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EFEITOS DO CONSUMO DE ÁCIDOS GRAXOS n-3, n-6 E *TRANS* SOBRE ASPECTOS BIOQUÍMICOS E MOLECULARES EM UM MODELO ANIMAL DE MANIA

TESE DE DOUTORADO

Fabíola Trevizol

Santa Maria, RS, Brasil

2014

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Fabíola Trevizol

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Orientadora: Profª. Dra. Marilise Escobar Bürger

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elaborada por
Fabíola Trevizol

como requisito parcial para obtenção do grau de
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Santa Maria, 18 de julho de 2014.

Dedico esta tese ao Juliano

e a nossa amada Liz.

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“No dramático momento em que uma célula masculina, microscópica e serpenteante, encaminha-se para célula-ovo, muito maior, e liga-se a ela, um ser humano começa a existir e a nutrição tem início. Este período de desenvolvimento, quando as coisas podem ser definitivamente “certas” ou “erradas”, é de vital importância e a nutrição pode exercer uma profunda influência, que se estende por toda a vida.” **(Roger Willians)**

RESUMO

Tese de doutorado
Programa de Pós-Graduação em Farmacologia
Universidade Federal de Santa Maria, RS, Brasil

EFEITOS DO CONSUMO DE ÁCIDOS GRAXOS n-3, n-6 E *TRANS* SOBRE ASPECTOS BIOQUÍMICOS E MOLECULARES EM UM MODELO ANIMAL DE MANIA

Autora: Fabíola Trevizol
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Local e Data da Defesa: Santa Maria, 18 de julho de 2014.

Ácidos graxos (AG) são fosfolipídeos constituintes das membranas neuronais onde são fundamentais para o desenvolvimento e funcionamento do cérebro. Durante o pico do crescimento neuronal, o qual ocorre durante a última semana de gestação e período de aleitamento, há um rápido acúmulo de AG poliinsaturados de cadeia longa (AGPI-CL) para o desenvolvimento fetal e neonatal normal, garantindo o desenvolvimento de suas funções neurológicas. Durante as últimas décadas foram observadas mudanças nos hábitos alimentares, principalmente em países ocidentais, devido ao aumento do consumo de AG *trans* e ômega-6 (n-6) em detrimento do consumo de AG ômega-3 (n-3). Estas mudanças podem favorecer o desenvolvimento de processos oxidativos e alterar a neuroplasticidade neuronal, facilitando assim o desenvolvimento de doenças neuropsiquiátricas, e dentre estas, o transtorno bipolar (TB). Através de um modelo animal de mania induzido por anfetamina, avaliamos comparativamente a influência da suplementação diária de óleos ou gordura desde o período pré-concepcional até o desmame das ninhadas em regiões cerebrais dos filhotes de 1ª e de 2ª geração. Três grupos de ratas Wistar foram suplementadas diariamente (3g/kg/v.o.) desde uma semana antes da concepção, durante a gestação e aleitamento com óleo de peixe (OP, rico em AGPI n-3); óleo de soja (rico em AGPI n-6) ou gordura vegetal hidrogenada (GVH; rica em AGT). No período de desmame, filhotes de ambos os sexos foram mantidos sob a mesma suplementação original até 90 dias de idade. Enquanto os filhotes machos foram incluídos no estudo da 1ª geração, as fêmeas foram separadas e acasaladas nas mesmas condições de suplementação já descritas, obtendo-se assim, animais adultos de 2ª geração, os quais foram incluídos no 2º estudo e divididos em 3 experimentos. No estudo de 1ª geração, aos 90 dias de idade, metade de cada suplementação foi tratada com uma dose diária de anfetamina (4mg/Kg, ip) ou solução salina (controle), durante 14 dias, quando foram submetidos aos testes comportamentais e avaliações bioquímicas no córtex, estriado e hipocampo. A suplementação com GVH favoreceu a incorporação de AGT nas três estruturas cerebrais descritas, aumentou a atividade locomotora induzida por ANF e aumentou os danos oxidativos. Já a suplementação com OP permitiu um aumento da porcentagem de DHA, diminuindo a razão AGPI n6/n3 nas três regiões avaliadas, o que pode ter contribuído para uma maior fluidez das membranas neurais e menor incidência de danos oxidativos. Ratos machos adultos da 2ª geração foram também expostos ao modelo animal de mania induzido por anfetamina, sendo porém separados em dois experimentos distintos: no primeiro experimento, além de avaliações comportamentais relacionadas à memória, marcadores do status oxidativo e análises moleculares foram feitas no hipocampo, sendo observado um prejuízo destes parâmetros no grupo suplementado com GVH, enquanto o grupo OP mostrou efeitos benéficos. No segundo experimento, além do comportamento locomotor, análises bioquímicas e moleculares foram feitas no córtex, quando novamente, a suplementação com GVH mostrou efeitos deletérios. No terceiro experimento avaliamos a influência da suplementação de gordura *trans* em animais de 1ª e 2ª geração sobre o mesmo modelo animal de mania e a resposta farmacológica ao carbonato de lítio (droga estabilizadora do humor), quando observamos que o lítio foi capaz de reverter todos efeitos induzidos pela ANF. Tomados em conjunto, os dados apresentados nesta tese sugerem que o consumo aumentado de alimentos industrializados, os quais são ricos em grodura *trans*, podem estar envolvidos no aumento da incidência de doenças neuropsiquiátricas. Contrariamente, uma alimentação balanceada, a qual inclui fontes de omega-3, reduz a suscetibilidade para o desenvolvimento de tais condições, em decorrência das possíveis alterações na composição fosfolipídica das membranas neurais.

PALAVRAS CHAVE: AGPI n-3 e n-6. Gordura trans. Estresse oxidativo. Neuroplasticidade. Transtorno bipolar

ABSTRACT

Doctoral Thesis
Post-Graduate Program in Pharmacology
Federal University of Santa Maria, RS, Brasil

EFFECTS OF THE CONSUMPTION OF N-3, N-6 AND *TRANS* FATTY ACIDS ON BIOCHEMICAL AND MOLECULAR ASPECTS IN AN ANIMAL MODEL OF MANIA

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Date and place of defense: July 18th, 2014, Santa Maria

Fatty acids (FA) are constituents of neuronal phospholipid membranes, where they are essential for the development and functioning of the brain. During the peak of neuronal growth, occurring during the last week of gestation and lactation, there is a rapid accumulation of long-chain polyunsaturated FA (LC-PUFAs), which are synthesized from a physiologically appropriate supply of essential fatty acids (EFA) for normal fetal and neonatal development , ensuring the development of neurological functions. During the last decades, there occurred changes in dietary habits in Western countries, mainly with increased consumption of *trans* fatty acids (TFA) and omega-6 (n-6) at the expense of consumption of omega-3 (n-3). These changes may increase oxidative damage and alter neuronal neuroplasticity, thereby facilitating the development of neuropsychiatric diseases such as bipolar disorder (BD). Through a model of amphetamine-induced mania in rats, we evaluated comparatively the influence of daily supplementation of different fats since pre-conception until weaning of 1st and 2nd generation litters on behavioral parameters in conjunction with biochemical changes in brain regions. Three groups of female *Wistar* rats were supplemented (3g/kg; p.o. per day) from one week before conception through pregnancy and breast-feeding with fish oil (FO, rich in n-3 PUFA), soybean oil (SO; rich in PUFA n-6) or hydrogenated vegetable fat (HVF; rich in TFA). During weaning, pups of both sexes were kept under the original supplementation until 90 days of age. While male offspring were included in the study of the 1st generation, females were mated and maintained in the same supplementation, thus obtaining animals of the 2nd generation, which were used in the 2nd study and divided into 3 experiments. In the study of the 1st generation at 90 days of age, one half of each supplementation group was treated with a daily dose of amphetamine (AMPH 4mg/kg, ip) or saline (control) for 14 days, when they were subjected to behavioral tests and biochemical assessments in the cortex, striatum and hippocampus. HVF supplementation was associated with TFA incorporation in the three structures, increased AMPH-induced locomotor activity, and increased oxidative damage. Since FO supplementation increased DHA percentage and decreased the n6/n3 ratio in the three regions analyzed, it may have improved membrane fluidity and reduced oxidative stress in such animals. Adult male rats born from the 2nd generation were also exposed to an animal model of mania induced by amphetamine and used in two different experiments. In the first experiment, animals were evaluated for memory behavior and biochemical and molecular analysis in the hippocampus, in which these parameters were decreased by HVF supplementation and improved by FO supplementation. In the second experiment, animals were evaluated regarding hyperactivity and biochemical and molecular analyses in the cortex, where again HVF supplementation was associated with loss in some parameters. In a third experiment we evaluated the influence of *trans* fat supplementation in rats exposed to the same mania animal model and the response to lithium (a mood stabilizing drug) treatment in 1st and 2nd generation animals. Lithium was able to reverse all AMPH-induced effects. Taken together, our findings suggest that the increased consumption of processed foods, which are rich in *trans* fat, may be related to an increased incidence of neuropsychiatric conditions. Conversely, a balanced diet, which includes omega-3 sources , reduces susceptibility to developing such conditions, possibly by changing the composition of the neuronal phospholipid membrane.

Keywords: PUFAs n-3 and n-6. T*rans* fat. Oxidative stress. Neuroplasticity. Bipolar disorder.

LISTA DE ILUSTRAÇÕES

INTRODUÇÃO

MANUSCRITO 1

MANUSCRITO 2

MANUSCRITO 3

APÊNDICE

RESULTADOS PRELIMINARES

LISTA DE TABELAS

LISTA DE ABREVIATURAS

AA – ácido araquidônico
AG – ácidos graxos
AGE – ácidos graxos essenciais
AGPI – ácidos graxos poliinsaturados
AGT – ácidos graxos *trans*
AGI – ácidos graxos insaturados
AGS – ácidos graxos saturados
AGMI – ácidos graxos monoinsaturados
ALA – ácido α-linoléico
ANF – anfetamina
BDNF – fator neurotrófico derivado do cérebro
CAT – catalase
COX – ciclooxigenase
DA – dopamina
DAT – transportador de dopamina
DHA – ácido docosahexaenóico
EO – estresse oxidativo
EPA – ácido eicosapentaenoico
ER – espécies reativas
EROs – espécies reativas de oxigênio
GPx – glutationa peroxidase
GSH – glutationa reduzida
GVH – gordura vegetal hidrogenada
IL-8 – interleucina 8
LA – ácido linoleico
Li – lítio
LOX – lipooxigenase
LPO – peroxidação lipídica ou lipoperoxidação
LT – leucotrienos
n-3 – ômega-3
n-6 – ômega-6
PG – prostaglandinas
PGI3 – prostaglandina I3
PLA2 – fosfolipase A_2
RL – radicais livres
SNC – sistema nervoso central
SOD – superóxido dismutase
TB – transtorno bipolar
TBARS – substâncias reativas ao ácido tiobarbitúrico
TNF – fator necrose tumoral
TRκB – receptor tirosina quinase B
TX – tramboxanos
TXA2 – tramboxano A2
VIT C – vitamina C
5-LOX – lipooxigenase 5

LISTA DE APÊNDICES

SUMÁRIO

APRESENTAÇÃO

Esta tese está estruturada em seções dispostas da seguinte forma: Introdução, Objetivos, Produção Científica (Artigo 1, Manuscritos Científicos 1, 2 e 3), Discussão, Conclusões, Referências Bibliográficas e Apêndice 1.

Os itens Materiais e Métodos, Resultados, Discussão e Referências encontram-se inseridos nos próprios artigo e manuscritos na seção **PRODUÇÃO CIENTÍFICA** e representam a íntegra deste estudo.

Ao fim encontram-se os itens **DISCUSSÃO e CONCLUSÃO**, nos quais há interpretações e comentários gerais dos manuscritos científicos contidos neste estudo.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem nos itens **INTRODUÇÃO e DISCUSSÃO**.

O **APÊNDICE 1** refere-se aos experimentos executados durante realização de Doutorado Sanduíche.

1. INTRODUÇÃO

1.1 Nutrição Materna e Desenvolvimento Cerebral

O desenvolvimento e a manutenção das funções psicomotoras e cognitivas, no decorrer da vida, sofrem influência decisiva da nutrição principalmente no período perinatal. Considerando que a mãe é a principal fonte nutricional do feto (através da placenta) e lactente (RAO et al., 2007), o impacto da alimentação materna sobre a prole é uma área de pesquisa que requer mais atenção (BUCKLEY et al., 2005).

Embora todos os nutrientes sejam importantes para o desenvolvimento estrutural do Sistema Nervoso Central (SNC), alguns lipídeos, como os Ácidos Graxos Poliinsaturados de Cadeia Longa (AGPI-CL), podem influenciar decisivamente em determinadas fases do desenvolvimento neuronal (GEORGIEFF, 2006). Não só a quantidade, mas o tipo de ácido graxo materno consumido durante a gestação e aleitamento, vem sendo considerada como principal determinante do crescimento, do desenvolvimento visual e neurológico (STORLIEN et al., 1991; INNIS, 2007).

Durante o desenvolvimento embrionário e pós-natal, o maior acréscimo de AGPI ocorre durante o último trimestre de gestação e os primeiros 2 anos após o nascimento nos seres humanos (MARTINEZ, 1992; CLANDININ et al., 1980), e até os primeiros 15 dias após o nascimento em ratos (DOBBING; SANDER, 1979). É neste período que ocorrem os processos de neurodesenvolvimento mais importantes - mielinização, organização de sistemas neurotransmissores, arborização dendrítica e gênese sináptica (LAURITZEN et al., 2001; SALIBA; MARRET, 2001). Neste momento, o cérebro é especialmente vulnerável a quaisquer deficiências nutricionais, em função de ser o período em que os processos implicados no desenvolvimento cerebral ocorrem com maior rapidez (ALMEIDA et al., 2002; MORGANE et al., 2002).

1.2 Ácidos Graxos

Os ácidos graxos (AG) e os seus derivados, como os triacilgliceróis e fosfolipídeos, são exemplos de lipídios. Eles compõem um conjunto de moléculas biológicas que têm a característica comum de serem insolúveis em água e solúveis em solventes orgânicos. AG são fundamentais em muitos processos vitais com funções de reserva energética, secreção hormonal, composição das membranas celulares e um importante papel na sinalização celular e atividade pró/anti-inflamatórias (RIEMER et al., 2010; ALBERTS et al., 2004; COOPER; HAUSMAN, 2007).

Os ácidos graxos são formados por uma cadeia hidrocarbonada (2 a 20 ou mais átomos de carbono), e possuem duas extremidades quimicamente distintas: uma delas possui uma carboxila (COOH, região que se ioniza em solução, extremamente hidrofílico e reativo quimicamente); e a outra uma metila (CH3, região hidrofóbica e sem muita reatividade química) (ALBERTS et al., 2004). Na nomenclatura dos AG, o primeiro número indica o comprimento da cadeia de carbono, o segundo número, após os dois pontos, refere-se ao número de duplas ligações e o terceiro número, depois do n, representa o número de carbonos a partir da metila terminal da molécula para a primeira dupla ligação (WAINWRIGHT, 1992).

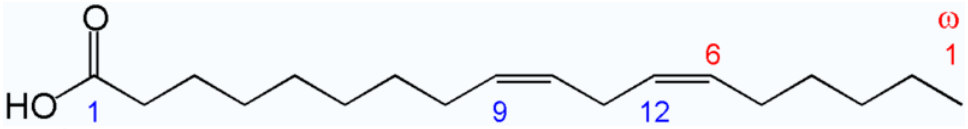
Quanto à extensão da cadeia, eles classificam-se em AG de cadeia curta com cauda alifática de menos de 6 átomos de carbono; de cadeia média, com cauda alifática de 6 a 12 carbonos; de cadeia longa, com cauda alifática de mais de 12 carbonos; e de cadeia muito longa, com cauda alifática contendo mais de 22 átomos de carbono. Quando se trata de ácidos graxos essenciais (AGE) costuma-se usar uma terminologia ligeiramente diferente: os de cadeia curta possuem 18 carbonos e os de cadeia longa têm 20 ou mais átomos de carbono (LEHNINGER; NELSON; COX, 2002).

Os AG também podem ser classificados de acordo com as ligações moleculares presentes (LEHNINGER; NELSON; COX, 2002). Assim, os ácidos graxos podem ser classificados em saturados (AGS, com ausência de dupla ligação), monoinsaturados (AGM, com apenas uma dupla ligação) ou poli-insaturados (AGPI, apresentando mais de uma dupla ligação) (BOTHAM; MAYES, 2007).

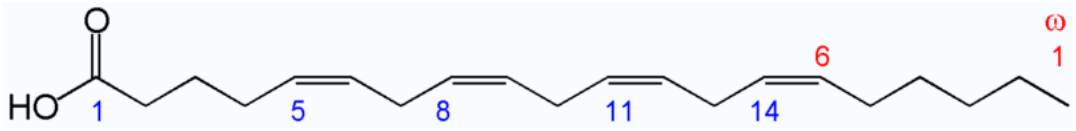
1.2.1 Ácidos graxos essenciais

Os AGPI das séries ômega-3 (n-3) e ômega-6 (n-6) se diferenciam na posição da primeira dupla ligação contando a partir do grupo metílico terminal da cadeia. Os AGPI n-3 e n-6 são considerados ácidos graxos essenciais (AGE) por não serem biossintetizados em mamíferos, suas enzimas não podem inserir duplas ligações em nenhuma posição mais próxima ao grupo metila terminal, e serem necessários para compor as membranas fosfolipídicas neurais (SOLFRIZZI et al., 2005; YEHUDA et al., 2005; HONSTRA, 2001), portanto, devem ser obtidos a partir da dieta (HEIRD; LAPILLONNE, 2005).

O ácido linoléico (LA, 18:2 n-6) (figura 1), representante da série n-6, está presente em abundância nos óleos vegetais de girassol, milho, soja, algodão entre outros (SANGIOVANNI; CHEW, 2005). Os AG da série n-3 como o ácido α-linolênico (ALA, 18:3 n-3) (figura 2) são encontrado em quantidades apreciáveis nas nozes e sementes oleaginosas como a linhaça, a canola e a chia (DZIEZAK, 1989; HULBERT et al., 2004). Já os ácidos eicosapentaenóico (EPA, 20:5 n-3) (figura 2) e docosahexaenóico (DHA, 22:6 n-3) (figura 2), os quais são considerados AG poliinsaturados de cadeia longa (AGPI-CL), são encontrados em algas, microalgas, fitoplâncton e peixes (salmão, sardinha, truta...) de águas salgadas frias e profundas (WAINWRIGHT, 1992; HULBERT et al., 2005).

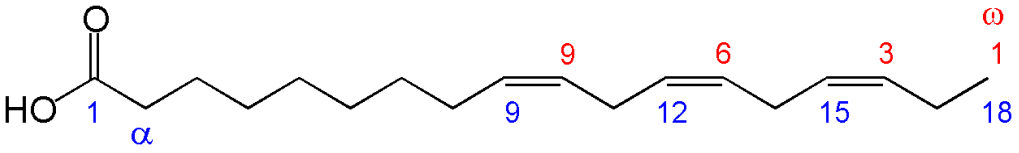


Ácido Linoléico (LA) (18:2 n-6)

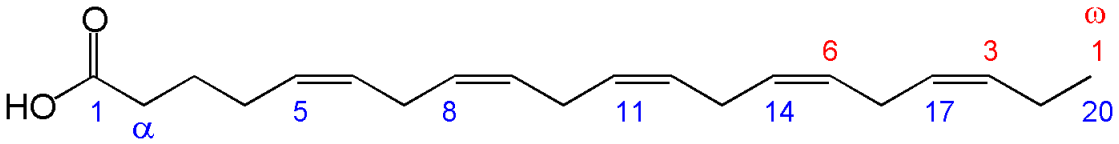


Ácido Araquidônico (AA) (20:4 n-6)

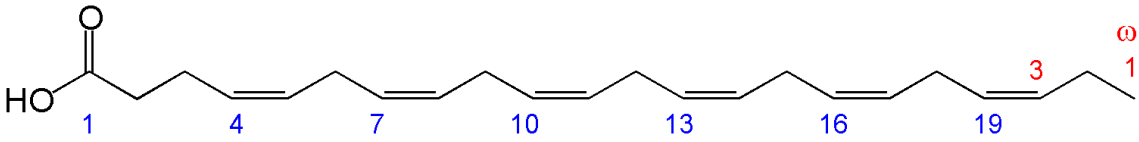
Figura 1. Ácidos Graxos Essenciais Poliinsaturados Ômega 6 (n-6).



Ácido alfa-linolênico (ALA) (18:3 n-3)



Ácido eicosapentaenóico (EPA) (20:5 n-3)



Ácido docosahexaenóico (DHA) (22:6 n-3)

Figura 2. Ácidos Graxos Essenciais Poliinsaturados Ômega 3 (n-3).

Os ácidos linoléico e α-linolênico, quando consumidos, podem ser alongados e dessaturados em AGPI-CL (Figura 3). O LA pode ser metabolizado em outros AG da série n-6, incluindo o ácido araquidônico (AA, 20:4 n-6). O ALA é metabolizado em outros AG n-3, entre eles o EPA e o DHA, tendo sido demonstrado que os níveis teciduais de EPA podem ser aumentados com a maior ingestão de alimentos ricos em ALA, porém os níveis de DHA não são alterados (MANTZIORIS et al., 2000). Este processo metabólico é mediado por enzimas presentes no fígado e astrócitos do sistema nervoso central (SNC) conhecidas como elongases (adição de duas unidades de carbono) e dessaturases (adição de dupla ligação), resultando em uma competição metabólica entre os dois grupos (SALEM, 1999). Como resultado dessa competição, EMKEN et al. (1994) mostraram que a conversão de ALA em metabólitos AGPI-CL pelas dessaturases, é reduzido em ≈ 50% quando o consumo de LA é duplicado, considerando o consumo total de energia diária. Neste sentido, um excesso de LA poderá dificultar a transformação de ALA nos derivados EPA e DHA, e vice-versa. O equilíbrio do consumo desses dois sub-tipos de ácidos graxos é necessário para a manutenção adequada de suas diferentes funções fisiológicas (SALEM, 1999).

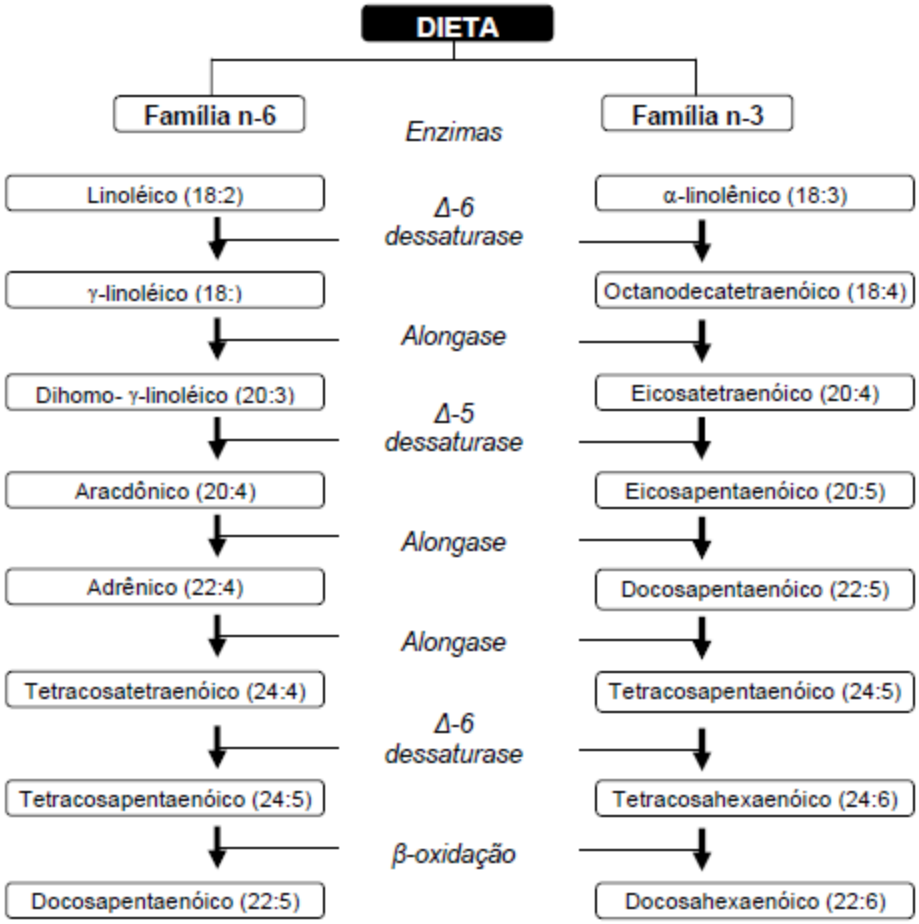


Figura 3. Etapas bioquímicas para síntese dos AGPI-Cl das séries n-3 e n-6.
Fonte: LAURITZEN et al., (2001).

1.2.1.1 Ácidos graxos essenciais e funções cerebrais

O cérebro necessita de um aporte adequado de ácidos graxos para manter sua integridade estrutural e consequentemente suas funções normais, principalmente por apresentar elevado teor de lipídeos de membranas, que corresponde à 50% do seu peso seco, e entre estes, 35% são AGEs (MARTEINSDOTTIR et al., 1998; DAS; VADDADI, 2004; UAUY; DANGOUR, 2006),

Muitas funções fisiológicas são atribuídas aos AGEs. Dentre estas podemos destacar a participação na composição das membranas fosfolipídicas celulares (HÖGYES et al., 2003; HICHAMI et al., 2007; SUMIYOSHI et al., 2008), a modulação da expressão gênica

(HORROCKS; FAROOQUI, 2004; MAZZA et al., 2007), o envolvimento nos processos de transdução de sinais (FARKAS et al., 2002; McNAMARA e CARLSON, 2006), a geração de segundos mensageiros e a regulação de canais iônicos e de receptores (FAROOQUI; HORROCKS; FAROOQUI, 2000). Além disso, os AGEs podem modular a atividade da proteína-kinase C, a síntese de radicais livres e óxido nítrico (YEHUDA, 2003; DAS, 2003); agregação plaquetária (CLARKE et al., 2005) e a geração de eicosanóides (CLARKE et al., 2005; MAZZA et al., 2007).

Sabe-se que o AA é essencial para o crescimento normal, devido sua função na sinalização e divisão celular, e também por desempenhar um importante papel imunológico sendo precursor de mediadores inflamatórios como eicosanóides. Os eicosanoides são formados quando o AA é retirado das membranas pela ação da enzima fosfolipase A_2 (PLA2), sendo então metabolizado pelas enzimas ciclooxigenase (COX) e a lipoxigenase (LOX) (HEIRD; LAPILLONNE, 2005) dando origem a prostaglandinas (PG) e tromboxanos (TX) da série 2 e leucotrienos da série 3, os quais além de potentes agentes pró-inflamatórios também desempenham funções na transmissão sináptica (ELIAS; INIS, 2001).

As prostaglandinas e tromboxanos da série 2 e leucotrienos da série 4 formados a partir do EPA (AGPI-CL n-3) são estruturalmente semelhantes aos conhecidos eicosanóides derivados do AA, mas com uma fraca ação inflamatória e de agregação plaquetária (LAYÉ, 2010; MARSZALEK; LODISH, 2005; SEE LEE et al., 1984). Isto contrasta com a biossíntese e as ações dos membros das famílias de resolvinas e protectinas que apresentam ações biológicas potentes, já demonstradas *in vitro* e *in vivo*. Os AGPI-CL n-3 são mediadores de pró-resolvinas que incluem: resolvinas da série E sintetizadas a partir do EPA; resolvinas da série D sintetizadas a partir do DHA e as neuroprotectinas / protectinas sintetizadas do DHA. LUKIW et al. (2005) mostraram que o derivado endógeno do DHA (neuroprotectina D1- NPD1) exerce atividade regulatória, neuroprotetora e antiinflamatória neuronal, além de estar envolvido na programação dos genes de expressão e atividade anti-apoptótica, promovendo a sobrevivência dos neurônios que estão sob estresse oxidativo.

Diferentes mecanismos têm sido propostos para explicar os efeitos protetores dos AGPI n-3 em condições oxidativas. Alguns estudos têm enfatizado a propriedade antioxidante dos AGPI n-3 (OKUYAMA; ORIKASA; NISHIDA, 2008; TARDIVEL et al., 2009; SAW et al., 2010) sobre as EROs. Da mesma forma, pesquisas com animais de laboratório demonstraram que o DHA e EPA são antioxidantes nutricionais indiretos e reduzem peróxidos de lipídios no cérebro (BARCELOS et al., 2010; CHOI-KWON et al., 2004; HOSSAIN et al., 1999) e no fígado de ratos (YILMAZ et al., 2004). A atividade

neuroprotetora do DHA também foi evidenciada através das propriedades antioxidantes indiretas *in vivo* (BAZAN, 2005; CALON et al., 2004; HASHIMOTO et al., 2002; WU; YING; GOMEZ-PINILLA, 2004; YAVIN et al., 2002), através do aumento da atividade da glutationa redutase (HASHIMOTO, 2002), diminuição da oxidação de proteínas (TREVIZOL et al., 2011; CALON et al., 2004; WU; YING; GOMEZ-PINILLA, 2004) e dos níveis de peróxidos de lipídios e EROs (HASHIMOTO et al., 2002; 2006).

Estudos demonstraram também que o DHA participa diretamente da modulação da expressão gênica, em processos que envolvem estresse oxidativo, sinalização e divisão celular, crescimento e apoptose (SIMOPOULOS, 2006; YAVIN, 2006). A presença do DHA na membrana celular aumenta a sua fluidez, e por sua vez, modifica a mobilidade das proteínas e a atividade das enzimas cerebrais e retinianas, fundamentais para a transdução dos sinais nervosos (plasticidade sináptica) (MITCHELL et al., 2003; LAURITZEN et al., 2001; MURPHY, 1990). DHA e EPA podem ainda influenciar as funções cerebrais por afetar a produção e a função de neurotransmissores como a serotonina e a dopamina (DU BOIS et al., 2006; FENTON et al., 2000) e inibir a fosfolipase A_2 (BENNET; HORROBIN, 2000).

O conteúdo de DHA em muitas regiões cerebrais é 30 a 50% maior que o AA, exercendo um papel crítico no funcionamento do sistema nervoso central (particularmente membrana sináptica) e retina. Estudos realizados em recém-nascidos primatas (não humanos) e humanos indicam que o DHA é essencial para o desenvolvimento e o funcionamento normal da retina e do cérebro (BIRCH et al., 2002; 2002; CHAMPUX et al., 2002; SANGIOVANNI et al., 2000; NEURINGER, 2000). Acredita-se que deficiências de DHA durante desenvolvimento pré e pós-natal afetam a acuidade visual, as funções cognitivas e, possivelmente, o comportamento e a suscetibilidade às desordens psiquiátricas (FUGH-BERMAN; COTT, 1999).

1.2.1.2 Doenças associadas à carência de AGEs

Como já mencionado, AGEs exercem importantes funções celulares, principalmente em nível cerebral, desde a infância até o envelhecimento, estando envolvidos no desenvolvimento de muitas doenças. Alguns sintomas da deficiência de AGEs incluem fadiga, problemas dermatológicos, deficiência imune, desordens gastrointestinais, problemas cardíacos e circulatórios, retardo no crescimento e esterilidade (MARSZALEK; LODISH,

2005; YEHUDA; MOSTOFSKY, 1997, TAPIERO et al., 2002). Deficiências de PUFAs parecem estar implicadas também no desenvolvimento ou agravamento da artrite reumatóide, asma, câncer de mama e de próstata (SHAPIRO, 2003).

Com ênfase nas funções do SNC, tem sido evidenciado que a carência de n-3 está associada à depressão (FERRAZ et al., 2008), desordens de hiperatividade (BURGESS et al., 2000), processo de envelhecimento, aprendizado e memória (BOURRE, 2004). Ainda existem poucos estudos sobre os efeitos da deficiência de AG n-3 a longo prazo, mas sugere-se uma contribuição para o desenvolvimento de hipertensão arterial (ARMITAGE et al., 2003), doença de Huntington (CLIFFORD et al., 2002), esquizofrenia (DAS, 2004) e transtorno bipolar (STOLL et al., 1999; PARKER et al., 2006). Por outro lado, a obtenção de ômega 3 a partir da dieta pode agir sobre parâmetro oxidante/antioxidante em regiões dopaminérgicas cerebrais (SARSILMAZ et al., 2003) e o hipotálamo (SONGUR et al., 2004), sugerindo proteção em doenças neurológicas, motoras, bem como nas desordens neuropsiquiátricas (BLACK et al., 1984).

1.2.2 Ácidos graxos *trans*

Nos reinos animal e vegetal, os AG geralmente possuem insaturações na forma *cis*, que ocorrem quando os hidrogênios ligados aos carbonos da dupla ligação encontram-se no mesmo lado da cadeia carbônica (CHRISTIE, 2003; MARTIN et al.,2007). Na forma *trans*, estes átomos de hidrogênio localizam-se em lados opostos da cadeia carbônica, formando uma molécula linear, semelhante à de um ácido graxo saturado (AGS) (figura 4). A conformação linear é o estado de menor energia e permite um melhor empacotamento das moléculas, ficando mais próximas umas das outras e aumentando a interação entre elas. A consequência disso é o aumento do ponto de fusão dos AGS e AGT, em relação aos ácidos graxos insaturados *cis* (CURI, 2002).

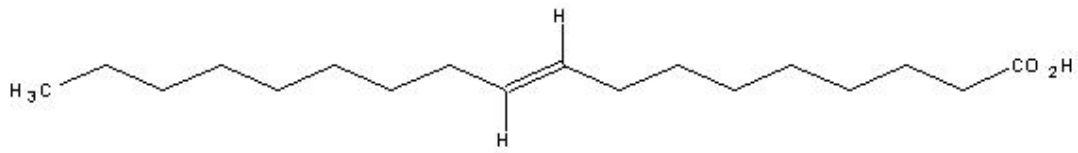


Figura 4. Estrutura do ácido elaídico (C18:1 9t), representante de AGT.

Os ácidos graxos trans (AGT) podem ser produzidos industrialmente, mas também ocorrem naturalmente. Industrialmente, eles são formados durante a hidrogenação parcial de óleos vegetais, quando um hidrogênio é adicionado à dupla ligação carbono-carbono dos AG na presença de catalisadores, o que converte óleos vegetais líquidos em gorduras semi-sólidas (MOZAFFARIAN et al., 2006). Naturalmente são encontrados– em pequenas quantidades (menos de 5% do total de AG) no leite e seus derivados, carne e gordura de mamíferos ruminantes, resultantes da biohidrogenação de ácidos graxos polinsaturados pela bactéria anaeróbia *Butyrivibrio fibrisolvents* (PADOVESE; MANCINI FILHO, 2002; JAKOBSEN et al., 2006).

O processo de hidrogenação parcial concede algumas propriedades físicas das gorduras saturadas aos óleos vegetais, tais como consistência mais firme, maior ponto de fusão e estado físico semi-sólido à temperatura ambiente. Tais mudanças aumentam a estabilidade oxidativa destes óleos, conferindo maior resistência frente à deteriorização dos sabores e aromas. A presença destes óleos nos alimentos também melhora a estabilidade das gorduras durante a fritura, prolongando a vida de prateleira dos alimentos (CURI, 2002; TARRAGO-TRANI et al., 2006; ECKEL et al., 2007).

Embora o consumo destes isômeros possa ocorrer pela ingestão de produtos lácteos ou que contenham gordura e carne de origem bovina e/ou caprina, foi com a maior variedade de produtos à base de gordura parcialmente hidrogenada no mercado que então, se verificou um aumento no consumo de AGT, já que a presença desses AG nesses produtos pode chegar a 60% (PADOVESE; MANCINI FILHO, 2002; BAYARD, 1995). Os AGT estão presentes em quantidades variadas nos alimentos processados com óleos parcialmente hidrogenados, como recheios de biscoitos, formulações de bases para sopas e cremes, sorvetes, batata frita, salgadinhos de pacotes, pipoca para microondas, margarinas, empanados de frango, pães, lanches de preparação rápida (“fast-foods”), bolos, tortas industrializadas e assados (SEMMA, 2002).

Apesar de sua utilidade tecnológica, os efeitos do consumo desses ácidos graxos nos

alimentos têm sido objeto de grande controvérsia no que diz respeito aos aspectos do seu metabolismo, absorção, acúmulo no organismo e os seus efeitos nas funções enzimáticas, formação de prostaglandinas, transporte e deposição de colesterol nas artérias, doenças cardíacas e câncer. (MANCINI; CHEMIN, 1996).

1.2.2.1 Os ácidos graxos t*rans* e funções celulares

Os AGT da dieta são absorvidos e transportados até as células, onde são utilizados como fonte de energia ou depositados nos tecidos para utilização futura (CURI, 2002). Nos tecidos humanos, a absorção, o transporte, a incorporação e a excreção dos AGT ocorrem de forma similar a outros AG da dieta (IOM, 2002), competindo inclusive pelos mesmos sistemas enzimáticos envolvidos na síntese de AGPI-CL (MAHFOUZ; KUMMEROW, 1999) Aparentemente, as concentrações de AGT incorporados aos tecidos refletem seu consumo. Estudos relacionados a incorporação dos AGT em tecidos de ratos, mostraram que os teores de isômeros *trans* adicionados nas dietas foram suficientemente incorporados e metabolizados, alterando o perfil de AG nos tecidos desses animais (LOÏ et al., 2000; SABARENSE; MANCINI-FILHO, 2003; TEIXEIRA et al., 2011; 2012).

Os AGT quando incorporados aos fosfolipídios de membranas celulares são capazes de modular a função celular, alterando a fluidez de membrana (menos fluída) e consequentemente as respostas dos receptores de membrana (GURR; HARWOOD, 1991; ROACH et al., 2004). Em uma revisão sobre possíveis mecanismos moleculares dos AGT, foi sugerido que os AGT poderiam afetar as funções e repostas celulares devido à sua capacidade de ligação e modulação dos receptores nucleares, os quais regulam a transcrição de genes (MOZAFFARIAN et al., 2006).

A relação entre os AGT e os mecanismos pró-inflamatórios e pró-apoptóticos não estão bem estabelecidos. Teoricamente, os AGT podem modular as respostas de macrófagos e monócitos em humanos, aumentando a produção do fator de necrose tumoral (TNF) e interleucina-6, bem como os níveis de proteína quimio-tática de monócito 1 (indicadores de inflamação). Os AGT também afetam a função vascular, por aumentar os níveis circulantes de biomarcadores da disfunção endotelial, bem como influenciar o metabolismo de AG nos adipócitos (MOZAFFARIAN et al., 2006). Estudos tem associado positivamente a alta

ingestão de AGT com o aumento da atividade do sistema TNF e o aumento de interleucina-6 e proteína C reativa. A alta ingestão de AGT parece estar relacionada com maiores níveis de marcadores da disfunção endotelial (E-selectina), sugerindo que uma alta ingestão de AGT pode afetar adversamente a função celular endotelial (LOPEZ-GARCIA e cols, 2005). Além disso, foi observado que os AGT podem inibir a reação de dessaturação e enlongação dos LA e ALA para AA, DHA e EPA, e também podem se tornar substrato alternativo das dessaturases resultando na formação de eicosanoides sem atividade biológica (INNIS; GREEN; HALSEY, 1999; INNIS, 2006). Todavia, esse último efeito dos AGT ocorrerá se as concentrações de AGE estiverem baixas, ou seja, ingestões inadequadas dos AGE permitem maior incorporação dos isômeros *trans*.

1.2.2.2 Ácidos graxos *trans* e o desenvolvimento de doenças

Como já citado, evidências recentes indicam que os AGT promovem inflamação. A inflamação é um fator de risco para aterosclerose, morte cardíaca súbita e diabetes. Estudos sugerem que seus efeitos pró-inflamatórios podem causar disfunção endotelial (MAZAFFARIAN et al., 2006), e essa tem papel importante no desenvolvimento e progressão da aterosclerose e outras doenças cardiovasculares (LOPEZ-GARCIA et al., 2005; ZAPOLSKA-DOWNAR; KOSMIDER; NARUSZEWICZ, 2006). Nesse contexto, dados da literatura mostraram um alto consumo de AGT aumenta os riscos de doenças cardiovasculares, aumenta as concentrações plasmáticas de LDL e triglicerídeos e reduz significativamente a concentrações do HDL (MOZZAFARIAN; CLARK, 2009; SUN et al., 2007; CÓLON-RAMOS et al., 2006; WILLETT, 2006; MENSINK et al., 2003). Além disso, estudos mostram uma associação positiva entre a ingestão de AGT e os riscos para diabetes em adultos e ratos de mães que consumiram essa gordura durante a gestação e lactação (OSSO et al., 2008; PISANI et al., 2008; ALBUQUERQUE et al. 2006; SALMERÓN et al., 2001; HU et al., 2001). Embora seja sugerido que os AGT aumentem o risco para o câncer, seus efeitos na etiologia desta patologia ainda são contraditórios (STENDER; DYERBERG; 2004; KOHLMEIER et al., 1997).

