PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

LISIANI SAUR

ESTRESSE PÓS-TRAUMÁTICO E TRATAMENTO COM CETAMINA EM RATOS WISTAR: ANÁLISE COMPORTAMENTAL, HISTOFISIOLÓGICA, BIOQUÍMICA E NEUROMETABÓLICA.

Porto Alegre
2017

LISIANI SAUR

ESTRESSE PÓS-TRAUMÁTICO E TRATAMENTO COM CETAMINA EM RATOS WISTAR: ANÁLISE COMPORTAMENTAL, HISTOFISIOLÓGICA, BIOQUÍMICA E NEUROMETABÓLICA.

Tese apresentada como requisito para a obtenção do grau de Doutor pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientador:

Prof. Dr. Léder Leal Xavier

Porto Alegre

2017

Ao meu marido, Piter Zapparoli Dal-Ri, por ser meu porto seguro.

Agradecimentos

A minha família, pelo amor e por me ensinar o valor do estudo e do trabalho.

Ao meu orientador e amigo, Professor Léder Leal Xavier. Meu sincero agradecimento pela dedicação, conselhos, ensinamentos e principalmente pela confiança ao longo desses anos de convívio. Aqui se encerra uma fase, mas a amizade e a admiração ficarão para sempre.

À Raquel, técnica do Laboratório de Biologia Celular e Tecidual, obrigada pelo auxílio durante todas as etapas do mestrado e do doutorado, mas principalmente, muito obrigada pela amizade.

Às minhas amigas Pamela, Maricota e Mari, obrigada por tornarem meus dias mais felizes e divertidos. Nosso período de convivência no laboratório jamais será esquecido.

Aos colegas e amigos do Laboratório de Biologia Celular e Tecidual, pelo companheirismo e dedicação em todas as etapas deste estudo.

Aos colegas e amigos do Centro de Pesquisa pré-clínica do Instituto do Cérebro (INSCER) do Rio Grande do Sul - PUCRS por me auxiliar a desenvolver este trabalho.

Às colegas do Programa de Pós-Graduação em Ciências Biológicas – Neurociências da UFRGS pela disponibilidade e ajuda.

Ao meu marido Piter Zapparoli Dal-Ri, por absolutamente tudo.

Aos professores do Programa de Pós-Graduação em Biologia Celular e Molecular da PUCRS pela contribuição na minha formação.

A todos que de alguma forma contribuíram para a realização desse trabalho.

À CAPES, CNPq, FAPERGS e PUCRS pelo financiamento ao longo desses 4 anos.

RESUMO

O transtorno do estresse pós-traumático (*Post-Traumatic Stress Disorder* - PTSD) é uma doença neuropsiquiátrica relacionada à exposição a um acontecimento traumático. O PTSD é caracterizado clinicamente por uma série de sintomas debilitantes incluindo a revivência frequente do evento traumático, fuga de estímulos associados ao trauma, hipervigilância, mudanças de cognição e humor entre outros. Nem todos os indivíduos expostos a graves experiências traumáticas desenvolvem PTSD, esta psicopatologia afeta uma subpopulação de indivíduos vulneráveis cuja exposição a uma experiência estressante excede sua capacidade de adaptação. Neste sentido, foi argumentado que o PTSD pode, pelo menos em parte, ser um distúrbio relacionado ao medo. Assim, as regiões encefálicas mais amplamente estudadas nesta psicopatologia são o córtex pré-frontal, o hipocampo e a amígdala, pois são regiões relacionadas com os processos de armazenamento e consolidação de novas memórias incluindo memórias emocionais, tais como de medo e raiva. Devido à complexa apresentação clínica do PTSD e a sua sintomatologia muito variável esta ainda é uma doença classificada como de difícil tratamento. A cetamina é um fármaco antagonista de receptores NMDA que tem demonstrado promissores efeitos no tratamento da depressão, entretanto, por ser uma droga que produz efeitos dissociativos e psicóticos, existe a preocupação de que esta droga possa estar relacionada com o aumento dos sintomas do PTSD. Um dos modelos animais mais amplamente utilizados para mimetizar as alterações comportamentais e neuroquímicas do PTSD é o choque inescapável único. Na primeira parte do trabalho observamos importantes alterações comportamentais e histológicas provocadas pela exposição ao choque nas patas. Os animais com PTSD apresentaram um maior comportamento de "*freezing*" quando reexpostos ao mesmo contexto

aversivo, bem como maior produção de bolos fecais. Além disso, importantes alterações foram observadas nos astrócitos da região CA1 do hipocampo destes animais como: diminuição na densidade, arborização e quantidade de processos primários. Ademais, a polaridade astrocitária também foi alterada em relação aos animais controle. Na segunda parte do trabalho analisamos os efeitos da cetamina sobre este modelo animal. Como nem todos os animais apresentam a mesma resposta durante a reexposição ao contexto aversivo, ou seja, os animais apresentam diferenças individuais na sua susceptibilidade ao estresse traumático, nós decidimos separar os animais com PTSD em dois grupos: aqueles que apresentaram uma resposta comportamental exagerada (*Extreme Behavioral Response* - EBR) e aqueles que apresentaram uma resposta comportamental mínima (*Minimal Bahavioral Response* - MBR). Além disso, o metabolismo da glicose e os níveis de BDNF no córtex frontal, hipocampo e amígdala foram analisados oito e nove dias após a indução do PTSD, respectivamente. Os níveis de BDNF foram analisamos através ensaios bioquímicos e o metabolismo da glicose foi analisado por meio do ^{18}F-FDG-microPET que avalia o consumo da glicose nos tecidos. Observamos que os animais com PTSD classificados como EBR apresentaram um aumento no comportamento de *freezing* e que o tratamento com cetamina piorou a resposta comportamental, ou seja, a cetamina agravou os sintomas do PTSD. Entretanto, nenhuma alteração foi observada nos níveis de BDNF e no metabolismo da glicose. Estes resultados demonstram que o choque nas patas, como modelos de PTSD em animais, induz importantes alterações comportamentais e astrocitárias. Observamos também que a cetamina piora os sintomas do PTSD e que, tal qual observado em humanos, nem todos os indivíduos expostos a um estresse traumático desenvolvem os sintomas. Contudo, este modelo experimental de PTSD parece não estar relacionado com alterações de longo-prazo nos níveis de BDNF e metabolismo da glicose.

ABSTRACT

Post-traumatic stress disorder (PTSD) is a neuropsychiatric condition related to exposure to a traumatic event. It is clinically characterized by several debilitating symptoms including frequent re-experiencing of the traumatic event, avoidance bahavior, hypervigilance, and cognitive and mood changes among others. Not all individuals exposed to severe traumatic experiences develop PTSD. This psychopathology affects a vulnerable subpopulation of individuals confronted with a stressful experience that exceeds their capacity to cope. In this sense, it has been argued that PTSD can be considered a fear-related disorder. Thus, the brain regions most widely studied in this psychopathology are the prefrontal cortex, the hippocampus and the amygdala, because they are related to the storage and consolidation of new memories including emotional memories, such as fear and anger. Due to the complex clinical presentation of PTSD and its very variable symptoms, it is considered a condition to treat. Ketamine is an NMDA receptor antagonist that has shown promising effects in the treatment of depression. However, because it produces dissociative and psychotic effects, there is a concern this drug might be related with increased PTSD symptoms. One of the most widely used animal models to mimic the behavioral and neurochemical changes of PTSD is the inescapable footshock. In the first part of this study, we observed significant behavioral and histological changes caused by exposure to footshock. Animals with PTSD exhibited longer freezing bouts when re-exposed to the same aversive context, as well as increased pellet production. In addition, important changes were observed in astrocytes from the hippocampal CA1 region of these animals, such as decreased astrocytic density, ramification and fewer primary processes. Furthermore, the polarity of the astrocytes was also changed when compared to control

animals. In the second part of this study, we analyzed the effects of ketamine on this animal model. Since not all animals have the same response during the re-exposure to aversive environment, that is, the animals have individual differences in their susceptibility to traumatic stress, we decided to separate the animals with PTSD into two groups: those with an extreme behavioral response (EBR) and those with a minimal behavioral response (MBR). Furthermore, the glucose metabolism and the BDNF levels in the frontal cortex, hippocampus and amygdala were analyzed 8 and 9 days after PTSD induction, respectively. BDNF levels were analyzed through biochemical assays and glucose metabolism was analyzed by ^{18}F-FDG-microPET, which measures the glucose uptake in tissues, in other words, the metabolic demand. We observed that animals with PTSD classified as EBR showed an increase in freezing behavior and the treatment with ketamine worsened that behavioral response, that is, the ketamine worsened the PTSD symptoms. However, no changes were observed in BDNF levels or glucose metabolism. These results demonstrate that footshock as an animal model of PTSD induced significant behavioral and astrocytic alterations. We also observed that ketamine worsened the PTSD symptoms and that, as is the case with humans, not all individuals that are exposed to a traumatic stress develop PTSD. However, this animal model does not seem to be related to long-term changes in BDNF levels or glucose metabolism.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

^{18}F-FDG – ^{18}F-2-fluoro-2-deoxy-glucose

AMPA – Ácido α-amino-3-hidroxi-5-metil-4-isoxazol propiônico

BDNF – Fator neurotrófico derivado do encéfalo

BrdU – Bromodesoxiuridine

CIU – Choque inescapável único

DSM-5 – Diagnóstico e estatística para transtornos mentais

EBR – Resposta comportamental extrema – "*extreme behavioral response*"

eEF2 – Fator de elongação eucariótico 2

ERK – Quinases relacionadas à sinalização extracelular

GDNF – Fator neurotrófico derivado da glia

GFAP – Proteína glial fibrilar ácida

GFAP+ – positivo para GFAP

GSK-3 – Quinase de glicogênio sintase 3

HPA – Hipotálamo-hipófise-adrenal

KeV – Kilo-eletro-volt

MBR – Resposta comportamental mínima – *"minimal bahavioral response*"

microPET – Tomografia por emissão de pósitrons para pequenos animais

mPFC – Córtex pré-frontal medial

mRNA – RNA mensageiro

mTOR – *Mammalian target of rapamycin*

NGV – Fator de crescimento do nervo

NMDA – N-metil D-aspartato

PET – Tomografia por emissão de pósitrons

PFC – Córtex pré-frontal

PKB/Akt – Proteína quinase B

PTSD – Transtorno do estresse pós-traumático

SNS – Sistema nervoso simpático

SNC – Sistema nervoso central

Trk B – Quinase do receptor de tropomiosina B

vmPFC – Córtex pré-frontal ventro-medial

SUMÁRIO

Capítulo I

1. INTRODUÇÃO

2. IMPORTÂNCIA E JUSTIFICATIVA

3. OBJETIVOS

1. INTRODUÇÃO

1.1. Estresse

O estresse é um estado de ameaça à homeostase que produz diferentes alterações fisiológicas e comportamentais, dependendo do tipo, gravidade e duração deste (Ravindran et al., 2005). A exposição a condições estressoras resulta em uma série de respostas adaptativas, no intuito de que organismo possa lidar com a ameaça, aumentando assim a probabilidade de sobrevivência (Carrasco e Van de Kar 2003; Grosman et al., 2011).

O estresse começa com um estímulo de origem interna ou externa ao organismo que ativa o eixo hipotálamo-hipófise-adrenal (HPA) e o sistema nervoso simpático (SNS). A estimulação destes sistemas de emergência é a principal resposta do organismo ao estresse e tem como objetivo principal restabelecer a homeostase (Reber 2012).

A ativação do eixo HPA resulta na liberação de glicocorticoides – cortisol em humanos e corticosterona em roedores – pelo córtex da suprarrenal, por sua vez a estimulação do SNS resulta na liberação de adrenalina pela porção medular da suprarrenal para o sistema circulatório (Reber 2012). As mudanças fisiológicas associadas à ativação do SNS em resposta ao estresse são: mobilização de energia para manter as funções cerebrais e musculares, atenção aguçada e focada da ameaça percebida, aumento das taxas de perfusão e da utilização da glicose encefálica, aumento da função cardiovascular e da respiração, redistribuição do fluxo sanguíneo, diminuição do apetite, entre outros (Carrasco e Van de Kar 2003).

Embora a resposta ao estresse seja essencial e adaptativa às mudanças no meio ambiente, quando esta resposta é exagerada ou sustentada, pode causar

comprometimento neural, que é observado após a exposição ao estresse prolongado (McIntosh e Sapolsky 1996). Portanto, é essencial limitar a duração de tempo durante a qual os tecidos corporais são expostos aos glicocorticoides, a fim de minimizar os efeitos catabólicos, antirreprodutivos, imunossupressores e neurodegenerativos do cortisol/corticosterona que podem causar danos fisiológicos além de aumentar a suscetibilidade para o desenvolvimento de doenças neuropsiquiátricas (Musazzi et al., 2010; Tsigos e Chrousos 2002) (Figura 1).

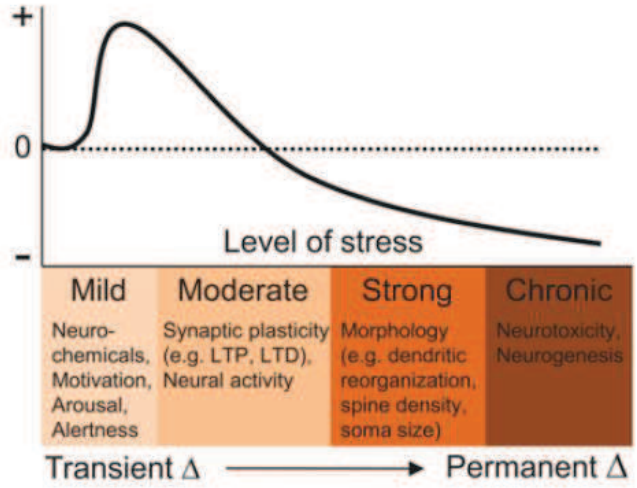


Figura 1. Efeitos biológicos do estresse sobre o SNC. Com o aumento da gravidade do estresse, alterações nos neurotransmissores, na plasticidade sináptica, na citoarquitetura das células e na atividade neural ocorrem em diferentes regiões do SNC envolvidas na resposta ao estresse e que podem influenciar funções cognitivas como aprendizagem e memória. + e – representam um aumento e diminuição nas funções neurais, respectivamente (Retirado de Kim et al., 2015).

1.2. A memória do medo

O medo é um mecanismo adaptativo que visa à sobrevivência do indivíduo quando este é confrontado com circunstâncias que ameaçam a vida. Na verdade, as memórias de medo são formadas durante situações perigosas para facilitar reações defensivas apropriadas, no intuito de reduzir o perigo ou lesão (Steimer 2002).

Enquanto as memórias do medo possuem um propósito adaptativo auxiliando a sobrevivência, elas também podem revelar-se patológicas quando inadequadamente evocadas em condições seguras. É esse tipo de condição que pode, em alguns indivíduos, levar ao surgimento de patologias psiquiátricas como o transtorno do estresse pós-traumático (PTSD) (Grupe e Nitschke 2013; Giustino e Maren 2015).

Em modelos animais, a memória do medo tem sido estudada, principalmente, através do condicionamento Pavloviano e/ou do condicionamento instrumental inibitório. No condicionamento Pavloviano, um estímulo aversivo (geralmente um choque nas patas), associado ou não a outro estímulo (geralmente acústico), adquire a capacidade de provocar um medo condicionado ao contexto. Se o estímulo acústico não for utilizado, os animais aprendem a vincular o próprio contexto com os choques nas patas, ou seja, quando os animais forem novamente expostos ao mesmo contexto aversivo, eles irão apresentar o comportamento padrão do medo, chamado de "*freezing*", isto é, uma imobilidade generalizada (com exceção dos músculos usados na respiração) causada por uma resposta generalizada da musculatura esquelética dos animais. Enquanto isso, o condicionamento instrumental inibitório é geralmente realizado através do teste de esquiva inibitória no qual os animais aprendem a permanecer no compartimento seguro (plataforma de madeira) e a não descer para o compartimento no qual, anteriormente, eles receberam um choque nas patas (Izquierdo et al., 2016; Lopresto et al., 2016).

Além de prejuízos na extinção do medo condicionado (Jovanovic e Ressler 2010; Wessa e Flor 2007) e hiper-condicionabilidade (ou seja, medo condicionado anormalmente forte) (Orr et al., 2000), estudos recentes têm demonstrado que a generalização do medo condicionado é um dos principais sintomas dos distúrbios de ansiedade e, em particular, do PTSD (Lissek et al., 2005). Generalização é definida

como a transferência do medo experimentado durante um evento traumático para outas situações (American Psychiatric Association 2013). Enquanto indivíduos saudáveis são capazes de discernir novas situações inócuas de eventos aversivos anteriores e, consequentemente, responder de forma apropriada, os indivíduos com PTSD exibem elevada reatividade, mesmo na presença de sinais que transmitem segurança (Lissek et al., 2005).

1.3. O transtorno do estresse pós-traumático (PTSD -"Post-Traumatic Stress Disorder")

Os transtornos de ansiedade são um problema comum em todo o mundo, e um deles é o transtorno do estresse pós-traumático (Borghans e Homberg 2015). O PTSD é listado no Manual Diagnóstico e Estatístico de Transtornos Mentais *(Diagnostic and Statistical Manual of Mental Disorders* - DSM-5) como, um trauma relacionado à exposição a um acontecimento traumático. A característica definidora de uma experiência traumática é a sua capacidade de provocar impotência, medo ou horror. Geralmente isso ocorre em resposta a um evento de ameaça de morte, lesão grave ou perda de integridade física (Diehl et al., 2007; Liberzon et al., 2005). Não somente a exposição direta ao trauma leva ao desenvolvimento do PTSD, mas testemunhar uma lesão violenta ou morte não natural também pode constituir uma experiência traumatogênica (Liberzon et al., 2005).

Por ser uma patologia associada ao circuito do medo (Shin e Handwerger 2009; Shvil et al., 2013), é possível que os pacientes com PTSD apresentem uma persistente resposta condicionada ao medo e/ou resistência à extinção da memória (Pitman 1988).

O DSM-5 define ainda os critérios que devem embasar o diagnóstico de PTSD: 1- Exposição a um evento traumático; 2- Revivência frequente do evento traumático

através de *"flashbacks"*, pesadelos ou situações associadas ao trauma; 3- Fuga persistente de estímulos associados ao trauma; 4- Mudanças negativas na cognição e humor, bem como outros efeitos indiretos, tais como aumento da irritabilidade, insônia, diminuição da capacidade de concentração e da interação social; 5- Resposta exagerada ao susto e hipervigilância; 6- Os sintomas apresentados devem ser persistentes ao longo do tempo; 7- Os sintomas devem afetar substancialmente o funcionamento do indivíduo; 8- Outros fatores que podem causar tais sintomas devem ser excluídos.

Os dados sobre a prevalência dessa psicopatologia variam de 3 a 8% na população em geral, este número pode dobrar em populações afetadas por conflitos, pode chegar a 30% em veteranos de guerra e a 50% em sobreviventes de abuso sexual (Kessler et al., 2005; Bisson et al., 2015; Breslau et al., 1998; Yehuda 2002). Assim, o PTSD é a quarta desordem psiquiátrica mais comum no mundo e causa uma incapacidade substancial nas esferas sociais, ocupacionais e interpessoais (Breslau et al., 1998; Yehuda 2002).

Nem todos os indivíduos expostos a graves experiências traumáticas desenvolvem PTSD, esta psicopatologia afeta uma subpopulação de indivíduos vulneráveis cuja exposição a uma experiência estressante excede sua capacidade de adaptação. Assim como os humanos, animais também apresentam diferenças individuais na sua susceptibilidade ao estresse traumático. Alguns animais apresentam reações de curta duração que não levam ao estresse prolongado e alguns, apesar de terem sido submetidos ao mesmo protocolo estressor, exibem respostas exageradas ao estresse (Cohen et al., 2014). Mesmo em linhagens laboratoriais isogênicas, animais geneticamente idênticos podem ser consideravelmente mais ou menos susceptíveis a manipulações experimentais idênticas (Krishnan et al., 2007). A neurobiologia

individual bem como experiências passadas estão entre os fatores que contribuem para o desenvolvimento da doença. (Diehl et al., 2007) (Figura 2).

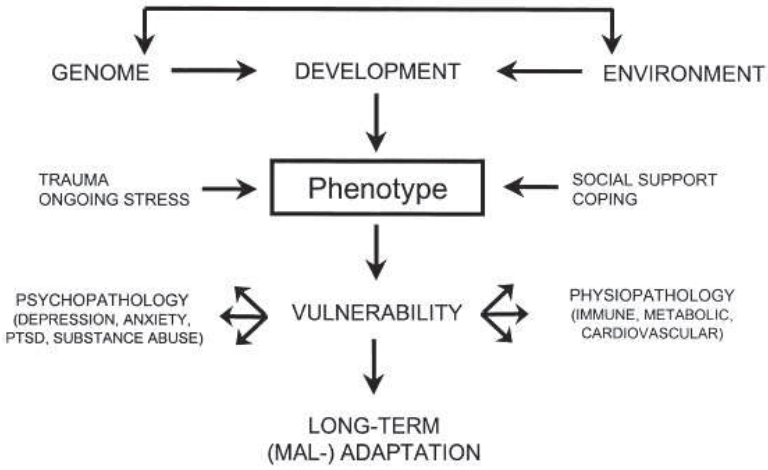


Figura 2. Modelo proposto da interação entre a predisposição genética e o ambiente (experiências passadas) levando a um fenótipo vulnerável. A exposição subsequente ao estresse ou trauma pode induzir à exacerbação da resposta comportamental e consequentemente ao desenvolvimento de patologias associadas ao estresse como o PTSD. O apoio social ou o estilo de enfrentamento (positivo ou negativo) adotado pelo indivíduo também determinam a vulnerabilidade individual (Retirado de Heim e Nemeroff, 2001).

O PTSD também manifesta uma elevada comorbidade com outros transtornos psiquiátricos. O estudo realizado por Kessler et al. (1995) identificou que cerca de 80% dos pacientes com PTSD do sexo masculino e 70% dos do sexo feminino foram diagnosticados com, pelo menos, uma outra condição psiquiátrica. Assim, um percentual significativo de pacientes com PTSD sofrem de transtornos de humor, transtornos de ansiedade, depressão, estresse, bem como abuso de substâncias e/ou dependências (Liberzon et al., 2005). A fim de compreender a neurobiologia do PTSD, modelos animais desta doença são utilizados, no intuito de estudar os diferentes aspectos desta psicopatologia.

1.3.1. Relação entre as alterações comportamentais em humanos e em modelos animais com PTSD

Os modelos animais de PTSD têm se concentrado na sua capacidade de reproduzir os sintomas biológicos e comportamentais observados em humanos. Dentre os sintomas psicológicos de roedores estão: 1- A resposta exagerada ao susto, provocado por um estímulo sonoro que pode representar o sintoma de hipervigilância; 2- A resposta intensificada de medo condicionado (por exemplo, quando o animal é exposto a um lembrete situacional), pode representar sintomas de revivência do evento traumático; 3- A diminuição da exploração em campo aberto representa sintomas de neofobia e fuga à situação relacionada à memória aversiva; 4- A diminuição na resposta por recompensas, ou uma resposta diferencial para analgesia e anestesia, pode indicar um entorpecimento emocional. Dentre esses sintomas, o susto e medo condicionado são, provavelmente, as analogias mais diretas e, portanto, mais extensivamente estudadas nos modelos animais de PTSD (Liberzon et al., 2005).

1.4. Regiões encefálicas envolvidas na psicopatologia do PTSD

As alterações neuroanatômicas associadas a exposição ao estresse traumático foram demonstradas em regiões como o cíngulo anterior, ínsula, locus coeruleus e área tegmental ventral. Contudo, a maioria dos estudos sobre as anormalidades bioquímicas, neuroanatomicas e estruturais observadas em pacientes e modelos animais de PTSD está focada no hipocampo, amígdala e córtex pré-frontal (PFC) (Burbiel 2015; Wilson et al., 2014; Moore et al., 2001; Corral-Frias et al., 2013; Bingham et al., 2013; Rauch et al., 1996; Shin et al., 1999).

O hipocampo é uma das mais importantes regiões envolvidas no PTSD, uma vez que está relacionada com os processos de armazenamento e consolidação de novas

memórias e também por seu *feedback* inibitório sobre o eixo HPA. O hipocampo parece interagir com a amígdala durante a codificação das memórias emocionais, tais como de medo e raiva. Enquanto o córtex pré-frontal desempenha um papel de controle sobre as estruturas subcorticais para regular as respostas comportamentais adequadas (Giustino e Maren 2015) (Figura 3).

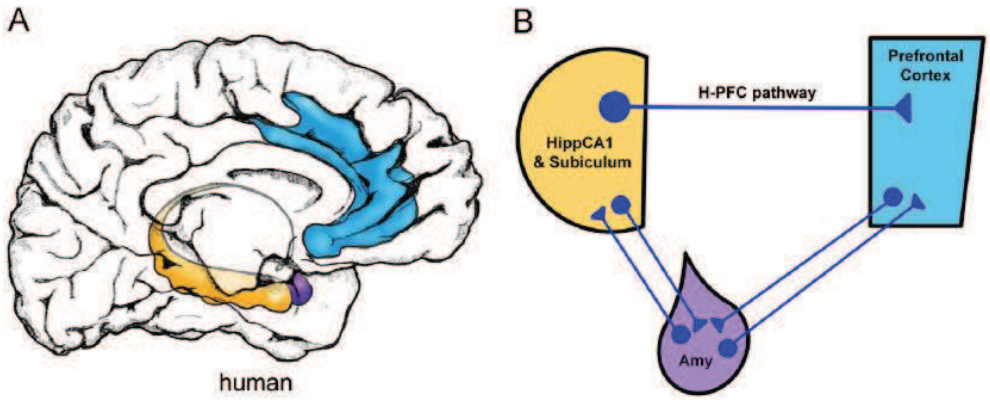


Figura 3. (A) Desenho esquemático de um cérebro humano na vista sagital mostrando o hipocampo (amarelo), a amígdala (roxo) e o PFC (azul) que são as três principais regiões envolvidas na psicopatologia do PTSD. (B) Esquema mostrando as conectividades gerais entre estas regiões (Retirado de Godsil et al., 2013).

1.4.1. Hipocampo e PTSD

Estudos recentes demonstram que o hipocampo desempenha um papel crítico nos distúrbios psiquiátricos relacionados ao estresse. Esta estrutura medial-temporal apresenta uma grande concentração de receptores para glicocorticoides que são responsáveis pela modulação da liberação desses hormônios através dos efeitos inibidores sobre o eixo HPA (McEwen 2008; Sapolsky et al., 2000; Sapolsky 2003). Além disso, o hipocampo é uma das regiões responsáveis pela formação, consolidação e processamento de memórias emocionais (Leuner e Gould 2010). Portanto, essa região

constitui peça fundamental de integração cognitiva, neuro-hormonal e neuroquímica em resposta ao estresse (Bremner 1999).

Em dois modelos diferentes de PTSD (estresse social e choque nas patas) foram observadas diminuições no número de células Bromodesoxiuridina (BrdU) positivas no giro denteado (Gould et al., 1998) e na zona subgranular (Kikuchi et al., 2008) do hipocampo de ratos. O número reduzido de células BrdU positivas pode ser reflexo tanto da diminuição na proliferação como na sobrevivência de células recém-geradas. Além disso, o número de células BrdU positivas se correlaciona negativamente com os sintomas de hipervigilância/hiperexcitação nos animais com PTSD (Kikuchi et al., 2008), indicando que a diminuição destas células pode ser relevante para os sintomas comportamentais observados nesta doença.

Alterações morfológicas também foram observadas nas células do hipocampo. Em outro modelo animal de PTSD (exposição a um predador) foi observado que, oito dias após a indução, os animais apresentaram diminuições na complexidade dendrítica, no comprimento dos dendritos, no número de ramificações e na densidade de espinhos dendríticos nos neurônios de CA1, CA3 e giro denteado do hipocampo (Cohen et al., 2014). Do mesmo modo o estresse crônico de contenção (21 dias) causa uma diminuição na densidade e no comprimento dos dendritos na região CA3 do hipocampo (Watanabe et al., 1992), além de induzir alterações na estrutura do terminal sináptico na mesma região (Magariños et al., 1997). Estes dados estão de acordo com dados a respeito da intensa exposição do hipocampo aos glicocorticoides. Sabe-se que o estresse aumenta os níveis de glicocorticoides no sistema circulatório (Kao et al., 2015; Reber 2012). Neste sentido, alguns estudos que avaliaram os efeitos da administração crônica de níveis elevados de corticosterona em ratos adultos demonstraram uma diminuição da ramificação e do comprimento dos dendritos (Woolley et al., 1990) e uma diminuição

no número absoluto de neurônios da região CA3 (Sapolsky et al., 1985). Portanto, esses dados indicam que a intensa exposição das células aos glicocorticoides pode afetar negativamente a estrutura do hipocampo.

Alterações a nível molecular também foram observadas nos neurônios do hipocampo de animais com PTSD. Em um estudo que utilizou microscopia eletrônica de transmissão, foram observadas as seguintes alterações: encolhimento celular, condensação da cromatina, núcleo irregular e entalhes na membrana nuclear (Li et al., 2010a). Outro estudo que utilizou a mesma técnica detectou um aumento na fragmentação do DNA nos neurônios do hipocampo de animais com PTSD (Li et al., 2010b). Todas essas anormalidades na estrutura neuronal são condizentes com a morfologia apoptótica, indicando um aumento nas taxas de apoptose nos neurônios do hipocampo desses animais. Importante salientar que, em ambos os estudos, o número de células apoptóticas atingiu o nível máximo sete dias após a indução do PTSD e então diminuíram gradualmente até o dia 28. Além disso, alterações na estrutura do terminal sináptico também foram observadas em camundongos com PTSD. Herrmmann e colaboradores (2012) relataram uma diminuição nas proteínas pré-sinápticas sinapsina e sinaptofisina além de uma diminuição no neurofilamento H que é um indicador de plasticidade axonal.

