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PREFERÊNCIA CONDICIONADA A PSICOESTIMULANTE EM RATOS JOVENS APÓS SUPLEMENTAÇÃO COM DIFERENTES ÁCIDOS GRAXOS

DISSERTAÇÃO DE MESTRADO

Fábio Teixeira Kuhn

Santa Maria, RS, Brasil
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PREFERÊNCIA CONDICIONADA A PSICOESTIMULANTE EM RATOS JOVENS APÓS SUPLEMENTAÇÃO COM DIFERENTES ÁCIDOS GRAXOS

Fábio Teixeira Kuhn

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Farmacologia**.

Orientadora: Profª. Drª. Marilise Escobar Bürger

Santa Maria, RS, Brasil
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Universidade Federal de Santa Maria
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A comissão Examinadora, abaixo assinada, aprova a Dissertação de Mestrado

PREFERÊNCIA CONDICIONADA A PSICOESTIMULANTE EM RATOS JOVENS APÓS SUPLEMENTAÇÃO COM DIFERENTES ÁCIDOS GRAXOS

Elaborada por
Fábio Teixeira Kuhn

Como requisito parcial para obtenção do grau de
Mestre em Farmacologia

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Santa Maria, 06 de fevereiro de 2013.

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A Deus, aquele que me guia, me protege e me faz melhor a cada dia.

A verdadeira viagem de descobrimento não consiste em procurar novas paisagens,
e sim em ter novos olhos.

(Marcel Proust)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Farmacologia
Universidade Federal de Santa Maria

PREFERÊNCIA CONDICIONADA A PSICOESTIMULANTE EM RATOS JOVENS APÓS SUPLEMENTAÇÃO COM DIFERENTES ÁCIDOS GRAXOS

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Data e Local da Defesa: Santa Maria, 06 de fevereiro de 2013.

A dieta ocidental sofreu grandes modificações nas últimas décadas, principalmente em decorrência do grande consumo de alimentos industrializados e *fast foods*, que contém uma grande proporção de gorduras ricas em ácidos graxos *trans* (AGT). Ácidos graxos poliinsaturados (AGPI) são componentes essenciais nas membranas neuronais, sendo que os principais AGPI são os da série ômega-3 (n-3) e ômega-6 (n-6), os quais são obtidos exclusivamente da dieta. Os ácidos graxos *trans* (AGT) podem inibir a atividade de enzimas como as dessaturases e elongases, e também competir com os AGPI pela incorporação nas membranas neuronais, modificando sua fluidez e afetando as funções fisiológicas neuroquímicas cerebrais.

A qualidade das gorduras consumidas durante os períodos de gestação e lactação determina uma significativa transferência de AGPI e AGT através da placenta e do leite materno. Diferentes mecanismos relacionados à alteração da composição dos AG podem provocar alterações comportamentais, as quais podem ser especialmente refletidas sobre a neurotransmissão dopaminérgica, aumentando assim a ansiedade e a hedonia, os quais podem exacerbar o efeito aditivo de drogas psicoestimulantes.

A adolescência é o período em que há grande busca por novidades, o que aumenta a probabilidade para o início de experiências que podem culminar em adição. O protocolo de preferência condicionada de lugar (PCL) é um modelo animal bastante utilizado para a compreensão dos mecanismos envolvidos no abuso de drogas psicoativas, servindo também como uma ferramenta de estudos que permitam a prevenção e/ou tratamento da adição.

O objetivo deste estudo foi avaliar a influência da suplementação de diferentes gorduras ricas em ácidos graxos n-3 (óleo de peixe-OP), n-6 (óleo de soja-OS) e ácidos graxos *trans*-AGT (gordura vegetal hidrogenada-GVH) desde o período pré-concepcional, gestação e lactação, e mantidas até a adolescência sobre parâmetros de ansiedade e de preferência à anfetamina (ANF) em ratos jovens, os quais foram avaliados em conjunto com análises do status oxidativo cerebral.

Durante 6 semanas, 24 ratas Wistar adultas foram suplementadas com OS, OP e GVH, compreendendo os períodos desde a concepção, prenhez até o final da amamentação. Após o desmame, apenas 1 filhote macho de cada ninhada foi separado e mantido com a mesma suplementação da projenitora até atingir 40 dias de idade, quando foram submetidos ao protocolo de condicionamento (8 dias) com ANF a fim de conduzir os testes comportamentais e bioquímicos. GVH aumentou a preferência de lugar condicionado por ANF e apresentou sintomas de ansiedade durante a abstinência, indicando um maior potencial de abuso neste grupo experimental. A suplementação de GVH durante o desenvolvimento e crescimento

também foi associada aos maiores danos oxidativos e reduzida atividade antioxidante, enquanto o grupo suplementado com OP mostraram menores níveis de proteína carbonil. Este estudo permitiu observar a influência deletéria do consumo de AGT durante o período perinatal e durante o desenvolvimento, a qual pode ser especialmente evidenciada através dos sintomas de preferência à psicoestimulantes, ansiedade e danos oxidativos no cérebro.

Palavras-chave: Ácidos graxos *trans*, anfetaminas, ômega-3, drogas de abuso, adição

ABSTRACT

Master Dissertation
Graduate Program in Pharmacology
Federal University of Santa Maria

CONDITIONED PREFERENCE FOR PSYCHOSTIMULANT IN YOUNG RATS AFTER SUPPLEMENTATION WITH DIFFERENT FATTY ACIDS

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ADVISOR: PhD. MARILSE ESCOBAR BÜRGER
Date and place of defense: February 6, 2013, Santa Maria

Western diet has undergone major changes in recent decades, mainly due to the large consumption of processed foods and *fast foods*, which contains a large proportion of fats rich in *trans* fatty acids (TFA). Polyunsaturated fatty acids (PUFA) are essential components of the neuronal membranes, and the main PUFAs are the omega-3 series (n-3) and omega 6 (n-6), which are obtained solely from the diet. *Trans* fatty acids (TFAs) can inhibit the activity of enzymes such as desaturases and elongases, and also compete with PUFA for incorporation into neuronal membranes, modifying its fluidity and affecting physiological functions in neurochemical brain.

The quality of fat consumed during periods of pregnancy and lactation determines a significant transfer of PUFA and TFA through the placenta and breast milk. Different mechanisms related to the change in composition of fatty acids can cause behavioral changes which can especially be reflected on dopaminergic neurotransmission, thus increasing anxiety and hedonia, which may exacerbate the additive effect of psychostimulant drugs.

Adolescence is the period in which there is a great novelty seeking, which increases the likelihood for early experiences that may culminate in addition. The protocol conditioned place preference (CPP) is a widely used animal model for understanding the mechanisms involved in the abuse of psychoactive drugs, also serving as a research tool, enabling the prevention and/or treatment of addiction.

The aim of this study was to evaluate the influence of supplementation of different fats rich in n-3 fatty acids (fish oil-FO), n-6 (soybean oil-SO) and *trans* fatty acids-TFA (hydrogenated vegetable fat-HVF) since pre-conception, pregnancy and lactation, and maintained until adolescence on anxiety parameters and preference to amphetamine (AMPH) in young rats, which were evaluated with analyzes of cerebral oxidative status.

During 6 weeks, 24 female Wistar rats were supplemented with SO, FO and HVF, comprising the periods from conception, pregnancy until the end of breastfeeding. After weaning, only 1 male puppy from each litter was separated and kept with the original supplementation until 40 days of age when they were subjected to the conditioning protocol (8 days) with AMPH to conduct behavioral and biochemical tests. HVF increased conditioned place preference by AMPH and showed anxiety-like symptoms during withdrawal, indicating a greater potential for abuse in this experimental group. The HVF supplementation during development and growth was also associated with higher oxidative damage and reduced antioxidant activity, while the FO supplemented group showed lower protein carbonyls. This study showed the deleterious influence of consumption of TFA during the perinatal period and during

development, which can be evidenced by symptoms especially preference at psychostimulants, anxiety and oxidative damage in the brain.

Key-words: *Trans* fatty acids, amphetamine, omega-3, addiction

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ARTIGO CIENTÍFICO

LISTA DE ABREVIATURAS E SIGLAS

AA - ácido araquidônico

AG - ácido graxo

AGE - ácidos graxos essenciais

AGM - ácidos graxos monoinsaturados

AGPI - ácidos graxos poliinsaturados

AGT - ácidos graxos trans

ALA - ácido α-linolênico

ANF - anfetamina

DHA - ácido docosahexaenóico

DPA - ácido docosapentaenóico

EPA - ácido eicosapentaenóico

LA - ácido linoleico

MET - metanfetamina

OP - óleo de peixe

OS - óleo de soja

PCL - preferência condicionada de lugar

SNC - sistema nervoso central

SUMÁRIO

APRESENTAÇÃO

Esta dissertação apresenta os métodos, resultados e discussão inseridos em um artigo científico que encontra-se na secção **ARTIGO CIENTÍFICO**, sob a formatação da revista para a qual foi submetido para publicação.

Ao fim desta dissertação encontra-se o item **CONCLUSÕES FINAIS**, nos quais há interpretações e comentários gerais sobre o manuscrito científico.

As referências referem-se às citações que aparecem no item **INTRODUÇÃO** desta dissertação.