Um estudo realizado por ACAR et al. (2003), mostrou que uma pequena incorporação de AGT no cérebro foi capaz de causar uma forte modificação nos níveis endógenos de dopamina. A alteração na composição dos fosfolipídios de membrana neuronal também está

associada com a fisiopatologia de doenças neurológicas e psiquiátricas, incluindo hiperatividade, esquizofrenia, depressão e transtorno bipolar (COTT, 1999). Desta forma, a incorporação dos AGT nos fosfolipídios da membrana neuronal pode estar relacionada com o desenvolvimento de desordens neurológicas. Nesse contexto, um estudo do nosso laboratório realizado em ratos mostrou que a suplementação com AGT no período pós-desmame aumentou a suscetibilidade desses animais a desenvolverem hiperatividade e alterações bioquímicas relacionadas ao modelo animal de mania (TREVIZOL et al., 2011). No entanto, estas relações carecem de mais estudos em termos de mecanismos.

1.3 Fator neurotrófico Derivado do Encéfalo

O fator neurotrófico derivado do encéfalo (BDNF, brain derived neurotrophic factor), é considerado a principal neurotrofina do cérebro, e tem grande expressão no hipocampo, córtex, amígdala e cerebelo (STRAKOWSKI et al., 2005; SHIMIZU et al., 2003), áreas cerebrais relacionadas com o controle do humor, emoção e cognição (QIAN et al, 2007; STRAKOWSKI et al., 2005; ERNFORS et al., 1990). O BDNF exerce diversos efeitos sobre a plasticidade neural e sináptica, induzindo estímulo à maturação, nutrição, crescimento e integridade neuronal (LOU et al, 2008; DECHANT; NEUMANN, 2002).

Após ser transcrito o BDNF é traduzido na forma de seu precursor o pró-BDNF, o pró-BDNF é levado ao retículo endoplasmático sendo então envolto pelo complexo de golgi e empacotado em vesículas secretórias. As vesículas podem ser agrupadas para liberação espontânea ou, mais frequentemente, liberadas frente a um estímulo (CHEN et al, 2006). O pró-BDNF secretado por neurônios e células de Schawnn, é posteriormente convertido em BNDF maduro por proteases extracelulares (tais como plasmina e fator ativador de plasminogênio extracelular) (PANG et al., 2004). O pró-BDNF, além de regular a liberação de BDNF maduro, também tem a capacidade de ligar-se ao receptor p75, acarretando em apoptose celular, e também promovendo a regulação da depressão de longa duração hipocampal (KANDEL, 2001, REICHARDT, 2006). O BDNF tem pouca afinidade pelo receptor p75, porém o receptor tirosina quinase B (TrkB) se liga especificamente ao BDNF maduro e sua ativação está ligada a processos de crescimento e sobrevivência neuronal (LU, B, PANG e WOO, 2005).

A ação do BDNF através de sua ligação aos receptores denominados Trk-B promovem uma cascata de sinalizadores intracelulares e de transcrição em vários sistemas neuroquímicos (MAPK, PI3-K, PLC) (REICHARDT, 2006). A ativação do Trk-B e p75 promove e suprime, respectivamente, o crescimento dendrítico, assim como uma potenciação ou depressão sináptica. Esse balanço entre as ligações BDNF-TrkB e pró-BDNF-p75 são importantes para as alterações das estruturas sinápticas e densidade das espículas dendríticas (LU; PANG; WOO, 2005). Este balanço é fundamental para a chamada plasticidade sináptica.

Os efeitos produzidos pelo BDNF podem variar conforme a fase do desenvolvimento. No início da fase fetal, o BDNF é importante para a formação e maturação dos neurônios em geral. Na fase adulta, tem papel fundamental no processo de consolidação da memória episódica (POST, 2007). O BDNF promove, ainda, a sobrevivência de vários neurônios do SNC incluindo hipocampais e corticais (LINDHOM et al., 1996; GHOSH, CARNAHAN; GREENBERG, 1994), colinérgicos (ALDERSON et al., 1990), dopaminérgicos (HYMAN et al., 1991) e serotoninérgicos (RUMAJOGEE et al., 2004). Essa multiplicidade de ações em diferentes tipos de neurônios tem embasado pesquisas científicas associando as diversas doenças neuropsiquiátricas e alterações do BDNF (REIS et al., 2008; POST, 2007; AYDEMIR et al., 2005).

1.4 Transtorno Bipolar

O transtorno bipolar (TB) é uma doença mental crônica que acomete aproximadamente 2,4% da população mundial (MERIKANGAS et al., 2011). Entretanto, quando são também consideradas formas mais leves deste transtorno (o chamado espectro bipolar), estudos indicam uma prevalência de até 6% na população geral (GAZALLE et al., 2005). A organização mundial da saúde (OMS) considera o TB como uma das dez principais causas de incapacitação do mundo (LOPEZ; MURRAY, 1998), e talvez a mais letal das doenças mentais, com uma taxa de suicídio 30 vezes maior do que a encontrada na população geral para casos não tratados (HILTY; BRADY; HALES, 1999).

O termo bipolar expressa dois polos do humor ou de estados afetivos que se alternam: a depressão e seu oposto, a mania. Seu curso clínico é crônico, usualmente caracterizado por períodos de exacerbação dos sintomas (episódios agudos) intercalados por períodos sub-sindrômicos e períodos de remissão da doença (eutimia).

O diagnóstico de TB baseia-se pela ocorrência de pelo menos um episódio maníaco ou hipomaníaco durante a vida, na qual a presença de um ou mais episódios maníacos ou a alternância de humor com pelo menos um episódio de depressão maior, confere o diagnóstico de TB tipo I, enquanto que a presença de um ou mais episódios de depressão maior e pelo menos um episódio hipomaníaco confere o diagnóstico de TB tipo II (MÜLLER-OERLINGHAUSEN; BERGHÖFER; BAUER, 2002). A presença de um episódio maníaco é definida por uma elevação persistente do humor (humor eufórico ou irritável), acompanhado por pelo menos três dos seguintes sintomas: aumento da autoconfiança e grandiosidade, pressão por falar, diminuição da necessidade do sono, pensamento acelerado ou fuga de ideias, distratibilidade, alteração do comportamento dirigido para atividades prazerosas, frequentemente imprudentes ou perigosas, ou agitação psicomotora (American Psychiatric Association, 2000). Além disso, o episódio deve ser suficientemente severo para causar prejuízo significativo no âmbito familiar, social ou ocupacional, ou ter presença de sintomas psicóticos com necessidade de hospitalização.

As medicações de maior evidência no tratamento de episódios agudos de depressão e mania, e no tratamento de manutenção e/ou prevenção são: lítio, ácido valpróico e carbamazepina, bem como alguns medicamentos de segunda escolha como antipsicóticos típicos (clorpromazina e haloperidol) e atípicos (olanzapina e risperidona) (YATHAM et al., 2005). Entretanto, os índices de recorrência e de resistência aos medicamentos de primeira linha são bastante elevados (POSTE et al., 2003; DENNEHY et al., 2005). Isso se deve, pelo menos em parte, ao pouco conhecimento sobre os mecanismos fisiopatológicos envolvidos na ação destas drogas, o que é essencial para o desenvolvimento de terapias específicas, que devem ser mais efetivas, agir rapidamente e ser mais toleráveis que as terapias existentes.

Estudos neurofuncionais, baseados em ressonância magnética e tomografia por emissão de pósitrons, mostraram uma diminuição significativa no metabolismo do córtex pré-frontal durante a depressão com subsequente aumento em algumas regiões da mesma estrutura durante a fase maníaca (MALHI et al., 2004; STRAKOWSKI; DELBELLO; ADLER, 2005). Além disso, estas ações parecem estar acompanhadas de um aumento no metabolismo da amígdala e do estriado, sugerindo que alterações em regiões dopaminérgicas como o córtex pré-frontal, sistema límbico e gânglios da base podem estar associadas à fisiopatologia do TB.

A fisiopatologia do TB está relacionada à genética, às vias neuro-hormonais, vias de transdução de sinal, regulação da expressão gênica (ZARATE Jr. et al., 2006), atividade diminuída da bomba sódio-potássio ATPase (Na^+K^+ATPase) (EL-MALLAKH; WYATT,

1995; MÜLLER-OERLINGHAUSEN et al., 2002; HERMAN et al., 2007), alterações nas vias de sinalização que regulam a neuroplasticidade e a sobrevivência celular (BDNF, PKC, PKA, MAPK/ERK CREB, , caspase3 e 6...) (EINAT et al., 2003; MANJI et al.., 2003; GOULD et al., 2004; PICCHINI et al., 2004; GRANDE et al., 2010; BERK et al., 2011) e estresse oxidativo (ZARATE Jr. et al., 2006; STECKERT et al., 2010; BERK et al., 2011).

Nos últimos anos estudos tem mostrado que disfunções mitocondriais e, consequentemente a geração de radicais livres (RL), estão envolvidos em distúrbios neuro-psiquiátricos como o TB (KATO; KATO,, 2000; KULOGLU et al., 2002; SUN et al., 2006; ANDREZZA et al., 2006; FREY et al., 2006c; MACHADO–VIEIRA et al., 2007). A disfunção mitocondrial resulta em anormalidades no metabolismo energético celular e aumento da produção de EROs. KATO; KATO (2000) encontrou mutações no DNA mitocondrial em córtex cerebral *post mortem* de pacientes com TB, enquanto outros pesquisadores observaram estes danos no DNA no sangue de pacientes com TB (ANDREZZA et al., 2006). Coerentemente com esses achados, foi observado uma redução dos níveis plasmáticos das enzimas superóxido dismutase (SOD), glutationa peroxidase (GPx) e catalase (CAT) (enzimas antioxidantes), e um aumento dos níveis de espécies reativas ao ácido tiobarbitúrico (TBARS, produto da peroxidação lipídica) em pacientes com TB em diferentes fases da doença (mania, depressão e eutimia) (KULOGLU et al., 2002; OZCAN et al., 2004; RANJEKAR et al., 2003), sendo que o tratamento com lítio foi capaz de reverter esses parâmetros (MACHADO-VIEIRA et al., 2007).

1.4.1 Modelo animal de mania

O TB é uma doença particularmente desafiadora, no sentido de desenvolver um modelo animal adequado. Até o presente, não existe um modelo animal ideal para o estudo desse transtorno (GOULD E EINAT, 2007; EINAT E MANJI, 2006; MACHADO-VIEIRA et al., 2004), uma vez que apresenta padrão crônico e oscilatório (mania, depressão e estados mistos). Consequente tem sido desenvolvido modelos animais que reproduzam determinados aspectos do quadro clínico do TB. Embora existam vários modelos animais de depressão (NESTLER et al., 2002), a maioria dos modelos animais de TB tem focalizado o quadro clínico de mania, o qual possui como dificuldade principal, a reprodução do sintoma central, que é o humor elevado ou euforia. Sendo assim, os modelos animais de mania geralmente

avaliam a atividade locomotora, a agressividade ou comportamento de risco ou recompensa (EINAT et al., 2000; MACHADO-VIEIRA et al., 2004). O modelo de hiperatividade induzido por psicoestimulantes (usualmente anfetamina e cocaína) é considerado o modelo animal de mania mais bem estabelecido e aceito até o momento (MACHADO-VIEIRA et al., 2004; GOULD et al., 2004; NESTLER et al., 2002, FREY et al., 2006 a, b, c, d).

Nesse contexto, estudos com modelo animal de mania induzido por administrações repetidas de ANF (FREY et al., 2006 a,b,c) mostraram também que drogas estabilizadoras do humor são capazes de reverter e/ou prevenir a hiperatividade, bem como a redução das atividades das enzimas antioxidantes (SOD, GPx e CAT), o aumento da peroxidação lipídica e carbonilação de proteínas e a redução dos níveis do BDNF induzidos por esse psicoestimulante (GOULD et al., 2001; FREY et al., 2006d,e).

Já foi demonstrado que a administração de drogas que aumentam os níveis de dopamina no SNC, como anfetamina (ANF) e L-dopa, induzem sintomas maníacos tanto em pacientes com TB, quanto em indivíduos saudáveis (MURPHY et al.,1971; GERNER et al., 1976; JACOBS e SILVERSTONE, 1986). A ANF aumenta significativamente a concentração de dopamina (DA) na fenda sináptica, principalmente por facilitar a liberação de DA das vesículas pré-sinápticas, mas também por bloquear a recaptação de DA a partir dos transportadores neuronais de dopamina (DAT) pré-sinápticos (SULZE et al., 1995). O DAT é o principal responsável pelo término da transmissão dopaminérgica, pois faz a recaptação do DA de volta para o terminal neuronal.. Este aumento de DA, aumentaria também a desaminação oxidativa, catalisada pela enzima monoamino oxidase (MAO) bem como sua auto-oxidação. Este metabolismo leva à respectiva formação de H_2O_2 e dopamino-quinonas, que pode ser convertidos ao radical hidroxil, altamente tóxico, via reação de Fenton. (GRAHAM, 1978; CHIUEH et al., 1992). Estes radicais iniciam processos oxidativos nos lipídeos de membranas celulares (peroxidação lipídica), bem como a oxidação de cadeias de aminoácidos das proteínas e danificam o DNA e outras biomoléculas vitais (FINKEL et al., 2000; VALKO et al., 2004; 2007).

Estudos recentes sugerem que a variação da expressão gênica do transportador de DA pode estar envolvido na suscetibilidade ao desenvolvimento do TB (GREENWOOD et al., 2006). Níveis elevados de DA foram encontrados em pacientes na fase maníaca (JOYCE et al., 1995). Sabe-se também, que drogas que bloqueiam os receptores dopaminérgicos D2 são consideradas agentes de primeira escolha no tratamento da mania aguda (YATHAM et al., 2005). No presente estudo, adotamos a administração repetida de ANF como um modelo animal de mania, para o estudo do TB.

1.5 Mudanças nos Hábitos Alimentares

Pesquisas recentes mostram que alterações no processo de industrialização de alimentos, particularmente nos países desenvolvidos, são umas das principais causas nas mudanças dos hábitos alimentares da população, os quais levaram a uma redução na ingesta de AGPI n-3 e um aumento no consumo de AGPI n-6 e AGT (BORSONELO; GALDURÓZ, 2008).

Estudos nutricionais sugerem que a dieta ocidental está deficiente em AGPI-CL n-3, Em particular, a razão n-6/n-3 aumentou de 2-4:1 no século passado para mais que 20:1 atualmente (TIEMEIER et al., 2003). Não há um consenso a respeito da proporção homeostática celular ideal para que ambos tenham seu melhor aproveitamento pelo organismo, porém alguns autores afirmam que a razão ideal n-6/n-3 seria de 3:1 (DAS, 2002; OLLIS; MEYER; HOWE, 1999), ja outros defendem a razão de 6:1 (MARSZALEK; LODISH, 2005; WIJENDRAN; HAYES, 2004).

A ingestão de gorduras aumentou mais de 60% durante os últimos 35 anos (WELLS; BUZBY, 2008) e representa, atualmente, entre 1.7 a 8% do total de ingestão mundial de lipídios (OSSO et al., 2008). Segundo LARQUÉ; ZAMORA; GIL (2001), os alimentos contendo gordura parcialmente hidrogenada contribuem com cerca de 80% a 90% da ingestão diária de AGT. Para os alimentos provenientes dos animais ruminantes, esta contribuição é bem menor, em torno de 2 a 8% (STENDER; ASTRUP; DYERBERG, 2008), já os óleos refinados apresentam em torno de 1% a 1,5% de AGT, mas quando reutilizado para frituras estes valores podem contribuir significativamente para ingestão diária desse AG.

Considerando o alto consumo de alimentos industrializados nos países ocidentais, onde também se observa uma perda de AGEs devido à destruição do LA e ALA durante a hidrogenação parcial dos óleos vegetais e durante o cozimento e o processamento dos alimentos (DAS, 2006), existe uma real diminuição dos teores de ácidos graxos essenciais como LA e principalmente de ALA na dieta (INNIS, 2006). Vários estudos epidemiológicos têm reportado baixas concentrações plasmáticas de DHA e EPA em indivíduos com esquizofrenia, déficits de atenção, dislexia, desordens de personalidade, depressão e transtorno bipolar (PEET; STOKES, 2005; RICHARDSON, 2006; LIN; HUANG; SU, 2010). O declínio da ingesta de AG n-3 nas últimas décadas pode estar relacionado com o aumento da prevalência dessas desordens neuropsiquiátricas (HAAG, 2003).

Sabendo que a dieta materna influencia o perfil lipídico tecidual da prole, e que estudos mostram que durante a gestação os AGT são transferidos para o feto e também são secretados no leite, demonstrando uma exposição do feto aos AGT presentes na dieta materna (MOORE; DHOPESHWARKAR, 1980), torna-se importante investigar a influência da suplementação materna com GVH, rica em AGT, em comparação com AG n-3 e n-6 sobre o desenvolvimento de desordens neuropsiquiátricas em proles de primeira e segunda geração.

2. OBJETIVOS

2.1 Objetivo geral

Verificar a influência da suplementação de diferentes gorduras ricas em ácidos graxos n-6, n-3 e *trans* sobre alterações comportamentais, bioquímicas e moleculares em animais de 1ª e 2ª gerações, submetidos à um modelo animal de mania.

2.2 Objetivos Específicos

- Quantificar o teor qualitativo e quantitativo dos ácidos graxos (AG) contidos nos diferentes óleos e gorduras incluídos nos protocolos experimentais;
- Quantificar o teor relativo de incorporação de diferentes AG no córtex, hipocampo e estriado, e observer a influência dos diferentes ácidos graxos sobre parâmetros de comportamento e status oxidativo dos animais adultos de 1ª geração relacionados ao modelo animal de mania induzidos pela anfetamina;;
- Quantificar o teor relativo de incorporação de diferentes ácidos graxos, correlacionar possíveis alterações no conteúdo de AG cerebrais e memória de reconhecimento e avaliar o status oxidativo e marcadores moleculares em hipocampo dos animais adultos de 2ª geração após indução do modelo animal de mania;
- Quantificar o teor relativo de incorporação de diferentes ácidos graxos, avaliar o status oxidativo e marcadores no córtex e comportamento de hiperatividade dos animais adultos de 2ª geração após indução do modelo animal de mania;
- Avaliar a possível influência da suplementação de GVH em animais de 1ª e 2ª geração sobre os efeitos farmacológicos do carbonato de lítio.

3. PRODUÇÃO CIENTÍFICA

Os resultados inseridos nesta tese apresentam-se sob a forma de artigo científico e manuscritos 1, 2 e 3, os quais se encontram aqui estruturados. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se no próprio artigo e nos manuscritos, os quais estão dispostos da mesma forma que foram publicado (1) e submetidos (1, 2 e 3).

3.1 Artigo 1

INFLUENCE OF LIFELONG DIETARY FATS ON THE BRAIN FATTY ACIDS AND AMPHETAMINE INDUCED BEHAVIORAL RESPONSES IN ADULT RAT

F. Trevizol, K. Roversi, V.T. Dias, Kr. Roversi, C.S. Pase, R.C.S. Barcelos, D.M. Benvengnú, F.T. Kuhn, G.S. Dolci, D.H. Ross, J.C. Veit, J. Piccolo, T. Emanuelli, M.E. Bürger

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3.2 Manuscrito 1

CROSS-GENERATIONAL *TRANS* FAT INTAKE MODIFIES BDNF mRNA IN THE HIPPOCAMPUS: IMPACT ON MEMORY LOSS IN A MANIA ANIMAL MODEL

Fabíola Trevizol, Verônica T Dias, Katiane Roversi, Raquel CS Barcelos, Fabio T Kuhn, Karine Roversi, Camila S Pase, Ronaldo Golombieski, Juliana C Veit, Jaqueline Piccolo, Tatiana Emanuelli, João BT Rocha, Marilise E Bürger

Periódico: **Hipocamppus**

Status: **em fase de revisão**

Cross-generational *trans* fat intake modifies BDNF mRNA in the hippocampus: Impact on memory loss in a mania animal model

Running-title: Trans-fat intake impairs memory and BDNF mRNA in hippocampus

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Abstract

Recently, we have described the influence of dietary fatty acids (FA) on mania-like behavior of first generation animals. Here, two sequential generations of female rats were supplemented with soybean oil (SO, rich in n-6 FA, control group), fish oil (FO, rich in n-3 FA) and hydrogenated vegetable fat (HVF, rich in trans FA) from pregnancy and during lactation. In adulthood, half of each group was exposed to an amphetamine (AMPH)-induced mania animal model for behavioral, biochemical and molecular assessments. FO supplementation was associated with lower reactive species (RS) generation and protein carbonyl (PC) levels and increased dopamine transporter (DAT) levels, while HVF increased RS and PC levels, thus decreasing catalase (CAT) activity and DAT levels in hippocampus after AMPH treatment. AMPH impaired short- (1h) and long- (24h) term memory in the HVF group. AMPH exposure was able to reduce hippocampal BDNF- mRNA expression, which was increased in FO. While HVF was related to higher *trans* FA (TFA) incorporation in hippocampus, FO was associated with increased percentage of n-3 polyunsaturated FA (PUFA) together with lower n-6/n-3 PUFA ratio. Interestingly, our data showed a positive correlation between brain-derived neurotrophic factor (BDNF) mRNA and short- and long-term memory (r^2=0.53; *P*=0.000/ r^2=0.32; *P*=0.011, respectively), as well as a negative correlation between PC and DAT levels (r^2=0.23; *P*=0.015). Our findings confirm that provision of n-3 or TFA during development over two generations is able to change the neuronal membrane lipid composition, protecting or impairing the hippocampus, respectively, thus affecting neurothrophic factor expression such as BDNF mRNA. In this context, chronic consumption of *trans* fats over two generations can facilitate the development of mania-like behavior, so leading to memory impairment and emotionality, which are related to neuropsychiatric conditions.

Key-words: Neural membrane phospholipid; omega-3; oxidative damage; Dopamine transporter.

Introduction

Changes in dietary habits for the past 35 years have increased the consumption of processed foods, rich in *trans* fat, by 60% (Wells and Buzby, 2008). Additionally, animal research has shown that an inadequate maternal nutrition can change both morphological and physiological parameters of pups, because maternal intake of fatty acids (FA) determines the transfer of essential and non-essential fatty acids (EFA) through the placenta and milk (Innis, 2007). From a functional point of view, EFA play a key role in fetal growth, whose deficit can compromise the development of the central nervous system (CNS) (Herrera, 2002).

The term "essential" means that these FA cannot be naturally synthesized in mammals, and therefore they must be supplied from diet. Thus, EFA are polyunsaturated fatty acids (PUFA) identified in two families: omega-6 (n-6 FA) and omega-3 (n-3 FA), whose source are vegetable oils in the form of linoleic acid (LA; 18:2 n-6) and cold-water fish and oil seeds in the alpha-linolenic acid (ALA; 18:3 n-3) form, respectively. After absorption, both LA and ALA are desaturated and elongated in long chain polyunsaturated fatty acid (LC-PUFA) such as arachidonic acid (AA; 20:4 n-6), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) respectively, which are incorporated into membrane phospholipids. LC-PUFA are fundamental for the neuronal membrane because they modulate the brain's physiological functions (Haag, 2003). Furthermore, while EPA and DHA are precursors of prostaglandins of series 3 (PG-3) of reduced pro-inflammatory activity, AA originates prostaglandins of series 2 (PG-2), which are recognized for generating reactive species and pro-inflammatory cytokines (Calder, 2006). On the other hand, chronic intake of processed foods, which is rich in TFA, allows also incorporation of these FA into neuronal membranes, thus reducing the fluidity of the neuronal membranes and altering its biochemical properties and the functionality of trans-membrane proteins (Grandgirard et al., 1998; Morgado et al., 1998; Larqué et al., 2003). In fact, increased consumption of *trans* fat rather than EFA may cause an imbalance between n-6 and n-3 PUFA (Simopoulos, 2006), mainly because n-6 FA and TFA are able to compete with n-3 FA for the activity of both desaturases and elongases. These metabolic pathways favor the generation of AA and *trans* isomers rather than EPA and DHA (Chardigny et al, 1996). Furthermore, TFA derivatives have been described as inhibiting the activity of δ-6 and δ-5 desaturases (Phivilay et al., 2009), whose consequences in the CNS are still poorly known.

Particularly important, some studies have shown a relationship between consumption of processed foods and an increased susceptibility to develop neuropsychiatric diseases, such as

hyperactivity, autism, schizophrenia and bipolar disorder (BD) (Hamazaki K et al., 2009; Trevizol et al., 2011; 2013). Bipolar disorder (BD) is a severe mood disorder of great morbidity and high likelihood of recurrence (McIntyre et al., 2007), whose pathophysiology merits further studies. The pathophysiology of BD has been related to changes in energy metabolism (Kato and Kato, 2000), oxidative stress development (Steckert et al., 2010) and alterations in brain neurotransmission pathways and plasticity (Machado-Vieira et al., 2007). In addition, cognitive impairments and functional decline has been described in BD patients (Goldstein et al., 2009), indicating that BD may be also related to memory impairments besides depression and mania (Millan et al., 2012).

A number of studies have reported a reduction of brain-derived neurotrophic factor (BDNF) blood levels in BD patients during mania (Machado-Vieira et al., 2007), depression (Yoshimura et al., 2006) and even in euthymic states. BDNF is strongly expressed in the hippocampus, where it has been associated with learning processes through neurogenesis and neuroplasticity (Duman and Vaidya, 1998).

Recent studies by our group showed that TFA supplementation in pups born from mothers also supplemented with these fats from pregnancy and lactation presented increased oxidative damage in mesocorticolimbic brain areas. Conversely, FO supplementation was able to exert an evident protective role (Trevizol et al., 2011; 2013), reducing as well oxidative damage in different brain areas related to neuropsychiatric diseases. So far, no studies involving cognitive dysfunction and TFA consumption have been published, and we wondered if prolonged *trans* fat consumption could modify memory parameters and BDNF mRNA expression in the hippocampus. In order to find out, we compared parameters of memory, brain oxidative status, BDNF mRNA expression and their interaction with FA incorporation in hippocampus of 2nd generation rats submitted to a mania animal model. These animals were born and grew under the same original supplementation rich in n-6 (Control), n-3 or *trans* FA (TFA) as their mothers and grandmothers.

Experimental Procedures

Animals

All animal procedures were approved by the Ethical Research Committee of the Federal University of Santa Maria. Animals were maintained and used in accordance with the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA). Animals

were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23°C±1) and on a 12 h-light/dark cycle throughout the experimental period. One week before mating, female adult *Wistar* rats (n=8) were supplemented (3g/Kg; p.o.) (Kuhn et al., 2013; Pase et al., 2013) with either soybean oil (SO-C), which was an isocaloric control group, fish oil (FO) or hydrogenated vegetable fat (HVF), and maintained under the same supplementation during pregnancy and lactation. One female pup of each litter was maintained on the same supplementation until adulthood, when they were mated. These dams were kept on the same original supplementation until weaning of the litter of the second generation, when one male pup from each litter (n=8) and the same supplementation was grouped (four per cage) and kept under the same oral treatment until 90 days of age, when they were submitted to an animal model of mania, which is described below. SO and HVF were purchased in a local supermarket and FO was donated by Herbarium® (Curitiba, Brazil).

Animal model of mania

At 90 days of age, one half of each experimental group was submitted to an animal model of mania, while the other half was treated with saline. Rats received a single daily injection of amphetamine/saline (AMPH- 4 mg/kg/ip) for 14 days (Trevizol et al., 2011) and 24h after the last injection animals were submitted to behavioral observations.

Behavioral assessments

Novel object recognition task (NORT)

This paradigm is related to the animals' natural motivation to explore novelties, which is considered an innate instinct they use to recognize their environment (Heldt et al., 2007), where a higher score implies a higher recognition rate, indicating better memory. The NORT was carried out in the same open-field arena 24h after the locomotor status observations. Recognition memory was assessed as previously described (De Lima et al. 2005): the arena floor was covered with sawdust (from bedding material) during the recognition memory training and test trials. On the first day, rats were given one training trial in which they were exposed to two identical objects (A1 and A2, double Lego toys), which were positioned in two adjacent corners and the rats were allowed to freely explore the objects for 5 min (training session). Testing of short-term memory (STM) and long-term memory (LTM) was

performed 1h and 24 h, respectively, after the training session. The rats were allowed to explore the open field for 5 min in the presence of two objects: the familiar object A and a second novel object B or C, which were placed at the same locations as in the training session. All objects presented similar textures, colors, and sizes, but distinctive shapes. Between trials, the objects were cleaned with a 5% alcohol solution; exploration was defined as sniffing or touching the object with the nose. A recognition index calculated for each animal was expressed by the ratio TN/(TF + TN) (TF = time spent exploring the familiar object; TN = time spent exploring the novel object).

Tissue preparations

Following (24h) the last behavioral evaluations, all animals were anesthetized (sodium pentobarbital, 50 mg/kg body weight ip) and euthanized by cervical decapitation. Their brains were removed and cut coronally at the caudal border of the olfactory tubercle to remove the hippocampus (Paxinos and Watson, 2007). The tissue was separated into three parts, one of which was homogenized in 10 volumes (w/v) of 0.1 M Tris-HCl, pH 7.4 and centrifuged at 3500 x g (15 min), and the supernatant used for biochemical assay. Another part of each hippocampus was used to determine the fatty acids profile, and the third part was stored in freezer at -80 for subsequent molecular analysis.

Fatty acids profile in brain tissues

The fat was extracted from brain samples using chloroform and methanol as described by Bligh and Dyer, 1959, and used for determination of the FA profile. To prevent lipid oxidation during and after extraction, 0.02% butyl hydroxy toluene was added to the chloroform used. FA composition was determined by gas chromatography. Fat was saponified in methanolic KOH solution and then esterified in methanolic H_2SO_4 solution (Hartmann eLago, 1973). Methylated fatty acids were analyzed using a gas chromatograph (Agilent Technologies, HP 6890N) equipped with a capillary column DB-23 (60 m x 0.25 mm x 0.25 μm) and flame ionization detector. The temperature of the injector port was set at 280°C and the carrier gas was nitrogen (0.9 mL/min). After injection (1 μL, split ratio 50:1), the oven temperature was held at 160°C for 1 min, then it was increased to 240°C at 4°C/min and held at this temperature for 9 min. Standard FA methyl esters (37-component FAME Mix, C 22:5n3 and PUFA no. 2 from Sigma, Saint Louis, MO, USA and C 22:5n-6 from NuChek Prep. Inc., Elysian, MN, USA) were run under the same conditions and the subsequent

retention times were used to identify the FA. FA were expressed as percentage of the total FA content.

Biochemical assessments

Protein carbonyl determination

The oxidative damage to proteins was assessed in brain tissues by determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) as previously described (Levine et al., 1994). Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH. The quantification of protein carbonyls in the samples was determined in the absorbance of 370 nm.

Reactive Species (RS) generation with DCH (dichlorofluorescein-reactive species, DCH-RS)

RS levels were measured using the oxidant sensing fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCHF-DA) (Hempel et al., 1999). The oxidation (DCHF-DA) to fluorescent dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. An aliquot of 10 µM (DCHF–DA) in ethanol was added to the supernatants, and the fluorescence intensity from DCF was measured for 300 s and expressed as a percentage of the untreated control group. The protein content was normalized by quantification according to Lowry (Lowry et al., 1951).

Catalase (CAT) activity

Catalase activity was spectrophotometrically quantified in tissues by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the presence of the cell homogenate (pH 7.0 at 25ºC) at 240 nm. The enzymatic activity was expressed in µmol H_2O_2/min/g tissue.

Molecular assessments

Quantification of dopamine transporter (DAT) levels by western blot

Hippocampal tissue was homogenized in a lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM PMSF, 10 ug/ml^{-1} aprotinin, 0.1 mM benzethonium chloride, 0.5 mM sodium vanadate. The homogenates were then centrifuged; the supernatants collected and total protein concentration was determined according to

MicroBCA procedure (Pierce, IL, USA), using bovine serum albumin as standard. Levels of DAT and actin were analyzed by western blot. Briefly, protein samples were separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to a PVDF membrane (Millipore, MA, USA). Non-specific binding sites were blocked in Tris-buffered saline (TBS), overnight at 4 °C, with 2% BSA and 0.1% Tween-20. Membranes were rinsed in buffer (0.05% Tween-20 in TBS) and then incubated with primary antibodies: anti-actin (1:1000; Santa Cruz Biotechnology, CA, USA) and anti-DAT (1:500; Santa Cruz Biotechnology, CA, USA), followed by anti-goat (1:25.000; Santa Cruz Biotechnology, CA, USA) and anti-rabbit (1:8000; Santa Cruz Biotechnology, CA, USA) IgG horseradish peroxidase conjugate, respectively. After rinsing with buffer, the immunocomplexes were visualized using the 3,3',5,5' – Tetramethylbenzidine (TMB) (Sigma, USA) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using ImageJ software. Actin was used as an internal control for western blot such that data were standardized according to actin values.

Quantification of gene expression by real-time polymerase chain reaction (RT-PCR)

To quantify expression of BDNF mRNA, a quantitative RT-PCR assay was performed. Total RNA from hippocampus was extracted with Trizol Reagent (Invitrogen) according to the manufacturer´s suggested protocol. The cDNA was synthesized with M-MLV reverse transcriptase enzyme, using total RNA and random primer (Invitrogen) according to the manufacturer´s suggested protocol. For analysis of rat BDNF, we measured total BDNF mRNA using the following sense and antisense primers: sense 5′-GCG GCA GAT AAA AAG ACT GC -3′; and antisense: 5′-GTA GTT CGG CAT TGC GAG TT -3′. For analysis of the housekeeping gene tubulin as an internal standard, the sense primer 5′-CAT GAA CAA CGA CCT CAT CG -3′ and the antisense primer 5′-TGT GGA CAC CAT CAC GTT CT -3′ were used. Quantitative real-time polymerase chain reaction was performed in 20 µL PCR mixtures containing 1x PCR Buffer, 25µM dNTPs, 0.2µM of each primer, 3mM MgCl2, 0.1x SYBR Green I (Molecular Probes) and 0.5U Platinum Taq DNA polymerase (Invitrogen). The thermal cycle was carried out in Illumina (ECO Software Version 4.0) and the following protocol was used: activation of Taq DNA polymerase at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 15s and extension at 72° C for 20s, fluorescence was acquired at the end of each extension phase. SYBR fluorescence was analyzed by ECO software version 4.0 (Applied Illumina), and the

CT value for each sample was calculated and reported using the $2^{-\Delta\Delta CT}$ method. For each well, analyzed in biological quadruplicate, ΔC_T value was obtained by subtracting the Tubulin C_T value from the C_T value of the BDNF gene. The ΔC_T mean value obtained from the control group of each gene was used to calculate the $\Delta\Delta C$t of the respective gene ($2^{-\Delta\Delta Ct}$), according to Bustin et al., 2009.

Statistical Analysis

The hippocampal FA content was analyzed by one-way ANOVA followed by Duncan's test. Behavioral and biochemical evaluations were analyzed by two-way ANOVA [3(SO-C, FO and HVF)] x [2(vehicle/amphetamine)], followed by Duncan's multiple range test, when appropriate. As the expression of hippocampal BDNF mRNA and DAT levels showed no parametric data, they were analyzed by Kruskal–Wallis test followed by two-tailed Mann–Whitney *U*-test. Value of $P<0.05$ was considered as significant in all comparisons made.

Results

Time spent on the novel object recognition test (NORT) evaluated in open field is shown in Figure 1.

Two-way ANOVA of time spent in the NORT after 1h revealed a significant main effect of drug [$F(1,42)=8.02$;$P<0.05$] and a significant supplementation x drug interaction [$F(2,42)=7.01$;$P<0.05$]. Two-way ANOVA of time spent in the NORT after 24h revealed a significant main effect of supplementation and drug [$F(2,42)=11.94$ and $F(1,42)=5.86$;$P<0.05$, respectively] and a significant drug x supplementation interaction [$F(2,42)=4.72$;$P<0.05$].

Post hoc test showed that the different supplementations had no influence on both short- (1h) and long- term (24h) memory. After 1h, AMPH treatment increased time spent in the NORT by FO-supplemented animals, while this behavior was decreased in the HVF group (Fig. 1A). AMPH administration also increased long-term memory, which was observed by time spent in the NORT after 24h, in both SO-C and FO, but not in HVF group, whose recognition index was lower than in those groups (Fig. 1B).

The fatty acids composition quantified in hippocampus is shown in Table 1.

One-way ANOVA followed by Duncan's test showed that, in hippocampus, FO supplementation decreased MUFA by 8.41%, LA by 25.71% and n-6 PUFA by 10.77%, while it increased n-3 PUFA by 27.42% and DHA by 34.01%. Overall, FO supplementation showed a lower n-6/n-3 FA ratio by 29.9% in comparison to SO-C. On the other hand, HVF reduced MUFA by 11.32%, allowing also a TFA incorporation of 0.21% in this brain area, which was not observed in FO and SO-C groups.

The influence of different supplementations on the reactive species (RS) generation, protein carbonyl (PC) levels and catalase (CAT) activity in hippocampus is shown in Figure 2.

Two-way ANOVA of RS generation revealed a significant main effect of supplementation and drug [$F(2,42)=6.62$; $F(1,42)=11.62$; $P<0.05$, respectively]. Two-way ANOVA of PC levels revealed a significant main effect of supplementation and drug [$F(2,42)=11.42$; $F(1,42)=17.84$; $P<0.05$, respectively]. Two-way ANOVA of CAT activity revealed a significant main effect of supplementation [$F(2,42)=9.54$; $P<0.001$].

Duncan's test showed that FO supplementation was associated with lower RS generation when compared to both SO-C and HVF groups, whose values were similar to each other. AMPH treatment increased the generation of this oxidative marker in FO and HVF groups, but no differences were observed between the supplementations after drug administration (Fig. 2A).

Post-hoc test showed that among groups treated or not with amphetamine, FO supplementation was related to lower PC levels in hippocampus when compared to SO-C and HVF groups. In fact, AMPH treatment was able to increase PC levels in the HVF group only (Fig. 2B).

HVF-supplemented animals showed decreased CAT activity in hippocampus per se, while FO and SO-C groups showed similar values to each other. AMPH treatment had no effect on this enzyme, but the HVF group showed decreased CAT activity in relation to the FO group after AMPH treatment (Fig. 2C).

The influence of different supplementations on DAT levels in hippocampus is shown in Figure 3.

Kruskal-Wallis analysis of variance revealed a significant difference ($H= 18.45$, $p=0.024$) in DAT levels in hippocampus. While FO supplementation increased DAT levels *per se* of vehicle-injected animals, AMPH administration did not affect this molecular marker, however DAT levels were higher in FO and lower in HVF than in SO-C.

The influence of different supplementations on the quantitative expression of BDNF mRNA in hippocampus is shown in Figure 4.

Kruskal-Wallis analysis of variance revealed significant differences (H= 16.25, p=0.006) in hippocampal BDNF mRNA.

Although the different supplementations had no effect *per se* on the BDNF mRNA of vehicle-treated groups, AMPH administration was able to change this marker in both FO and HVF groups, whose mRNA expression was increased and reduced, respectively. In fact, comparisons between AMPH-treated groups showed increased BDNF mRNA in FO and decreased BDNF mRNA in HVF as compared to SO-C. In fact, HVF supplementation was able to reduce the expression of this molecular marker in relation to SO-C group.

Additional statistical analyses revealed a significant negative correlation between PC and DAT levels (r^2=0.23, P=0.015; Fig. 5A), as well as positive correlations of hippocampal BDNF mRNA with short (r^2=0.53, P=0.000; Fig. 5B) and long-term memory (r^2=0.31, P=0.011; Fig. 5C).

Discussion

Our current aim was to evaluate the influence of both fish oil (FO) and hydrogenated vegetable fat (HVF) supplementation on memory loss related to a mania animal model, thus assessing oxidative and molecular markers in hippocampus of rats exposed or not to AMPH in adulthood. All supplemented fats were isocaloric, including the control group, soybean oil (SO-C), which is the most widely consumed form of oil in Western societies today. The supplementations were started from pregnancy of the 1st generation of rats and maintained until adulthood of the 2nd generation. This experimental design was adopted due to the importance of the perinatal period for a more direct incorporation of FA in brain membranes (Herrera, 2002), affecting their structure, function and neurotransmission (Fernstrom, 1999). At present, the incidence of neuropsychiatric diseases is a matter of concern, especially in Western countries, where chronic consumption of *trans* fats can be an additional risk factor for the progress of these conditions. In this connection, we have recently demonstrated increased susceptibility of animals born and/ or grown under the influence of *trans* fat in the development of memory impairment, movement disorders and hyperlocomotion related to manic behavior (Teixeira et al., 2011; 2012; Trevizol et al., 2011; 2013), which were associated to a small but significant incorporation of TFA in brain areas. *Trans* fat also

increased preference for psychostimulant drugs (Kuhn et al., 2013), facilitating the development of anxiety-like and fear symptoms in 2nd generation young rats (Pase et al., 2013). Inversely, adequate provision of n-3 FA showed preventive effects on movement disorders (Barcelos et al., 2010; 2011), depression-like behavior (Ferraz et al., 2008), and improvement of memory performance (Trevizol et al., 2013). In fact, n-3 FA are able to contribute for increased fluidity and plasticity of brain neuronal membranes (Hashimoto et al., 2002), being able to reduce risks for neuropsychiatric and/or neurodegenerative diseases (Richardson and Ross, 2000).