Em humanos, a atrofia hipocampal é uma das alterações morfológicas mais comumente observadas em pacientes com PTSD (Kitayama et al., 2005; Lindauer et al., 2006; Bremner et al., 2008; Bremner et al., 1995; Shin et al., 2004a). Ademais, alguns estudos demonstram que as reduções no volume hipocampal estão correlacionadas com a gravidade da doença e o grau de déficit cognitivo observado nesses pacientes (Bremner 1999; Bremner 2002). Alterações nos processos de formação e evocação de memórias também constituem um aspecto importante do quadro clínico de pacientes

portadores de psicopatologias relacionadas ao estresse, uma vez que estes indivíduos muitas vezes apresentam déficits importantes de memórias declarativas (de fatos e eventos), e fragmentação de memórias, tanto autobiográficas como relacionadas ao trauma (Brewin et al., 1996).

1.4.2. Amígdala e PTSD

Estudos, tanto em humanos como em modelos animais, têm demonstrado que a amígdala, uma das regiões-chave do sistema límbico, possui um papel importante na memória emocional (Han et al., 2014; Harding et al., 2002). A amígdala modula a consolidação da memória com o armazenamento de informações emocionalmente relevantes tendo, portanto, um papel crítico na formação da memória aversiva de longo prazo. Várias evidências indicam que a amígdala está criticamente envolvida no circuito neural do estresse e desempenha um papel crucial na codificação e recuperação das memórias de medo condicionado, bem como na modulação dos comportamentos de ansiedade (Shekhar et al., 2005; Ebner et al., 2004; Carter et al., 2004; Davis 2006; Roozendaal e McGaugh 1996).

O complexo amigdaloide é comporto de três subgrupos distintos: o núcleo central, o núcleo basolateral e o núcleo medial (Harding et al., 2002). Dados sobre as alterações observadas na amígdala variam dependendo do núcleo analisado e do protocolo estressor empregado. Por exemplo, o estresse crônico por imobilização (um modelo animal de PTSD) causa um aumento na arborização dendrítica e uma maior densidade de espinhos dendríticos na amígdala basolateral (Cohen et al., 2014; Cui et al., 2008; Lucassen et al., 2014; Vyas et al., 2002; Mitra et al., 2005), além disso, essas alterações não são revertidas após um período de recuperação (Lucassen et al., 2014). Em contraste, um protocolo de estresse agudo produziu uma pronunciada

desramificação e retração dendrítica específicas ao hemisfério direito da amígdala basolateral. Entretanto, assim como no estresse crônico, o estresse agudo também aumentou a densidade de espinhos dendríticos, embora esta alteração seja menos pronunciada do que as mudanças na morfologia dendrítica (Maroun et al., 2013).

Estudos que analisaram o núcleo medial da amígdala de ratos mostraram que uma única sessão com duração de 1h de estresse por contenção foi capaz de provocar uma diminuição na densidade de espinhos dendríticos, entretanto, quando o estímulo tornou-se mais vigoroso (6 horas de imobilização em um único dia) ou repetido (6 horas de imobilização por dia, durante 28 dias), nenhuma diferença na densidade de espinhos dendríticos foi observada (Marcuzzo et al., 2007). Todavia, outro estudo que avaliou os efeitos da imobilização crônica (6 horas de imobilização por dia, durante 21 dias) sobre os neurônios de camundongos, demonstrou uma redução de aproximadamente 20% na densidade de espinhos dendríticos na amígdala medial (Bennur et al., 2007).

Quando a amígdala foi analisada como um todo, ou seja, sem distinção de um núcleo específico, nenhuma alteração no comprimento dos dendritos apicais e na densidade de espinhos dendríticos foi observada em camundongos expostos a um protocolo de estresse crônico de contenção (Kassem et al., 2013). Neste caso, pode ser que a heterogeneidade das alterações observadas nos diferentes núcleos da amígdala, seja responsável pela ausência de alterações significativas ao longo de toda a amígdala.

Alterações nos processos de apoptose na amígdala de ratos com PTSD também foram relatadas. Um estudo observou uma alteração na taxa das proteínas Bax/Bcl-2, indicando um aumento na apoptose celular na amígdala basolateral desses animais (Ding et al., 2010). A Bax é uma proteína pró-apoptótica, enquanto a Bcl-2 é uma proteína anti-apoptótica, portanto, alterações nas taxas de Bax/Bcl-2 desempenham um papel importante na regulação da sobrevivência ou morte das células. Deste modo, a

apoptose das células da amígdala pode ser parte da patogênese do PTSD. Além disso, alterações a nível intracelular também foram observadas em neurônios da amígdala de ratos. Um estudo recente que utilizou a técnica de microscopia eletrônica de transmissão demonstrou que um estresse prolongado único induziu: inchaço e perda das cristas mitocondriais, condensação da cromatina, fragmentação do núcleo, desaparecimento do nucléolo e que ao mesmo tempo, essas alterações podem refletir em danos funcionais aos neurônios da amígdala dos animais (Han et al., 2014).

Em humanos, estudos morfométricos da amígdala revelaram resultados divergentes: aumento (Kuo et al., 2012), diminuição (Weninger et al., 2008; Morey et al., 2012) ou nenhuma alteração (Bremner et al., 1997; Lindauer et al., 2004; Lindauer et al., 2005; Schmahl et al., 2009) no volume da amígdala de pacientes com PTSD. Ademais, Veer e colaboradores (2015) relataram uma diminuição somente no volume da amígdala direita de pacientes com PTSD quando comparado com os controles e que essa diminuição parece se originar dos núcleos basolateral e medial da amígdala. Um estudo sugeriu ainda que a redução do volume da amígdala direita está intimamente associada aos sintomas de ansiedade e excitação em veteranos de guerra (Pietrzak et al., 2015). Além disso, alguns estudos relataram um aumento no fluxo sanguíneo na amígdala de pacientes com PTSD durante a apresentação de imagens visuais ou auditivas relacionadas ao trauma indicando uma hiper-responsividade da amígdala nesses pacientes (Liberzon et al, 1999; Shin et al., 2004b).

Em contraste com as alterações hipocampais observadas tanto em humanos como em modelos animais de PTSD que mostram, em sua grande maioria, uma atrofia acentuada, as alterações observadas na amígdala são paradoxais e ainda não há consenso a este respeito. O que parece bastante evidente é que os diferentes núcleos da amígdala apresentam diferentes alterações, indicando que cada núcleo amigdaloide

possui uma função distinta no circuito neural do estresse (Cohen et al., 2014). Em uma tentativa de traduzir esses dados conflitantes para a área clínica, uma amígdala hiperativa talvez faça parte do processo que sustenta os sintomas do PTSD como medo e ansiedade, por sua vez, a atrofia observada em alguns núcleos da amígdala e no hipocampo pode ser responsável pelas falhas nos processos de armazenamento, evocação e extinção da memória relacionada ao trauma (Cohen et al., 2014).

1.4.3. Córtex pré-frontal e PTSD

O córtex pré-frontal (PFC) é uma estrutura do lóbulo frontal anterior que medeia funções cognitivas críticas, incluindo a flexibilidade cognitiva, que é a capacidade de um organismo para, de forma adaptativa, alterar o comportamento de acordo com mudanças nas exigências. Em indivíduos saudáveis, o PFC age filtrando e suprimindo informações no intuito de mudar a atenção para informações e respostas relevantes (Carrion e Wong, 2012). Este é um processo cognitivo fundamental e está intimamente relacionado ao processo de aprendizagem (George et al., 2015). No entanto, os indivíduos com PTSD têm dificuldades em manter a atenção e podem ser facilmente distraídos. Eles também têm dificuldades em suprimir memórias intrusivas do trauma (por exemplo, flashbacks, pesadelos) e em extinguir as respostas de medo. Estas manifestações comportamentais e de deficiência de aprendizagem observadas na fisiopatologia do PTSD são, em parte, resultado das anormalidades observadas no PFC (Carrion e Wong, 2012).

Foi proposto que a desregulação da função do PFC observada no PTSD contribui para os sintomas da doença, ao alterar a capacidade dos indivíduos em contextualizar adequadamente estímulos relacionados com o stress (Liberzon e Sripada, 2008). Pesquisas de neuroimagem indicam que o PFC é hiporresponsivo no PTSD e que

a hiporresponsividade é inversamente proporcional à gravidade dos sintomas (Shin et al., 2006). A ativação diminuída do PFC foi demonstrada em resposta a diversos estímulos como: sons e imagens relacionados com o trauma (Bremner et al., 1999a; Yang et al., 2004; Hou et al., 2007), palavras relacionadas ao combate, em veteranos de guerra (Shin et al., 2004b; Shin et al., 2001) e palavras relacionadas ao abuso (Bremner et al., 2004; Bremner et al., 1999b). Da mesma forma, a hiporresponsividade do PFC no PTSD tem sido associada a estímulos emocionais não relacionados ao trauma, tais como, fotografias aversivas (Phan et al., 2006), imagens de ameaças (Kim et al., 2008), expressões faciais de medo (Rauch et al., 2000; Shin et al., 2005; Williams et al., 2006) e até mesmo durante situações de repouso, na qual nenhum estímulo foi aplicado (Molina et al., 2010). Outra importante alteração relatada no PTSD é a redução na espessura do PFC (Sussman et al., 2016; Bing et al., 2013; Milad et al., 2005; Kühn et al., 2011; Hartley et al., 2011).

Estudos em modelos animais têm demonstrado que o estresse também causa importantes alterações a nível celular no PFC. Os principais resultados destes estudos relataram uma redução significativa na complexidade dendrítica, no comprimento dendrítico total e uma diminuição na ramificação e densidade dos espinhos dendríticos apicais dos neurônios piramidais do PFC medial (Radley et al., 2004; Radley et al., 2005; Czéh et al., 2008; Goldwater et al., 2009). Entretanto, parece que estas alterações são plásticas e não degenerativas, porque elas revertem espontaneamente após um período de recuperação (Goldwater et al., 2009; Radley et al., 2005), pelo menos quando os animais são jovens, pois um estudo como ratos de meia-idade e idosos não conseguiu mostrar esta remodelação dendrítica reversível (Bloss et al., 2010).

O PFC é uma região com abundância de receptores de glicocorticoides e estes efeitos induzidos pelo estresse são mediados, em parte, pela ativação destes receptores,

isso porque os níveis artificialmente elevados de glicocorticoides resultam em alterações morfológicas semelhantes às observadas após a exposição ao estresse (Czeh et al., 2008; Popoli et al., 2011).

Diversos estudos sobre o PFC têm demonstrado que o impacto do estresse em uma região do encéfalo pode se espalhar para outras áreas que são sinapticamente ligadas (Sousa e Almeida 2012) como, por exemplo, a via que conecta o PFC ventro-medial (vmPFC) à amígdala, que apresenta-se fisiologicamente alterada no PTSD (Gilboa et al., 2004). Nos seres humanos, o vmPFC tem uma influência inibitória sobre a amígdala semelhante a de roedores (Delgado et al., 2008). Assim, a alteração na via vmPFC-amígdala encontrada no PTSD pode ser uma das causas dos sintomas desta doença.

Todas essas alterações descritas podem afetar a capacidade do PFC em modular as subsequentes respostas ao estresse, e parece claro que o estresse está associado com alterações morfofuncionais do PFC.

1.5. Astrócitos e PTSD

Os astrócitos representam o principal elemento celular do sistema homeostático no SNC, sendo responsáveis pela maior parte dos aspectos do suporte metabólico, regulação da concentração de íons e neurotransmissores, manutenção da barreira hematoencefálica e preservação da integridade dos tecidos após lesão (Catalani et al., 2002, Lo et al., 2003, Petty e Lo 2002, Park et al., 2003, Theodosis et al., 2008). Evidências recentes também sugerem que os astrócitos influenciam vários aspectos da transmissão sináptica e, assim como os neurônios, participam ativamente do processamento da informação nas sinapses “tripartite” (Perea et al., 2009) (Figura 4).

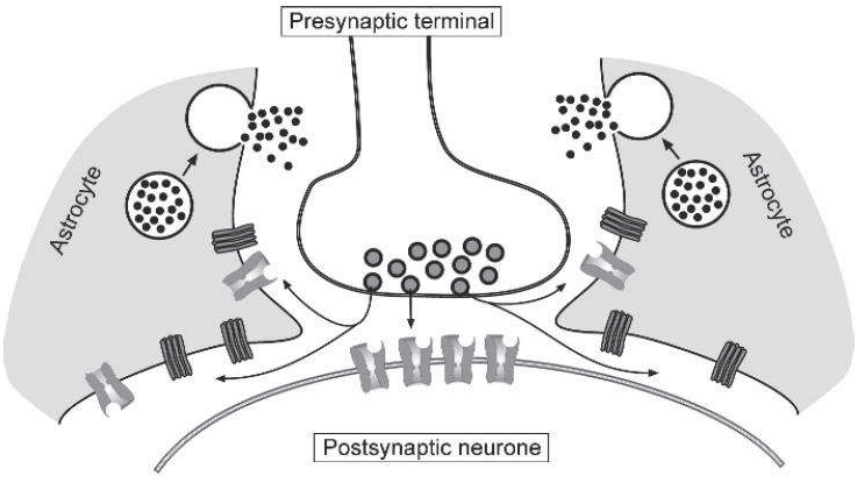


Figura 4. Sinapse tripartite. A sinapse tripartite é constituída de um terminal pré-sináptico, uma membrana neuronal pós-sináptica e os astrócitos adjacentes. O neurotransmissor liberado no terminal pré-sináptico interage com receptores específicos localizados tanto na membrana pós-sináptica neuronal como na membrana astroglial. Os astrócitos também podem sinalizar para os neurônios a partir da liberação de gliotransmissores (Retirado de Verkhratsky e Butt 2007).

Em regiões como o hipotálamo e o hipocampo, foi demonstrado que os astrócitos participam ativamente da plasticidade sináptica, ou seja, os astrócitos desempenham um papel importante na formação, manutenção e eliminação das sinapses, processos estes que constituem a base da adaptação do encéfalo para as constantes mudanças no ambiente externo e são essenciais para aprendizagem e memória, e que até recentemente acreditavam-se ser de responsabilidade exclusiva dos neurônios (Araque e Navarrete 2010; Ullian et al., 2001; Verkhratsky e Butt 2007). Além disso, a remodelação dos processos astrocíticos está intimamente associada às alterações morfológicas em neurônios vizinhos (Theodosis et al., 2008), portanto, estudos recentes tem demonstrado o papel crítico desempenhado pelos astrócitos nos transtornos psiquiátricos.

Pesquisas em modelos animais de PTSD têm demonstrado que a exposição a um estresse traumático provoca uma diminuição no número de astrócitos positivos para a proteína glial fibrila ácida (GFAP) que é um importante marcador astrocitário responsável pela manutenção das funções cito-arquitetônicas dos astrócitos. Ademais, os astrócitos de animais com PTSD apresentaram uma diminuição na expressão de GFAP, além de corpos celulares atrofiados e diminuição na espessura dos prolongamentos (Xia et al., 2013; Han et al., 2015). Anormalidades a nível molecular também foram relatadas: inchaço e vacuolização das mitocôndrias e expansão do retículo endoplasmático foram descritas nos astrócitos do hipocampo de ratos com PTSD (Han et al., 2015).

Consistente com esses resultados, outros trabalhos que avaliaram os efeitos do estresse crônico em modelos animais demonstraram importantes alterações nos astrócitos. O estresse crônico por imobilização (6 horas por dia durante 3 semanas) reduz a expressão de GFAP na substancia cinzenta periaquedutal de ratos (Imbe et al., 2012). O mesmo modelo de estresse também foi capaz de provocar importantes alterações em três principais parâmetros morfológicos: 40% de redução no comprimento dos processos astrocíticos, 56% de redução no volume dos processos e 58% de redução no número de processos astrocíticos, além de uma redução de 38% na expressão de GFAP no córtex pré-frontal de ratos (Tynan et al., 2013). Os processos astrocíticos constituem o maior sítio de concentração de GFAP, portanto, essa atrofia observada nos astrócitos é extremamente importante, uma vez que a GFAP é uma proteína do citoesqueleto fundamental para a remodelação dos astrócitos em resposta a diferentes situações fisiológicas e patológicas (Allen e Barres 2005).

A exposição a um estresse crônico imprevisível (modelo animal de depressão) foi capaz de diminuir em 19% o número de células GFAP positivas no córtex pré-

frontal de ratos (Banasr e Duman 2008). Por sua vez, a exposição a um estresse psicossocial provocou uma redução tanto na densidade de astrócitos (25%) como no volume do soma astrocitário (25%) no hipocampo de musaranhos, além disso, essas alterações se correlacionam com a diminuição do volume hipocampal observada neste estudo (Czéh et al., 2006).

Ademais, estudos de neuroimagem estrutural revelaram que o volume do hipocampo é reduzido em pacientes com PTSD e pelo fato dos astrócitos serem a maior população de células, não só no hipocampo, como também em todo o SNC, é plausível que as mudanças na densidade e estrutura dos astrócitos sejam, ao menos em parte, responsáveis pela atrofia hipocampal observada nesses pacientes. Avaliações histológicas quantitativas *post-mortem*, em pacientes que sofriam de transtornos de humor, corroboram esses achados, uma vez que demonstram uma diminuição do número de células gliais no córtex pré-frontal e na amígdala de pacientes com transtorno depressivo (Öngür et al., 1998; Rajkowska et al., 1999; Bowley et al., 2002; Cotter et al., 2002; Hamidi et al., 2004).

Essa diminuição no número de astrócitos bem como na expressão de GFAP pode induzir deficiências funcionais na atividade neuronal. Isso por que os astrócitos são responsáveis por sintetizar e liberar muitos dos fatores neurotróficos vitais para a saúde neuronal, tais como o fator neurotrófico derivado do encéfalo (BDNF), fator neurotrófico derivado da glia (GDNF) e fator de crescimento do nervo (NGF) (Friedman et al., 1998; Althaus e Richter-Landsberg 2000). Estes fatores neurotróficos regulam o crescimento neuronal além de serem essenciais para a plasticidade neural, e a sua disponibilidade reduzida pode resultar no aumento da vulnerabilidade celular ou até mesmo na morte celular (Czéh et al., 2006). Além disso, os astrócitos contribuem para a neuroproteção e a sobrevivência dos neurônios, portanto, qualquer disfunção astrocítica

pode afetar seriamente a viabilidade neuronal (Takuma et al., 2004). Assim, os astrócitos parecem ter um papel muito mais importante em processos como formação e evocação de memórias, emoção e cognição do que se acreditava anteriormente.

1.6. Cetamina no tratamento do PTSD

Desde que o PTSD foi incluído no Manual Diagnóstico e Estatístico de Transtornos Mentais (*Diagnostic and Statistical Manual of Mental Disorders* – DSM) elaborado pela Associação Americana de Psiquiatria na década de 80, centenas de ensaios clínicos têm procurado identificar métodos de amenizar seus sintomas. Esses métodos variam desde abordagens farmacológicas, que tratam diretamente o PTSD e seus sintomas, até os tratamentos cognitivo-comportamentais, que são baseados em princípios de condicionamento e aprendizagem através de psicoterapia. Embora as terapias psicológicas apresentem uma relativa eficácia no tratamento do PTSD, essa patologia permanece classificada como de difícil tratamento (Sofuoglu et al., 2014; Cukor et al., 2009).

A cetamina é um anestésico administrado intravenosamente, especialmente em pacientes vítimas de queimaduras. Normalmente ela é utilizada em concomitância com componentes opioides, servindo para diminuir a quantidade de opioides necessária para controlar a dor de forma eficaz. A cetamina é uma droga multifuncional que afeta vários receptores, incluindo os receptores NMDA, receptores opioides, e receptores monoaminérgicos (Hirota e Lambert 1996). A cetamina também é utilizada na anestesia venosa total, onde funciona como um analgésico ou como anestésico, dependendo da concentração plasmática (Hirota e Lambert 1996).

Estudos recentes têm demonstrado o grande potencial da cetamina no tratamento de doenças psiquiátricas, apresentando um rápido efeito antidepressivo

depois da aplicação de apenas uma única dose subanestésica. A administração endovenosa (0.5mg/Kg) de cetamina em pacientes bipolares ou com depressão maior, tem efeito antidepressivo e antissuicida nestes pacientes (Price et al., 2009; Aan het Rot et al., 2010; Zarate et al., 2012). Ademais, o estudo realizado por Zarate et al. (2012) demonstrou que esses efeitos surgem somente cerca de uma hora após a administração da cetamina e que podem durar por até 3 dias (com uma média de recaída após 2 dias). Ao mesmo tempo, poucos efeitos adversos foram relatados pelos pacientes, o mais comum deles foi o efeito dissociativo que ocorreu 40 minutos após a administração da droga (Zarate et al., 2012).

A teoria mais aceita sugere que o mecanismo de ação subjacente ao rápido efeito antidepressivo da cetamina é dependente do aumento da atividade da rota da *mammalian target of rapamycin* (mTOR) (Figura 5). Em condições normais, o glutamato, liberado pelo neurônio pré-sináptico, liga-se tanto aos receptores N-metil D-aspartato (NMDA) como aos receptores ácido α-amino-3-hidroxi-5-metil-4-isoxazol propiônico (AMPA) localizados na membrana pós-sináptica. A ativação do receptor NMDA leva a um aumento da fosforilação do fator de elongação eucariótico 2 (eEF2) silenciando, assim, a tradução do BDNF. O bloqueio dos receptores NMDA pela cetamina faz com que o glutamato ligue-se mais intensamente aos receptores AMPA. Isso leva a uma diminuição da fosforilação do eEF2 e, consequentemente, reinstaura a tradução do BDNF. A tradução do BDNF desencadeia a sinalização da quinase do receptor de tropomiosina B (Trk B) que leva a ativação da quinase relacionada a sinalização extracelular (ERK) e da proteína quinase B (PKB/Akt) e suprimindo a quinase de glicogênio sintase 3 (GSK-3). Esta cascata de sinalização ativa a mTOR levando ao aumento de proteínas sinápticas como a sinapsina I, PSD95 e GluR1 e ao aumento da densidade de espinhos dendríticos. O resultado final desta cascata de

eventos é a remodelagem dos botões sinápticos e, consequentemente, os efeitos comportamentais positivos em modelos animais de depressão. Este é considerado o possível mecanismo pelo qual a cetamina induz os seus efeitos antidepressivos (Naughton et al., 2014).

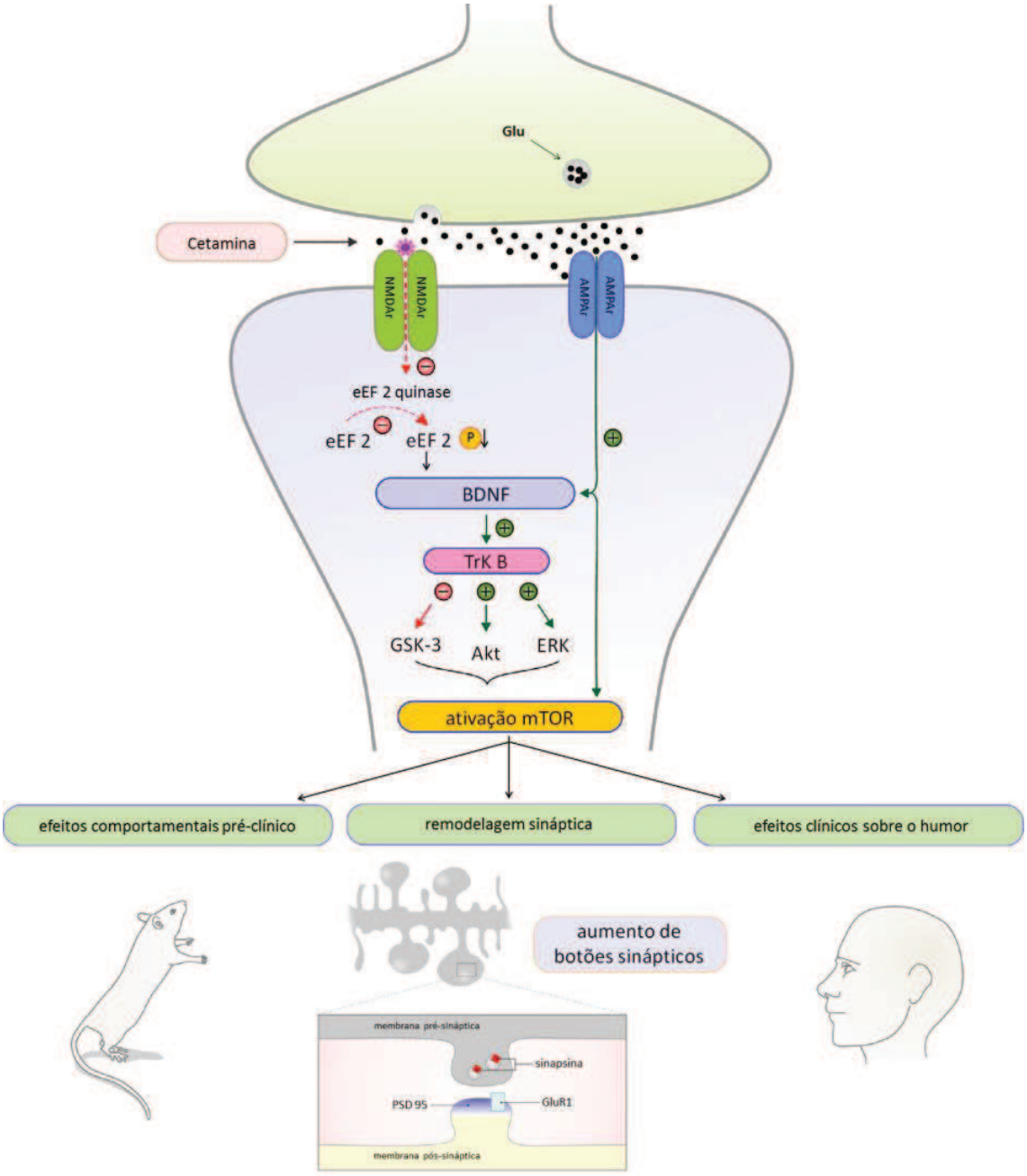


Figura 5: Mecanismo proposto através do qual a cetamina produz seus efeitos antidepressivos (Adaptado de Naughton et al., 2014).

Atualmente existem poucos estudos sobre o uso de cetamina para o tratamento do PTSD e, pelo fato desta droga estar associada com a dissociação e a psicose, há uma preocupação de que ela possa resultar em maiores taxas desta doença (Cukor et al., 2009).

Um estudo avaliou o prontuário de 147 soldados internados com queimaduras graves em um centro médico militar nos Estados Unidos, e comparou 119 pacientes que receberam cetamina durante a internação e 28 que não receberam. Os resultados demonstraram que os pacientes tratados com cetamina apresentaram taxas significativamente mais baixas de incidência de PTSD quando comparados aos indivíduos não tratados com cetamina (26,9% vs 46,4%, respectivamente) mesmo apesar destes pacientes terem queimaduras maiores, maior pontuação de gravidade da lesão, passando por mais operações e mais tempo na UTI. (McGhee et al., 2008). Outro estudo realizado com 41 pacientes com mais de 10 anos de histórico de PTSD grave demonstrou que os pacientes que receberam cetamina apresentaram uma redução nos sintomas do PTSD nas primeiras 24 horas após a administração da droga, quando comparados com os pacientes que receberam midazolam. Além disso, em sete pacientes o efeito durou pelo menos 14 dias (Feder et al., 2014). D'Andrea e Sewell (2013) relataram o caso de um veterano de guerra de 23 anos com PTSD que havia sido tratado com diversos medicamentos incluindo fluoxetina, citalopran, escitalopran, bupropion, olanzapina, valproato, e risperidona entre outros, sem apresentar melhora em seu quadro clínico. O veterano foi então tratado com cetamina. Uma única dose de 35mg aplicada juntamente com 30mg de propofol e a resposta inicial foi imediata. Ele se mostrou motivado, feliz, interessado na família e relatou também melhoras durante o sono. As melhoras duraram 15 dias. Este relato de caso indica que a cetamina intravenosa pode

ser eficaz mesmo para casos de PTSD extremamente resistentes ao tratamento (D'Andrea e Sewell 2013).

Entretanto, estes resultados são controversos, pois o estudo realizado por Zeng e colaboradores (2013) em pacientes com histórico de abuso sexual e abuso físico não demonstrou nenhum efeito da cetamina sobre os sintomas do PTSD. Outro estudo demonstrou que 15 indivíduos expostos a queimaduras graves com PTSD e que receberam cetamina/midazolam como um tratamento analgésico/sedativo apresentavam sintomas de PTSD significativamente mais graves do que os indivíduos que não receberam o mesmo tratamento (Winter e Irle 2004). Resultados semelhantes foram descritos em dois estudos envolvendo vítimas de acidentes moderados. Os pacientes que receberam cetamina durante o tratamento inicial na emergência mostraram um aumento nos sintomas de revivência, dissociação e fuga quando comparado com doentes que receberam outra medicação opioide durante o tratamento inicial (Schönenberg et al., 2005; Schönenberg et al., 2008).

No entanto, pelo fato de, na maioria dos casos relatados, a cetamina ter sido administrada juntamente com outras medicações deve-se ter cuidado na interpretação dos resultados. Por isso é importante estudar os efeitos da cetamina separadamente. Neste sentido, a realização de estudos pré-clínicos que demonstrem os efeitos da cetamina aplicada de forma isolada de outras medicações é de extrema importância.