1. INTRODUÇÃO

1.1 A modificação da bi-camada fosfolipídica das membranas neuronais e as possíveis conseqüências fisiopatológicas em relação à adição

Ácidos graxos (AG) são compostos orgânicos formados por uma cadeia de hidrocarbonetos ligada a um grupo carboxílico (COOH) em um extremo da cadeia e uma metila (CH_3) no outro, formando acilgliceróis (mono, di ou triacilgliceróis). Dependendo da natureza da cadeia de hidrocarbonetos, os ácidos graxos podem ser saturados (ligações simples) ou insaturados (duplas ligações), esses podem ser monoinsaturados (AGM) ou poliinsaturados (AGPI) (LUNN e THEOBALD, 2006; LEHNINGER, NELSON e COX, 2002). Os AG são as maiores fontes fornecedoras de energia de todos os macronutrientes, são componentes essenciais na estrutura das membranas celulares fosfolipídicas dos mamíferos e abundantes no sistema nervoso central (SNC) (GOMEZ-PINILLA, 2008; SUMIYOSHI et al., 2008; HORROBIN et al., 1991).

Muitos AG podem ser sintetizados pelos seres humanos, porém, há um grupo de AGPI, denominados de ácidos graxos essenciais (AGE), os quais o corpo humano não consegue sintetizar, ou seja, são obtidos exclusivamente da dieta, os AG das séries ômega-3 (n-3) e ômega-6 (n-6), cujas designações n-3 e n-6 representam a posição da primeira dupla ligação, que por sua vez define as duas famílias de AGPI (RUBIO-RODRÍGUEZ et al., 2010). Um dos principais representantes da série n-6 é o ácido linoleico (C18:2 n-6, LA), é abundante nos óleos de sementes como soja, girassol e milho (HULBERT et al., 2005); o ácido α-linolênico (C18:3 n-3, ALA), é encontrado tanto em algas e fitoplâncton como em frutos do mar, peixes e crustáceos (GIBSON, 2004; WAINWRIGHT, 1992) sendo o AG mais abundante da série n-3. Dentre os ácidos graxos da série ômega-6 está o ácido araquidônico (C:20:4n-6, AA) que é produzido por enzimas elongases e dessaturases a partir do LA, envolvendo uma série de passos de elongação e dessaturação (SPRECHER, 1981), o ácido eicosapentaenóico (C20:5n-3, EPA), ácido docosapentaenóico (C22:5n-3, DPA) e o ácido docosahexaenóico (C22:6n-3, DHA), são formados a partir do ALA através das mesmas enzimas (Figura 1) (GUILLOU et al., 2010; MOON et al., 2001).

Atualmente os hábitos alimentares na sociedade ocidental são caracterizados por um elevado consumo de alimentos contendo carnes, óleos de sementes, *fast food* (pizzas, hambúrgueres) e alimentos industrializados (bolos, biscoitos), que contêm uma grande quantidade de ácidos graxos saturados, gorduras *trans* e uma baixa proporção de AGPI (FERNÁNDEZ-SANJUAN, 2000). Assim como no ocidente, as dietas dos países orientais e mediterrâneos vêm sofrendo as mesmas alterações nos últimos anos, especialmente entre os jovens (MAYNERIS-PERXACHS et al., 2010; BALDINI et al., 2009; KONTOGIANNI et al., 2008).

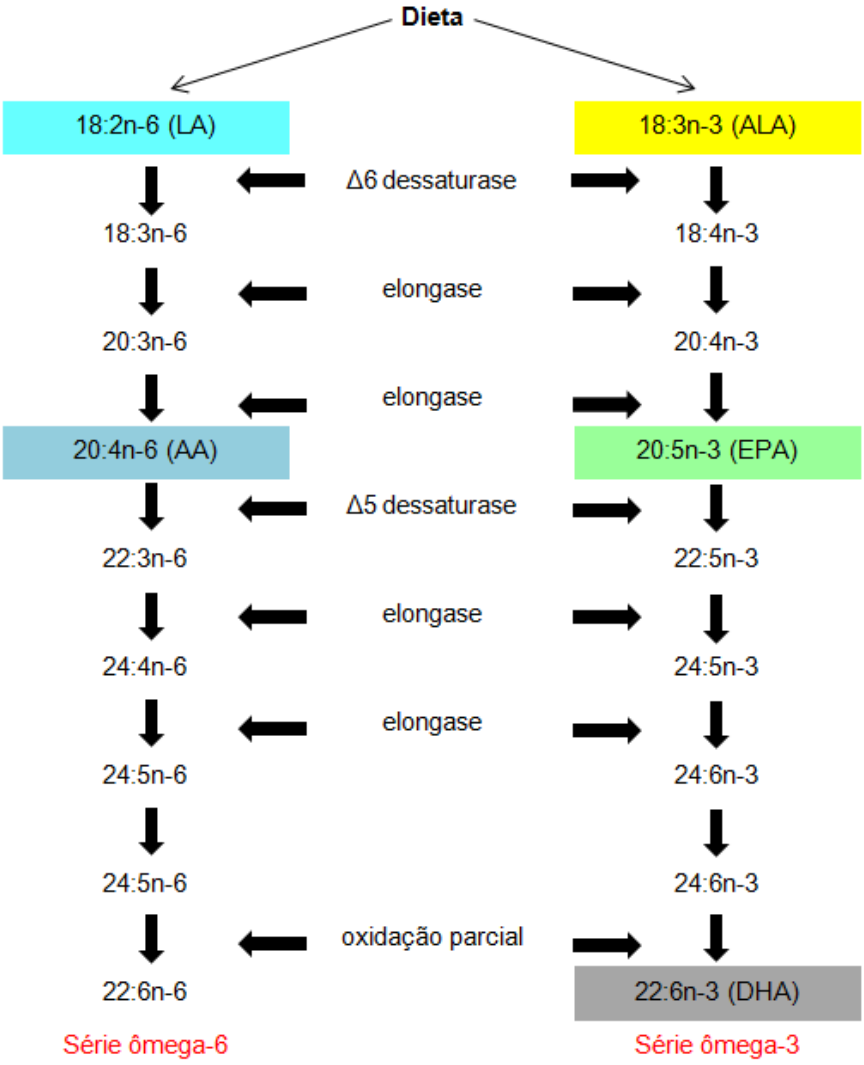

Figura 1: Metabolização dos ácidos graxos poliinsaturados (adaptado de BORSONELO e GALDURÓZ, 2008).

Ácidos graxos *trans* (AGT) são derivados principalmente de óleos vegetais hidrogenados (STENDER, ASTRUP e DYERBERG, 2008), seu uso extenso na produção de alimentos industrializados deve-se ao objetivo de prolongar a vida de prateleira e tornar esses alimentos mais palatáveis (OSSO et al., 2008), sua cadeia carbônica apresenta insaturação

com hidrogênios em posição *trans*, o que fornece maior estabilidade à ligação (Figura 2). Os AGT também podem ser obtidos de fontes naturais, através de alimentos como carne, leite, manteiga e queijo, que são de origem animal, em que o produto primário é obtido de ruminantes (PADOVESE; MANCINI-FILHO, 2002). Alguns vegetais como coco, palma e dendê também são geradores de AGT (CARVALHO et al. 2003; SALEM, 1999).

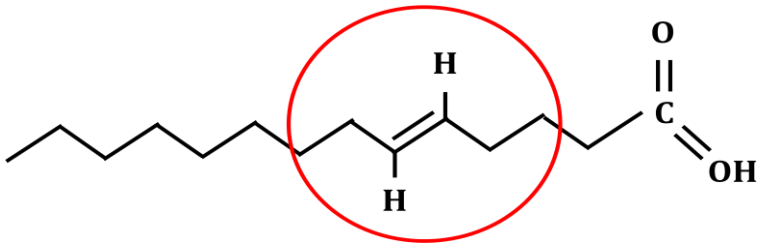

Figura 2: Representação de um ácido graxo com insaturação em posição *trans*.

Alguns autores compararam o consumo de *fast food*, consumida principalmente por jovens, com a culinária japonesa típica (KAMEI et al., 2002) ou a mediterrânica tradicional (AMBRING et al., 2006), ambas com uma grande quantidade de peixes. Essas pesquisas concluíram que a proporção de AG n-6:n-3 está próxima de 2:1, enquanto que em pessoas que consomem *fast food*, pode atingir valores de até 25:1, muito mais elevado do que o ideal que é determinado em 2-4:1 (SIMOPOULOS, 2002), sendo aceitável uma proporção de até 10:1 (SIMOPOULOS, 1991).

O consumo materno de AG durante a gravidez e lactação determina a transferência de AGPI e AGT, através da placenta e do leite materno (INNIS, 2007). O perfil materno de AGPI é um importante determinante para a saúde durante a infância e a vida adulta dos descendentes. Fetos e recém-nascidos requerem um fornecimento adequado de ácidos graxos n-6 e n-3, principalmente DHA e AA para o desenvolvimento de estruturas neurológicas e para o crescimento e desenvolvimento normal de outros tecidos (SOUZA, ROCHA, e CARMO, 2012).

Os AG constituem cerca de 60% da matéria seca do cérebro, e em particular, fosfolipídios constituem 80% da composição de todas as membranas celulares (juntamente com canais iônicos e receptores). Alguns estudos investigaram os efeitos de AGT na composição de AG do cérebro. Estes estudos realizados em animais suplementados com AGT

demonstraram proporções elevadas de AG da série n-6 e diminuição de DHA (PETTERSEN e OPSTVEDT, 1992), sugerindo que a composição de AGPI no cérebro é influenciada pelo consumo de AGT, provavelmente pela inibição da Δ6 e Δ5 dessaturases (LARQUE et al., 2003; KIRSTEIN, HOY e HOLMER, 1983). Além disso, os AGT podem ser incorporados em fosfolipídios de membrana, alterando a fluidez, propriedades bioquímicas, e a função das células (SALEM et al., 2001). Neste contexto, AG e fosfolipídios desempenham papéis críticos e dinâmicos no desenvolvimento cerebral, assim como no processo de envelhecimento e doenças, eles participam na transdução de sinal, plasticidade da membrana sináptica, transcrição de genes e fluxo sanguíneo cerebral, e modulam as respostas do cérebro a ação de drogas, neuroinflamação e isquemia (GOMEZ-PINILLA, 2008; WU, YING e GOMEZ-PINILLA, 2004).

