premature death, major social and economic consequences (Kessler et al., 1994). Major depressive disorder accounts for 4.4% of the total overall global disease burden, a contribution similar to that ischemic heart disease or diarrheal diseases (Mann, 2005). Among persons with major depression, 75-85% have recurrent episodes (Mueller, 1999; Keller, et al. 1986) and 10-30% recovery incompletely and have persistent, residual depressive symptoms (Mann, 2005).

Recently, there are evidences that reactive oxygen species (ROS) play an important role in the pathogenesis of many diseases, particularly in neurological and psychiatric diseases, like major depression and bipolar disorder, due to the central nervous system vulnerability to oxidative stress because of its high oxygen utilization, the reducing potential of certain neurotransmitters, its modest antioxidant defenses, its lipid-rich constitution that provides ready substrates for oxidation and the presence of redox-catalytic metals such as iron and cooper (Bilici et al, 2001; Takuma et al, 2004; Frey et al., 2006a; Halliwell, 2006; Valko et al., 2007). Several brain structures are involved in the stress response, e.g., hypothalamus, frontal cortex and hippocampus and these structures may be affected by chronic exposure to stress (McEwen, 2005; Pacak and Palkovits, 2001). Under normal conditions, mitochondria is the major source of ROS, which are produced in several complexes of the electron transport chain (Mattiasson, 2004). The extensive production of ROS leads to superoxide and products of lipid peroxidation formation and consequently leads to loss of fluidity in cell membranes, falls in membrane potential and eventual rupture and releasing of cell and organelle contents

and, consequently, in various neurotransmitter systems (serotonin and noradrenaline) which are related to major depression (Esterbauer et al., 1991; Maes et al., 1996).

Recently, our group demonstrated that in a dopaminergic animal model of mania induced by amphetamine increased the production of oxidative stress products and showed that lithium and valproate exert protective effects against amphetamine-induced oxidative stress in prefrontal and hippocampus. This finding supports the hypothesis that oxidative stress may be associated with the pathophysiology of bipolar disorder (Frey et al, 2006a). We also demonstrated that rats chronically exposed to amphetamine (dopaminergic animal model of mania) showed increase of thiobarbituric acid reactive substances (TBARS) and superoxide production in submitochondrial particles of prefrontal cortex and hippocampus (Frey et al, 2006b). Manoli et al (2000) demonstrated that chronic mild stress induces an increase in oxidative stress. In hypothalamus a decreased lipoperoxidation was observed, however total radical-trapping potential showed no difference. In hippocampus no difference was observed. This study concluded that prolonged stress induces oxidative stress which varies selectively with the brain region.

The literature reveal that emotional changes, such as exposure to stressful situations can influence feeding behavior and several studies have demonstrated that rats exposed to chronic stressors may alter they food intake pattern as well as they body weight (Katz et al., 1981; Willner et al., 1987; Willner, 1991). Ely et al. (1997) observed that animals repeatedly stressed by restraint increased food ingestion. It should be pointed out, however, that there is a certain degree of predictability of the stressor in studies using only one stressor when compared to other models using different stressors (Katz et al., 1981; Willner et al., 1987; Willner, 1991). In addition, the use of chronic mild stressors has been proposed as an animal model of depression (Pucilowski et al., 1993; Katz et al., 1981; Willner, 1991). In this model, rats are exposed to different weak stressors for several days. The response to rewarding

stimuli is diminished, as demonstrated by tests showing reduced sucrose consumption, which is interpreted as anhedonia. Thus, different models of stress can lead to different effects concerning feeding behavior.

In this context, we studied the effects of chronic mild stress on the generation of ROS in submitochondrial particles in the rat brain. More specifically, we decided to investigate the prefrontal cortex, cortex and hippocampus because alterations in these brain regions are thought to be associated with psychiatry symptoms (Antonova et al., 2004; Frey et al, 2006b).

MATERIALS AND METHODS

Animals

Male Wistar rats (3-4 months, 220-310 g) were obtained from our breeding colony (UNESC). The animals were housed 5 to a cage with food and water available *ad libitum* (except for the stressed group during the period when the stressor applied required no food or no water) and were maintained on a 12-h light/ dark cycle (lights on at 7:00 am). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

Experimental Procedure

Chronic mild stress (CMS) protocol was adapted from the procedure described by Gamaro and collaborators (2003). The animals were divided in two groups: control and stressed. Control were kept undisturbed in their home cages during the 40 days of treatment

receiving only ordinary daily care with daily supports of food and water. The 40-days chronic mild stress paradigm was used for the animals in the stressed group. Individual stressors and length of time applied each day. The following stressors were used: (i) 24h of food deprivation; (ii) 24h of water deprivation; (iii) 1-3h of restraint, as described later, (iv) 1,5-2h of restraint at 4°C; (v) forced swimming during 10 or 15 min, as described later; (vi) flashing light during 120-210min; (vii) isolation (2-3 days). Stress was applied at different times everyday, in order to minimize its predictability.

Restraint was carried out by placing the animal in a 25cm x 7cm plastic tube and adjusting it with plaster tape on the outside, so that the animal was unable to move. There was a 1cm hole at the far end for breathing. Forced swimming was carried out by placing the animal in a glass tank measuring 50cm x 47cm 40cm with 30cm of water at 23±2°C. Exposure to flashing light was made by placing the animal in a 60cm x 60cm x 25cm plywood made box divided in 16 cells of 15cm x 15cm x25cm with a frontal glass wall. A 40w lamp, flashing at frequency of 60 flashes/min, was used.

Sweet food consumption

After 40 days of treatment, consumption of sweet food was measured in 30 animals (15 controls and 15 stressed) to verify anhedonia. The animals were placed in a lightened rectangular box (40 cm × 15 cm × 20 cm) with a glass ceiling, floor and side walls made of wood and divided into 12 equal rectangles by black lines. Froot loops (10 Kellogg's® pellets of wheat and corn starch and sucrose) were placed in one extremity of the box. Animals were submitted to 5 sessions of 3 min each one, once a day, in order to become familiarized with this food. After being habituated, the animals were exposed to two test sessions, 3 min each

one, when the number of ingested pellets and locomotor activity (crossings of the black lines and rearings) were measured (Gamaro et al, 2003). A protocol was established so that when the animal ate part of the Froot loops (e.g. 1/3 or 1/4) this fraction was considered. These two evaluations were made with the animals submitted to fasting (during a period of 22 h prior to the behavioral task) or with animals fed ad libitum. These evaluations were made since food deprivation, which is used in many behavior tasks as a motivating stimulus, may also be an acute stressor (Katz et al, 1981; Gamaro et al, 2003)

Body weight was measured at the beginning and after stress protocol. After consumption of sweet food, the animals were killed by decapitation. The hippocampus, prefrontal cortex and cortex (cerebral cortex minus the prefrontal cortex) were immediately isolated by hand dissection and stored at -80ºC for posterior analyses for oxidative stress in submitochondrial particles.

Oxidative stress in submitochondrial particles

As an index of uncoupling of electron transporter chain (ETC), the generation of mitochondrial superoxide ($O2^{-}$) was measured as previously described (Poderoso et al., 1996). In brief, superoxide anion production was determined in washed submitochondrial particles (SMP) using a spectrophotometric assay based on superoxide-dependent oxidation of epinephrine to adrenochrome at 37 °C (E480 nm = 4.0 mM cm). Mitochondria (1 mg/ml) were treated for 10 min, at 37 °C. SMP were obtained by freezing and thawing (three times) the mitochondria solution, washed (twice) with 140 mM KCl, 20 mM Tris–HCl (pH 7.4) and suspended in the same medium. The reaction medium consisted of 230 mM mannitol, 70 mM

sucrose, 10mMHEPES–KOH (pH 7.4), 4.2mMsuccinate, 0.5mM KH2PO4, SMP (1.0 mg protein/ml), 0.1 μM catalase and 1 mM epinephrine.

Superoxide dismutase (E.C. 1.15.1.1.) was used at 0.1–0.3 μM final concentration as a negative control to confirm assay specificity. As a marker of lipid peroxidation, we measured the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, as previously described (Esterbauer and Cheeseman, 1990). Briefly, the samples were mixed with 1ml of trichloroacetic acid 10% and 1ml of thiobarbituric acid 0.67%, and then heated in a boiling water bath for 15 min. TBARS were determined by the absorbance at 535 nm. All the results were normalized by the protein content, using bovine albumin as standard (Lowry et al., 1951).

Statistical analysis

Results were presented as mean ± SEM. Differences among experimental groups were determined by Student's *t*-test. *P*-values <0.05 were considered to indicate statistical significance.

RESULTS

Figure 1 illustrated the effects of the CMS in sweet food intake, locomotor activity and body weight. As depicted in **Figure 1A**, stressed group decreased sucrose intake when compared with control Group (t=3.978; df=21.979 P=0.001). Interestingly, the number of crossing (t=0.235; df=28; P=0.0816) and rearing (t=0.113; df=28; P=0.911) were not statistically significant when comparing stressed and control groups as showed **Figure 1B**. **Figure 1C** illustrated the effects of the CMS in body weight**.** After the chronic mild stress

protocol, stressed rats displayed lack of body weight gain in comparison with body weight gain in control group (t=5.899; df=28; P=0.0001)

The **Figure 2** showed the effects of the CMS in generation of ROS in submitochondrial particles in the rat brain. CMS protocol increased superoxide geration in the prefrontal cortex (t=6.160; df=8; P=0.0001), cortex (t=8.087; df=8; P=0.0001) and hippocampus (t=4.747; df=8; P=0.001) as demonstrated in **Figure 2A**, as well increased the TBARS generation (**Figure 2B**) in the cortex (t=4.086; df=6; P=0.006).

DISCUSSION

The present study demonstrated that: (1) CMS rats displayed reduced sweet food intake, without significant changes in locomotor activity; (2) CMS rats showed decreased body weight compared to non-stressed rats; (3) increased superoxide (prefrontal, cortex and hippocampus) and products of lipid peroxidation (cortex) formation in stressed rats compared to control.