São raros os estudos em modelos animais de PTSD tratados com cetamina. Um destes, realizado em camundongos expostos a um choque nas patas como modelo de PTSD, demonstrou que o tratamento crônico (18 dias) com doses de 0.65, 1.25 e 2.5mg/kg de cetamina aplicada i.p. alivia o comportamento de *freezing* dos animais quando reexpostos ao contexto aversivo. Além disso, o tratamento com cetamina reverteu todas as alterações comportamentais relacionadas com a ansiedade nestes

animais: numero de entrada e porcentagem de tempo gasto nos braços abertos no teste de *plus-maze* elevado. Ademais, as doses de 1.25 e 2.5mg/kg de cetamina foram capazes de aumentar os níveis de BDNF no hipocampo (Zhang et al., 2015). Entretanto, o outro estudo realizado em ratos expostos a um predador como modelo de PTSD demonstrou que a administração de cetamina (0.5, 5 e 15mg/kg) durante 3 dias, iniciando a primeira dose uma hora após a exposição ao trauma, foi ineficaz na prevenção dos sintomas comportamentais de longo-prazo do PTSD, pelo contrário, quando avaliados 31 dias após a exposição ao trauma, os animais tratados com cetamina (todas as doses) apresentaram resposta de *freezing* maior do que os animais tratados com veículo (Juven-Wetzler et al., 2014). Portanto, parece que devido à complexa apresentação clínica do PTSD e a sua sintomatologia muito variável ainda não se conseguiu estabelecer dados consistentes sobre o tratamento com cetamina nesta doença neuropsiquiátrica.

1.7. Tomografia por emissão de pósitrons em pequenos animais (microPET)

A tomografia por emissão de pósitrons (PET) é uma técnica de medicina nuclear não invasiva que permite análises in vivo de processos fisiológicos e bioquímicos. A tomografia por emissão de pósitrons em pequenos animais (microPET) funciona da mesma maneira que a PET com alta resolução (aproximadamente 1mm), funcionando como um método de imagem pré-clínico (Virdee et al., 2012; Lancelot e Zimmer 2010). O microPET utiliza traçadores conjugados com radionuclídeos emissores de pósitrons, formando os radio-traçadores. Os traçadores podem ser quantificados em termos de processos fisiológicos específicos tais como o fluxo sanguíneo encefálico, a taxa metabólica encefálica ou a disponibilidade de receptores no encéfalo. Os radio-traçadores são administrados intravenosamente e seguem a

distribuição normal da molécula marcada acumulando-se em diferentes regiões corporais conforme a sua farmacodinâmica. O radionuclídeo conjugado decai e libera um pósitron de uma determinada energia cinética. Esse pósitron viaja alguns milímetros e colide com um elétron em um processo que se chama aniquilação liberando, simultaneamente, dois fótons com energia de 511 kilo-eletro-volt (KeV) em direções opostas (180º) que são captados pelos detectores no equipamento de microPET. Um computador então refaz o trajeto destes dois fótons e reconstrói a distribuição molecular do radio-traçador em uma estrutura 3D. Os radio-traçadores distribuem-se por todo o corpo, mas acumulam-se em regiões onde são mais utilizados ou que tenham maior afinidade, portanto quanto mais fótons gama forem liberados de uma determinada região, maior a quantidade de traçador neste local (Virdee et al., 2012; Lancelot e Zimmer 2010) (Figura 6).

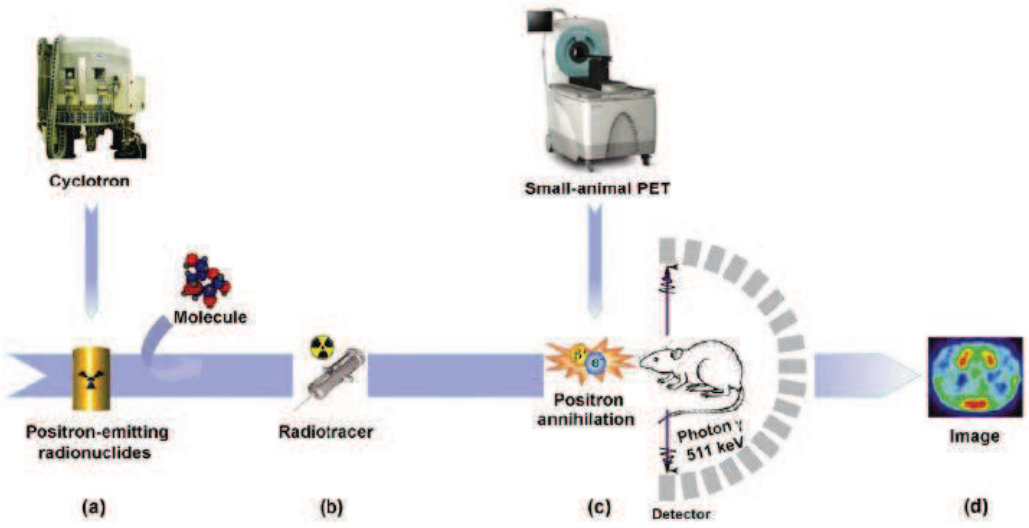


Figura 6. Representação esquemática dos princípios do PET. (A) O cíclotron, um acelerador de partículas, produz radionuclídeos que emitem pósitron. (B) Estes radionuclídeos são conjugados a moléculas e tornam-se um radiotraçador. (C) Para o escaneamento do microPET o radiotraçador é administrado a um animal de pequeno porte que é colocado, anestesiado, dentro do equipamento. O radiotraçador irá acumular nos tecidos a serem estudados, e o decaimento do seu radionuclídeos libera os pósitrons. Estes pósitrons percorrem alguns milímetros dentro do tecido até colidirem com um elétron. Nesta colisão as partículas são aniquiladas e liberam dois raios de fótons gama em direções opostas, com energia de 511 keV. Estes fótons são captados pelo anel de detectores no equipamento. (D) Com base na percepção temporal dos fótons, um computador calcula a rota dos fótons e reconstrói uma imagem tridimensional que mostra a distribuição temporal-espacial da molécula emissora de pósitrons (Retirado de Lancelot e Zimmer, 2010).

O uso no microPET tem-se mostrado especialmente informativo em estudos pré-clínicos de doenças neurodegenerativas e distúrbios neuropsiquiátricos, pois torna possível a quantificação de diversos processos fisiológicos e bioquímicos no organismo vivo. Além disso, esta técnica permite realizar estudos longitudinais com o mesmo animal minimizando o número de animais utilizados e diminuindo o número de repetições de um mesmo experimento. Esta abordagem é especialmente importante em estudos de progressão de um modelo de doença, bem como no estudo de novas estratégias de tratamento, pois cada animal serve como seu próprio controle. A

utilização do microPET pode acelerar inclusive o desenvolvimento de novas drogas revelando informações sobre biomarcadores em diferentes patologias, os mecanismos de ação e a biodisponibilidade de novas drogas (Virdee et al., 2012; Lancelot e Zimmer 2010).

2. IMPORTÂNCIA E JUSTIFICATIVA

Quando o diagnóstico de PTSD foi introduzido em 1980, acontecimentos traumáticos suficientes para induzir esta condição eram considerados raros (Schnurr et al., 2002). Desde então, estudos epidemiológicos têm documentado ampla incidência e prevalência dessa doença. A violência urbana, o alto índice de acidentes de trânsito, bem como o aumento no número de sequestros e assaltos criaram um cenário propício para o desenvolvimento desse transtorno. Estima-se que de 50% a 90% da população mundial seja exposta a algum evento traumático ao longo da sua vida (Chang et al., 2017; Kessler et al., 1995; Breslau et al., 1998) e que aproximadamente 7 a 8% dessas pessoas desenvolverão PTSD (Chang et al., 2017; Kessler et al., 1995).

Assim, fornecer uma avaliação detalhada da resposta comportamental ao estresse, delinear mecanismos neurais específicos e principalmente, avaliar potenciais compostos terapêuticos são ações essenciais para o melhor prognóstico dos pacientes acometidos por esse transtorno. Neste sentido, a cetamina tem se mostrado uma alternativa terapêutica no tratamento de algumas doenças neuropsiquiátricas como a depressão e o transtorno bipolar demonstrando potente efeito antidepressivo e antisuicida cerca de uma hora após a sua administração (aan het Rot et al., 2010; Zarate et al., 2012). Contudo os efeitos desta droga no tratamento do PTSD ainda são pouco estudados. Nossa hipótese é que a cetamina pode ser uma importante alternativa no tratamento do PTSD e atuar como coadjuvante no tradicional tratamento psicológico.

Entretanto, pouco se sabe sobre os efeitos morfofisiológicos associados ao uso dessa droga tanto em pacientes como em modelos animais de PTSD. Este estudo pretende auxiliar na compreensão dos efeitos da cetamina com uso do choque inescapável único como modelo animal de PTSD.

3. OBJETIVOS

Com base nas informações descritas, os objetivos desta tese foram subdivididos da seguinte maneira:

3.1. Objetivos Gerais

Artigo Científico 1

a) Avaliar, em ratos Wistar, os efeitos comportamentais e neurohistofisiológicos do protocolo de Choque Inescapável Único, como modelo experimental de PTSD em relação aos animais controle.

Artigo Científico 2

b) Analisar os efeitos comportamentais, neurometabólicos e bioquímicos do tratamento com cetamina neste modelo experimental.

3.2. Objetivos Específicos

Artigo Científico 1

a) Avaliar as alterações no peso corporal em animais com PTSD experimental em relação aos animais controle;

b) Avaliar a duração dos episódios de *freezing* como medida de medo condicionado ao contexto nestes animais;

c) Quantificar o número de bolos fecais produzidos durante o teste de medo condicionado ao contexto nestes animais;

d) Estimar a densidade de astrócitos imunorreativos para proteína glial fibrilar ácida (GFAP), com uso de morfometria planar, no hipocampo (*stratum radiatum*) e amígdala medial nestes animais;

e) Avaliar a densidade óptica regional e celular dos astrócitos GFAP positivos no hipocampo (*stratum radiatum*) e amígdala medial nestes animais;

f) Analisar a polaridade e grau de ramificação dos processos, nos astrócitos GFAP positivos, com o uso da técnica dos círculos concêntricos de Sholl, no hipocampo nestes animiais;

g) Relacionar os achados acima com os sintomas do PTSD.

Artigo Científico 2

a) Avaliar a duração dos episódios de *"freezing"* como medida de medo condicionado ao contexto em animais com PTSD experimental em relação aos animais controle;

b) Avaliar as diferenças no padrão comportamental dos animais com PTSD e subdividi-los em "*extreme behavioral response*" (EBR) e "*minimal behavioral response*" (MBR);

c) Verificar as alterações metabólicas utilizando ^{18}F-FDG através da técnica de microPET no córtex frontal, hipocampo e amígdala nestes animais;

d) Dosar os níveis de Brain-Derived Neurotrophic Factor (BDNF) no hipocampo e córtex frontal nestes animais;

e) Relacionar os achados acima com a possível reversão do quadro de estresse pós-traumático induzida pela cetamina.

4. MODO DE APRESENTAÇÃO DOS MÉTODOS E RESULTADOS

Os métodos e resultados são constituídos pelos capítulos II e III:

Capítulo II – Primeiro artigo da tese publicado no periódico *Neurochemical Research.*

Capítulo III – Segundo artigo da tese submetido ao periódico *Neuroscience Letters.*

Capítulo II

"Experimental post-traumatic stress disorder decreases astrocyte density and changes astrocytic polarity in the CA1 hippocampus of male rats"

Artigo publicado no periódico
Neurochemical Research, 2015.

Neurochem Res
DOI 10.1007/s11064-015-1770-3

ORIGINAL PAPER

Experimental Post-traumatic Stress Disorder Decreases Astrocyte Density and Changes Astrocytic Polarity in the CA1 Hippocampus of Male Rats

Lisiani Saur[1] · Pedro Porto Alegre Baptista[1] · Pamela Brambilla Bagatini[1] · Laura Tartari Neves[1] · Raquel Mattos de Oliveira[1] · Sabrina Pereira Vaz[1] · Kelly Ferreira[1] · Susane Alves Machado[1] · Régis Gemerasca Mestriner[1] · Léder Leal Xavier[1]

Received: 31 July 2015 / Revised: 20 October 2015 / Accepted: 11 November 2015

Abstract Post-traumatic stress disorder (PTSD) is a psychiatric condition resulting from exposure to a traumatic event. It is characterized by several debilitating symptoms including re-experiencing the past trauma, avoidance behavior, increased fear, and hyperarousal. Key roles in the neuropathology of PTSD and its symptomatology have been attributed to the hippocampus and amygdala. These regions are involved in explicit memory processes and context encoding during fear conditioning. The aim of our study was to investigate whether PTSD is capable of altering the morphology, density and expression of glial fibrillary acidic protein (GFAP) in astrocytes from the CA1 region of the hippocampus and the medial amygdala and correlate the data obtained with the orientation index of the polarity of astrocytes. Thirty male rats were divided in two groups: control (n = 15) and PTSD (n = 15). The inescapable shock protocol, in which the animals are exposed to a single episode of footshock, was used to induce PTSD. Our results show that, in the hippocampus, PTSD is capable of decreasing the density of GFAP+ astrocytes as well as altering astrocytic morphology, as shown by the reductions observed in the total number of primary processes, in the number of primary processes in the lateral quadrants, and the degree of branching in the lateral quadrants. The analysis of the orientation index indicates that PTSD alters the polarity of hippocampal astrocytes. No alterations were observed in the amygdala astrocytes. Therefore, this study demonstrates notable changes in hippocampal astrocytes, supporting the concept that these cells play an important role in PTSD symptomatology.

Keywords PTSD · Astrocyte · Morphology · GFAP · Hippocampus · Amygdala

Introduction

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder that develops following exposure to trauma [1]. The defining characteristic of a traumatic experience is its capacity to provoke feelings of fear, helplessness and/or horror. This usually occurs in response to experiencing, confronting or even witnessing the threat of death, serious injury, or the loss of physical integrity [2]. According to the most recent edition of The Diagnostic and Statistical Manual of Mental Disorders [3], a diagnosis of PTSD necessitates exposure to a traumatic event, intrusive symptoms, the avoidance of trauma-associated stimuli, negative cognitions/mood, hyperarousal, and significant social impairment. These symptoms must persist for at least 30 days and should not be due to illness, medication, or substance abuse [4].

Traumatic stress has multiple effects on the physiology, neurochemistry, and behavior of humans and animals. Decreased food intake, weight loss, increased conditioned fear and an increase in fecal pellets are some examples [5–12].

Inescapable single footshock is one of the models most widely employed to mimic PTSD in animals. Exposure to stressful events affects the subsequent sensitivity to fear. Within this context, freezing behavior indicates a sense of

✉ Léder Leal Xavier
llxavier@pucrs.br

[1] Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Avenida Ipiranga, 6681, Prédio 12C, Sala 104, Porto Alegre, RS CEP 90619-900, Brazil

Published online: 17 November 2015

immediate threat and intense horror, thus constituting a useful measure of conditioned fear [13–15]. These symptoms correlate with the dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis and several neurotransmitter systems [16, 17] as well as producing functional or structural changes in the hippocampus, amygdala, prefrontal cortex, locus coeruleus, and ventral tegmental area [18–22]. The hippocampus is one of the most important regions involved in the development of PTSD, as it is related to explicit memory processes and context encoding during fear conditioning. It also appears to interact with the amygdala during the encoding of emotional memories, such as of fear and rage, which is a highly relevant process in the study of trauma and PTSD [23].

Polarity is a key feature of a wide variety of biological cell processes, such as asymmetric cell division and cell migration and is notorious in epithelial cells and neurons [24]. Recent evidence has shown polarization also exists in glial cells [24–30]. The term astrocyte polarization refers to the fact that astrocytes are endowed with processes that differ in regard to structure, function and membrane molecules [30]. In this study, cell polarity is defined as the morphological orientation of the astrocytic processes along an axis.

Thus, using an animal model of PTSD, the goals of our study were to analyze: (1) the possible alterations in the astrocytic density and expression of glial fibrillary acidic protein (GFAP) in hippocampal astrocytes in the *stratum radiatum* within the CA1; (2) the possible alterations in the astrocytic density and expression of GFAP in astrocytes from the medial amygdala (MeA); and (3) the possible changes in polarity of hippocampal astrocytes through morphological evaluation.

Materials and Methods

Animals

The animals were obtained from the *Centro de Reprodução e Experimentação de Animais de Laboratório* of the *Universidade Federal do Rio Grande do Sul* and maintained in controlled environmental conditions with food and water ad libitum, under a 12/12 h dark/light schedule. Thirty male Wistar rats weighing about 300–350 g and aged about 3 months were used. The animals were divided into two groups: control (n = 15) and PTSD (n = 15). The rats were acclimatized to their new housing cage over a period of 7 days. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institute of Health (USA). All efforts were made to minimize animal suffering and reduce the number of animals needed.

Animal Model of PTSD: Inescapable Shock

This protocol involves a single exposure to footshock without an opportunity of escape [10, 31]. The footshock apparatus consists of a 50 × 25 × 25 cm box divided into two compartments separated by a door. The whole apparatus has a transparent acrylic front wall for visualization of the animal. The first compartment has a linoleum floor, and the second compartment has a floor consisting of 1 mm bronze bars spaced every 10 mm. The animals were individually placed in the first compartment and, after 2 min, the door separating the two compartments was opened. The moment the animal crossed into the second compartment the door was closed and then a 1 mA 60 Hz footshock was applied for 20 s. The animals in the control group underwent the same described procedure, but did not receive the footshock.

Exposure to a Situational Reminder (SR)

For the SR exposure, 1 week after the initial footshock protocol, the animals were placed in the first compartment of the apparatus for 2 min (no footshocks) and the freezing behavior was recorded. At this time, the door remained closed, and the animals were not allowed to cross to the second compartment. The purpose of the SR is to re-expose the animal to the same aversive context in order to verify if the footshock protocol during the PTSD-induction was effective. The duration of the freezing behavior is a measure of conditioned fear.

Freezing is defined as a lack of every movement except those required for breathing. The freezing response, as a measure of conditioned fear, in the re-exposure to the aversive environment, reflects the response to the related-trauma cues [12]. The SR exposure and the footshock protocol were performed in the morning between 8:00 and 11:00 a.m. To reduce possible errors, the freezing behavior was evaluated by two experienced researchers, and the corresponding results from the evaluators were averaged to provide the value for each animal. The total number of pellets produced during the SR was quantified by counting individual *pellet* in the apparatus at the end of the 2 min.

Weight

The animals were weighed twice: once before exposure to the inescapable shock and again, 1 week later, before exposure to the SR.

Sample Preparation

All animals were euthanized and the brain tissue collected 24–28 h after the situational reminder, perfusion was

conducted alternating animals from the different experimental groups to avoid time bias (for example: 1st animal: CTRL group, 2nd animal: PTSD group, 3rd animal: CTRL group, 4th animal: PTSD group,…). Eight rats per group were used for the immunohistochemical study. Animals were deeply anesthetized with ketamine (90 mg/kg) and xylazine (15 mg/kg) (i.p.) and injected with 1 ml heparin (Cristalia, Brazil). Using a peristaltic pump (Milan, Brazil, 50 mL/min), the animals were perfused through the left cardiac ventricle with 200 mL of saline solution followed by 200 mL of fixative solution of 4 % paraformaldehyde diluted in 0.1 M phosphate buffer (PB), pH 7.4. Brains were dissected from the skull, post-fixed for 4 h in the same fixative solution at room temperature, cryoprotected in 30 % sucrose solution in PB at 4 °C until they sank (about 24 h), frozen in liquid nitrogen, and then stored in a freezer (−20 °C). Coronal brain sections (50 μm) were obtained using a cryostat (Leica, Germany) and one in every three sections was collected for analysis.

GFAP Immunohistochemistry

The main rostral anatomic reference to obtain the analyzed section was the complete presence of the dentate gyrus granule cell layer (approx. Bregma −2.40 mm, interaural 6.60 mm). The analyses were performed until the appearance of the ventral hippocampus (approx. Bregma −4.08 mm, interaural 4.92 mm) [32]. Brain coronal sections were collected in phosphate-buffered saline (PBS) and processed for GFAP immunohistochemistry. Free floating sections were washed and blocked with 2 % bovine serum albumin (BSA) in PBS containing 0.4 % Triton X-100 (PBS-Tx, Sigma Chemical Co., USA) for 30 min. They were then incubated with polyclonal GFAP antiserum raised in rabbit (Dako, UK—Ref:Z0334), diluted 1:500 in 0.3 % of PBS-Tx for 48 h at 4 °C. After being washed twice with PBS-Tx, the sections were incubated in anti-rabbit IgG whole molecule peroxidase-conjugated antibody produced in goat (Sigma, USA), diluted 1:3000 in PBS-Tx at room temperature for 2 h.

The reaction was developed by incubating the sections in a medium containing 0.06 % 3,3′-diaminobenzidine (DAB, Sigma-Chemical Co., USA) dissolved in PBS for 10 min and in the same solution containing 1 μL of 3 % H_2O_2 per mL of DAB medium for an additional 10 min. Immediately after the DAB + H_2O_2 reaction, the sections were rinsed in PBS, dehydrated in a series of increasing ethanol concentrations (70, 90 and 100 %, 2 min each) cleared with xylene and covered with Permount and coverslips. As a control to rule out unspecific binding, in a few sections, the primary antibody was omitted and replaced by PBS. In order to minimize differences in the staining of astrocytes and in background levels, the brains in both experimental groups were fixed and post-fixed in identical solutions for the same length of time, processed at the same time and incubated in the same immunostaining medium for the same length of time.

Astrocytic Density Estimation and GFAP Immunoreactivity Evaluation in the Hippocampus

The number of GFAP-immunoreactive astrocytes per mm^2 in the *stratum radiatum* of the CA1 was estimated using an Olympus BX 50 microscope coupled to an Opton camera and Image Pro Plus software (Image Pro-Plus 6.1, Media Cybernetics, Silver Spring, USA).

This analysis was performed in accordance with the following studies [33–36]. For this analysis, three digitized images (20X) from selected areas were obtained from each section. Altogether, five sections from each animal were analyzed. Thus, 15 images were analyzed per animal. Three randomized areas of interest (AOIs) measuring 4945 μm^2 were overlaid on each image. The astrocytes located inside this square or intersected by the upper and/or right edges of the square were counted. Astrocytes intersected by the lower and/or left edges of the square were not counted. Only GFAP immunopositive astrocytes with a defined cell soma were counted. The investigators who analyzed the images were blinded to the analysis.

The intensity of GFAP immunoreactivity was measured using semi-quantitative densitometric analysis [37] with the same software employed to estimate the astrocytic density. The same images used to estimate astrocytic density were used in the analysis of regional optical density (OD). The images were converted to an 8-bit gray scale (256 gray levels) and three AOIs (4945 μm^2) were overlaid on each image.

For the analysis of cellular OD, three digitized images (40x) were obtained from each section. Altogether, five sections from each animal were analyzed. In general, each image showed only one astrocyte, thus, fifteen images (astrocytes) were analyzed from each animal. The images were converted to gray scale and one AOI measuring 4.36 μm^2 was placed over the astrocytic cell bodies in each image. Cellular GFAP expression was only measured in the glial soma, immunoreactivity in the processes was not measured.

All lighting conditions and magnifications were kept constant during the process of capturing the images. Blood vessels and histological artifacts were avoided and the background correction was performed in accordance with our previously published protocol [37].

The OD was calculated using the following formula:

$$OD(x,y) = -\log[(INT(x,y)-BL)/(INC-BL)]$$

where "OD(*x*,*y*)" is the optical density at pixel (*x*,*y*), "INT(x,y)" or intensity is the intensity at pixel (*x*,*y*), "BL"

or black is the intensity generated when no light passes through the material, and "INC" is the intensity of the incidental light, which is completely white.

Astrocytic Density Estimation and GFAP Immunoreactivity Evaluation in the Medial Amygdala

The number of GFAP-immunoreactive astrocytes per mm^2 in the medial amygdala was estimated as described above, except that in this region, one digitized image (20X) from the MeA was obtained from each section. Altogether, five sections from each animal were analyzed. Thus, five images were analyzed per animal.

The intensity of regional and cellular GFAP immunoreactivity was also measured as described above, except that in the MeA, the cellular OD was measured by overlaying the AOI (4.36 μm^2) on one astrocyte within the 20X digitized image, thus, five images (astrocytes) were analyzed from each animal.

Morphological Analysis of Hippocampal Astrocytes

The morphological analysis was conducted using the same images employed to measure cellular optical density. For the analysis of astrocytic branching, an adaptation of Sholl's concentric circles technique was used [38]. Briefly, seven virtual circles set at 3.91 μm intervals were drawn around each astrocyte and the number of astrocytic processes intersecting the lines in each virtual circle in both the central (superior and inferior) and lateral (right and left) quadrants were counted. The central and lateral quadrants were defined as the portions perpendicular and parallel, respectively, in relation to the *stratum pyramidale* (see Fig. 2). Primary process quantification was performed by counting the processes extending directly from the soma in both the lateral and central quadrants of astrocytes in the same sections. The longest primary process in each quadrant and the area of the astrocytic soma were measured by tracing the processes and cell bodies using a manual measurement tool available in the Image Pro Plus software. These morphological analyses were not carried out in the MeA astrocytes because, in this region, astrocytes are located very close to each other, and it is very difficult to distinguish astrocytic processes individually. Then, in order to avoid misleading results we decided not to perform the morphological analysis in the MeA.

Degree of Polarity in Hippocampal Astrocytes

The degree of polarity was calculated in accordance with a previous study [39]. The degree of polarity is determined by the quotient of the number of lateral intersections of the astrocytic processes divided by the number of central intersections of the astrocytic processes. Smaller numbers indicate a fusiform shape with a preferred orientation perpendicular to the *stratum pyramidale* and larger numbers indicate a more stellate shape with a preferred orientation parallel to the *stratum pyramidale*. This quotient is referred as the orientation index.

Statistical Analysis

The statistical analyses were performed using SPSS 11.0 (Statistical Package for the Social Sciences, USA), and the G*Power 3.1 software (*Institut Für Experimentelle Psychologie, Heinrich Heine Universitat*, Germany) was used to calculate the statistical power [40]. One-way ANOVA for repeated measures was used to analyze the weight and an unpaired *t* test was used in all other analyses. Pearson's correlation coefficient was performed to correlate the data from each of the observers in the analysis of duration of freezing behavior during the SR, as well as correlate the data from the freezing behavior and fecal pellets. The data are expressed as mean ± SD and the results were considered significant when $p \leq 0.05$.

Results

Statistical Power Analysis

The statistical power was calculated using the means and standard deviation of each analyzed parameter. The findings were: for the duration of freezing behavior, 100 %; the number of fecal pellets, 99 %; hippocampal astrocytic density, 77 %; the orientation index, 94 %; and the morphological analyses, 95 %.

Body Weight

There is no statistical difference or correlation between the groups (Fig. 1a).

Conditioned Fear and Fecal Pellets Count

The effect of an exposure to an inescapable footshock, used as a PTSD inducing protocol, on conditioned fear of the animal models is shown in Fig. 1b, which displays the duration of freezing behavior during the SR. The statistical analysis showed the inescapable shock protocol had significant effect on the PTSD group, as denoted by a longer duration of freezing behavior when compared to the control group ($p < 0.001$). No foot-skin alterations were noted following electric shock. A Pearson's Correlation was employed to verify the concordance of the data from each

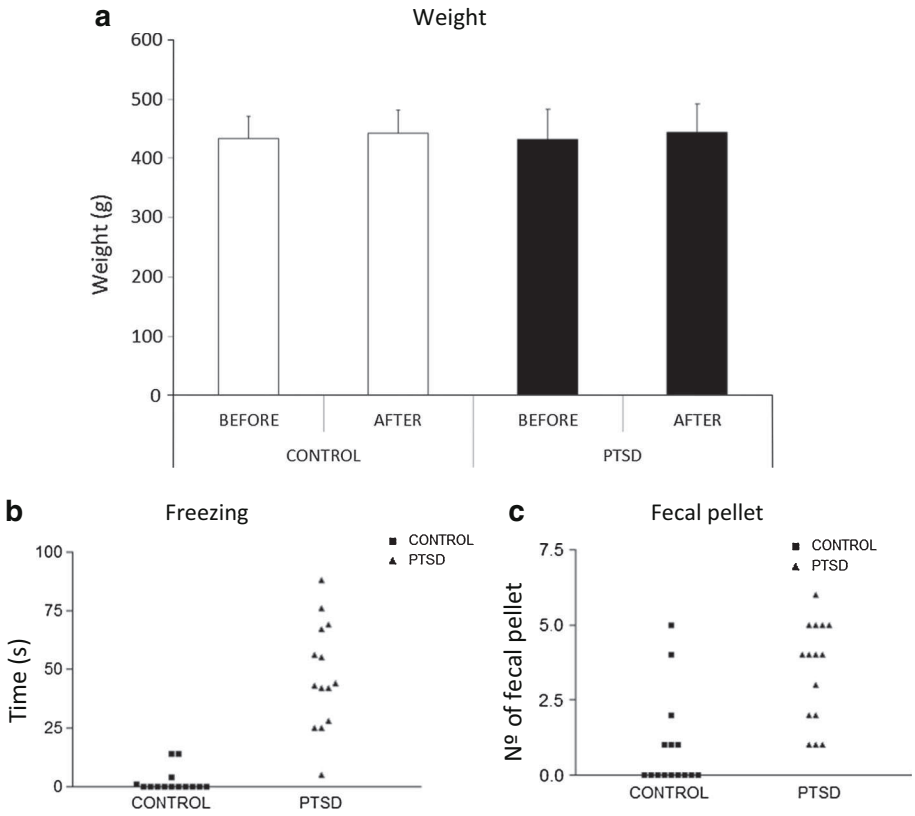


Fig. 1 Effect of a PTSD animal model on weight and behavior. **a** Weight of animals before and 7 days after exposure to shock. There were no differences in the weight of the animals in the control and PTSD groups. **b** Duration of freezing behavior during the situational reminder 7 days after shock exposure. Animals from the PTSD group showed longer duration of freezing behavior when compared to the control group animals ($p < 0.001$) (control = 2 ± 4.7 s; PTSD = 47 ± 21.9 s). **c** Quantification of *fecal pellets* during the situational reminder. Animals from the PTSD group produced more *fecal pellets* than those from the control group ($p < 0.001$) (control = 0.93 ± 1.5 fecal pellets; PTSD = 3.4 ± 1.6 fecal pellets)

observer. This analysis showed a strong correlation between both observers (r = 0.983; $p < 0.001$).