Em recente estudo, Santos et al. (2012) analisou os perfis de AG de 80 mães adolescentes saudáveis, através de amostra de sangue pós-parto, tanto da mãe quanto do sangue do cordão umbilical no momento do nascimento, o qual mostrou que LA estava presente em proporções maiores no cordão umbilical do que no plasma materno, enquanto que AA estava presente em proporções maiores nos lipídios totais do sangue e menores no cordão umbilical. Os AGT encontrados no plasma materno apresentaram uma correlação negativa com o ácido oleico e LA, indicando que AGT interferem na proporção de AGPI, enquanto que ALA teve uma correlação positiva com o perímetro encefálico ao nascimento, mostrando que o desenvolvimento, principalmente dos tecidos cerebrais, podem estar associados com uma quantidade adequada de AGPI.

Em outro estudo, Pimentel et al. (2012) avaliou a prole de ratas suplementadas durante a gestação e a lactação com gordura vegetal hidrogenada (GVH), rica em AGT, obteve como resultado a elevação dos níveis de endotoxinas e de fatores desencadeantes de inflamação, também redução da saciedade através dos sensores da via hipotalâmica nos filhotes com 90 dias, tendo como consequência distúrbios metabólicos como obesidade, mesmo após a retirada do fator causal, ou seja, o efeito permaneceu nos descendentes após o consumo da dieta contendo AGT.

O metabolismo de AG e fosfolipídios é anormal em um grande número de doenças cerebrais humanas, incluindo acidente vascular cerebral, doenças de Parkinson e de Alzheimer, esclerose múltipla, demência associada ao HIV-1, transtorno bipolar (TB), e depressão, há evidências de que o consumo de alimentos com proporções elevadas n-6:n-3 bem como AGT poderia desempenhar um papel na fisiopatologia dessas perturbações

psiquiátricas (SINCLAIR et al., 2007; YOUNG e CONQUER, 2005). Comportamentos agressivos e distúrbios de abuso de drogas podem estar associados com uma ingestão insuficiente de AGPI, sugerindo ser um dos fatores que contribuem para o aumento de comportamentos violentos em alguns indivíduos (MERIKANGAS, MEHTA e MOLNAR, 1998). Diferentes mecanismos provocados pela alteração na composição dos AG podem provocar modificações comportamentais, tendo um papel na redução dos escores de comportamento de ansiedade e raiva, o que mostra que um aumento no consumo de DHA pode aumentar a serotonina do cérebro (HIBBELN et al., 1998). Reduções significativas nos escores de ansiedade, após um tratamento de três meses, com suplementação diária de EPA, DHA e outros AGPI da série n-3 foram observados em pacientes em tratamento para abstinência de drogas de abuso (BUYDENS-BRANCHEY, BRANCHEY e HIBBELN, 2008; BUYDENS-BRANCHEY, 2003).

Há dados que indicam que em jovens cuja ingestão dietética de DHA e de peixe foi adequada, houve uma menor probabilidade de comportamentos hostis (IRIBARREN et al., 2004). Foi mostrado ainda, através de um estudo, que a suplementação com cápsulas de óleo contendo DHA impediu um aumento da agressividade em momentos de estresse entre estudantes japoneses (HAMAZAKI et al., 1996)

Yehuda, Rabinovitz e Mostofsky (2005) investigaram os efeitos da administração de uma mistura de AGPI n-3 e n-6 sobre a ansiedade em estudantes universitários onde observaram uma significativa melhora no humor, concentração, fadiga, e sono. Eles também observaram uma diminuição no cortisol que é o hormônio que está associado ao estresse quando em níveis elevados. Green et al., (2006) observou diminuição dos níveis de AGPI nas membranas de células vermelhas do sangue de pacientes com transtornos de ansiedade.

Anggadiredja et al. (2003) em seu estudo, mostrou que a dependência de drogas de abuso como canabinóides e opióides pode estar associada com a cascata do AA no cérebro, pois demonstrou que com a retirada do THC houve uma diminuição nos níveis de prostaglandinas, com a consequente diminuição da ativação da cascata do AA, outros tipos de drogas também podem estar associadas com esse mecanismo para o desenvolvimento de dependência.

Há uma vasta literatura sobre diferentes aspectos dos AGPI publicada nos últimos anos, principalmente sobre seu papel na saúde humana, porém são limitados os estudos envolvendo AGT e sua influência nas membranas neuronais e mais especificamente no abuso de drogas. Uma vez que alterar os hábitos nutricionais de uma sociedade como um todo é

muito difícil, muitos produtos enriquecidos com AG n-3, tal como suplementos nutricionais ou alimentos funcionais têm sido desenvolvidos para suplementar a dieta e alcançar uma boa proporção de AG n-6:n-3 sem alterar a dieta em demasiado.

1.2 Psicoestimulantes anfetamínicos e adolescência

Os psicoestimulantes são amplamente utilizados em todo o mundo, e apresentam como efeito principal o aumento do estado de alerta e vigília, promovendo ânimo e motivação. Um dos principais grupos de estimulantes centrais são as anfetaminas (ANF). Sintetizadas pela primeira vez em 1887, foram produzidas por meio de modificação da estrutura química da efedrina, com efeitos farmacológicos de estimulação do SNC através de ação simpaticomimética, foram utilizadas primeiramente como descongestionante nasal, broncodilatadores e para o tratamento da narcolepsia (HUNT, KUCK e TRUITT, 2007; NORDAHL, SALO e LEAMON, 2003).

Durante a Segunda Guerra Mundial as ANF foram usadas pelos militares da Alemanha, Japão e Estados Unidos, para aumentar o estado de vigília, reduzir a fadiga e suprimir o apetite. Os militares americanos também fizeram o uso durante as Guerras da Coréia e do Vietnã (VEARRIER et al. 2012; HUNT, KUCK e TRUITT, 2007). Em meados dos anos 1940 o uso das ANF se difundiu na sociedade, grandes quantidades foram produzidas licitamente e a Metanfetamina (MET) foi extensivamente prescrita para inúmeras indicações. Donas de casa, motoristas de caminhão, estudantes e profissionais utilizaram para aumentar a vigília, melhorar o humor e a atenção, e redução de peso. Grande parte dessas pessoas desenvolveram tolerância e adição (VEARRIER et al. 2012; CICCARONE, 2011; HUNT, KUCK, e TRUITT, 2007).

A mistura racêmica da ANF (mistura equivalente de anfetamina dextrógira e levógira) foi utilizada em meados de 1936 para reduzir o apetite de indivíduos obesos, no tratamento do déficit de atenção e na doença de Parkinson (SEIDEN e SABOL, 1993). Nessa época já havia relatos de graves efeitos adversos à saúde envolvendo as anfetaminas. Um estudo inicial relatou que, mesmo com a dose terapêutica recomendada de anfetamina inalada, a maioria dos indivíduos apresentaram severos efeitos cardíacos como aumento da frequência e pressão arterial, palpitações, taquicardia e dores no peito (NORDAHL, SALO e LEAMON, 2003). O

uso contínuo de ANF também conduz a distúrbios neurológicos e psicóticos tipicamente caracterizados por alucinações e delírios, bem como transtornos de humor e ansiedade (GOOD e RADCLIFFE, 2011). A partir dos vários efeitos danosos que foram revelados pelo uso das ANF, tomou-se conhecimento que seu uso não era seguro e medidas restritivas de controle para a obtenção dessas substâncias foram tomadas pelos governos.

O mecanismo de ação das ANF está principalmente relacionado ao sistema dopaminérgico, promovendo o aumento da liberação neuronal de dopamina (DA), inibição da sua recaptação pelo transportador de DA, e bloqueio da ação da enzima responsável pela regulação das monoaminas na fenda sináptica monoamina oxidase (MAO) que faz o catabolismo das catecolaminas, e que apresenta papel fundamental no equilíbrio da atividade do sistema dopaminérgico, noradrenérgico e serotoninérgico periférico e central (NORDAHL, SALO e LEAMON, 2003; ROTHMAN et al., 2001; BROWN, HANSON e FLECKENSTEIN, 2000). A sinalização dopaminérgica está envolvida em vários circuitos cerebrais: funções endócrinas, olfativas e comportamentais. Entre os comportamentos dependentes de sinalização dopaminérgica estão a locomoção, cognição, motivação, aprendizagem e apetite (THANOS et al., 2010; VIGGIANO, RUOCCO e SADILE, 2003; MISSALE et al., 1998).

Em alguns países as ANF ainda são utilizadas na terapêutica médica, mas também usadas para fins não medicamentosos de forma ilícita (MACHALOVA et al., 2012; MURRAY, 1998). No Brasil ainda são permitidas a venda, com retenção de receita, do Metilfenidato (Ritalina®) usado no tratamento do transtorno do déficit de atenção e hiperatividade (TDAH), e da Sibutramina (Sibus®) como inibidor do apetite. Em 2011 foi proibida a comercialização bem como fabricação, pela Agência Nacional de Vigilância Sanitária – ANVISA, dos medicamentos anfepramona, femproporex e mazindol, anfetamínicos supressores do apetite também chamados de anorexígenos (BRASIL - RDC No- 52/2011).

O abuso de ANF vem tornando-se um problema global, principalmente através dos medicamentos. Estimativas mostram uma alta prevalência do consumo de psicoativos anfetamínicos entre motoristas nos últimos anos que são usados por esses condutores para aumentar a sua atenção durante as viagens de longa distância (SOUZA et al., 2012; DAVEY, FREEMAN e LAVELLE, 2009). O consumo de anfetaminas aumentou drasticamente na última década nos Estados Unidos, assim como em outros países desenvolvidos, contrariamente a tendência de retração que vinha ocorrendo.