The CMS paradigm, which was originally described by Willner et al., 1987, is a model of depression obtained by using chronic unpredictable mild stressors (for a review see: Willner, 2005). In the CMS model, both consumption of and preference for sucrose intake as well as decreased intracranial self stimulation behavior have served as markers of generalized decrease in sensitivity to reward, which behavior is quite related to anhedonia (Willner et al, 1987; Gamaro et al, 2003; Berkris et al, 2005). In accordance with the literature, present data confirm that rats exposed to CMS procedure consume less sweet food compared to non-stressed rats.

Literature data support the fact that stressed rats had severe loss of body weight as well as behavioral alterations (Matthews et al, 1995; Gamaro et al, 2003; Berkris et al, 2005). Emotional changes, such as exposure to stressful situations can influence feeding behavior and some studies have demonstrated that chronic exposure to stressors may alter rats' body weight (Dess et al, 1988). Relative to baseline, there is typically a 3% of body weight loss compared to an 8% gain in isolated controls and this loss is reduced, but still persists even when food deprivation is omitted from the CMS schedule (Matthews et al, 1995). Gamaro et al (2003) demonstrate that there was none alteration in the habitual rat chow intake in chronically-stressed rats after chronic mild stress protocol. Reduced saccharin intake persists when the water and food deprivation components of the CMS schedule is omitted (Matthews et al, 1995). In accordance with the literature, our findings also demonstrated lack of body weight gain in stressed rats compared to body weight gain in non-stressed rats.

Some studies (Dess et al., 1988; Konarska et al., 1990; Harro et al., 2001) have reported that chronic exposure to stressors of a certain severity decreases food intake and body weight of rats. Despite this, the type, duration or severity of stress and the predictability of the stressor applied may modify the responses to stress (Pucilowski et al., 1993). Morley et al. (1986) proposed that stress can lead to either decreased or increased feeding, depending on the nature of the stressor. For instance, repeated restraint stress leads to alterations in feeding behavior with increased food ingestion (Ely et al., 1997), which is interpreted as an expression of increased levels of anxiety in chronically stressed animals, since this effect is reversed by diazepam (Ely et al., 1997).

In this context, oxidative stress in rat brain structures may play a role in the pathogenesis of depression (Erem et al, 2007). In humans, Bilici et al (2001) demonstrated elevated ROS in plasma of patients with major depression, especially in those with melancholic type. Other studies have reported numerous oxidative disturbances parameters in

patients with major depression, including oxidative damage in erythrocytic membranes (suggested by the depletion of omega-3 fatty acids) (Peet et al., 1998); elevated lipid peroxidation products (Sarandol et al., 2007; Selley, 2004); elevated superoxide anion (O_2^-) generation (Henrotin et al, 2005) and oxidative DNA damage (Forlenza and Miller, 2006). It has been postulated that mutations in mitochondrial DNA (mtDNA) could lead to increased ROS generation and vice versa (de Grey, 2005). Interestingly, recent studies have suggested that ROS generation may play a role in the increased mtDNA mutations observed in neuropsychiatric disorders (Munakata et al, 2005). Our findings demonstrated an increase of submitochondrial particles formation (i.e. superoxide in prefrontal, cortex, hippocampus and lipoperoxidation in cortex) in stressed rats compared to control.

In an animal model of mania induced by amphetamine was observed an increased oxidative stress parameters in submitochondrial particles of prefrontal cortex and hippocampus and showed that lithium and valproate exerted protective effects against amphetamine-induced oxidative stress (Frey et al, 2006a). This finding supports the hypothesis that oxidative stress may be associated with the pathophysiology of bipolar disorders (Frey et al, 2006b). One reason for these discrepancies may be fact the basal activities of various antioxidant enzymes, such as superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase are highly variable across brain regions (Carvalho et al., 2001).

In humans, when measured by the serum, superoxide dismutase activity, superoxide dismutase /glutathione peroxidase plus catalase ratio were also increased in manic and depressed patients. On the other hand, TBARS levels were increased in bipolar patients regardless of the phase of the disorder, suggesting a potential oxidative damage in bipolar disorder (Andreazza et al., 2007).

There are four major sources of ROS: (i) oxidative burst (i.e., activation of immune cells by different causes); (ii) oxidative process (e.g., electron transport chain, cytochrome P450 activation, and increased monoamine oxidation); (iii) lipid peroxidation; and (iv) oxidative stress (e.g., trauma, ischemia). The formation submitochondrial particles also may be related to electron transport chain alteration (for a review see: Koopman et al, 2005; Bilici et al, 2001). Madrigal et al (2001) demonstrated that in adult male rats, stress (immobilization for six hours during 21 days) inhibits the activities of the first complexes of the mitochondrial respiratory chain (inhibition of 69% in complex I-III and of 67% in complex II-III), without affecting complex IV activity, ATP production and oxygen consumption. This sustained mitochondrial inhibition would potentiate ONOO2 formation, leading to the depletion of antioxidant defenses (Radi et al., 1991) as well as increasing lipid peroxidation (Darley-Usmar et al. 1992). Lipid peroxidation could cause structural damage to membranes, including those which form mitochondria, and potentiate their dysfunction (Darley-Usmar et al. 1992).

Although stress may induce a decrease in glucose uptake, this effect is not sufficient to affect the energy metabolism of these cells. Torres et al (2001) showed that after 50 days of chronic stress increased blood glucose levels. However, glucose utilization, measured as CO_2 production in hippocampal and cerebral cortex slices, was the same in stressed and control groups.

Fontella et al (2004) verify the effect of repeated restraint stress on several parameters of oxidative stress in the hippocampus of adult Wistar rats, was evaluated the lipid peroxide levels, the production of free radicals, the total radical-trapping potential and the total antioxidant reactivity levels, and antioxidant enzyme activities in hippocampus of rats. The results showed that repeated restraint stress induced an increase in lipid peroxide levels and in antioxidant enzyme activity, while total antioxidant reactivity was reduced.

Studies showed that, activation of immune cells, present in major depression, especially polymorphonuclear leukocytes, leads to overproduction of ROS, interfering with the structure and ratio of polyunsaturated fatty acids (Deger et al., 1996) thus, causing loss of fluidity in biological membranes. Additionally, it was demonstrated that catecholamine metabolism increase in depression and this process leads to overproduction of ROS (Maher and Davis, 1996). Indeed, this process results in reduced catecholamine concentrations in cerebral cortex (Linscheer and Vergroesen, 1988). It was concluded that overproduction of ROS might result in destruction of phospholipids and reduce viscosity of cell membranes (McIntyre, 1988). Alterations in membrane viscosity may influence several steps in biogenic amine function, such as density or function of serotonergic or catecholaminergic receptors (Van-der-Vliet and Bast, 1992).

In conclusion, this study demonstrated that decreased sweet food intake, which reflects an anhedonia-like behavior in rats, was observed in our rats exposed to the CMS paradigm, increased superoxide generation in the all brain structures analyzed as well increased thiobarbituric acid reactive substances. These observations support the view that the CMS paradigm is an animal model of depression which mimics alterations observed in depressive patients and is also a useful model to test the hypothesis that altered brain energy metabolism is associated to neuropsychiatric disorders.

Acknowledgements: This research was supported by grants from CNPq (ECG, FDP and JQ), FAPESC (ECG and JQ), Instituto Cérebro e Mente (FDP, ECG and JQ) and UNESC (ECG, FDP and JQ). FDP and JQ are CNPq Research Fellows. SSV is holders of a CAPES studentship, and CMC is holder of a CNPq studentship.

REFERENCES

Andreazza AC, Cassini C, Rosa AR, Leite MC, de Almeida LM, et al. Serum S100B and antioxidant enzymes in bipolar patients. **Journal of Psychiatric Research** 2007; 41:523-9

Antonova E, Sharma T, Morris R, Kumari V. The relationship between brain structure and neurocognition in schizophrenia: a selective review. **Schizophrenia Research** 2004; 70:117–145.

Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. **Behavioural Brain Research** 2005; 161:45-59.

Bilici M, Efe H, Köroğlu MA, Uydu HA, Bekaroğlu M, Değer O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. **Journal of Affective Disorders** 2001; 64:43-51.

Carvalho F, Fernandes E, Remião F, Gomes-Da-Silva J, Tavares MA, Bastos MD. Adaptative response of antioxidant enzymes in different areas of rat brain after repeated d-amphetamine administration. **Addiction Biology** 2001; 6:213-221.

Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. **Trends in Pharmacological Sciences** 2002; 23:238–45.

Eren I, Naziroglu M, Demirdas A. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. **Neurochemical Research** 2007; 32, 1188–1195.

Darley-Usmar VM, Hogg H, O'Learly VJ, Wilson MT, Moncada S. The simultaneous generation of superoxide and nitric oxide can initiate lipid peroxidation in human low density lipoprotein. **Free Radical Research Communications** 1992; 17:9–20.

Deger O, Bekaroglu M, Orem A, Orem S, Uluutku N, Soylu C. Polymorphonuclear (PMN) elastase levels in depressive disorders. **Biological Psychiatry** 1996; 39, 357–363.

de Grey AD. Reactive oxygen species production in the mitochondrial matrix: implications for the mechanism of mitochondrial mutation accumulation. **Rejuvenation Research** 2005; 8:13–17.

Dess NK, Raizer J, Chapmen CD, Garcia J. Stressors in the learned helplessness paradigm: effects on body weight and conditioned taste aversion in rats. **Physiology & Behavior** 1988; 44:483–490.

Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA et al. Superoxide activates mitochondrial uncoupling proteins. **Nature** 2002; 415:96–99.

Ely DR, Dapper V, Marasca J, Corrêa JB, Gamaro GD, Xavier MH et al. Effect of restraint stress on feeding behavior of rats. **Physiology & Behavior** 1997; 61:395–398.