The number of fecal pellets produced during the SR was also significantly higher in the PTSD group (Fig. 1c; $p \leq 0.001$). We also correlated the data between the duration of freezing and the number of fecal pellets. The results showed the data were significantly correlated (r = 0.662; $p = 0.005$).

Immunohistochemical Qualitative Analysis

In our study, we analyzed astrocytes from the *stratum radiatum* within the CA1 region of the hippocampus and from the medial amygdala of Wistar rats (Figs. 2, 3). In the hippocampal qualitative analysis, we observed that the astrocytic soma and primary processes react strongly to GFAP immunohistochemistry while the reaction in the secondary processes is weaker. Moreover, we observed that few tertiary processes are reactive on astrocytes stained by this technique. We noted that most of the astrocytes have a fusiform shape, extending their processes to the pyramidal layer in the upper region and to the *stratum lacunosum moleculare* in the lower region. The animals in the control group seem to have a higher GFAP+ astrocyte density. In the qualitative analysis of the MeA, many astrocytes were found to be reactive to GFAP. However, being located very close to each other, it was very difficult to distinguish individual astrocytic processes, as was possible in the hippocampus. Moreover, it seems that the astrocytes in the MeA have fewer branches than those in the hippocampus.

Thus, we performed a quantitative evaluation involving estimating cell density and semi-quantitative evaluations involving regional and cellular ODs in the hippocampus and medial amygdala. Additionally, we also evaluated hippocampal astrocytic morphology.

Astrocytic Density and Regional and Cellular Optical Density in the Hippocampus

In the animals from the PTSD group, there was a decrease in GFAP+ astrocytic density (Fig. 4a; $p \leq 0.05$) when compared to the control group. No differences were observed in the regional (Fig. 4b) or cellular (Fig. 4c) GFAP immunoreactions.

Astrocytic Density and Regional and Cellular Optical Density in the Medial Amygdala

No differences were observed in astrocytic density (Fig. 4d; $p \leq 0.05$), regional OD (Fig. 4e) and cellular OD (Fig. 4f) in the MeA.

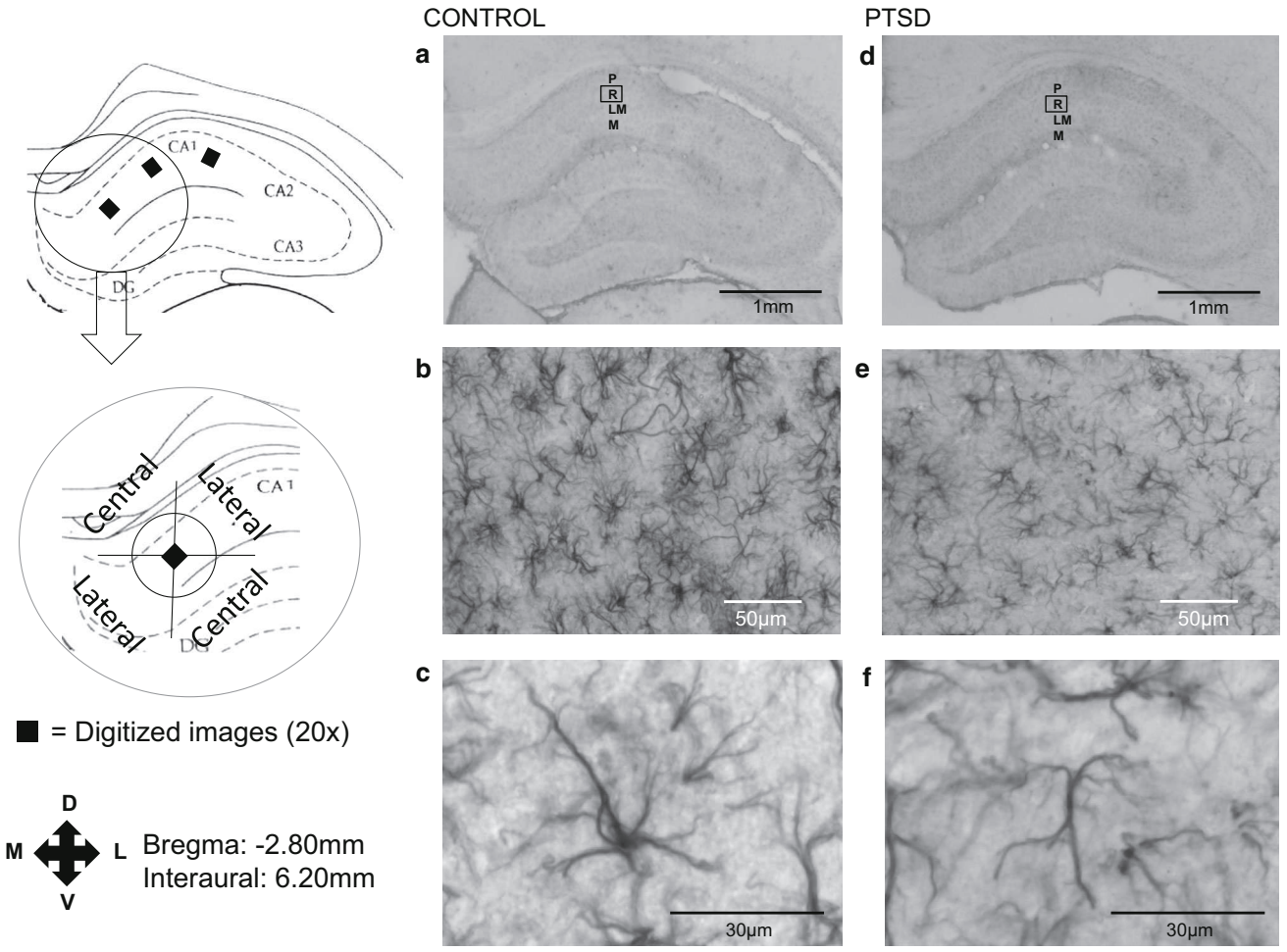


Fig. 2 Digitalized images of the hippocampus after GFAP immunohistochemistry showing the CA1 region. **a**–**c** Control; **d**–**f** PTSD. **a**, **d** Digitalized images at 1×; **b**, **e** digitalized images at 20×; **c**, **f** digitalized images at 40×. *Square* areas of capture at 20×. P = *stratum pyramidale*; R = *stratum radiatum*; LM = *stratum lacunosum moleculare* and M = *stratum moleculare*. The central and lateral quadrants were defined in relation to the *stratum pyramidale*. Adapted from [32]

Morphological Analyses in Hippocampal Astrocytes

Analysis of Astrocytic Branching and Degree of Polarity

In relation to astrocytic branching, the PTSD group presented a decrease in the number of intersections in the lateral quadrants when compared to the control group (Fig. 5c; $p \leq 0.05$). No differences were observed in the central quadrants (Fig. 5b) or in the total (Fig. 5d) number of intersections.

In the analysis of the orientation index, the PTSD group presented a lower orientation index when compared to the control group (control: 0.62 ± 0.14; PTSD: 0.42 ± 0.09; $p < 0.05$).

Analysis of the Number of Primary Processes

Both the number of total primary processes and the number of primary processes in the lateral quadrants were lower in the PTSD group (Fig. 5g; $p \leq 0.01$, Fig. 5f; $p \leq 0.05$, respectively). No difference was observed in the central quadrants (Fig. 5e).

Length of the Primary Processes

There were no differences in the comparison of the length of the longest primary processes (Fig. 5h, i).

Area of the Astrocytic Soma

There were no differences in the comparison of the area of the astrocytic soma (Fig. 5j).

Discussion

In our study, we have demonstrated that exposure to an inescapable footshock, a PTSD protocol for animal models, induces behavioral changes and can alter the density and

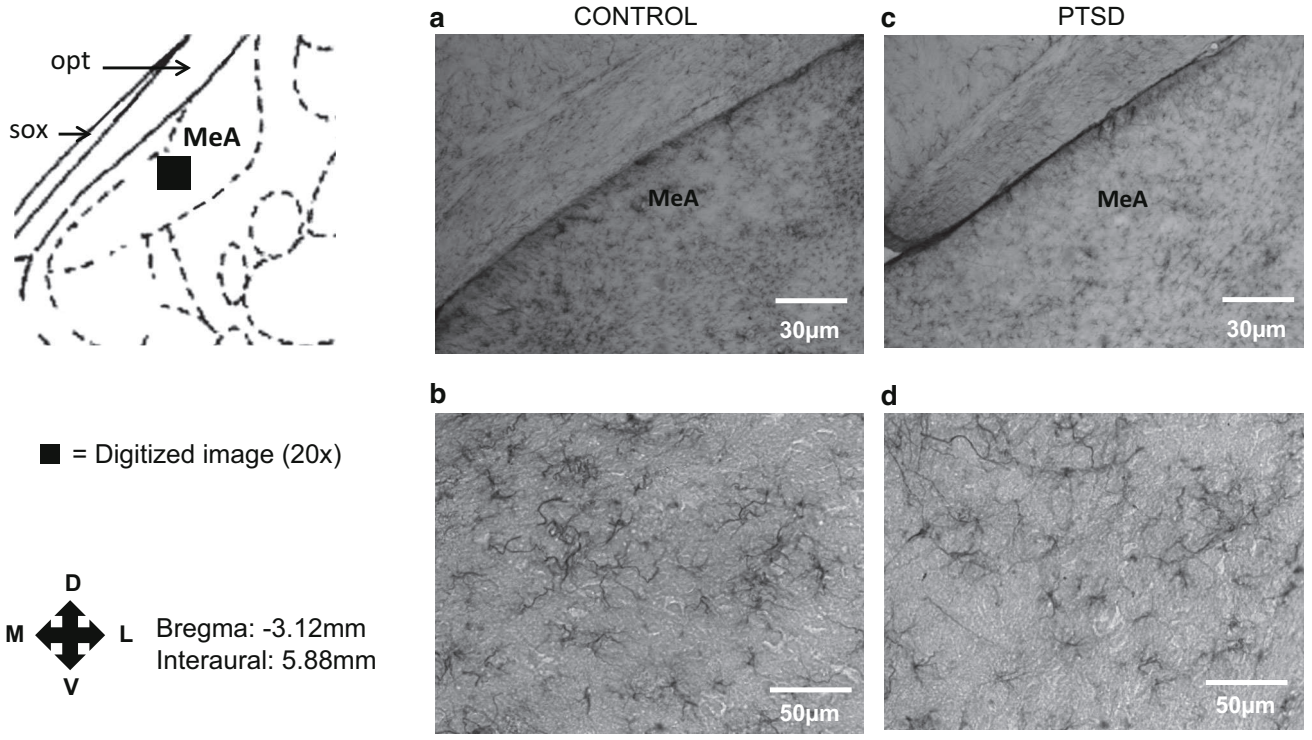


Fig. 3 Digitalized images of the medial amygdala after GFAP immunohistochemistry showing the MeA region. **a**, **b** Control; **c**, **d** PTSD. **a**, **c** Digitalized images at 4×; **b**, **d** digitalized images at 20x; *square* areas of capture at 20×. *MeA* medial amygdala, *opt* optic tract, *sox* supraoptic decussation. Adapted from [32]

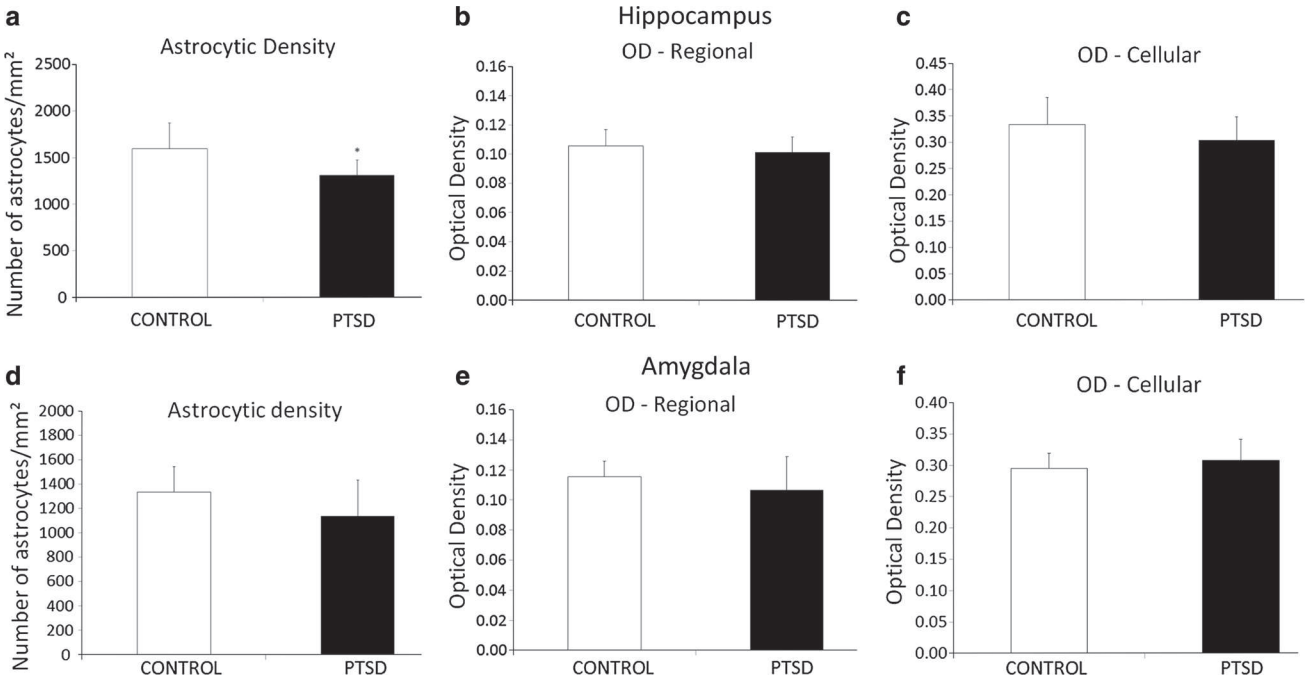


Fig. 4 Effects of a PTSD animal model on astrocytic density and GFAP expression in the hippocampus and medial amygdala. **a**–**c** Control; **d**–**f** PTSD. **a**, **d** Astrocytic density; **b**, **e** regional optical density; and **c**, **f** cellular optical density. *$p < 0.05$; (mean ± SD); *OD* optical density

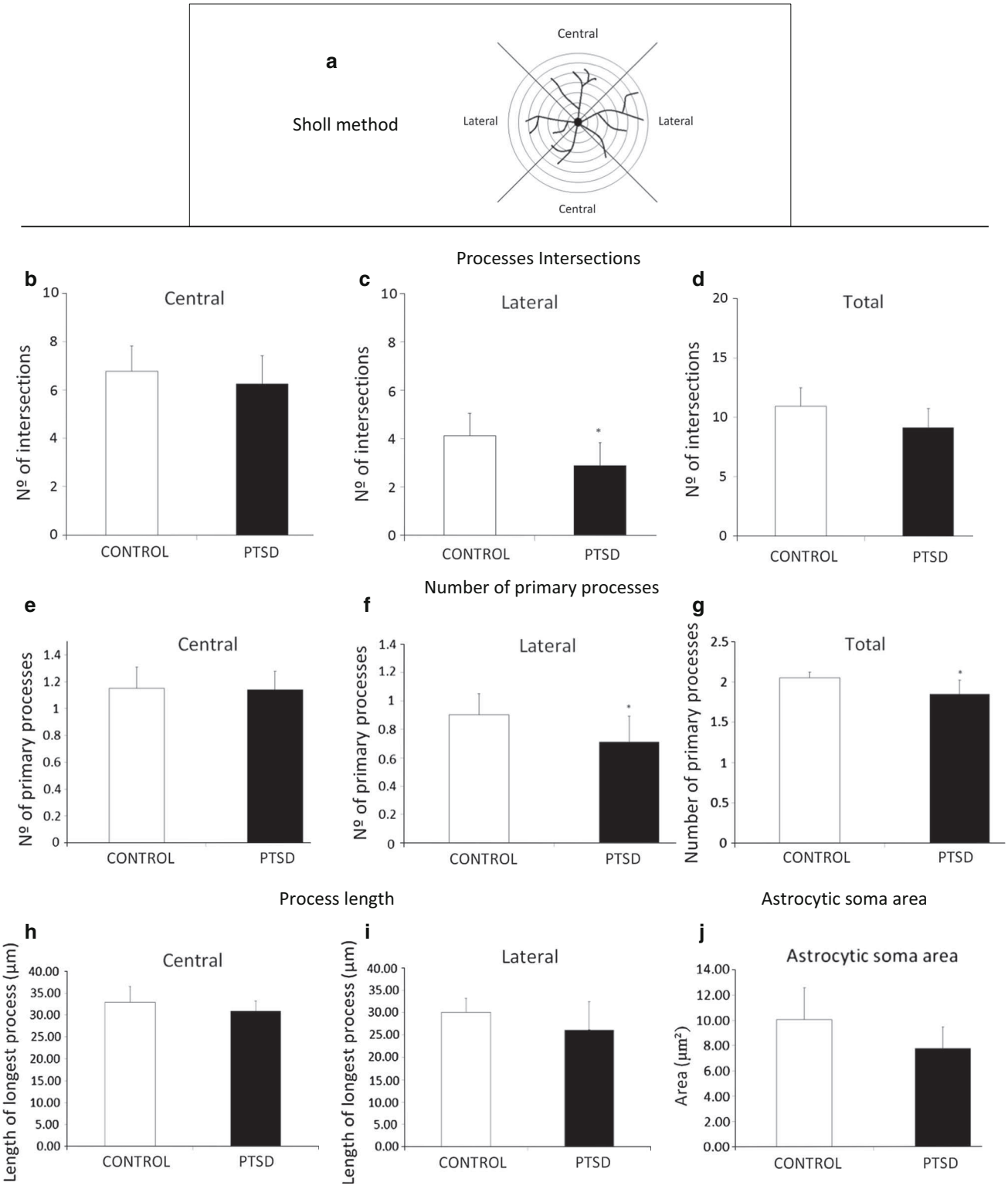


Fig. 5 Morphological analyses. **a** A schematic representation of Sholl's concentric circles method, **b–d** number of intersections with the *circles* in the central and lateral quadrants and total number of intersections, respectively; **e–g** primary processes in the central and lateral quadrants and total number of primary processes, respectively; **h**, **i** length of the longest primary process in the central and lateral quadrants respectively; **j** astrocytic soma area $^{*}p < 0.05$; (mean ± SD)

polarity of GFAP+ astrocytes in the *stratum radiatum* within the CA1 region of the hippocampus.

During the 7 days between exposure to the shock and the SR, we observed no differences in the body weight between the animals from the PTSD and control groups (Fig. 1a). Although some studies have demonstrated weight loss in rats following different stress-inducing situations, such as restraint stress [41–44], chronic cold stress [45], tail shock [42, 46, 47], social defeat [48], and the predator exposure psychosocial stress [49], it was not the case in the present study. The differences between the findings of the present study and those reported in the above-mentioned studies might be related to differences in the type, duration and intensity of the stressors.

We also observed an increase in the production of fecal pellets during the SR in the PTSD group (Fig. 1c). The same result has been described in previous studies involving the use of cold-restraint stress [8], water avoidance stress [9], and restraint stress [41]. The fecal pellet count is not a precise measure of stressor aversiveness. However, combined with the other measures (duration of freezing behavior and weight) it provides a complementary measure [41].

Our study also found an increase in the duration of freezing behavior in the PTSD animals during the SR (Fig. 1b). Freezing behavior has been used and proven to be a useful measure in a number of animal-based PTSD studies such as: footshock [10–12, 50, 51], exposure to the predator [52, 53] single prolonged stress followed by footshock [54, 55], footshock followed by forced swimming [56] and a combination of shock and predator odor [57]. Freezing behavior indicates a sense of immediate threat and intense fear, and the fact that this behavior was remembered 7 days after stress exposure in our study—or more, as previously demonstrated [10, 12, 50, 51]—implies that a memory process related to a contextual stimulus must have occurred.

We also analyzed the relationship between behavioral responses to PTSD and changes in GFAP+ astrocytes from the hippocampus and medial amygdala. The amygdala modulates memory consolidation with the storage of emotionally relevant information and plays a critical role in fear and anxiety [58], while the hippocampus activity has mainly been associated with trauma-related memory [59–62]. Although, hippocampal and amygdalar neuronal changes have been well described in PTSD [63–73], few studies have assessed the astrocytic changes in either humans or animal models of PTSD. While damage to the hippocampus in PTSD is usually related to atrophy and apoptosis [74–78], the amygdala has often been found to be hyper-responsive to trauma or threat-related stimuli in PTSD [68, 69, 71].

In our study, we observed a decrease in astrocytic density in the *stratum radiatum* within the CA1 region of the hippocampus (Fig. 4a). Our results are in accordance with previous studies that have evaluated astroglial changes in animal models of chronic stress and demonstrated a decrease of 25 % in GFAP+ cells in the hippocampus [79]. Moreover, hippocampal atrophy is one of the most common morphological changes observed in patients with PTSD [74–78]. Because astrocytes are the largest population of cells in the hippocampus, it is plausible to suggest this atrophy might be due, at least in part, to the loss of astrocytes observed in our PTSD animal model [80]. This decrease in the number of astrocytes may induce hippocampal functional impairments. Astrocytes are responsible for synthesizing and releasing many of the neurotrophic factors vital for neuronal health, such as brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF) and nerve growth factor (NGF) [81, 82]. These neurotrophic factors regulate neuronal growth and are essential for neural plasticity. Their reduced availability could result in increased cellular vulnerability or even in cell death [79]. Neuronal alterations have also been described in hippocampus. A previous study reported a decrease in dendritic length and dendritic number in neurons from CA1 region and dentate gyrus of the hippocampus in an animal model of PTSD [83]. In other animal stress models, such as restrain stress, decreases in synaptic spine density and dendritic length in CA1 neurons and atrophy of apical dendrites in CA3 neurons of the hippocampus were demonstrated [43, 65]. These results suggest astrocytes and neurons may modify simultaneously in order to better interact and adapt to the stressful condition.

There is evidence indicating different amygdaloid nuclei are critically involved with fear, anxiety, stress responses, and the consolidation of aversive memories [84–87]. Thus, the MeA has been demonstrated to be associated with stressful situations [70, 72]. Despite the changes observed in astrocytic density in the hippocampus, no changes were observed in the MeA (Fig. 4a–c). Although data on astrocytic changes in the amygdala are scarce, some neuronal alterations have been observed in human and animal models of PTSD. The changes in amygdala neurons seem to vary depending on the specific nuclei being analyzed. For example, single prolonged stress (that is another animal model of PTSD) and chronic restraint stress, have been reported to induce a significant increase in dendritic branching, dendritic length and spine density in the basolateral amygdala [68, 69, 71], while a significant reduction in the density of spines was observed in the MeA neurons [70, 72]. However, there is currently no consensus regarding this data because it was previously demonstrated that acute stress produces dendritic debranching and retraction, specifically in the right basolateral amygdala of rats [73]. Volumetric studies of amygdala in patients with

PTSD reveal different results: unchanged [88–91], smaller [92, 93], and larger [94] amygdala volumes in patients with PTSD. While volumetric and neuronal changes observed in the amygdala are still controversial, no changes have been described in amygdala astrocytes in PTSD. In our study, no alteration in GFAP+ astrocytes was detected in the medial amygdala, which may be due to the fact that, at least in MeA, astrocytes are less vulnerable to the effects of PTSD than in the hippocampus. However, it is very difficult to determine the mechanism involved, due to the absence of studies on astrocytic changes in the amygdala in humans and in animal models of PTSD.

In this study, we also observed morphological alterations in the hippocampal GFAP-positive astrocytes analyzed using the Sholl method. We observed that the degree of branching (Fig. 5c) and the number of primary processes (Fig. 5f) in the lateral quadrants were reduced in the PTSD group. Other studies have reported similar findings regarding astrocyte morphology. It was demonstrated that animals subjected to a single prolonged stress, a different model of PTSD, presented thin astrocytic processes in the hippocampus and the anterior cingulate cortex [80]. Furthermore, chronic stress can reduce process length by 40 %, process volume by 56 %, and the number of astrocytic process branches in the prefrontal cortex by 58 % [95]. The astrocytic processes contain the largest concentration of GFAP, and the atrophy observed in these cells is critical since GFAP is an essential cytoskeletal protein for astrocytic remodeling in response to different physiological and pathological situations [95, 96]. While a previous study suggests that PTSD alters the area of astrocytic bodies [80], in our study, we found no such alteration (Fig. 5j), which may be explained by the different PTSD protocols used in each study. Another important point is that the soma area is not a very reliable morphometric parameter, because as astrocytic soma can be sectioned in different regions, the observers may visualize a different sized area depending on the region in which it was sectioned.

A recent study by our group found evidence to support the hypothesis that, like epithelial cells and neurons, hippocampal astrocytes may be polarized [26, 33, 39]. In fact, some studies have demonstrated that astrocytes are endowed with processes that differ regarding structure, function, and membrane molecules, such as aquaporin-4 [24–30]. As mentioned above, in this study, cell polarity is defined as an asymmetric structure of the astrocytic processes along a polarity axis. Therefore, we also analyzed the polarity of astrocytes in the *stratum radiatum* of the hippocampus. We observed that, in animals from the PTSD group, the orientation index decreased in comparison to the control group. This result indicates that astrocytes from the PTSD group changed their polarity and acquired a more fusiform shape.

Most astrocytes in the *stratum radiatum* of the CA1 region of the hippocampus have a fusiform shape, almost perpendicular to the *stratum pyramidale*. This orientation starts to appear about 3 weeks postnatally and remains stable into adulthood [39, 97]. Studies have demonstrated that positive interventions such as physical exercise [33] and environmental enrichment [34] increase the degree of astrocytic branching in the lateral quadrants with astrocytes adopting a more stellate shape. In this study, we observed that astrocytes also changed their morphology in response to an adverse stimulus, but interestingly, in the opposite manner, adopting a more fusiform shape.

Other studies have also demonstrated alterations in the polarity of astrocytes under different interventions. In an in vitro experiment, it was observed that, in the presence of 0.5 mM valproate, astrocytes in the CA1 region were less likely to adopt a fusiform shape [98]. Furthermore, the astrocytic processes were found to be less complex and their number and length were reduced. Astrocytes also became polarized following experimental ischemic and hemorrhagic stroke, where they were found to extend long processes towards the wound and migrate to participate in the formation of the glial scar in the sensorimotor cortex and striatum [36]. Blood vessels also appear to influence astrocytic morphology. In an attempt to reach a vessel, astrocytes can also change the orientation of their processes [97]. Probably, in these studies, as in ours, the distribution of molecules may be anisotropic along the axis and possibly influence the astrocytic morphology.

The pyramidal neurons in the CA1 region project their apical dendrites through the *stratum radiatum* [99]. These dendrites may also alter their morphology, thus causing the astrocytes to adapt and modify their morphology. Therefore, despite not having a clearly polarized morphology, as seen in neurons, where the dendrites are clearly discernable from axons, astrocytes present some degree of assymetry in the *stratum radiatum* of the hippocampus. Like the baso–apical polarity of the epithelial cells and the dendrite–axon polarity of neurons, the polarity of astrocytes could be a key factor in the functionality of the cell.

Conclusions

Our study provides promising new information on the hippocampal astrocytic changes induced by a well-established PTSD animal model. The main findings of our study are that PTSD alters the morphology, changes the polarity and decreases the density of GFAP+ hippocampal astrocytes. However, no changes were observed in the amygdala astrocytes. Therefore, this study greatly reinforces the ongoing hypothesis that hippocampal astrocytes are involved in the pathophysiology of PTSD.

Acknowledgments This research was supported by the Brazilian funding agencies: *Conselho Nacional de Pesquisa e Desenvolvimento* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Apoio à Pesquisa do Estado do Rio Grande do Sul* (FAPERGS). Lisiani Saur was supported by a Doctorate scholarship from CAPES. Dr. Léder Leal Xavier is a CNPq investigator.