In this context, most neuropsychiatric diseases, including BD, are closely related to cognitive impairments (Goldstein et al., 2009), which involve the BDNF-dependent hippocampal cognitive function, whose reduction has been linked to impairments in learning and memory of animals (Cirulli et al., 2004). In fact, BDNF acts as a memory molecule able to increase long-term potentiation (LTP) (Bliss and Collingridge, 1993), serving as a neurochemical substrate for synaptic plasticity and memory formation (Moser et al., 1998). In this study, we observed that 2nd generation rats treated with *trans* fat showed impairments in both short- and long-term memory after AMPH exposure, indicating that the prolonged consumption of these fats can modify cellular signaling in an unpredictable fashion. Furthermore, our study also showed significant TFA incorporation in hippocampus, with higher values than those observed in 1st generation rats also supplemented with TFA (Trevizol et al., 2013). Interestingly, these 1st generation animals did not show the memory impairments observed here, which occurred concomitantly with a decreased expression of BDNF mRNA in the hippocampus, so explaining the decreased cognitive performance. In fact, the positive correlation found for both times of memory performance with BDNF mRNA reinforce the hypothesis proposed in this study.

As in this experimental group we also observed an increase in RS generation accompanied by an increase in PC levels following AMPH treatment, we hypothesized that: i) TFA present in neural membranes, even in small amounts, is a precursor of proinflammatory mediators such as cytokines and interleukines (Okada et al., 2013), favoring RS generation; ii) AMPH increases dopamine (DA) output from neurons, whose autooxidation also favors RS generation; iii) Oxidative processes can affect proteins from membranes, as observed by increased PC levels; iv) Such protein damage seems to have impaired dopamine reuptake, as observed by reduced DAT levels . The reduced reuptake of DA increments DA levels in the synaptic cleft, whose autoxidation generates more pro-oxidative metabolites; v) TFA present in membranes may be interfering with DA signaling as well, exacerbating reactive species

generation, whose levels were not adequately controlled by the antioxidant defense system; vi) TFA in neural membranes may affect the synaptic plasticity (Larqué et al., 2003), modifying neurotransmission; and v) Taken together, these processes may all be damaging the DNA, as observed by reduced levels of BDNF mRNA, impairing memory performance.

On the other hand, the importance of n-3 LC-PUFA supplementation during the perinatal period has pointed to an association between percentage of maternal plasma DHA and cognitive functions of the neonate (Larqué et al., 2012). Consistent with this, changes to DHA-induced neuronal membrane properties have been correlated with learning abilities (Hashimoto et al., 2005). In this study, FO caused the highest hippocampal DHA incorporation together with the best memory performance, confirming recent reports of the neuroprotective role of n-3 PUFA in the same mania animal model (Trevizol et al., 2013). Indeed, FO was associated with lower RS generation *per se*, whose level was increased by AMPH treatment, but such increase was not sufficient to cause damage to proteins, as observed by lower hippocampal PC levels. We hypothesized that this apparent protection to proteins can be due to the presence of n-3 LC-PUFA in neural membranes, which are precursors of prostaglandins of series 3, which are less pro-inflammatory metabolites than are n-6 and TFA derivatives (prostaglandins of series 2 and inflammatory cytokines) (Bagga et al., 2003, Okada et al., 2013), generating lower oxidative damage (Hashimoto et al., 2002, 2005). In addition, n-3 PUFA are also precursors of neuroprotectins and resolvins (Bazan 2007), which have been related to anti-inflammatory properties (Mukherjee et al., 2004). While RS generation has been related to reduced levels of BDNF (Wu et al., 2004), our study showed that FO was able to increase BDNF mRNA expression after AMPH treatment alongside better memory. Thus, FO intake during pregnancy may be preventive of cognitive impairments associated with neuropsychiatric conditions, possibly by reducing hippocampal oxidative damage, thus preserving BDNF expression. The presence of n-3 FA in membranes is also associated with greater neural membrane fluidity, which may affect the density and activity of transmembrane receptors. In addition, our study showed an increased DAT level *per se*, which was not altered by AMPH treatment in the FO supplemented group.

Other studies have suggested that TFA consumption might impair some neurological functions, raising further concerns about the nutritional role of TFA during the perinatal period (Decsi and Boehm, 2013). Our results indicate that *trans* fat supplementation over two generations may cause significant changes in signaling pathways related to such neurothrophic factors as BDNF, whose impairment was observed in both short and long memory function in the mania animal model. However, FO not only improved the cognitive

function, but also increased hippocampal BDNF mRNA, ameliorating the oxidative status as well. We suggest that, taken together, these events may be related to signaling pathways, resulting in improvement of synaptic plasticity and the brain's cognitive abilities. On this basis, recommended dietary guidelines on perinatal TFA intake should be reviewed and widely publicized.

Disclosure

All authors reported no biomedical financial interests or potential conflicts of interest

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References

Aebi H 1984. Catalase in vitro. Methods Enzymol 105:121–126.

Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST 2003. Differential effects of prostaglandina derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proc Natl Acad Sci U S A 100:1751–1756.

Barcelos RCS, Benvegnú DM, Boufleur N, Reckziegel P, Muller LG, Pase C et al.(2010. Effects of ω-3 essential fatty acids (ω-3 EFAs) on motor disorders and memory dysfunction typical neuroleptic-induced: behavioral and biochemical parameter. Neurosci Res 17:228–237.

Barcelos RCS, Benvegnú DM, Boufleur N, Pase C, Teixeira AM, Reckziegel P et al 2011. Short term dietary fish oil supplementation improves motor deficiencies related to reserpine-induced parkinsonism in rats. Lipids 46:143–149.

Bazan NG 2007. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Clin Nutr Metab Care 10:136–41.

Bligh EG, Dyer WJ 1959. A rapid method of total lipid extraction and purification. Can J BiochemPhysiol 37:911–917.

Bliss TV, Collingridge GL 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39.

Bustin SE, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M,et al. 2009. The mike guidelines: *m*inimum *i*nformation for publication of *q*uantitative real-time pcr *e*xperiments. Clin Chem, 55(4):1-12.

Calder PC 2006. Polyunsaturated fatty acids and inflammation. Prost Leukotrienes and Essen Fatty Acids 75:197-202.

Chardigny JM, Sébédio JL, Grandgirard A, Martine L, Berdeaux O, Vatéle JM 1996. Identificationof novel trans isomers of 20:5 n-3 in liver lipids of rats fed heated oil. Lipids 31:165-168.

Cirulli F, Berry A, Chiarotti F, Alleva E 2004. Intra hippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze. Hippocamp 14:802–807.

De Lima MN, Laranja DC, Caldana F, Brombergg E, Roesler R, Schröder N 2005. Reversal of age-related déficits in object recognition memory in rats with l-deprenyl. Exp Gerontol 40:506-511.

Desci T, Boehm G 2013. *trans* isomeric fatty acids are inversely related to the availability of long-vhain PUFAs in the perinatal period. Am J Clin Nutr 98:543S-548S.

Duman RS, Vaidya VA 1998. Molecular and cellular actions of chronic electroconvulsive seizures. J ECT 14:181–93.

Fernstrom JD 1999. Effects of dietary polyunsaturated fatty acids on neuronal function. Lipids 34:161–169.

Ferraz AC, Delattre AM, Almendra RG, Sonagli M, Borges C, Araujo P, Andersen ML, Tufik S, Lima MMS 2011. Chronic Ω-3 Fatty Acids Supplementation Promotes Beneficial Effects on Anxiety, Cognitive and Depressive-like Behaviors in Rats Subjected to a Restraint Stress Protocol. Behav Brain Res 219:116–122.

Goldstein TR, Birmaher B, Axelson D, Goldstein BI, Gill MK, Esposito-Smythers C, et al. 2009. Psychosocial functioning among bipolar youth. Journal of Affective Disorders 114:174-183.

Grandgirard A, Piconneaux A, Sebedio JL, Julliard F 1998. Trans isomers of long-chain polyunsaturated fatty acids in tissue lipid classes of rats fed with heated linseed oil. Reprod Nutr Dev 38:17–29.

Haag M 2003. Essential fatty acids and the brain. Can J Psychiatr 48:195–203.

Hamazaki K, Choi KH, Kim HY 2009. Phospholipid profile in the postmortem hippocampus of patients with schizophrenia and bipolar disorder: no changes in docosahexaenoic acid species. J Psychiatr Res 44(11):688-693.

Hartman L, Lago BC 1973. A rapid preparation of fatty methyl esters from lipids. Lab Pract 22:475–477.

Hashimoto M, Hossain S, Shimada T, Sugioka K, Yamasaki H, Fujii Y et al 2002. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. J Neurochem 81:1084–1091.

Hashimoto M, Tanabe Y, Fujii Y, Kikuta T, Shibata H, Shido O 2005. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid beta-infused rats. J Nutr 135:549–555.

Heldt AS, Stanek L, Chhatwal JP, Ressler KJ 2007. Hippocampus specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. Mol Psychiatry 12:656-670.

Hempel SL, Buettner GRO, Malley YQ, Wessels DA, Flaherty DM 1999. Dihydrofluoresceindiacetate is superior for detecting intracellular oxidants: comparison with 20, 70-dichloro dihydrofluoresceindiacetate, 5 (and 6)- carboxy-20, 70-dichloro dihydrofluoresceindiacetate, and dihydrorhodamine 123. Free Radical Biol Med 27:146–59.

Herrera E 2002. Implication of dietary fatty acids during pregnancy on placental, fetal and postnatal development -a review. Plecenta Suppl A: S9-19.

Innis SM 2007. Fatty acids and early human development. Early Hum Dev 83:761-766.

Kato T, Kato N 2000 Mitochondrial dysfunction in bipolar disorder. Bipolar Disord 2:180–190.

Kuhn FT, Roversi Kr, Antoniazzi CTD, Pase CS, Trevizol F, Barcelos RCS et al 2013. Influence of trans fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats. Pharmacol Biochem and Behavior 110:58–65.

Larqué E, Garcia-Ruiz PA, Perez-Llamas F, Zamora S, Gil A 2003. Dietary *trans* fatty acids alter the compositions of microsomes and mitochondria and the activities of microsome delta-6 fatty acid desaturase and glucose-6-phosphatase in livers of pregnant rats. J Nutr 133: 2526–2531.

Larqué E, Gil-Sa´nchez A, Prieto-Sa´nchez MT, Koletzko B 2012. Omega3 fatty acids, gestation and pregnancy outcomes. Br J Nutr 107(2):S77-84.

Levine RL, Williams JA, Stadtman ER, Shacter E 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951. Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275.

Machado-Vieira R, Dietrich MO, Leke R, Cereser VH, Zanatto V, Kapczinski K et al 2007. Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. Biol Psychiatry 61:142–144.

McIntyre RS, Soczynska JK, Beyer JL, Woldeyohannes HO, Law CW, Miranda A et al 2007. Medical comorbidity in bipolar disorder: reprioritizing unmet needs. Current Opinion in Psychiatry 20:406–416.

Millan MJ, Agid Y, Brune M, Bullmore ET, Carter CS, Clayton NS et al 2012. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. Nat Rev Drug Discov 11(2):141–168.

Morgado N, Galleguillos A, Sanhueza J, Garrido A, Nieto S, Valenzuela A 1998. Effect of the degree of hydrogenation of dietary fish oil on the trans fatty acid content and enzymatic activity of rat hepatic microsomes. Lipids 33:669 –673.

Moser EI, Krobert KA, Moser MB, Morris RG 1998. Impaired spatial learning after saturation of long-term potentiation. Science 281:2038–2042.
Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG 2004. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc Natl Acad Sci USA 101: 8491–8496.

Okada Y, Tsuzuki Y, Sato H, Narimatsu N, Hokari R, Kurihara C, Watanabe C, Tomita K, Komoto S, Kawaguchi A, Nagao S, Miura S 2013. Trans fatty acids exacerbate dextran sodium sulphate-induced colitis by promoting the up-regulation of macrophage-derived proinflammatory cytokines involved in T helper 17 cell polarization. Clinical e Experimental Immunology 174 (3) 459–471.

Pase CS, Roversi Kr, Trevizol F, Roversi K, Kuhn FT, Schuster AJ et al 2013. Influence of perinatal trans fat on behavioral responses and brain oxidative status of adolescent rats acutely exposed to stress. Neurosc 247:242–252

Paxinos G, Watson C. The rat brain in stereotaxic coordinates, sixth ed. 2007, Elsevier, Amsterdam.

Phivilay A, Julien C, Tremblay C, Berthiaume L, Julien P, Giguere Y et al 2009. High dietary consumption of trans fatty acids decreases brain docosahexaenoic acid but does not alter amyloid-b and tau pathologies in the 3xTg- AD model of Alzheimer's disease. Neurosc 159:296–307.

Richardson AJ, Ross MA 2000. Fatty acid metabolism in neurodevelopmental disorder: a new perspective on associations between attention-deficit/hyperactivity disorder, dyslexia, dyspraxia and the autistic spectrum. Prostagl Leukot Essent Fatty
Acids 63:1–9.

Simopulos AP 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. Biomed e Pharmacother 60:502-507.

Steckert AM, Valvassori SS, Moretti M, Dal Pizzol F, Quevedo J 2010. Role of oxidative stress in the pathophysiology of bipolar dosorder. Neurochem Res 35: 1295-1301.

Teixeira AM, Pase CS, Boufleur N, Roversi K, Barcelos RCS, Benvegnú DM, Segat HJ et al 2011. Exercise affects memory acquisition, anxiety-like symptoms and activity of membrane-bound enzyme in brain of rats fed with different dietary fats: impairments of *trans* fat. Neuroscience 195: 80–88.

Teixeira AM, Dias VT, Pase CS, Roversi K, Boufleur N, Barcelos RCS et al 2012. Could dietary trans fatty acids induce movement disorders? Effects of exercise and its influence on Na^+K^+-ATPase and catalase activity in rat striatum. Behaviour Brain Res 226:504–510.

Trevizol F, Benvegnú DM, Barcelos RCS, Boufleur N, Dolci GS, Müller LG et al 2011**.** Comparative study between n-6, trans and n-3 fatty acids on repeated amphetamine exposure: A possible factor for the development of mania. Pharmacol Biochem and Behavior 97**:**560–565.

Trevizol F, Roversi K, Dias VT, Roversi KR, Pase CS, Barcelos RCS et al 2013. Influence of lifelong dietary fats on the brain fatty acids and amphetamine-induced behavioral responses in adult rat. Progress in Neuro-Psychopharmacol e Biological Psych 45:215–222.

Wells HF, Buzby JC. Dietary assessment of major trends in U.S. food consumption (1970-2005). USDA Econ Inform Bull, 2008; 33.

Wu A, Ying Z, Gomez-Pinilla F 2004. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, andcounteract learning disability after traumatic brain injury in rats. J Neuritrauma 21:1457–1467.

Yoshimura R, Nakano Y, Hori H, Ikenouchi A, Ueda N, Nakamura J 2006. Effect of risperidone on plasma catecholamine metabolites and brain-derived neurotrophic factor in patients with bipolar disorders. Hum Psychopharmacol 21:433–438.

Figure captions

Figure 1. Influence of supplementation of different fatty acids on recognition memory. Animals were born of dams (and grandmothers) treated with different fats from gestation/lactation and maintained in the same supplementation until 90 days of age, when they were treated with amphetamine (AMPH-4mg/kg) or vehicle for 14 days. Short-term memory (STM) and long-term memory (LTM) retention tests were performed 1h (A) and 24 h (B) after training, respectively. Data are expressed as mean±S.E.M. Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between the supplementations in the same treatment ($P<0.05$).

Figure 2. Influence of supplementation of different fatty acis on ROS generation (A), protein carbonyl levels (B), and catalase activity (C) in hippocampus of rats born from dams (and grandmothers) treated with different fats from gestation/lactation, and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. Data are expressed as mean±S.E.M. Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between the supplementations in the same treatment; ($P<0.05$). *Indicates significant difference from vehicle in the same supplementation ($P <0.05$).

Figure 3. Influence of supplementation of different fatty acids on DAT levels in hippocampus of rats born of dams (and grandmothers) treated with different fats from gestation/lactation and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. The median is the midline; boxes represent the interval from the first (25%) to the last (75%) quartile and the line the maximum and minimum values. Abbreviations: SO-C: soybean oil; FO: fish oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between supplementations in the same treatment; ($P<0.05$).

Figure 4. Influence of supplementation of different fatty acids on BDNF mRNA levels in hippocampus of rats born of dams (and grandmothers) treated with different fats from gestation/lactation and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. The median is the midline; boxes represent the interval from the first (25%) to the last (75%) quartile and the line the

maximum and minimum values. Abbreviations: SO-C: soybean oil; FO: fish oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between supplementations in the same treatment; ($P<0.05$). *Indicates significant difference from vehicle in the same supplementation ($P<0.05$).

Figure 5. Linear regression analysis between PC levels and DAT levels (A), recognition index 1h (B), recognition index 24h (C) and BDNF mRNA levels in hippocampus of rats born from dams (and grandmothers) treated with different fats from gestation/lactation, and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. Statistical analysis revealed the following *P* significance levels for the r^2 values: 0.23, 0.53 and 0.31 respectively.

Table 1

Fatty acids composition of hippocampus of rats supplementing with different oil/fat over second generation (% of total fatty acids identified)

Fatty acid	Mean (±SD) Hippocampus		
	Soybean oil	Fish oil	Hydrogenated vegetable fat
16:0	20.29 ±0.11	20.64 ±0.13	20.18 ±0.23
18:0	18.28 ±0.55[b]	19.74 ±0.18[a]	19.56 ±0.10[a]
Σ SFA	40.40 ±0.62	42.04 ±0.01	41.20 ±0.44
15:1 n-5	2.58 ±0.00[b]	2.56 ±0.08[b]	2.83 ±0.00[a]
16:1 n-7	0.69 ±0.04[a]	0.51 ±0.05[b]	0.58 ±0.03[ab]
17:1 n-7	4.23 ±0.00[b]	4.84 ±0.12[a]	4.51 ±0.00[c]
18:1 n-7	3.73 ±0.21	3.39 ±0.21	3.35 ±0.03
18:1 n-9	21.04 ±0.19[a]	18.04 ±0.36[b]	17.55 ±0.38[b]
18:1 n-9t	n.d.	n.d.	0.21 ±0.01
20:1 n-9	0.78 ±0.00[a]	0.62 ±0.05[b]	0.73 ±0.01[a]
Σ MUFA	33.64 ±0.27[a]	30.81 ±0.23[b]	29.83 ±0.62[b]
18:2 n-6	5.40 ±0.11[a]	3.25 ±0.15[b]	5.38 ±0.36[a]
20:4 n-6	9.73 ±0.34	10.05 ±0.16	9.80 ±0.60
22:4 n-6	2.77 ±0.01[a]	2.51 ±0.05[b]	2.94 ±0.11[a]
22:5 n-3	n.d.	n.d.	n.d.
22:6 n-3	8.87 ±0.55[b]	11.15 ±0.08[a]	9.74 ±0.03[b]
Σ PUFA	27.09 ±0.54	27.14 ±0.24	26.85 ±0.54
Σ n-3	8.75 ±0.02[b]	11.15 ±0.08[a]	8.99 ±0.18[b]
Σ n-6	17.92 ±0.01[a]	15.99 ±0.16[b]	17.17 ±0.03[a]
Σ *trans* FA	n.d.	n.d.	0.21 ±0.01
n6/n3 ratio	2.04 ±0.01[a]	1.43 ±0.00[b]	1.94 ±0.02[a]

The following fatty acids were found at concentrations lower than 0.5% and for this reason are not shown: C14:0, C15:0, C16:1n7, C17:0, C20:0, C18:3 n-3, C20:3n6, C22:0, C22:1 n-6, C22:1 n-9, C24:0 e C24:1 n-9. The following fatty acids were not detected in the analyzed samples: C20: n-3 e C22:2 n-6.
Different lowercases indicate significant difference among SO, FO and HVF (*P*_0.05).
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

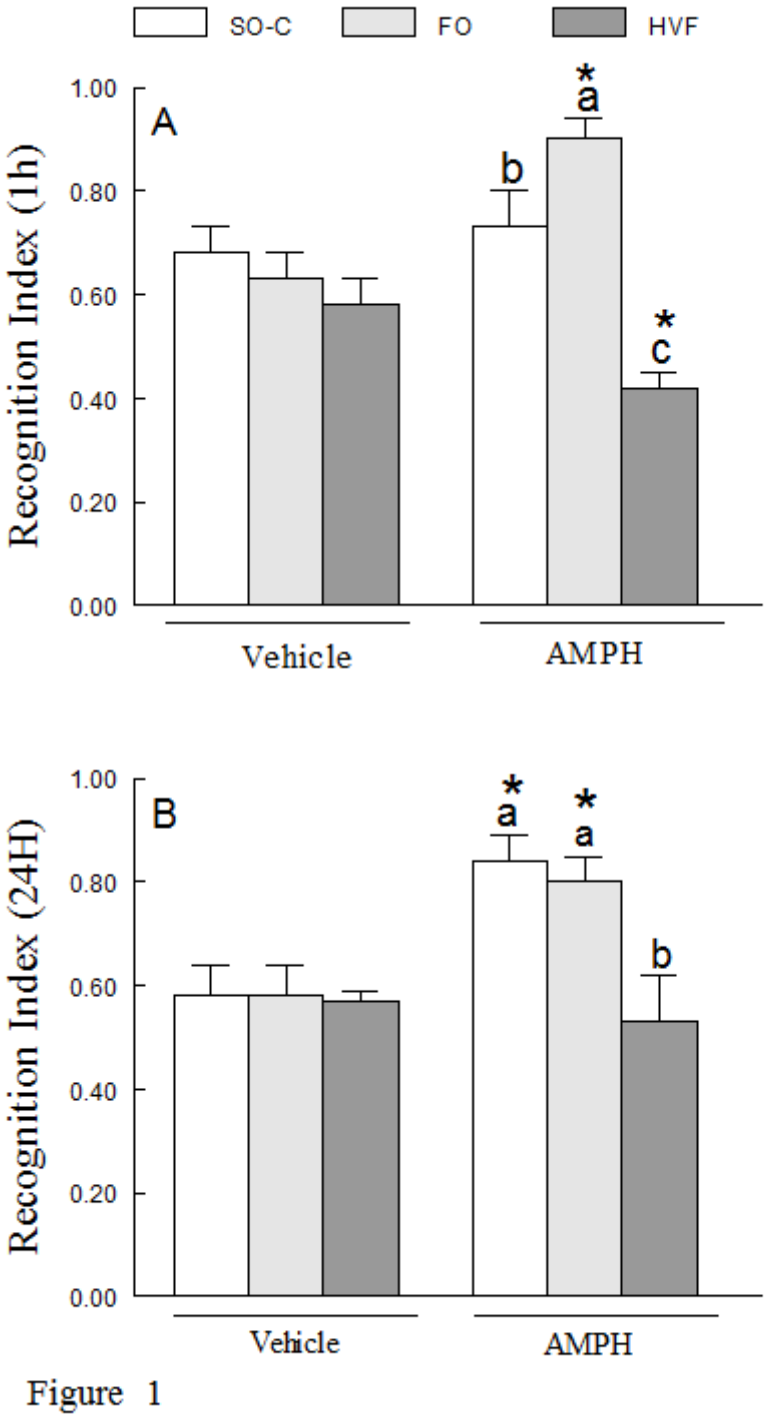
SO-C
FO
HVF
A
Recognition Index (1h)
1.00
0.80
0.60
0.40
0.20
0.00
b
*a
*c
Vehicle
AMPH
B
Recognition Index (24H)
1.00
0.80
0.60
0.40
0.20
0.00
*a
*a
b
Vehicle
AMPH

Figure 1

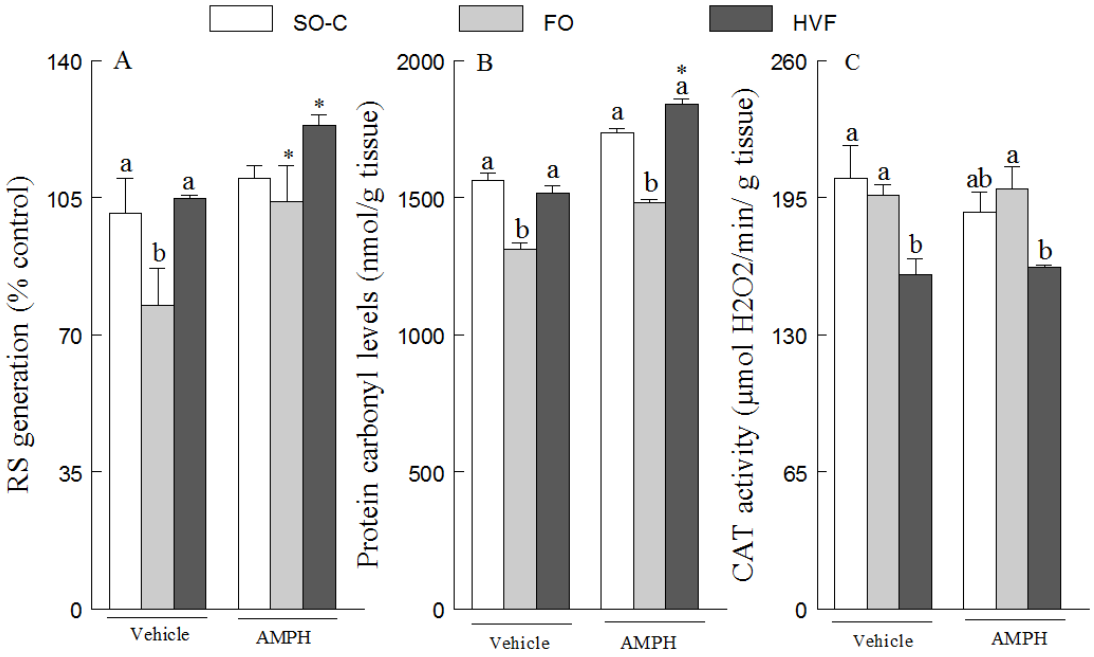
SO-C
FO
HVF
A
B
C
RS generation (% control)
Protein carbonyl levels (nmol/g tissue)
CAT activity (µmol H2O2/min/ g tissue)
140
105
70
35
0
2000
1500
1000
500
0
260
195
130
65
0
a
b
a
a
*
a
a
b
a
a
*
a
a
b
a
ab
a
b
Vehicle
AMPH
Vehicle
AMPH
Vehicle
AMPH

Figure 2

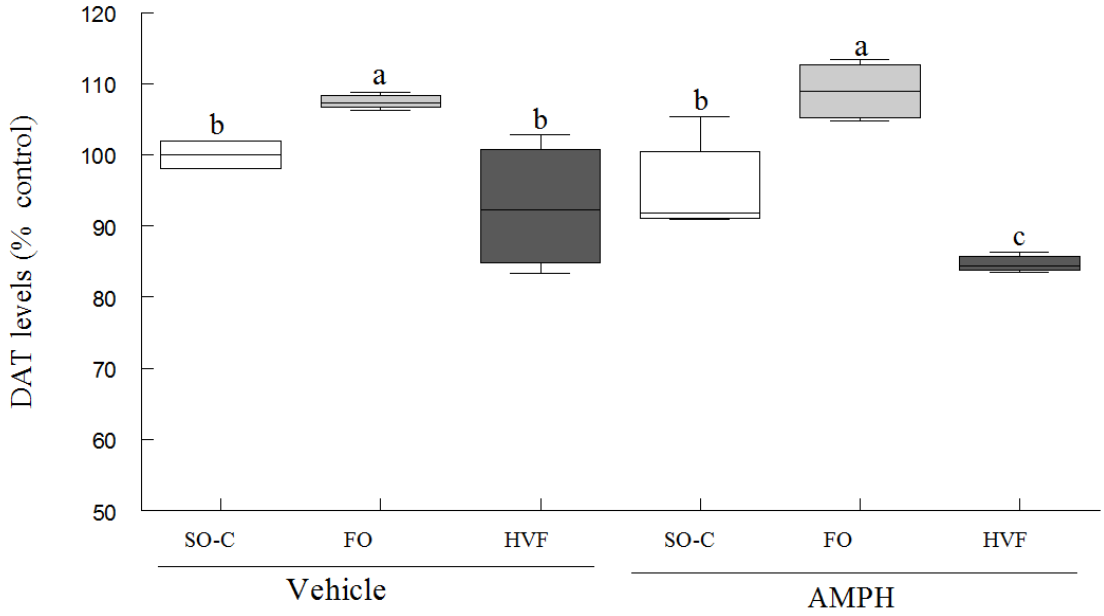
120
110
100
90
80
70
60
50
DAT levels (% control)
b
a
b
b
a
c
SO-C
FO
HVF
SO-C
FO
HVF
Vehicle
AMPH

Figure 3

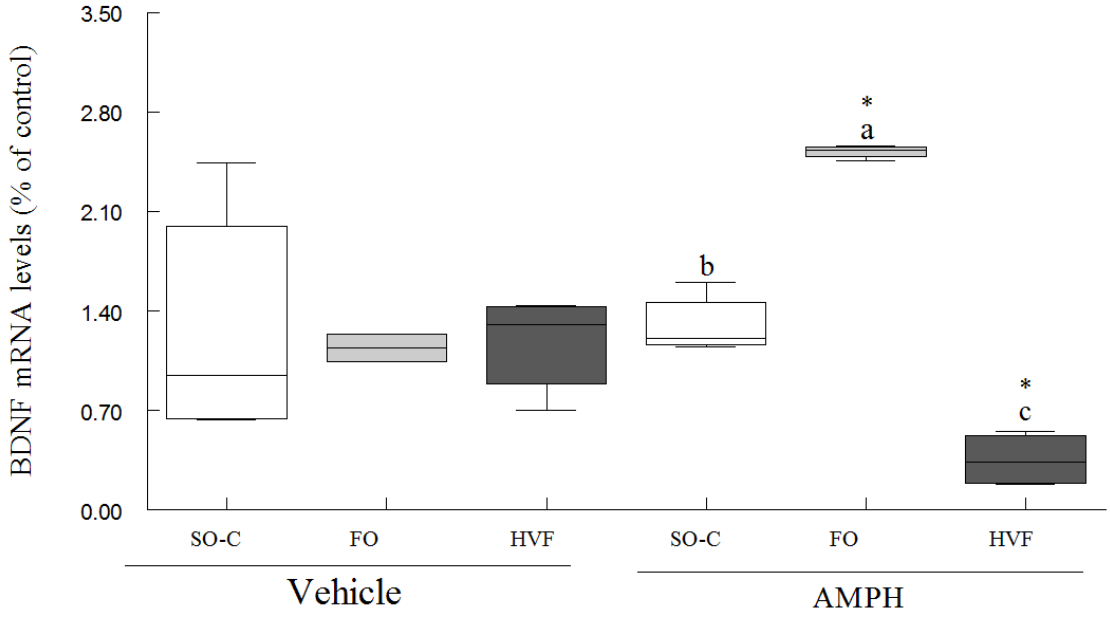
BDNF mRNA levels (% of control)
3.50
2.80
2.10
1.40
0.70
0.00
*
a
b
*
c
SO-C
FO
HVF
SO-C
FO
HVF
Vehicle
AMPH

Figure 4

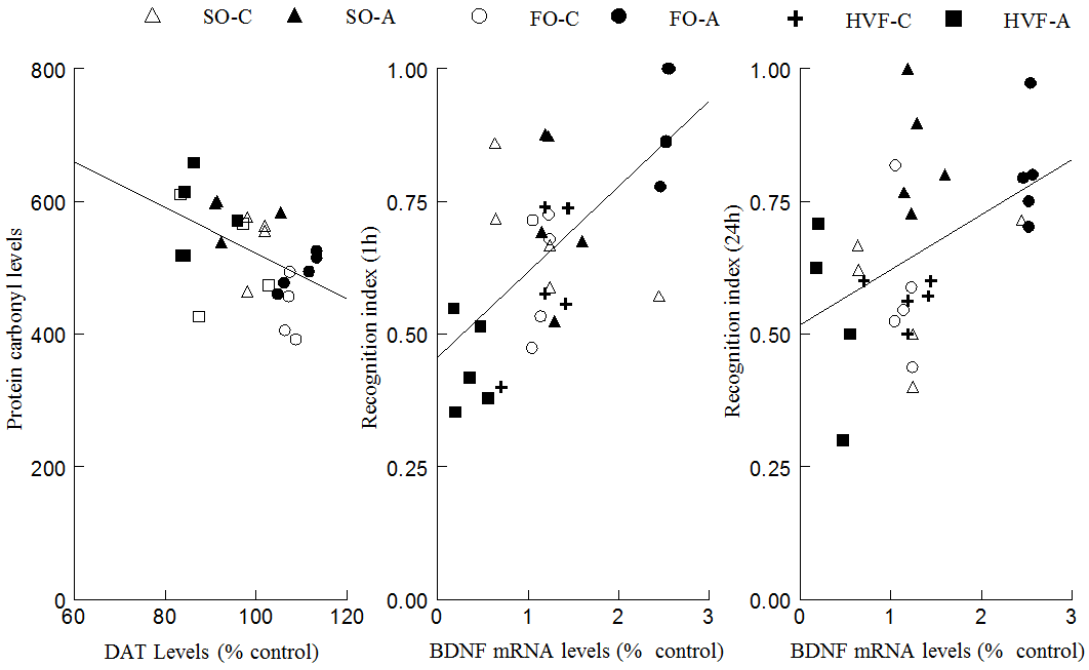
SO-C
SO-A
FO-C
FO-A
HVF-C
HVF-A
Protein carbonyl levels
DAT Levels (% control)
Recognition index (1h)
BDNF mRNA levels (% control)
Recognition index (24h)
BDNF mRNA levels (% control)

Figure 5

3.3 Manuscrito 2

CROSS-GENERATIONAL *TRANS* FAT INTAKE FACILITATES MANIA-LIKE BEHAVIOR: OXIDATIVE AND MOLECULAR MARKERS IN BRAIN CORTEX

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Status: **Em fase de submissão**

Cross-generational *trans* fat intake facilitates mania-like behavior: oxidative and molecular markers in brain cortex

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Abstract

The growing consumption of fast food in Western countries has raised concerns about eating habits and disease development. This study evaluated the influence of *trans* fat consumption on behavioral, biochemical and molecular changes in brain cortex of second generation rats exposed to animal model of mania. Two sequential generations of female rats were supplemented with soybean oil (SO, rich in n-6 FA, control group), fish oil (FO, rich in n-3 FA) and hydrogenated vegetable fat (HVF, rich in *trans* FA) from pregnancy, during lactation to adulthood. In adulthood, a part of each group was treated with amphetamine (AMPH-4mg/kg-i.p., once a day, for 14 days) in order to induce mania-like behavior. AMPH-treatment increased locomotor and exploratory activities in all experimental groups, which were higher in the HVF group. While the FO group showed increased n-3 PUFA incorporation and a reduced n-6/n-3 PUFA ratio in cortex, HVF allowed a small but significant incorporation of TFA and increased n-6/n-3 PUFA ratio in this brain area. In fact, the FO group showed minor AMPH-induced hyperactivity, decreased reactive species (RS) generation *per se*, and caused no changes in protein carbonyl (PC) levels and dopamine transporter (DAT). FO supplementation showed some molecular changes, since proBDNF was increased *per se* and was reduced by AMPH, thus decreasing the BDNF level following drug treatment. On the other hand, HVF supplementation was related to increased hyperactivity, higher PC levels *per se* and higher AMPH-induced PC levels, reflecting on DAT, whose levels were decreased *per se* as well as in AMPH-treated groups. In addition, while HVF supplementation increased BDNF-mRNA *per se*, AMPH-treatment reduced this value, acting on BDNF, whose level was lower in the same experimental group treated with AMPH. ProBDNF levels were influenced by HVF supplementation, but it was not sufficient to modify BDNF levels. Our findings are consistent with previous studies from our laboratory, reinforcing that prolonged consumption of *trans* fat allows TFA incorporation in cortex, which may increase the vulnerability to development of mania-like behavior, oxidative damage and too molecular changes. Our study is a warning about cross-generational, long-term consumption of processed food, since high *trans* fat food may facilitate the development of neuropsychiatric conditions, including BD.

Keywords: *trans* fat; oxidative damage; DAT, BDNF; mania animal model; bipolar disorder

1. INTRODUCTION

Mania is a cardinal feature of bipolar disorder (BD), a debilitating psychiatric condition which can cause disabling impairment in social and occupational functions (Magalhaes et al., 2012). Many hypotheses have been postulated to explain the exact neurochemical mechanism underlying this pathophysiology, which has been related to different signaling pathways, abnormalities in neural plasticity and the body's neurochemical systems (Schloesser et al., 2008; Martinowich et al., 2009). More recently, some studies have reported the involvement of oxidative stress (OS) in the pathogenesis of mania, holding oxidative damage to lipid and proteins as possible factors related to neuronal and glial impairment in BD (Kunz et al., 2008; Andreazza et al., 2010a,b; Steckert et al., 2010).

Intrauterine and early postnatal growth are characterized by a very fast deposition of fatty acids (FA) in the fetal tissues. In fact, the fetus and the newborn require large amounts of essential fatty acids (EFA) that cannot be synthesized "de novo" by mammals, and therefore must be supplied from maternal diet and provided by placental transfer or breastmilk. Among these, long-chain polyunsaturated fatty acids (LC-PUFA) belonging to the n-3 and n-6 series, such as docosahexaenoic acid (DHA, 22:6 n-3) and n-6 FA arachidonic acid (AA, 20:4 n-6), respectively, are rapidly incorporated in the retina and brain nervous tissue during the brain's growth spurt, mainly from the last trimester of pregnancy up to 2 years of age in humans (Martinez, 1992; Clandinin et al., 1980a; Clandinin et al., 1980b; Dobbing and Sands, 1973). So these early development periods are critical for the brain and visual functions (Martinez, 1992). In addition n-3 and n-6 FA may influence brain function throughout life by modifying neuronal membrane fluidity, membrane activity-bound enzymes, number and affinity of receptors, function of neuronal membrane ionic channels, and production of neurotransmitters and brain peptides (Yehuda, 2003).

Given the importance of EFA to the fetus and newborn, concerns arose about the widespread changes in eating habits and the increased consumption of n-6 and trans fatty acids (TFA) to the detriment of n-3 FA, altering the n-6/n3 ratio (Simopoulous, 2006). Dietary TFA are derived mainly from hydrogenated vegetable oils (Wolff et al., 1998; Koletzko and Müller, 1990; Lichtenstein, 1995; Stender et al., 2008), whose consumption has been steadily increasing since the 20th century and now accounts for 1.7-8% of the world dietary fat intake (Osso et al., 2008) through processed and fast food (Stender et al., 2008; Allison et al., 1999; Van de Vijver et al., 2000). It is well documented that dietary TFA can be quickly incorporated into membrane phospholipids, thus decreasing membrane fluidity and

altering the biochemical properties as well the functionality of their proteins (Grandgirard et al., 1998; Morgado et al., 1998; Larqué et al., 2003). Considering the growing consumption of processed food in Western countries, reports have associated its chronic intake to the pathophysiology of neurological and psychiatric disorders such as hyperactivity, autism, schizophrenia and bipolar disorder (Richardson and Ross, 2000; De Leon et al., 2002; Cott J., 1999; Hamazaki K et al., 2009).

Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophin family. It is initially synthesized as a precursor protein (preproBDNF) in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF is transported to the Golgi aparatus for sorting into either constitutive or regulated secretory vesicles. BDNF is known to exert diverse influences on neural plasticity, such as neuronal survival, neuritic growth, neuronal differentiation, synaptic plasticity, and nerve repair (Dechant and Neumann, 2002). This neurotrophin has been found throughout the brain, with particular abundance in the hippocampus and cortex, which are brain areas thought to be critical for the control of mood, emotion, and cognition (Ernfors et al., 1990). Lower serum BDNF levels have been observed in both manic and depressive phases of BD compared with control and euthymic states, and showed a negative correlation with the severity of manic episode (Cunha et al., 2006; Machado-Vieira et al., 2007). On the other hand, Barbosa et al. (2010) reported increased BDNF during bipolar manic state in comparison to healthy people. So BDNF changes in the peripheral blood and their relationship with BD are not always consistent, besides depending on the state, phenotype, brain region and cell type involved (Matrisciano et al., 2009; Young, 2009). Consistent with these brain functions, BDNF was suggested to exert an important role in the pathophysiology and treatment of unipolar and bipolar disorder (Kapzinski et al., 2008; Post, 2007).

Recent experimental studies of our group showed that *trans* fat supplementation from post-natal (Trevizol et al., 2011) and trans-natal development until adulthood (Trevizol et al., 2013) in rats were interestingly related to behavioral impairments and oxidative damage in brain tissues, while fish oil, rich in n-3 FA, was found to be beneficial in the same parameters related to animal model of mania. This study was designed to comparatively evaluate the effect of FO versus *trans* fat supplementation on behavioral changes and oxidative and molecular parameters in brain cortex of second generation rats exposed to a mania animal model.