Esterbauer H, Schaur RJ, Zollner H. Chemistry and Biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. **Free Radical Biology & Medicine** 1991; 11, 81–128.

Fontella FU, Siqueira IR, Vasconcellos APS, Tabajara AS, Netto CA, Dalmaz C. Repeated restraint stress induces oxidative damage in rat hippocampus. **Neurochemical Research** 2005; 30:105-111.

Frey BN, Valvassori SS, Réus GZ, Martins MR, Petronilho FC, Bardini K et al. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. **Journal of Psychiatry Neuroscience** 2006a; 31:326-32.

Frey BN, Valvassori SS, Gomes KM, Martins MR, Dal-Pizzol F, Kapczinski F et al. Increased oxidative stress in submitochondrial particles after chronic amphetamine exposure. **Brain Research** 2006b; 1097:224-9.

Forlenza MJ, Miller GE. Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. **Psychosomatic Medicine** 2006; 68, 1–7.

Gamaro GD, Manoli LP, Torres IL, Silveira R, Dalmaz C. Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. **Neurochemistry International** 2003; 42:107-14.

Halliwell B. Oxidative stress and neurodegeneration: where are we now? **Journal of Neurochemistry** 2006; 97:1634-1658.

Harro J, Tonissaar M, Eller M, Kask A, Oreland L. Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. **Brain Research** 2001; 899:227–239.

Holmes PV. Rodent models of depression: reexamining validity without anthropomorphic inference. **Critical Reviews in Neurobiology** 2003;15:143–74.

Katz RJ, Roth KA, Carroll BJ. Animal models and human depressive disorders. **Neuroscience and Biobehavioral Reviews** 1981; 5:231–246.

Keller MB, Lavori PW, Rice J, Coryell W, Hirschfeld RM. The persistent risk of chronicity in recurrent episodes of nonbipolar major depressive disorder: a prospective follow-up. **The American Journal of Psychiatry** 1986;143:24-28.

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. **Archives of General Psychiatry** 1994; 51:8-19.

Konarska M, Stewart RE, McCarty R. Predictability of chronic intermittent stress: effects on sympathetic-adrenal medullary responses of laboratory rats. **Behavioral and Neural Biology** 1990 53:231–243.

Koopman WJ, Verkaart S, Visch HJ, van der Westhuizen FH, Murphy MP, van den Heuvel LW et al. Inhibition of complex I of the electron transport chain causes O2-. -mediated mitochondrial outgrowth. **American Journal of Physiology** 2005; 288:C1440-50.

Liang HW, Xia Q, Bruce IC. Reactive oxygen species mediate the neuroprotection conferred by a mitochondrial ATP-sensitive potassium channel opener during ischemia in the rat hippocampal slice. **Brain Research** 2005; 1042:169-75.

Linscheer WG, Vergroesen AJ. Lipids. In: Shils ME., Young VR., editors. Modern Nutrition in Health and Disease. Lea and Febiger: Philadelphia PA, 1988. p. 72–107.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. **The Journal of Biological Chemistry** 1951; 193:265–275.

Madrigal JL, Olivenza R, Moro MA, Lizasoain I, Lorenzo P, Rodrigo J et al. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. **Neuropsychopharmacology** 2001; 24:420-9.

Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H. Fatty acid composition in major depression decreased v3 fractions in cholesteryl esters and increased C20, 4v6/C20, 5v3 ratio in cholesteryl esters and phospholipids. **Journal of Affective Disorders** 1996; 38:35–46.

Maher P, Davis JB. The role of monoamine metabolism in oxidative glutamate toxicity. **Journal of Neuroscience** 1996; 16:6394–6401.

Mann JJ. The medical management of depression. **New England Journal of Medicine** 2005; 353:1819-34.

Manoli LP, Gamaro GD, Silveira PP, Dalmaz C. Effect of chronic variate stress on thiobarbituric-acid reactive species and on total radical-trapping potential in distinct regions of rat brain. **Neurochemical Research** 2000; 25:915-921.

Matthews K, Forbes N, Reid IC. Sucrosse consumption as an hedonic measure following chronic unpredictable mild stress. **Physiology & Behavior** 1995; 57:241-248.

Mattiasson G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S et al. Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. **Nature Medicine** 2003; 9:1062–1068.

McEwen BS. Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. **Metabolism** 2005; 54:20–23.

Morley JE, Levine AS, Willenbring ML. Stress induced feeding disorders. In: Carruba, M.O., Blundell, J.E. (Eds.), Pharmacology of Eating Disorders: **Theoretical and Clinical Developments. Raven, New York**, pp. 71–99, 1986.

McIntyre N. Familial LCAT deficiency and fish-eye disease. **Journal of Inherited Metabolic Disease** 1988; 11:45–56.

Mueller TI, Leon AC, Keller MB, Solomon DA, Endicott J, Coryell W et al. Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. **The American Journal of Psychiatry** 1999; 156:1000-1006.

Munakata K, Iwamoto K, Bundo M, Kato T. Mitochondrial DNA 3243A>G mutation and increased expression of LARS2 gene in the brains of patients with bipolar disorder and schizophrenia. **Biological Psychiatry** 2005; 57:525–532.

Murphy MP, Echtay KS, Blaikie FH, Asin-Cayuela J, Cocheme HM, Green K et al. Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-N-tert-butylnitrone. **Journal of Biological Chemistry** 2003; 278:48534–48545.

Pacak K, Palkovits M. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. **Endocrine Reviews** 2001; 22:502–548

Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. **Biological Psychiatry** 1998; 43: 315–319.

Poderoso JJ, Carreras MC, Lisdero C, Riobo N, Schopfer F, Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. **Archives of Biochemistry and Biophysics** 1996; 328: 85–92.

Pucilowski O, Overstreet DH, Rezvani AH, Janowsky DS. Chronic mild stress-induced anhedonia: greater effect in a genetic rat model of depression. **Physiology & Behavior** 1993; 54:1215–1220.

Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite oxidation of sulfhydryls. The citotoxic potential of superoxide and nitric oxide. **Journal of Biological Chemistry** 1991; 266:4244– 4250.

Sarandol A, Kirli S, Akkaya C, Altin A, Demirci M, Sarandol E Oxidative-antioxidative systems and their relation with serum S100 B levels in patients with schizophrenia: Effects of short term antipsychotic treatment. **Progress in Neuropsychopharmacology and Biological Psychiatry** 2007; 1:1164–1169.

Selley ML. Increased (E)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression. **Journal of Affective Disorders** 2004; 80:249–256.

Takuma K, Baba A, Matsuda T. Astrocyte apoptosis: implications for neuroprotection. **Progress in Neurobiology** 2004; 72:111–127.

Torres ILS, Gamaro GD, Silveira-Cucco SN, Michalowski MB, Corrêa JB, Perry MLS et al. Effect of acute and repeated restraint stress on glucose oxidation to CO_2 in hippocampal and cerebral cortex slices. **Brazilian Journal of Mediiological Research** 2001; 34:111-116.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. **International Journal of Biochemistry and Cell Biology** 2007; 39:44-84.

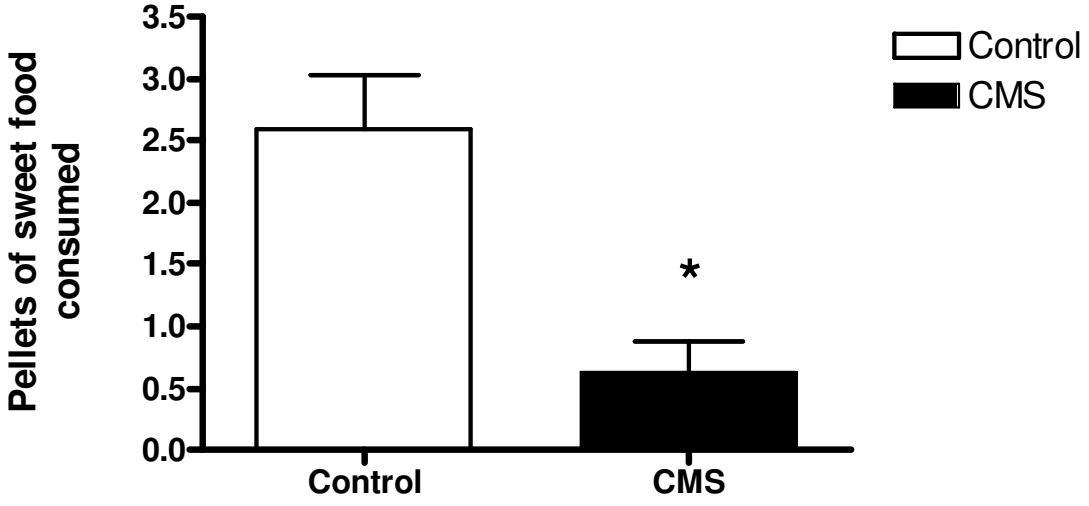
Van-der-Vliet A, Bast A. Effect of oxidative stress on receptors and signal transmission. **Chemico-Biological Interactions** 1992; 85:95–116.

Willner P, Towell A, Sampson D, Sophokclous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. **Psychopharmacology** 1987; 93:358-364.