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Lapiz-Bluhm MD, Peterson AL (2014) Neurobehavioral mechanism of traumatic stress in post-traumatic stress disorder. Curr Top Behav Neurosci 18:161–190
2. Liberzon I, Khan S, Young EA (2005) Animal models of post-traumatic stress disorder. Handb Stress Brain 15:231–250
3. American PA (2013) Diagnostic and statistical manual of mental disorders: DSM-5, 5th edn. APA, Washington
4. Wilson CB, McLaughlin LD, Ebenezer PJ, Nair AR, Francis J (2014) Valproic acid effects in the hippocampus and prefrontal cortex in an animal model of post-traumatic stress disorder. Behav Brain Res 268:72–80
5. Harris RBS, Palmondon J, Leshin S, Flatt WP, Richard D (2006) Chronic disruption of body weight but not of stress peptides or receptors in rats exposed to repeated restraint stress. Horm Behav 49:615–625
6. Harris RBS, Mitchell TD, Simpson J, Redmann SM Jr, Youngblood BD, Ryan DH (2002) Weight loss in rats exposed to repeated acute restraint stress is independent of energy or leptin status. Am J Physiol Regul Integr Comp Physiol 282:77–88
7. Harris RBS, Zhou J, Youngblood BD, Rybkin II, Smagin GN, Ryan DH (1990) Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets. Am J Physiol 258:329–337
8. Barone FC, Deegan JF, Price WJ, Fowler PJ, Fondacaro JD, Ormsbee HS 3rd (1998) Cold-restraint stress increases rat fecal pellet output and colonic transit. Am J Physiol 275:1928–1938
9. Liu Y, Luo H, Liang C, Xia H, Xu W, Chen J, Chen M (2013) Actions of hydrogen sulfide and ATP-sensitive potassium channels on colonic hypermotility in a rat model of chronic stress. PLoS One 8(2):e55853
10. Diehl LA, Silveira PP, Leite MC, Crema LM, Portella AK, Billodre MN, Nunes E, Henriques TP, Fidelix-da-Silva LB, Heis MD, Gonçalves CA, Quillfeldt JA, Dalmaz C (2007) Long lasting sex-specific effects upon behavior and S100b levels after maternal separation and exposure to a model of post-traumatic stress disorder in rats. Brain Res 1144:107–116
11. Li S, Murakami Y, Wang M, Maeda K, Matsumoto K (2006) The effects of chronic valproate and diazepam in a mouse model of posttraumatic stress disorder. Pharmacol Biochem Behav 85:324–331
12. Siegmund A, Wotjak CT (2007) A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. J Psychiatr Res 41:848–860
13. Yu H, Watt H, Kesavan C, Johnson PJ, Wergedal JE, Mohan S (2012) Lasting consequences of traumatic events on behavioral and skeletal parameters in a mouse model for post-traumatic stress disorder (PTSD). PLoS One 7:e42684
14. Ryoke R, Yamada K, Ichitani Y (2014) Long-term effects of traumatic stress on subsequent contextual fear conditioning in rats. Physiol Behav 129:30–35
15. Juven-Wetzler A, Cohen H, Kaplan Z, Kohen A, Porat O, Zohar J (2014) Immediate ketamine treatment does not prevent post-traumatic stress responses in an animal model for PTSD. Eur Neuropsychopharmacol 24:469–479
16. McEwen BS (2008) Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol 583:174–185
17. Sapolsky RM (2003) Stress and plasticity in the limbic system. Neurochem Res 28:1735–1742
18. Moore H, Rose HJ, Grace AA (2001) Chronic cold stress reduces the spontaneous activity of ventral tegmental dopamine neurons. Neuropsychopharmacology 24:410–419
19. Corral-Frias NS, Lahood RP, Edelman-Vogelsang KE, French ED, Fellous JM (2013) Involvement of the ventral tegmental area in a rodent model of post-traumatic stress disorder. Neuropsychopharmacology 38:350–363
20. Bingham BC, Sheela Rani CS, Frazer A, Strong R, Morilak DA (2013) Exogenous prenatal corticosterone exposure mimics the effects of prenatal stress on adult brain stress response systems and fear extinction behavior. Psychoneuroendocrinology 38:2746–2757
21. Rauch SL, van der Kolk BA, Fisler RE, Alpert NM, Orr SP, Savage CR, Fischman AJ, Jenike MA, Pitman RK (1996) A symptom provocation study of posttraumatic stress disorder using positron emission tomography and script-driven imagery. Arch Gen Psychiatry 53:380–387
22. Shin LM, McNally RJ, Kosslyn SM, Thompson WL, Rauch SL, Alpert NM, Metzger LJ, Lasko NB, Orr SP, Pitman RK (1999) Regional cerebral blood flow during script-driven imagery in childhood sexual abuse-related PTSD: a PET investigation. Am J Psychiatry 156:575–584
23. Huang ZL, Liu R, Bai XY, Zhao G, Song JK, Wu S, Du GH (2014) Protective effects of the novel adenosine derivative WS0701 in a mouse model of posttraumatic stress disorder. Acta Pharmacol Sin 35:24–32
24. Etienne-Manneville S (2008) Polarity proteins in glial cell functions. Curr Opin Neurobiol 18:488–494
25. Anders S, Minge D, Griemsmann S, Herde MK, Steinhäuser C, Henneberger C (2014) Spatial properties of astrocyte gap junction coupling in the rat hippocampus. Philos Trans R Soc Lond B 369:20130600
26. Derouiche A, Pannicke T, Haseleu J, Blaess S, Grosche J, Reichenbach A (2012) Beyond polarity: functional membrane domains in astrocytes and müller cells. Neurochem Res 37:2513–2523
27. Lavi E, Wang Q, Stieber A, Gonatas NK (1994) Polarity of processes with Golgi apparatus in a subpopulation of type I astrocytes. Brain Res 647:273–285
28. Steiner E, Enzmann GU, Lin S, Ghavampour S, Hannocks MJ, Zuber B, Rüegg MA, Sorokin L, Engelhardt B (2012) Loss of astrocyte polarization upon transient focal brain ischemia as a possible mechanism to counteract early edema formation. Glia 60:1646–1659
29. Wolburg H, Noell S, Wolburg-Buchholz K, Mack A, Fallier-Becker P (2009) Agrin, aquaporin-4, and astrocyte polarity as an important feature of the blood-brain barrier. Neuroscientist 15:180–193
30. Yang J, Lunde LK, Nuntagij P, Oguchi T, Camassa LM, Nilsson LN, Lannfelt L, Xu Y, Amiry-Moghaddam M, Ottersen OP, Torp R (2011) Loss of astrocyte polarization in the tg-ArcSwe mouse model of Alzheimer's disease. J Alzheimers Dis 27:711–722
31. Diehl LA, Alvares LO, Noschang C, Engelke D, Andreazza AC, Gonçalves CA, Quillfeldt JA, Dalmaz C (2012) Long-lasting effects of maternal separation on an animal model of post-traumatic stress disorder: effects on memory and hippocampal oxidative stress. Neurochem Res 37:700–707

32. Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. Academic Press, San Diego
33. Saur L, Baptista PP, de Senna PN, Paim MF, Nascimento PD, Ilha J, Bagatini PB, Achaval M, Xavier LL (2014) Physical exercise increases GFAP expression and induces morphological changes in hippocampal astrocytes. Brain Struct Funct 219:293–302
34. Viola GG, Rodrigues L, Américo JC, Hansel G, Vargas RS, Biasibetti R, Swarowsky A, Gonçalves CA, Xavier LL, Achaval M, Souza DO, Amaral OB (2009) Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice. Brain Res 1274:47–54
35. Mestriner RG, Pagnussat AS, Boisserand LS, Valentim L, Netto CA (2011) Skilled reaching training promotes astroglial changes and facilitated sensorimotor recovery after collagenase-induced intracerebral hemorrhage. Exp Neurol 227:53–61
36. Mestriner RG, Saur L, Bagatini PB, Baptista PP, Vaz SP, Ferreira K, Machado SA, Xavier LL, Netto CA (2015) Astrocyte morphology after ischemic and hemorrhagic experimental stroke has no influence on the different recovery patterns. Behav Brain Res 278:257–261
37. Xavier LL, Viola GG, Ferraz AC, Da Cunha C, Deonizio JM, Netto CA, Achaval M (2005) A simple and fast densitometric method for the analysis of tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and in the ventral tegmental area. Brain Res Brain Res Protoc 16:58–64
38. Sholl DA (1953) Dendritic organization in the neurons of the visual and motor cortices of the cat. J Anat 87:387–406
39. Nixdorf-Bergweiler BE, Albrecht B, Heinemann U (1994) Developmental changes in the number, size, and orientation of GFAP-positive cells in the CA1 region of rat hippocampus. Glia 12:180–195
40. Faul F, Erdfelder E, Lang A, Buchner A (2007) GnPower3:a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39:177–191
41. Zhang W, Hetzel A, Shah B, Atchley D, Blume SR, Padival MA, Rosenkranz JA (2014) Greater physiological and behavioral effects of interrupted stress pattern compared to daily restraint stress in rats. PLoS One 9:e102247
42. Jiang X, Zhang ZJ, Zhang S, Gamble EH, Jia M, Ursano RJ, Li H (2011) 5-HT2A receptor antagonism by MDL 11,939 during inescapable stress prevents subsequent exaggeration of acoustic startle response and reduced body weight in rats. J Psychopharmacol 25:289–297
43. Kassem MS, Lagopoulos J, Stait-Gardner T, Price WS, Chohan TW, Arnold JC, Hatton SN, Bennett MR (2013) Stress-induced grey matter loss determined by MRI is primarily due to loss of dendrites and their synapses. Mol Neurobiol 47:645–661
44. Valles A, Marti O, Garcia A, Armario A (2000) Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats. Am J Physiol Regul Integr Comp Physiol 279:1138–1144
45. Roth MK, Bingham B, Shah A, Joshi A, Frazer A, Strong R, Morilak DA (2012) Effects of chronic plus acute prolonged stress on measures of coping style, anxiety, and evoked HPA-axis reactivity. Neuropharmacology 63:1118–1126
46. Ottenweller JE, Servatius RJ, Natelson BH (1994) Repeated stress persistently elevates morning, but not evening, plasma corticosterone levels in male rats. Physiol Behav 55:337–340
47. Jia M, Meng F, Smerin SE, Xing G, Zhang L, Su DM, Benedek D, Ursano R, Su YA, Li H (2012) Biomarkers in an animal model for revealing neural, hematologic, and behavioral correlates of PTSD. J Vis Exp 68:e3361
48. Meerlo P, Overkamp GJ, Daan S, Van Den Hoofdakker RH, Koolhaas JM (1996) Changes in behaviour and body weight following a single or double social defeat in rats. Stress 1:21–32
49. Zoladz PR, Conrad CD, Fleshner M, Diamond DM (2008) Acute episodes of predator exposure in conjunction with chronic social instability as an animal model of post-traumatic stress disorder. Stress 11:259–281
50. Tulogdi A, Sörös P, Tóth M, Nagy R, Biró L, Aliczki M, Klausz B, Mikics E, Haller J (2012) Temporal changes in c-Fos activation patterns induced by conditioned fear. Brain Res Bull 88:359–370
51. Yu H, Watt H, Kesavan C, Johnson PJ, Wergedal JE, Mohan S (2012) Lasting consequences of traumatic events on behavioral and skeletal parameters in a mouse model for post-traumatic stress disorder (PTSD). PLoS One 7:e42684
52. Juven-Wetzler A, Cohen H, Kaplan Z, Kohen A, Porat O, Zohar J (2014) Immediate ketamine treatment does not prevent post-traumatic stress responses in an animal model for PTSD. Eur Neuropsychopharmacol 24:469–479
53. Campos AC, Piorino EM, Ferreira FR, Guimarães FS (2013) Increased nitric oxide-mediated neurotransmission in the medial prefrontal cortex is associated with the long lasting anxiogenic-like effect of predator exposure. Behav Brain Res 256:391–397
54. Mirshekar M, Abrari K, Goudarzi I, Rashidy-Pour A (2013) Systemic administrations of β-estradiol alleviate both conditioned and sensitized fear responses in an ovariectomized rat model of post-traumatic stress disorder. Neurobiol Learn Mem 102:12–19
55. Wang W, Liu Y, Zheng H, Wang HN, Jin X, Chen YC, Zheng LN, Luo XX, Tan QR (2008) A modified single-prolonged stress model for post-traumatic stress disorder. Neurosci Lett 441:237–241
56. Ryoke R, Yamada K, Ichitani Y (2014) Long-term effects of traumatic stress on subsequent contextual fear conditioning in rats. Physiol Behav 129:30–35
57. Hawley WR, Grissom EM, Belkin MN, James TF, Dohanich GP (2013) Decreased sexual motivation and heightened anxiety in male Long-Evans rats are correlated with the memory for a traumatic event. Arch Sex Behav 42:659–668
58. Cui H, Sakamoto H, Higashi S, Kawata M (2008) Effects of single-prolonged stress on neurons and their afferent inputs in the amygdala. Neuroscience 152:703–712
59. Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashan T, Staib LH, Soufer R, Charney DS (2003) Neural correlates of declarative memory for emotionally valenced words in women with posttraumatic stress disorder related to early childhood sexual abuse. Biol Psychiatry 53:879–889
60. Shin LM, Shin PS, Heckers S, Krangel TS, Macklin ML, Orr SP, Lasko N, Segal E, Makris N, Richert K, Levering J, Schacter DL, Alpert NM, Fischman AJ, Pitman RK, Rauch SL (2004) Hippocampal function in posttraumatic stress disorder. Hippocampus 14:292–300
61. Thomaes K, Dorrepaal E, Draijer NP, de Ruiter MB, Elzinga BM, van Balkom AJ, Smoor PL, Smit J, Veltman DJ (2009) Increased activation of the left hippocampus region in Complex PTSD during encoding and recognition of emotional words: a pilot study. Psychiatry Res 171:44–53
62. Brohawn KH, Offringa R, Pfaff DL, Hughes KC, Shin LM (2010) The neural correlates of emotional memory in posttraumatic stress disorder. Biol Psychiatry 68:1023–1030
63. Woolley CS, Gould E, McEwen BS (1990) Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res 531:225–231
64. Watanabe Y, Gould E, McEwen BS (1992) Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res 588:341–345
65. Magariños AM, Verdugo JM, McEwen BS (1997) Chronic stress alters synaptic terminal structure in hippocampus. Proc Natl Acad Sci USA 94:14002–14008

66. Uno H, Lohmiller L, Thieme C, Kemnitz JW, Engle MJ, Roecker EB, Farrell PM (1990) Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I. Hippocampus. Brain Res Dev Brain Res 53:157–167
67. Gould E, Tanapat P, McEwen BS, Flügge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci USA 95:3168–3171
68. Cohen H, Kozlovsky N, Matar MA, Zohar J, Kaplan Z (2014) Distinctive hippocampal and amygdalar cytoarchitectural changes underlie specific patterns of behavioral disruption following stress exposure in an animal model of PTSD. Eur Neuropsychopharmacol 24:1925–1944
69. Cui H, Sakamoto H, Higashi S, Kawata M (2008) Effects of single-prolonged stress on neurons and their afferent inputs in the amygdala. Neuroscience 152:703–712
70. Bennur S, Shankaranarayana Rao BS, Pawlak R, Strickland S, McEwen BS, Chattarji S (2007) Stress-induced spine loss in the medial amygdala is mediated by tissue-plasminogen activator. Neuroscience 144:8–16
71. Lucassen PJ, Pruessner J, Sousa N, Almeida OF, Van Dam AM, Rajkowska G, Swaab DF, Czéh B (2014) Neuropathology of stress. Acta Neuropathol 127:109–135
72. Marcuzzo S, Dall'oglio A, Ribeiro MF, Achaval M, Rasia-Filho AA (2007) Dendritic spines in the posterodorsal medial amygdala after restraint stress and ageing in rats. Neurosci Lett 424:16–21
73. Maroun M, Ioannides PJ, Bergman KL, Kavushansky A, Holmes A, Wellman CL (2013) Fear extinction deficits following acute stress associate with increased spine density and dendritic retraction in basolateral amygdala neurons. Eur J Neurosci 38:2611–2620
74. Kitayama N, Vaccarino V, Kutner M, Weiss P, Bremner JD (2005) Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. J Affect Disord 88:79–86
75. Lindauer RJ, Olff M, van Meijel EP, Carlier IV, Gersons BP (2006) Cortisol, learning, memory, and attention in relation to smaller hippocampal volume in police officers with posttraumatic stress disorder. Biol Psychiatry 59:171–177
76. Bremner JD, Elzinga B, Schmahl C, Vermetten E (2008) Structural and functional plasticity of the human brain in posttraumatic stress disorder. Prog Brain Res 167:171–186
77. Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB (1995) MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 152:973–981
78. Shin LM, Shin PS, Heckers S, Krangel TS, Macklin ML, Orr SP, Lasko N, Segal E, Makris N, Richert K, Levering J, Schacter DL, Alpert NM, Fischman AJ, Pitman RK, Rauch SL (2004) Hippocampal function in posttraumatic stress disorder. Hippocampus 14:292–300
79. Czéh B, Simon M, Schmelting B, Hiemke C, Fuchs E (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31:1616–1626
80. Xia L, Zhai M, Wang L, Miao D, Zhu X, Wang W (2013) FGF2 blocks PTSD symptoms via an astrocyte-based mechanism. Behav Brain Res 256:472–480
81. Friedman WJ, Black IB, Kaplan DR (1998) Distribution of the neurotrophins brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 in the postnatal rat brain: an immunocytochemical study. Neuroscience 84:101–114
82. Althaus HH, Richter-Landsberg C (2000) Glial cells as targets and producers of neurotrophins. Int Rev Cytol 197:203–277
83. Cohen H, Kozlovsky N, Matar MA, Zohar J, Kaplan Z (2014) Distinctive hippocampal and amygdalar cytoarchitectural changes underlie specific patterns of behavioral disruption following stress exposure in an animal model of PTSD. Eur Neuropsychopharmacol 24:1925–1944
84. Carter RN, Pinnock SB, Herbert J (2004) Does the amygdala modulate adaptation to repeated stress? Neuroscience 126:9–19
85. Davis M (2006) Neural systems involved in fear and anxiety measured with fear-potentiated startle. Am Psychol 61:741–756
86. Ebner K, Rupniak NM, Saria A, Singewald N (2004) Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxietyrelated behavior in rats. Proc Natl Acad Sci USA 101:4280–4285
87. Roozendaal B, McGaugh JL (1996) Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. Neurobiol Learn Mem 65:1–8
88. Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, Capelli S, McCarthy G, Innis RB, Charney DS (1997) Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse—a preliminary report. Biol Psychiatry 41:23–32
89. Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, den Heeten GJ, Gersons BP (2004) Smaller hippocampal volume in Dutch police officers with posttraumatic stress disorder. Biol Psychiatry 56:356–363
90. Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, Den Heeten GJ, Gersons BP (2005) Effects of psychotherapy on hippocampal volume in out-patients with post-traumatic stress disorder: a MRI investigation. Psychol Med 35:1421–1431
91. Schmahl C, Berne K, Krause A, Kleindienst N, Valerius G, Vermetten E, Bohus M (2009) Hippocampus and amygdala volumes in patients with borderline personality disorder with or without posttraumatic stress disorder. J Psychiatry Neurosci 34:289–295
92. Weniger G, Lange C, Sachsse U, Irle E (2008) Amygdala and hippocampal volumes and cognition in adult survivors of childhood abuse with dissociative disorders. Acta Psychiatr Scand 118:281–290
93. Morey RA, Gold AL, LaBar KS, Beall SK, Brown VM, Haswell CC, Nasser JD, Wagner HR, McCarthy G (2012) Amygdala volume changes in posttraumatic stress disorder in a large case-controlled veterans group. Arch Gen Psychiatry 69:1169–1178
94. Kuo JR, Kaloupek DG, Woodward SH (2012) Amygdala volume in combat-exposed veterans with and without posttraumatic stress disorder: a cross-sectional study. Arch Gen Psychiatry 69:1080–1086
95. Tynan RJ, Beynon SB, Hinwood M, Johnson SJ, Nilsson M, Woods JJ, Walker FR (2013) Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. Acta Neuropathol 126:75–91
96. Allen NJ, Barres BA (2005) Signaling between glia and neurons: focus on synaptic plasticity. Curr Opin Neurobiol 15:542–548
97. Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 *stratum radiatum* occupy separate anatomical domains. J Neurosci 22:183–192
98. Fennrich S, Ray D, Nau H, Schlosshauer B (1998) Radial astrocytes: toxic effects induced by antiepileptic drug in the developing rat hippocampus in vitro. Eur J Cell Biol 77:142–150
99. Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J (2007) The hippocampus book. Oxford University Press, New York

Capítulo III

"Ketamine promotes increased freezing behavior in rats with experimental PTSD without changes in brain glucose metabolism (^{18}F-FDG) or BDNF"

Artigo submetido ao periódico *Neuroscience Letters*.

Elsevier Editorial System(tm) for Neuroscience Letters

Manuscript Draft

Manuscript Number:

Title: Ketamine promotes increased freezing behavior in rats with experimental PTSD without changes in brain glucose metabolism (18F-FDG) or BDNF

Article Type: Research paper

Keywords: PTSD; frontal cortex; hippocampus; amygdala; microPET; BDNF

Corresponding Author: Miss Lisiani Saur, MSc.

Corresponding Author's Institution: Pontifícia Universidade Católica do Rio Grande do Sul

First Author: Lisiani Saur, MSc.

Order of Authors: Lisiani Saur, MSc.; Laura T Neves; Samuel Greggio, Dr.; Gianina T Venturin, Dr.; Cristina M Jeckel, Dr.; Jaderson C Da Costa, Dr.; Karine Bertoldi; Bruna Schallenberger; Ionara R Siqueira, Dr.; Leder L Xavier, Dr.

Abstract: Post-traumatic stress disorder (PTSD) is a psychiatric condition caused by exposure to a traumatic event. However, not all individuals exposed to severe traumatic experiences develop PTSD. Ketamine has recently been reported to be efficacious in depression and related psychological disorders. However, since ketamine produces transient dissociation and psychotic state, there is concern that this drug may be correlated with sustained symptoms of PTSD. In this study, we employed inescapable footshock as an animal model of PTSD and classified the animals according to the degree of their individual behavioral responses as either an "extreme behavioral response" (EBR) or "minimal behavioral response" (MBR). The study goals were to evaluate the glucose metabolism with micoPET and quantify BDNF protein in the hippocampus, frontal cortex, and amygdala, which are regions that have important roles in the neuropathology of PTSD. Sixty male rats were initially divided into four groups: Control+Saline (CTRL+SAL), Control+Ketamine (CTRL+KET), PTSD+Saline (PTSD+SAL) and PTSD+Ketamine (PTSD+KET). Our results show that animals classified as EBR exhibited increased freezing behavior and that ketamine treatment was associated with greater severity of freezing behavior in PTSD animals. The analysis of the glucose metabolism and the BDNF protein levels showed no significant differences. These results suggest that ketamine might aggravate PTSD symptoms and that this effect is unrelated to alterations in glucose metabolism or BDNF protein levels.

Suggested Reviewers: Israel Liberzon
liberzon@med.umich.edu

Ronald Kessler
kessler@hcp.med.harvard.edu

Rachel Yehuda
rachel.yehuda@med.va.gov

Joseph Zohar
joseph.zohar@sheba.health.gov.il

Eva Irle
eirle@gwdg.de

Michael Schönenberg
michael.schoenenberg@uni-tuebingen.de

Hagit Cohen
hagitc@bgu.ac.il

Ann Rasmusson
ann.rasmusson@yale.edu

Cover Letter

December 14th, 2016

S.G. Waxman
Editor-in-Chief
Neuroscience Letters

Dear Dr. S.G. Waxman

Please find attached the article entitled "Ketamine promotes increased freezing behavior in rats with experimental PTSD without changes in brain glucose metabolism (^{18}F-FDG) or BDNF", whose authors are: Lisiani Saur, Laura Tartari Neves, Samuel Greggio, Gianina Teribele Venturin, Cristina Maria Moriguchi Jeckel, Jaderson Costa DaCosta, Karine Bertoldi, Bruna Schallenberger, Ionara Rodrigues Siqueira, Léder Leal Xavier. This article is a significantly revised resubmission of the previously rejected paper NSL-161962.

Recent studies have highlighted the role of ketamine in the treatment of some neuropsychiatric diseases, such as depression. However, little has been published about PTSD and ketamine. Our study provides new information regarding the effects of ketamine on PTSD treatment and we also analyze the BDNF protein and glucose metabolism in the hippocampus, frontal cortex and amygdala.

We would greatly appreciate it if you would consider this paper for publication in Neuroscience Letters.

All the authors have read, approved the manuscript, and agreed to have their names listed as authors and have no conflicts to disclose. This research has not been previously published, nor is under consideration by another journal.

Thank you for your attention
We are looking forward to hearing from you.
Sincerely yours,

Lisiani Saur, MSc.
Laboratório de Biologia Celular e Tecidual
Departamento de Ciências Morfofisiológicas
Faculdade de Biociências
PUCRS
Avenida Ipiranga, 6681 Prédio 12 Sala 144
Porto Alegre, RS, Brazil
E-mail: lisiani.saur@acad.pucrs.br
Telephone: (55) (51) 33203545

***Highlights**

Highlights

1) Ketamine increases freezing behavior in rats with experimental PTSD
2) PTSD and ketamine treatment do not alter BDNF protein levels in rat brain
3) PTSD and ketamine treatment do not alter glucose metabolism in rat brain

Ketamine promotes increased freezing behavior in rats with experimental PTSD without changes in brain glucose metabolism (^{18}F-FDG) or BDNF

Lisiani Saur[a,*], Laura Tartari Neves[a], Samuel Greggio[b], Gianina Teribele Venturin[b], Cristina Maria Moriguchi Jeckel[b], Jaderson Costa Da Costa[b], Karine Bertoldi[c], Bruna Schallenberger[c], Ionara Rodrigues Siqueira[c], Léder Leal Xavier[a]

[a]Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Faculdade de Biociências. Pontifícia Universidade Católica do Rio Grande do Sul. Avenida Ipiranga, 6681, 90619-900, Porto Alegre, RS, Brasil.

[b]Centro de Pesquisa Pré-Clínica, Instituto do Cérebro do Rio Grande do Sul - Brain Institute (BraIns); Laboratório de Neurociências, Instituto do Cérebro do Rio Grande do Sul - Brain Institute (BraIns), Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brasil.

[c]Departamento de Farmacologia, Instituto de Ciências Básicas da Saúde (ICBS). Universidade Federal do Rio Grande do Sul. Rua Sarmento Leite 500, Porto Alegre, RS, Brasil.

*Corresponding author: Lisiani Saur, MSc.

E-mail address: lisiani.saur@acad.pucrs.br

Phone: 55 51 33203545 Fax: 55 51 33203612

Address: Departamento de Ciências Morfofisiológicas, Laboratório de Biologia Celular e Tecidual, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS). Av. Ipiranga 6681, Prédio 12C, Sala 104. CEP 90619-900, Porto Alegre, RS, Brazil.

ABSTRACT

Post-traumatic stress disorder (PTSD) is a psychiatric condition caused by exposure to a traumatic event. However, not all individuals exposed to severe traumatic experiences develop PTSD. Ketamine has recently been reported to be efficacious in depression and related psychological disorders. However, since ketamine produces transient dissociation and psychotic state, there is concern that this drug may be correlated with sustained symptoms of PTSD. In this study, we employed inescapable footshock as an animal model of PTSD and classified the animals according to the degree of their individual behavioral responses as either an “extreme behavioral response” (EBR) or “minimal behavioral response” (MBR). The study goals were to evaluate the glucose metabolism with micoPET and quantify BDNF protein in the hippocampus, frontal cortex, and amygdala, which are regions that have important roles in the neuropathology of PTSD. Sixty male rats were initially divided into four groups: Control+Saline (CTRL+SAL), Control+Ketamine (CTRL+KET), PTSD+Saline (PTSD+SAL) and PTSD+Ketamine (PTSD+KET). Our results show that animals classified as EBR exhibited increased freezing behavior and that ketamine treatment was associated with greater severity of freezing behavior in PTSD animals. The analysis of the glucose metabolism and the BDNF protein levels showed no significant differences. These results suggest that ketamine might aggravate PTSD symptoms and that this effect is unrelated to alterations in glucose metabolism or BDNF protein levels.

Keywords: PTSD; frontal cortex; hippocampus; amygdala; microPET; BDNF

INTRODUCTION

The Diagnostic and Statistical Manual for Mental Disorders (DSM-5) listed PTSD as a condition related to exposure to a traumatic experience. A traumatic experience is characterized by its ability to induce helplessness, horror and/or fear. Generally, it happens after a life-threatening event, loss of physical integrity or serious injury [1,2]. Some of the better known psychophysiological symptoms of PTSD include exaggerated startle, impaired sleep, intrusive memories or flashbacks and the persistent avoidance of trauma associated situations/stimuli [2]. Not all individuals confronted with severe traumatic events develop PTSD. Data on the PTSD prevalence in the general population vary from 3 to 8% [3,4]. However, Kessler *et al.*, [5] reported that 50% of women and 60% of men will have a traumatic experience at some point of their lives. Therefore, this pathology affects a subpopulation of vulnerable individuals exposed to a traumatic experience that exceeds their capacity to cope [1,2]. Like humans, animals show individual differences in their susceptibility to traumatic stress. Some animals exhibit shorter duration reactions that do not induce prolonged stress responses and some, in spite of being submitted to similar stress situations, exhibit an exacerbated stress response [6].

Ketamine is a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor for glutamate [7]. It has been used clinically since the 1960s as a dissociative anesthetic agent, although its usage has largely been discontinued due to undesired psychic effects (perceptual alterations such as dissociative experiences) occurring in approximately 12% of patients [8].

However, more recently, ketamine has been shown to produce rapid antidepressant effects as well as a decrease in suicidal ideation following only a single sub-anesthetic dose in depressed patients [9,10]. In PTSD patients, the results obtained with ketamine are controversial. Some studies have demonstrated positive effects [11-14], while others have demonstrated negative effects [7,15-17] of ketamine treatment. However, in most cases, ketamine was administered concomitantly with other medications (benzodiazepines, for example). Therefore, it is important to undertake pre-clinical studies to evaluate the independent effects of ketamine.

In this study, we employed the inescapable footshock protocol as an experimental model of PTSD and the population of stress exposed rodents was classified in accordance with the level of their individual behavioral response [6]. We classified the PTSD animals into two distinct groups: “extreme behavioral response” (EBR) and “minimal behavioral response” (MBR). The goals of this study were to evaluate the ^{18}F-2-fluoro-2-deoxy-glucose (^{18}F-FDG) uptake and quantify brain derived neurotrophic factor (BDNF) protein in the frontal cortex, hippocampus and amygdala, three important regions involved in the stress response.