2. EXPERIMENTAL PROCEDURES

2.1 Animals

All animal procedures were approved by the Ethics Committee of Animal Use (CEUA) of the Federal University of Santa Maria. Animals were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23°C±1) and on a 12 h-light/dark cycle throughout the experimental period. One week before mating, female adult *Wistar* rats (n=8) were supplemented (3g/Kg; p.o.) (Kuhn et al., 2013; Pase et al., 2013) with soybean oil (SO-C, isocaloric control group), fish oil (FO, rich in n-3 FA) or hydrogenated vegetable fat (HVF, rich in *trans* fatty acids), and maintained under the same supplementation during pregnancy and lactation. One female pup of each litter was maintained on the same supplementation until adulthood, when they were mated. These dams were kept on the same original supplementation until weaning of the litter of the second generation, when one male pup from each litter (n=8) and the same supplementation was grouped (four per cage) and kept under the same oral treatment until 90 days of age, when they were submitted to an animal model of mania, which is described below. SO (Camera®, Ijuí, Brazil) and HVF (Primor®, Ijuí, Brazil) were purchased in a local supermarket and FO was donated by Herbarium® (Curitiba, Brazil).

2.2 Animal model of mania

At 90 days of age, half of each experimental group was submitted to an animal model of mania, while the other half was treated with saline. Rats received a single daily injection of amphetamine/saline (AMPH- 4 mg/kg/ip) for 14 days (Trevizol et al., 2011) and 2h after the last injection animals were submitted to behavioral observations.

2.3 Behavioral assessments

2.3.1 Open-field test

Animals were placed individually in the center of a circular open-field arena (50 cm of diameter) enclosed by matte white walls and a white floor divided into squares, adapted from Kerr et al., 2005. The numbers of crossings (horizontal squares crossed) and rearings (vertical movements) shown by the animals for 5 minutes were recorded and used as measures of spontaneous locomotor activity and exploratory behavior, respectively. Briefly,

locomotor activity was evaluated 2h after the last AMPH or vehicle injection (Frey et al., 2006, Trevizol et al., 2011). The open field arena was cleaned with a 5% alcohol solution between the sessions.

2.4 Tissue preparations

One day after the last behavioral evaluation, all of the animals were anesthetized (sodium pentobarbital, 50 mg/kg body weight ip) and euthanized by cervical decapitation. Their brains were removed and cut coronally at the caudal border of the olfactory tubercle to remove the cortex (Paxinos and Watson, 2007). The tissue was separated into three parts, one of which was homogenized in 10 volumes (w/v) of 0.1 M Tris-HCl, pH 7.4 and centrifuged at 3500 x g (15 min), and the supernatant used for biochemical assay. Another part of each cortex was used to determine the fatty acids profile, and the third part was stored in freezer at -80 for subsequent molecular analysis.

2.5 Fatty acids profile in brain tissue

The fat was extracted from brain samples using chloroform and methanol as described by Bligh and Dyer, 1959, and used for determination of the FA profile. To prevent lipid oxidation during and after extraction, 0.02% butyl hydroxy toluene was added to the chloroform used. FA composition was determined by gas chromatography. Fat was saponified in methanolic KOH solution and then esterified in methanolic H_2SO_4 solution (Hartmann eLago, 1973). Methylated fatty acids were analyzed using a gas chromatograph (Agilent Technologies, HP 6890N) equipped with a capillary column DB-23 (60 m x 0.25 mm x 0.25 μm) and flame ionization detector. The temperature of the injector port was set at 280°C and the carrier gas was nitrogen (0.9 mL/min). After injection (1 μL, split ratio 50:1), the oven temperature was held at 160°C for 1 min, then increased to 240°C at 4°C/min and held at this temperature for 9 min. Standard FA methyl esters (37-component FAME Mix, 22:5n3 methylester and PUFA methylester no. 2 from Sigma, Saint Louis, MO, USA and 22:5n-6 from NuChek Prep. Inc., Elysian, MN, USA) were run under the same conditions and the subsequent retention times were used to identify the FA. FA were expressed as percentage of the total FA content.

2.6 Biochemical assessments

2.6.1 Protein carbonyl levels determination

The oxidative damage to proteins was assessed in cortex by determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) as previously described (Levine et al., 1994). Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH. The quantification of protein carbonyls in the samples was determined in the absorbance of 370 nm.

2.6.1 Reactive species (RS) generation with DCH (dichlorofluorescein-reactive species, DCH-RS)

Reactive species levels were measured using the oxidant sensing fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCHF-DA) (Hempel et al., 1999). The oxidation (DCHF-DA) to fluorescent dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. An aliquot of 10 μM (DCHF–DA) in ethanol was added to the supernatants, and the fluorescence intensity from DCF was measured for 300 s and expressed as a percentage of the untreated control group. The protein content was normalized by quantification according to Lowry (Lowry et al., 1951).

2.7 Molecular assessments

2.7.1 Immunoblotting analysis of dopamine transporter (DAT) density

Cortical tissue was homogenized in a lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM PMSF, 10 ug/ml^{-1} aprotinin, 0.1 mM benzethonium chloride, 0.5 mM sodium vanadate. The homogenates were then centrifuged; the supernatants collected and total protein concentration was determined according to MicroBCA procedure (Pierce, IL, USA), using bovine serum albumin as standard. Levels of DAT and actin were analyzed by western blot. Briefly, protein samples were separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to a PVDF membrane (Millipore, MA, USA). Non-specific binding sites were blocked in Tris-buffered saline (TBS), overnight at 4 °C, with 2% BSA and 0.1% Tween-20. Membranes were rinsed in buffer (0.05% Tween-20 in TBS) and then incubated with primary antibodies: anti-actin (1:1000; Santa Cruz Biotechnology, CA, USA) and anti-DAT (1:500; Santa Cruz Biotechnology, CA, USA), followed by anti-goat (1:25.000; Santa Cruz Biotechnology, CA, USA) and anti-rabbit (1:8000; Santa Cruz Biotechnology, CA, USA) IgG horseradish peroxidase conjugate, respectively. After rinsing with buffer, the immunocomplexes were visualized using the 3,3’,5,5’ – Tetramethylbenzidine (TMB) (Sigma, USA) according to the

manufacturer's instructions. The film signals were digitally scanned and then quantified using ImageJ software. Actin was used as an internal control for western blot such that data were standardized according to actin values.

2.7.2 Quantification of BDNF mRNA expression by real-time polymerase chain reaction (RT-PCR)

To quantify expression of BDNF mRNA, a quantitative RT-PCR assay was performed. Total RNA from cortex was extracted with Trizol Reagent (Invitrogen) according to the manufacturer´s suggested protocol. The cDNA was synthesized with M-MLV reverse transcriptase enzyme, using total RNA and random primer (Invitrogen) according to the manufacturer´s suggested protocol. For analysis of rat BDNF, we measured total BDNF mRNA using the following sense and antisense primers: sense 5′-GCG GCA GAT AAA AAG ACT GC -3′; and antisense: 5′-GTA GTT CGG CAT TGC GAG TT -3′. For analysis of the housekeeping gene tubulin as an internal standard, the sense primer 5′-CAT GAA CAA CGA CCT CAT CG -3′ and the antisense primer 5′-TGT GGA CAC CAT CAC GTT CT -3′ were used. Quantitative real-time polymerase chain reaction was performed in 20 µL PCR mixtures containing 1x PCR Buffer, 25µM dNTPs, 0.2µM of each primer, 3mM MgCl2, 0.1x SYBR Green I (Molecular Probes) and 0.5U Platinum Taq DNA polymerase (Invitrogen). The thermal cycle was carried out in Illumina (ECO Software Version 4.0) and the following protocol was used: activation of Taq DNA polymerase at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 15s and extension at 72° C for 20s, fluorescence was acquired at the end of each extension phase. SYBR fluorescence was analyzed by ECO software version 4.0 (Applied Illumina), and the CT value for each sample was calculated and reported using the $2^{-\Delta\Delta CT}$ method. For each well, analyzed in biological quadruplicate, $^{\Delta}C_T$ value was obtained by subtracting the Tubulin C_T value from the C_T value of the BDNF gene. The $^{\Delta}C_T$ mean value obtained from the control group of each gene was used to calculate the $^{\Delta\Delta}C$t of the respective gene ($2^{-\Delta\Delta Ct}$), according to Bustin et al., 2009.

2.7.3 Immunoblotting analysis of proBDNF and BDNF density

Cortex was dissected out and homogenized in a 5 % SDS solution containing a protease inhibitor cocktail (Sigma, São Paulo/SP, Brazil) and frozen at -20 ºC. After defrost, the protein content was determined using the bicinchoninic acid assay (Pierce, São

Paulo/Brazil). Sample extracts were diluted to a final protein concentration of 2 μg/μL in Protein Sample Loading Buffer and boiled in 95°C for 5 min. Protein (60, 100 and 80 μg, for hippocampus, cortex and striatum respectively) was separated by 4-14% SDS-PAGE together with pre-stained molecular weight standards (Bio-Rad, São Paulo/Brazil), and transferred onto a nitrocellulose membrane (Amersham, São Paulo/Brazil) for 1.5 hour at 150 mA. The membrane was blocked with 5% skimmed milk diluted in Tris-buffered saline containing 0.1 % Tween-20 (TBS-T) for 1 hour, and then probed with rabbit polyclonal anti-proBDNF antibody (1:2000, ab72440, Abcam, USA) or rabbit polyclonal anti-BDNF antibody (1:1000, ab72439, Abcam, USA) at 4°C overnight. Mouse monoclonal β-tubulin (1:20.000 or 1:40.000 dilution, sc-53140, Santa Cruz, USA) was used as control. The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature (anti-rabbit, 1:10.000 or anti-mouse, 1:20.000, and visualized by an ECL method using ECL Western Blotting Substrate (Pierce, São Paulo/Brazil) and Kodak film. The X-ray film was scanned and the bands were quantified using ImageJ software (NIH, US).

2.8 Statistical Analysis

The cortical FA content in brain cortex was analyzed by one-way ANOVA followed by Duncan's test. Behavioral (locomotor index), biochemical (RS and PC levels) and molecular (DAT, BDNF and proBDNF) evaluations were analyzed by two-way ANOVA [3(SO-C, FO and HVF)] x [2(vehicle/amphetamine)], followed by Duncan's multiple range test, when appropriate. Non-parametric data (cortical BDNF mRNA expression) were analyzed by Kruskal–Wallis test followed by two-tailed Mann–Whitney *U*-test. $P<0.05$ was considered as significant in all comparisons made.

3. Results

3.1. Locomotor and exploratory activities evaluated in Open Field (Figure 1).

Two-way ANOVA of crossing number revealed a significant main effect of supplementation and drug [$F(2,42)=58.58$; $P<0.001$; $F(1,42)=4.80$; $P<0.05$]. Two-way ANOVA of rearing frequency revealed a significant main effect of supplementation, drug, and a significant supplementation x drug interaction [$F(2,42)=15.74$; $P<0.001$; [$F(1,42)=6.05$; $P<0.05$; $F(2,42)=5.64$; $P<0.05$, respectively].

Duncan's test showed no effects of supplementation per se on locomotor status; AMPH administration increased the crossing number in all experimental groups, which was higher in HVF group (Fig. 1A). AMPH administration also increased the rearing frequency in both SO-C and HVF, but not in FO. In fact, rearing frequency was higher in HVF than in SO-C, and it was higher in both these groups than in FO (Fig. 1B).

3.2. Fatty acids composition in brain cortex (Table 1).

One-way ANOVA followed by Duncan's test showed that FO supplementation reduced AA (20:4 n-6) by 5.64% and adrenic acid (22:4 n-6) by 15.19%, and increased docosapentaenoic acid (DPA, 22:4 n-3) by 100% and DHA (22:6 n-3) by 18.47%. Among these, a reduction of Σ n-6 by 5.52% was observed, reflecting on the n-6/n-3 FA ratio, which was decreased by 20.45% in comparison to SO group. In addition, HVF supplementation allowed a low, but significant TFA incorporation (0.11%) in this brain area ($P<0.05$ for all comparisons).

3.3. Influence of the fat supplementations on reactive species (RS) generation and protein carbonyl (PC) levels (Figure 2).

Two-way ANOVA of RS generation revealed a significant main effect of supplementation and a significant supplementation x drug interaction [$F(2,42)=5.31$; $P<0.05$] [$F(2,42)=7.44$; $P<0.05$; $F(2,42)=11.65$; $P<0.05$, respectively].
Two-way ANOVA of PC level revealed a significant main effect of supplementation [$F(2,42)=11.65$; $P<0.001$].

Fish oil supplementation reduced RS generation *per se* in comparison to SO-C and HVF, whose values were similar to each other. AMPH reduced RS generation in SO-C group, while all AMPH-treated groups showed similar RS generation in cortex (Fig. 2A).

Post-hoc test showed that HVF supplementation was related to higher PC level *per se*, as well in AMPH-treated animals in comparison to SO-C and FO groups. AMPH treatment did not change this oxidative marker in brain cortex (Fig. 2B).

3.4 The influence of different supplementations on dopamine transporter (DAT) levels in cortex (Figure 3).

Two-way ANOVA of DAT level revealed a significant main effect of supplementation, drug and a significant drug x supplementation interaction [$F(2,42)=28.31$; $P<0.001$; $F(1,42)=10.63$; $P<0.05$ and $F(2,42)=6.92$; $P<0.05$, respectively].

Duncan's test showed that HVF was able to reduce DAT levels per se in comparison to SO-C and FO groups. AMPH decreased DAT levels in SO-C and FO group, while in HVF group this transporter was not changed, remaining lower than in those other experimental groups.

3.5 The influence of different supplementations on the quantitative expression of BDNF mRNA in cortex (Figure 4).

Kruskal-Wallis analysis of variance revealed significant differences ($H= 20.81$, $p=0.0009$) in cortex BDNF mRNA.

Hydrogenated vegetable fat supplementation increased BDNF mRNA *per se* as compared with SO-C and FO supplementations, and the BDNF mRNA of FO group was also higher than in the vehicle-treated SO-C group. AMPH administration was able to change this marker in both SO-C and HVF groups, whose mRNA expression was increased and reduced, respectively. In fact, comparisons across AMPH-treated groups showed decreased BDNF mRNA in HVF as compared to SO-C and FO groups.

3.6 Influence of different supplementations on the BDNF and proBDNF levels in cortex (Figure 5).

Two-way ANOVA of BDNF levels revealed a significant main effect of supplementation and drug [$F(2,42)=7.75$; $P<0.05$; $F(1,42)=32.22$; $P<0.001$, respectively]. Two-way ANOVA of proBDNF levels revealed a drug x supplementation interaction [$F(2,42)=11.47$; $P<0.001$].

Post-hoc test showed that BDNF levels of HVF group were lower than in FO group. Amphetamine treatment reduced BDNF levels in FO and HVF, while this last experimental group showed lower BDNF levels than SO-C and FO groups did (Fig. 5A).

Duncan's test showed that FO and HVF supplementations increased proBDNF levels per se, whose values were similar each other, but higher than those of SO-C. Differently, AMPH treatment increased proBDNF levels in SO-C, which was decreased in FO

supplementation. In fact, among AMPH-treated rats, both FO and HVF showed decreased proBDNF levels that SO-C (Fig. 5B).

4. Discussion

In this study, hyperlocomotion (mania animal model), cortical FA composition, and oxidative and molecular status were evaluated in a second generation of adult rats supplemented with SO-C (control, rich in n-6 FA), FO (rich in n-3 FA) or HVF (rich in TFA) from pregnancy and lactation to weaning and adulthood.

The different supplementations were able to change the composition of neural membranes in the brain cortex. While FO supplementation increased n-3 and reduced n-6 PUFA incorporation, these changes decreased the n-6/n-3 PUFA ratio, which was lower than in the other experimental groups. These data may at least partly explain some of the beneficial influences in the FO group as n-3 FA (EPA and DHA) are also substrate for cyclooxygenase-2 (Bagga et al., 2003) giving rise to prostaglandins of series 3 (PGE3) (Bagga et al., 2003). PGE3 derivatives are less pro-inflammatory than PGE2 derivatives (from AA), which are known to generate reactive species and pro-inflammatory cytokines (Calder, 2006), leading to increased presence of n-3 FA related to decreased inflammatory processes, which may provide some protection against oxidative damage, as observed here. In fact, despite higher incorporation of n-3 PUFA in the cortical membranes, here we did not observe all of the beneficial effects of FO consumption reported previously (Trevizol et al., 2011; Trevizol et al., 2013). Here, FO supplementation was able to prevent RS generation *per se*, which was however lost in AMPH-treated group, showing also no oxidative damage to proteins, as observed by PC levels. In addition, the FO group did not show changes to DAT levels, BDNF-mRNA expression and BDNF levels, but was able to increase proBDNF *per se*, as well to reduce this molecular precursor following AMPH treatment.

Adequate consumption of n-3 FA is important because increased n-3 intake to the detriment of n-6 LC-PUFA has been related to a lower incidence of both unipolar and bipolar depression (Hibbeln et al., 2006). In line with this, an experimental study showed that n-3 FA deficient diets may alter monoamine systems in limbic structures known to control mood (Chalon, 2006). Furthermore, the mania animal model used here showed that supplementations *per se* were not able to change the evaluated behavioral parameters, but while HVF supplementation was associated with higher locomotion and exploration, FO showed some protective influence on exploratory behavior induced by AMPH. These

findings are consistent with other recent studies from our laboratory, where HVF supplementation was related to increased susceptibility to develop mania-like behavior (Trevizol et al., 2011; 2013), thus reinforcing the predictability of this animal model, as AMPH administration may precipitate mania-like symptoms in normal human volunteers and in BD subjects (Anand et al., 2000).

Besides the relative beneficial influence of FO supplementation, the primary target of this study was *trans* fat provided through HVF supplementation. Such supplementation during two sequential generations of rats allowed a small but significant TFA incorporation in cortex, which was also related to an increased n-6/n-3 PUFA ratio. Taken together, these events are able to change the composition of brain membranes, affecting the locomotor status, as already commented above, thus changing oxidative status and molecular markers. Indeed, besides AMPH-induced hyperactive behavior, the HVF group showed increased PC levels *per se* and after AMPH. Such damage to proteins may have exerted influences on the dopamine transporter (DAT), whose level was decreased in this experimental group. Interestingly, such influence was not significantly reflected on the cortical BDNF and proBDNF levels, as AMPH-treatment was able to reduce BDNF levels in the HVF group with no influences on proBDNF.

Acar et al. (2003) suggested that besides the imbalance generated by their presence, *trans* FA may directly act on the increased DA neurotransmission, suggesting that *trans* PUFA could act on gene expression and protein transcription, which are involved in DA metabolism. Here, we also observed molecular changes in the HVF-supplemented group, when DAT levels (protein responsible for removal of DA in the synaptic cleft) were reduced *per se* and following AMPH treatment, confirming Acar's hypothesis (Acar et al., 2003). Interestingly, the cortical TFA incorporation observed in the HVF group may also be related to an increase of BDNF-mRNA expression *per se*, which was accompanied by increased proBDNF kevels, but no change in BDNF levels. As a whole, these findings indicate that the molecular signalizing cascade suffers influences from TFA incorporation in the brain membranes, but apparently this neuroadaptation is not enough to reach the activation threshold for BDNF synthesis. Additionally, a radical reduction in the BDNF-mRNA expression following AMPH-treatment was observed in the HVF group, which occurred together with lower levels of both BDNF and proBDNF. This finding reinforces our initial hypothesis related to mania development, when prolonged consumption of *trans* fat may facilitate behavioral changes, as well as reduce the cortical BDNF levels, which is associated with BD development (Cunha et al., 2006). Additional evidence suggested BDNF

involvement in BD (Frey et al., 2006; Gluck et al., 2001; Hashimoto et al., 2004), considering that clinical studies have shown a significant negative correlation between serum lipid peroxidation markers and BDNF levels in BD patients (Walz et al., 2007), thus suggesting that oxidative damage may be mechanistically associated with this disease (Walz et al., 2007).

Overall, our findings support the notion that cross-generational long-term consumption of HVF may be related to an increased vulnerability to develop neuropsychiatric conditions, an idea that is further supported by previous studies from our laboratory where animals chronically supplemented with *trans* fat had movement disorders (Teixeira et al. 2012), memory impairments (Teixeira et al., 2011), increased preference for addictive drugs (Kuhn et al., 2013), and anxiety-like and fear behavior after stress exposure (Pase et al., 2013).

Considering that nutrition during pregnancy and lactation determines the transfer of EFA and TFA to the fetus through the placenta and milk (Innis, 2007), our study showed that TFA incorporation in brain membranes was related to behavioral and biochemical changes, which were reflected on molecular neuroadaptations *per se* and following a mania animal model. These findings reinforce impairments from prolonged consumption of *trans* fat, especially during early life periods, whose consequences may be manifested through neuropsychiatric vulnerabilities in adulthood.

References

Acar N, Chardigny JM, Darbois M, Pasquis B, Sébédio JM (2003), Modification of the dopaminergic neurotransmitters in striatum, frontal cortex and hippocampus of rats fed for 21 months with trans isomers of a-linolenic acid. Neurosci Res 45:375–382.

Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, Heimbach JT (1999), Estimated intakes of trans fatty and other faty acids in the US population. J Am Diet Assoc 99:166–174.

Anand A, Verhoeff P, Seneca N, Zoghbi SS, Seibyl JP, Charney DS, Innis RB (2000), Brain SPECT imaging of amphetamine-induced dopamine release in euthymic bipolar disorder patients. Am J Psychiatry 157:1108–1114.

Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST (2003), Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proc Natl Acad Sci U S A 100:1751–1756.

Barbosa IG, Huguet RB, Mendonca VA, Neves FS, Reis HJ, Bauer ME, et al. (2010). Increased plasma levels of brain-derived neurotrophic factor in patients with long-term bipolar disorder. Neurosci Lett 475:95–98.

Calder PC (2006), n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83:1505S-1519S.

Chalon S (2006), Omega-3 fatty acids and monoamine neurotransmission. Prostaglandins Leukot Essent Fatty Acids 75:259-269.

Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW (1980a), Extrauterine fatty acid accretion n in infant brain:implications for fatty acid requirements. Early Hum. Dev. 4:131–138.

Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW (1980b), Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements, Early Hum Dev 4:121–129.

Cott, J (1999). Omega-3 fatty acids and psychiatric disorders. Alternative Therapies in women's health 1(13):97-104.

Cunha AB, Frey BN, Andreazza AC, Goi JD, Rosa AR, Goncalves CA, Santin A, Kapczinski F (2006), Serum brain derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. Neurosci Lett 398:215–219.

De Leon J, Tracy J, McCann E, McGrory A (2002), Polydipsia and schizophrenia in a psychiatric hospital: a replication study. Schizophr Res 57:293–301.

Dobbing J, Sands J (1973), Quantitative growth and development of human brain. Arch Dis Child 48:757–767.

Fernstrom JD (1999). Effects of dietary polyunsaturated fatty acids on neuronal function. Lipids 34: 161–9.

Frey BN, Valvassori SS, Réus GZ, Martins MR, Petronilho FC, Bardini K, Dal-Pizzol F, Kapczinski F, Quevedo J (2006), Changes in antioxidant defense enzymes after d-amphetamine exposure: implications as an animal model of mania. Neurochem Res 31:699–703.

Frey BN, Martins MR, Petronilho FC, Dal-Pizzol F, Quevedo J, Kapezinski F. Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania. Bip Disord 2006; 8:275–280.

Gluck MR, Moy LY, Jayatilleke E, Hogan KA, Manzino L, Sonsalla PK (2001), Parallel increases in lipid and protein oxidative markers in several mouse brain regions after methamphetamine treatment. J Neurochem 79:152–160.

Hamazaki K, Choi KH, Kim HY (2009). Phospholipid profile in the postmortem hippocampus of patients with schizophrenia and bipolar disorder: no changes in docosahexaenoic acid species. J Psychiatr Res 44(11):688-693.

Hashimoto K, Shimizu E, Iyo M (2004), Critical role of brainderived neurotrophic factor in mood disorders. Brain Res Rev 24:104–114.

Dechant G, Neumann , H (2002), Neurotrophins, Adv Exp Med Biol 513: 303–334.

Grandgirard A, Piconneaux A, Sebedio JL, Julliard F (1998), Trans isomers of long-chain polyunsaturated fatty acids in tissue lipid classes of rats fed with heated linseed oil. Reprod Nutr Dev 38:17–29.

Hartman L and Lago BC (1973), A rapid preparation of fatty methyl esters from lipids. Lab Pract 22:475–477.

Hempel SL, Buettner GRO, Malley YQ, Wessels DA, Flaherty DM (1999), Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 20, 70-dichloro dihydrofluorescein diacetate, 5 (and 6)- carboxy-20, 70-dichloro dihydrofluorescein diacetate, and dihydrorhodamine 123. Free Radical Biol Med 27:146–159.

Hibbeln JR, Nieminen LR, Blasbalg TL, Riggs JA, Lands WE (2006), Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. Am J Clin Nutr 83:1483S-1493S.

Innis SM (2007), Dietary (n-3) fatty acids and brain development. J Nutr 137:855–859.

Kapczinski F, Frey BN, Kauer-Sant'Anna M, Grassi-Oliveira R (2008), Brain-derived neurotrophic factor and neuroplasticity in bipolar disorder. Expert Rev Neurother 8:1101–1113.

Kerr DS, Bevilaqua LR, Bonini JS, Rossato JI, Kohler CA, Medina JH, Izquierdo I, Cammarota M (2005), Angiotensin II blocks memory consolidation through an AT2 receptor dependent mechanism. Psychopharmacology 179:529–535.

Koletzko B, Müller J (1990), Cis- and trans-isomeric fatty acids in plasma lipids of newborn infants and their mothers. Biol Neonate 57:172–178.

Kuhn FT, Roversi KR, Antoniazzi CT, Pase CS, Trevizol F, Barcelos RC, Dias VT, Roversi K, Boufleur N, Benvegnú DM, Piccolo J, Emanuelli T, Bürger ME (2013), Influence of trans fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats. Pharmacol Biochem Behav 110:58-65.

Larqué E, Garcia-Ruiz PA, Perez-Llamas F, Zamora S, Gil A (2003), Dietary trans fatty acids alter the compositions of microsomes and mitochondria and the activities of microsome delta 6-fatty acid desaturase and glucose-6-phosphatase in livers of pregnant rats. J Nutr 133:2526–2531.

Levine RL, Williams JA, Stadtman ER, Shacter E (1994), Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–57.

Lichtenstein AH (1995), Trans fatty acid and hydrogenated fats. What do we know? Nutr Today 30:102–107.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951), Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275.

Machado-Vieira R, Dietrich MO, Leke R, Cereser VH, Zanatto V, Kapczinski F, Souza DO, Portela LV, Gentil V (2007), Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. Biol Psychiatry 61:142–144.

Magalhaes PV, Kapczinski F, Nierenberg AA, Deckersbach T, Weisinger D, Dodd S, et al. (2012), Illness burden and medical comorbidity in the systematic treatment enhancement program for bipolar disorder. Acta Psychiatr Scand 125:262–263.

Martinez M (1992), Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 120:S129–S138.

Martinowich K, Schloesser RJ, Manji HK (2009), Bipolar disorder: from genes to behavior pathways. J Clin Invest 119:726–736.

Matrisciano F, Bonaccorso S, Ricciardi A, Scaccianoce S, Panaccione I, Wang L, Ruberto A, Tatarelli R, Nicoletti F, Girardi P, Shelton RC (2009), Changes in BDNF serum levels in patients with major depression disorder (MDD) after 6 months treatment with sertraline, escitalopram, or venlafaxinee. J Psychiatr Res 43:247–254.

Morgado N, Galleguillos A, Sanhueza J, Garrido A, Nieto S, Valenzuela A (1998), Effect of the degree of hydrogenation of dietary fish oil on the trans fatty acid content and enzymatic activity of rat hepatic microsomes. Lipids 33:669–673.

Osso FS, Moreira AS, Teixeira MT, Pereira RO, Tavares do Carmo MD, Moura AS (2008), Trans fatty acids in maternal milk lead to cardiac insulin resistance in adult offspring. Nutrition 24:727-732.

Pase CS, Roversi K, Trevizol F, Roversi K, Kuhn FT, Schuster AJ, Vey LT, Dias VT, Barcelos RC, Piccolo J, Emanuelli T, Bürger ME (2013), Influence of perinatal trans fat on behavioral responses and brain oxidative status of adolescent rats acutely exposed to stress. Neuroscience 247:242-252.

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. 6th ed. Amsterdam:Elsevier.

Post RM (2007), Role of BDNF in bipolar and unipolar disorder: clinical and theoretical Implications. J Psychiatr Res 41:979–990.

Richardson AJ, Ross MA (2000), Fatty acid metabolism in neurodevelopmental disorder: a new perspective on associations between attention-deficit/hyperactivity disorder, dyslexia, dyspraxia and the autistic spectrum. Prostaglandins Leukot Essent Fatty Acids 63:1–9.

Schloesser RJ, Huang J, Klein PS, Manji HK (2008), Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. Neuropsychopharmacol 33:110–133.

Simopoulos AP (2006), Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. T Biomed Pharmacother 60:502-507.

Steckert AM, Valvassori SS, Moretti M, Dal-Pizzol F, Quevedo J (2010), Role of oxidative stress in the pathophysiology of bipolar disorder. Neurochem Res 35:295–301.

Stender S, Astrup A, Dyerberg J (2008), Ruminant and industrially produced trans fatty acids: health aspects. Food Nutr Res 52:1–8.

Teixeira AM, Pase CS, Boufleur N, Roversi K, Barcelos RCS, Benvegnú DM, Segat HJ et al (2011). Exercise affects memory acquisition, anxiety-like symptoms and activity of membrane-bound enzyme in brain of rats fed with different dietary fats: impairments of *trans* fat. Neuroscience 195: 80–88.

Teixeira AM, Dias VT, Pase CS, Roversi K, Boufleur N, Barcelos RCS et al (2012). Could dietary trans fatty acids induce movement disorders? Effects of exercise and its influence on Na^+K^+-ATPase and catalase activity in rat striatum. Behaviour Brain Res 226:504–510.

Trevizol F, Roversi K, Dias VT, Roversi K, Pase CS, Barcelos RC, Benvegnu DM, Kuhn FT, Dolci GS, Ross DH, Veit JC, Piccolo J, Emanuelli T, Bürger ME (2013), Influence of lifelong dietary fats on the brain fatty acids and amphetamine-induced behavioral responses in adult rat. Prog Neuropsychopharmacol Biol Psychiatry 45:215-222.

Trevizol F, Benvegnú DM, Barcelos RCS, Boufleur N, Dolci GS, Müller LG Pase CS, Reckziegel P, Dias VT, Segat H, Teixeira AM, Emanuelli T, Rocha JB, Bürger ME (2011), Comparative study between n6, trans and n3 fatty acids on repeated amphetamine exposure: a possible factor for the development of mania. Pharmacol Biochem Behav, 97:560–565.

Van de Vijver LP, Kardinaal AF, Couet C, Aro A, Kafatos A, Steingrimsdottir L, Amorim Cruz JA, Moreiras O, Becker W, van Amelsvoort JM, Vidal-Jessel S,Salminen I, Moschandreas J, Sigfússon N, Martins I, Carbajal A, Ytterfors A, Poppel G (2000), Association between trans fatty acid intake and cardiovascular risk factors in Europe: the transfair study. Eur J Clin Nutr 54:126–135.

Walz JC, Andreazza AC, Frey BN Cacilhas AA, Ceresér KM, Cunha AB, Weyne F, Stertz L, Santin A, Gonçalves CA, Kapczinski F. (2007) Serum neurotrophin- 3 is increased during manic and depressive episodes in bipolar disorder. Neurosci Lett 415:87–89.

Wolff RL, Precht D, Molkentin J (1998), Occurrence and distribution profiles of trans-18:1 acids in edible fats of natural origin. In: Sebedio JL, ChristieWW, editors. Trans fatty acids in human nutrition. Dundee: The Oily Press 1–33.

Young W (2009), Review of lithium effects on brain and blood Cell Transplant. 18: 951–975.

Yehuda S (2003), Omega-6/omega-3 ratio and brain-related functions. World Rev Nutr Diet 92:37–56.

Figure captions

Figure 1. Influence of different supplemented fats on locomotor and exploratory activities. Animals were born of dams (and grandmothers) treated with different fats from gestation/lactation and maintained in the same supplementation until 90 days of age, when they were treated with amphetamine (AMPH-4mg/kg) or vehicle for 14 days. Data are expressed as mean±S.E.M. Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between the supplementations in the same treatment (P<0.05). *Indicates significant difference from vehicle in the same supplementation (P <0.05).

Figure 2. Influence of different supplemented fats on RS generation (A) and protein carbonyl levels (B) in cortex of rats born from dams (and grandmothers) treated with different fats from gestation/lactation, and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. Data are expressed as mean±S.E.M. Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between the supplementations in the same treatment; (P<0.05). *Indicates significant difference from vehicle in the same supplementation (P <0.05).

Figure 3. Influence of different supplemented fats on DAT levels in cortex of rats born of dams (and grandmothers) treated with different fats from gestation/lactation and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. Data are expressed as mean±S.E.M. Abbreviations: SO-C: soybean oil; FO: fish oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between supplementations in the same treatment; (P<0.05).

Figure 4. Influence of different supplemented fats on BDNF mRNA levels in cortex of rats born of dams (and grandmothers) treated with different fats from gestation/lactation and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. The median is the midline; boxes represent the interval from the first (25%) to the last (75%) quartile and the line the maximum and minimum values. Abbreviations: SO-C: soybean oil; FO: fish oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between supplementations in the

same treatment; (P<0.05). *Indicates significant difference from vehicle in the same supplementation (*P*<0.05).

Figure 5. Influence of different supplemented fats on BDNF (A) and proBDNF (B) levels in cortex of rats born of dams (and grandmothers) treated with different fats from gestation/lactation and maintained in the same supplementation until 90 days of age, when they were treated with AMPH (4mg/kg) or vehicle for 14 days. Data are expressed as mean±S.E.M. Abbreviations: SO-C: soybean oil; FO: fish oil; HVF: hydrogenated vegetable fat. Lowercase indicates significant difference between supplementations in the same treatment; (P<0.05).

Table 1

Fatty acids composition of cortex of rats supplementing with different oil/fat over second generation (% of total fatty acids identified).

Fatty acid	Mean (±SD)		
	Cortex		
	Soybean oil	Fish oil	Hydrogenated vegetable fat
16:0	21.51 ±0.02	21.32 ±0.06	21.56 ±0.22
18:0	20.80 ±0.07	20.12 ±0.09	20.20 ±0.03
Σ SFA	44.58 ±0.52	43.15 ±0.07	43.54 ±0.32
15:1 n-5	2.32 ±0.03^{b}	2.42 ±0.01^{a}	2.38 ±0.00^{ab}
16:1 n-7	0.36 ±0.00	0.41 ±0.01	0.37 ±0.33
17:1 n-7	4.97 ±0.09^{b}	5.04 ±0.04^{a}	4.93 ±0.03^{c}
18:1 n-7	3.16 ±0.00	3.16 ±0.01	3.17 ±0.01
18:1 n-9	15.87 ±0.08^{b}	15.74 ±0.05^{b}	16.35 ±0.13^{a}
18:1 n-9t	n.d.	n.d.	0.11 ±0.03
20:1 n-9	0.89 ±0.02^{a}	0.86 ±0.01^{a}	0.80 ±0.02^{b}
Σ MUFA	27.89±0.04	27.92 ±0.01	27.77 ±0.19
18:2 n-6	1.74 ±0.15	1.59 ±0.09	1.73 ±0.05
20:4 n-6	10.82 ±0.07^{a}	10.21 ±0.07^{b}	10.97 ±0.03^{a}
22:4 n-6	3.16 ±0.05^{a}	2.68 ±0.04^{b}	3.30 ±0.04^{a}
22:5 n-3	0.15 ±0.01^{b}	0.30 ±0.04^{a}	0.22 ±0.00^{b}
22:6 n-3	11.37 ±0.09^{b}	13.47 ±0.17^{a}	11.71 ±0.02^{b}
Σ PUFA	27.60 ±0.20^{c}	28.93 ±0.08^{a}	28.25 ±0.21^{b}
Σ n-3	11.80 ±0.30^{b}	13.96 ±0.04^{a}	11.91 ±0.01^{b}
Σ n-6	15.57 ±0.00^{a}	14.71 ±0.27^{b}	15.89 ±0.03^{a}
Σ *trans* FA	n.d.	n.d.	0.11 ±0.03
n6/n3 ratio	1.33 ±0.00^{b}	1.07 ±0.00^{c}	1.37 ±0.02^{a}

The following fatty acids were found at concentrations lower than 0.5% and for this reason are not shown: C14:0, C15:0, C16:1n7, C17:0, C20:0, C18:3 n-3, C20:3n6, C22:0, C22:1 n-6, C22:1 n-9, C24:0 e C24:1 n-9. The following fatty acids were not detected in the analyzed samples: C20: n-3 e C22:2 n-6.
Different lowercases indicate significant difference among SO, FO and HVF (*P*_0.05).
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

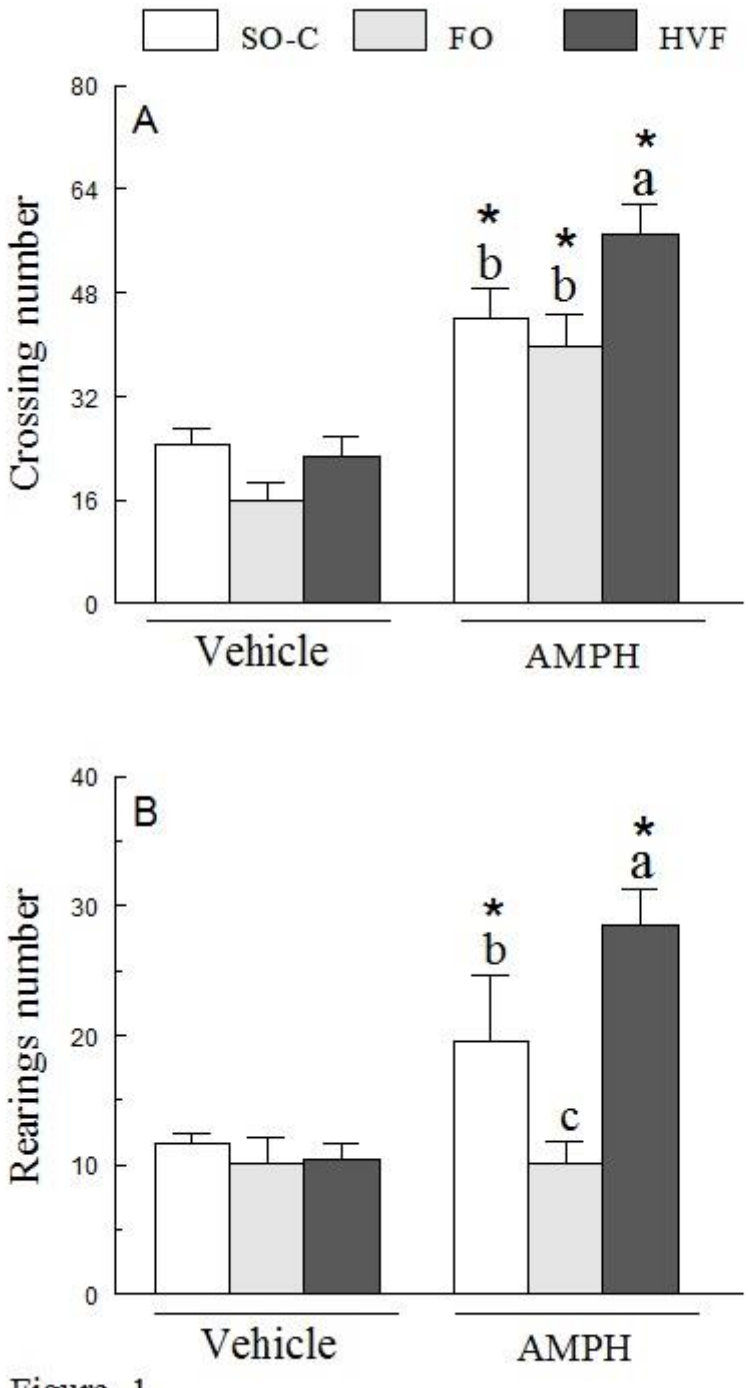
SO-C
FO
HVF
A
Crossing number
80
64
48
32
16
0
*
b
*
b
*
a
Vehicle
AMPH
B
Rearings number
40
30
20
10
0
*
b
c
*
a
Vehicle
AMPH