A dependência de drogas é provocada pelo uso repetido de substâncias com potencial de abuso, sendo que a principal característica é uma reduzida capacidade de regular o desejo de exposição à droga, independente dos riscos envolvidos. (FEIL et al. 2010; HYMAN e MALENKA; 2001).

Os últimos dados publicados no relatório das Nações Unidas – *International Narcotic and Control Board* (*Office on drugs and crimes – Amphetamines and ecstasy 2008*) sobre a situação das ANF no mundo mostra que o consumo de medicamentos estimulantes do tipo ANF ficou bem acima da média mundial na América do Sul, indicando um uso excessivo desses psicoestimulantes, a partir de fontes lícitas. Entre o período de 2000/02 e do período de 2004/06, o consumo de psicoestimulantes sintéticos do tipo anfetamínicos aumentou de 7 doses diárias (DD) por 1.000 habitantes para a 11 DD, um aumento de 57%, sendo que Argentina e Brasil foram primeiro e terceiro países do mundo com maiores números de prescrições, com 17 e 10 DD/ 1.000 habitantes, respectivamente. Este aumento é preocupante e mostra o excessivo número de prescrições que podem estar associados ao uso indevido (UNITED NATIONS, 2008). Os fármacos prescritos perdem apenas para a maconha como substância de abuso. Além disso, há um aumento de duas vezes em mortes induzidas pelas drogas nos últimos 10 anos, impulsionado pelos medicamentos (UNITED NATIONS, 2012).

Os custos humanos, sociais e econômicos do abuso de drogas na América do Norte têm sido elevados. De acordo com as estimativas mais recentes, mais de 45 mil pessoas morrem na região por causas relacionadas a drogas a cada ano. O Centro Canadense de Abuso de Substâncias estimou os custos de uso de drogas ilícitas para a economia canadense em mais de 8 bilhões de dólares por ano.

Há uma fase de desenvolvimento que está associada a uma tendência a assumir mais riscos que indivíduos de outras faixas etárias, como o uso de drogas de abuso, tabaco, álcool e substâncias ilícitas, que é a adolescência, definida como o período de desenvolvimento entre a infância e a fase adulta, marcada pela busca por novidades (WILEY, JONES e WRIGHT, 2011; DOREMUS-FITZWATER, VARLINSKAYA e SPEAR, 2010; ARNETT, 1992). Essa fase é compartilhada por seres humanos e outras espécies de mamíferos, como os roedores (DAHL, 2004; SPEAR, 2000).

Os adolescentes são menos sensíveis que os adultos aos psicoestimulantes em exposição ao fármaco pela primeira vez, ou seja, a transição para a dependência de drogas de abuso é mais rápida do que nos adultos (WU e SCHLENGER, 2003). Além disso, o risco de abuso de drogas na idade adulta é maior em indivíduos que tiveram o primeiro contato na

adolescência (MERLINE, 2004), sugerindo que a exposição à psicoestimulantes nessa fase pode ter efeitos únicos e duradouros sobre a sensibilidade à psicoestimulantes na idade adulta.

As vias neurais que medeiam a busca por novidades, pertencem principalmente ao sistema dopaminérgico (BARDO, DONOHEW e HARRINGTON, 1996), também são locais de ação de psicoestimulantes, indicando que uma remodelação do desenvolvimento deste circuito pode ser a base do diferencial de vulnerabilidade de adolescentes e adultos às drogas de abuso (ERNST, ROMEO e ANDERSEN, 2009). As mudanças no circuito mesocorticolímbico na adolescência são semelhantes em pessoas (WEICKERT et al., 2007) e roedores (MOLL et al., 2000) e incluem aumento do transportador de dopamina e dos receptores de dopamina. Em roedores, a adolescência começa logo após o desmame e pode ser dividida em três fases: início da adolescência que abrange o período após o desmame e antes da puberdade, que dura cerca de 13 dias (21 dias pós-natal - DPN) a DPN34. O período médio da adolescência engloba o tempo pouco antes e depois da puberdade, a partir de DPN 34 a DPN 45, e final da adolescência que começa em DPN 45 e dura até DPN 60, quando os ratos atingem a maturidade sexual (TIRELLI, LAVIOLA e ADRIANI, 2003).

A preferência condicionada de lugar (PCL) proporciona uma medida da busca pela droga através da avaliação da preferência de um animal (ou aversão) a um determinado ambiente associado aos efeitos de determinada droga. Usando este procedimento, a substância é pareada com um ambiente neutro, ou seja, o animal receberá a droga em um ambiente onde previamente não foi observada preferência, enquanto que um segundo ambiente é exclusivamente pareado com veículo (CARLEZON, MAGUE e ANDERSEN, 2003). Após o condicionamento, por determinado período, o animal move-se livremente entre os dois ambientes, em um estado livre de drogas, e o tempo gasto em cada ambiente é medido. A medida do tempo no ambiente pareado com a droga é interpretada como uma preferência, ao passo que o tempo gasto longe do ambiente é interpretado como uma aversão (BRENHOUSE e ANDERSEN, 2008). A PCL positiva está fortemente correlacionada com a auto-administração de drogas em ratos, prevê efeitos de recompensa e avalia o potencial de abuso de uma substância (MCCORVY et al., 2011).

Poucos estudos examinaram os sintomas de adição e as consequências do abuso de drogas em relação ao consumo de diferentes ácidos graxos. Esta é uma importante questão pelo potencial dos ácidos graxos *trans* de alterarem as membranas neuronais e com isto serem capazes de exacerbar os efeitos de drogas de abuso e induzir distúrbios de ansiedade em seres humanos.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito da suplementação de diferentes ácidos graxos em ratos jovens nascidos de projenitoras também suplementadas durante a gestação e lactação sobre parâmetros comportamentais e bioquímicos relacionados à preferência por anfetamina.

2.2 Objetivos Específicos

- Avaliar os efeitos de diferentes óleos ricos em AG n-3, n-6 e gordura rica em AGT, suplementados desde os períodos gestacional, amamentação e desenvolvimento sobre a preferência de lugar condicionado por ANF em ratos jovens;

- Avaliar a influência dos diferentes AG sobre parâmetros locomotores, exploratórios e de ansiedade em ratos jovens previamente expostos à ANF;

- Avaliar a influência dos diferentes AG sobre o desenvolvimento de estresse oxidativo (oxidação de proteínas) e o status antioxidante (atividade da catalase), avaliados no córtex, estriado e hipocampo, bem como os níveis da glutationa redutase no plasma e eritrócitos dos ratos jovens previamente expostos à ANF;

3. ARTIGO CIENTÍFICO

Os resultados inseridos nesta dissertação apresentam-se sob a forma de artigo científico, o qual se encontra aqui estruturado. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se no próprio manuscrito.

O referido artigo foi submetido para publicação na revista *Pharmacology, Biochemistry and Behavior* e encontra-se sob revisão.

Influence of *trans* fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats

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Influence of *trans* fat and omega-3 on the preference of psychostimulant drug in the first generation of young rats

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Abstract

Adolescence is related to seeking behavior, with a higher probability of trying out addictive drugs. The current Western diet often provides considerable amounts of saturated and *trans* fatty acids (TFA), whose incorporation into neuronal membranes has been implicated in changes of brain neurochemical functions. Such influence has caused concerns due to precipitation of neuropsychiatric disorders, whose data are still unclear. Here we evaluated the influence of different fats on preference parameters for amphetamine (AMPH): adolescent rats were orally supplemented with fish oil (FO, rich in n-3 FA), soybean oil (SO, rich in n-6 FA) and hydrogenated vegetable fat (HVF, rich in saturated and *trans* FA) from weaning, which were born of dams supplemented with the same fat from pregnancy and lactation. AMPH preference, anxiety-like symptoms and locomotor index were evaluated in conditioned place preference (CPP), elevated plus maze (EPM) and open-field (OF), respectively, while brain oxidative status was determined in cortex, striatum and hippocampus. HVF increased AMPH-CPP and was associated with withdrawal signs, as observed by increased anxiety-like symptoms. Moreover, FO and SO were not associated with AMPH preference, but only FO-supplemented rats did not show any anxiety-like symptoms or increased locomotion. FO supplementation was related to lower oxidative damages to proteins and increased CAT activity in all brain areas evaluated, as well as increased GSH levels in blood, while HVF was related to higher oxidative status. In conclusion, our study showed the harmful influence of TFA on AMPH-CPP and drug craving symptoms, which can be related to dopaminergic neurotransmission.

Keywords: *Trans* fatty acid, omega-3, amphetamine, drugs abuse, addiction

1. Introduction

Adolescence is a life period related to novelty seeking and risky behavior, which may lead to the use of psychoactive drugs and eventually to drug addiction (Gladwin et al., 2011). Addiction is characterized by compulsion, craving and seeking for drugs that persist despite adverse consequences. Recent literature data have suggested that only cannabis surpassed the use of prescription drugs (SAMHSA, 2011), whose abuse has been linked to a twofold increase in deaths in the last 10 years, representing an elevated human, social and economic cost. Although some psychostimulants such as methylphenidate and amphetamine salts have been used to treat attention deficit hyperactivity disorder (ADHD) and narcolepsy, such drugs are also illegally available for non-medical use (Clegg-Kraynok et al., 2011). In this context, amphetamine (AMPH) and its derivatives are intensely used worldwide for recreational purposes, and its use is related to increasing alertness, euphoria and insomnia, generally accompanied by accelerated speech and irritability. Besides the above-mentioned drugs, this chemical group includes such other psychostimulants as diethylpropion and fenproporex, which are legally used as appetite suppressants in some countries (Souza et al., 2012). AMPH and its correlates inhibit vesicular reuptake of dopamine (DA) and norepinephrine (NE) and increase their neuronal release (Brown et al., 2000). As a result, the dopaminergic signaling affects different brain circuits related to sight, smell and behavioral functions, as well as parameters affecting mobility, cognition, motivation, learning and appetite (Viggiano et al., 2003). AMPH-induced reward and anxiety-like symptoms have been experimentally studied (Häggkvist et al., 2009), and drug seeking behavior has been evaluated in conditioned place preference (CPP) (Mathews et al., 2010), where classical conditioning is used to pair with an unconditioned stimulus, i.e, a drug is associated with a neutral environmental stimulus, which is strongly correlated with self-administration of drugs in rats (Bardo and Bevins, 2000). Thus, CPP is an animal model that provides a measure of drug seeking behavior assessed by

cues of environmental preference or aversion associated with drug effects. A positive CPP can be defined as an increased preference for the drug-paired environment, and is able to predict rewarding effects and assess potential drug abuse (McCorvy et al., 2011).