EXPERIMENTAL PROCEDURES

Animals

All procedures were previously approved by the university’s ethical committee (CEUA 13/00350-PUCRS) and were conducted in accordance with

the University's guideline. Sixty male Wistar rats, 12 weeks old, were obtained from the *Centro de Modelos Biológicos Experimentais (CeMBE)* of the *Pontifícia Universidade Católica do Rio Grande do Sul.* The rats were maintained in standard laboratory conditions with freely available food and water*,* and 12:12h dark/light cycle. In the first part of this study, animals were divided into four groups of 15 animals each: 1-Control+Saline (CTRL+SAL); 2-Control+Ketamine (CTRL+KET); 3-PTSD+Saline (PTSD+SAL); 4-PTSD+Ketamine (PTSD+KET). Then, after the situational reminder test, the animals from groups 3 and 4 were divided into EBR or MBR according to the behavioral criterion. In the PTSD+SAL group, eight animals were classified as EBR and seven animals were MBR and in the PTSD+KET group, ten animals were classified as EBR and five animals as MBR.

PTSD experimental model

In this experimental model, the animals are subjected to a single footshock without the possibility of escape [1]. The apparatus consists of a 50x25x25 cm box separated into two compartments by a removable door. To induce PTSD, the animals were individually positioned in the first compartment, which has a wooden floor. After two minutes, the door was opened and we waited until the animal crossed to the second compartment, in which the floor is a bronze grid. At that moment, the door was closed and then a 1mA 60Hz footshock was delivered for 20 seconds. The animals in the control groups were subjected to the same procedure, but no shock was applied.

Drug Administration

A single intraperitoneal injection was applied on the night of the 6th day of the experiment (Fig. 1). The ketamine group animals received 10 mg/kg of ketamine (Cristália, Brazil), while the saline group animals received 0.5 ml of saline solution. This ketamine dose has demonstrated positive effects in the treatment of depression [18,19].

Situational reminder (SR)

One week after the initial footshock protocol (7th day of the experiment – Fig. 1), the animals were submitted to the SR exposure. The animals were positioned in the first compartment of the apparatus for two minutes, but the door dividing the compartments remained closed. In this behavioral test, two experienced researchers evaluated the length of the freezing bout (the average of both time periods provided the value for each animal), which is a measure of conditioned fear.

Cut-off behavioral criterion

Individual animals from the PTSD+SAL and PTSD+KET groups were classified as having “extreme” or “minimal” behavioral responses according to a pre-established criterion. Here, the average length of the freezing bout of all the animals in the PTSD+SAL group (mean=14.23 seconds) was used as the cut-

off criterion: animals that froze for over 14.23 seconds were classified as EBR and animals that froze for less than 14.23 seconds were classified as MBR.

^{18}F-FDG MicroPET Scan

Only animals that were classified as EBR in the PTSD+SAL and PTSD+KET groups were submitted to the microPET scanning procedure (5 per group). On day 8 (Fig. 1), the animals received an intravenous injection of 1 mCi of ^{18}F-FDG, and were scanned 40 minutes after conscious tracer uptake. List mode static acquisitions were acquired for 30 minutes using a TriumphTM microPET system (LabPET-4, TriFoil Imaging, Northridge, CA, USA). During the scan, the animals were kept under inhalatory anesthesia (induction at 3–4% isoflurane and medical oxygen, and 2–3% for maintenance dose), with body temperature maintained at 36ºC. The field of view (FOV; 3.75cm) was centered on the rat's head. Data were reconstructed using the 3D-MLEM algorithm (3D maximum likelihood estimation method) with 20 iterations. The images were not corrected for attenuation. The Fusion Toolbox (PMOD v3.5, PMOD Technologies, Zurich, Switzerland) was used to spatially normalize the microPET images into an ^{18}F-FDG template. An MRI rat brain VOI template, previously coregistered to the microPET image database, was used to overlay the normalized images [20]. The ^{18}F-FDG uptake in the frontal cortex, hippocampus and amygdala were expressed as standard uptake values (SUVs).

Sample extraction and BDNF determination

For the BDNF analysis, on day 9 of the experiments (Fig. 1), the animals were decapitated without anesthesia. The rat brains were immediately removed and washed in saline solution. The frontal cortex and hippocampus were dissected out, placed in liquid nitrogen and stored at -80ºC until used. For technical reasons, the amygdala was not dissected. The BDNF concentration was determined by ELISA according to the manufacturer's instructions (Millipore, Sandwich ELISA Kit, ChemiKine^{TM}).

Data analysis

The data were analyzed using one-way ANOVA and Pearson's correlation was used to correlate the data from both observers during the SR. We used the SPSS 11.0 software (Statistical Package for the Social Sciences, USA) and the results are shown as mean±SE. Significance was set at $p \leq 0.05$.

RESULTS

Freezing Behavior

Freezing behavior during the SR is shown in Fig. 2. The analysis of Pearson's correlation coefficient revealed a strong correlation between the observers ($r = 0.981$; $p<0.001$). The statistical analysis showed that the EBR animals (from both the PTSD+SAL and PTSD+KET groups) exhibited longer

freezing bouts when compared with all the other groups. Moreover, animals from the PTSD+KET(EBR) group exhibited longer freezing bouts when compared with animals from the PTSD+SAL(EBR) group.

^{18}F-FDG-MicroPET

The glucose metabolism induced by PTSD, as well as the effect of ketamine treatment are shown in Fig. 3. The ^{18}F-FDG SUVs showed no significant statistical differences between the groups in the frontal cortex (Fig. 3a and 3b), hippocampus (Fig. 3d and 3e) or amygdala (Fig 3g and 3h).

BDNF protein levels

The effects of PTSD and Ketamine treatment on BDNF protein levels are shown in Fig. 3. No significant differences were observed between the groups in the frontal cortex (Fig. 3c) or hippocampus (Fig. 3f).

DISCUSSION

In this study, we observed that an inescapable footshock protocol as an experimental model of PTSD and treatment with ketamine did not alter the resting glucose metabolism or BDNF protein in the frontal cortex, hippocampus or amygdala of Wistar rats. Moreover, we noted that ketamine treatment increased freezing behavior in the EBR animals.

Our results demonstrate that, as observed in humans, animals show consistent individual differences in their behavioral and physiological response patterns to environmental demands. In freezing behavior, we observed that the PTSD animals classified as MBR presented similar responses to the CTRL+SAL group (Fig. 2). As observed in other studies, not all stressed animals responded similarly. Some remained unaffected, showing little fear sensitization [21,22]. Even in highly inbred laboratory strains, genetically identical subjects may be considerably more or less susceptible to similar experimental manipulations [23]. The reasons for these individual differences remain largely unknown, although individual neurobiology and past experiences are considered a major risk factor for the development of the disease.

Unlike the MBR animals, the EBR animals (both PTSD+SAL and PTSD+KET) showed longer freezing behavior during the SR when compared to all other groups (Fig. 2). Freezing behavior is used as measure of conditioned fear and indicates a sense of intense horror or immediate threat. This behavior suggests the animals developed long-lasting anxiety, which is one of the main features of PTSD, because it was remembered 7 days after the exposure to the footshock. Moreover, the animals from the PTSD+KET group exhibited longer freezing bouts when compared to the PTSD+SAL group. This data demonstrates that, at least in animals exposed to stress, ketamine worsens anxiety-related behavior. The same result has been described in an experimental PTSD study involving predator-scent stress, which showed that exposed rats treated with 3 different doses of ketamine (0.5, 5 and 15mg/kg administered one hour following stress exposure) exhibited a significant increase in freezing behavior [7]. A human study demonstrated that 15 trauma-

exposed burn subjects who received ketamine/midazolam as an analgesic/sedative treatment presented significantly more severe PTSD symptoms than subjects who were not given the treatment [15]. Similar results have been described in previous studies involving victims of moderate accidents. Patients who received ketamine during their initial emergency treatment showed an increase in reexperiencing symptoms, elevated dissociative symptoms and heightened avoidance when compared to patients who received opioid medication during their initial treatment [16,17]. Ketamine is an anesthetic administered especially in military hospitals to burns patients. This drug is associated with psychosis and dissociation, leading to the concern that it may increase the rates of PTSD development [24]. Moreover, some authors suggest that antagonizing the NMDA receptor increases the vulnerability of stressed patients to develop PTSD, because clinical studies propose that NMDA antagonists may transiently stimulate cortico-limbic glutamate release and produce symptoms resembling dissociative states [15,25]. However, there is no consensus regarding these data, because studies conducted in humans and animals have demonstrated beneficial effects of ketamine in the treatment of PTSD symptoms [11-14].

Because the frontal cortex, hippocampus and amygdala are the three major regions involved in the stress response, we decided to analyze the glucose metabolism and BDNF levels in these regions. BDNF is an important neurotrophin involved in the development, growth, function and maintenance of several neuronal systems [26]. Thus, BDNF is involved in synaptic plasticity, a process necessary for long-term learning and memory, mainly in the hippocampus, amygdala and prefrontal cortex for the formation of emotional

memories, including fear memories [26]. Recent evidence suggests that BDNF might mediate the pathophysiology of mood disorders, including PTSD [14]. In our study, we found no change in BDNF expression in any of the analyzed regions nine days after the stress exposure. Our results are in accordance with those reported by previous studies that analyzed the effects of PTSD on long- and short-term BDNF mRNA expression, which showed alterations in BDNF levels are transient. Rasmusson *et al.*, [27], demonstrated a decrease in BDNF mRNA levels in the *dentate gyrus,* 60 min after exposure to footshock stress. However, the effect proved to be transient, since BDNF mRNA levels returned to normal within 48h. Moreover, another PTSD animal model demonstrated a persistent (30 days) decrease in BDNF mRNA expression only in the CA1 region, while changes in other brain areas (CA3, dentate gyrus and frontal cortex) were transient [28]. In the same way, a maternal separation paradigm found a transient increase in BDNF expression in the prefrontal cortex and hippocampus when measured at post-natal day 17, which was not observed at post-natal day 35 [29]. Another study demonstrated that inescapable tailshock increased BDNF mRNA levels only in males and only immediately after the stress session [30]. A study involving PTSD patients found higher BDNF serum levels in the PTSD group. However, that study also showed that patients who were subjected to trauma exposure in the previous year maintained that difference, while those patients with more remote trauma (more than 1 year) did not [31]. A study that evaluated individuals who did or did not develop PTSD following a sexual trauma observed no difference between patients with and without PTSD in terms of BDNF serum levels [32] and the only study exploring BDNF levels in the cerebrospinal fluid of PTSD patients found no difference

when compared with controls [33]. In our study, we evaluated the BDNF levels 9 days after the initial stress. Therefore, it is possible to speculate that our animals presented changes in BDNF expression. However, those changes were restricted to an hour or a few days and there were no apparent long-term changes. Taken together, the above findings suggest that stress events may dysregulate BDNF signaling, even transiently, interfering with the normal functioning of the brain and contributing to the potential development of PTSD.

The brain glucose metabolism was analyzed using the ^{18}F-FDG-microPET technique, which measures the glucose uptake in tissues. In our study, no apparent alterations were observed in the frontal cortex, hippocampus or amygdala. Extant data demonstrating ^{18}F-FDG-microPET or even cerebral blood flow (CBF) abnormalities in PTSD patients/animal models are limited and conflicting. For example: increased [34-37], decreased [34,38,39] and unchanged [40,41] glucose metabolism and CBF have been reported in different nuclei within the amygdala. Similar conflicting results have also been found in different areas of the PFC, with increased [37,42] and decreased [37,39-42] glucose metabolism and CBF being reported. The only consensus seems to be with respect to the hippocampus, in which only decreases in glucose metabolism have been reported [34,39,40]. These contradictory results can be attributed to many factors, such as differences in time since the onset of PTSD, heterogeneity of trauma exposure and differences in the paradigms in which the analyses were performed (resting, sleep, trauma-related, trauma-unrelated and neutral scripts). Therefore, it seems that due to PTSD's complex clinical presentation and quite variable symptomatology, it is difficult to establish consistent data about ^{18}F-FDG in this neuropsychiatric condition.

The main findings of this study are that ketamine treatment increases freezing behavior in PTSD animals. However, this alteration is not related to changes in glucose metabolism or BDNF levels in the hippocampus, frontal cortex or amygdala.

ACKNOWLEDGEMENTS

This research was supported by the Brazilian funding agencies: *Conselho Nacional de Pesquisa e Desenvolvimento* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Apoio à Pesquisa do Estado do Rio Grande do Sul* (FAPERGS). Lisiani Saur was supported by a Doctorate scholarship from CAPES. Dr. Léder Leal Xavier, Dr. Jaderson Costa Da Costa and Dr. Ionara Rodrigues Siqueira are CNPq investigators.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

[1] Diehl LA, Silveira PP, Leite MC, Crema LM, Portella AK, Billodre MN, Nunes E, Henriques TP, Fidelix-da-Silva LB, Heis MD, Gonc¸alves CA, Quillfeldt JA, Dalmaz C (2007) Long lasting sex-specific effects upon behavior and S100b levels after maternal separation and exposure to a model of post-traumatic stress disorder in rats. Brain Res 1144:107–116

[2] Liberzon I, Khan S, Young EA (2005) Animal models of posttraumatic stress disorder. Handb Stress Brain 15:231–250

[3] Bisson JI, Cosgrove S, Lewis C, Robert NP (2015) Post-traumatic stress disorder. BMJ 26;351:h6161

[4] Yehuda R (2002) Post-traumatic stress disorder. N Engl J Med 346(2):108-14

[5] Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB (1995) Posttraumatic stress disorder in the National Comorbidity Survey. Arch Gen Psychiatry 52(12):1048-1060

[6] Cohen H, Kozlovsky N, Matar MA, Zohar J, Kaplan Z (2014) Distinctive hippocampal and amygdalar cytoarchitectural changes underlie specific patterns of behavioral disruption following stress exposure in an animal model of PTSD. Eur Neuropsychopharmacol 24:1925–1944

[7] Juven-Wetzler A, Cohen H, Kaplan Z, Kohen A, Porat O, Zohar J (2014) Immediate ketamine treatment does not prevent posttraumatic stress responses in an animal model for PTSD. Eur Neuropsychopharmacol 24(3):469-79

[8] Annetta MG, Iemma D, Garisto C, Tafani C, Proietti R (2005) Ketamine: new indications for an old drug. Curr Drug Targets 6(7):789-94

[9] aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS, Mathew SJ (2010) Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. Biol Psychiatry 67(2):139-145

[10] Zarate CA Jr, Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A, Selter J, Marquardt CA, Liberty V, Luckenbaugh DA (2012) Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. Biol Psychiatry 71(11):939-946

[11] McGhee LL, Maani CV, Garza TH, Gaylord KM, Black IH (2008) The correlation between ketamine and posttraumatic stress disorder in burned service members. J Trauma 64:S195-199

[12] Feder A, Parides MK, Murrough JW, Perez AM, Morgan JE, Saxena S, Kirkwood K, Aan Het Rot M, Lapidus KA, Wan LB, Iosifescu D, Charney DS (2014) Efficacy of intravenous ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. JAMA Psychiatry 71(6):681-8

[13] D'Andrea D, Sewell RA (2013) Transient resolution of treatment-resistant posttraumatic stress disorder following ketamine infusion. Biol Psychiatry 74(9):e13-4

[14] Zhang LM, Zhou WW, Ji YJ, Li Y, Zhao N, Chen HX, Xue R, Mei XG, Zhang YZ, Wang HL, Li YF (2015) Anxiolytic effects of ketamine in animal models of posttraumatic stress disorder. Psychopharmacology 232(4):663-72

[15] Winter H, Irle E (2004) Hippocampal volume in adult burn patients with and without posttraumatic stress disorder. Am J Psychiatry 161(12):2194-200

[16] Schönenberg M, Reichwald U, Domes G, Badke A, Hautzinger M (2005) Effects of peritraumatic ketamine medication on early and sustained posttraumatic stress symptoms in moderately injured accident victims. Psychopharmacology 182(3):420-5

[17] Schönenberg M, Reichwald U, Domes G, Badke A, Hautzinger M (2008) Ketamine aggravates symptoms of acute stress disorder in a naturalistic sample of accident victims. J Psychopharmacol 22(5):493-7

[18] Kristal JH, Sanacora G, Duman RS (2013) Rapid-actin glutamatergic antidepressant: the path to ketamine and beyond. Biol Psychiatry 73:1133-1141

[19] Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS (2010) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329:959–964

[20] Baptista PP, Saur L, Bagatini PB, Greggio S, Venturin GT, Vaz SP, Ferreira Kdos R, Junqueira JS, Lara DR, DaCosta JC, Jeckel CM, Mestriner RG, Xavier LL (2015) Antidepressant Effects of Ketamine Are Not Related to ^{18}F-FDG Metabolism or Tyrosine Hydroxylase Immunoreactivity in the Ventral Tegmental Area of Wistar Rats. Neurochem Res 40(6):1153-64

[21] Cohen H, Zohar J, Matar M (2003) The relevance of differential response to trauma in an animal model of posttraumatic stress disorder. Biological Psychiatry 53(6):463–73

[22] Cohen H, Zohar J, Matar MA, Zeev K, Loewenthal U, Richter-Levin G (2004) Setting apart the affected: the use of behavioral criteria in animal models of post traumatic stress disorder. Neuropsychopharmacology 29(11):1962–70

[23] Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131(2):391-404

[24] Cukor J, Spitalnick J, Difede J, Rizzo A, Rothbaum BO (2009) Emerging treatments for PTSD. Clin Psychol Rev 29(8):715-726

[25] Chambers RA, Bremner JD, Moghaddam B, Southwick SM, Charney DS, Krystal JH (1999) Glutamate and post-traumatic stress disorder: toward a psychobiology of dissociation. Semin Clin Neuropsychiatry (4):274-81

[26] Andero R, Ressler KJ (2012) Fear extinction and BDNF: translating animal models of PTSD to the clinic. Genes Brain Behav 11(5):503-12

[27] Rasmusson AM, Shi L, Duman R (2002) Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. Neuropsychopharmacology 27(2):133-42

[28] Kozlovsky N, Matar MA, Kaplan Z, Kotler M, Zohar J, Cohen H (2007) Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. Int J Neuropsychopharmacol 10(6):741-58

[29] Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA (2004) Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol Psychiatry 55:708–714

[30] Bland ST, Schmid MJ, Der-Avakian A, Watkins LR, Spencer RL, Maier SF (2005) Expression of c-fos and BDNF mRNA in subregions of the prefrontal cortex of male and female rats after acute uncontrollable stress. Brain Res 1051(1-2):90-9

[31] Hauck S, Kapczinski F, Roesler R, de Moura Silveira E Jr, Magalhães PV, Kruel LR, Schestatsky SS, Ceitlin LH (2010) Serum brain-derived neurotrophic factor in patients with trauma psychopathology. Prog Neuropsychopharmacol Biol Psychiatry 34(3):459-62

[32] Simsek S, Uysal C, Kaplan I, Yuksel T, Aktas H (2015) BDNF and cortisol levels in children with or without post-traumatic stress disorder after sustaining sexual abuse. Psychoneuroendocrinology 56:45-51

[33] Bonne O, Gill JM, Luckenbaugh DA, Collins C, Owens MJ, Alesci S, Neumeister A, Yuan P, Kinkead B, Manji HK, Charney DS, Vythilingam M (2011) Corticotropin-releasing factor, interleukin-6, brain-derived neurotrophic factor, insulin-like growth factor-1, and substance P in the cerebrospinal fluid of civilians with posttraumatic stress disorder before and after treatment with paroxetine. J Clin Psychiatry 72(8):1124-8

[34] Yehuda R, Harvey PD, Golier JA, Newmark RE, Bowie CR, Wohltmann JJ, Grossman RA, Schmeidler J, Hazlett EA, Buchsbaum MS (2009) Changes in relative glucose metabolic rate following cortisol administration in aging

veterans with posttraumatic stress disorder: an FDG-PET neuroimaging study. J Neuropsychiatry Clin Neurosci 21(2):132-43

[35] Ramage AE, Litz BT, Resick PA, Woolsey MD, Dondanville KA, Young-McCaughan S, Borah AM, Borah EV, Peterson AL, Fox PT (2016) Regional cerebral glucose metabolism differentiates danger- and non-danger-based traumas in post-traumatic stress disorder. Soc Cogn Affect Neurosci 11(2):234-42

[36] Zhu Y, Du R, Zhu Y, Shen Y, Zhang K, Chen Y, Song F, Wu S, Zhang H, Tian M (2016) PET Mapping of Neurofunctional Changes in a Post-traumatic Stress Disorder Model. J Nucl Med Mar 16. pii: jnumed.116.173443

[37] Vermetten E, Schmahl C, Southwick SM, Bremner JD (2007) Positron tomographic emission study of olfactory induced emotional recall in veterans with and without combat-related posttraumatic stress disorder. Psychopharmacol Bull 40(1):8-30

[38] Buchsbaum MS, Simmons AN, DeCastro A, Farid N, Matthews SC (2015) Clusters of Low (18)F-Fluorodeoxyglucose Uptake Voxels in Combat Veterans with Traumatic Brain Injury and Post-Traumatic Stress Disorder. J Neurotrauma 15;32(22):1736-50

[39] Stocker RP, Cieply MA, Paul B, Khan H, Henry L, Kontos AP, Germain A (2014) Combat-related blast exposure and traumatic brain injury influence brain glucose metabolism during REM sleep in military veterans. Neuroimage 99:207-14

[40] Molina ME, Isoardi R, Prado MN, Bentolila S (2010) Basal cerebral glucose distribution in long-term post-traumatic stress disorder. World J Biol Psychiatry (2 Pt 2):493-501

[41] Gold AL, Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, Metzger LJ, Dougherty DD, Alpert NM, Fischman AJ, Pitman RK (2011) Decreased regional cerebral blood flow in medial prefrontal cortex during trauma-unrelated stressful imagery in Vietnam veterans with post-traumatic stress disorder. Psychol Med 41(12):2563-72

[42] Rilling JK, Winslow JT, O'Brien D, Gutman DA, Hoffman JM, Kilts CD (2001) Neural correlates of maternal separation in rhesus monkeys. Biol Psychiatry 49(2):146-57

LEGENDS

FIGURE 1 – Timeline depicting the experimental procedure.

FIGURE 2 – Duration of freezing bouts during exposure to the situational reminder 7 days after PTSD induction. Longer freezing bouts were observed in the PTSD+SAL(EBR) and PTSD+KET(EBR) groups when compared to all other groups. Moreover, the PTSD+KET(EBR) group exhibited longer freezing bouts when compared to the PTSD+SAL(EBR) group. (a) $p \leq 0.01$, when compared to the CTRL+SAL, CTRL+KET, PTSD+SAL(MBR) and the PTSD+KET(MBR) groups; (b) $p \leq 0.001$, when compared to the CTRL+SAL, CTRL+KET, PTSD+SAL(MBR) and PTSD+KET(MBR) groups; (c) $p \leq 0.01$ when compared to the PTSD+SAL(EBR) group.

FIGURE 3 – ^{18}F-FDG-microPET and BDNF quantification. Effects of PTSD and ketamine treatment on glucose metabolism and BDNF protein levels in the frontal cortex, hippocampus and amygdala. (a, d, g) Normalized brain image in coronal view showing the frontal cortex (orange), hippocampus (purple) and amygdala (red), respectively. Areas were defined based on Paxinos coordinates using a rat-ROI-template in the PMOD software (Color figure online). (b, e, h) ^{18}F-FDG uptake in the frontal cortex, hippocampus and amygdala, respectively. (c, f) BDNF protein quantification in the frontal cortex and hippocampus, respectively.

Figure 1

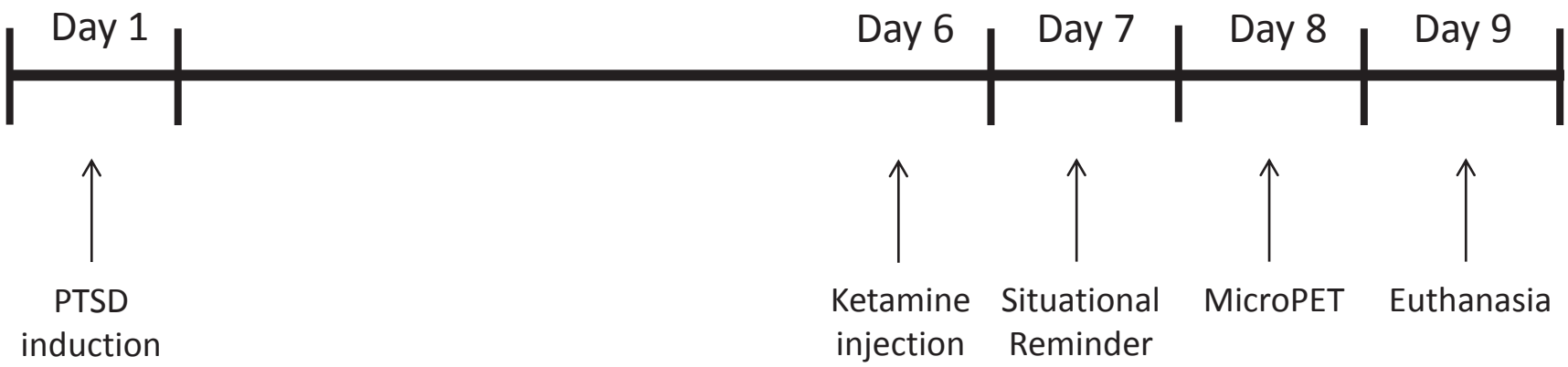

Day 1
Day 6
Day 7
Day 8
Day 9
PTSD induction
Ketamine injection
Situational Reminder
MicroPET
Euthanasia

Figure 2

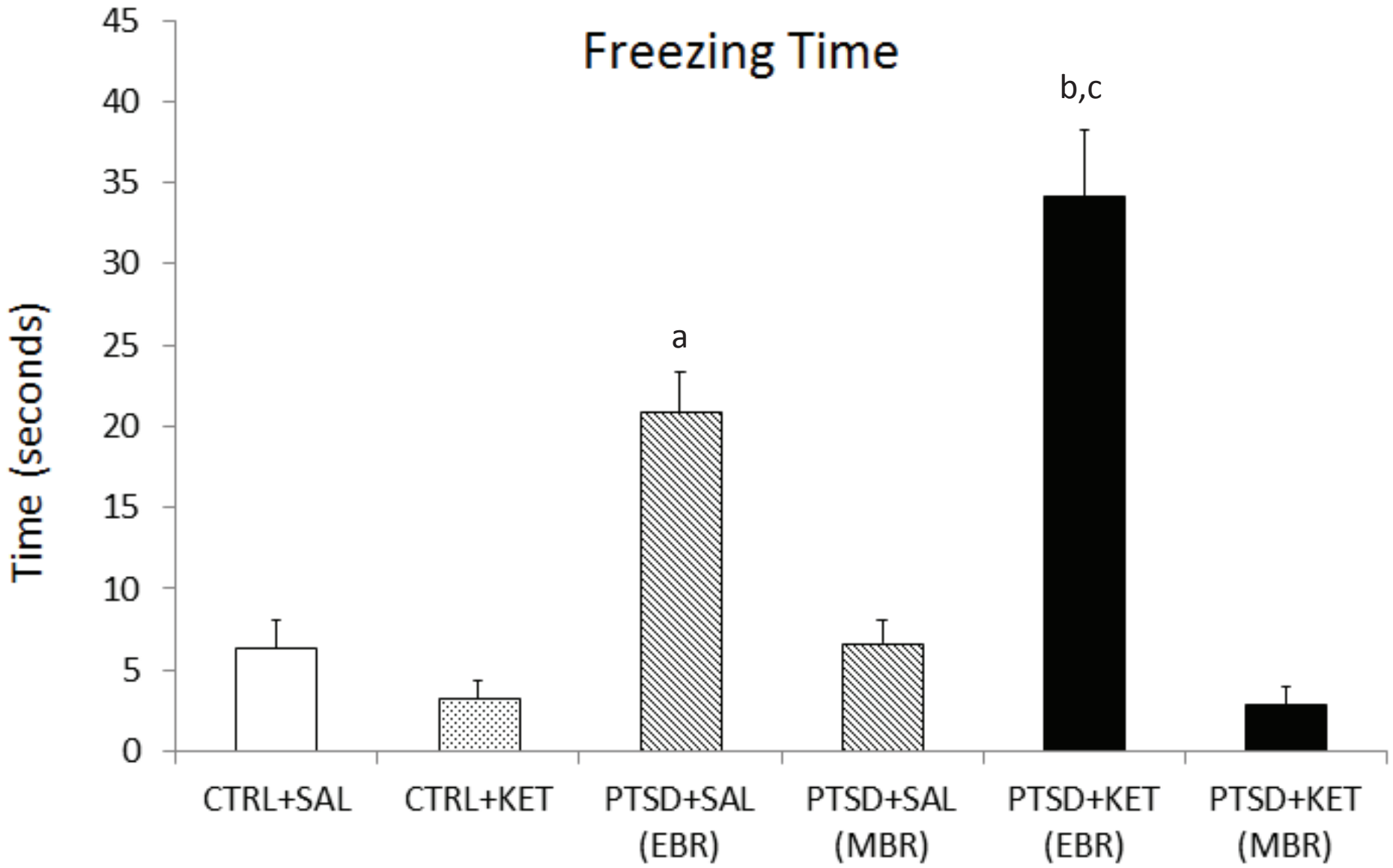
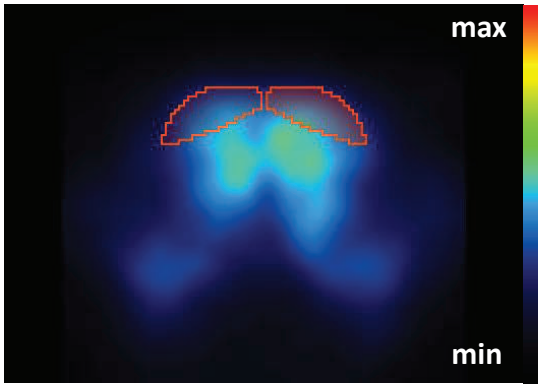
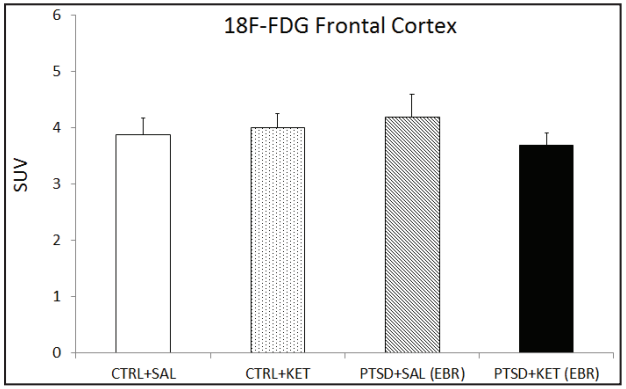
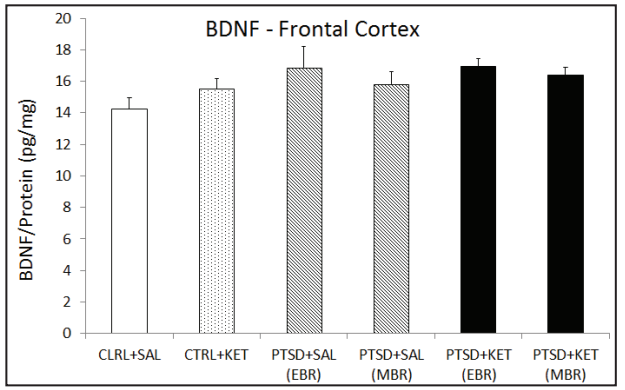
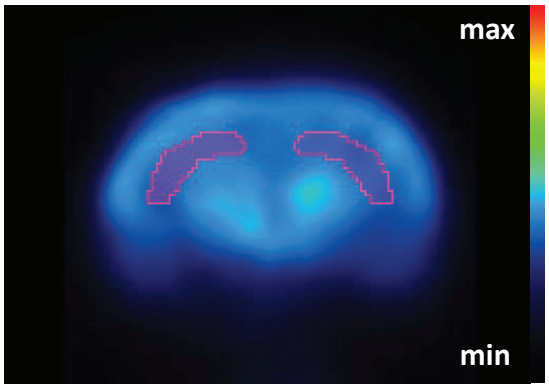