Figure 1

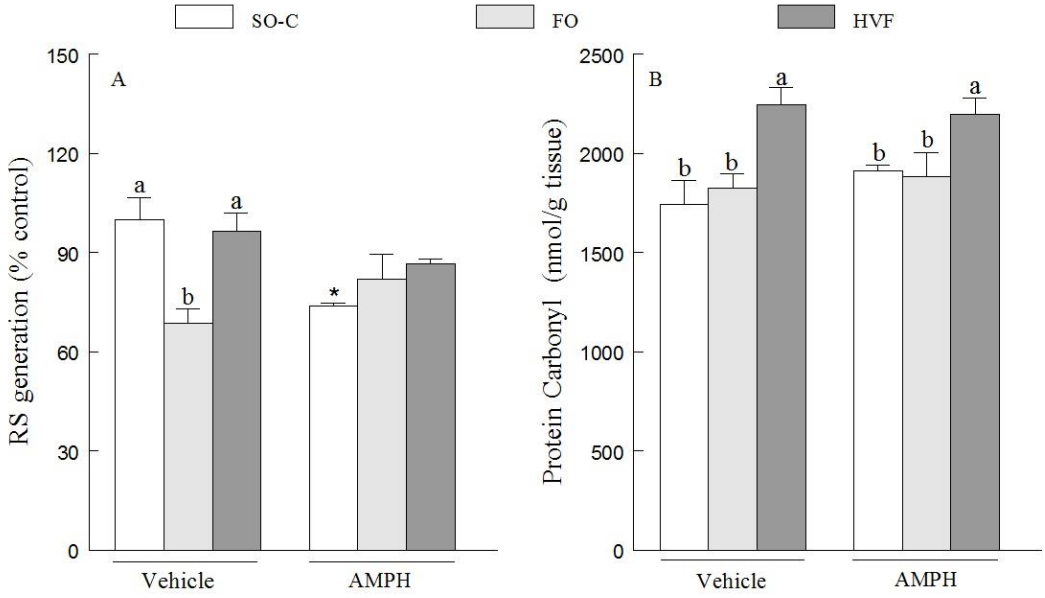
SO-C
FO
HVF
A
RS generation (% control)
150
120
90
60
30
0
a
b
a
*
Vehicle
AMPH
B
Protein Carbonyl (nmol/g tissue)
2500
2000
1500
1000
500
0
b
b
a
b
b
a
Vehicle
AMPH

Figure 2

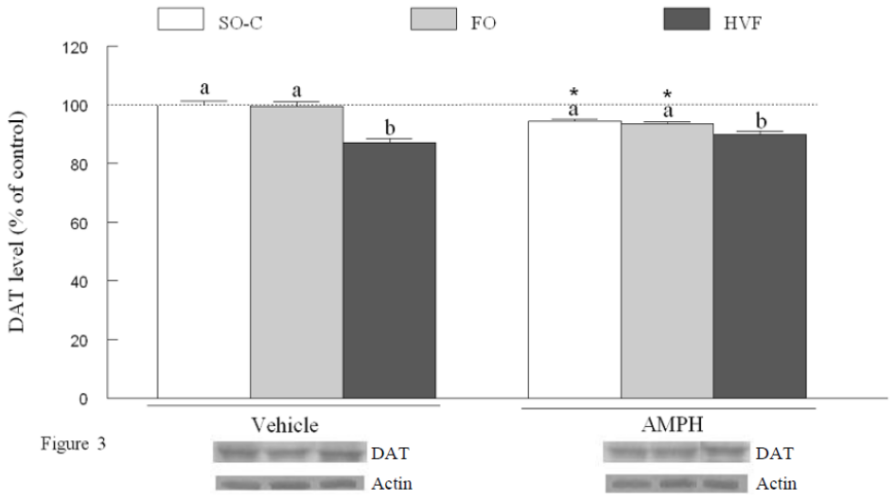
SO-C
FO
HVF
DAT level (% of control)
120
100
80
60
40
20
0
a
a
b
*
a
*
a
b
Vehicle
AMPH
DAT
Actin
DAT
Actin

Figure 3

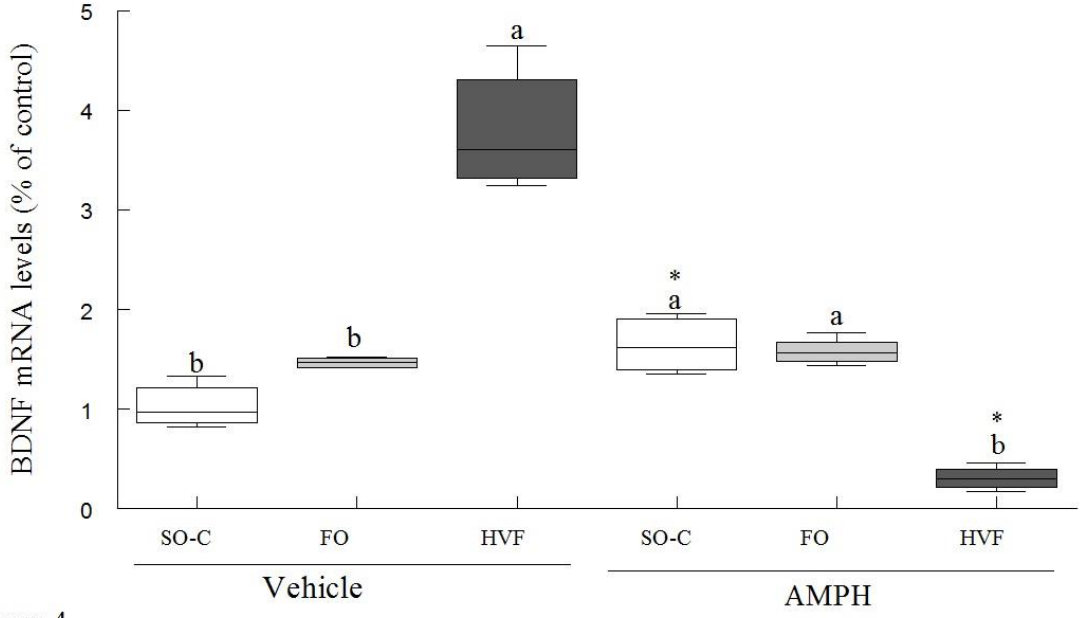
BDNF mRNA levels (% of control)
5
4
3
2
1
0
a
b
b
*
a
a
*
b
SO-C
FO
HVF
SO-C
FO
HVF
Vehicle
AMPH

Figure 4

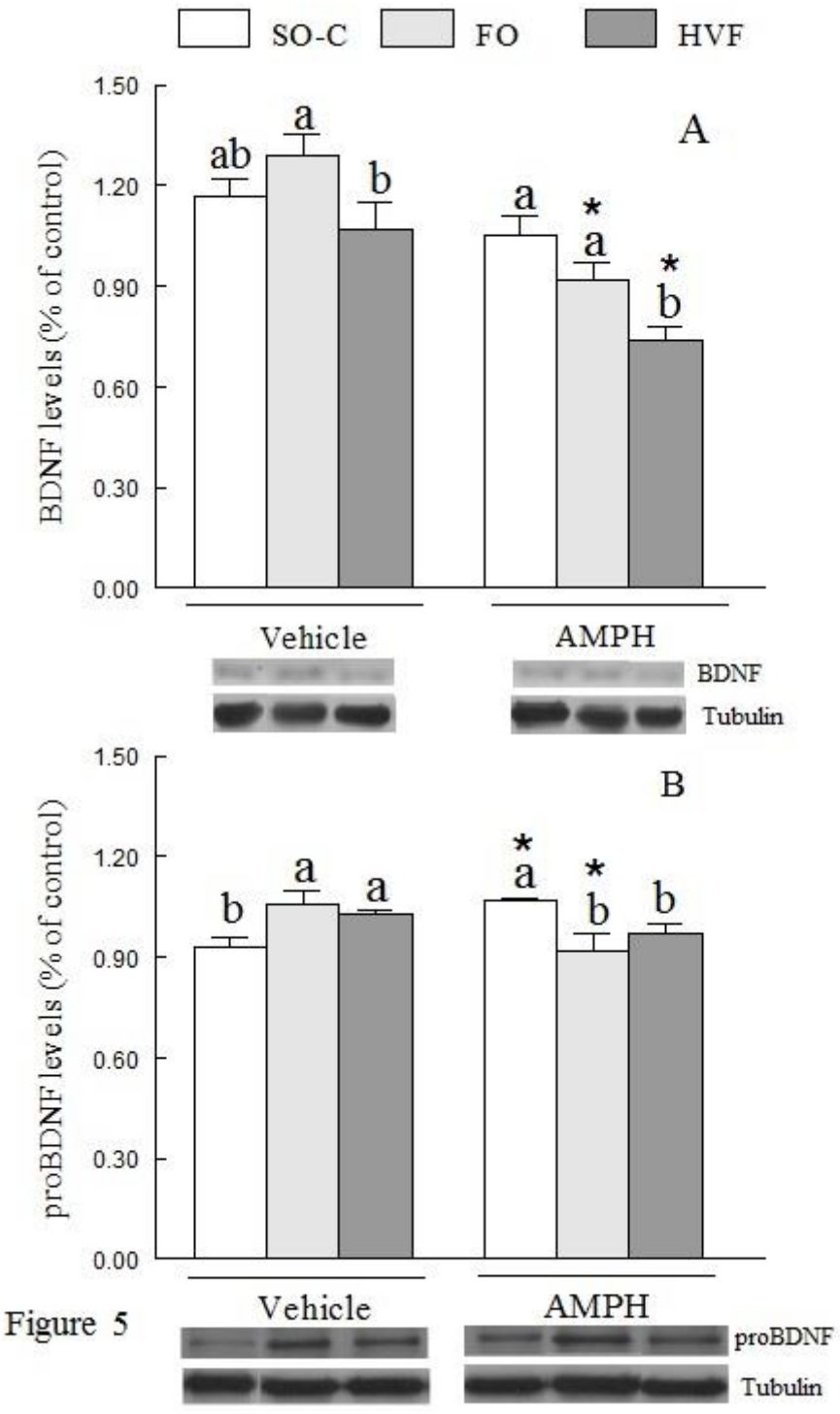
SO-C
FO
HVF
A
BDNF levels (% of control)
1.50
1.20
0.90
0.60
0.30
0.00
ab
a
b
a
*a
*b
Vehicle
AMPH
BDNF
Tubulin
B
proBDNF levels (% of control)
b
a
a
*a
*b
b
Vehicle
AMPH
proBDNF
Tubulin

Figure 5

3.4 Manuscrito 3

TRANS FAT SUPPLEMENTATION OVER TWO GENERATIONS OF RATS EXACERBATES BEHAVIORAL AND BIOCHEMICAL DAMAGES IN A MODEL OF MANIA: CO-TREATMENT WITH LITHIUM

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Trans fat supplementation over two generations of rats exacerbates behavioral and biochemical damages in a model of mania: Co-treatment with lithium

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Abstract

We investigated whether *trans* fat supplemented over two generations of rats could alter neuronal membranes and influence mania-like behaviors, as well as the effects of lithium (Li). Two sequential generations of female rats were supplemented with soybean oil (SO-C, rich in *n-6* fatty acids - FA), an isocaloric control group, and hydrogenated vegetable fat (HVF, rich in *trans* fatty acids - TFA). Male rats born from 1st and 2nd generations were maintained in the same original supplementation until adulthood, when they were exposed to an animal model of mania amphetamine (AMPH)-induced and co-treated with Li or not.

AMPH increased locomotion of both generations and this influence was higher in the HVF than in the SO-C group. Conversely, AMPH increased long-term memory in SO-C group of 2nd generation. All behavioral parameters were leveled out by Li co-treatment in both generations. HVF supplementation allowed hippocampal TFA incorporation in rats of both generations (0.1 and 0.2%, respectively). Oxidative parameters indicated higher levels of protein carbonyl (PC) in HVF group with no changes in catalase (CAT) activity of 1st generation. In the 2nd generation of animals, AMPH increased PC levels of both experimental groups, whereas CAT activity was lower *per se* in HVF group only. The co-treatment with Li leveled out PC levels and CAT activity indicating a significant neuroprotective role. These findings suggest that chronic HVF consumption allows a rising incorporation of TFA in the brain, which may be reflected on the neuropsychiatric conditions related to mania, whereas the effects of Li are not modified in the course of this harmful dietary habit.

Keywords: Generations, fat supplementation, animal model of mania, oxidative stress.

Introduction

Brain membrane phospholipids consist basically of fatty acids (FA) provided by the diet. In developmental periods such as pregnancy and infancy, this FA supply is particularly critical (Bourre *et al.*, 1989, Wauben *et al.*, 2001). Also, the type of FA incorporated into membranes modifies their fluidity, signal transduction and transcription factors (Passos *et al.*, 2012, Karr *et al.*, 2011, Acar *et al.*, 2003, Jump, 2002). Clinical studies have showed that prior to birth the different FA accumulated by the fetus must originate from maternal circulation through placental transfer, and after birth they must derive from milk and later from complementary foods. Thus, the deficiency of essential lipids during initial stages of life may have long-term consequences for neural development (Innis, 2007, 2005, 2003).

The concern regarding the Western dietary habits has risen due to the increase in consumption of processed foods, rich in *trans* fatty acids (TFA), which is starting in early years (Simopoulos, 2002, 1999, Allison *et al.*, 1999, Martinetz, 1992). TFA are produced by the hydrogenation of vegetable oils and their excessive consumption has been related to biological impairments such as significant loss of essential fatty acids (EFA) (Pettersen and Opstvedt, 1992), suppression of *delta-6* and *delta-5* desaturases (Mahfouz *et al.*, 1984), which affect the synthesis of long-chain polyunsaturated fatty acids (LC-PUFA) (Souza, Rocha and Carmo, 2012, Larqué *et al.* 2003, 2001, Cook, 1981). Moreover, animal studies in our group revealed the influence of TFA supplementation on stress (Pase *et al.*, 2013), drug preference (Kuhn *et al.*, 2013) and the development of neuropsychiatric disorders (Trevizol *et al.*, 2013, 2011, Teixeira *et al.*, 2012), including bipolar disorder (BD).

This neuropsychiatric condition is characterized by recurrent cycles of mania and depression, often associated with co-morbidities and suicide, thereby undermining the social and professional life (Kupfer 2005, Judd and Akiskal, 2003, Tohen *et al.*, 2003). The BD pathophysiology remains unclear, and different stages are associated with different neurobiological mechanisms (Einat and Manji, 2006). The neurotransmission imbalance is a neurobiological basis that has gained force due to its relation to other pathways proposed to explain this illness, such as oxidative stress (OS), inflammation (arachidonic acid - AA cascade) and neurotrophic factors (Berk *et al.*, 2011, Jump, 2002). The animal model of mania amphetamine (AMPH)-induced is well documented (Trevizol *et al.* 2013, 2011, da-Rosa *et al.* 2012, Frey *et al.*, 2006 a,b) and induces mania-like behaviors in animals, such as sensitization and hyperactivity, which can be attenuated or reversed by classical mood-stabilizing drugs such as lithium (Li).

Taking into account that dietary FA were reported as one of the major factors capable of modulating OS in the brain (Virgili *et al.,* 1996), and may preferentially deplete key FA (Jacka *et al.,* 2010b), this could result in further vulnerability to psychiatric illnesses in general. We designed this study to assess the influence of hippocampal FA incorporation over two generations of rats, and whether this incorporation may facilitate the development of an animal model of mania AMPH-induced and has effects on Li activity according to behavioral and biochemical parameters.

Materials and Methods

Animals

Male and female *Wistar* rats were used in the experimental protocols. They were housed four per cage in Plexiglass cages with free access to food and water in a room with controlled temperature (23° C±1° C), on a 12-hour light/ 12-hour dark cycle throughout the experimental period. All procedures with animals were approved by the Ethics Research Committee of Universidade Federal de Santa Maria (UFSM-24/2010), which is affiliated to the National Council for the Control of Animal Experiments (CONCEA), following international norms of care and animal maintenance, and all efforts were made to minimize the number of animals used and their suffering.

Experimental design

Protocol I – *Effects of different supplementations on the first generation of rats submitted to an animal model of mania AMPH-induced and co-treated with lithium or not.*

One week prior to mating, female adult *Wistar* rats (n=6) were divided into two experimental groups supplemented (3g/Kg; p.o.) (Pase *et al.*, 2013, Kuhn *et al.*, 2013, Trevizol *et al.* 2013) with soybean oil (SO-C), an isocaloric control group due to its adequate levels of polyunsaturated FA, *n-6/n-3* ratio within acceptable limits and its elevated consumption worldwide (Teixeira *et al.*, 2011, 2012, Viola and Viola, 2009, Yehuda *et al.*, 2005, Simopoulos, 2002) or hydrogenated vegetable fat (HVF), rich in TFA. The FA profiles of supplementations are described by Trevizol *et al.* 2013. Female rats were maintained under the same supplementation throughout pregnancy and lactation. From weaning (postnatal day

21), one male pup from each litter (n=6) was grouped and maintained under the same oral treatment until 90 days of age when they were submitted to an animal model of mania described as follows.

Protocol II – *Effects of different supplementations on the second generation of rats submitted to an animal model of mania AMPH-induced and co-treated with lithium or not.*

Animals were maintained under the same supplementation and environmental conditions as in *protocol I* until the birth of first generation. One female pup of each litter was maintained on the same supplementation until adulthood, when they were mated. One male pup from each litter (n=6) from those dams was grouped and maintained under the same oral treatment until 90 days of age when they were submitted to the animal model of mania.

Animal model of mania

At 90 days of age, half of each experimental group supplemented with SO-C or HVF was submitted to an animal model of mania. Male rats received a single daily injection of AMPH (4mg/kg/ip) or vehicle (saline) for 14 days (adapted from Frey *et al.*, 2006a,b). From the 8^{th} to the last day (14^{th} day) of AMPH administration half of the animals in each group were co-treated with Li (35mg/kg/ip) or vehicle (saline) twice a day. Twenty-four hours after the last injection, animals were submitted to behavioral assessments followed by euthanasia for the biochemical assays.

Behavioral assessments

Open-field test

Animals were placed individually in the center of an open-field arena (40 x 40 x 30 cm) enclosed by matte black walls and floor divided into squares, as previously described by Kerr *et al.*, 2005. The number of crossings (horizontal squares crossed) was recorded for 5 minutes and used as measures of spontaneous locomotor activity. Locomotor activity was briefly assessed 2 hours after the last AMPH or vehicle injection (Frey *et al.*, 2006a, b). The open field arena was cleaned with a 5% alcohol solution between the sessions.

Novel object recognition task (NORT)

This paradigm is related to the natural motivation of animals to explore novelties, being considered an innate instinct they use to recognize their environment (Heldt *et al.*, 2007). The NORT was carried out in the same open-field arena 24 hours after the locomotor status observations. Recognition index was assessed as previously described (De Lima *et al*. 2005; Schröder *et al*., 2003). The arena floor was covered with sawdust (from bedding material) during the recognition memory training and test trial. On the first day, rats were given one training trial in which they were exposed to two identical objects (A1 and A2, double Lego toys) positioned in two adjacent corners, 9cm from the walls, and were allowed to freely explore the objects for 10min (training session). The long-term memory test was performed 24 hours after the training session. The rats were allowed to explore the arena for 5min in the presence of two objects: the familiar object A and a second novel object C, placed at the same locations as in the training session. All objects had similar textures, colors, and sizes, but distinctive shapes. The objects were cleaned between trials with a 5% alcohol solution. Exploration was defined as sniffing or touching the object with the nose. A recognition index calculated for each animal was expressed by the ratio TN/(TF + TN). (TF = time spent exploring the familiar object; TN = time spent exploring the novel object).

Tissue preparations

Animals of each experimental group were anesthetized (sodium pentobarbital, 50mg/kg body weight ip) and euthanized by cervical decapitation. Their brains were immediately removed, maintained on ice, and cut coronally at the caudal border of the olfactory tubercle for hippocampus removal (Paxinos and Watson, 2007). One half of each tissue was homogenized with 0.1 M Tris-HCl, pH 7.4 and centrifuged at 3000xg (10 min), and the supernatants were used for biochemical assays. Another half of each hippocampus was used to determine the FA profile showed in Table 1.

Fatty acids profile of hippocampus

Fat was extracted from tissue samples using chloroform and methanol as described by Bligh and Dyer 1959. In order to prevent lipid oxidation during and after extraction, 0.02% butyl hydroxy toluene was added to the chloroform used. FA composition was determined by

gas chromatography. Fat was saponified in methanolic KOH solution and then esterified in methanolic H_2SO_4 solution (Hartmann e Lago, 1973). Methylated fatty acids were analyzed using an Agilent Technologies gas chromatograph (HP 6890N) equipped with a capillary column DB-23 (60 m x 0.25 mm x 0.25 μm) and flame ionization detector. The temperature of the injector port was set at 280°C and the carrier gas was nitrogen (0.9 mL/min). After injection (1 μL, split ratio 50:1), the oven temperature was held at 160°C for 1 min, then it was increased to 240°C at 4°C/min and held at this temperature for 9 min. Standard FA methyl esters (37-component FAME Mix, C 22:5n3 and PUFA no. 2 from Sigma, Saint Louis, MO, USA, and C 22:5n-6 from NuChek Prep. Inc., Elysian, MN, USA) were run under the same conditions and the subsequent retention times were used to identify the FA. FA were expressed as percentage of the total FA content.

Biochemical assays

Catalase (CAT) activity

The hippocampal enzyme activity was spectrophotometrically quantified by the method of Aebi (1984), which includes monitoring the disappearance of H_2O_2 in the presence of the cell homogenate (pH 7.0 at 25°C) at 240 nm. The enzymatic activity was expressed in nmol H_2O_2/min/g tissue.

Protein carbonyl (PC) determination

The oxidative damage to hippocampal proteins was assessed with the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) as previously described (Yan *et al.*, 1995, Levine *et al.*, 1994,), with some modifications. Proteins were briefly precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH. The quantification of protein carbonyls in the samples was determined in the absorbance of 370 nm. The results were expressed in nmol/g tissue.

Statistical analysis

The FA content measured in hippocampus was analyzed by one-way ANOVA followed by Duncan's test. Behavioral and biochemical assessments were analyzed by two-way ANOVA followed by Duncan's multiple range test when appropriate. Value of $P<0.05$ was considered significant for all comparisons made.

Results

Locomotor activity of first and second generation of rats assessed in Open Field is shown in Figure 1.

Duncan's test showed no changes of supplementation *per se* in locomotor activity of animals of 1st and 2nd generation (Fig. 1A, B). AMPH administration increased crossing in SO-C and HVF groups of both generations, although HVF presented higher increase than SO-C group (Fig. 1A, 1B). The co-treatment with Li caused a reduction in locomotor activity in both groups of both generations (Fig. 1A, 1B).

Long-term memory of first and second generation of rats assessed in the novel object recognition test (NORT) is shown in the Figure 2.

The post hoc test showed that no influence of neither supplementation nor AMPH-administration was observed in the recognition index among animals of 1st generation (Fig. 2A), however, Li co-treatment increased this index regardless the supplementation. Regarding the animals of the 2nd generation (Fig. 2B), the post hoc test showed no differences in the supplementation *per se*, but AMPH administration increased the recognition index only in SO-C group. While SO-C group co-treated with Li showed recognition index at baseline level, Li could improve the recognition index in HVF group, and was higher than that quantified in SO-C group.

Hippocampal FA profile of first and second generation of rats is shown in Table 1.

HVF group showed a growing incorporation of TFA (0.1 and 0.2%, respectively) among animals of 1st and 2nd generation exclusively, what was not observed in SO-C group. HVF supplementation decreased ΣMUFA by 11.3% in rats of 2nd generation. Comparisons between both generations revealed a decreased incorporation of ΣSFA (5.6 and 3.0%) and Σn-6 PUFA (5.3 and 9.0%) in SO-C and HVF groups, respectively. Additionally, SO-C supplementation during two sequential generations allowed an increase of ΣMUFA (17%) as

well as a decrease in ΣPUFA (3.2%) and in Σn-3 PUFA (4.4%), while HVF supplementation was related to an increase in ΣTFA (100%) and a decrease in n-6/n-3 PUFA ratio (9.5%), compared to animals of the 1st generation in the same supplementation.

The influence of different supplementations on protein carbonyl (PC) levels in hippocampus of first and second generation of rats are shown in Figure 3.

Duncan's test showed that the different supplementation did not change PC levels *per se* in both generations of rats (Fig. 3A and 3B). Among animals of 1st generation, AMPH administration increased this oxidative parameter in HVF group (Fig 3A). Among animals of 2nd generation, AMPH increased PC levels in both SO-C and HVF groups, and the values were similar (Fig. 3B). Interestingly, Li co-treatment leveled out PC levels in SO-C and HVF groups of both 1st and 2nd generations (Fig. 3A and 3B).

The influence of different supplementations on catalase (CAT) activity in hippocampus of first and second generation of rats is shown in Figure 4.

Regarding animals of the 1st generation, the different supplementations exerted no effects *per se* in CAT activity. In addition, while AMPH administration caused no significant changes in CAT activity of both supplemented groups, Li co-treatment decreased the activity of this enzyme and values were similar in both groups (Fig. 4A).

In the 2nd generation of rats, HVF supplementation was related to a reduced CAT activity *per se* compared to SO-C group. While AMPH administration caused no significant changes in this antioxidant defense in both supplemented groups, Li co-treatment decreased CAT activity in SO-C and no changes occurred in HFV group, whose values remained the same (Fig. 4B).

Discussion

The main purpose of this study was to assess whether the supplementation with *trans* fat during two sequential generations of rats could alter the composition of hippocampal membranes, thus affecting the susceptibility to develop mania-like symptoms AMPH-induced, as well as Li effects. Our current findings showed that the type of fat or oil supplemented from mating to adulthood of two generations of rats could alter the FA profile

in the hippocampus of both generations, thus affecting behavioral and biochemical parameters related to mania development with no changes on Li activity.

It is important to highlight that studies regarding Western societies have shown a growing incidence of neuropsychiatric diseases (Jacka *et al.,* 2010a, 2010b, Hibbeln *et al.,* 2006, Narrow, 2002), raising concerns about their etiology (Arnett, 1999; Sevgi, 2006) including dietary habits. As a matter of fact, dietary factors appear to be closely related to additional risks of progress of those diseases. Remarkably, the chronic consumption of *trans* fat may be of substantial interest considering that it has increased lately in early years (Bauer and Waldrop, 2009, Osso *et al.,* 2008, Innis, 2007, Allison *et al.,* 1999,) and studies do not agree in findings regarding the adequate amount of *trans* FA in diets as well as EFA (Rocha e Souza, 2012).

The experimental sensitization AMPH-induced has been used as a well-documented animal model of mania (Frey *et al.,* 2006a,b; Trevizol *et al.,* 2011, 2012). The interesting fact in the current study was that HVF supplementation was related to an increased hyperlocomotion induced by AMPH, which has been considered a mania-like symptom. However Li treatment was able to control this behavioral parameter regardless the supplementation and the generation.

Few studies have investigated the effects of *trans* FA consumption in early years and its consequences in adult life regarding memory parameters (Allison *et al.,* 1999, Collison *et al.,* 2010, Gibson *et al.,* 2011). An interesting and innovative finding was observed in animals of 2nd generation: when HVF supplementation hindered the improvement of memory AMPH-induced, this improvement occurred in SO-C group. Li treatment leveled out memory performance in animals of both generations regardless the supplementation. Literature lacks studies that refer to the consumption of *trans* FA in early years and its consequences on behaviors related to neuropsychiatric conditions, including BD. In fact, previous studies of our group showed the impairments caused by *trans* fat on this same animal model of mania, when consumed from weaning and during the growth of pups (Trevizol *et al.,* 2011), as well as during pregnancy and lactation (Trevizol *et al.,* 2013). However, no comparative study between sequential generations of animals supplemented with *trans* fat and its influence on mania-like behavior and Li effects was developed.

The exact mechanism related to these behavioral impairments observed in animals supplemented with HVF is unknown although it is possible to suggest the involvement of TFA incorporation in hippocampal membranes. In fact, our findings showed the incorporation of TFA in hippocampus of the group supplemented with HVF in a time-dependent manner,

once rats from 2nd generation showed twice the incorporation in relation to the ones of 1st generation. We can hypothesize that prolonged consumption of *trans* fat allowed a small but significant TFA incorporation in the hippocampus, indicating that this type of FA may be modifying the membrane fluidity and neurotransmitters release, thus impairing locomotion and blocking the increased memory performance induced by AMPH. Of particular importance to our findings are the significant changes in the synaptic plasticity and fluidity of membranes due to TFA incorporation in the brain (Larqué *et al.*, 2003) as well as its affecting dopamine (DA) neurotransmission (Acar *et al.*, 2002), which have been reported.

It is well-known that DA is a pivotal neurotransmitter in brain areas related to behaviors such as locomotion and memory, especially in the mesolimbic connections, which include the hippocampus (Furini *et al.*, 2014, Mallo *et al.*, 2007, Baik *et al.* 1995). Considering DA release, it is important to emphasize that in addition to inhibiting the vesicular monoamine transporter (DAT2), AMPH enhances DA releases from vesicular storage pools, along with the inhibition of the activity of monoamine oxidase (MAO), thus reducing cytosolic DA metabolism (Sulzer, 2011) and increasing the neurotransmitter level. The excess of extracellular DA favors its autoxidation and consequently the generation of reactive species (RS) (Graham *et al.*, 1978), whose accumulation may be hazardous to neurons and tissues.

Considering that OS is one of the pathways implicated in the pathophysiology of BD (Kuloglu *et al.*, 2002, Ozcan *et al.*, 2004, Machado-Vieira *et al.*, 2007, Brunning *et al.*, 2012, Trevizol *et al.*, 2013), and AMPH may increase the general screening of oxidative status, we verified that in our study. AMPH administration increased the PC levels in HVF-supplemented animals of both generations, and exerted no influences *per se*. Similarly, the type of supplementation *per se* did not change the hippocampal CAT activity in animals of 1st generation, but HVF supplementation decreased the activity of this antioxidant in rats of 2nd generation. Again, our results revealed that Li co-treatment could stabilize oxidative markers such as PC levels and CAT activity, regardless the supplementation or the generation, confirming its fundamental role in mania-like disorders. Our study is in accordance with Frey (2006b), who suggested that Li exerts neuroprotective effects on oxidative damages induced by AMPH, and also acts against inflammatory processes (Bazinet *et al.*, 2006, Lee *et al.*, 2008, Goldstein *et al.*, 2009). In this sense, Li could act directly on the AA cascade, decreasing the AA mobilization from brain membranes, which is most likely related to the generation of less pro-inflammatory metabolites. Therefore, Li may minimize this cascade,

exerting protection against the development of oxidative damage by both pathways (Berk *et al.*, 2012, Kim *et al.*, 2011, Rapoport et al., 2009, Rao and Rapoport, 2009).

Conclusion

In summary, we observed that TFA from processed foods might be significantly incorporated into brain areas such as hippocampus through generations, thus facilitating the development of neuropsychiatric conditions, such as mania. As a consequence, AMPH effects were altered increasing the susceptibility to hyperactivity and impairing long-term memory. Besides behavioral changes, TFA incorporation in the brain may interfere with the generation of more pro-inflammatory metabolites affecting the oxidative pathway, reducing the antioxidant defenses and favoring the development of oxidative damages. In addition, our findings showed that hippocampal incorporation of TFA exerted no influences on Li effects, which could balance both behavioral and biochemical parameters regardless the generation. Our initial hypothesis was partially confirmed and the outcome suggests that prolonged consumption of *trans* fat in early years may increase the vulnerability to the development of behavioral disturbances related to neuropsychiatric disorders in adulthood.

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References

Acar N. et al. Modification of the dopaminergic neurotransmitters in striatum, frontal cortex and hippocampus of rats fed for 21 months with *trans* isomers of a-linolenic acid. Neurosci Res 2003; 45: 375–82.

Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–126. Allison DB. et al. Estimated intakes of *trans* fatty and other fatty acids in the US population. J Am Diet Assoc 1999; 99: 166–74.

Amminger et al. Long-Chain ^{6}_w-3 Fatty Acids for Indicated Prevention of Psychotic Disorders. Arch Gen Psychiatric 2010; 67.

Arnett JJ. Adolescent storm and stress, reconsidered. Am Psychol 1999; 54: 317–26.

Bagga D. et al. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proc Natl Acad Sci 2003; 100: 1751-56.

Baik JH. et al. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 1995; 377: 424–8.

Bauer LR, Waldrop j. *Trans* fat intake in children: risks and recommendations. Pediatr Nurs. 2009; 35: 346-51.

Bazinet, R.P., et al. Chronic carbamazepine decreases the incorporation rate and turnover of arachidonic acid but not docosahexaenoic acid in brain phospholipids of the unanesthetized rat: relevance to bipolar disorder. Biol. Psychiatry 2006; 59: 401–407.

Bazinet RP. Is the brain arachidonic acid cascade a common target of drugs used to manage bipolar disorder? Biochem. Soc. Trans. 2009; 37: 1104–1109.

Berk et al. Pathways underlying neuroprogression in bipolar disorder: Focus on inflammation, oxidative stress and neurotrophic factors Neuroscience and Biobehavioral Reviews 2011; 35: 804–17.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37: 911–7.

Bourre JM. et al. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. J Nutr 1989; 119: 1880–92.

Bruning et al. Diphenyl diselenide ameliorates behavioral and oxidative parameters in na animal model of mania induced by ouabain. Progress in Neuro-Psychopharmacology e Biological Psychiatry 2012; 38: 168–174.

Collison et al. Dietary *trans*-fat combined with monosodium glutamate induces dyslipidemia and impairs spatial memory. Physiology e Behavior 2010; 99: 334–342.
Cook HW. The influence of *trans*-acids on desaturation and elongation of fatty acids in developing brain. Lipids 1981; 16: 920–6.

Cott J. Omega-3 fatty acids and psychiatric disorders. Alternat Therap women's health 1999; 1: 97-104.

Da-Rosa DD. et al. Effects of lithium and valproate on oxidative stress and behavioral changes induced by administration of m-AMPH. Psychiatry Research 2012; 198: 521–26.

De Lima MN. et al. Reversal of age-related deficits in object recognition memory in rats with L-deprenyl. Exp Gerontol 2005; 40: 506–11.

Delas I. Benefits and hazards of fat-free diets Trends in Food Science & Technology 2011; 22: 576-582.

Einat H, Manji HK. Cellular Plasticity Cascades: Genes-To-Behavior Pathways in Animal Models of Bipolar Disorder. Biol Psychiatry 2006; 59: 1160 –71.

Fernstrom JD. Effects of dietary polyunsaturated fatty acids on neuronal function. Lipids 1999; 34: 161–9.

Ferraz AC. et al. Chronic Ω-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol. Behav Brain Res 2011; 219: 116–22.

Frey et al. Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania. Bipolar Disorders 2006a; 8: 275–280.

Frey et al. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J Psychiatry Neurosci 2006b;

Furini CRG. et al. D1 and D5 dopamine receptors participate on the consolidation of two different memories. Behavioural Brain Research 2014; 271, 212–217.

Gibson RA, Muhlhausler B, Maria Makrides M. Conversion of linoleic acid and alphalinolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. Maternal and Child Nutrition 2011; 7: 17–26.

Goldstein BI. et al. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. J. Clin. Psychiatry 2009; 70: 1078–1090.

Graham DG. et al. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. Mol Pharmacol 1984; 14: 644–53.

Greiner RS. Rats with Low Levels of Brain Docosahexaenoic Acid Show Impaired Performance in Olfactory-Based and Spatial LearningTask. Lipids 1999; 34.

Hamazaki K, Choi KH, Kim HY. Phospholipid profile in the postmortem hippocampus of patients with schizophrenia and bipolar disorder: no changes in docosahexaenoic acid species. J Psychiatr Res 2009.

Hartman L, Lago BC. A rapid preparation of fatty methyl esters from lipids. Lab Pract 1973; 22: 475–7.

Heldt AS. et al. Hippocampus specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. Mol Psychiatry 2007; 12: 656–70.

Hibbeln JR. et al. Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. Am J Clin Nutr 2006; 83: 1483–93.

Igarashi M. et al. Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain. J Lipid Res 2007; 48: 2463– 70.

Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr 2003; 143: 1–8.

Innis SM. Essential Fatty Acid Transfer and Fetal Development. Placenta 2005; 26: 70-75.

Innis SM. Dietary (n−3) fatty acids and brain development. J Nutr 2007; 137: 855–9.
Jacka FN. et al. Association of Western and traditional diets with depression and anxiety in women. Am J Psychiatry 2010a; 167: 305–11.

Jacka FN. et al. The role of omega-3 fatty acids in the prevention and treatment of the common mental disorders. J. Affect Disord 2010.

Judd LL, Akiskal HS. The prevalence and disability of bipolar spectrum disorders in the US population: Re-analysis of the ECA database taking into account subthreshold cases. J Affect Disord 2003; 73:123–31.

Jump DB. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr Opin Lipidol 2002; 13: 155–64.

Karr JE, Alexander JE, Winningham RG. Omega-3 polyunsaturated fatty acids and cognition throughout the lifespan: a review. Nutr Neurosci 2011; 14: 216-25.

Kavraal S. Maternal intake of Omega-3 essential fatty acids improves long term potentiation in the dentate gyrus and Morris water maze performance in rats. Brain Research 2012; 1482:32–39.

Kerr DS. et al. Angiotensin II blocks memory consolidation through an AT2 receptor dependent mechanism. Psychopharmacology 2005; 179: 529–35.

Kim HJ, Thayer SA. Lithium Increases Synapse Formation between Hippocampal Neurons by Depleting Phosphoinositides Mol Pharmacol 2009; 75: 1021–1030.
Kim SJ. et al. Omega-3 and omega-6 fatty acids suppress ER- and oxidative stress in cultured neurons and neuronal 2010.

Kuhn FT. et al. Influence of *trans* fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats. Pharmacology, Biochemistry and Behavior 2013; 110: 58–65.

Kuloglu M. et al. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. Cell Biochem Funct 2002; 20: 171–5.

Kupfer DJ. The increasing medical burden in bipolar disorder. JAMA 2005; 293: 2528 –30.

Larqué E, Zamora S, Gil A. Dietary *trans* fatty acids in early life: a review. Early Hum Dev 2001; 65: 31–41.

Larqué E. et al. Dietary *trans* fatty acids alter the compositions of microsomes and mitochondria and the activities of microsome delta-6-fatty acid desaturase and glucose-6-phosphatase in livers of pregnant rats. J Nutr 2003; 133: 2526–31.

Lee HJ. et al. Chronic administration of lamotrigine downregulates COX-2 mRNA and protein in rat frontal cortex. Neurochem. Res. 2008; 33: 861–866.

Levine RL. et al. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 1994; 233: 346–57.

Luchtman & Song. Cognitive enhancement by omega-3 fatty acids from child-hood to old age: Findings from animal and clinical studies. Neuropharmacology 2013; 64: 550-565.

Machado-Vieira R. et al. Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. Neurosci Lett 2007; 421: 33–6.

Mahfouz MM, Smith T, Kummerow FA. Effect of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat liver microsomes. Lipids 1984; 19: 214–22.

Mallo T. et al. Rats with persistently low or high exploratory activity: Behaviour in tests of anxiety and depression, and extracellular levels of dopamine. Behavioural Brain Research 2007; 177: 269–281.

Martinetz M. Tissue levels of polyunsaturated fatty acids during early human development, J Pediatr 1992; 120: 129–38.

McCormick CM, Ibrahim FN. Locomotor activity to nicotine and Fos immunoreactivity in the paraventricular nucleus of the hypothalamus in adolescent socially-stressed rats. Pharmacol Biochem Behav 2007; 86: 92-102.

Narrow WE. et al. Revised prevalence estimates of mental disorders in the United States. Arch Gen Psychiatry 2002; 59: 115–23.

Neuringer M, Anderson GJ, Connor WE. The essentiality of n−3 fatty acids for the development and function of the retina and brain. Annu Rev Nutr 1988; 8: 517–41.

Osso FS. et al. *Trans* fatty acids in maternal milk lead to cardiac insulin resistance in adult offspring. Nutrition 2008; 24: 727–732.

Ozcan ME. et al. Antioxidant enzyme activities and oxidative stress in affective disorders. Int Clin Psychopharmacol 2004; 19: 89–95.

Ozkul A. et al. Effects of Lithium and Lamotrigine on Oxidative-Nitrosative Stress and Spatial Learning Deficit After Global Cerebral Ischemia. Neurochem Res. 2014; 25. [Epub ahead of print]

Parker G. et al. Omega-3 fatty acids and mood disorders. Am. J. Psychiatry 2006; 163: 969–978.

Pase CS. et al. Influence of perinatal *trans* fat on behavioral responses and brain oxidative status of adolescent rats acutely exposed to stress. Neuroscience 2013; 247: 242–52.

Passos PP. et al. Dopaminergic cell populations of the rat substantia nigra are differentially affected by essential fatty acid dietary restriction over two generations. Journal of Chemical Neuroanatomy 2012; 44: 66–75.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. Amsterdam: Elsevier; 2007.

Pettersen J, Opstvedt J. Fatty acid composition of lipids of the brain and other organs in suckling piglets. Lipids 1992; 27: 761-69.

Qiu X. Biosynthesis of docosahexaenoic acid (DHA, 22:6-4, 7,10,13,16,19): two distinct pathways Prostaglandins, Leukotrienes and Essential Fatty Acids 2003; 68: 181–186.

Rao JS, Rapoport SI. Mood-Stabilizers target the brain arachidonic acid cascade Curr Mol Pharmacol 2009; 2: 207-214.

Rapoport SI. et al. Bipolar disorder and mechanisms of action of mood stabilizres. Brain Res Rev. 2009; 61: 185–209.

Rocha MS, Souza AS. Effects of a normolipidic diet containing *trans* fatty acids during perinatal period on the growth, hippocampus fatty acid profile, and memory of young rats according to sex. Nutrition 2012; 28: 458-464.