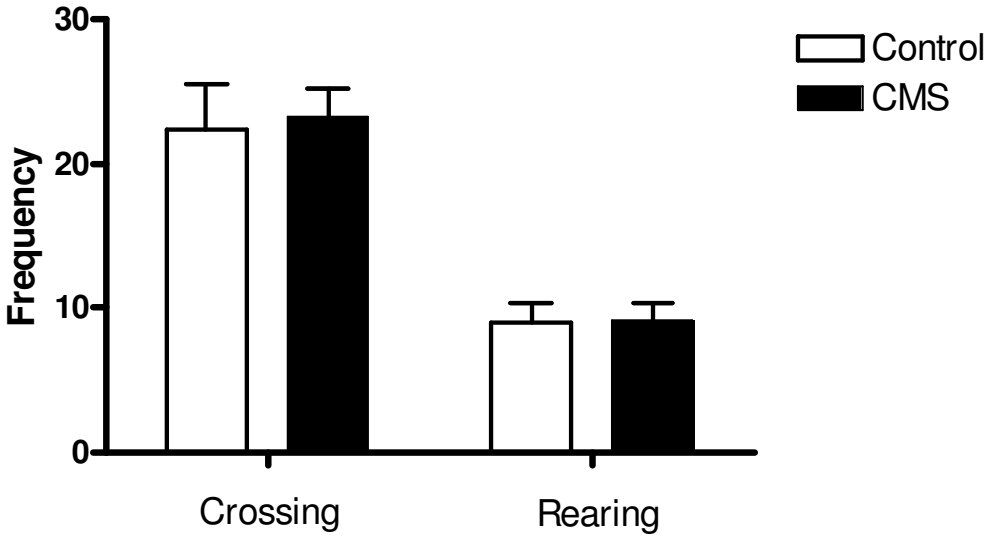
Wilner P. Chronic mild stress (CMS) revisited: consistency and behavioral-neurobiological concordance in the effects of CMS. **Neuropsychobiology** 2005; 52:90-110.

FIGURE 1

A)


Pellets of sweet food consumed
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
CMS
Control
CMS

B)


Frequency
30
20
10
0
Crossing
Rearing
Control
CMS

C)

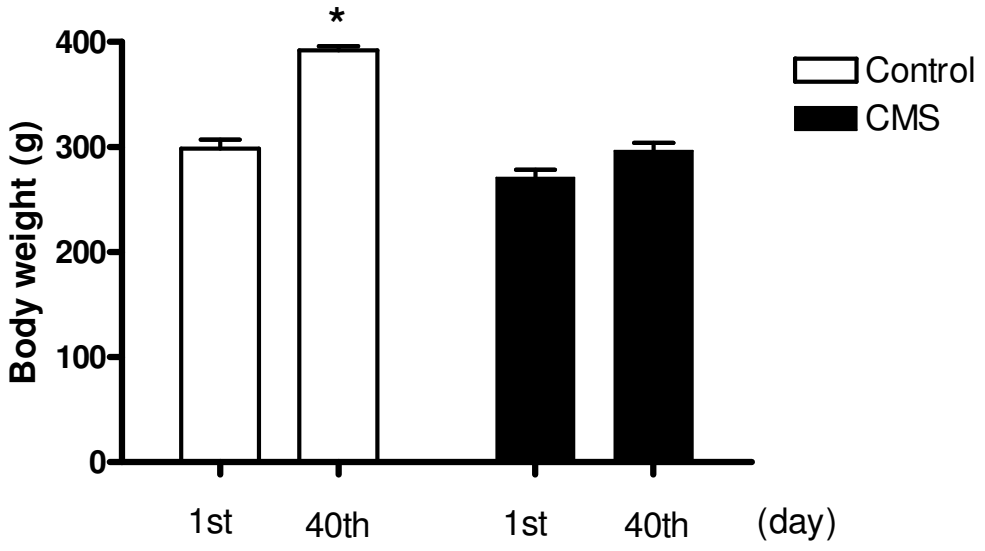
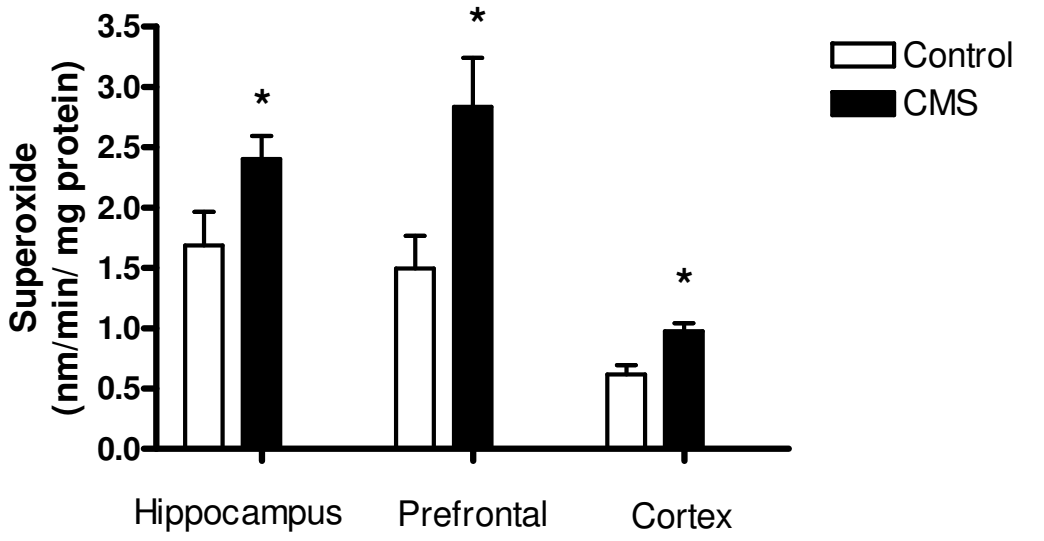
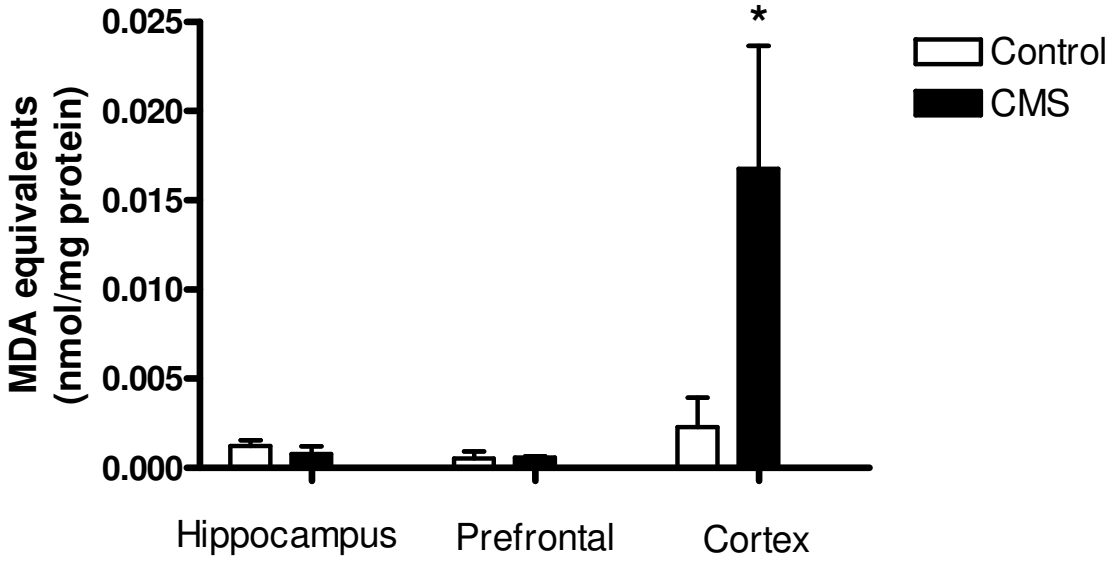

*
400
300
200
100
0
Body weight (g)
Control
CMS
1st
40th
1st
40th
(day)

FIGURE 2

A)


Superoxide
(nm/min/ mg protein)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
Control
CMS
Hippocampus
Prefrontal
Cortex

B)


MDA equivalents
(nmol/mg protein)
0.025
0.020
0.015
0.010
0.005
0.000
*
Control
CMS
Hippocampus
Prefrontal
Cortex

LEGENDS OF FIGURES

Figure 1 – Effect of CMS paradigm on sweet food consumption (A), number of crossings and rearings of rats subjected to the open-field test (B) and rat body weight in the 1st and 40th day of experiment (C). Bars represent means ± S.E.M. of 15 rats. * $p<0.05$ vs. control according to Student's t-test.

Figure 2 - Effect of CMS paradigm on generation of ROS in submitochondrial particles in superoxide (A) and TBARS (B) generation in rat brain. Bars represent means ± S.E.M. of 15 rats. * $p<0.05$ vs. control according to Student's t-test.

PARTE III

1. DISCUSSÃO

A presente dissertação composta por três artigos teve a intenção de aprofundar o conhecimento das bases biológicas dos transtornos depressivos que estão ainda na sua pré-história evolutiva. Entre os objetivos avaliados estão a replicação do modelo de ECLV no laboratório de neurociências da Universidade do Extremo Sul Catarinense, avaliação da atividade locomotora, peso corporal, peso da glândula adrenal e dosagens dos níveis da proteína do BDNF no hipocampo, sangue e liquor.

A anedonia, medida pela redução do consumo de sacarose, foi induzida nos ratos expostos ao modelo de ECLV em nosso laboratório quando comparado com os controles, sendo assim, replicado com sucesso o modelo animal de depressão. Não foi encontrada alteração na atividade locomotora dos animais expostos aos estressores em comparação com o grupo controle. Os dados comportamentais gerados por esse estudo confirmam e expandem os achados prévios de que a exposição de ratos a estressores crônicos leves e variados gradualmente induz um estado anedônico acessado pela redução do consumo de comida doce - sacarose (Gamaro et al., 2003; Stout et al., 2000; Willner, 1997).

Em relação ao peso corporal total no final do estudo, os ratos submetidos ao protocolo de estresse demonstraram uma perda de ganho ponderal enquanto os controles ganharam peso no mesmo período de tempo. Nossos achados estão de acordo com a literatura onde ratos expostos ao modelo de ECLV apresentam perda ou ausência de ganho ponderal mesmo quando a privação de comida é retirada do protocolo de estresse (Hagan et al., 1997 e Matthews et al., 1995). Assim, a alteração do peso é conseqüência do estresse em geral e não unicamente pela privação temporária de comida (Vollmayr et al., 2003 e Willner, et al.,

1996). Nos indivíduos com depressão melancólica a perda de peso é um dos critérios diagnósticos (American Psychiatric Association, 2000).