Freezing Time
Time (seconds)
45
40
35
30
25
20
15
10
5
0
a
b,c
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+SAL (MBR)
PTSD+KET (EBR)
PTSD+KET (MBR)

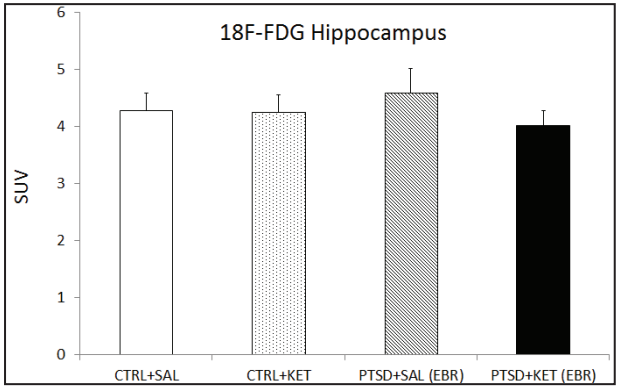
Figure 3

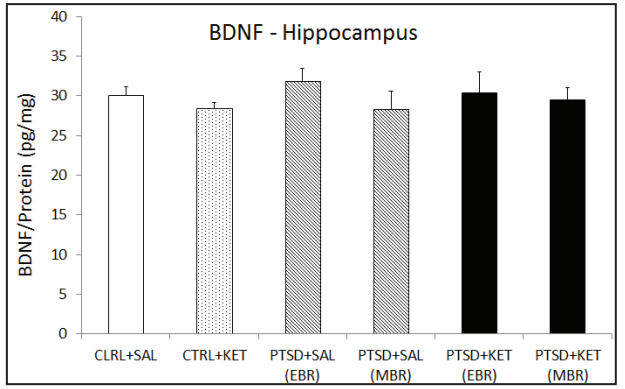

a
max
min

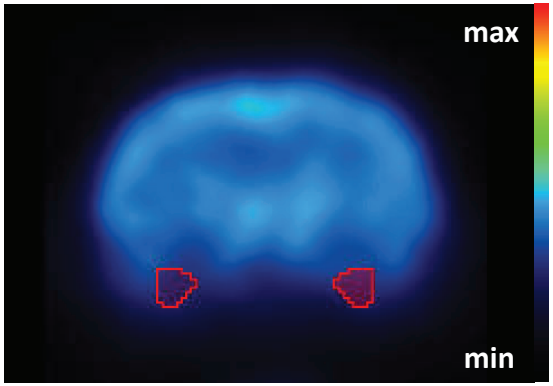

b
18F-FDG Frontal Cortex
SUV
0
1
2
3
4
5
6
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+KET (EBR)

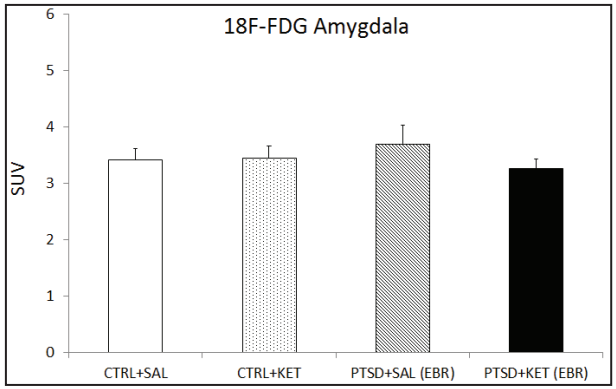

c
BDNF - Frontal Cortex
BDNF/Protein (pg/mg)
0
2
4
6
8
10
12
14
16
18
20
CLRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+SAL (MBR)
PTSD+KET (EBR)
PTSD+KET (MBR)


d
max
min


e
18F-FDG Hippocampus
SUV
0
1
2
3
4
5
6
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+KET (EBR)


f
BDNF - Hippocampus
BDNF/Protein (pg/mg)
0
5
10
15
20
25
30
35
40
CLRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+SAL (MBR)
PTSD+KET (EBR)
PTSD+KET (MBR)


g
max
min


h
18F-FDG Amygdala
SUV
0
1
2
3
4
5
6
CTRL+SAL
CTRL+KET
PTSD+SAL (EBR)
PTSD+KET (EBR)

Capítulo IV

5. CONSIDERAÇÕES FINAIS

6. PERSPECTIVAS

7. REFERÊNCIAS

5. CONSIDERAÇÕES FINAIS

5.1. Considerações a respeito do modelo experimental

As pesquisas em animais têm desempenhado um papel significativo na compreensão atual dos fatores biológicos e comportamentais envolvidos na psicopatologia do PTSD. Embora estudos clínicos sejam indiscutivelmente importantes, eles são limitados por questões éticas e práticas. Assim, os estudos em modelos animais são imprescindíveis para a compreensão das respostas comportamentais desencadeadas, dos mecanismos neurais específicos envolvidos, dos aspectos relevantes que contribuem para o desenvolvimento da doença, bem como do desenvolvimento de possíveis compostos terapêuticos que possuam eficácia no tratamento desta psicopatologia. Entretanto, os modelos animais ainda enfrentam uma série de desafios, principalmente no que concerne aos componentes subjetivos ou experienciais do PTSD, que por estarem relacionados à neurobiologia individual e às experiências passadas, são difíceis de modelar em animais. Devido à variedade de sintomas do PTSD, os cientistas estão desenvolvendo modelos animais que mimetizem um seleto grupo deles. Neste sentido, Yehuda e Antelman (1993) elaboraram uma lista que continua a ser utilizada até hoje para validar um modelo animal de PTSD. De acordo com essa lista, quatro critérios devem estar presentes para que um modelo experimental seja comparável ao PTSD em humanos: (1) Mesmo estressores muito breves devem ser capazes de induzir os sintomas biológicos e comportamentais do PTSD; (2) O estressor deve ser capaz de induzir os sintomas de uma forma dose-dependente; (3) As alterações biológicas produzidas devem persistir ou tornarem-se mais pronunciadas ao longo do tempo; (4) Deve haver variabilidade individual na resposta ao estresse. Postula-se que outro aspecto importante da exposição ao estresse

traumático, que contribui fortemente para o desenvolvimento subsequente dos sintomas, é o baixo grau de controle e previsibilidade dos estímulos de estresse (Foa et al., 1992).

Neste sentido, vários modelos experimentais foram desenvolvidos para atender a estes requisitos e mimetizar o PTSD. Embora seja praticamente impossível recriar todas as características de um transtorno psiquiátrico, alguns modelos conseguem reproduzir com sucesso as características-chave desta doença. Um dos mais utilizados modelos experimentais de PTSD é a exposição a um choque inescapável único (CIU). Neste modelo, os animais são expostos a um episódio único de choque nas patas sem a possibilidade de escapar (Liberzon et al., 2005). Este modelo é capaz de produzir diferentes características do PTSD, incluindo o aumento do medo condicionado ao contexto (Maier 1990; Diehl et al., 2007), o aumento da neofobia (Job e Barnes 1995), a diminuição da interação social (possivelmente análoga a comportamentos de esquiva) (Short e Maier 1993), a redução no comportamento exploratório, o aumento da ansiedade (Diehl et al., 2007) e a diminuição do consumo de alimentos saborosos (Griffiths et al., 1992; Krolow et al., 2013). Ademais, a exposição ao CIU também produz uma analgesia opioide análoga à observada em pacientes humanos com PTSD (van der Kolk et al., 1985; Maier 1989). Mais interessante ainda é que algumas dessas alterações foram observadas muito tempo após a exposição ao evento estressante (até um mês após a exposição ao CIU), demonstrando que esse modelo de PTSD apresenta efeitos duradouros sobre o comportamento e fisiologia dos animais. Deste modo, o CIU é considerado um excelente modelo para mimetizar os efeitos, a curto e longo prazo, da exposição a um estressor grave, tal qual o observado em seres humanos (Liberzon et al., 2005).

5.2. Discussão

Na primeira parte deste estudo foi demonstrado que a exposição a um choque nas patas, como modelo de PTSD, induz importantes alterações comportamentais além de alterar a densidade, a arborização e a polaridade dos astrócitos GFAP+ no *stratum radiatum* da região CA1 do hipocampo. Este modelo animal provocou um aumento na duração do comportamento de *freezing* nos animais durante a exposição ao lembrete situacional. O comportamento de *freezing* indica uma sensação de ameaça ou medo intenso e é amplamente utilizado como medida de medo em vários modelos animais de PTSD (Diehl et al., 2007; Siegmund e Wotjak, 2007; Li et al., 2006; Tulogdi et al., 2012; Juven-Wetzler et al., 2014; Ryoke et al., 2014; Mirshekar et al., 2013; Wang et al., 2008). Além disso, observamos um aumento na produção de bolos fecais durante o lembrete situacional. A contagem de bolos fecais não é uma medida precisa de medo (Zhang et al., 2014), no entanto, combinado com a medida de tempo de *freezing* fornece um dado complementar. Este tipo de resposta demonstra que, através de um processo de aprendizagem associativa, os animais apresentaram um comportamento de medo condicionado. O medo condicionado é uma das características mais marcantes dos distúrbios de ansiedade, em particular do PTSD (Lissek et al., 2005). Outra característica marcante do PTSD é a generalização do medo, ou seja, a transferência do medo experimentado durante uma situação para outras situações, aparentemente inócuas. É o caso, por exemplo, de um ex-prisioneiro que, ao entrar em um elevador, ou em uma sala fechada, pode se lembrar de ser levado para a sala de tortura. Os estudos que avaliam o medo condicionado, bem como a generalização do medo remontam do início do século passado (Pavlov 1927; Watson e Rayner 2000). Desde então, diversos outros trabalhos têm tentado entender os mecanismo por trás destes dois marcantes sintomas do PTSD.

A neurocircuitaria do medo condicionado e da generalização tem sido associada ao córtex pré-frontal, hipocampo e amígdala (Giustino e Maren 2015; Lopresto et al., 2016), da seguinte maneira:

- Déficits na identificação de contextos não perigosos mediados pelo hipocampo.
- A hiporresponsividade do PFC leva a déficits na extinção, no aprendizado seguro e na supressão da resposta a estímulos associados ao trauma.
- A hiper-responsividade da amígdala leva aos sintomas de hiperexcitação e explica a qualidade indelével da memória emocional do evento traumático.

Portanto o PTSD é uma doença complexa que não está associada a déficits em uma região específica, mas sim em várias. Assim, os dois artigos desta tese focaram-se no estudo destas três regiões.

No primeiro artigo desta tese, observamos também uma diminuição na densidade de astrócitos no *stratum radiatum* da região CA1 do hipocampo. A atrofia do hipocampo é uma das alterações morfológicas mais comumente observadas em pacientes com PTSD (Kitayama et al., 2005; Lindauer et al., 2006; Bremner et al., 2008; Bremner et al., 1995; Shin et al., 2004a) e pelo fato dos astrócitos serem a maior população de células do hipocampo (Xia et al., 2013), é possível sugerir que esta atrofia possa ser ocasionada, pelo menos em parte, pela perda de astrócitos nesta região. Os astrócitos são responsáveis pela síntese e libertação de muitos dos fatores neurotróficos vitais para a saúde neural, tais como o BDNF, fator neurotrófico derivado da glia (GDNF) e fator de crescimento do nervo (NGF) (Friedman et al., 1998; Althaus e Richter-Landsberg 2000). Esses fatores neurotróficos regulam o crescimento neural e são essenciais para a plasticidade e a sua disponibilidade reduzida pode resultar em aumento da vulnerabilidade celular ou mesmo em morte celular (Czéh et al., 2006).

Apesar das alterações observadas nos astrócitos hipocampais, nenhuma alteração foi observada nos astrócitos da amígdala medial, isto pode ser devido ao fato de que, ao menos nesta região, os astrócitos sejam menos vulneráveis aos efeitos do PTSD do que no hipocampo. Neste estudo também observamos que o PTSD induziu alterações na morfologia dos astrócitos GFAP+ do *stratum radiatum* da região CA1 do hipocampo, que foi analisada através do método dos círculos concêntricos de Sholl. A maioria dos astrócitos nesta região possui um formato fusiforme, quase perpendicular ao *stratum pyramidale*, com os prolongamentos orientados paralelamente aos dendritos descendentes dos neurônios da camada piramidal (Nixdorf-Bergweiler et al., 1994; Bushong et al., 2002). A orientação dos astrócitos no *stratum radiatum*, em ratos, começa a tornar-se polarizada com aproximadamente 3 semanas de idade e permanece assim até a vida adulta (Nixdorf-Bergweiler et al., 1994). A orientação dos astrócitos é baseada na determinação do quociente entre o número de intersecções laterais, dividido pelo número de intersecções centrais, dos processos astrocíticos, com os círculos de Sholl. Números menores indicam um formato fusiforme com uma orientação perpendicular ao *stratum pyramidal*e e números maiores indicam um formato mais estrelado, com uma orientação mais paralela ao *stratum pyramidale*. Este quociente determina o grau de polaridade dos astrócitos e é referido como índice de orientação. Nossos resultados, a respeito da morfologia dos astrócitos, mostraram que o PTSD provocou uma diminuição no grau de ramificação e no número de processos primários dos astrócitos nos quadrantes laterais, mas não nos quadrantes centrais. Portanto esta diminuição na ramificação lateral indica que o PTSD induziu uma mudança na morfologia dos astrócitos nesta região, fazendo com que eles adotassem um formato mais fusiforme. O índice de orientação variou de 0.62 ± 0.14 nos animais controle para 0.42 ± 0.09 nos animais com PTSD. Esta alteração parece ser bastante importante, pois

estudos que avaliaram alterações na ramificação astrocítica demonstraram que intervenções positivas como exercício físico (Saur et al., 2014), e enriquecimento ambiental (Viola et al., 2009) induzem os astrócitos a adotar uma forma mais estrelada, principalmente através do aumento no número de ramificações laterais, enquanto intervenções negativas como a presença de valproato (Fennrich et al., 1998) e o acidente vascular cerebral (Mestriner et al., 2011) induzem uma diminuição nas ramificações dos astrócitos, tal qual observado em nosso estudo. Assim, parece que os processos astrocíticos são extremamente plásticos e alteram a sua estrutura em resposta às demandas ambientais (Allen e Barres 2005). Algumas evidências mostram que o remodelamento dos processos astrocíticos está intimamente ligado a atividade neuronal e frequentemente ocorre em sincronia com mudanças morfológicas em neurônios vizinhos, um tipo de plasticidade astrócitos-neurônio (Theodosis et al., 2008). O próprio conceito de sinapse tripartite sugere que os processos astrocíticos influenciam a atividade sináptica e reflete a cooperação entre neurônios e astrócitos no SNC (Theodosis et al., 2008).

Na segunda parte do estudo nós avaliamos se a cetamina poderia trazer algum efeito benéfico no tratamento do PTSD. A cetamina é um bloqueador de receptor NMDA e tem demonstrado resultados promissores no tratamento de outras doenças neuropsiquiátricas como a depressão e o transtorno bipolar (Price et al., 2009; Aan het Rot et al., 2010; Zarate et al., 2012). Outra questão que decidimos abordar nesta parte do estudo foi o padrão de resposta comportamental entre os animais. Tal qual o observado em humanos, os animais apresentam diferenças individuais e respondem de maneira diferente a estímulos semelhantes. Assim, decidimos classificar os animais PTSD em dois grupos: "*extreme behavioral response*" (EBR) e "*minimal bahavioral response*" (MBR) de acordo com o comportamento apresentado no teste de medo

condicionado ao contexto. Deste modo, observamos que os animais com PTSD classificados como MBR apresentam comportamento semelhante aos animais controle. Estes animais apresentam uma reação de curta duração que não leva a uma resposta prolongada ao estresse, ou seja, apresentam uma resposta comportamental resiliente (são capazes de se adaptar). Respostas comportamentais semelhantes foram observadas em outros estudos com diferentes modelos animais de PTSD como a exposição a um predador (Cohen et al., 2003; Mitra et al., 2005), exposição à urina de um predador (Cohen et al., 2004), experiência de "quase" afogamento (Cohen et al., 2004) e derrota social (Krishnan et al., 2007). Além disso, alguns tipos de estressores provocam uma resposta comportamental mais extrema que outros, é o caso da experiência de "quase" afogamento, que provoca uma resposta comportamental mais severa do que a exposição à urina de um predador (Cohen et al., 2004). Decidimos realizar este tipo de abordagem após verificar que, o modelo experimental de PTSD utilizado nesta tese, assim como os modelos experimentais acima citados, produzem nos animais respostas comportamentais diferentes. Além disso, baseado na premissa de que o nosso modelo experimental de PTSD é válido, parece razoável abordar a análise dos dados de uma forma que mais se assemelhe à abordagem utilizada pelo DSM-5, cujo diagnóstico de PTSD baseia-se em critérios de inclusão e exclusão. Do mesmo modo, este tipo de abordagem é conceitualmente mais equivalente aos critérios propostos por Yehuda e Antelman (1993) sobre a validade de modelos experimentais de PTSD. Ademais, Cohen e colaboradores (2003) sugeriram que os estudos em modelos experimentais de PTSD utilizem não mais a expressão "todos os animais expostos a um evento traumático", mas sim "os animais expostos a um evento traumático que desenvolveram mudanças comportamentais específicas" (Cohen et al., 2003).

Para diferenciar os animais resilientes daqueles susceptíveis, utilizamos um critério comportamental pré-definido que, nesta tese, foi: a média de tempo de *freezing* de todos os animais do grupo PTSD+Salina. Outros estudos utilizaram outros critérios baseados, por exemplo, no comportamento nos testes de *plus-maz*e e *open-field* e na resposta de sobressalto acústica (Cohen et al., 2003, Cohen et al., 2004, Ardi et al., 2016). Entretanto deve-se ter bastante cuidado na interpretação dos resultados gerados. Isso porque, como os modelos experimentais de transtornos psicológicos não refletem com precisão as doenças em humanos, mas somente aproximam-se em certos aspectos, seria presunçoso supor que os critérios aplicados neste estudo, e em outros, reflitam com exatidão os critérios utilizados no diagnóstico do PTSD em humanos (Cohen et al., 2003). As razões para as diferenças individuais no tipo de resposta apresentada por cada indivíduo ainda são desconhecidas. Embora, alguns autores sugiram que fatores genéticos, bem como os eventos adversos durante o desenvolvimento inicial estejam entre os principais fatores que contribuam para a vulnerabilidade aos efeitos do estresse, fazendo com que alguns indivíduos estejam mais predispostos a desenvolver o PTSD do que outros (Heim e Nemeroff 2001; McEwen 2008; Mitra et al., 2005; Adamec et al., 2012). Dados recentes apontam inclusive para um distinto padrão de mudança citoarquitetural nos neurônios do hipocampo entre as diferentes categorias de respostas comportamentais apresentadas pelos animais (resiliente ou susceptível). Um estudo realizado por Cohen e colaboradores (2014) demonstrou que, oito dias após a exposição a um predador como modelo experimental de PTSD, a quantidade, a complexidade e o comprimento dos dendritos, bem como a densidade de espinhos dendríticos dos neurônios tanto na camada granular do giro denteado como dos neurônios piramidais da região CA1 do hipocampo são menores nos animais EBR em relação aos animais MBR (Cohen et al., 2014). Na amígdala, o comprimento e a complexidade dos dendritos

neuronais são maiores nos animais EBR quando comparados com os animais MBR. Além disso, essas alterações foram seguidas pelo aumento na expressão de receptores de glicocorticoides no giro denteado e em CA1 somente nos animais EBR (Cohen et al., 2014). Assim, estudos que visem fornecer uma visão mais ampla de neurotransmissores e mediadores celulares, potencialmente envolvidos na mediação das respostas ao estresse, e que estão envolvidos na adaptação ou na má adaptação do indivíduo são especialmente importantes no intuito de identificar os fatores de risco biológicos que tornam determinados indivíduos especialmente vulneráveis a transtornos relacionados ao estresse.

Outro importante achado deste estudo foi que o tratamento com cetamina aumentou o comportamento de *freezing* durante o teste de medo condicionado. Esta é uma preocupação que existia com relação ao uso da cetamina no tratamento do PTSD, visto que esta droga está associada a efeitos dissociativos e psicóticos semelhantes aos associados ao PTSD podendo, portanto, aumentar os sintomas desta doença. As orientações psiquiátricas atuais para o tratamento de transtornos psiquiátricos como a depressão e o PTSD ainda não incluem a cetamina (Canadian Agency for Drugs and Technologies in Health, 2014), entretanto, baseados em evidencias pré-clínicas do envolvimento do sistema glutamatérgico nos transtornos de humor, estudos foram realizados para testar as propriedades antidepressivas da cetamina. Vários estudos bem controlados demonstraram que a cetamina pode ter rápidos efeitos antidepressivos (Price et al., 2009; Aan het Rot et al., 2010; Zarate et al., 2012). Entretanto os resultados a respeito do uso da cetamina no tratamento do PTSD ainda são bastante controversos. Alguns estudos evidenciaram que a cetamina tem um efeito positivo na melhora dos sintomas de PTSD. Em um destes, pacientes com queimaduras graves receberam cetamina durante o processo cirúrgico ao qual foram submetidos (McGhee et al., 2008),

em outro, um paciente que tinha sido diagnosticado com PTSD há 3 anos também apresentou melhora nos sintomas da doença após ser tratando com cetamina (D'Andrea e Sewell 2013), por fim, um estudo realizado com pacientes com mais de 10 anos de histórico de PTSD demonstrou efeitos benéficos do tratamento com esta droga (Feder et al., 2014). Em contrapartida, naqueles estudos que encontraram uma piora nos sintomas do PTSD, a cetamina havia sido administrada durante o tratamento inicial após o evento traumático (Juven-Wetzler et al., 2014; Schönenberg et al., 2005; Schönenberg et al., 2008). Levando isso em consideração, pode-se especular que a administração de cetamina pode ter funções aditivas na resposta ao estresse em andamento quando administrada em uma janela de tempo precoce após o trauma inicial, provavelmente como resultado do aumento simultâneo do glutamato (provocado pela cetamina) e de glicocorticoides (provocado pelo estresse) no SNC (Schönenberg et al., 2005). Portanto, aspectos de segurança, possíveis mecanismos de ação e direções futuras da pesquisa sobre o uso da cetamina em condições psiquiátricas ainda devem ser amplamente investigados, especialmente porque a cetamina continua a ser amplamente analisada como alternativa no tratamento da depressão, doença que, com certa frequência, está associada com histórico de trauma (Schönenberg et al., 2005).

Neste estudo avaliamos também o metabolismo da glicose através da técnica ^{18}F-FDG-microPET. O ^{18}F-FDG (^{18}F-2-fluoro-2-deoxy-glucose) é um dos radio-traçadores mais comumente usados em estudos de PET e é utilizado para medir o consumo da glicose nos tecidos. Neste trabalho, nenhuma alteração foi observada no córtex frontal, hipocampo e amígdala nos animais com PTSD tampouco nos animais submetidos ao tratamento com cetamina. Os dados a respeito do metabolismo da glicose tanto em humanos como em modelos animais de PTSD são bastante conflitantes. Por exemplo, foi relatado aumento (Yehuda et al., 2009; Ramage et al., 2016; Zhu et al.,

2016), diminuição (Buchsbaum et al., 2015; Stocker et al., 2014; Yehuda et al., 2009) e nenhuma alteração (Molina et al., 2010) no metabolismo da glicose em diferentes núcleos da amígdala. Resultados conflitantes também foram observados em diferentes regiões do córtex pré-frontal: aumento (Rilling et al., 2001) e diminuição (Stocker et al., 2014; Rilling et al., 2001; Molina et al., 2010) na distribuição de ^{18}F-FDG foram relatados. O único consenso parece ser com relação ao hipocampo, no qual somente diminuições no metabolismo da glicose foram relatadas (Yehuda et al., 2009; Stocker et al., 2014; Molina et al., 2010). Estes resultados contraditórios podem ser atribuídos a diversos fatores que variam amplamente de estudo para estudo, como, por exemplo, diferenças no tamanho das amostras, variação do tempo em que o PTSD teve início, heterogeneidade dos tipos de traumas, comorbidade com outras doenças e diferenças no paradigma sob o qual a análise foi realizada (repouso, sono, apresentação de uma imagem relacionada ao trauma, apresentação de imagens traumatogênicas, porém não relacionadas ao trauma e exposição a imagens neutras). Além disso, em nosso estudo, (pelo fato de ser tecnicamente impossível realizar este tipo de análise em outras condições), os animais estavam sob efeito de anestesia durante a captura do PET, condição que pode influenciar os resultados. Neste sentido é bastante difícil fazer algum tipo de inferência a partir destes resultados. Portanto, parece que devido à complexa apresentação clínica do PTSD e a sua sintomatologia muito variável, ainda não se estabeleceu dados consistentes sobre o metabolismo encefálico nesta doença neuropsiquiátrica (Virdee et al., 2012).

Analisamos também os níveis de BDNF no hipocampo e na amígdala nove dias após a indução do estresse. O BDNF é um membro da família das neurotrofinas que desempenha um papel importante no crescimento, desenvolvimento e manutenção de vários sistemas neurais e também está envolvido nos processos de plasticidade sináptica

e liberação de neurotransmissores. O BDNF é especialmente importante no hipocampo, amígdala e córtex pré-frontal, que são regiões envolvidas nos processos de aprendizagem e memória, incluindo as memórias de medo (Andero e Ressler 2012). A expressão de BDNF é regulada pelo estresse e evidências recentes sugerem que esta neurotrofina possui um papel chave na patogênese dos transtornos de humor (Bennett et al., 2016; Angelucci et al., 2014; Martinotti et al., 2015). Além disso, os mecanismos de ação dos agentes terapêuticos utilizados no tratamento de vários transtornos neuropsiquiátricos estão associados ao reestabelecimento dos níveis normais de BDNF circulante (Manji e Duman 2001; Rasmusson et al., 2002).

Entretanto, neste estudo, não observamos nenhuma alteração na expressão de BDNF nas regiões analisadas. Estudos anteriores que avaliaram os efeitos de curto e longo prazo do PTSD sobre a expressão desta proteína demonstraram que as alterações nos níveis de BDNF nesta doença são transitórios e restritos a horas ou poucos dias, em modelos animais. Entretanto, enquanto alguns demonstraram uma diminuição inicial dos níveis de BDNF, outros demonstraram um aumento de BDNF nos estágios iniciais após o estresse. Por exemplo, Rasmusson e colaboradores (2002) evidenciaram uma diminuição nos níveis de BDNF mRNA no giro denteado 60 minutos após a exposição a um choque na cauda, e que esses níveis retornaram ao normal após 48 horas. Foi demonstrado também que a exposição a um predador provoca uma diminuição persistente (30 dias) nos níveis de BDNF mRNA somente na região CA1 do hipocampo, enquanto que as diminuições encontradas em outras regiões (CA3, giro denteado e córtex frontal) foram transitórias. Além disso, esta diminuição foi relatada somente nos animais cujo comportamento foi mais severamente afetado pelo estresse (Kozlovsky et al., 2007). Outros estudos, no entanto, demonstraram um aumento inicial nos níveis de BDNF após o estresse. Roceri e colaboradores (2004), utilizando a

separação materna como modelo de estresse, relataram um aumento na expressão de BDNF mRNA no hipocampo e no córtex pré-frontal 17 dias pós-natal, contudo, a expressão de BDNF retornou aos níveis normais 35 dias pós-natal. Em outro estudo foi evidenciado um aumento nos níveis de BDNF mRNA somente imediatamente após um choque na cauda e que foram restaurados aos níveis normais 60 minutos após o estresse (Bland et al., 2005). Por fim, um estudo realizado em humanos demonstrou que pacientes com PTSD possuem níveis séricos de BDNF mais elevados quando comparado com o grupo controle. Entretanto, este efeito foi observado somente em pacientes cujo trauma era recente (menos de um ano). Em pacientes com traumas mais remotos, os níveis de BDNF eram iguais aos do grupo controle (Hauck et al., 2010).