Different studies have related dependence and withdrawal symptoms from abuse substances to neuronal inflammatory processes, which involve the arachidonic acid (AA) cascade as an essential component of such reactions (Yamamoto et al., 2004). Thus, the increased consumption of processed foods in Western countries in the last decades has concerned health and food safety authorities, because fast foods are rich in saturated (Baggio and Bragagnolo, 2006) and *trans* fats (Allison et al., 1999) and poor in essential fatty acids (EFA). Chronic consumption of *trans* FA (TFA) can represent a loss of EFA and modify the composition of neuronal membranes phospholipids, thus affecting the proinflammatory cascade in the brain (Yamamoto et al., 2004). Of particular importance, EFA are so denominated due to mammals' inability to synthesize them, and thus these FA must be obtained from the diet (Hulbert et al., 2002). In fact, α-linolenic acid (LNA, 18:3) is an EFA of the omega-3 series and originates docosahexaenoic (DHA, 22:6-n3) and eicosapentaenoic (EPA, 20:5-n3), while linoleic acid (LA 18:2) is an EFA of the omega-6 series and a precursor of arachidonic acid (AA, 20:4-n6). Of particular importance, DHA and EPA compete with AA for its incorporation into the lipid cell membranes and serve as a substrate for cyclooxygenase-2 (COX-2) (Bagga et al., 2003).This enzyme acts on n-6 PUFA and generates prostaglandins (PG) of series 2 (PGE2), while its action on n-3 PUFA generates PGE of series 3 (PGE3), which is less pro-inflammatory than the former. In this sense, the type of FA incorporated into neuronal membranes phospholipids is strongly regulated by diet composition (Abbott et al., 2012), showing that TFA can change this composition and affect physiological functions, cell permeability and synaptic membrane fluidity of the brain (Jump 2002; Yehuda et al., 2002).

While previous studies showed that dietary deficits of PUFA during early life are linked to increased n-6:n-3 FA ratio in the brain, significant abnormalities of DA neurotransmission in adult rats have been reported (Chalon, 2006). A human pilot study performed in substance abusers showed beneficial influence of n-3 PUFA on anger and anxiety scores, which were reduced in these patients (Buydens-Branchey et al., 2008). In fact, literature is abundant when it comes to deficiency of omega-3 FA, but up to now few studies have evaluated the influence of chronic consumption of saturated and *trans* fats on the DA neurotransmission (Acar et al., 2003). Of particular importance, no study reported the influence of saturated and TFA on DA neurotransmission and its relationship with addiction. Recent studies by our research group showed the development of AMPH-induced mania (Trevizol et al., 2011), anxiety-like symptoms and memory-loss (Teixeira et al., 2011) in rats supplemented with TFA, reflecting its influence on the development of psychiatric abnormalities. Consequently, further detailed investigations about it are urgently needed to establish the nutritional safety of TFA in humans (Wandall, 2008).

In this study we evaluated the influence of dietary FA on the development of reward symptoms consequent to AMPH in rats chronically supplemented with hydrogenated vegetable fat (HVF, rich in TFA), fish oil (FO, rich in n-3 FA) and soybean oil (SO, rich in n-6 FA) from pregnancy and lactation of their dams, diets which were maintained until adolescence. We believe that this is an emergent issue because drug abuse has the potential to exacerbate or induce different degrees of neuropsychiatric disorders in humans (He et al., 2005).

2. Methods

2.1. Animals and experimental procedure

Twenty-four female adult Wistar rats (200-220g) were placed in Plexiglas cages (groups of two animals) with free access to food and water in a room with controlled temperature (23 ± 1 °C) and on a 12 h light/dark cycle with lights on at 7:00 a.m. Animals were supplemented once a day by gavage (3g/kg) (Ferraz et al., 2011) with fish oil (FO), hydrogenated vegetable fat (HVF) or soybean oil (SO), which was considered an isocaloric control. The supplementations were initiated six weeks before mating and continued during pregnancy, which was confirmed by presence of sperm in vaginal smear, being maintained until postnatal day 21 (PND 21), when litters were weaned. The profile of the FA of each supplemented fat was determined by gas chromatography (Hartman and Lago, 1973) (Table 1). In order to exclude genetic factors, after weaning only one male pup of each litter was included in each experimental group (n=8), which were maintained under the same supplementation as theirs dams until PND 40 (Figure 1). The experimental protocol was approved by the Animal Ethical Committee (Universidade Federal de Santa Maria-UFSM-24/2010), which is affiliated to the Council for Control of Animal Experiments (CONCEA), following international norms of care and animal maintenance.

2.2. Drugs and Solutions

DL-amphetamine (Merck, Germany) 4 mg/mL, which was adapted from Hiroi and White (1991) and Bowling and Bardo (1994). These authors used a dose of 2 mg/kg of D-AMPH, and therefore in our study the dose of DL-AMPH was duplicated in order to maintain the same stimulant response in the animals.

2.3. Behavioral evaluations

2.3.1. Conditioned place preference (CPP) procedure

The CPP apparatus had three compartments separated by manual guillotine doors: two boxes of equal size (45 × 45 × 50 cm) and equivalent intensity of light, but with different textures. One compartment had a smooth white floor and striped walls, while the other had a striped floor and smooth white walls. These two preference compartments were accessible by a central compartment (18x 36x 50 cm) which was gray with a smooth floor. The boxes were indirectly illuminated by incandescent light (60 Watts) of equal intensity at all times. The apparatus was cleaned with alcohol 20% using wet sponge and paper towel before the introduction of each animal.

Habituation of rats in the CPP was achieved with the following steps: habituation, pretest, conditioning, and test. For habituation, animals were lead to a room of behavioral evaluation, when the supplementations were stopped. At PND 41 every animal was placed in the both compartments (black and white) for 15 min so that it could settle down. The purpose of this procedure was to exclude exploratory behavior that is common in new environments during both the pretest and conditioning phases, thus avoiding misinterpretations. At PND 42, the pretest was conducted to determine initial chamber preference, when animals were placed in the neutral compartment of the CPP with free access to the entire apparatus for 15 min, after which the animals were returned to their home cages. The time spent in each compartment was recorded. From these results, the chamber in which each rat spent more time during the pretest was defined as the initially preferred chamber; conversely, the chamber in which the rat spent less time was elected for drug conditioning. Thus, at PND 43, using baseline preference measurements from pretests, rats were given AMPH (4mg/Kg i.p.) in the opposite chamber of preference. AMPH was administered in alternation with saline at 4h intervals. Immediately after each injection, rats were placed in the non-preferred and preferred compartment of CPP, respectively. Rats were allowed to access either chamber

during each conditioning session of 25 min. This procedure was continued for 8 consecutive days (PND 43-50), when AMPH is exclusively paired with a previously neutral environment whereas vehicle is exclusively paired with a second environment (Carlezon et al., 2002).

After the conditioning phase, rats were placed in the middle chamber with doors open for 15 min and given access to both chambers. Time spent in the drug-paired environment is interpreted as preference, whereas time spent away from that environment is interpreted as aversion (Brenhouse and Andersen, 2008).

2.3.2. Elevated plus maze (EPM)

The apparatus consisted of a platform elevated 50 cm from the floor. Two opposite arms (50x10 cm) were enclosed by 40 cm high walls whereas the other two arms had no walls. The four arms had at their intersection a central platform (10x 10 cm), which gave access to any of the four arms. At the beginning of each test, the rat was placed in the central platform facing an open arm. Time spent in the open arms, and number of open arm entries was registered during the five-minute test, with the test apparatus being disinfected after each animal. On PND51, animals were observed in EPM, which is based on the innate fear rodents have for open and elevated spaces (Montgomery, 1955). In this test, rats display a variety of behaviors: while the time spent and entries number in the open arms are related to lower anxiety-like symptoms, behaviors of fear and risk are quantified by time spent in the closed arms (Rodgers et al., 1997).

2.3.3. Open-field (OF) test

The OF apparatus consisted of an arena (42x 42x 28 cm) divided into twelve quadrants, where the animals were placed individually for 5 min (Kerr et al. 2005). The OF

was disinfected after each test. Locomotor activity was quantified by the crossing number, while exploratory behavior was evaluated by the rearing number (Henderson et al., 2004).

2.4 Biochemical measurements

At PND54, animals were anesthetized with xylazine/ ketamine (20 and 100 mg/Kg, i.p., respectively) and euthanized by exsanguinations. The collected blood (collected by cardiac puncture in heparinized tubes) was centrifuged at 1300 x g for 15 min for plasma and used for glutathione peroxidase (GSH) assay. Brains were immediately removed and cut coronally at the caudal border of the olfactory tubercle. Cortex, striatum and hippocampus were dissected out and homogenized in 10 volumes (w/v) of 10mM tris-HCl buffer (pH 7.4) for determination of protein carbonyl (PC) levels, which estimate oxidative damages to proteins, as well catalase (CAT) and reduced glutathione (GSH) levels, which estimate the antioxidant defenses.