Encontramos um aumento do peso médio da glândula adrenal dos ratos estressados em comparação com o grupo controle. O estímulo prolongado e intensificado do ACTH nas células do córtex da adrenal promove uma hipertrofia da glândula com aumento do seu peso (Gamaro et al., 2003; Harro et al., 2001 e Konarska et al., 1990). Este é um parâmetro indireto da avaliação de hiper-ativação do eixo HHA.

A hiper-ativação desse eixo promove conseqüências deletérias aos neurônios podendo representar uma das causas de redução numérica e volumétrica de distintas regiões cerebrais encontradas em estudos de neuroimagem de pacientes com transtorno depressivo grave recorrente (Gonçalves et al., 2006; Sheline et al., 2003; Rajkowska, 2000; Soares et al., 1997 e Elkis et al., 1995). O hipocampo é a área mais estudada e lesões desta região explicariam o prejuízo da memória declarativa de pacientes com TDM (Bremner, 1999).

Alguns pacientes com TDM apresentam anomalias de hiper-ativação do eixo HHA e falham em suprimir esta ativação com o teste de dexametasona (Carrol et al., 2007; Gillespie et al., 2005 e Froger et al., 2004). O uso de antidepressivos reverte essa hiper-ativação em pacientes que remitem os sintomas depressivos (Gillespie et al., 2005 e Froger et al., 2004). Gamaro et al. (2003) encontrou níveis reduzidos de monoaminas (serotonina, noradrenalina e dopamina) em tecido cerebral de ratos Wistar após protocolo de ECLV. A serotonina promove a ativação da transcrição gênica do RG que é necessário para inibir, por *feedbak* negativo, a secreção de CRH no hipotálamo e do ACTH na hipófise anterior (Shelton, 2007 e Stout et al., 2000). Estudos utilizando o modelo de ECLV em ratos encontraram redução na concentração de RG no hipocampo, mas não em córtex após a aplicação do modelo (Froger et

al., 2004). A redução dos RG subseqüente ao estresse crônico explicaria a hiper-ativação do eixo HHA pela falha de sua inibição (Froger et al., 2004).

Os níveis séricos de ACTH e cortisol dos ratos expostos ao modelo de ECLV variam muito entre os trabalhos. Autores (Gillespie et al., 2005) encontram níveis aumentados nos animais com anedonia e outros (Gronli et al., 2004 e Stout et al., 2000) não encontram diferenças estatísticas entre o grupo controle e o grupo estressado. Stout et al. (2000) encontrou aumento da secreção de CRH em distintas regiões cerebrais de ratos submetidos ao modelo de ECLV.

Eventos traumáticos ou estressantes apresentam associação causal com transtornos depressivos e transtornos de ansiedade (Kaufman et al., 2000). A exposição ao estresse crônico pode conduzir a atrofia e perda neuronal como vista anteriormente. O hipocampo além de expressar e regular o eixo HHA por ativação dos RG também expressa níveis elevados de mRNA e proteína do BDNF no rato adulto normal (Conner et al., 1997). Diversos estudos demonstraram que o estresse crônico reduz a expressão do mRNA do BDNF no hipocampo (Duman et al., 2006; Shirayama et al., 2002; Russo-Neustadt et al., 2001 e Smith et al., 1995). Alterações na síntese do BDNF têm sido propostas como base da fisiopatologia dos transtornos depressivos (Tapia-Arancibia et al., 2004). Os ratos expostos ao protocolo de ECLV não apresentaram alterações na concentração da proteína do BDNF no hipocampo. De forma análoga, dois outros artigos anteriores que utilizaram protocolos de estresse que induziram anedonia nos animais não encontraram alterações na concentração da proteína do BDNF em hipocampo de ratos estressados quando comparados aos controles (Gronli et al., 2006 e Rosenbrock et al., 2005). Entretanto, Gronli et al. (2006) evidenciaram reduções nos níveis da proteína do BDNF quando analisado somente a região do giro denteado do hipocampo, porém quando toda estrutura era analisada essa redução não era estatisticamente

significativa. Assim, acredita-se que a concentração da proteína do BDNF apresente variações entre as distintas regiões cerebrais.

Estudos em humanos com TDM não medicados apresentam redução dos níveis séricos da proteína do BDNF (Bergström et al., 2008). Ainda, baixos níveis séricos de BDNF estão associados com maior vulnerabilidade para desenvolver quadros depressivos em indivíduos saudáveis (Karege et al., 2002). Entretanto, nos ratos expostos ao modelo do ECLV nós observamos uma tendência ao aumento nos níveis séricos do BDNF quando comparados com os controles. Todavia, essa ausência de redução dos níveis séricos de BDNF no grupo estressado poderia ser devido à (1) diferença entre espécies em desenvolver comportamento anedônico e (2) à possibilidade da existência de um mecanismo neuroprotetor que poderia estar ativado nos animais em situações de estresse e (3) variações entre as diferentes estruturas cerebrais. Ratos expostos ao ECLV e resistentes em desenvolver anedonia, promoviam um aumento do mRNA de BDNF (Shirayama et al., 2002). Este foi o primeiro artigo onde os níveis de proteína do BDNF foram mensurados no sangue e liquor de ratos submetidos ao ECLV. Diferentemente de outros estudos, que mensuravam o mRNA, nós dosamos a concentração da proteína do BDNF. Dessa forma, ao contrário de pacientes com TDM não medicados, ratos expostos ao protocolo de ECLV não demonstraram qualquer alteração estatisticamente significativa nos níveis séricos ou liquóricos da proteína do BDNF.

Em virtude do fato que as bases biológicas dos transtornos depressivos ainda não estão completamente compreendidas, outros objetivos deste trabalho foram avaliar os parâmetros do estresse oxidativo no cérebro dos ratos.

Foi demonstrado em nosso estudo que os ratos submetidos ao paradigma de ECLV apresentaram (1) aumento da carbonilação protéica nas regiões pré-frontal, hipocampo, estriado e córtex, (2) peroxidação lipídica no cerebelo e estriado, (3) aumento da atividade da

enzima catalase (CAT) em cerebelo, hipocampo, estriado e córtex e (4) redução da atividade da enzima superóxido dismutase (SOD) nas regiões pré-frontal, hipocampo, estriado e córtex em ratos estressados comparado aos controles.

O estresse oxidativo exerce participação na patogênese da depressão (Eren et al., 2007). O cérebro humano é particularmente suscetível ao dano oxidativo, pois utiliza cerca de 20% do oxigênio corporal, possui limitada capacidade antioxidante e possui quantidade relativamente alta de ácidos graxos peroxidáveis (Floyd, 1999). Um trabalho que fez uso de um modelo de depressão baseado no estresse causado pela restrição repetida induziu um aumento nos níveis de TBARS no hipocampo dos ratos estressados (Fontella et al., 2005). Outro estudo utilizando como estressor a imobilização promoveu aumento da peroxidação lipídica no córtex cerebral, cerebelo e hipocampo comparado com os ratos do grupo sem aplicação do estresse (Liu et al., 1996). Além disso, o mesmo trabalho aumentou a oxidação protéica no córtex, hipotálamo e estriado (Liu et al., 1996). Um estudo com pacientes portadores de TDM, principalmente do subtipo melancólico, apresentavam aumento das espécies reativas do oxigênio no plasma (Bilici et al., 2001).

Pamplona et al. (2006) realizou um estudo onde os ratos eram expostos a um protocolo de restrição calórica onde ficavam recebendo quantidade restrita de alimento e no final do estudo apresentavam falta de ganho ponderal, assim como os ratos dos nossos estudos quando expostos ao protocolo de ECLV. Entretanto, ao contrário dos nossos resultados, os ratos expostos à restrição calórica e com perda do ganho ponderal apresentavam redução da geração de ROS. Portanto, o aumento dos parâmetros de estresse oxidativo não seriam melhores explicados pela perda de ganho ponderal e restrição calórica.

Outros trabalhos encontraram diversas alterações dos parâmetros que avaliam o estresse oxidativo em pacientes com TDM, como dano às membranas de eritrócitos (Peet et

al., 1998), aumento da geração do ânion superóxido (Sarandol et al., 2007) e dano oxidativo à molécula do DNA (Forlenza et al., 2006). Nossos achados referentes ao aumento da peroxidação lipídica e carbonilação protéica são consistentes com resultados prévios sugerindo que o estresse oxidativo possui um papel importante na fisiopatologia dos transtornos depressivos, sendo que as defesas antioxidantes variam nas distintas regiões cerebrais (Frey et al., 2006a e Frey et al., 2006b).

O desequilíbrio entre as atividades enzimáticas da SOD e CAT são produzidas por alterações do estado redox e podem acarretar estresse oxidativo (Andrades et al., 2005 e Klamt et al., 2001). Um estudo recente com pacientes com TDM encontrou aumento da atividade das enzimas antioxidantes (SOD e CAT) no plasma (Bilici et al., 2001). Em situações em que a atividade da enzima SOD está aumentada sem um concomitante aumento na atividade da CAT, o produto intermediário peróxido de hidrogênio pode acumular e gerar radicais hidroxilas. Esses radicais causam dano oxidativo importante em proteínas (Halliwell, 2006). Em nosso trabalho encontramos aumento da atividade da catalase em cerebelo, hipocampo, estriado e córtex, bem como a redução da atividade da enzima SOD nas regiões pré-frontal, hipocampo, estriado e córtex. Porém, várias enzimas além da SOD geram peróxido de hidrogênio incluindo xantina, urato, glicose, lisil, monoamino e d-aminoácido oxidases (Halliwell, 2006).

O nosso terceiro artigo evidenciou que: (1) ratos expostos ao modelo de ECLV desenvolveram anedonia sem alterar a atividade locomotora; (2) ratos expostos ao protocolo do ECLV apresentaram falta de ganho ponderal quando comparados com os controles; (3) aumento do superóxido (pré-frontal, córtex e hipocampo) e aumento da formação dos produtos derivados da peroxidação lipídica (córtex) nos animais estressadas comparados com os controles.