Schroder N, J. O'Dell S, Marshall JF. Neurotoxic Methamphetamine Regimen Severely Impairs Recognition Memory in Rats. Synapse 2003; 49: 89–96.

Sevgi S, Ozek M, Eroglu L. L-NAME prevents anxiety-like and depression-like behavior in rats exposed to restraint stress. Methods Find Exp Clin Pharmacol 2006; 28: 95–99.
Shao et al. The effect of mood stabilizer lithium on expression and activity of glutathione stransferase izoenzymes. Neuroscience 2008; 151: 518–524.

Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 1999; 70: 560-69.

Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 2002; 56: 365–79.

Songur A. et al. Hypothalamic superoxide dismutase, xanthine oxidase, nitric oxide, and malondialdehyde in rats fed with x-3 fatty acids. Prog Neuropsychopharmacol Biol Psychiatry 2004; 28: 693–698.

Souza AS, Rocha MS, Carmo MGT. Effects of a normolipidic diet containing *trans* fatty acids during perinatal period on the growth, hippocampus fatty acid profile, and memory of young rats according to sex. Nutrition 2012; 28: 458–64.

Sprecher H. Biochemistry of essential fatty acids. Prog Lipid Res 1981; 20: 13–22.
Sprecher H, Chen Q, Yin FQ. Regulation of the biosynthesis of 22:5n-6 and 22:6n-3: a complex intracellular process. Lipids 1999; 34: 153–156.

Sulzer D. How Addictive Drugs Disrupt Presynaptic Dopamine Neurotransmission. Neuron 2011; 69: 628–649.

Teixeira AM. et al. Exercise affects memory acquisition, anxiety-like symptoms and activity of membrane-bound enzyme in brain of rats fed with different dietary fats: impairments of *trans* fat. Neuroscience 2011; 195: 80–88.

Teixeira AM. et al. Could dietary *trans* fatty acids induce movement disorders? Effects of exercise and its influence on Na+K+-ATPase and catalase activity in rat striatum. Behav Brain Res 2012; 226: 504–10.

Trevizol F. et al. Comparative study between n-6, *trans* and n-3 fatty acids on repeated amphetamine exposure: a possible factor for the development of mania. Pharmacol Biochem Behav 2011; 97: 560–65.

Trevizol F. et al. Influence of lifelong dietary fats on the brain fatty acids and amphetamineinduced behavioral responses in adult rat. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2013; 45: 215–22.

Tohen M. et al. The McLean-Harvard First-Episode Mania Study: Prediction of recovery and first recurrence. Am J Psychiatry 2003; 160: 2099 –2107.

Viola P, Viola M. Virgin olive oil as a fundamental nutritional component and skin protector. Clin Dermatol 2009; 27: 159–165.

Virgili F. et al. Dietary fatty acids composition modulates oxidative stress and cellular injury in the liver of CBrCl intoxicated rats. Nutr Res 1996; 16: 1679–88.

Wauben IP. et al. Dietary *trans* fatty acids combined with a marginal essential fatty acid status during the pre- and postnatal periods do not affect growth or brain fatty acids but may alter behavioral development in B6D2F(2) mice. J Nutr 2001; 131: 1568–73.

Yan LJ, Traber MG, Packer L. Spectrophotometric method for determination of carbonyls in oxydatively modified apolipoprotein B of human low-density lipoproteins. Anal Biochem 1995; 228: 349–51.

Yehuda S, Rabinovitz S, Mostofsky DI. Essential fatty acids and the brain: from infancy to aging. Neurobiol Aging 2005; 26: 98–102.

Youdim KA, Martin A, Joseph AMJ. Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci 2000; 18: 383–99.

Figure captions

Figure 1. Locomotor activity of first (1A) and second (1B) generation of rats supplemented with SO-C or HVF was assessed in an open field test. Male rats were treated with AMPH (4mg/kg) or vehicle for 14 days. From the 8th to the last day (14th) of AMPH administration half of the animals in each group were co-treated with Li (35mg/kg/ip) or vehicle (saline) twice a day. Behavioral assessments were carried out 2 hours after the last AMPH injection. Data are expressed as mean±S.E.M. Abbreviations: SO-C: soybean oil-control; HVF: hydrogenated vegetable fat. Different lowercase letters indicate differences between supplementations in the same drug treatment (P<0.05). * indicates significant differences from vehicle in the same supplementation (P<0.05). # indicates significant differences from AMPH-administered animals in the same supplementation (P<0.05).

Figure 2. Recognition index of first (2A) and second (2B) generation of rats supplemented with SO-C or HVF was assessed on time spent on the novel object recognition test. Male rats were treated with AMPH (4mg/kg) or vehicle for 14 days, from the 8th to the last day (14th) of AMPH administration half of the animals in each group were co-treated with Li (35mg/kg/ip) or vehicle (saline) twice a day. Long-term memory retention test was performed 24 hours after training. Data are expressed as mean±S.E.M. Abbreviations: SO-C: soybean oil-control; HVF: hydrogenated vegetable fat. Different lowercase letters indicate differences between supplementations in the same drug treatment (P<0.05). * indicates significant differences

from vehicle in the same supplementation (P<0.05). # indicates significant differences from AMPH-administered animals in the same supplementation (P<0.05).

Figure 3**.** Influence of SO-C or HVF supplementation on hippocampal protein carbonyl levels of first (3A) and second (3B) generation of rats. They were treated with AMPH (4mg/kg) or vehicle for 14 days, from the 8th to the last day (14th) of AMPH administration half of the animals in each group were co-treated with Li (35mg/kg/ip) or vehicle (saline) twice a day. Data are expressed as mean±S.E.M. Abbreviations: SO-C: soybean oil-control; HVF: hydrogenated vegetable fat. Different lowercase letters indicate differences between supplementations in the same drug treatment (P<0.05). * indicates significant differences from vehicle in the same supplementation (P<0.05). # indicates significant differences from AMPH-administered animals in the same supplementation (P<0.05).

Figure 4. Influence of SO-C or HVF supplementations on hippocampal catalase activity of first (4A) and second (4B) generation of rats. They were treated with AMPH (4mg/kg) or vehicle for 14 days, from the 8th to the last day (14th) of AMPH administration half of the animals in each group were co-treated with Li (35mg/kg/ip) or vehicle (saline) twice a day. Data are expressed as mean±S.E.M. Abbreviations: SO-C: soybean oil-control; HVF: hydrogenated vegetable fat. Different lowercase letters indicate differences between supplementations in the same drug treatment (P<0.05). * indicates significant differences from vehicle in the same supplementation (P<0.05). # indicates significant differences from AMPH-administered animals in the same supplementation (P<0.05).

Table 1. Hippocampal profile of fatty acids of rats supplemented with different oil/fat in the first and second generation (% of total fatty acids identified).

	First generation		Second generation	
Fatty acid	**SO-C**	**HVF**	**SO-C**	**HVF**
Σ SFA	42.8 ±0.3	42.5 ±0.0	40.4 ±0.6*	41.2 ±0.4*
Σ MUFA	28.7±0.6	27.9 ±0.5	33.6 ±0.3a*	29.8 ±0.6b
Σ PUFA	28.0 ±0.1	27.1 ±0.3	27.1 ±0.5*	26.8 ±0.5
Σ n-3	9.1 ±0.0	8.9 ±0.0	8.7 ±0.0*	8.9 ±0.2
Σ n-6	18.9 ±0.1	18.9 ±0.3	17.9 ±0.0*	17.2±0.0*
Σ TFA	n.d.	0.1 ±0.0	n.d.	0.2 ±0.0*
n-6/n-3 ratio	2.1	2.1	2.0	1.9*

Different lowercases indicate significant difference between SO-C and HVF groups in first or second generation ($P<0.05$). * Indicate significant difference between 1st and 2nd generation in the same supplementation ($P<0.05$). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: *trans* fatty acids.

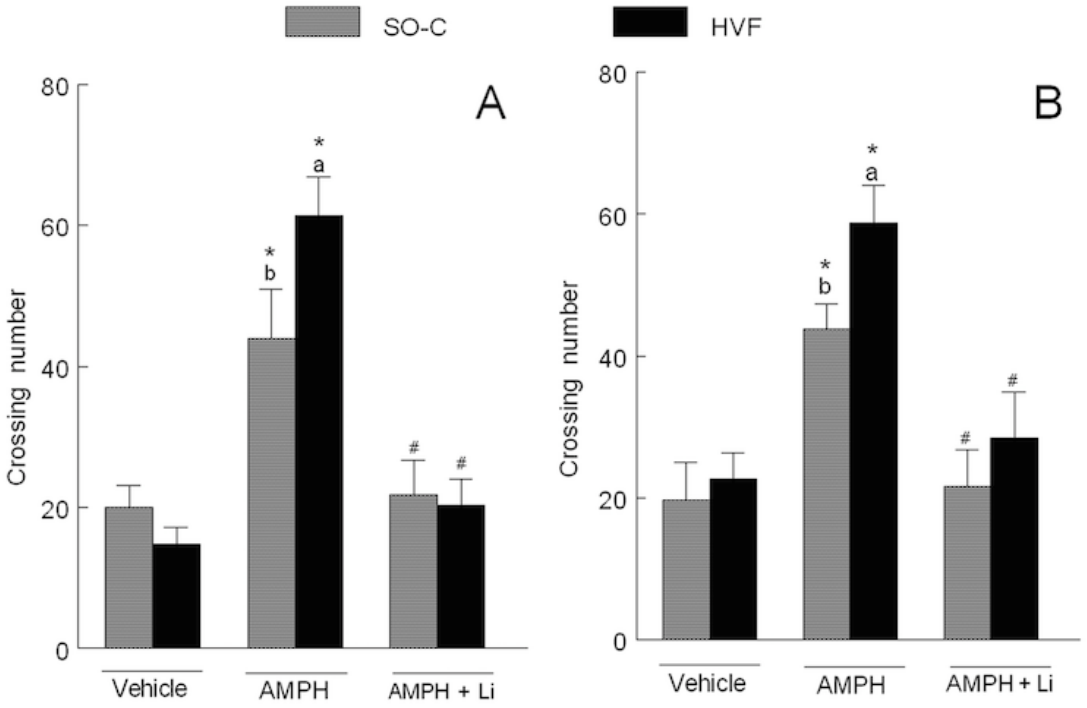
SO-C
HVF
A
B
Crossing number
80
60
40
20
0
*
a
*
b
#
Vehicle
AMPH
AMPH + Li

Figure 1

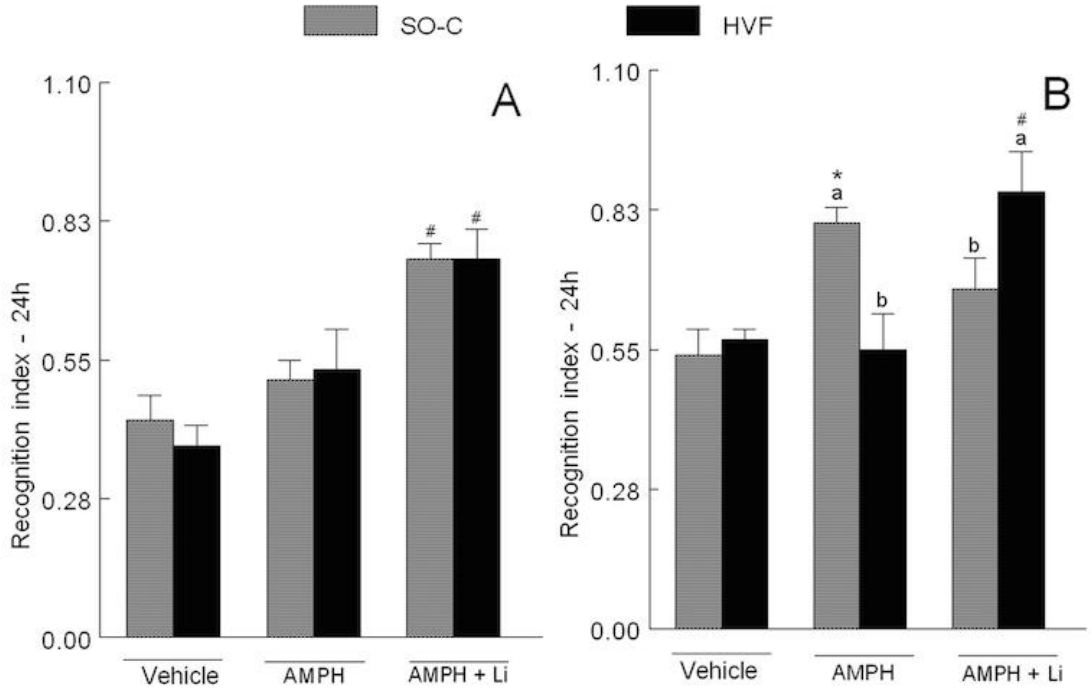
SO-C
HVF
A
B
Recognition index - 24h
1.10
0.83
0.55
0.28
0.00
Vehicle
AMPH
AMPH + Li
#
*
a
b

Figure 2

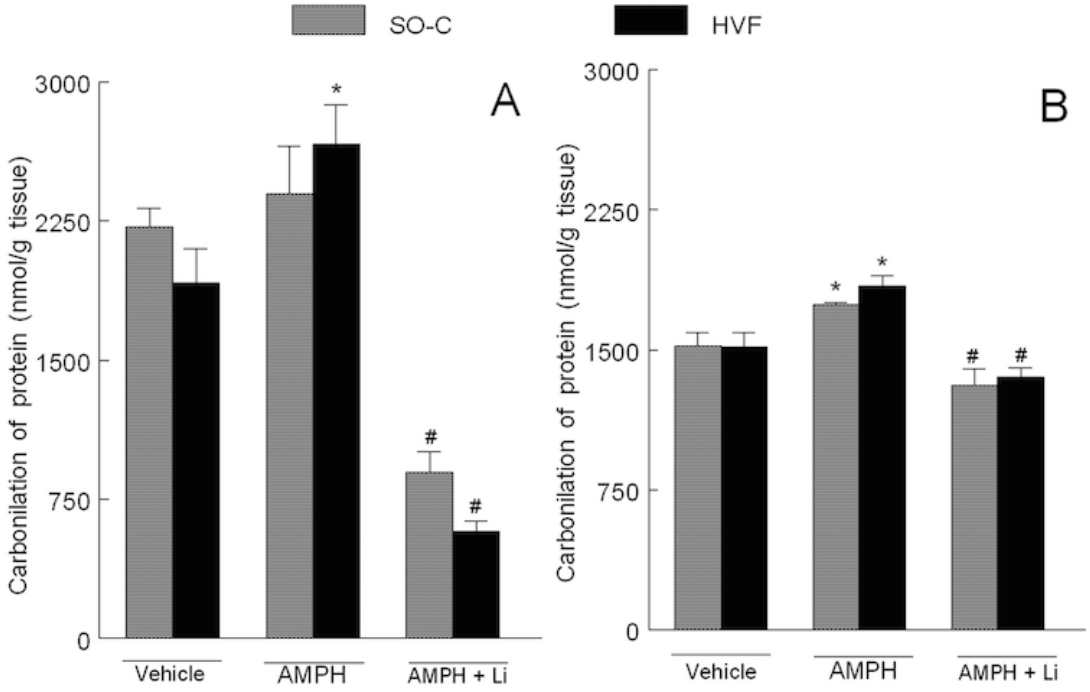
SO-C
HVF
A
B
Carbonilation of protein (nmol/g tissue)
3000
2250
1500
750
0
*
#
Vehicle
AMPH
AMPH + Li

Figure 3

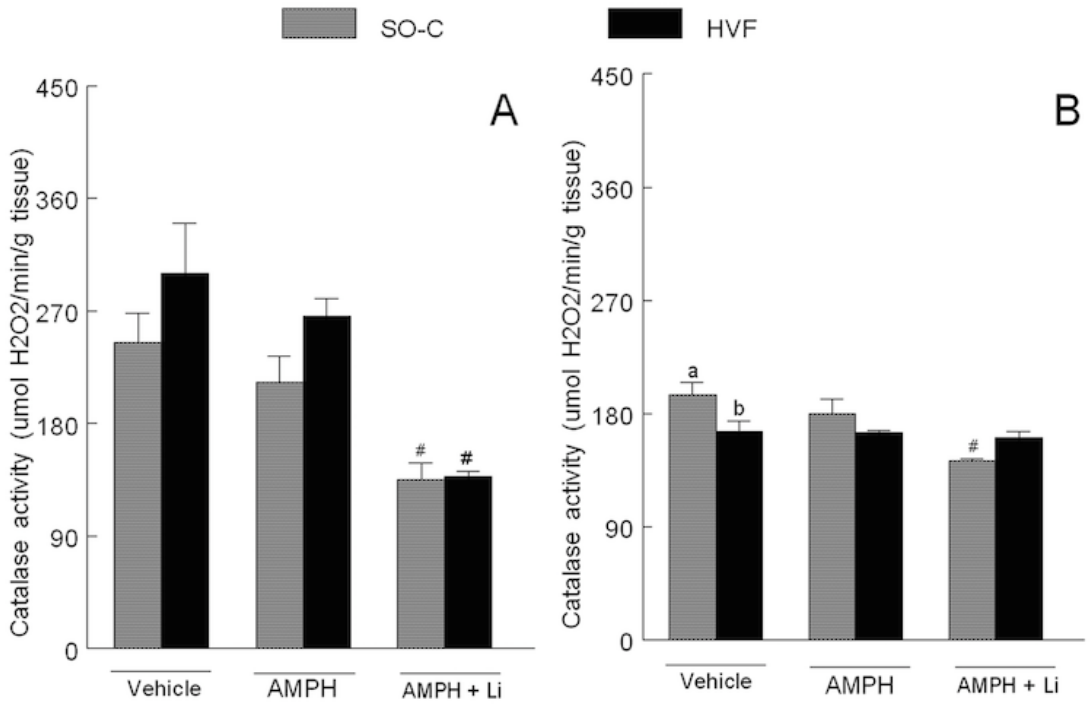
SO-C
HVF
A
B
Catalase activity (umol H2O2/min/g tissue)
450
360
270
180
90
0
#
a
b
Vehicle
AMPH
AMPH + Li

Figure 4

4. DISCUSSÃO

Em um estudo prévio no nosso grupo de pesquisa avaliamos a influência da suplementação com óleo de soja (grupo controle, rico em AGPI n-6), óleo de peixe (OP, rico em AGPI n-3) e gordura vegetal hidrogenada (GVH, rica em AG *trans*) em ratos *Wistar* desde o desmame até a idade adulta, e observamos que as diferentes suplementações foram capazes de alterar o comportamento e os parâmetros bioquímicos em um modelo animal de mania induzido por anfetamina (TREVIZOL et al., 2011). Considerando nosso estudo anterior e a importância da dieta materna durante a gestação e lactação (HERRERA, 2002), fases cruciais para o desenvolvimento do sistema nervoso central do feto e recém-nascido, e períodos nos quais ocorre maior incorporação de AGE no SNC (INNIS, 2007; MARTINEZ e MOUGAN, 1998), no presente estudo avaliamos a influência destes mesmos óleos e gordura em ratos *Wistar* machos ao longo de duas gerações sobre o modelo animal de mania induzido por ANF, padronizado anteriormente.

A partir dos experimentos descritos no artigo 1, foi possível observar que uma suplementação oral de óleos e gordura desde a concepção até a idade adulta (90 dias), permitiu uma incorporação significativa dos diferentes AG no córtex, estriado e hipocampo dos animais. Considerando que os AGPI n-3 estão em constante competição metabólica com os AGPI n-6, e que a maior oferta de AG de uma série pode inibir as reações de síntese (dessaturação e elongação) de AG presentes em menor proporção (PAWLOSKY et al., 2003), este estudo mostrou que a maior incorporação de DHA e ΣAGPI n-3 bem como uma menor incorporação de AGPI n-6 ocorreram nas três estruturas cerebrais do animais suplementados com OP, enquanto o contrário ocorreu com os animais suplementados com óleo de soja. Além disto, AGT são capazes de inibir os sistemas enzimáticos responsáveis pela síntese de AG de cadeia longa, interferindo negativamente na biossíntese de AGPI n-3 e n-6 (BLOND et al., 1995; BOURRE et al., 1990; KUMMEROW et al., 2004; LARQUÉ et al., 2001; 2003). Tal evento foi observado apenas através da redução de DHA e aumento da razão n-6/n-3 AGPI no estriado dos animais suplementados com GVH. Além disto, a incorporação de AGT foi observada em todas as áreas cerebrais avaliadas, exclusivamente neste grupo experimental.

Evidências mostram o envolvimento de espécies reativas de oxigênio (EROs) nos transtornos neuropsiquiátricos devido a elevada vulnerabilidade do sistema nervoso central ao estresse oxidativo (EO) (BEN-SCHACHAR, 2002; CALABRESE et al., 2001; TAKUMA,

BABA e MATSUDA 2004). Além disso, estudos demonstram alterações na atividade das enzimas antioxidantes de pacientes com TB acompanhadas por um aumento de danos oxidativos (MACHADO-VIEIRA, 2007; OZCAN et al., 2004; RANJEKAR et al., 2003; KULOGLU et al., 2002). Este estudo mostrou que a incorporação de AGT nas diferentes áreas cerebrais dos animais suplementados com GVH foi associada ao aumento da geração de espécies reativas (ER) e oxidação proteica nos grupos tratados ou não com ANF. Tais alterações bioquímicas foram acompanhadas de uma maior atividade locomotora e exploratória após o tratamento com ANF. Assim, acreditamos que a incorporação de AGT nas referidas áreas cerebrais foram suficientes para causar alterações funcionais no SNC, indicando também suas relações com danos oxidativos, frequentemente associados a diferentes condições neuropsiquiátricas. Por outro lado, a suplementação com OP foi relacionada com uma menor geração de ER e níveis de proteína carbonil (PC) *per se*, prevenindo a elevação destes parâmetros após a administração de ANF. Paralelamente, a suplementação de OP foi capaz de prevenir o aumento da atividade exploratória induzido pela ANF, melhorando também a memória de longa duração, a qual foi avaliada no teste de reconhecimento de objeto novo, parâmetro este que não foi alterado pela ANF. Neste contexto, é possível estabelecer uma relação entre estes resultados e a geração de metabólitos dos AGPI n-3, os quais apresentam menor atividade pró-inflamatória e pró-apoptótica (BAZAN, 2005; 2007; CHALON et al., 2001; YEHUDA, 2003) nos tecidos cerebrais dos animais suplementados com OP, cujos metabólitos são diferentes daqueles gerados pelo metabolismo dos AGPI n-6 e/ou AGT (BORSONELO e GALDURÓZ, 2008). Assim, a redução dos danos cerebrais observados no presente estudo, poderia ser atribuída, pelo menos em parte, aos eicosanoides, docosanóides, neuroprotectinas e resolvinas resultantes do metabolismo dos AGPI n-3 (CHALON et al., 2001; MASSARO et al., 2008; YEHUDA, 2003).

Seguindo esta linha de raciocínio, decidimos verificar se a suplementação com os óleos e a gordura por um tempo mais prolongado (2ª geração) poderia aumentar a incorporação dos diferentes AG, os quais estão presentes nas diferentes suplementações. Adicionalmente às observações comportamentais e bioquímicas também observadas na 1ª geração, os estudos descritos nos manuscritos 1 e 2, incluem algumas análises moleculares, as quais formam desenvolvidas em busca de mecanismos mais específicos que possam explicar os eventos aqui mostrados. A suplementação com GVH ao longo de duas gerações dobrou a incorporação de AGT no hipocampo, enquanto a razão de AGPI n-6/n-3 não foi alterada, cujo efeito já havia sido observado em estudos anteriores, confirmando que o efeito inibitório dos

AGT sobre as dessaturases não ocorre se as quantidades de AGE na dieta são suficientes (DE SOUZA, 2012; KURATA e PRIVETT, 1981). A presença de AGT na região hipocampal foi relacionada com um prejuízo de memória curta e longa duração juntamente com maiores danos oxidativos, os quais foram evidenciados pelo aumento da geração de espécies reativas e níveis aumentados de carbonilação de proteína. Além destas, a presença de AGT no hipocampo também pode ser associada a um menor nível do transportador de dopamina (DAT) e expressão RNAm do BDNF, resultados estes obtidos após a administração de ANF. Consistente com esses achados WU, YING e GOMEZ-PINILLA (2004), forneceram evidências de que o BDNF e o EO se inter-relacionam para afetar a plasticidade sináptica e a função cognitiva. Sendo assim, a presença de AGT na dieta pode estar relacionada com perdas cognitivas em doenças neuropsiquiátricas. Neste mesmo estudo, observamos que a suplementação OP por duas gerações sequenciais não foi capaz de aumentar significativamente a presença de AGPI n-3 no hipocampo, mas continua apresentado a maior incorporação de DHA e a menor razão DE AGPI n-6/n-3 dos três grupos experimentais. Além disto, a maior presença de AGPI n-3 nas membranas do hipocampo foi associada a uma melhor memória de curta duração e menor EO, bem como ao aumento dos níveis de DAT e da expressão do RNAm-BDNF após o modelo animal de mania. O conjunto dos resultados apresentados até aqui, juntamente com outros dados da literatura, sugerem que a maioria das doenças neuropsiquiátricas, incluindo TB, estão intimamente relacionados com deficiências cognitivas (GOLDSTEIN et al., 2009; MILLAN et al., 2012). Neste sentido, uma ingestão adequada de AGPI n-3, fartamente presentes no OP, especialmente durante o período perinatal, pode ser um fator preventivo para deficiências cognitivas associadas às condições neuropsiquiátricas, possivelmente por reduzir danos oxidativos no hipocampo, preservando assim, a expressão de BDNF.

No Manuscrito 2, observamos que a suplementação de OP durante duas gerações foi capaz de aumentar a incorporação de DHA e ΣAGPI n-3, bem como reduzir a razão de AGPI n-6/n-3 no córtex cerebral. Esta suplementação manteve o efeito preventivo sobre o aumento da atividade exploratória induzido pela ANF, porém, seus efeitos benéficos sobre o status oxidativo e molecular observados nos estudos anteriores já descritos nesta tese, foram menos expressivos, já que a ANF causou danos mais sutis no córtex dos animais de 2ª geração. Apesar disto, o consumo de óleos ricos em AGPI n-3 continua sendo importante, uma vez que estudos relacionaram níveis reduzidos de DHA em sangue ou tecido cerebral post-mortem na doença de Alzheimer, TB e outros distúrbios neuropsiquiátricos (CONQUER et al., 2000; LUKIW; BAZAN, 2000; McNAMARA et al., 2008; 2010). Seguindo essa linha, algumas

pesquisas já mostraram os benefícios da suplementação de AG n-3 no controle de sintomas de pacientes com TB (FREEMAN, 2000; TURNUBULL; CULLEN-DRILL; SMALDONE, 2008). Ainda, como mostrado no manuscrito 2 desta tese, a suplementação de gordura *trans* ao longo de duas gerações não aumentou a incorporação de AGT no córtex, quando comparada aos animais de 1ª geração, sendo apenas observado um aumento na razão de AGPI n-6/n-3 nesta área do cérebro. Da mesma forma, a atividade locomotora e exploratória dos animais induzida pela ANF, também não foi aumentada neste grupo experimental de 2ª geração, confirmando assim a hipótese de que a incorporação de AGT em áreas cerebrais envolvidas na atividade motora podem estar relacionadas a alterações comportamentais observadas neste modelo animal de mania. Além disso, a presença de AGT na região cortical aumentou significativamente a expressão do RNAm-BDNF, a qual não se refletiu nos níveis do BDNF e muito sutilmente nos níveis de pro-BDNF. Sendo assim, é possível sugerir que o expressivo aumento do RNAm-BDNF pode configurar uma tentativa do organismo para compensar uma tradução deficiente, em decorrência da presença dos AGT no córtex destes animais. Esta presença de AGT também tornou este tecido cerebral mais vulnerável às ações da ANF, já que esta droga reduziu drasticamente a expressão do RNAm-BDNF, juntamente com menores níveis de BNDF e pro-BDNF. A partir disto, pode-se sugerir que a suplementação de gordura *trans* pode facilitar o desenvolvimento de desordens neuropsiquiátricas, uma vez que outros estudos já sugeriram o envolvimento destas com alterações na plasticidade neural e envolvimento do BDNF, especialmente no TB (SCHLOESSER, R.J., et al, 2008). Considerando a presença dos AGT em áreas cerebrais, ACAR et al. (2003) relacionou este evento com um aumento da neurotransmissão dopaminérgica, sugerindo que os AGPI *trans* podem atuar sobre a expressão gênica e transcrição de proteínas envolvidas no metabolismo da DA. No presente estudo, a presença de AGT foi relacionada com a redução do níveis do DAT, BDNF e proBDNF, cujas alterações podem também estar relacionadas aos danos oxidativos, os quais foram observados a partir dos maiores níveis de PC no córtex deste grupo experimental.

Todos estes estudos de suplementação de gorduras *trans* ao longo de gerações (1ª e 2ª) permitiram observar que a presença de AGT de maneira tecido-dependente, é capaz de modificar parâmetros comportamentais, bioquímicos e moleculares relacionados ao modelo animal de mania aqui utilizado. Entretanto, um questionamento foi levantado em relação à uma possível influência da suplementação desta gordura sobre tratamentos farmacológicos empregados no controle dos sintomas do TB, tal como o lítio. A fim de preencher esta lacuna, o experimento seguinte (Manuscrito 3) foi desenvolvido para avaliar a influência da

suplementação de gordura *trans* ao longo de duas gerações em um modelo animal de mania juntamente com carbonato de lítio, que é uma droga amplamente utilizada no tratamento do TB. A ANF, usada neste estudo como um modelo animal de mania, aumenta a liberação e reduz a recaptação de DA na fenda sináptica pelo DAT, aumentando a auto-oxidação do neurotransmissor e gerando espécies reativas (SULZE et al., 1995). Enquanto a presença de AGPI *trans* em membranas cerebrais foi suficiente para causar danos na neurotransmissão dopaminérgica (ACAR et al., 2003), o presente estudo mostrou que a presença de AGT no hipocampo de ratos de 1ª e 2ª geração suplementados com gordura *trans* foi relacionada com uma maior suscetibilidade dos animais para desenvolver comportamento hiperativo em decorrência do modelo animal de mania. Além disto, tal incorporação também causou prejuízos de memória nos animais de 2ª geração expostos ao modelo animal de mania, o que pode ser relacionado aos danos oxidativos, também observados neste grupo experimental. Por outro lado, a presença de AGT no hipocampo dos animais de 1ª e 2ª geração não alterou a ação farmacológica do carbonato de lítio, droga esta que reverteu todos os efeitos induzidos pela administração de ANF. Neste sentido, o efeito neuroprotetor do lítio já foi demonstrado em outros estudos (AGHDAM; BARGER, 2007; CAMINS et al., 2009; FREY et al., 2006d; 2006e; ROWE; CHUANG, 2004), desde que seu mecanismo de ação conta com a redução da expressão da fosfolipase A_2, a qual é responsável pelo "turnover" do AA nas membranas fosfolipídicas neurais, e a inibição da COX_2, a qual é responsável pela síntese de eicosanoides pró-inflamatórios (BOSETTI et AL., 2002; RAPOPORT; BOSETTI, 2011).

Algumas alterações observadas no metabolismo de AGEs foram descritas como um fator de risco para desordens relacionadas ao neurodesenvolvimento (RICHARDSON; PURI, 2000), enquanto uma razão de AGPI n-6/n-3 aumentada, foi observada no soro de crianças com suspeita de alterações neurológicas (CORTÉS et al., 2013). Tomados em conjunto com os dados apresentados nesta tese, é possível sugerir que a ingestão prolongada de alimentos processados, os quais são ricos em gordura *trans*, em detrimento de óleos vegetais e/ou animais ricos em AGPI n-3, podem aumentar a suscetibilidade ao desenvolvimento de desordens neuropsiquiátricas, entre elas o Transtorno Bipolar, que por analogia ou coincidência, têm aumentado nas últimas décadas.

5. CONCLUSÕES

- ✓ A suplementação de óleo de soja, óleo de peixe e gordura vegetal hidrogenada desde o período perinatal até a idade adulta, bem como no decorrer da 1ª e 2ª gerações de ratos, permitiu a maior incorporação de AG n-6, n-3 e *trans*, respectivamente, em diferentes tecidos cerebrais, mostrando que o perfil lipídico das membranas neurais reflete rapidamente a oferta exógena proveniente de diversas fontes como a ingestão direta, via placenta e leite materno;
- ✓ A incorporação de AG n-3 e *trans* provenientes das respectivas suplementações refletem diferentes respostas sobre o status oxidativo e molecular cerebral de ratos tratados ou não com anfetamina, modificando também a resposta comportamental de hiperatividade e memória;
- ✓ Os resultados apresentados nesta tese mostram que apesar do cérebro apresentar uma grande seletividade para incorporar AGE, permitiu apenas uma pequena incorporação de AGT. Entretanto este tipo de AG é capaz de modificar processos fisiológicos normais relacionados aos aspectos comportamentais, bioquímicos e moleculares, o que pode facilitar o desenvolvimento de doenças neuropsiquiátricas;
- ✓ A suplementação de GVH aumentou a predisposição dos animais para o desenvolvimento de comportamento hiperativo, devido ao modelo animal de mania, porém não alterou a atividade farmacológica do lítio.

6. REFERÊNCIAS

ACAR N., et al. Modification of the dopaminergic neurotransmitters in striatum, frontal cortex and hippocampus of rats fed for 21 months with trans isomers of α-linolenic acid. **Neurosc. Res**, v. 45, p.375-382, 2003.

AEBI, U., CHIU, W., MILLIGAN, R. Role of catalase on antioxidative defenses. **J Struct Biol**, v.2, p.117-118, 1995.

AGALE, S., et al. Maternal caloric restriction spares fetal brain polyunsaturated fatty acids in Wistar rats. **Brain Dev.**, v. 32, p. 123-129, 2010.

AGHDAM, S.Y., BARGER, S.W. Glycogen Synthase Kinase-3 in neurodegeneration and neuroprotection: Lessons from lithium. **Curr Alzheimer Res**, v.4, p.21-31, 2007.

AL, M. D., et al. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. **Br. J.Nutrition**, v. 68, p.74-55, 1995

ALBERTS, B.; JOHNSON, A.; LEWIS, J.; RAFF, M.; ROBERTS, K.; WALTER, P. **Biologia Molecular da Célula**. 2ed. Porto Alegre: Artmed, 2004.

ALBUQUERQUE, K.T., et al. Intake of *trans* fatty acid rich hydrogenated fat during pregnancy and lactation inhibits the hypophagic effect of central insulin in adult offspring. **Nutrition**, v.22, p.820-829, 2006.

ALDERSON, R.F., et al. Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. **Neuron**, v. 5, p. 297–306, 1990.

ALMEIDA, S.S., et al. Nutrition and brain function: a multidisciplinary virtual symposium nutritional. **Neurosciences**, v. 5(5), p. 311-20, 2002.

American Psychiatric Asociation. Diagnostic and Statistical Manual of Mental Disorders, fourth editon – Text Revision. American Psychiatric Association; Washington, D.C., 2000.

AMUSQUIVAR, E., HERRERA, E. Influence of changes in dietary fatty acids during pregnancy on placental and fetal fatty acid profile in the rat. **Biol. Neonate**, v.83, p.136-45, 2003.

ANDREAZZA, A.C., et al. DNA damage in bipolar disorder. **Psychiatry Research,** v.153, p. 27-32, 2006.

ARMITAGE, J. A., et al. Developmental programming of aortic and renal structure in offspring of rats fed fat-rich diets in pregnancy. **J.Physiol.**, v.565, p.171-84, 2005.

ARMITAGE, J.A., et al. Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency. **Lipids**, v.38, p.459-464, 2003.

ATKINSON A.; GATENBY AD.; LOWE AG. The determination of inorganic orthophosphate in biological systems. **Biochim Biophys Acta**,v. 320, p.195–204, 1973.

AYDEMIR, O.; DEVECI, A.; TANELI, F. The effect of chronic antidepressant treatment on serum brain-derived neurotrophic factor levels in depressed patients: a preliminar study. **Prog Neuropychopharmacol Biol Psychiatry**,v.29, p.261-265, 2005.

BALLABRIGA A. Life style, environment and childhood diseases. **Annais Esp Pediatr**, v.3, v.42:1-19, 1990.

BAZAN, N.G. Neuroprotectin D1 (NPD1): a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress. **Brain Pathalogy**, v. 15, n. 2, p. 159-266, 2005.

BEN-SCHACHAR, D. Mitochondrial dysfunction in schizophrenia: a possible linkage to dopamine**. J Neurochem**, v.83, p.1241–1251, 2002.

BENNETT, C.N.; HORROBIN, D.F. Gene targets related to phospholipids and fatty acid metabolism in schizophrenina and other psychiatric disorders. An update. **Prostaglandins Leukotrienes & Essential Fatty Acids**, v.63, p.7-59, 2000.

BERK, M.; et al. Pathways underlying neuroprogression in bipolar disorder: Focus on inflammation, oxidative stress and neurotrophic factors. **Neurosc and Biobehav Rev**, v.35, p.804-817, 2011.

BIRCH, E.E.; et al. A randomized controlled trial of long chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. **Am J Clin Nutr**, v. 75 (3), p. 570-580, 2002.

BLACK, K.L.; et al. Eicosapentaenoic acid: effect on brain prostaglandins, cerebral blood flow and edema in ischemic gerbils. **Stroke**, v.15: p.65-69, 1984.

BLASS, J.P., GIBSON, G.E. Cerebrometabolic aspects of delirium in relationship to dementia. **Dement Geriatr Cogn Disord**, v.10, p.335-338, 1999.

BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. **Can J Physiol**, v.37, p.911-17, 1959.

BLOND, J.P. Effects of dietary 18:3 n-3 trans isomers on the dela-6 desaturation of alfa-linolenic acid. **J food lipids**, v.2, p.99-109, 1995.

BORSONELO, E.C.; GALDURÓZ, J.C.F. The role of polyunsaturated fatty acids (AGPI-CLs) in development, aging and substance abuse disorders: Review and propositions. **Prostaglandins Leucotrienes & Essential fatty Acids**, v.78, p.237-245, 2008.

BOSETTI, F., et al. Chronic lithium downregulates cyclooxygenase-2 activity and prostaglandin E_2 concentration in rat brain. **Molecular Psychiatry**, v.7, p.845-850, 2002.

BOTHAM, K. M.; MAYES, P. A. Biossíntese dos ácidos graxos e eicosanóides. In: MURRAY, R. K.; GRANNER, D. K.; RODWELL, V. W. **Harper Bioquímica Ilustrada**. 27ed. São Paulo: McGraw-Hill, 2007.

BOURRE, J.M. Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during aging. **Journal Nutrition Health Aging**, v.8, p.163-174, 2004.

BOURRE, J. M. et al. Dietary linolenic acid and polyinsaturated fatty acids in rat brain and other organs: minima requeriment of linolenic acid. **Lipids**, v. 25, n8, p. 465-472, 1990.

BUCKLEY, A. J. Altered body composition and metabolism in the male offspring of high fatfed rats. Metabolism, v. 54, p. 500-507, 2005.

BURGESS, J.R.; et al. Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder. **Am. J. Clin. Nutrition**, v.71, p.327S-330S, 2000.

BUSTIN SE., et al. The miqe guidelines: *m*inimum *i*nformation for publication of *q*uantitative real-time pcr *e*xperiments. **Clin Chem**, v. 55(4), p. 1-12, 2009.

CALABRESE, V., et al. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. **Neurochem Res**, v.26, p.739–764, 2001.

CALDER, P.C. Dietary modification of inflammation with lipids. **Proc Nutr Soc**, v.61 (3), p. 345-358, 2002.

CALON, F. et al. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. **Neuron**, v. 43, p. 633-645, 2004.

CALON, F.; COLE, G. Neuroprotective action of omega-3 polyunsaturated fatty acids against neurodegenerative diseases: Evidence from animal studies. **Prostaglandins Leukot Essent Fatty Acids**, v.77, p. 287-293, 2007.

CAMINS, A., et al. Potential Mechanisms Involved in the Prevention of the Neurodegenerative Diseases by Lythium. **Neuroscience & Therpeuti**cs, v.15, p.333-344, 2009.

CASTRÉN, E. Is mood chemistry? **Nature Reviews Neuroscience,** v.6, p.241-246, 2005.

CHAMPOUX, M.; et al. Fatty acid formula supplementation and neuromotor development in rhesus monkey neonates. **Pediatr Res**, v. 51(3), p. 273-281, 2002.

CHEN ZY, JING D, BATH KG, IERACI A, KHAN T, SIAO CJ, HERRERA DG, TOTH M, YANG C, MCEWEN BS, HEMPSTEAD BL, LEE FS. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. **Science**, v.314 (5796), p.140-143, 2006.

CHEN, G.; et al. Enhancement of hippocampal neurogenesis by lithium. **Journal neurochemistry**, v. 75, p. 1729-1734, 2000.

CHIUEH, C.C.; et al. Intracranial microdialysis of salicylic acid to detect hydroxyl radical generation through dopamine autooxidation in the caudate nucleus. **Free Radic Biol Med,** v. 13, p.581–583, 1992.