2.4.1 Protein carbonyl (PC) quantification

PC was quantified by the method of Yan et al. (1995), with some modifications. Soluble protein was mixed with 2,4-dinitrophenylhydrazine (DNPH; 10mM in 2M HCl) or HCl (2 M) and incubated at room temperature for 1 h. Denaturing buffer (150mM sodium phosphate buffer, pH 6.8, with 3% sodium dodecyl sulfate), ethanol (99.8%) and hexane (99.5%) were added, mixed by shaking and centrifuged. The protein isolated from the interface was washed twice with ethyl acetate/ethanol 1:1 (v/v) and suspended in denaturing buffer. Each DNPH sample was read at 370nm in a spectrophotometer against the corresponding HCl sample (blank). The results were expressed as nmol carbonyl/g tissue.

2.4.2 Catalase (CAT) activity

CAT activity was spectrophotometrically quantified by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the presence of cell homogenate (pH 7 at 25°C) at 240 nm for 120 s. The enzymatic activity was expressed in µmol H2O2/min/g tissue.

2.4.3 Reduced glutathione (GSH) levels

Plasma and blood red cells GSH content was determined after reaction with 5,5'-dithiobis-(2-nitrobenzoic acid); the yellow color developed was read at 412 nm, in accordance with (Boyne and Ellman 1972). A standard curve using glutathione was constructed in order to calculate the content of GSH, expressed as nmol GSH/mL plasma.

2.5 Statistical analysis

Homogeneity of the data was analyzed by Levene's test. Behavioral assessments and biochemical evaluations were analyzed by one-way ANOVA followed by Duncan's multiple range test, when appropriate (Software package Statistica 8.0 for Windows was used). Values of $P<0.05$ were considered statistically significant for all comparisons made.

3. Results

3.1. Behavioral evaluations

3.1.1 The development of preference for AMPH evaluated in CPP is shown in Figure 2:

Analysis of variance (ANOVA) followed by Duncan's test showed that HVF significantly increased AMPH preference in comparison with others groups (FO and SO), which showed no preference for any compartment.

3.1.2 Anxiety-like symptoms evaluated in Elevated Plus Maze (EPM) are shown in Figure 3: Duncan's test showed that FO and SO groups spent more time in the open arms when compared to the HVF (Fig. 3A). Besides spending less time in the closed arms (Fig. 3B), FO and SO groups showed higher number of entries in the open arms than the HVF group (Fig. 3C).

3.1.4 Locomotor activity and exploratory performance evaluated in Open Field (OP) are shown in Figure 4: Post hoc tests showed that crossing number and rearing frequency were lower in FO than in both SO and HVF, which showed comparable locomotion (Fig. 4A and 4B).

3.2 Biochemical evaluations

3.2.1 Estimation of oxidative damage and antioxidant defenses measured by protein carbonyl (PC) levels and catalase (CAT) activity in cortex, striatum and hippocampus are shown in Figure 5: HVF supplemented animals showed increased PC levels in cortex and hippocampus in relation to other groups (Fig. 5A and 5C). In striatum, FO supplementation decreased PC levels when compared to HVF and SO groups, whose values were similar to each other (Fig. 5B).

HVF supplementation decreased CAT activity in cortex and hippocampus when compared to FO and SO groups (Fig. 5D and 5F). Interestingly, FO increased CAT activity in striatum in relation to SO and HVF groups (Fig. 5E), which were comparable to each other.

3.2.2 Estimation of antioxidant defenses determined by reduced glutathione (GSH) levels in plasma and blood red cells (BRC) is shown in Table 2: Rats supplemented with FO showed

higher levels of GSH in both plasma and BRC when compared to SO and HVF groups, whose levels were similar to each other.

4. Discussion

In this study, pups born of dams supplemented with isocaloric fats from six weeks before conception and during gestation and lactation were maintained under the same experimental protocol until adolescence and exposed to AMPH for eight consecutive days. Animals supplemented with HVF, which is rich in TFA, showed AMPH-CPP. Furthermore, HVF group showed augmented anxiety-like symptoms and development of oxidative damages, which was observed by higher PC levels and reduced antioxidant activity in both cortex and hippocampus. On the other hand, SO- and FO-supplemented groups did not show AMPH preference or behavioral withdrawal symptoms, but only FO showed lower oxidative status and higher antioxidant protection, which were observed in striatum and blood.

Literature is lacking in studies about influences of chronic dietary TFA on brain neuronal membranes and their relationship with addictive drug preference, which may be predictive of addiction. On the other hand, efforts have been made to evaluate the influence of n-3 deficient diet on mechanisms related to drug abuse. McNamara et al. (2008) reported that omega-3 deficient mice showed increased sensitization to AMPH, which was related to selective changes in the mesolimbic DA pathway. As n-3 PUFA are precursors of EPA and DHA, their incorporation into membrane phospholipids can have a significant impact on DA neurotransmission (Miranda et al., 2007), and affect both the production of neurotransmitters as DA and its receptor function (Fenton et al., 2000). On the other hand, dietary TFA may also be incorporated into neuronal membrane phospholipids, changing its physical properties as permeability and fluidity (Jump 2002). Such changes may consequently affect brain

physiological functions, which involve dopaminergic neurotransmission (Acar et al., 2003) as well as the enzymes activity (Teixeira et al. 2011, 2012). In fact, TFA availability may modify brain EFA status, since TFA exert inhibitory effects on both δ-5 and δ-6 desaturases (Larqué et al., 2003), impairing the desaturation and elongation of LNA (n-3) and LA (n-6) into its LC-PUFA derivatives (Barceló-Coblijn and Murphy, 2009), which should compose the membrane phospholipids of brain neurons. In line with this, a recent study has shown that despite very slight brain incorporation, dietary TFA was able to modify behavioral and biochemical parameters of experimental animals (Souza et al., 2012). In addition, even a smaller brain incorporation of TFA was associated with changes on dopaminergic neurotransmitters levels in striatum and hippocampus (Acar et al., 2003), suggesting that TFA can act on gene expression involved in the DA metabolism (Sessler and Ntambi, 1998). On the other hand, n-3-PUFA deficiency was able to affect dopaminergic neurotransmission, such as the vesicular pool of DA and serotonin, and modify receptors in specific brain areas (Chalon, 2006).Whereas TFA represents a loss of EFA, our findings of AMPH-CPP may be related to changes in dopaminergic neurotransmission resulting from the incorporation of TFA into neuronal brain membranes, which for the moment can just be hypothesized because incorporation of FA into neuronal brain membranes was not evaluated in the present study. In fact, similarly to what was stated above, recent studies of our group confirmed that a small TFA brain incorporation is sufficient to change enzymes activity in brain areas, which were related to movement disorders (Teixeira et al., 2012) and anxiety-like symptoms (Teixeira et al., 2011), reinforcing the hypothesis raised here.

While data in the literature have shown a relationship between high intake of saturated FA and aggressive behavior in rodents (Raygada et al., 1998), n-3 PUFA enriched diets have been related to reduced anxiety symptoms (Ikemoto et al., 2001). In the present study, HVF supplemented animals showed higher degree of anxiety and increased locomotor index, which

are consistent with AMPH craving symptoms. On the other hand, while both FO and SO groups did not show anxiety-like symptoms, FO supplementation was related to reduced locomotor index, pointing to a reduced degree of seeking-behavior from the psychostimulant. Furthermore, a recent study of our group showed that HVF-supplemented rats, which were treated or not with AMPH, showed an increased locomotor index and higher oxidative status in brain tissues (Trevizol et al., 2011), suggesting that TFA *per se* is able to increase brain oxidative damages and also exacerbate AMPH effects. In fact, AMPH is an addictive drug, whose action mechanism is based on increased DA neuronal release as well as inhibition of DA vesicular uptake (Brown et al., 2000). This exacerbated dopaminergic transmission results in DA autoxidation and deamination (by MAO), which increase ROS production. In the present study, all animals were treated with AMPH, but rats supplemented with HVF showed higher oxidative damages and lower antioxidant defenses, as observed by increased levels of protein carbonyl and reduced CAT activity in both cortex and hippocampus. Inversely, FO- and SO- treated rats did not show increase of this oxidative marker or antioxidant defense in these brain areas, indicating that the provision of n-3 EFA was sufficient to prevent such damages. In addition, FO-treated group present reduced oxidative damages and increased antioxidant defenses, which was evidenced by CAT activity in striatum and GSH levels in both plasma and BRC. Taken together, these findings in brain and blood antioxidant status suggest that n-3 EFA can maintain and increase, respectively, the antioxidant defenses in response to AMPH-induced ROS generation (Topalca et al., 1999). In addition, different literature studies have indicated that intake of foods rich in TFA is able to increase inflammation markers and reduce antioxidant defenses against oxidative stress (Sánchez-Moreno et al., 2004). As already mentioned, a metabolite of n-6 EFA (AA) competes with the derivatives of n-3 EFA (EPA and DHA), which are substrate for cyclooxygenase-2 (COX-2). In fact, this enzyme generates PGE of series 2 (PGE2) and 3 (PGE3), respectively (Ueta et al.,

2010), but the latter is less pro-inflammatory than the former. In our findings, SO supplementation did not cause the same impairing effects as HVF implementation did on the brain oxidative status, but its elevated content of n-6 EFA did not contribute either to ameliorate the antioxidant defenses, as observed in the FO group. Intriguingly, so far no TFA derivatives resulting from COX-2 activity were identified; nevertheless, TFA affect per se the incorporation of LC-PUFA into neuronal membranes, as described above, whose consequences are still unknown. So far, there are no comparative studies on the influence of supplementation of different dietary FA from gestation and lactation to adolescence and young adulthood on AMPH preference, behavioral symptoms of craving and oxidative status in brain.