O estresse oxidativo novamente se evidencia em cérebro de animais com anedonia demonstrando o papel dos radicais livres na etiopatogenia da depressão (Erem et al, 2007). Estudos em humanos, Bilici et al (2001) demonstrou aumento das ERO no plasma de pacientes com TDM, especialmente naqueles com subtipo melancólico. Outros estudos relatam diversos distúrbios oxidativos em pacientes com TDM, incluindo dano oxidativo de membrane de eritrócitos (Peet et al., 1998); aumento dos produtos de degradação lipídica (Sarandol et al., 2007; Selley, 2004); aumento nas concentrações do ânion superóxido (O_2^-) (Henrotin et al, 2005) e dano oxidativo em molécula de DNA (Forlenza and Miller, 2006). Nossos achados encontraram um aumento na formação de partículas sub-mitocondriais (i.e. superoxido em córtex pré-frontal, córtex, hipocampo e lipoperoxidação em córtex) nos animais com anedonia.

Assim, a anedonia em ratos aumenta a geração do anion superóxido em todas estruturas cerebrais analisadas bem como aumento na formação de TBARS. Estes resultados sustentam a hipótese a alteração do metabolismo energético tem papel relevante na etiopatogenia do TDM.

Por fim, as bases biológicas dos transtornos depressivos ainda não estão totalmente esclarecidas permanecendo uma imensa lacuna no conhecimento dessa área. Este trabalho procurou acrescentar ao arsenal científico atual mais informações e questionamentos sobre as alterações neuroquímicas dos estados depressivos. Para isso foi utilizado e replicado um modelo animal de depressão baseado no estresse com boa validade aparente e preditiva. O modelo do estresse crônico leve variado induziu estado anedônico nos animais aferido pela redução do consumo de sacarose. Sendo o TDM um problema crônico e que promove lesão celular e volumétrica cerebral, procuramos, através da avaliação dos parâmetros de estresse oxidativo, avaliar possíveis agentes causais para tais lesões. Encontramos assim, alterações de dano oxidativo em distintas regiões cerebrais de ratos com anedonia. Estas alterações são

possivelmente responsáveis, em parte, pela degradação de moléculas importantes para a manutenção da homeostase celular e resposta antidepressiva. Além disso, aferimos que, na região mais estudada e confirmada de lesão celular nos quadros depressivos, o hipocampo, os níveis de proteína do BDNF não estavam alterados. O BDNF tem papel importante na manutenção da homeostase, proteção, resposta antidepressiva e plasticidade neuronal. As causas e conseqüências desses achados necessitam ser elucidadas em novas pesquisas.

Assim, esperamos que novos estudos possam ser feitos com a intenção de elucidar os mecanismos neurobiológicos subjacente a essas alterações, encontrar novas terapêuticas e, então, maximizar a qualidade de vida dos pacientes portadores de transtornos depressivos.

REFERÊNCIAS

ADLER, V; YIN, Z; TEW, KD; RONAI, Z. Role of redox potential and reactive oxygen species in stress signaling. **Oncogene 18(45):6104-11, 1999.**

AKIN, D; MANIER, DH; SANDERS-BUSHM, E; SHELTON, RC. Decreased serotonin 5-HT2A receptor-stimulated phosphoinositide signaling in fibroblasts from melancholic depressed patients. **Neuropsychopharmacology 29(11):2081-7, 2004.**

AKIN, D; MANIER, DH; SANDERS-BUSHM, E; SHELTON, RC. Signal transduction abnormalities in melancholic depression. **Int J Neuropsychopharmacol 8(1):5-16, 2005.**

ALDRED, S; GRIFFITS, HR. Oxidation of protein in human low-density lipoprotein exposed to peroxyl radicals facilitates uptake by monocytes proptection by antioxidants in vitro. **Envirol Toxicol Pharmacol 15: 111-117, 2004.**

ALTAR, CA. Neurotrophins and depression. **Trends Pharmacol Sci 20(2):59-61, 1999.**

ANDRADES M, RITTER C, MOREIRA JC, DAL-PIZZOL, F. Oxidative parameters differences during non-lethal and lethal sepsis development. **J Surg Res 125(1):68–72, 2005.**

ANGELUCCI, F; ALOE, L; VASQUEZ, PJ; MATHE, AA. Mapping the differences in the brain concentration of brain-derived neurotrophic factor (BDNF) and nerve growth-factor (NGF) in an animal model of depression**. Neuroreport 11 (6): 1369-1373, 2000.**

AURIACOMBE, M; RENECIC, JP; MOAL, LM. Animal models of anhedonia. **Psychopharmacology 134:337–338, 1997.**

BARRET, TJ; VEDECKIS WV**.** Occupancy and composition of proteins bound to the AP-1 sites in the glucocorticoid receptor and c-jun promoters after glucocorticoid treatment and in different cell types. **Recept Signal Transduct 6(3-4):179-93, 1996**

BHANSALI, P; DUNNING, J; SINGER, SE; DAVID, L; SCHMAUSS, C. Early life stress alters adult serotonin 2C receptor pre-mRNA editing and expression of the alpha subunit of the heterotrimeric G-protein G q. **J Neurosci 27(6):1467-73, 2007**.

BELMAKER, RH; AGAM, G. Major depressive disorder. **NEJM 3: 55-68, 2008.**

BERGSTRÖM, A; JAYATISSA, MN; MORK, A; WIBORG, O. Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study. **Brain Res 1196:41-52, 2008.**

BILICI, M; EFE, H; KÖROĞLU, MA; UYDU, HÁ; BEKAROGLU, M; DEGER, O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. **J Affect Disord 64(1):43-51, 2001.**

BLENDY, JA. The role of CREB in depression and antidepressant treatment. **Biol Psychiatry 59: 1144-1150, 2006.**

BREMNER, JD. Does stress damage the brain? **Biol Psychiatry 45: 797-805, 1999.**

BROWN, ES; RUSH, AJ; MCEWEN, BS. Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. **Neuropsychopharmacology 21(4):474-84, 1999.**

BURKE, HM; DAVIS, MC; OTTE, C; MOHR DC. Depression and cortisol responses to psychological stress: a meta-analysis. **Psychoneuroendocrinology 30(9):846-56, 2005.**

CABIB, S. What is mild in mild stress? **Psychopharmacology 134: 344-346, 1997.**

CARROL, BJ; CASSIDY, F, NAFTOLOWITZ, D; TATHAM NE, WILSON WH, IRANMANESH A, LIU PY, VELDHUIS JD. Pathophysiology of hypercortisolism in depression. **Acta Psychiatr Scand 433: 90-103, 2007**.

CASAROTTO ,PC; ANDREATINI, R. Repeated paroxetine treatment reverses anhedonia induced in rats by chronic mild stress or dexamethasone. **Eur Neuropsychopharmacol 17(11):735-42, 2007.**

CASTRÉN, E; VÕIKAR, V; RANTAMÄKI, T. Role of neurotrophic factors in depression. **Curr Opin Pharmacol 7(1):18-21, 2007.**

CHEETA, S; RUIGT, G; VAN PROOSDIJ, J; WILLNER, P. Changes in sleep architecture following chronic mild stress. **Biol Psychiatry 41(4):419-27, 1997.**

COLEMAN, NA. Antioxidants in critical care medicine. **Environ Toxicol and Pharmacol, 10: 183-188, 2001.**

CONNER, JM; LAUTERBORN, JC; YAN, Q; GALL, CM; VARON, S. Distribution of brain derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. **J Neurosci 17:2295–313, 1997.**

COULL, MA; LOWTHER, S; KATONA, CL; HORTON, RW. Altered brain protein kinase C in depression: a post-mortem study. **Eur Neuropsychopharmacol 10(4):283-8, 2000.**

DEOGRACIAS, R; ESPLIGUERO, G; IGLESIAS, T; RODRÍGUEZ-PEÑA, A. Expression of the neurotrophin receptor trkB is regulated by the cAMP/CREB pathway in neurons. **Mol Cell Neurosci 26(3):470-80, 2004.**

DRANOVSKY, A; HEN, R. Hippocampal neurogenesis: regulation by stress and antidepressants. **Biol Psychiatry 59: 1136-1143, 2006.**

DUFFAU, H. Brain plasticity: from pathophysiological mechanisms to therapeutic applications. **J Clin Neurosci 13(9):885-97, 2006.**

DUMAN, RS. Depression: a case of neuronal life and death? **Biol Psychiatry 56: 140-145, 2004.**

DUMAN, RS; MALBERG, J; NAKAGAWA, S; D'AS, C. Neuronal plasticity and survival in mood disorders. Biol **Psychiatry 48: 732-739, 2000.**

DUMAN, RS; MONTEGGIA, LM. A neurotrophic model for stress-related mood disorders. **Biol Psychiatry 59:1116–1127, 2006.**

DWIVEDI, Y; CONLEY, RR; ROBERTS, RC; TAMMINGA, CA; PANDEY, GN. [(3)H]cAMP binding sites and protein kinase a activity in the prefrontal cortex of suicide victims. **Am J Psychiatry 159(1):66-73, 2002.**

DWIVEDI, Y; RIZAVI, HS; SHUKLA, PK; LYONS, J; FALUDI, G; PALKOVITS, M; SAROSI, A; CONLEY, RR; ROBERTS, RC; TAMMINGA, CA; PANDEY, GN. Protein kinase A in postmortem brain of depressed suicide victims: altered expression of specific regulatory and catalytic subunits. **Biol Psychiatry 55(3):234-43, 2004.**

DWIVEDI, Y; RAO, JS; RIZAVI, HS; KOTOWSKI, J; CONLEY, RR; ROBERTS, RC; TAMMINGA, CA; PANDEY, GN. Abnormal expression and functional characteristics of

cyclic adenosine monophosphate response element binding protein in postmortem brain of suicide subjects. **Arch Gen Psychiatry 60(3):273-82, 2003**.