CHOIN-KWON, S.; et al. Temporal changes in cerebral antioxidant enzyme activities ischemia and reperfusion in a rat focal brain ischemia model: effect of dietary fish oil. **Dev Brain Res**, v. 152, n. 1, p. 11-18, 2004.

CHRISTIE, W.W. Lipid analysis: Isolation, separation, indentification and structural analysis of lipids. 3ª ed., The Oily Press, v. 15 in The Oily Press Lipid Library, Bridgwater, England,; 2003.

CLANDININ, M. T. et al. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. **Early Hum. Dev.**, v. 4, p. 121-129, 1980.

CLANDININ, M.T.; JUMPSEN, J.; SUCH, M. Relationship between fatty acid accretion, membrane composition, and biologic functions. **J Pediatric**, v.125, p. S25-S32, 1994.

CLARKE, J.; et al. Dietary supplementation of omega-3 polyunsaturated fatty acids worsens forelimb motor function after intracerebral hemorrhage in rats. **Experimental Neurology**, v. 191, p. 119-127, 2005.

CLIFFORD, J.J.; et al. Essential fatty acids given from conception prevent topographies of motor deficits in a transgenic model of Huntington's disease. **Neuroscience**, v.109, p. 81-88, 2002.

CÓLON-RAMOS, U.; BAYLIN, A.; CAMPOS, H. The relation between *trans* fatty acid levels and increased risk of myocardial infarction does not hold at lower levels of *trans* fatty acids in the Costa Rican food supply. **The J of Nutrit**, v. 136, p. 2887-2892, 2006.

CONNOR, W.E. Importance of n-3 Fatty Acids in Health and Disease. **Am J Clin Nu**., v.7, p.171S-5S, 2000.

CONQUER, J.A.; et al. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other type of dementia, and cognitive impairment. **Lipids**, v.35, p.1305-1312, 2000.

COOPER, G. M.; HAUSMAN, R. **A célula. Uma abordagem molecular**. 3ed. Porto Alegre: Artmed, 2007.

CORTÉS, E., et al. Índice elevado de ácidos grasos ômega 6/ômega 3 em ninños com neuropatías causa o efecto. **Nutrición Hospitalaria**, v.28(3), p.1165-1170, 2013.

COTT, J. Omega-3 fatty acids and psychiatric disorders. **Alternative Therapies in women's health**; v. 1(13), p. 97-104, 1999.

COYLE, J.T., MANJI, H.K. Getting balance: drugs for bipolar disorder share target. **Nature Medicine v.8**, p.557-558, 2002.

COYLE, J.T., DUMAN, R.S. Finding the intracellular signaling pathways affected by mood disorder treatments. **Neuron**, **v.**38, p.157-160, 2003.

CURI, R., et al. Entendendo a gordura - os ácidos graxos. 1ª ed., Ed. Manole, São Paulo, 2002.

DAS, U.N. Long-chain polyunsaturated fatty acids in the growth and development of brain and memory. **Nutrition**, v.19, p.62-65, 2003.

DAS, U.N. Can perinatal supplementation of long-chain polyunsaturated fatty acids prevent schizophrenia in adult life? **Med Sci Monit**, v. 10, p. 33-7, 2004.

DAS, U.N.; VADDADI, K.S. Essential fatty acids in Huntington's disease. **Nutrition**, v.20, p. 942-947, 2004.

DE LIMA M.N., et al. Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. **Neuroscience Letters.** v.405, p.142-146, 2006.

DE LIMA M.N., et al. Differential effects of low and high doses of topiramate on consolidation and retrieval of novel object recognition memory in rats. **Epilepsy and Behavior**, v.10 (1), p.32-37, 2007.

DE SOUZA, A.S., et al. Effects of a normolipidic diet containing *trans* fatt acids during perinatal period on the grouth, fatty acids profile, and memory of young rats according to sex. **Nutrition**, v.28, p.458-464, 2012.

DECHANT, D., NEUMANN, H. Neurotrophins. **Adv. Exp. Med. Biol**., v. 513, p.303–334, 2002.

DENNEHY, E.B.,et al. Does provider adherence to a treatment guideline change clinical outcomes for patients with bipolar disorder? Results from the Texas medications Alghorithm Poject. **Psychological Medicine, v.** 35, p.1695-1706, 2005.

DU BOIS, T.M.; et al. fatty acids differentially affect serotonin receptor and transporter binding in the rat brain. **Neuroscience**, v.139. p. 1397-1403, 2006.

DZIEZAK, J.D. Fats , oils and fat substitutes. **Food Technol**, v.43(7), p.66-74, 1989.

ECKEL, R.H., et al. Understanding the complexity of *trans* fatty acid reduction in the American diet. **Circulation**, v. 115, p.2231-2246, 2007

EINAT, H.; KOFMAN, O.; BELMAKER, R.H. Animal models of bipolar disorder: from a single episode to progressive cycling models. In: Myslobodsky M, winer O. (Eds.), Contemporary issues in modeling psychopharmacology. Boston: Kluwer Academic pulishers, pp. 164-180, 2000.

EINAT, H.; YUAN, P.; GOULD, T.D.; LI, J.; DU, J.; ZHANG, L.; MANJI, H.K.; CHEN, G. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. **J Neurosci**, v.23, p.7311-7316, 2003.

EINAT, H.; MANJI, H.K. Cellular plasticity cascades: genes-to-behavior pathways in animal models of bipolar disorder. **Biological Psychiatry**, v.59, p.1160-1171, 2006.

ELIAS, S.L.; INNIS, S.M. Infant plasma *trans*, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. **Am J Clin Nutr**, v.73, p.807-14, 2001.

EL-MALLAKH, R.S.; WYATT, R.J. The Na, K-ATPase hypothesis for bipolar illness. **Biol Psychiatry**, v.37, p.235-244, 1995.

EMKEN, E.A.; ADLOT, R.O.; GULLEY, R.M. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. **Biochim Biophys Acta**, v.1213, p.277-288, 1994.

FARKAS, E.; et al. Dietary long chain PUFAs differentially affect hippocampal muscarinic 1 and serotonergic 1 A receptors in experimental cerebral hypoperfusion. **Brain Research**, v. 954, p. 32-41, 2002.

FAROOQUI, A.A.; HORROCKS, L.A.; FAROOQUI, T. Glycerophospholipids in brain: their metabolism, incorporation into membranes, function, and involvement in neurological disorders. **Chemistry and Physic of Lipids**, v. 106, p. 1-29, 2000.

FENTON, W.S.; HIBBLEN, J.; KNABLE, M. Essential fatty acids, lipid membrana abnormalities and the diagnosis and treatment of schizophrenia. **Biol Psychiatry**, v.47, p.8-21, 2000.

FERRAZ, A.C.; et al. The antidepressant role of dietary long-chain polyunsaturated n-3 fatty acids in two phases in the developing brain. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, v.78, p. 183-188, 2008.

FINKEL, T.; HOLBROOK, N.J. Oxidants, oxidative stress and the biology of ageing. **Nature**, v. 408, p. 239–247, 2000.

FREY, B.N.; et al. Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania. **Bipolar Disorders**, v. 8, p. 275-280, 2006a.

FREY, B.N.; et al. Changes in antioxidant defense enzymes after D-amphetamine exposure: implications as an animal model of mania. **Neurochem Res**; v. 31, p. 699-703, 2006b.

FREY, B.N.; et al. Increased oxidative stress in submithochondrial particles after chronic amphetamine exposure. **Brain Research**, v.1097 (1), p.224-229, 2006c.

FREY, B.N.; et al. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. **Journal and Psychiatry and Neuroscience**, v.31 (5), p. 326-332, 2006d.

FREY, B.N.; et al. Effects of mood stabilizers on hippocampus BDNF levels in an animal of mania. **Life Sci**, v. 79, p. 281-286, 2006e.

FREEMAN, M.P. Omega-3 fatty acids in psychiatry: a review. Ann Clin Psychiatry, v.12 (3), p.159-165, 2000.

FRIDOVICH, I. Superoxide dismutases. **Annual Rev of Biochem**, v.44, p.147-159, 1975.

FRIESEN, R.; INNIS, S. M. *Trans* fatty acids in human milk in Canada declined with the introduction of trans fat food labeling. **J. Nutr.**, v. 136, p. 2558-2561, 2006.

FUGH-BERMAN, A.; COTT, J.M. Dietary supplementation and natural products as psychotherapeutic agents. **Psychosom Med**, v. 61(5), p. 712-718, 1999.

GALLEY, H.; DAVIES, M.J.; WEBSTER, N.R. Ascorbil radical formation in patients with sepsis: effects of ascorbate loading. **Free Radical Bil Med**, v.20, p.139-43, 1996.

GAZALLE, F.K.; et al. Clinical impact of late diagnose of bipolar disorder. **J. Affect. Disord**, v. 86, p. 313–316, 2005.

GEORGIEFF, M.K. Early brain growth: macronutrients for the developing brain. **Neoreviews**, V. 7(7), p. 323-334, 2006.

GERNER, R.H.; POST, R.M.; BUNNEY JR., W.E. A dopaminérgico mechanism in mania. **American journal of Psychiatry**, v.133, p.1177-1180, 1976.

GHOSH, P. et al. Abnormal aortic fatty acid composition and small artery function in offspring of rats fed a high fat diet in pregnancy. **J. Physiol.,** v. 22, p. 533-815, 2001.

GHOSH A, CARNAHAN J, GREENBERG ME. Requirement for BDNF in activitydependent survival of cortical neurons. **Science**, v.263, p.1618–1623, 1994.

GOLDSTEIN, T.R., et al. Psychosocial functioning among bipolar youth. **Journal of Affective Disorders**, v.114, p.174-183, 2009.

GOULD, T.D.; et al. AR-A014418, a selective GSK-3 inhibitor, produces antidepressant-like effects in the forced swim test. **International Journal of Neuropsychopharmacology**, v.7, p.387-390, 2004.

GOULD, T.D.; EINAT, H. Animal Models of bipolar disorder and mood stabilizer efficacy: A critical need for improvement. **Neuroscience and behavioral Reviews**, v.31, p.825-831, 2007.

GRAHAM, D.G. Oxidative pathways for catecholamines in the genesisof neuromelanin and cytotoxic quinines. **Mol Phatmacol**, v.14, p. 633-643, 1978.

GRANDE, I.; et al. The role of BDNF as a mediator of neuroplasticity in bipolar disorder. **Psychiatry Investig**, v.7, p.243-250, 2010.

GREENWOOD, T.A.; et al. Identification of additional variants within the human dopamine transporter gene provides further evidence for an association with bipolar disorder in two independent samples. **Molecular Psychiatry**, v.11, p.125-133, 2006.

GURR, M.I.; HARWOOD, J.L. Lipid Biochemistry – an introduction. 4ª ed., Great Britain, Chaoman & Hall, 1991.

HAAG, M. Essential fatty acids and the brain. **Can. J. Psychiatr**, v. 48, p.195-203, 2003.

HASHIMOTO, M. Chronic administration of docosahexaenóico acid ameliorates the impaitment of spatial cognition learning ability in amyloid {beta}-infused rats. **J Nutr**, v. 135, p. 549-555, 2005.

HASHIMOTO, M. et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. **J Neurochem**, v. 81, p. 1084-1091, 2002.

HARTMAN, L., e LAGO, B.C. A rapid preparation of fatty methyl esters from lipids. **Lab. Pract.**, v. 22, p. 475–477, 1973.

HEIRD, W.C., e LAPILLONNE, A. The role of essential fatty acids in development. **Annu Rev Nutr.**, v. 25, p. 549-571, 2005.

HERRERA, E. Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal developmentda review. **Placenta**, v.23, p.9S–19, 2002.

HERMAN, L.; HOUGLAND, T.; EL-MALLAKH, R.S. Mimicking human bipolar ion dysregulation models mania in rats. **Neurosci Biobehav Rev**, **v.**31, p.874-881, 2007.

HICHAMI, A.; et al. Olfactory discrimination ability and brain expression of c fos, Gir and Glut1 mRNA are altered in *n*−3 fatty acid-depleted rats. **Behavioural Brain Research**, v. 184, p. 1-10, 2007.

HILTY, D.M.; BRADY, K.Y.; HALES, R.E. A Review of Bipolar Disorders Among Adults. **Psychiatric Services**, v. 50, p. 201-213, 1999.

HÖGYES, E.; et al. Neuroprotective effect of developmental docosahexaenoic acid supplement against excitotoxic brain damage in infant rats. **Neuroscience**, v. 119, p. 999-1012, 2003.

HONSTRA G. Importence of polyunsaturated fatty acids of the n-6 and n-3 families for early human development. **Eur J Lipid Sci Technol**, v.103, p.379-389, 2001.

HORNSTRA, G. et al. Essential fatty acids in pregnancy and early human development. **Eur. J. Obstet. Gynecol. Reprod. Biol**., v. 61, p. 57-62, 1995.

HORROCKS, L.A.; FAROOQUI, A.A. Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, v. 70, p. 361-372, 2004.

HOSSAIN, M.S.; et al. Antioxidative effects of docosahexaenóico acid in the cerebrum versus cerebellum an Brainstem of aged hypercholesterolemic rats. **J Neurochem**, v. 72 (3), p. 1133-1138, 1999.

HU, F.B.; MANSON, J.E.; STAMPFER, M.J. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. **N Engl J Med**, v. 345, p. 790-797, 2001.

HULBERT, A.J.; et al. Dietary fats and membrane function: implications for metabolism and disease. **Biology Reviews**, v. 80, p. 155-169, 2005.

INNIS, S.M. Fatty acids and early human development. **Early Hum Dev**, v.83, p.761–766, 2007.

INNIS, S. Trans fatty intakes during pregnancy, infancy and early childhood. **Atheroscier Suppl**, v.7, p.17-20, 2006.

INNIS, S. M. Essential fatty acid transfer and fetal development. **Placenta**, v. 26, p. S70-5, 2005.

IOM – Institute of Medicine. Letter report on dietary reference intakes for *trans* fatty acids, Food Nutrition Board, 2002. Disponível em Http://www.nap.edu. Acessado em 12/11/2009.

JACOBS, D.; SILVERSTONE, T. Dextroamphetamine-indiced arousal in human subjects as a model of mania. **Psychological Medicine**, v.16, p.323-329, 1986.

JACQUES-SILVA, M.C.; et al. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. **Toxicol Appl Pharmacol**, v.88, p.119–125, 2001.

JAKOBSEN, M.U.; et al. Intake of ruminant *trans* fatty acids and risk of coronary heart disease – An overview. **Atherosclerosis Supplements**, v. 7, p. 9-11, 2006.

JOYCE, P.R.; et al. Urinary catecholamines and plasma hormones predict mood state in rapid cycling bipolar affective disorder. Journal of Affective Disorders, v. 33 (4), p. 233-243, 1995.

KANDEL ER. The molecular biology of memory storage: a dialogue between genes and synapses. **Science**, v. 294, p.1030-1038, 2001.

KATAN, M.B., ZOCK, P.L., MENSINK, R.P. Effects of fats an fatty acid on blood lipids in humans: an overview. **American Journal of Clinical Nutrition**, v.60, p.S-1017 S-1022, 1994.

KATO, T.; KATO, N. Mitochondrial dysfunction in bipolar disorder. **Bipolar Disorders**, v.2, p.180-190, 2000.

KHOSLA, P.; HAYES, K.C. Dietary *trans* monounsaturated fatty acids negatively impact plasma lipids in humans: critical review of the evidence. **Journal American College Nutrition**, v.15 (4), p.325-339, 1996.

KOHLMEIER, L.; et al. Adipose tissues *trans* fatty acids and breast cancer in the European Community Mlticenter Study on antioxidants, myocardial infarction, and breast cancer. **Cancer Epidemiol Biomarkers Prev**, v. 6, p. 705-710, 1997.

KRAUSS-ETSCHMANN, S. et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. **Am. J. Clin. Nutr.**, v. 85, p.1392-400, 2007

KULOGLU, M.; et al. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. **Cell Biochemistry and Function**, v.20, p.171-175, 2002.

KUMMEROW, F. A. et al. *Trans* fatty acids in hydrogenated fat inhibited the synthesis of the polyunsaturated fatty acids in the phospholipid of arterial cells. **Life Sci.**, v. 74, p. 2707-2723, 2004.

KURATA, N., PRIVETT, O. Effects of dietary trans acids on the biosynthesis of arachidonic acid in rat liver microsomes. **Lipids**, v.15, p.1029-1036, 1981.

LACHMAN, H.M.; PAPOLOS, D.F. A molecular model for bipolar affective disorder. **Med Hypotheses**, v.45, p.255-264, 1995.
and retina. **Progress in Lipid Research**, v. 40, p. 1-94, 2001.

LARQUÉ, E.; ZAMORA, S.; GIL, A. Dietary *trans* fatty acids in early life: a review. **Early Hum Dev**, v. 65, p. S31-S41, 2001.

LAURITZEN, L.; et al. The essentiality of long chain *n*-3 fatty acids in relation to development and function of the brain. **Prog Lipid Res**., v. 40(1-2), p.1-94, 2001.

LAYE, S. Polyunsaturated fatty acids, neuroinflammation and well being. **Prostaglandins Leukot Essent Fatty Acids**, v.82, p.295-303, 2010.

LEHNINGER, A.; NELSON, D. L.; COX, M. M. Lehninger: princípios de bioquímica. 3. ed. São Paulo: Sarvier, 2002. 975p.

LEPAGE, G.; ROY, C. C. Direct transesterification of all classes of lipids in a one-step reaction. **J Lip Res,** v.27, p. 14-20, 1986.

LEVANT, B., OZIAS, M. K., CARLSON, S. E. Specific brain regions of female rats are differentially depleted of docosahexaenoic acid by reproductive activity and an (n-3) fatty acid-deficient diet1,2. **J. Nut.**, v. 137, p. 130-134, 2007.

LEVANT, B. et al. Differential effects of modulation of docosahexaenoic acid content during development in specific regions of rat brain. **Lipids**, v. 407, p. 14-41, 2006.

LEVANT, B.; RADEL, J. D.; CARLSON, S. E. Decreased brain docosahexaenoic acid during development alters dopamine-related behaviors in adult rats are differentially affected by dietary remediation. **Behavioural Brain Research**. V. 152, p. 49-57, 2004.

LINDHOLM, D.; et al. Autocrineparacrine regulation of hippocampal neuron survival by IGF-1 and the neurotrophins BDNF, NT-3 and NT-4. **Eur J Neurosci**, v.8, p.1452–1460, 1996.

LIN PY. HUANG SY. SU KP. A meta-Analytic reviewof polyunsaturated fatty acid compositions in patients with depression. **Biolog Psych**., v.68, p. 140-147, 2010.

LIVAK KJ; SCHMITTGEN TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. **Methods**, v. 25 (4), p. 402-408, 2001.

LOÏ, C.; et al. Incorporation and metabolism of dietary *trans* isomers of linolenic acid alter the fatty acis profile of rat tissues. **Journal Nutrition**, v. 130, p. 2550-2555, 2000.

LOPEZ, A.D.; MURRAY, C.J.L. The global burden of disease, 1990-2020. **Nature Medicine**, v.4, p.1241-1243, 1998.

LOPEZ-GARCIA, E.; et al. consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. **The Journal of Nutrition**, v. 135, p. 562-566, 2005.

LOU SJ, LIU JY, CHANG H, CHEN PJ. Hippocampal neurogenesis and gene expression depend on exercise intensity in juvenile rats. **Brain Res**, v.1210, p.48-55, 2008.

LOWRY OH.; et al. Protein measurement with the Folin phenol reagent. **J Biol Chem**,v.193, p. 265–75, 1951.

LU, B.; PANG, P.T.; WOO, N.H. The yin and yang of neurotrophin action. **Nat Ver Neurosci**, v.6, p.603–14, 2005.

LUKIW, W.J.; et al. A role for docosahexaenoic acid-derived neuoprotection D1 in neural cell survival and Alzheimer disease. **J Clin Invest**, v.115(10), p. 2774-2783, 2005.

LUKIW, W.J.; BAZAN, N.G. Neuroinflammatory signaling upregulation in Alzheimer's disease. **Neurochem Res**, v.25, p.1173-1184, 2000.

MACHADO-VIEIRA, R.; KAPCZINSKI, F.; SOARES, J.C. Perspectives for the development of animal models of bipolar disorder. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v.28, p.209-224, 2004.

MACHADO-VIEIRA, R.; et al. Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. **Biol Psychiatry**, v. 15;61(2), p.142-4, 2007.

MAHFOUZ, M.M.; KUMMEROW, F.A. Hydrogenated fat high in trans monoenes with an adequate level of linoleic acid has no effect on prostaglandin synthesis in rats. **Journal Nutrition**, v.129, p.15-24, 1999.

MALHI, G.S.; et al. Bipolaroids: functional imaging in bipolar disorder. **Acta Psychiatrica Scandinavica**, v.110(422), p.46-54, 2004.

MANCINI, J.; CHEMIM, S. **Implicações nitricionais dos ácidos graxos *trans*.** In: Seminários "Gorduras Modificadas com Baixos Teores de Ácidos Graxos *trans*: aspectos nutricionais e tecnológicos". Sociedade Brasileira de Óleos e Gorduras, São Paulo, 1996

MANJI, H.K.; LENOX, R.H. Signaling cellular insight sinto the pathophysiology of bipolar disorder. **Biological Psychiatry**, v. 48 (6), p. 518-530, 2000.

MANJI, H.K.; et al. Neuroplasticity and cellular resilience in mood disorders. **Mol Psych,** v.5, p.578-593, 2001.

MANJI, H.K.; QUIROZ, J.A.; PAYNE, J.L.; SINGH, J.; LOPES, B.P.; VIEGAS, J.S.; ZARATE, C.A. The underlying neurobiology of bipolar disorder. **World Psychiatry**, v.2, p.136-146, 2003.

MANTZIORIS, F.; CLELAND, L. G.; GIBSON, R. A. Biochemical effects of a diet containing foods enriched with n-3 fatty acids. **Am J Clin Nutr**, v. 72, p. 42-48, 2000.

MARSZALEK, J.R. e LODISH, H.F. Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: breastmilk and fish are good for you. **Annu Rev Cell Biol**, v. 21, p. 633-657, 2005.

MARTEINSDOTTIR, I., et al. Changes in dietary fatty acids alter phospholipids fatty acid composition in selected regions of rat brain. **Prog. Neuro-Psychopharmacol. Boil. Psychiaty**, v.22, p.1007-1021, 1998.

MARTIN, C.A., et al. *Trans* fatty acid-forming process in food: a review. **Anais da Academia Brasileira de Ciências**, v. 79 (2), p. 343-350, 2007.

MARTINEZ, M., MOUGAN, I. Fatty acid composition of human brain phospholipds during normal development. **Jouranl Neirochem**, v.71(6), p.2528-2533, 1998.

MAZZA, M.; et al. Omega-3 fatty acids and antioxidants in neurological and psychiatric disease: An overview. **Progress in Neuro- Psychopharmacology & Biological Psychiatry**, v.31, p. 12-26, 2007.

McNAMARA, R.K.; et al. Selective déficits in erythrocyte docosahexaenoic acid composition in adult patients with bipolar disorder and major depressive disorder. **Journal of Affective Disorders**, v.126, p.303-311, 2010.

McNAMARA, R.K.; et al. Deficits in docosahexaenoic acidand associated elevations in the metabolism of arachidonic acid and saturated fatty acids in the postmortem orbitofrontal cortex of patients with bipolar disorder. **Psychiatry Res**, v.160, p.285-299, 2008

McNAMARA, R.K., CARLSON, S.E. Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, v. 75, p. 329- 349, 2006.

MELIKIAN H.E., et al. Human norepinephrine transporter. Biosynthetic studies using a site-directed polyclonal antibody. **J. Biol. Chem., v.** 269, p. 12290–12297, 1994.

MENSINK, R.P., et al. Effects of dietary acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. **The American Journal of Clinical Nutrition**, v. 77, p. 1146-1155, 2003.

MERIKANGAS KR, et al. Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative. **Arch Gen Psych, v.** 68(3), p. 241–51, 2011.

MILLAN, M.J., et al. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. **Nat Rev Drug Discov**, v.11(2), p.141–168, 2012.

MITCHELL, S.A., et al. The Apaf-1 internal ribosome entry segment attains the correct structural conformation for function via interactions with PTB and unr. **Mol Cell**, v. 11(3), p.757-771, 2003.

MORGANE, P.J., MOKLER, D.J., GALLER, J.R. Effects of Prenatal Protein Malnutrition on the Hippocampal Formation. **Neuroscience and Biobehavioral Rewiews**,v. 26,p. 471-483, 2002.

MARTINEZ, M. Tissue levels of polyunsaturated fatty acids during early human developmen. **J Pediatr**, v.120, p. S129–S138, 1992.

MORGANE, P.J., et al. Prenatal malnutrition and development of the brain. **Neurosci Biobehav Ver**, v.17, p. 91-128, 1993.

MOZZAFFARIAN, D.; CLARKE, R. Quantitative effects on cardiovascular risk fator and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. **European Journal Clinical Nutrition**, v.63(S2), p.S22-33, 2009.

MOZAFFARIAN, D.; et al. *Trans* fatty acids and cardiovascular disease. **The New England Jornal of Medicine**, v.354 (15), p. 1601-1613, 2006.

MÜLLER-OERLINGHAUSEN, B.; BERGHÖFER, A.; BAUER, M. Bipolar Disorder. **Lancet**, v.359, p.241–247, 2002.

MURPHY, D.L.; et al. Regular induction of hypomania by L-dopa in "bipolar" manic-depressive patients. **Nature**, v.229, p.135-136, 1971.

MURPHY, M.G. Dietary fatty acids and membrane protein function. **J Nutr biochem**, v.1, p.68-70, 1990.

MUSZBEK L, SZABÓ T, FÉSÜS L. A highly sensitive method for the measurement of the ATP-ase activity. **Anal Biochem**, v. 77, p. 286–8, 1977.

NESTLER, E.J.; et al. Neurobiology of depression. **Neuron**, v.34, p.13-25, 2002.

NEURINGER, M. Infant vision and retinal function in studies of dietary long-chain polyunsaturated fatty acids: methods, results, and implications. **Am J Clin Nutr**, v. 71, p. 256-267, 2000.

OHKAWA, H.; OHISHI, H.; YAGI, K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. **Anal Biochem**, v.95, p.351-358, 1979.

OKADA, Y., et al. Trans fatty acids exacerbate dextran sodium sulphate-induced colitis by promoting the up-regulation of macrophage-derived proinflammatory cytokines involved in T helper 17 cell polarization. **Clinical & Experimental Immunology**, v.174 (3), p.459–471, 2013.

OKUYAMA, H., ORIKASA, Y., NISHIDA, T. Significance of antioxidative functions of eicosapentaenoic and docosahexaenoic acids in marine microorganisms. **Appl. Environ. Microbiol.**, v. 74, p. 570-574, 2008.

OSSO, F.S. et al. *Trans* fatty acids in maternal milk lead to cardiac insulin resistance in adult offspring. **Nutrition**, v. 24, p. 727-32, 2008.

OTTO, S. J., et al. Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. **Am. J. Clin. Nutr.**, v. 302, p. 7-73, 2001.

OZCAN, M.E., et al. Antioxidant enzyme activities and oxidative stress in affective disorders. **International Clinical Psychopharmacology**, v.19, p.89-95, 2004.

OZANNE, S. E., et al. Maternal low protein diet in rats programmes fatty acid desaturase activities in the offspring. **Diabetologia**, v. 1337, p.41-42, 1998.

PADOVESE R.; MANCINI FILHO J. Ácidos graxos *trans*. In: CURI, R; POMPÉIA, C.; MIYASKA, C.K.; PROSCÓPIO, J>, cords. **Entendendo a gordura os ácidos graxos**. Barueri: Manoele, 2002. Cap.36, p.507-521.

PAWLOSKY, R. J. et al. Effects of beef- and fish-based diets on the kinetics of n-3 fatty acid metabolism in human subjects. **Am. J. Clin. Nutr.**, v. 565, p. 72-77, 2003.

PANG, P.T., et al. Cleavage of proBDNF by TPA/plasmin is essential for long-term hippocampal plasticity. **Science**, v.306, p.487-491, 2004.

PARKER, G., et al. Omega-3 fatty acids and mood disorders. Am J Psychiatry, v.163, p.969-978, 2006.

PEET, M., STOKES, C. Omega-3 fatty acids in the treatment of psychiatric disorders Drugs. **Nutr Neurosci**, v.65, p.1051-1059, 2005.

PICCHINI, A.M.; MANJI, H.K.; GOULD, T.D. GSK-3 and neurotrophic signaling: novel targets underlying the pathophysiology and treatment of mood disorders? **Drug Discovery Today: Disease Mechanisms,** v.1, p.419-428, 2004.

PISANI, L.P. et al. Hydrogenated fat intake during pregnancy and lactation modifies serum lipid profile and adipokine mRNA in 21 day-old-rats. **Nutrition**, v.24, P. 255-261, 2008.

POST, R.M. Role of BDNF in bipolar and unipolar disorder: clinical and theoretical implications. **J Psychiatr Research**, v.41(12), p.979-990, 2007.

POST, R.M.; et al. Morbidity in 258 bipolar outpatients followed for 1 year with prospective ratings on the NIMH life chart method. **Journal of Clinical Psychiatry**, v.64, p.680-690, 2003.

QIAN, L.; et al. Brain-derived neurotrophic factor and risk of schizophrenia: an association study and meta-analysis. **Biochem Biophys Res. Commun,** v.353 (3), p.738-743, 2007.

RANJEKAR, P.K.; et al. Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. **Psychiatry Research**, v.121, p.109-22, 2003.

RAO, S.S.; et al. Sensitivity of fetus and pups to excess levels of maternal intakes of alpha linolenic acid at marginal protein levels in Wistar rats. **Reprod. Toxicol.**, v. 24, p. 333-342, 2007.

RAPOPORT, S.I., BOSETTI, F. Do Lithium and Anticonvulsants Target the Brain Arachidonic Acid Cascade in Bipolar Disorder? Arch gen Psychiatry, v.59, p.592-596, 2002.

REICHARDT LF. Neurotrophin-regulated signaling pathways. **Philos Trans R Soc Lond B Biol Science**, v.361, p.545-564, 2006.

REIS, H.J.; et al. Increased serum levels of brain-derived neurotrophic fator in chronic institutionalized patients with schizophrenia. **Neurosci Letters**, v.439(2), p.157-159, 2008.

RICHARDSON, A.J. Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. **Int Rev Psychiatry**, v.18, p.155-172, 2006.

RIEMER, S.;MAES, M.;CHRISTOPHE, A.;RIEF, W. Lowered omega-3 PUFAs are related to major depression, but not to somatization syndrome. **J Affect Disord**, v.123, n.1-3, Jun, p.173-80. 2010

ROACH, C.; et al. Comparison of *cis* and *trans* fatty acid containing phosphatidylcholines on membrane properties. **Biochemistry**, v. 43, p. 6344-6351, 2004.

ROWE, M.K., CHUANG, D.M. Lithium neuroprotection: Molecular mechanisms and clinical implications. **Expert Rev Mol Med**, v.6, p.1-18, 2004.

RUMAJOGEE, P.; et al. Adaption of the serotoninergic neuronal phenotype in the absent of 5-HT autoreceptors or the 5-HT transporter: involvement of BDNF and cAMP. **Eur J Neurosci**, v.19, p.937-944, 2004

SABARENSE, C.M.; MANCINI-FILHO, J. Efeito da gordura vegetal parcialmente hidrogenada sobre a incorporação de ácidos graxos *trans* em tecidos de ratos. **Rev Nutr,** v. 16 (4), p. 399-407, 2003.

SALEM, N.Jr., Mechanisms of action of docosahexaenoic acid in the nervous system. **Lipids**, v.36 (9), p.945-959, 2001.

SALEM, J.N. Introduction to polyunsaturated fatty acids. **Backgrounder**, v.3(1), p.1-8, 1999.

SALIBA, E., MARRET, S. Cerebral white matter damage in the preterm infant: pathophysiology and risk factors. **Seminars in Neonatology,** v. 6(2), p. 121-133, 2001.

SALMERÓN, J.; et al. Dietary fat intake and risk of type 2 diabetes in women. **Am J Clin Nutr**, v. 73, p. 1019-1026, 2001.

SALVATORE M. F., APPARSUNDARAM S., GERHARDT G. A. Decreased plasma membrane expression of striatal dopamine transporter in aging. **Neurobiol. Aging, v.** 24, p. 1147–1154, 2003.

SANGIOVANNI, J.P.; et al. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they related t visual resolution acuity in healthy preterm infants. **Pediatrics**, v. 105 (6), p. 1292-1298, 2000.

SANIOVANNI, J.P.; CHEW, E.Y. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. **Progress in Retinal and Eye Research**, v. 24, p. 87-138, 2005.

SARSILMAZ, M.; et al. Potential role of dietary ϖ-3 essential fatty acids on some axidant/antioxidant parameters in rats' corpus striatum. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, v.69, p.253-259, 2003.

SAW, C. L. L., HUANG, Y., KONG, A.-N. Synergistic anti-inflammatory effects of low doses of curcumin in combination with polyunsaturated fatty acids: Docosahexaenoic acid or eicosapentaenoic acid. **Biochem. Pharmacol.**, v. 79, p. 421-430, 2010.

SCHILDKRAUT, J.J. The catecholamine hypothesis of affective disorders: a review of supporting evidence. **American Journal of Psychiatry**, v.122, p.509-522, 1965.

SCHLOESSER, R.J., et al. Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. **Neuropsychopharmacology**, v., p.110–133, 2008.

SCHMID, W. The micronucleus test. **Mutation research,** v.31, p. 9-15, 1975.

SCHRÖDER, N., et al. Neurotoxic methamphetamine regimen 40 severely impairs recognition memory in rats. **Synapse**, v.49(2), p.89-96, 2003.

SEUNG KIM, H.F.; et al. Inhibitory effects of omega-3 fatty acids on protein Kinase C activity in vitro. **Mol Psichiatry**, v.6, p.246-248, 2001.

SEMMA, M. *Trans* fatty acids: properties, benefits and risk. **Journal Health Science**, v. 48 (1), p. 7-13, 2002.

SHALTIEL, G.; CHEN, G.; MANJI, H.K.. Neurotrophic signaling cascades in the pathophysiology and treatment of bipolar disorder. **Curr.Opin.Pharmacol,** 2006.

SHAPIRO, H. Could n-3 polyunsaturated fatty acids reduce pathological pain by direct actions on the nervous system? **Prostaglandins, Leukotrienes, and Essential Fatty Acids**, v.68, p.219-224, 2003.

SIMOPOULOS, A.P. Essential fatty acids in health and chronic disease. **Am J Clin Nut**, v.70, p.560-569, 1999.

SIMOPOULOS, A.P. The importance of the ratio of omega-6/omega-3 essential fatty acids. **Biomed Pharmacother**, v.56, p.365-379, 2002.

SIMOPOULOS, A.P. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic disease. **Biomedicine & Pharmacotherapy**, v. 60, p. 502-507, 2006.

SOLFRIZZI, V.; et al. Dietary fatty acids intake: possible role in cognitive decline and dementia. **Experimental Gerontology**, v.40, p.257-270, 2005.

SONGUR, A.; et al. Hypothalamic superoxide dismutase, xanthine oxidase, nitric oxide and malondialdehyde in rats fed with fish n-3 fatty acids. **Prog Neuropsychopharmacol Biol Psychiatry**, v.28, p.693-698, 2004.

STECKERT, AM., et al. Role of oxidative stress in the pathophysiology of bipolar disorder. **Neuroch Res**, v. 35, p. 295-301, 2010.

STENDER, S.; DYEBERG, J. Influence of *trans* fatty acids on health. 4ª ed., A report from the Danish Nutrition Council. Publ. nº. 34, Compenhagen, 2003.

STENDER, S.; DYEBERG, J. Influence of *trans* fatty acids on health. **Annals of Nutrition & Metabolism**, v. 48, p. 61-66, 2004.

STOLL, A.L.; et al. Omega 3 Fatty Acids in Bipolar Disorder. **Arch Gen Psychiatry**, v.56, p.407-412, 1999.

STOLL, A.L.; et al. Neuroimaging in Bipolar Disorders: What have w learned? **Biological Psychiatry**, v. 49(1), p. 80, 2001.

STORLIEN, L. H. et al. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and x-3 fatty acids in muscle phospholipid**. Diabetes**, v. 40, p. 280-289, 1991.

STRAKOWSKI, S.M.; DELBELLO, M.P.; ADLER, C.M. The functional neuroanatomy of bipolar disorder: a review of neuroimaging findings. **Molecular Psychiatry**, v.10, p.105-116, 2005.

SULZE, R.D.; et al. Amphetamine redistributes dopamine from synaptic vesicles to the citosol and prometes reverse transport. **Journal of Neuroscience**, v.15, p.4102-4108, 1995.

SUMIYOSHI,T.; et al. Essential polyunsaturated fatty acids and social cognition in schizophrenia. **Psychiatry Research**, v. 157, p. 87-93, 2008.

SUN, X.; et al. Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. **Journal of Psychiatry and Neuroscience**, v.31, p.189-196, 2006.

SUN, Q.; MA, J.; et al. A prospective study of *trans* fatty acids in erythrocytes and risk of coronary heart disease. **Circulation**, v. 115, p. 1858-1865, 2007.

TAPIERO, H.; et al. Polyunsaturated fatty acods (PUFA) and eicosanoids in human health and patologies. **Biomedicine & Pharmacotherapy**, v.56(5), p.215-222, 2002.

TARDIVEL, S. et al. Protective effects of EPA and deleterious effects of DHA on eNOS activity in Ea hy 926 cultured with lysophosphatidylcholine. **Lipids**, v. 44, p. 225-235, 2009.

TARRAGO-TRANI, M.; et al. New and existing oils and fats used in products with reduced *trans*-fatty acid content. **Journal of the American Dietetic Association**, v. 106, p. 867-880, 2006.

TEIXEIRA, A.M. et al. Could dietary trans fatty acids induced movements disorders? Effects of exercise and its influence on $Na^{+}K^{+}$-ATPase and catalase activity in rat striatum. **Behav. Brain Res.**, v. 226, p. 504-510, 2012.

TEIXEIRA, A.M. et al. Exercise affects memory acquisition, anxiety-like symptoms and activity of membrane-bound enzyme in brain of rats fed with different dietary fats: impairments of *trans* fat. **Neuroscience**, v.195, p. 80-88, 2011.

TIEMEIER, H. Biological risk factors for late life depression. **Eur J Epidemiol**, v. 18, p. 745-750, 2003.

TREVIZOL, F. et al. Influence of lifelong dietary fats on the brain fatty acids and amphetamine-induced behavioral responses in adult rat. **Prog. Neuropsychopharmacol. Biol. Psychiatry**, v.45, p. 215-222, 20113.

TREVIZOL, F.; et al. Comparative study between n-6, trans and n-3 fatty acids on repeated amphetamine exposure: A possible factor for the development of mania. **Pharmacol. Biochem. and Behavior**, v.97, p.560-565, 2011.

UAUY, R.; DANGOUR, A.D. Nutrition in brain development and aging: role of essential fatty acids. **Nutr Rev**, v.64, p.24-33, 2006.

VALKO, M.; et al. Role of oxygen radicals in DNA damage and cancer incidence. **Mol Cell Biochem,** v. 266, p.37–56, 2004.

VALKO, M.; et al. Free radicals and antioxidants in normal physiological functions and human disease. **Int J Biochem Cell Biol**, v. 39, p.44–84, 2007.

WAINWRIGHT, P.E.; XING, H.C.; MUTSAERS, L.; MCCUTCHEON, D.; KYLE, D. Arachidonic acid offsets the effects on mouse brain and behavior of a diet with a low (n-6)/(n-3) ratio and very high levels of docosahexaenoic acid. **J. Nutr**, v.127, p.183, 1997.

WILLETT, W.C. Trans fatty acids and cardiovascular disease – epidemiological data. **Atherosclerosis Supplements**, v. 7, p. 5-8, 2006.

WAINWRIGHT, P.E. Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. **Proc Nutr Soc**, v.61, p.61-69, 2002.

WAINWRIGHT, P.E. Do essential rational use for n-3 fatty acids play a role in brain and behavioral development? **Neurosci Biobehav Rev**, v. 16, p. 193-205, 1992.

WEISSMAN, M.M.; et al. Cross-national epidemiology of major depression and bipolar disorder. **Journal of American Medical Association**, v.276, p.293-299, 1996.

WILLIAMS, R.S.; et al. A common mechanism of action for three mood-stabilizing drugs. **Nature**, v.417, p.292-295, 2002.

WU, C.W.; et al. Enhanced oxidative stress and aberrant mitochondrial biogenesis in human neuroblastoma SH-SY5Y cells during methamphetamine induced apoptosis. **Toxicol. Appl Pharmacol**, v. 220: p. 243-251, 2007.

WU, A., YING, Z., GOMEZ-PINILLA, F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. **European Journal of Neuroscience**, v.19. p.1699-1707, 2004.

WYSE, A.T.S.; WAJNER, M.; BRUSQUE, A. et al. Alanine reveses the inhibitory effect of phenylalanine and its metabolites on NA^{+},K^{+} -ATPase in synaptic plasma membranes from cerebral cortex of rats. **Biochem Soc Trans**, v. 23, p. 227S, 1995.