In conclusion, in this study we propose that chronic consumption of foods rich in TFA can modify factors related to preference for AMPH and its correlates. In addition, anxiety-like symptoms and locomotor restlessness related to seeking-behavior for AMPH as well as brain oxidative status may be exacerbated when TFA are present in the diet during the development and growing periods. Further experimental investigations are needed to establish the exact mechanism of the influence of TFA on the AMPH-CPP. Also, it becomes important to conduct epidemiological studies to determine a possible link between *trans* fat consumption and human drug addiction in the industrialized communities.

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Authors contribution

M.E.B and F.T.K were responsible for the study concept and design. K.R., N.B. and C.T.D.A contributed to the acquisition of animal data. C.S.P., V.T.D, J.P. and T.E. performed the biochemical analysis. R.C.S.B., F.T. and D.M.B. assisted with data analysis and interpretation of findings. M.E.B. and F.T.K. drafted the manuscript. T.E. and C.S.P. provided critical revision of the manuscript for important intellectual content. All authors critically reviewed the content and approved the final version for publication.

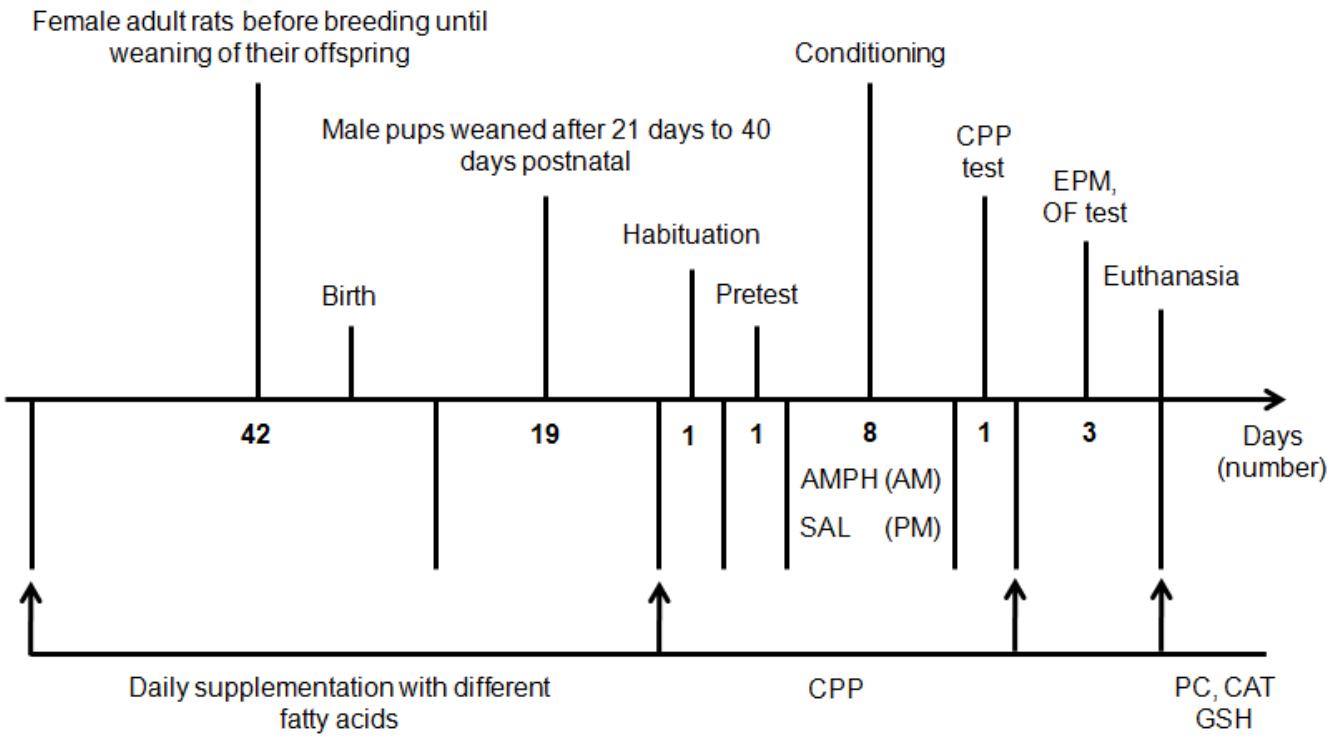

Fig. 1. Experimental design.

Abbreviations: CPP: conditioned place preference; EPM: elevated plus-maze; OF: open field; AMPH: amphetamine; SAL: saline.

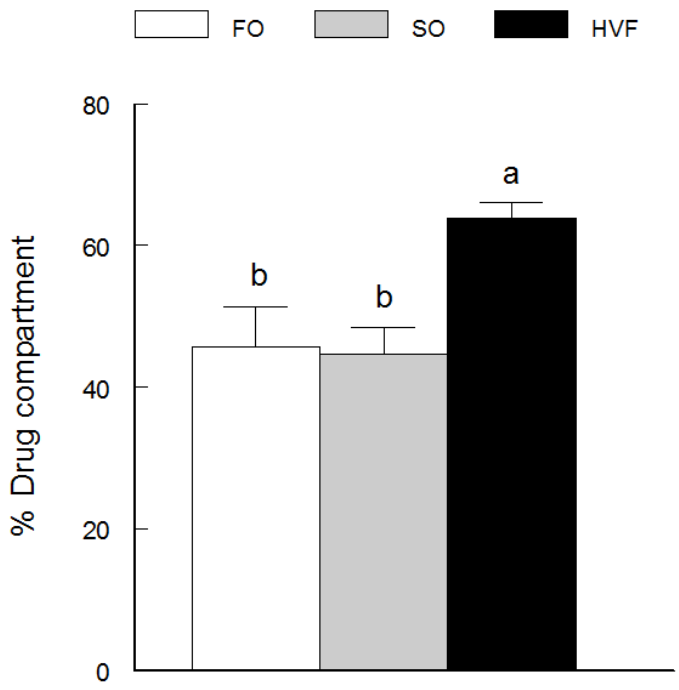

Figure 2

Fig. 2. Influence of supplementation of different fats on AMPH preference of young pups. Animals were born of dams treated with different fats from gestation/lactation and maintained with the same supplementation until 40 days of age, when they were conditioned with AMPH (4mg/kg for 8 days) and evaluated in conditioned place preference (CPP). Data are expressed as mean±S.E.M.

Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Different lowercase indicates significant difference between supplemented groups ($P<0.05$);

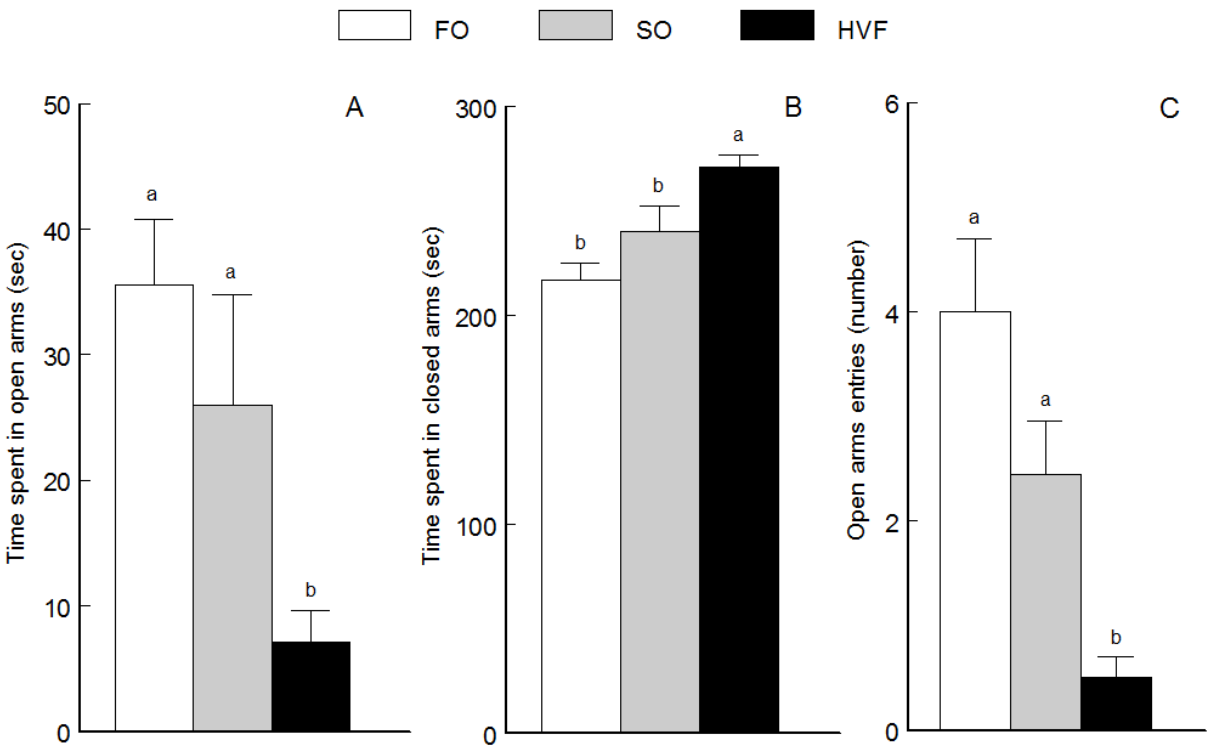

Figure 3

Fig. 3. Influence of supplementation of different fats on anxiety-like symptoms of young pups. Animals were born of dams treated with different fats from gestation/lactation and maintained in the same supplemented group until 40 days of age, when they were treated with amphetamine (AMPH-4mg/kg for 8 days) and submitted to behavioral assessments in EPM 4 h after the last AMPH injection. Data are expressed as mean±S.E.M.

Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Different lowercase indicates significant difference between supplemented groups ($P<0.05$), for A, B and C;

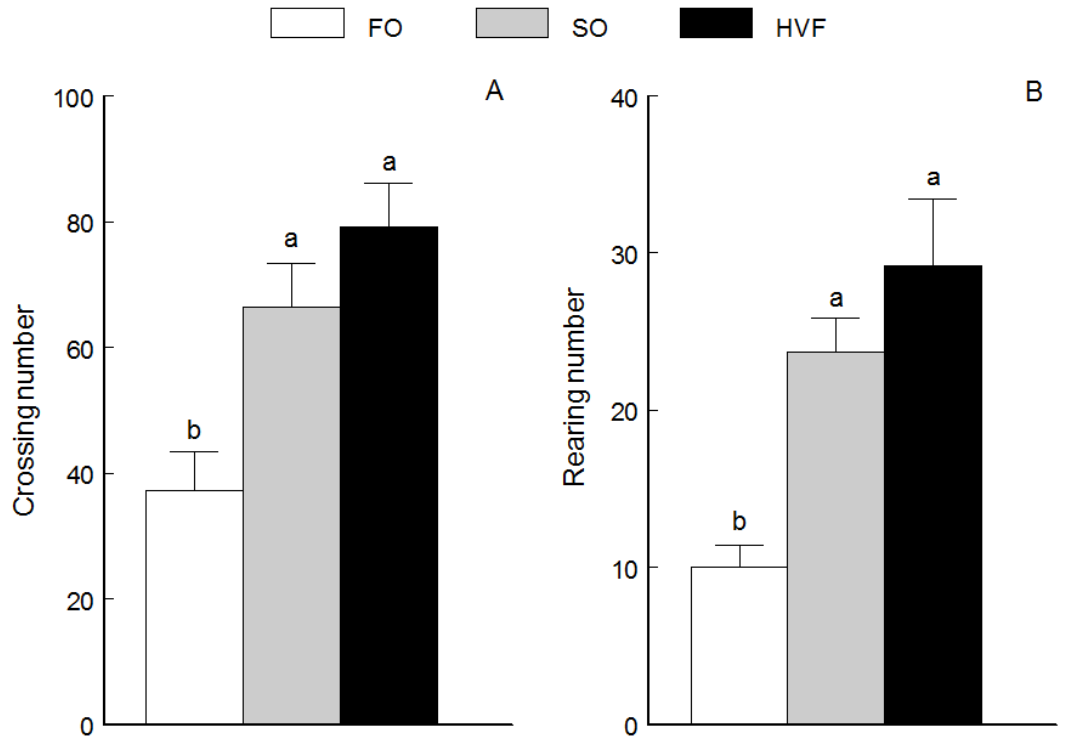

Figure 4

Fig. 4. Influence of supplementation of different fats on locomotor and exploratory activities of young pups. Animals were born of dams treated with different fats from gestation/lactation and maintained in the same supplemented group until 40 days of age, when they were treated with amphetamine (AMPH-4mg/kg for 8 days) and submitted to behavioral assessments in open field (OF) 4 h after the last AMPH injection. Data are expressed as mean±S.E.M.

Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Different lowercase indicates significant difference between supplemented groups ($P<0.05$), for A and B;

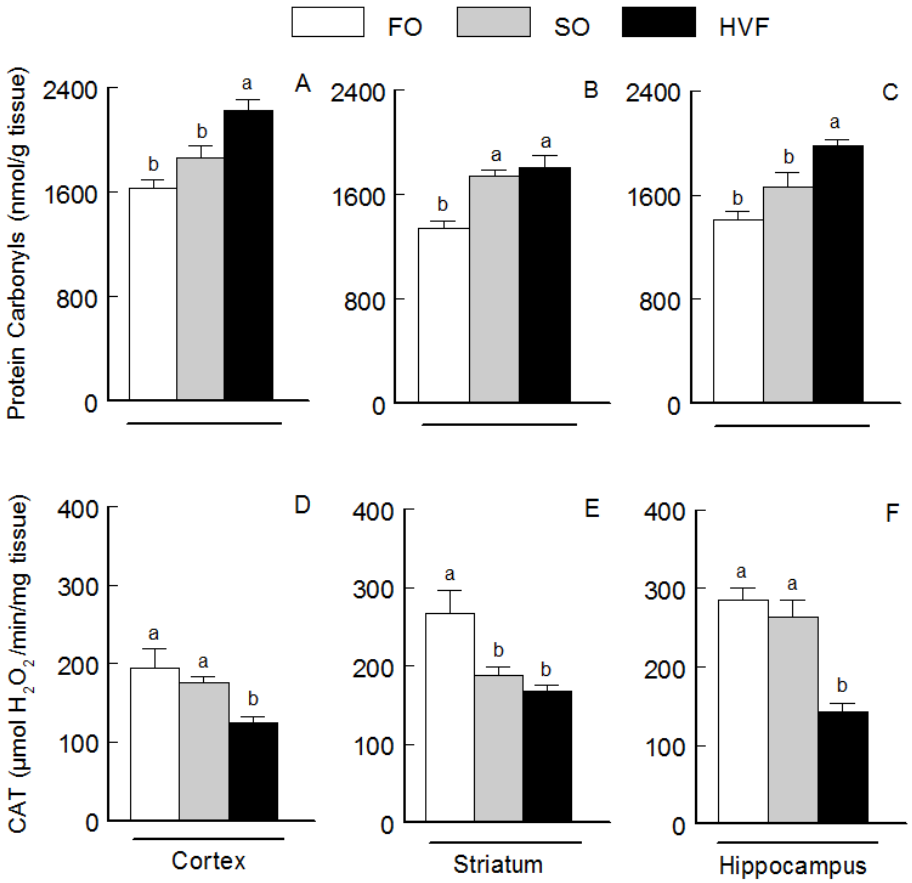

Figure 5

Fig. 5. Influence of supplementation of different fats on protein carbonyl levels (PC, Fig. A, B, C) and catalase activity (CAT, Fig. D, E, F) in cortex, striatum and hippocampus, respectively, of young male pups. Animals were born of dams treated with different fats from gestation/lactation and maintained in the same supplemented group until 40 days of age, when they were treated with amphetamine (AMPH-4mg/kg for 8 days). Biochemical evaluations were carried out 4 days after the last AMPH injection. Data are expressed as mean±S.E.M.

Abbreviations: FO: fish oil; SO: soybean oil; HVF: hydrogenated vegetable fat. Different lowercase indicates significant difference between supplemented groups ($P<0.05$), for all figures (A-F).

Table 1. Total percentage of fatty acids in the supplementation of different groups, and ratio Σn6:n3. Abbreviations: PUFA: polyunsaturated fatty acids; Sat: Saturated; Uns: Unsaturate.

Fatty acids	SO group	FO group	HVF group
Σ Saturated	10.85	17.56	15.49
Σ Monounsaturated	33.84	36.31	43.27
Σ Trans	0.38	0.29	11.72
Σ Polyunsaturated	54.03	45.62	29.52
Σ Sat:Uns ratio	0.12	0.21	0.19
Σ n-6 PUFA	49.98	21.23	27.58
Σ n-3 PUFA	4.79	18.7	1.94
Σ n6:n3 ratio	10:1	1:1	14:1

Table 2. Influence of supplementation of different fats on glutathione (GSH) levels in blood of young pups. Animals were born of dams treated with different fats from gestation/ lactation. Pups were maintained with the same supplementation until 40 days of age, when they were treated with amphetamine (4mg/kg) or its vehicle for 8 days.

Tissue	Groups (n=8)	GSH (nmol/g tissue)
Plasma	FO	1201.5±114.7 [a]
	SO	923.56±115.1 [b]
	HVF	783.04±41.3 [b]
BRC	FO	6398.3±655.9 [a]
	SO	3861.8±261.2 [b]
	HVF	3507.9±348.8 [b]

Abbreviations: GSH: glutathione; BRC: Blood red cells.

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4. CONCLUSÕES FINAIS

Através dos resultados experimentais obtidos neste estudo, podemos propor as seguintes conclusões:

1. O consumo crônico de AGT ofertado através da suplementação de GVH durante os períodos inicial e de crescimento dos animais, foi capaz de alterar parâmetros de preferência pela ANF, sugerindo que tal influência pode modificar a resposta hedônica frente à drogas psicoestimulantes, e facilitando assim o potencial de abuso destas drogas;

2. O consumo crônico de GVH foi associado ao aumento dos parâmetros de ansiedade observados após a exposição à ANF, o que sugere uma exacerbação de sintomas de abstinência no período pós-droga;

3. Contrariamente, os animais jovens suplementados com OP durante os períodos perinatal e de desenvolvimento apresentaram um reduzido índice locomotor durante o período pós-ANF, indicando que o aporte de AG n-3 em fases iniciais da vida pode ser refletido sobre a composição fosfolipídica das membranas neuronais, sugerindo maior estabilidade sobre a neurotransmissão;

4. A suplementação de OP mostrou potencial neuroprotetor, observado através dos menores danos oxidativos e também pela maior proteção antioxidante, a qual foi superior em relação ao grupos suplementados com OS e GVH.

5. PERSPECTIVAS

A partir dos resultados apresentados nesta dissertação, a continuidade dos estudos faz-se necessária, principalmente em busca do estabelecimento dos mecanismos moleculares envolvidos nestas respostas bioquímicas e comportamentais. Além disso, torna-se importante a realização de estudos epidemiológicos para determinar uma possível interação entre o consumo crônico e/ou excessivo de gordura *trans* e os parâmetros hedônicos envolvidos na adição, especialmente nas comunidades industrializadas.

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