EISCH, AJ; BOLAÑOS, CA; DE WIT, J; SIMONAK, RD; PUDIAK, CM; BARROT, M; VERHAAGEN, J; NESTLER, EJ. Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. **Biol Psychiatry 54(10):994-1005, 2003.**

ELKIS, H; FRIEDMAN, L; WISE, A; MELTZER, HY. Meta-analyses of studies of ventricular enlargement and cortical sulcal prominence in mood disorders. Comparisons with controls or patients with schizophrenia. **Arch Gen Psychiatry 52(9):735-46, 1995.**

EREN, I; NAZIROĞLU, M; DEMIRDAŞ, A; CELIK, O; UĞUZ, AC; ALTUNBAŞAK, A; OZMEN, I; UZ, E. Venlafaxine modulates depression-induced oxidative stress in brain and medulla of rat**. Neurochem Res 32(3):497-505, 2007.**

EREN, I; NAZIROGLU, M; DEMIRDAS, A. Protective effects of lamotrigine, aripiprazole and escitalopram on depression-induced oxidative stress in rat brain. **Neurochem Res 32(7): 1188–1195, 2007.**

FLOYD, RA. Antioxidants, oxidative stress, and degenerative neurological disorders. **Proc Soc Exp Biol Med 222(3):236-45, 1999.**

FONTELLA, FU; SIQUEIRA, IR; VASCONCELLOS, AP; TABAJARA, AS; NETTO, CA; DALMAZ, C. Repeated restraint stress induces oxidative damage in rat hippocampus. **Neurochem Res 30(1):105-11, 2005.**

FORLENZA, MJ; MILLAR, GE. Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. **Psychosom Med 68(1): 1–7, 2006.**

FRANKLIN, TB; PERROT-SINAL, TS. Sex and ovarian steroids modulate brain derived neurotrophic factor (BDNF) protein levels in rat hippocampus under stressful and non-stressful conditions. **Psychoneuroendocrinology 31 (1):38-48, 2006.**

FREY, BN; VALVASSORI, SS; GOMES, KM; MARTINS, MR; DAL-PIZZOL, F; KAPCZINSKI, F; QUEVEDO, J. Increased oxidative stress in submitochondrial particles after chronic amphetamine exposure. **Brain Res 1097(1):224-9, 2006a**.

FREY, BN; VALVASSORI, SS; RÉUS, GZ; MARTINS, MR; PETRONILHO, FC; BARDINI, K; DAL-PIZZOL, F; KAPCZINSKI, F; QUEVEDO, J. Changes in antioxidant defense enzymes after d-amphetamine exposure: implications as an animal model of mania. **Neurochem Res 31(5):699-703, 2006b**.

FROGER, N; PALAZZO, E; BONI, C; HANOUN, N; SAURINI, F; JOUBERT, C; DUTRIEZ-CASTELOOT, I; ENACHE, M; MACCARI, S; BARDEN, N; COHEN-SALMON, C; HAMON, M; LANFUMEY, L. Neurochemical and behavioral alterations in glucocorticoid receptor-impaired transgenic mice after chronic mild stress. **J Neurosci 24(11):2787-96, 2004.**

GACKOWSKI, D; ROZALSK,I R; SIOMEK, A; DZIAMAN, T; NICPON, K; KLIMARCZYK, M; ARASZKIEWICZ, A; OLINSKI, R. Oxidative stress and oxidative DNA damage is characteristic for mixed Alzheimer disease/vascular dementia. **J Neurol Sci 266(1-2):57-62, 2008.**

GAGE, FH. Structural plasticity: cause, result, or correlate of depression. **Biol Psychiatry 48(8):713-4, 2000.**

GAMARO, GD; MANOLI, LP; TORRES, ILS; SILVEIRA, R; DALMAZ, C. Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain strutures. **Neurochemistry International 42: 107-114, 2003.**

GILLESPIE, CF; NEMEROFF, CB. Hypercortisolemia and depression. **Psychosom Med 67:26-28, 2005.**

GONÇALVES, FA; COELHO, R. Depressão e tratamento: apoptose, neuroplasticidade e antidepressivos. **Acta Med Port 19: 9-20, 2006**.

GOODWIN, GM. Depression and associated physical diseases and symptoms. **Dialogues Clin Neurosci 8(2):259-65, 2006.**

GOULD, E; TANAPAT, P; MCEWEN, BS; FLÜGGE, G; FUCHS E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. **Proc Natl Acad Sci U S A 95(6):3168-71, 1998.**

GOULD, E; TANAPAT, P; RYDEL, T; HASTINGS, N. Regulation of hippocampal Neurogenesis in adulthood. **Biol Psychiatry 15;48(8):715-20, 2000.**

GRONLI, J; BRAMHAM, C; MURISON, R; KANHEMA, T; FISKE, E; BJORVATN, B; URSIN, R; PORTAS, CM. Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper. **Pharmacol Biochem Behav 85:842-849, 2006.**

GRONLI, J; MURISON, R; BJORVATN, B; SORENSEN, E; PORTAS, CM; URSUN, R. Chronic mild stress affects sucrose intake and sleep in rats. **Behavioural Brain Research, 150:139-147, 2004.**

GRONLI, J; MURISON, R; FISKE, E; BJORVATN, B; SORENSEN, E; PORTAS, CM; URSUN, R. Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. **Physiol Behav 84(4):571-7, 2005.**

HAGAN, JJ; HATCHER, JP. Revised CMS model. **Psychopharmacology 134:354–356, 1997.**

HALLIWELL, B. Oxidative stress and neurodegeneration: where are we now? **J Neurochemistry 97: 1634-1658, 2006.**

HAMMONDS, MD; SHIM, SS; FENG, P; CALABRESE, JR. Effects of subchronic lithium treatment on levels of BDNF, Bcl-2 and phospho-CREB in the rat. **Basic Clin Pharmacol Toxicol 100(5):356-9, 2007.**

HARRO, J; TONISSAAR, M; ELLER, M; KASK, A, ORELAND, L. Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. **Brain Res. 899: 227–239, 2001.**

JAYATISSA, MN; BISGAARD, C; TINGSTRÖM, A; PAPP, M; WIBORG, O. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. **Neuropsychopharmacology 31(11):2395-404, 2006.**

JÖHREN, O; FLÜGGE, G; FUCHS, E. Hippocampal glucocorticoid receptor expression in the tree shrew: regulation by psychosocial conflict. **Cell Mol Neurobiol 14(3):281-96, 1994.**

KAREGE, F; PERRET, G; BONDOLFI, G; SCHWALD, M; BERTSCHY, G; AUBRY, JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. **Psychiatry Res 15;109(2):143-8, 2002.**

KAREGE, F; SCHWALD, M; EL KOUAISSI, R. Drug-induced decrease of protein kinase a activity reveals alteration in BDNF expression of bipolar affective disorder. **Neuropsychopharmacology 29(4):805-12, 2004.**

KATZ, RJ. Animal model of depression: pharmacological sensitivity of a hedonic deficit. **Pharmacol Biochem Behav 16(6):965-8, 1982.**

KAUFMAN, J; PLOTSKY, PM; NEMEROFF, CB; CHARNEY, DS. Effects of early adverse experiences on brain structure and function: clinical implications. **Biol Psychiatry 48(8):778-90, 2000.**

KEMPERMANN, G; KRONENBERG, G. Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. **Biol Psychiatry 54(5):499-503, 2003.**

KESSING, LV. Epidemiology of subtypes of depression. **Acta Psychiatr Scand 433: 85-89, 2007.**

KITRAKI, E; KARANDREA, D; KITTAS, C. Long-lasting effects of stress on glucocorticoid receptor gene expression in the rat brain. **Neuroendocrinology 69(5):331-8, 1999.**

KLAMT, F; DAL-PIZZOL, F; CONTE DA FROTA, ML; JR WALZ, R; ANDRADES, ME; DA SILVA, EG; BRENTANI, RR; IZQUIERDO, I; FONSECA MOREIRA, JC. Imbalance of antioxidant defense in mice lacking cellular prion protein. **Free Radic Biol Med 30(10):1137–1144 259, 2001.**

KOPONEN, E; RANTAMÄKI, T; VOIKAR, V; SAARELAINEN, T; MACDONALD, E; CASTRÉN, E. Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. **Cell Mol Neurobiol 25(6):973-80, 2005.**

KOPONEN, E; VÕIKAR, V; RIEKKI, R; SAARELAINEN, T; RAURAMAA, T; RAUVALA, H; TAIRA, T; CASTRÉN, E. Transgenic mice overexpressing the full-length

neurotrophin receptor trkB exhibit increased activation of the trkB-PLCgamma pathway, reduced anxiety, and facilitated learning. **Mol Cell Neurosci 26(1):166-81, 2004.**

KONARSKA, M; STEWART, RE; MCCARTY, R. Predictability of chronic intermittent stress: effects on sympathetic-adrenal medullary responses of laboratory rats. **Behav. Neural. Biol. 53, 231–243,1990.**

LAWRENCE, MS; SAPOLSKY, RM. Glucocorticoids accelerate ATP loss following metabolic insults in cultured hippocampal neurons. **Brain Res 646(2):303-6, 1994.**

LIU, J; WANG, X; SHIGENAGA, MK; YÈO, HC; MORI, A; AMES, BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. **FASEB J 10(13):1532-8, 1996.**

MAGARIÑOS, AM; VERDUGO, JM; MCEWEN, BS. Chronic stress alters synaptic terminal structure in hippocampus. **Proc Natl Acad Sci U S A 94(25):14002-8, 1997.**

MAKINO, S; SMITH, MA; GOLD, PW. Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. **Endocrinology 136(8):3299-309, 1995.**

MANJI, HK; MOORE, GJ; CHEN, G. Lithium up-regulates the cytoprotective protein Bcl-2 in the CNS in vivo: a role for neurotrophic and neuroprotective effects in manic depressive illness. **J Clin Psychiatry 61 Suppl 9:82-96, 2000.**

MANJI, HK; QUIROZ, JA; SPORN, J; PAYNE, JL; DENICOFF, K; GRAY, NA; ZARATE, CA JR; CHARNEY, DS. Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. **Biol Psychiatry 53(8):707-42, 2003.**

MANN, JJ. The medical management of depression. **NEJM 353(27): 1819-1832, 2005**.