YAN, L.J.; TRABER, M.G.; PACKER, L. Spectrophotometric method for determination of carbonyls in oxidatively modified apolipoprotein B of human low-density lipoproteins. **Anal Biochem**, v.228, p. 349–351, 1995.

YAVIN, E.; BRAND, A.; GREEN, P. Docosahexaenoic abundance in the brain: a biodevice to combat oxidative stress. **Nutr Neurosci**, v. 5, p. 149-157, 2002.

YAVIN, E. Versatile roles of docosahexaenoic acid in the prenatal brain: from proand anti-oxidant features to regulation of gene expression. **Prostaglandins Leukot Essent Fatty Acids**, v. 75, p. 203-211, 2006.

YATHAM, L.N.; et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) guidelines for the management of patients with bipolar disorder: consensus and controversies. **Bipolar Disorders**, v.7(3), p.5-69, 2005.

YEHUDA,S.; MOSTOFSKY, D.I. Handbook of essential fatty acids biology: biochemistry, physiology, and behavioral neurobiology. New York: **Humane Press**, p. 427-452, 1997.

YEHUDA, S. Omega-6/omega-3 ratio and brain-related functions, world. **Rev. Nutr. Diet**, 92:37-56, 2003.

YEHUDA, S.; RABINOVITZ, S.; MOSTOFSKY, D. I. Essential fatty acids and the brain: From infancy to aging. **Neurobiology of Aging**, v. 26, p.98-102, 2005.

YILMAZ, H. R., et al. The effects of n-3 polyunsaturated fatty acids by gavage on some metabolic enzymes of rat liver. **Prostaglandins Leukot Essent Fatty Acids**, v. 71, p. 131-135, 2004.

ZAPOLKA-DOWNAR, D.; KOSMIDER, A.; NARUSZEWICZ, M. *Trans* fatty acids induce apoptosis in human endothelial cells. **Journal of Physiology and Pharmacology**, v. 56 (4), p. 611-625, 2005.

ZARATE, C.A. JR..; SINGH, J.; MANJI, H.K. Cellular plasticity cascades: targets for he development of novel therapeutics for bipolar disorder. **Biol Psychiatry**, v.59, p.1006- 1020, 2006.

7. APÊNDICE 1

O doutorado sanduíche que foi realizado no Instituto de Investigação do Medicamento e das Ciências Farmacêuticas (iMED.UL/CPM) da Faculdade de Farmácia da Universidade de Lisboa, na cidade de Lisboa/Portugal, e teve como objetivo principal o desenvolvimento de novas técnicas que poderão ser aplicadas no laboratório de origem, como também em ambientes futuros. De fato, durante este intercâmbio, ensaios *in vitro* relacionados à esclerose amiotrófica lateral foram desenvolvidos com óleo de peixe, o qual foi o mesmo utilizado no desenvolvimento dos experimentos realizados no Brasil.

O item **PROJETO** refere-se ao projeto no qual fiquei inserida durante a realização do doutorado sanduíche, o qual contém Introdução, Objetivos, Metodologia, Resultados Esperados e Referências bibliográficas.

O experimento realizado com óleo de peixe apresenta-se estruturado nos itens **MATERIAIS e MÉTODOS**, **RESULTADOS PARCIAIS e REFERÊNCIAS**.

7.1 PROJETO

Comunicação entre neurônios motores e células microgliais num modelo *in vitro* de esclerose amiotrófica lateral (ELA)

Introdução

A esclerose amiotrófica lateral (ELA) é uma doença neurodegenerativa fatal causada pela degeneração seletiva dos neurônios motores (NMs) na medula espinhal, tronco cerebral e córtex motor, causando deficiência a nível do andar, da fala, da deglutição e finalmente da função respiratória. A origem da doença não foi ainda estabelecida e não existem biomarcadores específicos, apesar de se encontrar associada a mutações ao nível da superóxido dismutase (SOD1) e da TDP-43. A neuroinflamação e a excitotoxicidade são tidas como estando associadas à doença. Mais recentemente estudos se referem que as células gliais se encontram envolvidas, havendo ativação de vias inflamatórias e produção de estresse oxidativo (Ince et al., 2011). Desta forma, considera-se que a ativação da microglia possa contribuir para o aparecimento e a progressão da ELA (Boillee et al., 2006), uma vez que as células além do seu papel protetor (tipo M2) podem, dependendo do seu estado de ativação e do estímulo realizado, potencializar o dano (tipo M1). Contudo, não se encontram identificados os fatores envolvidos e os seus alvos, o que contribui para a inexistência de biomarcadores fidedignos e de uma terapêutica eficaz.

Objetivos

O projeto visa o estudo dos efeitos causados pela indução dos fenótipos microgliais M1 e M2 (usando a linha microglial N9) sobre a viabilidade e plasticidade dos neurnios motores (linha celular NSC-34 expressando ou o tipo SOD1 selvagem, ou o mutado em G93A) a diferentes tempos de diferenciação celular que mimetizam desde os estádios iniciais até aos finais da doença, para se inferir acerca dos momentos e forma de modulação a ser induzida sobre uma micróglia que pode estar ineficiente e/ou excessivamente ativada. O aluno terá contato com técnicas de cultura celular, separação proteica por Western Blot, imunocitoquímica, RT-PCR, microscopia de fluorescência e citometria de fluxo.

Metodologia

1. Micróglia – linha cellular N9 obtida por imortalização de células microgliais obtidas de região cortical de camundongos CD1 (Righi et al., 1989);
2. Neurônios motores – linha celular NSC-34 transfetada com SOD1 humana, quer selvagem, quer mutada em G93A (G93A (NSC-34/hSOD1wt or NSC-34/hSOD1G93A, respectivamente) (Gomes et al., 2008);
3. A polarização da micróglia em M1 e M2 será realizada segundo o descrito por Durafourt et al. (Durafourt et al., 2012);
4. Os NMs serão cultivados entre 1 e 4 dias in vitro (DIV) após o que serão tratados com o meio condicionado microglial durante 24 h;
5. A ativação da metaloproteinase 9 e a produção de óxido nítrico serão avaliados no sobrenadante celular como o habitual no nosso laboratório (Silva et al., 2011);
6. A avaliação da morte neuronal será realizada através do iodeto de propídio e imunocitoquímica/citometria de fluxo (Vaz et al., 2011a);
7. A degeneração neuronal será avaliada por imunocitoquímica usando o Fluoro-JadeC®, a MAP-2 e o NeuN, por imunocitoquímica e Western blot (Fernandes et al., 2009, Silva et al., 2011, Vaz et al., 2011b);
8. A conectividade neuronal será avaliada pela expressão de proteínas sinápicas como a SNAP-25, a sinaptofisina e a SV-2, por Western blot (Silva et al., 2012)[1];
9. A HMBG1 será avaliada nos sobrenadantes dos neurônios e nas células por RT-PCR (Zurolo et al., 2011) e a citocina CX3CL1 por ELISA (Clark et al., 2009);[1]
10. A capacidade autofágica neuronal sera determinada por Western blot, com base na conversão de LC3-I em LC3-II (Palmela et al., 2012)[1].

Resultados antecipados

Espera-se que a microglia na fase M1 tenha uma papel primordial na disfunção dos neurônios NSC-34/hSOD1G93A, após 4 divisões e que a M2 previna a progressão da mesma. Se encontrarmos as vias de sinalização mais implicadas poderemos no futuro ensaias novos compostos terapêuticos capazes de as recuperar ou inibir, controlando de algum modo a evolução rápida da ELA.

Referências

Boillee, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., Kollias, G. and Cleveland, D. W. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312, 1389-1392.

Clark, A. K., Yip, P. K. and Malcangio, M. (2009). The liberation of fractalkine in the dorsal horn requires microglial cathepsin S. *J Neurosci* 29, 6945-6954.

Durafourt, B. A., Moore, C. S., Zammit, D. A., Johnson, T. A., Zaguia, F., Guiot, M. C., Bar-Or, A. and Antel, J. P. (2012). Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia* 60, 717-727.

Fernandes, A., Falcão, A. S., Abranches, E., Bekman, E., Henrique, D., Lanier, L. M. and Brites, D. (2009). Bilirubin as a determinant for altered neurogenesis, neuritogenesis, and synaptogenesis. *Dev Neurobiol* 69, 568-582.

Gomes, C., Palma, A. S., Almeida, R., Regalla, M., McCluskey, L. F., Trojanowski, J. Q. and Costa, J. (2008). Establishment of a cell model of ALS disease: Golgi apparatus disruption occurs independently from apoptosis. *Biotechnol Lett* 30, 603-610.

Ince, P. G., Highley, J. R., Kirby, J., Wharton, S. B., Takahashi, H., Strong, M. J. and Shaw, P. J. (2011). Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. *Acta Neuropathol* 122, 657-671.

Palmela, I., Sasaki, H., Cardoso, F. L., Moutinho, M., Kim, K. S., Brites, D. and Brito, M. A. (2012). Time-dependent dual effects of high levels of unconjugated bilirubin on the human blood-brain barrier lining. *Front Cell Neurosci* 6, 22.

Righi, M., Mori, L., De Libero, G., Sironi, M., Biondi, A., Mantovani, A ., Donini, S. D. and Ricciardi-Castagnoli, P. (1989). Monokine production by microglial cell clones. *Eur J Immunol* 19, 1443-1448.

Silva, S. L., Osório, C., Vaz, A. R., Barateiro, A., Falcão, A. S., Silva, R. F. and Brites, D. (2011). Dynamics of neuron-glia interplay upon exposure to unconjugated bilirubin. *J Neurochem* 117, 412-424.

Silva, S. L., Vaz, A. R., Diogenes, M. J., van Rooijen, N., Sebastiao, A. M., Fernandes, A., Silva, R. F. and Brites, D. (2012). Neuritic growth impairment and cell death by unconjugated bilirubin is mediated by NO and glutamate, modulated by microglia, and prevented by glycoursodeoxycholic acid and interleukin-10. *Neuropharmacology* 62, 2398-2408.

Vaz, A. R., Silva, S. L., Barateiro, A., Falcao, A. S., Fernandes, A., Brito, M. A. and Brites, D. (2011a). Selective vulnerability of rat brain regions to unconjugated bilirubin. *Mol Cell Neurosci* 48, 82-93.

Vaz, A. R., Silva, S. L., Barateiro, A., Fernandes, A., Falcão, A. S., Brito, M. A. and Brites, D. (2011b). Pro-inflammatory cytokines intensify the activation of NO/NOS, JNK1/2 and caspase cascades in immature neurons exposed to elevated levels of unconjugated bilirubin. *Exp Neurol* 229, 381-390.

Zurolo, E., Iyer, A., Maroso, M., Carbonell, C., Anink, J. J., Ravizza, T., Fluiter, K., Spliet, W. G., van Rijen, P. C., Vezzani, A. and Aronica, E. (2011). Activation of Toll-like receptor, RAGE and HMGB1 signalling in malformations of cortical development. *Brain* 134, 1015-1032.

7.2. MATERIAIS E MÉTODOS

7.2.1 Cultura de Células

Foram utilizadas células de neurônios motores da linha celular NSC-34 transfetada com SOD1 humana selvagem (nosso grupo controle, wild type –WT), ou mutada em G93A (grupo usado como modelo de esclerose amiotrófica lateral – G93A) (GOMES et al., 2008). Estas linhagens foram cultivadas em meio RPMI pH 7.2 (Sigma Aldrich®) suplementado com 5% de soro fetal bovino (SFB) (Sigma Aldrich®). No interior dos poços da placa de cultura (com 12 poços) foram colocadas lamínulas de vidro estéreis, previamente tratadas com 0,05% de poli-L-lisina (Sigma Aldrich®) por 30 minutos e o excesso foi retirado com água e após autoclavadas, em seguida deixadas secar na capela de fluxo laminar sob luz ultravioleta por 2 horas.

As células dos neurônios motores WT e G93A foram colocadas nos poços na concentração de 5.10^4 células/ml (quantidade suficiente para alcançar a confluência celular). Após 2 dias de crescimento celular em estufas com temperatura de 37ºC sob atmosfera de 5% de dióxido de carbono e 95% de ar, o meio de cultura de cada poço foi retirado, foram preparados meios de cultura com concentrações de 0, 370, 740 e 1000μg/ml de óleo de peixe (OP) em DMSO que foram adicionados aos poços (figura 1). As células foram colocadas novamente em estufas com temperatura e atmosfera controle como descrito anteriormente durante 4 dias, após este período os meios de cultura foram utilizados para determinação do óxido nítrico e quantificação das metaloproteinases, e as lamínulas com as células avaliadas por imunocitoquímica como descrito a seguir.

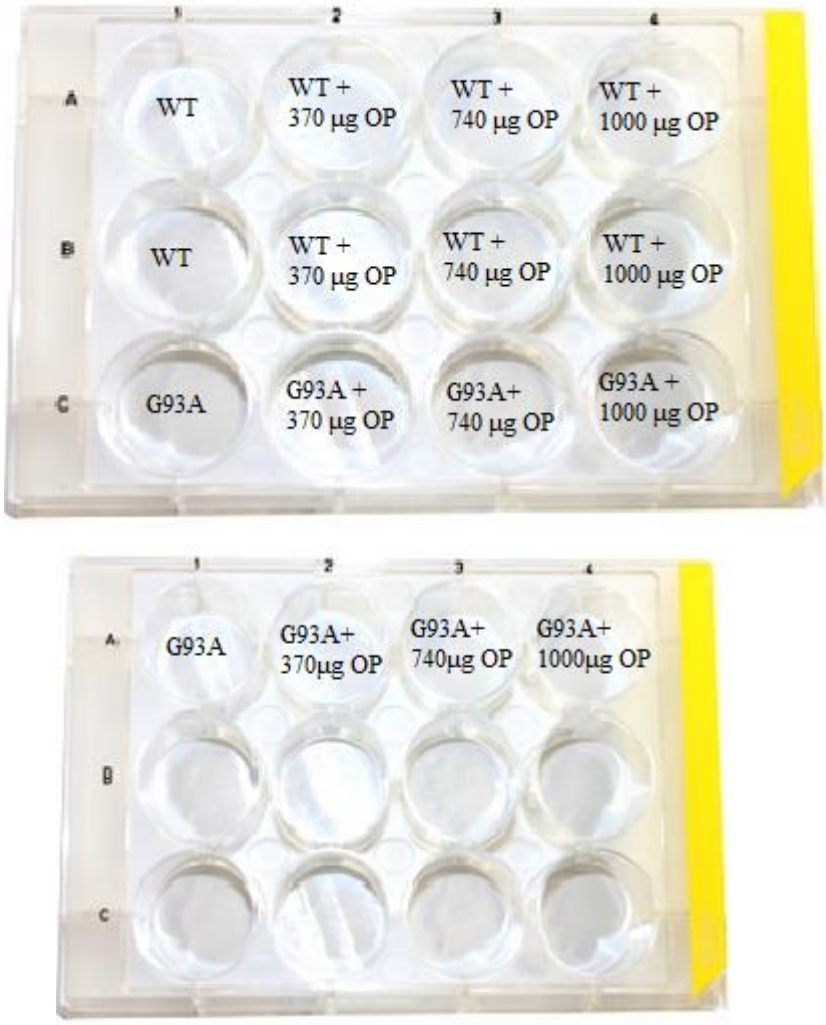

Figura 1. Esquema da disposição dos grupos nas placas.

7.2.2 Determinação de Óxido Nítrico (NO)

O Óxido Nítrico (NO) é um radical livre considerado um dos mais importantes mediadores de processos intra e intercelulares podendo desempenhar tanto um papel protetor quanto prejudicial, e é conhecido por contribuir para algumas condições patológicas (BAGASRA et al., 1995; VODOVOTZ et al., 1996; HAMID et al., 1993; SAKURAI et al., 1995). A produção de NO foi determinada indiretamente pela medida de nitritos (produto da reação do NO com o oxigênio) no sobrenadante da cultura celular como descrito por SILVA et al., 2011.

Em uma Placa de 96 poços foram acrescentados 100 ul do sobrenadante livre de células e 100 ul do reagente de Griesse (0,1% naphtylelthylenediamine e 1% sulfanilamida em 5% H_3PO_4). A absorbância foi determinada em leitor de microplaca com filtro de 540nm.

Os resultados são expressos em valores absolutos (μM), tendo em conta as absorbâncias das soluções da curva padrão.

7.2.3 Quantificação das Metaloproteinases de Matriz (MMP-2 e MMP-9) por Zimografia

As metaloproteinases de matriz (MMPs) são uma família de endopeptidases zinco-dependentes, relacionadas com a funcionalidade e estrutura dos tecidos por atuarem principalmente na degradação de componentes protéicos da matriz extracelular (KUPAI et al., 2010; SNOEK-VAN BEURDEN e VON DEN HOFF, 2005). Elas são classificadas em seis grupos de acordo com seu substrato específico e com a organização de seu domínio catalítico, o grupo das gelatinases é composto pelas MMP-2 e MMP-9 e é responsável por digerir a gelatina e desnaturar o colágeno IV (GEURTS et al., 2012; 2005 KUPAI et al., 2010; SNOEK-VAN BEURDEN e VON DEN HOFF). As MMPs são reguladores importantes dos processos fisiológicos e patológicos (GHAJAR et al., 2008; GHARAGOZLIAN et al., 2009; PAGE-McCAW et al., 2007), para descrever sua participação em diversas doenças é preciso detectar e quantificar sua atividade proteolítica.

Em 20μl do sobrenadante da cultura celular foi adicionado 20μl do tampão de carregamento (0,25 M Tris-base (pH6.8), 4% SDS, 40% glicerol, 0.2% azul de bromofenol), essa mistura foi corrida em gel eletroforese com 10% acrylamide e 0,1% de gelatin. Durante a eletroforese o SDS presente no gel desnatura e inativa as MMPs da amostras, assim, logo após a eletroforese as enzimas forão renaturadas pela remoção do SDS através da lavagem do gel com uma solução aquosa 2,5% do Triton X-100, um detergente não iônico. Após a renaturação das MMPs, o gel foi incubado por 18h a 37°C em uma solução contendo 50 mM Tris pH 7.4, 5 mM CaCl e 1 μM $ZnCl_2$, para que as endopeptidases degradem a gelatina copolimerizado no gel. Em seguida, o gel foi corado com uma solução de azul brilhante de Comassie 0,5% por 30 minutos, e descorado em solução de metanol 30% e acido acético 10% até a percepção de bandas incolores, em contraste com o fundo azul do gel, assim quanto mais clara a banda, maior é a concentração de MMPs da amostra. Uma vez revelados os géis, foram obtidas as imagens e feita a quantificação das bandas, sendo o resultado uma comparação entre as bandas referentes às amostras e as do controle (amostra sem tratamento), usando o software ImageJ.

7.2.4 Avaliação da Morte Neuronal Atravéz do Iodeto de Propídio e Hoechst

O uso de microscopia de fluorescência, associado a corantes nucleares como iodeto de propídio (PI) e Hoechst 33342 (Ho342), é utilizado para técnicas qualitativas de detecção de morte celular (SMITH et al., 1991). O iodeto de propídio prontamente entra e cora as células que perderam a integridade membranar (células necróticas e células apoptóticas em estado avançado), mas não pode atravessar a membrana de células viáveis (excluindo também as células apoptóticas, pois essas possuem membrana intacta), este corante se liga ao DNA de cadeia dupla e emite fluorescência vermelha (figura 2). O Corante Hoeschst 33342 penetra livremente em todas células, porém apenas permanece nas células mortas ou apoptóticas, visto que as vivas têm capacidade de bombeá-lo para fora destas, deste modo, é incorporado mais rapidamente e em maior quantidade pelas células apoptóticas que pelas células viáveis e os núcleos das células em apoptose ou necrose ficam marcados com uma cor azul fluorescente (figura 3) (BUNGO et al., 2006). A combinação de corante Hoechst 33342 (Ho342) que se difunde em membranas celulares intactas, e a solução de iodeto de propídio (PI), que não se difunde em membranas intactas, permite a identificação e a quantificação de células viáveis, apoptóticas e necróticas (SGONC, GRUBER, 1998).

As células aderentes não inpermeabilizadas que foram cultivadas sobre lamelas foram incubadas com uma solução de PI 75µm durante 15 minutos em temperatura ambiente na ausência de luz. Na sequência, as células foram fixadas com paraformaldeído a 4% durante 20 minutos à 37ºC, passado esse tempo as lamelas foram lavadas duas vezes com PBS e posteriormente incubadas com o corante Hoechst 33258 durante 12 horas a 4ºC, após este tempo foram novamente lavadas com PBS, sendo que as lâminas foram montadas com glicerol/PBS 1:1 e analisadas em microscópio de fluorescência. Observou-se a formação de corpos apoptóticos e morte celular pela presença da fluorescência.

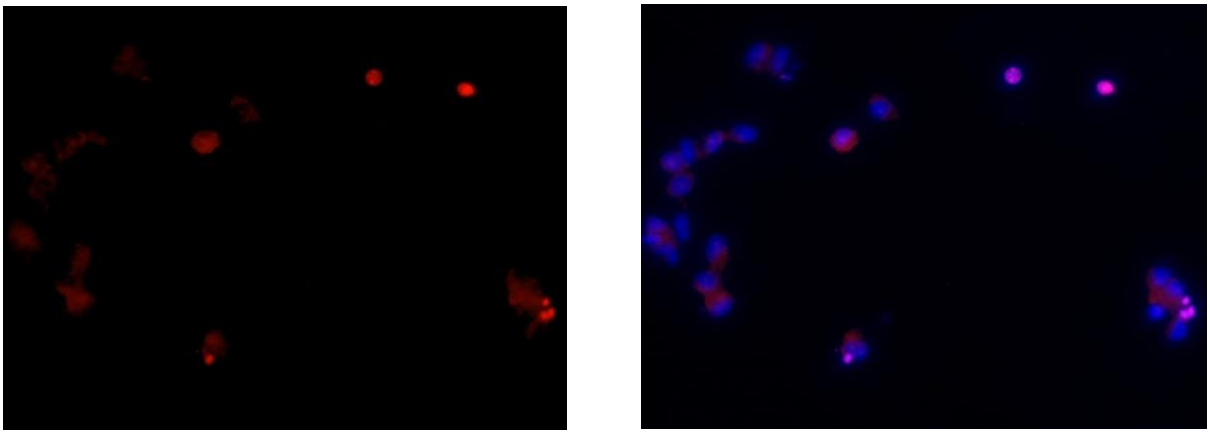

Figura 2. Células marcadas com iodeto de propídio.

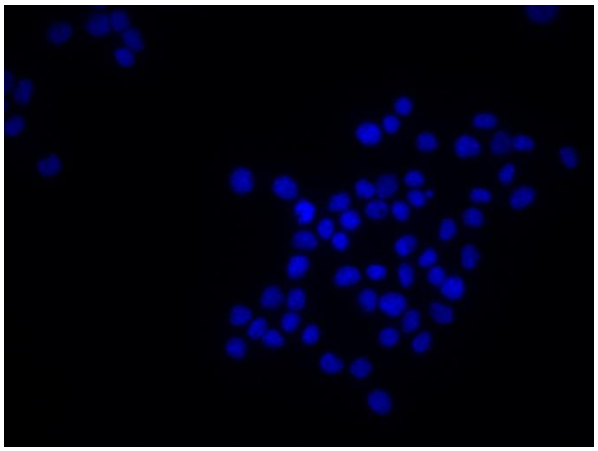

Figura 3. Células marcadas com Hoechst

7.2.5 Autofagia pela Técnica de Imunocitoquímica

Autofagia é um processo dependente de lisossomos que promove a reciclagem de componentes citoplasmáticos, como organelas e proteínas, visando à manutenção da homeostase celular. Este processo é ativado em resposta a diferentes estímulos, como falta de nutrientes, estresse oxidativo e lesões ao DNA. Deficiências nesta via estão intimamente relacionadas a diversas condições patológicas, como problemas cardíacos, câncer, neurodegeneração e envelhecimento. O LC3-I é uma proteína citosólica que durante os processos patológicos é conjugada a fosfatidiletanolamina para formar o LC3-II (LC3-

fosfatidiletanolamina conjugado), o LC3-II é recrutado pela membrana do autofagossoma e se funde ao lisossoma para formar autolisosomas, e os componentes presentes no autofagossoma são degradados por hidrolases lisossomais (ZIEGLER, U. e GROSCURTH, 2004). Assim, o turnover de lisossomas de autofagossomas marcados por LC3-II reflete a atividade autofágica.

As células aderentes que foram cultivadas sobre lamelas foram permeabilizadas com 500 μL (por poço) de Triton 0,2% em PBS por 20 min a temperatura ambiente, após esse tempo foram lavadas com PBS para realizar o bloqueio de sítios inespecíficos com soro bovino (BSA 3%) durante 30 minutos. Em seguida foi feita nova lavagem, e as lamelas forma incubadas com 50 μL de anticorpo primário (Anti LC3-I/II, 1:100) diluído em BSA 1% por 12 horas a temperatura de 4 ºC em câmera úmida. Após três lavagens com PBS as lamelas foram incubadas com 50 μL de anticorpo secundário (anti-mouse, 1:227) conjulgado com Alexa 488 (cor verde) durante 2 horas a temperature ambiente (Figura 4). Após três lavagens com PBS para retirada do anticorpo secundário as células foram marcadas com Hoechst 33342, e por fim, as lâminas foram montadas com glicerol/PBS 1:1 e analisadas em microscópio de fluorescência.

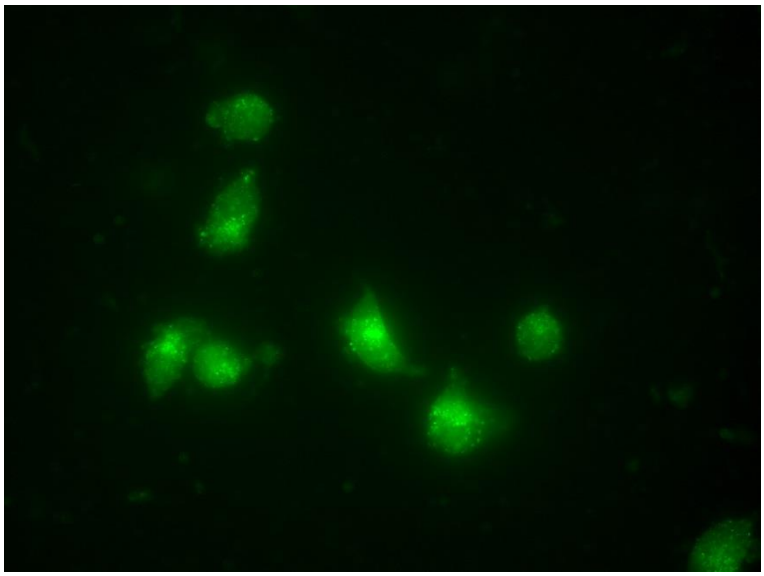

Figura 4. Células marcadas com anticorpo LC3-I/II e Alexa 488.

7.3 RESULTADOS PRELIMINARES

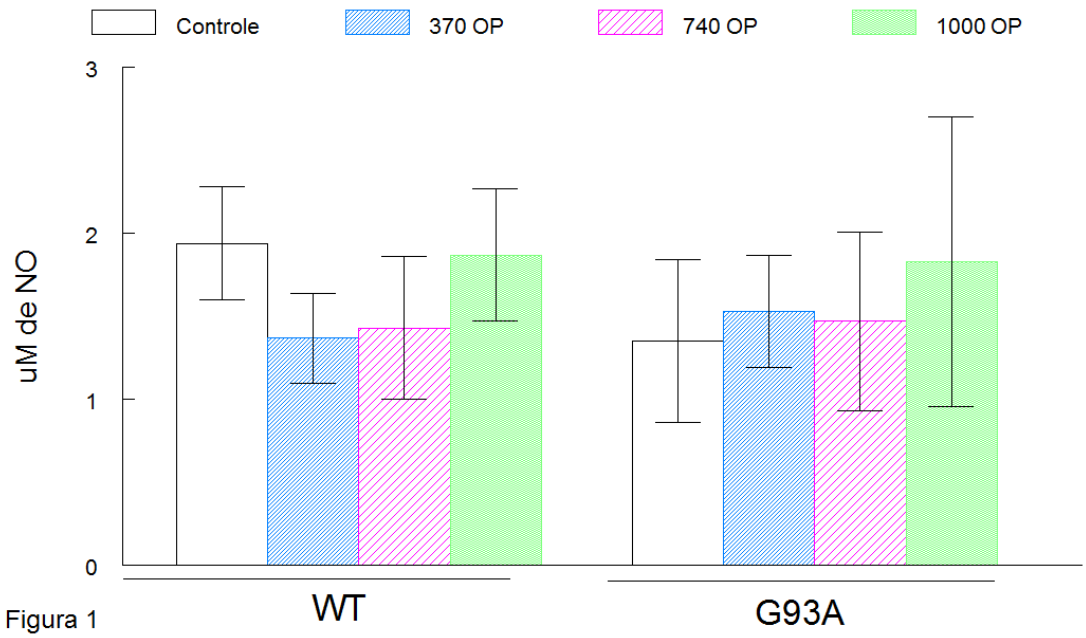

Figura 1. Formação de óxido nítrico nos meios de cultura de células motoras NSC-34 expostas a várias concentrações de óleo de peixe (0, 370, 740 e 1000µg/ml), resultado expressado µM de NO conforme curva padrão. Abreviaturas: NO: óxido nítrico; WT: wild tipe, grupo controle; G93A: célula motora mutada em G93A; OP: óleo de peixe.

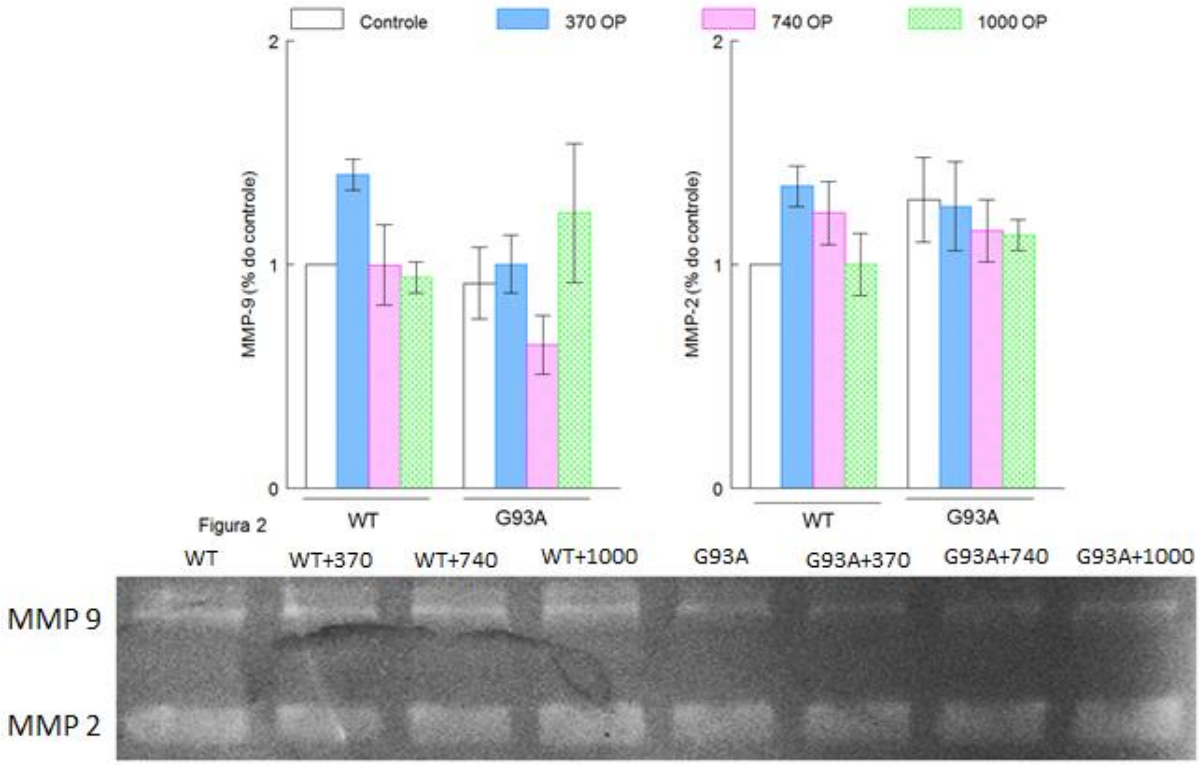

Figura 2. Quantificação de metaloproteínases 2 e 9 por zimografia nos meios de cultura de células motoras NSC-34 expostas a várias concentrações de óleo de peixe (0, 370, 740 e 1000µg/ml) e expressas em porcentagem do grupo controle (WT). Abreviaturas: MMP-2: metalloproteinase 2; MMP-9: metalloproteinase 9; WT: wild tipe, grupo controle; G93A: célula motora mutada em G93A; OP: óleo de peixe.

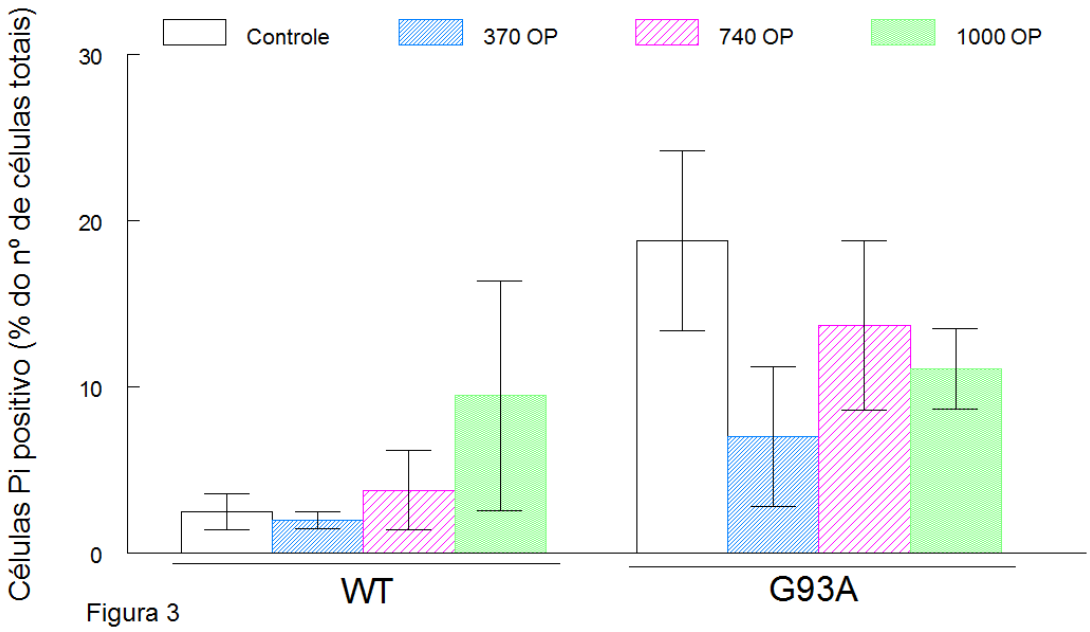

Figura 3. Número de células mortas (Pi positivas) em cultura de células motoras NSC-34 expostas a várias concentrações de óleo de peixe (0, 370, 740 e 1000µg/ml) e expressas em porcentagem do número de células totais. Abreviaturas: Pi: iodeto de propídio; WT: wild tipe, grupo controle; G93A: célula motora mutada em G93A; OP: óleo de peixe.

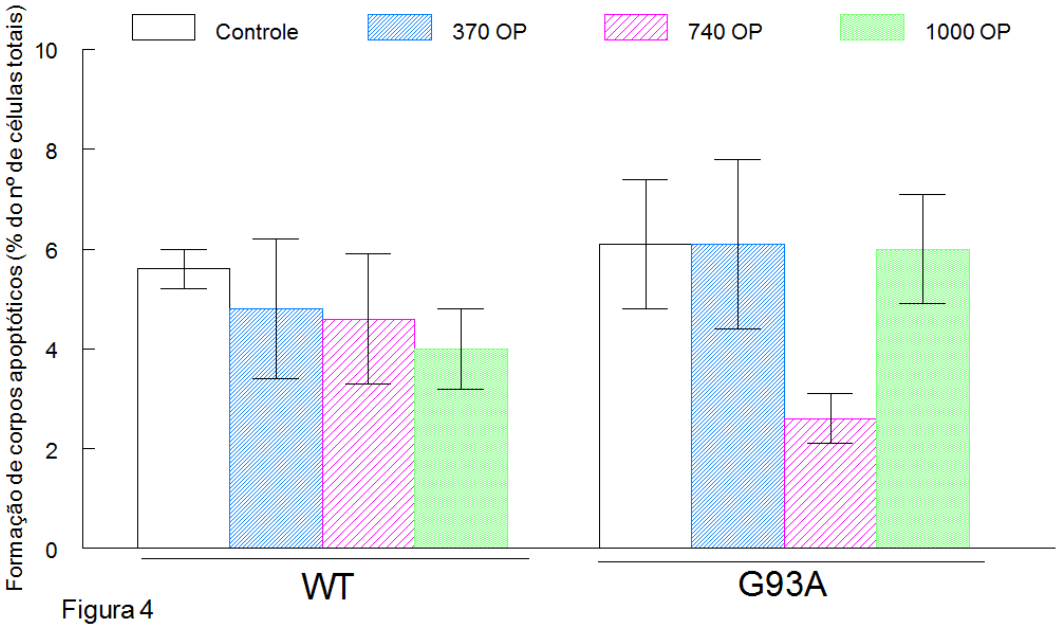

Figura 4. Porcentagem de células apoptóticas em cultura de células motoras NSC-34 expostas a várias concentrações de óleo de peixe (0, 370, 740 e 1000µg/ml) e expressas em porcentagem do número de células totais. Abreviaturas: WT: wild tipe, grupo controle; G93A: célula motora mutada em G93A; OP: óleo de peixe.

7.4 REFERÊNCIAS

BAGASRA, O., et al. Activation of inducible form of Inducible nitric oxide synthase in the brains of patients with multiple sclerosis. **Proc Natl Acad Sci**, v.92, p.12041-5, 1995.

BELLOC, F., et al. A Flow Cytometric Method Using Hoechst 33343 and Propidium Iodide for Simultaneous Cell Cycle Analysis and Apoptosis Determination in Unfixed Cells. **Cytometry**, v.17, p.69-65, 1994.

BUNGO, L., et al. Tulbaghia violácea inhibits growth and induces apoptosis in câncer cells in vitro. **African Journal of Biotechnology**, v.5, p.1936-1943, 2006.

GEURTS, N.; OPDENAKKER, G.; VAN DEN STEEN, P. E**.** Matrix Metalloproteinases as therapeutic targets in protozoan parasitic infections. **Pharmacology & Therapeutics**, v.133, p.257-279, 2012.

GHAJAR, C., M. et al. Matrix metalloproteinase control of capillary morphogenesis. **Crit Rev Eukaryot Gene Expr**, v.18, p.251-278, 2008.

GHARAGOZLIAN, S., et al. Matrix metalloproteinases in subjects with type 1 diabetes. **BMC Clin Pathol**, v.9, p.1-5, 2009.

GOMES, C., et al. Establishment of a cell model of ALS disease: Golgi apparatus disruption occurs independently from apoptosis. **Biotechnol Lett**, v.30, p.603-610, 2008.

HAMID, Q., et al. Induction of nitric oxide synthase is asthma. **Lancet**, v.342, p.1510-1513, 1993.

KUPAI, K., et al. Matrix metalloproteinase activity assays: Importance of zymography. **Journal of Pharmacological and toxicological methods,** v.61, p.205-209, 2010.

PAGE-McCAW, A,. et al. Matrix metalloproteinases and the regulation of tissue remodeling. **Nat Rev Mol Cell Biol**, v.8, p.221-233, 2007

SAKURAI, H., et al. Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthrides. **Journal Clin.Invest**, v.96, p.2357-63, 1995.

SILVA, S. L., et al. Dynamics of neuron-glia interplay upon exposure to unconjugated bilirubin. **J Neurochem**, v.117, p.412-424, 2011.

SNOEK-VAN BEURDEN; P. A. M; VON DEN HOFF, J. W. Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors**. Biotechniques**, v.38, p.73-83, 2005.

VODOVOTZ, Y., et al. Inducible nitric oxide synthase in tangle-bearing neurons of patients with Alzheimer's disease. **Journal Exp Med**, v.33, p.1425-33, 1996.

ZIEGLER, U.; GROSCURTH. Morphological features of cell death. **News Physiol Sci**, v.19, p.124-128, 2004.

CONCLUSÃO

O aprendizado das técnicas desenvolvidas em Portugal foi de extrema validade, já que a metodologia empregada nos ensaios de Western blotting foi padronizada no laboratório de origem brasileiro, imediatamente após o retorno do doutorado sanduíche. Tais dados estão incluídos nos manuscritos 1 e 2, como parte desta tese. Quanto aos resultados preliminares anteriormente mostrados, ainda não permitiram a redação de um manuscrito, já que o número de experimentos foi pequeno. Tais dados aguardam experimentos adicionais por parte do grupo que permanece em Portugal, o que deverá propiciar a redação de um manuscrito em sistema de colaboração Brasil-Portugal.