MATTHEWS, K; FORBES, N; REID, IC. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. **Physiol Behav 57(2):241-8, 1995.**

MERALI, Z; DU, L; HRDINA, P; PALKOVITS, M; FALUDI, G; POULTER, MO; ANISMAN, H. Dysregulation in the suicide brain: mRNA expression of corticotropin-

releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. **J Neurosci 24(6):1478-85, 2004.**

MIDDLETON, E; KANDASWANI, C; THEOHARIDES, TC. The effects of plant flavonoids on mammalian cell: implications for inflammation, heart diseases and cancer. **Pharmacological Reviews 52: 673-751, 2000.**

MINEUR, YS; BELZUNG, C; CRUSIO, WE. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. **Behav Brain Res 175: 43-50, 2006.**

MIZOGUCHI, K; ISHIGE, A; ABURADA, M; TABIRA, T. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. **Neuroscience 119(3):887-97, 2003.**

MIZOGUCHI, K; YUZURIHARA, M; ISHIGE, A; SASAKI, H; CHUI, DH; TABIRA, T. Chronic stress differentially regulates glucocorticoid negative feedback response in rats. **Psychoneuroendocrinology 26(5):443-59, 2001.**

MURRAY, CJ; LOPEZ AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. **Lancet 349(9064):1498-504, 1997.**

MURRAY, CJ; LOPEZ AD. Global mortality and disability and contribution of risk factors: Global Burden of Disease Study. **Lancet 349(9063):1436-42, 1997.**

NESTLER, EJ; GOULD, E; MANJI, H; BUNCAN, M; DUMAN, RS; GRESHENFELD, HK; HEN, R; KOESTER, S; LEDERHENDLER, I; MEANEY, M; ROBBINS, T; WINSKY, L; ZALCMAN, S. Preclinical models: status of basic research in depression. **Biol Psychiatry 52(6):503-28, 2002.**

NG, F; BERK, M; DEAN, O; BUSH, AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. **Int J Neuropsychopharmacol 21:1-26, 2008.**

PAMPLONA, R; BARJA, G. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. **Biochim Biophys Acta 1757(5-6):496-508, 2006.**

PANDEY, GN; DWIVEDI, Y. Focus on protein kinase A and protein kinase C, critical components of signal transduction system, in mood disorders and suicide. **Int J Neuropsychopharmacol 8(1):1-4, 2005.**

PANDEY, GN; DWIVEDI, Y; PANDEY, SC; CONLEY, RR; ROBERTSM RC; TAMMINGA, CA. Protein kinase C in the postmortem brain of teenage suicide victims. **Neurosci Lett 228(2):111-4, 1997.**

PANDEY, GN; DWIVEDI, Y; KUMARI, R; JANICAK, PG. Protein kinase C in platelets of depressed patients. **Biol Psychiatry 44(9):909-11, 1998.**

PANDEY, GN; DWIVEDI, Y; SRIDHARARAO, J; REN, X; JANICAK, PG; SHARMA, R. Protein kinase C and phospholipase C activity and expression of their specific isozymes is decreased and expression of MARCKS is increased in platelets of bipolar but not in unipolar patients. **Neuropsychopharmacology 26(2):216-28, 2002**.

PEET, M; MURPHY, B; SHAY, J; HORROBIN, D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. **Biol Psychiatry 43(5): 315–319, 1998.**

RAJKOWSKA, G. Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. **Biol Psychiatry 48(8):766-77, 2000.**

ROSENBROCK, H; KOROS, E; BLOCHING, A; PODHORNA, J; BORSINI, F. Effect of chronic intermittent restraint stress on hippocampal expression of marker proteins for synaptic plasticity and progenitor cell proliferation in rats. **Brain Res 1040(1-2):55-63, 2005.**

RUSSO-NEUSTADT, A; HA, T; RAMIREZ, R; KESSLAK, JP. Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. **Behav Brain Res 120:87–95, 2001.**

SAARELAINEN, T; HENDOLIN, P; LUCAS, G; KOPONEN, E; SAIRANEN, M; MACDONALD, E; AGERMAN, K; HAAPASALO, A; NAWA, H; ALOYZ, R; ERNFORS, P; CASTRÉN, E. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. **J Neurosci 23(1):349-57, 2003.**

SAPOLSKY, RM. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. **Biol Psychiatry 48: 755-765, 2000.**

SAPOLSKY, RM; PULSINELLI, WA. Glucocorticoids potentiate ischemic injury to neurons: therapeutic implications. **Science 229(4720):1397-400, 1985.**

SAPOLSKY, RM; UNO, H; REBERT, CS; FINCH, CE. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. **J Neurosci 10(9):2897-902, 1990.**

SARANDOL A, KIRLI S, AKKAYA C; ALTIN, A; DEMIRICI, M; SARANDOL,E. Oxidative antioxidative systems and their relation with serum S100 B levels in patients with schizophrenia: Effects of short term antipsychotic treatment. **Prog Neuropsychopharmacol Biol Psychiatry 1(6):1164–1169, 2007.**

SHALTIEL, G; CHEN, G; MANJI, HK. Neurotrophic signaling cascades in the pathophysiology and treatment of bipolar disorder. **Curr Opin Pharmacol 7(1):22-6, 2007.**

SCHILDKRAUT, JJ. The catecholamie hypothesis of affective disorders: a review of supporting evidence. **Am J Psychiatry 122(5):509-22, 1965.**

SHELINE, YI; GADO, MH; KRAEMER, HC. Untreated depression and hippocampal volum loss. **Am J Psychiatry 160(8): 1516-1518, 2003.**

SHELTON RC. The molecular neurobiology of depression. **Psychiatr Clin North Am 30(1):1-11, 2007.**

SHIEH, PB; HU, SC; BOBB, K; TIMMUSK, T; GHOSH, A. Identification of a signaling pathway involved in calcium regulation of BDNF expression. **Neuron 20(4):727-40, 1998.**

SHIMIZU, E; HASHIMOTO, K; OKAMURA, N; KOIKE, K; KOMATSU, N; KUMAKIRI, C; NAKAZATO, M; WATANABE, H; SHINODA, N; OKADA, S; IYO, M. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. **Biol Psychiatry 54(1):70-5, 2003.**

SHIRAYAMA, Y; CHEN, AC; NAKAGAWA, S; RUSSELL, DS; DUMAN, RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. **J Neurosci 22:3251–61, 2002.**

SMITH, MA; MAKINO, S; KVETNANSKY, R; POST, RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. **J Neurosci 15:1768–1777, 1995**.

SOARES. JC; MANN, JJ. The anatomy of mood disorders--review of structural neuroimaging studies. **Biol Psychiatry 41(1):86-106, 1997.**

STOUT SC, MORTAS P, OWENS MJ, NEMEROFF CB, MOREAU J. Increased corticotropin-releasing factor concentrations in the bed nucleus of the stria terminalis of anhedonic rats. **Eur J Pharmacol 401(1):39-46, 2000.**

TAPIA-ARANCIBIA, L; RAGE, F; GIVALOIS, L; ARANCIBIA, S. Physiology of BDNF: focus on hypothalamic function. **Front Neuroendocrinol 25:77-107, 2004.**

TSANKOVA, NM; BERTON, O; RENTHAL, W; KUMAR, A; NEVE, RL; NESTLER, EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. **Nat Neurosci 9(4):519-25, 2006.**

UNO, H; LOHMILLER, L; THIEME, C; KEMNITZ, JW; ENGLE, MJ; ROECKER, EB; FARRELL, PM. Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I. Hippocampus. **Brain Res Dev Brain Res 53(2):157-67, 1990**.

VOLLMAYR, B; HENN, FA. Stress model of depression. **Clinical Neuroscience research 3: 245-251, 2003.**

WILLNER, P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. **Neuropsychobiology 52(2):90-110, 2005.**

WILLNER, P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. **Psychopharmacology 134: 319-329, 1997**.

WILLNER, P; MITCHELL, PJ. The validity of animal models of predisposition to depression. **Behav Pharmacol 13(3):169-88, 2002.**

WILLNER, P; MOREAU, JL; NIELSEN, CK; PAPP, M; SLUZEWSKA, A. Decreased hedonic responsiveness following chronic mild stress is not secondary to loss of body weight. **Physiol Behav 60(1):129-34, 1996**.

WILLNER, P; TOWELL, A; SAMPSON, D; SOPHOKLEOUS, S; MUSCAT, R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. **Psychopharmacology 93(3):358-64, 1987.**

WISE, TN; FISHBAIN, DA; HOLDER-PERKINS, V. Painful physical symptoms in depression: a clinical challenge. **Pain Med Suppl 2:S75-82, 2007.**

WOOLLEY, CS; GOULD, E; MCEWEN, BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. **Brain Res 531(1-2):225-31, 1990**.

YEN, G; DUH, P; TSAI, H; HUANG, S. Pro-oxidative properties of flavonoids in human lymphocytes. **Biosci Biotechnol Biochem 67:1215-1222, 2003.**

ZHOU, R; GRAY, NA; YUAN, P; LI, X; ZCHEN, J; CHEN, G; DAMSCHRODER-WILLIAMS, P; DU, J; ZHANG, L; MANJI, HK. The anti-apoptotic, glucocorticoid receptor cochaperone protein BAG-1 is a long-term target for the actions of mood stabilizers. **J Neurosci 25(18):4493-502, 2005.**

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