Neste estudo, os níveis de BDNF foram analisados nove dias após a exposição ao estresse, portanto, podemos especular que nossos animais apresentaram alterações (diminuição/aumento) na expressão de BDNF, no entanto estas mudanças foram restritas a um curto período de tempo e não uma mudança de longo-prazo. Alguns autores sugerem que a diminuição nos níveis de BDNF observada após a exposição a um estresse pode ser um dos fatores responsáveis pela falha na codificação inicial da memória traumática, levando o indivíduo a desenvolver os sintomas da doença, além de ser um dos responsáveis pelos déficits cognitivos que alguns indivíduos com PTSD apresentam (Hauck et al., 2010; Rasmusson et al., 2002). Outros sugerem que o aumento inicial nos níveis de BDNF, observado após a exposição a um estresse, está relacionado a um mecanismo compensatório no intuito de proteger o encéfalo contra os efeitos deletérios dos níveis elevados de corticoides liberados após a exposição a um agente estressor (Faure et al., 2007).

Como observado, os resultados a respeito da expressão de BDNF no PTSD não são uniformes e são difíceis de comparar devido a diferenças metodológicas, incluindo

o tipo de estresse envolvido, a intensidade, o número de repetições, o tempo de exposição, as regiões analisadas, entre outros. Ainda assim, parece claro que o BDNF é um fator importante na resposta ao estresse e mesmo que as alterações sejam transitórias, podem contribuir para o desenvolvimento desta doença. Assim, é notável a necessidade de estudos que avaliem o tempo preciso durante o qual os níveis de BDNF permanecem alterados. Além disso, é importante saber se a repetição do estresse resulta em novas alterações transientes nos níveis de BDNF ou se esse efeito está sujeito à habituação.

Com base no descrito acima e nos achados dos dois artigos desta tese, conseguimos aprimorar um pouco mais o conhecimento a respeito das alterações comportamentais, histofisiológicas, bioquímicas e neurometabólicas que acompanham o PTSD e sobre a atuação da cetamina nesta doença neuropsiquiátrica.

6. PERSPECTIVAS

De acordo com a temática desta tese, as atividades listadas a baixo podem ser realizadas no intuito de aprimorar os conhecimentos sobre o assunto. Para isso, os seguintes parâmetros podem ser futuramente avaliados:

1) Avaliar os efeitos de longo prazo (30 dias) do PTSD sobre o comportamento de *freezing* dos animais.
    - Avaliar o comportamento de *freezing* a longo prazo é importante pois, postula-se que um aspecto importante do PTSD é que os sintomas devem persistir ao longo do tempo.

2) Avaliar os efeitos do choque nas patas, como modelo de PTSD, sob outros parâmetros comportamentais característicos da doença como ansiedade e hipervigilância através dos testes de *plus-maze* e do teste de sobressalto acústico.
    - Não só o tempo de *freezing* é utilizado para verificar a eficácia do modelo experimental de PTSD. Os resultados destes outros testes podem sem utilizados como critérios adicionais para separar os animais em EBR ou MBR.

3) Verificar se há diferenças entre as alterações astrocitárias observadas em animais com PTSD classificados como MBR e EBR.
    - No primeiro artigo desta tese, foram demonstradas alterações astrocitárias significativas induzidas pelo choque nas patas, entretanto, os

animais não foram classificados de acordo com a sua resposta comportamental.

4) Avaliar os efeitos da cetamina sobre os astrócitos do hipocampo e amígdala.
    - Foi demonstrado que os astrócitos são susceptíveis ao PTSD e que a cetamina piora os sintomas desta doença. Verificar se este agravamento na resposta comportamental está relacionado a alterações nos astrócitos destas duas regiões que estão amplamente relacionadas às memórias de medo.

5) Analisar as possíveis alterações de curto prazo do PTSD sobre os níveis de BDNF no hipocampo e córtex frontal com o uso deste modelo experimental.
    - Alguns estudos demonstraram alterações iniciais transitórias nos níveis de BDNF após a exposição a diferentes estressores, entretanto essas alterações foram restritas a algumas horas ou poucos dias. Além disso, nenhum deles utilizou o choque nas patas como modelo experimental de PTSD.

7. REFERÊNCIAS

Aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS, Mathew SJ (2010) Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. Biol Psychiatry 15;67(2):139-145

Adamec R, Toth M, Haller J, Halasz J, Blundell J (2012) A comparison of activation patterns of cells in selected prefrontal cortical and amygdala areas of rats which are more or less anxious in response to predator exposure or submersion stress. Physiol Behav 1;105(3):628-38

Allen NJ, Barres BA (2005) Signaling between glia and neurons: focus on synaptic plasticity. Curr Opin Neurobiol 15:542–548

Althaus HH, Richter-Landsberg C (2000) Glial cells as targets and producers of neurotrophins. Int Rev Cytol 197:203-277

Andero R, Ressler KJ (2012) Fear extinction and BDNF: translating animal models of PTSD to the clinic. Genes Brain Behav 11(5):503-12

Angelucci F, Ricci V, Gelfo F, Martinotti G, Brunetti M, Sepede G, Signorelli M, Aguglia E, Pettorruso M, Vellante F, Di Giannantonio M, Caltagirone C (2014) BDNF serum levels in subjects developing or not post-traumatic stress disorder after trauma exposure. Brain Cogn 84(1):118-22

Araque A, Navarrete M (2010) Glial cells in neuronal network function. Phil Trans R Soc B 365:2375–2381

Ardi Z, Albrecht A, Richter-Levin A, Saha R, Richter-Levin G (2016) Behavioral profiling as a translational approach in an animal model of posttraumatic stress disorder. Neurobiol Dis 88:139-47

Association AP (2013) Diagnostic and Statistical Manual of Mental Disorders, 5th edn. American Psychiatric Publishing, Arlington, VA

Banasr M, Duman RS (2008) Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry 15;64(10):863-870

Bennett MR, Hatton SN, Lagopoulos J (2016) Stress, trauma and PTSD: translational insights into the core synaptic circuitry and its modulation. Brain Struct Funct 221(5):2401-26

Bennur S, Shankaranarayana Rao BS, Pawlak R, Strickland S, McEwen BS, Chattarji S (2007) Stress-induced spine loss in the medial amygdala is mediated by tissue-plasminogen activator. Neuroscience 144:8–16

Bing X, Ming-Guo Q, Ye Z, Jing-Na Z, Min L, Han C, Yu Z, Jia-Jia Z, Jian W, Wei C, Han-Jian D, Shao-Xiang Z (2013) Alterations in the cortical thickness and the amplitude of low-frequency fluctuation in patients with post-traumatic stress disorder. Brain Res 15;1490:225-32

Bingham BC, Sheela Rani CS, Frazer A, Strong R, Morilak DA (2013) Exogenous prenatal corticosterone exposure mimics the effects of prenatal stress on adult brain stress response systems and fear extinction behavior. Psychoneuroendocrinology 38:2746–2757

Bisson JI, Cosgrove S, Lewis C, Robert NP (2015) Post-traumatic stress disorder. BMJ 26;351:h6161

Bland ST, Schmid MJ, Der-Avakian A, Watkins LR, Spencer RL, Maier SF (2005) Expression of c-fos and BDNF mRNA in subregions of the prefrontal cortex of male and female rats after acute uncontrollable stress. Brain Res 1051(1-2):90-9

Bloss EB, Janssen WG, McEwen BS, Morrison JH (2010) Interactive effects of stress and aging on structural plasticity in the prefrontal cortex. J Neurosci 2;30(19):6726-31

Borghans B, Homberg JR (2015) Animal models for posttraumatic stress disorder: An overview of what is used in research. World J Psychiatry 22;5(4):387-96

Bowley MP, Drevets WC, Ongür D, Price JL (2002) Low glial numbers in the amygdala in major depressive disorder. Biol Psychiatry 52(5):404-412

Bremner JD (1999) Does stress damage the brain? Biol Psychiatry 1;45(7):797-805

Bremner JD (2002) Neuroimaging studies in post-traumatic stress disorder. Curr Psychiatry Rep 4(4):254-263

Bremner JD, Elzinga B, Schmahl C, Vermetten E (2008) Structural and functional plasticity of the human brain in posttraumatic stress disorder. Prog Brain Res 167:171-186

Bremner JD, Narayan M, Staib LH, Southwick SM, McGlashan T, Charney DS (1999b) Neural correlates of memories of childhood sexual abuse in women with and without posttraumatic stress disorder. Am J Psychiatry 156(11):1787-95

Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB (1995) MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 152(7):973-981

Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, Capelli S, McCarthy G, Innis RB, Charney DS (1997) Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse—a preliminary report. Biol Psychiatry 41:23–32

Bremner JD, Staib LH, Kaloupek D, Southwick SM, Soufer R, Charney DS (1999a) Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. Biol Psychiatry 1;45(7):806-16

Bremner JD, Vermetten E, Vythilingam M, Afzal N, Schmahl C, Elzinga B, Charney DS (2004) Neural correlates of the classic color and emotional stroop in women with abuse-related posttraumatic stress disorder. Biol Psychiatry 15;55(6):612-20

Breslau N, Kessler RC, Chilcoat HD, Schultz LR, Davis GC, Andreski P (1998) Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. Arch Gen Psychiatry 55(7):626-632

Brewin CR, Dalgleish T, Joseph S (1996) A dual representation theory of posttraumatic stress disorder. Psychol Rev 103(4):670-686

Buchsbaum MS, Simmons AN, DeCastro A, Farid N, Matthews SC (2015) Clusters of Low (18)F-Fluorodeoxyglucose Uptake Voxels in Combat Veterans with Traumatic Brain Injury and Post-Traumatic Stress Disorder. J Neurotrauma 15;32(22):1736-50

Burbiel JC (2015) Primary prevention of posttraumatic stress disorder: drugs and implications. Mil Med Res 26;2:24

Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. J Neurosci 22:183–192

Canadian Agency for Drugs and Technologies in Health (2014) Intravenous Ketamine for the Treatment of Mental Health Disorders: A Review of Clinical Effectiveness and Guidelines

Carrasco GA, Van de Kar LD (2003) Neuroendocrine pharmacology of stress. Eur J Pharmacol 463(1-3):235-72

Carrion VG, Wong SS (2012) Can traumatic stress alter the brain? Understanding the implications of early trauma on brain development and learning. J Adolesc Health 51(2 Suppl):S23-8

Carter RN, Pinnock SB, Herbert J (2004) Does the amygdala modulate adaptation to repeated stress? Neuroscience 126:9–19

Catalani A, Sabbatini M, Consoli C, Cinque C, Tomassoni D, Azmitia E, Angelucci L, Amenta F (2002) Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus. Mech Ageing Dev 123:481-490

Chang JC, Yen AM, Chen HH, Chen SL, Chiu SY, Fann JC, Lee CS (2017) Comorbid diseases as risk factors for incident posttraumatic stress disorder (PTSD) in a large community cohort (KCIS no.PSY4). Sci Rep 27;7:41276

Cohen H, Kozlovsky N, Matar MA, Zohar J, Kaplan Z (2014) Distinctive hippocampal and amygdalar cytoarchitectural changes underlie specific patterns of behavioral disruption following stress exposure in an animal model of PTSD. Eur Neuropsychopharmacol 24:1925–1944

Cohen H, Zohar J, Matar M (2003) The relevance of differential response to trauma in an animal model of posttraumatic stress disorder. Biological Psychiatry 53(6):463–73

Cohen H, Zohar J, Matar MA, Zeev K, Loewenthal U, Richter-Levin G (2004) Setting apart the affected: the use of behavioral criteria in animal models of post traumatic stress disorder. Neuropsychopharmacology 29(11):1962–70

Corral-Frias NS, Lahood RP, Edelman-Vogelsang KE, French ED, Fellous JM (2013) Involvement of the ventral tegmental area in a rodent model of post-traumatic stress disorder. Neuropsychopharmacology 38:350–363

Cotter D, Mackay D, Chana G, Beasley C, Landau S, Everall IP (2002) Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. Cereb Cortex 12(4):386-394

Cui H, Sakamoto H, Higashi S, Kawata M (2008) Effects of single-prolonged stress on neurons and their afferent inputs in the amygdala. Neuroscience 152:703–712

Cukor J, Spitalnick J, Difede J, Rizzo A, Rothbaum BO (2009) Emerging treatments for PTSD. Clin Psychol Rev 29(8):715-726

Czéh B, Perez-Cruz C, Fuchs E, Flügge G (2008) Chronic stress-induced cellular changes in the medial prefrontal cortex and their potential clinical implications: does hemisphere location matter? Behav Brain Res 26;190(1):1-13

Czéh B, Simon M, Schmelting B, Hiemke C, Fuchs E (2006) Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 31(8):1616-1626

D'Andrea D, Sewell RA (2013) Transient resolution of treatment-resistant posttraumatic stress disorder following ketamine infusion. Biol Psychiatry 1;74(9):e13-4

Davis M (2006) Neural systems involved in fear and anxiety measured with fear-potentiated startle. Am Psychol 61:741–756

Delgado MR, Nearing KI, Ledoux JE, Phelps EA (2008) Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. Neuron 11;59(5):829-38

Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). Autor: American Psychiatric Association. Editora: American psychiatric - 5ª edição. Ano: 2013

Diehl LA, Silveira PP, Leite MC, Crema LM, Portella AK, Billodre MN, Nunes E, Henriques TP, Fidelix-da-Silva LB, Heis MD, Gonçalves CA, Quillfeldt JA, Dalmaz C (2007) Long lasting sex-specific effects upon behavior and S100b levels after maternal separation and exposure to a model of post-traumatic stress disorder in rats. Brain Res 4;1144:107-116

Ding J, Han F, Shi Y (2010) Single-prolonged stress induces apoptosis in the amygdala in a rat model of post-traumatic stress disorder. J Psychiatr Res 44(1):48-55

Ebner K, Rupniak NM, Saria A, Singewald N (2004) Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. Proc Natl Acad Sci U S A 23;101(12):4280-5

Faure J, Uys JD, Marais L, Stein DJ, Daniels WM (2007) Early maternal separation alters the response to traumatization: resulting in increased levels of hippocampal neurotrophic factors. Metab Brain Dis 22(2):183-95

Feder A, Parides MK, Murrough JW, Perez AM, Morgan JE, Saxena S, Kirkwood K, Aan Het Rot M, Lapidus KA, Wan LB, Iosifescu D, Charney DS (2014) Efficacy of

intravenous ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. JAMA Psychiatry 71(6):681-8

Fennrich S, Ray D, Nau H, Schlosshauer B (1998) Radial astrocytes: toxic effects induced by antiepileptic drug in the developing rat hippocampus in vitro. Eur J Cell Biol 77:142–150

Foa EB, Zinbarg R, Rothbaum BO (1992) Uncontrollability and unpredictability in post-traumatic stress disorder: an animal model. Psychol Bull 112(2):218-238

Friedman WJ, Black IB, Kaplan DR (1998) Distribution of the neurotrophins brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 in the postnatal rat brain: an immunocytochemical study. Neuroscience 84(1):101-114

George SA, Rodriguez-Santiago M, Riley J, Abelson JL, Floresco SB, Liberzon I (2015) Alterations in cognitive flexibility in a rat model of post-traumatic stress disorder. Behav Brain Res 1;286:256-64

Gilboa A, Shalev AY, Laor L, Lester H, Louzoun Y, Chisin R, Bonne O (2004) Functional connectivity of the prefrontal cortex and the amygdala in posttraumatic stress disorder. Biol Psychiatry 1;55(3):263-72

Giustino TF, Maren S (2015) The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear. Front Behav Neurosci 9;9:298

Godsil BP, Kiss JP, Spedding M, Jay TM (2013) The hippocampal-prefrontal pathway: the weak link in psychiatric disorders? Eur Neuropsychopharmacol 23(10):1165-81

Goldwater DS, Pavlides C, Hunter RG, Bloss EB, Hof PR, McEwen BS, Morrison JH (2009) Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. Neuroscience 1;164(2):798-808

Gould E, Tanapat P, McEwen BS, Flügge G, Fuchs E (1998) Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci U S A 17;95(6):3168-3171

Griffiths J, Shanks N, Anisman H (1992) Strain-specific alterations in consumption of a palatable diet following repeated stressor exposure. Pharmacol Biochem Behav 42(2):219-227

Grosman B, Shaik OS, Helwig BG, Leon LR, Doyle FJ 3rd (2011) A physiological systems approach to modeling and resetting of mouse thermoregulation under heat stress. J Appl Physiol 111(3):938-45

Grupe DW, Nitschke JB (2013) Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective. Nat Rev Neurosci 14(7):488-501

Hamidi M, Drevets WC, Price JL (2004) Glial reduction in amygdala in major depressive disorder is due to oligodendrocytes. Biol Psychiatry 55(6):563-569

Han F, Ding J, Shi Y (2014) Expression of amygdala mineralocorticoid receptor and glucocorticoid receptor in the single-prolonged stress rats. BMC Neurosci 19;15:77

Han F, Xiao B, Wen L (2015) Loss of Glial Cells of the Hippocampus in a Rat Model of Post-traumatic Stress Disorder. Neurochem Res 40(5):942-51

Harding AJ, Stimson E, Henderson JM, Halliday GM (2002) Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. Brain 125:2431-45

Hartley CA, Fischl B, Phelps EA (2011) Brain structure correlates of individual differences in the acquisition and inhibition of conditioned fear. Cereb Cortex 21(9):1954-62

Hauck S, Kapczinski F, Roesler R, de Moura Silveira E Jr, Magalhães PV, Kruel LR, Schestatsky SS, Ceitlin LH (2010) Serum brain-derived neurotrophic factor in patients with trauma psychopathology. Prog Neuropsychopharmacol Biol Psychiatry 34(3):459-62

Heim C, Nemeroff CB (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol Psychiatry 15;49(12):1023-39

Herrmann L, Ionescu IA, Henes K, Golub Y, Wang NX, Buell DR, Holsboer F, Wotjak CT, Schmidt U (2012) Long-lasting hippocampal synaptic protein loss in a mouse model of posttraumatic stress disorder. PLoS One 7(8):e42603

Hirota K, Lambert DG (1996) Ketamine: its mechanism(s) of action and unusual clinical uses. Br J Anaesth 77(4):441-444

Hou C, Liu J, Wang K, Li L, Liang M, He Z, Liu Y, Zhang Y, Li W, Jiang T (2007) Brain responses to symptom provocation and trauma-related short-term memory recall in coal mining accident survivors with acute severe PTSD. Brain Res 4;1144:165-74

Imbe H, Kimura A, Donishi T, Kaneoke Y (2012) Chronic restraint stress decreases glial fibrillary acidic protein and glutamate transporter in the periaqueductal gray matter. Neuroscience 223:209-218

Izquierdo I, Furini CR, Myskiw JC (2016) Fear Memory. Physiol Rev 96(2):695-750

Job RF, Barnes BW (1995) Stress and consumption: inescapable shock, neophobia, and quinine finickiness in rats. Behav Neurosci 109(1):106-116

Jovanovic T, Ressler K (2010) How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. Am J Psychiatry 167(6):648-62

Juven-Wetzler A, Cohen H, Kaplan Z, Kohen A, Porat O, Zohar J (2014) Immediate ketamine treatment does not prevent posttraumatic stress responses in an animal model for PTSD. Eur Neuropsychopharmacol 24(3):469-79

Kao CY, Stalla G, Stalla J, Wotjak CT, Anderzhanova E (2015) Norepinephrine and corticosterone in the medial prefrontal cortex and hippocampus predict PTSD-like symptoms in mice. Eur J Neurosci 41(9):1139-48

Kassem MS, Lagopoulos J, Stait-Gardner T, Price WS, Chohan TW, Arnold JC, Hatton SN, Bennett MR (2013) Stress-induced grey matter loss determined by MRI is primarily due to loss of dendrites and their synapses. Mol Neurobiol 47:645–661

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62(6):593-602

Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB (1995) Posttraumatic stress disorder in the National Comorbidity Survey. Arch Gen Psychiatry 52(12):1048-1060

Kikuchi A, Shimizu K, Nibuya M, Hiramoto T, Kanda Y, Tanaka T, Watanabe Y, Takahashi Y, Nomura S (2008) Relationship between post-traumatic stress disorder-like behavior and reduction of hippocampal 5-bromo-2'-deoxyuridine-positive cells after inescapable shock in rats. Psychiatry Clin Neurosci 62(6):713-720

Kim EJ, Pellman B, Kim JJ (2015) Stress effects on the hippocampus: a critical review. Learn Mem 18;22(9):411-6

Kim MJ, Chey J, Chung A, Bae S, Khang H, Ham B, Yoon SJ, Jeong DU, Lyoo IK (2008) Diminished rostral anterior cingulate activity in response to threat-related events in posttraumatic stress disorder. J Psychiatr Res 42(4):268-77

Kitayama N, Vaccarino V, Kutner M, Weiss P, Bremner JD (2005) Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. J Affect Disord 88(1):79-86

Kozlovsky N, Matar MA, Kaplan Z, Kotler M, Zohar J, Cohen H (2007) Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. Int J Neuropsychopharmacol 10(6):741-58

Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131(2):391-404

Krolow R, Noschang C, Arcego DM, Pettenuzzo LF, Weis SN, Marcolin ML, Huffell AP, Mota CS, Dalmaz C (2013) Isolation stress exposure and consumption of palatable diet during the prepubertal period leads to cellular changes in the hippocampus. Neurochem Res 38(2):262-272

Kühn S, Schubert F, Gallinat J (2011) Structural correlates of trait anxiety: reduced thickness in medial orbitofrontal cortex accompanied by volume increase in nucleus accumbens. J Affect Disord 134(1-3):315-9

Kuo JR, Kaloupek DG, Woodward SH (2012) Amygdala volume in combat-exposed veterans with and without posttraumatic stress disorder: a cross-sectional study. Arch Gen Psychiatry 69:1080–1086

Lancelot S, Zimmer L (2010) Small-animal positron emission tomography as a tool for neuropharmacology. Trends Pharmacol Sci 31(9):411-7

Leuner B, Gould E (2010) Structural plasticity and hippocampal function. Annu Rev Psychol 61:111-140

Li S, Murakami Y, Wang M, Maeda K, Matsumoto K (2006) The effects of chronic valproate and diazepam in a mouse model of posttraumatic stress disorder. PharmacolBiochem Behav 85:324–331

Li X, Han F, Liu D, Shi Y (2010a) Changes of Bax, Bcl-2 and apoptosis in hippocampus in the rat model of post-traumatic stress disorder. Neurol Res 32(6):579-586

Li XM, Han F, Liu DJ, Shi YX (2010b) Single-prolonged stress induced mitochondrial-dependent apoptosis in hippocampus in the rat model of post-traumatic stress disorder. J Chem Neuroanat 40(3):248-255

Liberzon I, Khan S, Young EA (2005) Animal models of posttraumatic stress disorder. Handbook of Stress and the Brain, Vol. 15. T. Steckler, N.H. Kalin and J.M.H.M. Reul (Eds.) pag:231-250

Liberzon I, Sripada C (2008) The functional neuroanatomy of PTSD: a critical review. Prog Brain Res 167:151–168

Liberzon I, Taylor SF, Amdur R, Jung TD, Chamberlain KR, Minoshima S, Koeppe RA, Fig LM (1999) Brain activation in PTSD in response to trauma-related stimuli. Biol Psychiatry 1;45(7):817-26

Lindauer RJ, Olff M, van Meijel EP, Carlier IV, Gersons BP (2006) Cortisol, learning, memory, and attention in relation to smaller hippocampal volume in police officers with posttraumatic stress disorder. Biol Psychiatry 15;59(2):171-177

Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, den Heeten GJ, Gersons BP (2004) Smaller hippocampal volume in Dutch police officers with posttraumatic stress disorder. Biol Psychiatry 56:356–363

Lindauer RJ, Vlieger EJ, Jalink M, Olff M, Carlier IV, Majoie CB, Den Heeten GJ, Gersons BP (2005) Effects of psychotherapy on hippocampal volume in out-patients with post-traumatic stress disorder: a MRI investigation. Psychol Med 35:1421–1431

Lissek S, Powers AS, McClure EB, Phelps EA, Woldehawariat G, Grillon C, Pine DS (2005) Classical fear conditioning in the anxiety disorders: a meta-analysis. Behav Res Ther 43(11):1391-424

Lo EH, Dalkara T, Moskowitz MA (2003) Mechanisms, challenges and opportunities in stroke, Nat Rev Neurosci 4:399-415

Lopresto D, Schipper P, Homberg JR (2016) Neural circuits and mechanisms involved in fear generalization: Implications for the pathophysiology and treatment of posttraumatic stress disorder. Neurosci Biobehav Rev 60:31-42

Lucassen PJ, Pruessner J, Sousa N, Almeida OF, Van Dam AM, Rajkowska G, Swaab DF, Czéh B (2014) Neuropathology of stress. Acta Neuropathol 127:109–135

Magariños AM, Verdugo JM, McEwen BS (1997) Chronic stress alters synaptic terminal structure in hippocampus. Proc Natl Acad Sci U S A 9;94(25):14002-14008

Maier SF (1989) Determinants of the nature of environmentally induced hypoalgesia. Behav Neurosci 103(1):131-143

Maier SF (1990) Role of fear in mediating shuttle escape learning deficit produced by inescapable shock. J Exp Psychol Anim Behav Process 16(2):137-149

Manji HK, Duman RS (2001) Impairments of neuroplasticity and cellular resilience in severe mood disorders: implications for the development of novel therapeutics. Psychopharmacol Bull 35(2):5-49

Marcuzzo S, Dall'oglio A, Ribeiro MF, Achaval M, Rasia-Filho AA (2007) Dendritic spines in the posterodorsal medial amygdala after restraint stress and ageing in rats. Neurosci Lett 424:16–21

Maroun M, Ioannides PJ, Bergman KL, Kavushansky A, Holmes A, Wellman CL (2013) Fear extinction deficits following acute stress associate with increased spine density and dendritic retraction in basolateral amygdala neurons. Eur J Neurosci 38:2611–2620

Martinotti G, Sepede G, Brunetti M, Ricci V, Gambi F, Chillemi E, Vellante F, Signorelli M, Pettorruso M, De Risio L, Aguglia E, Angelucci F, Caltagirone C, Di Giannantonio M (2015) BDNF concentration and impulsiveness level in post-traumatic stress disorder. Psychiatry Res 30;229(3):814-8

McEwen BS (2008) Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol 583(2-3):174-185

McGhee LL, Maani CV, Garza TH, Gaylord KM, Black IH (2008) The correlation between ketamine and posttraumatic stress disorder in burned service members. J Trauma 64:S195-199

McIntosh LJ, Sapolsky RM (1996) Glucocorticoids increase the accumulation of reactive oxygen species and enhance adriamycin-induced toxicity in neuronal culture. Exp Neurol 141(2):201-206

Mestriner RG, Pagnussat AS, Boisserand LS, Valentim L, Netto CA (2011) Skilled reaching training promotes astroglial changes and facilitated sensorimotor recovery after collagenase-induced intracerebral hemorrhage. Exp Neurol 227:53–61

Milad MR, Quinn BT, Pitman RK, Orr SP, Fischl B, Rauch SL (2005) Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. Proc Natl Acad Sci USA 26;102(30):10706-11

Mirshekar M, Abrari K, Goudarzi I, Rashidy-Pour A (2013) Systemic administrations of b-estradiol alleviate both conditioned and sensitized fear responses in an ovariectomized rat model of post-traumatic stress disorder. Neurobiol Learn Mem 102:12–19

Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S (2005) Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proc Natl Acad Sci U S A 28;102(26):9371-6

Molina ME, Isoardi R, Prado MN, Bentolila S (2010) Basal cerebral glucose distribution in long-term post-traumatic stress disorder. World J Biol Psychiatry (2 Pt 2):493-501

Moore H, Rose HJ, Grace AA (2001) Chronic cold stress reduces the spontaneous activity of ventral tegmental dopamine neurons. Neuropsychopharmacology 24:410–419

Morey RA, Gold AL, LaBar KS, Beall SK, Brown VM, Haswell CC, Nasser JD, Wagner HR, McCarthy G (2012) Amygdala volume changes in posttraumatic stress disorder in a large casecontrolled veterans group. Arch Gen Psychiatry 69:1169–1178

Musazzi L, Milanese M, Farisello P, Zappettini S, Tardito D, Barbiero VS, Bonifacino T, Mallei A, Baldelli P, Racagni G, Raiteri M, Benfenati F, Bonanno G, Popoli M (2010) Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants. PLoS One 5;5(1):e8566

Naughton M, Clarke G, O'Leary OF, Cryan JF, Dinan TG (2014) A review of ketamine in affective disorders: current evidence of clinical efficacy, limitations of use and pre-clinical evidence on proposed mechanisms of action. J Affect Disord 156:24-35

Nixdorf-Bergweiler BE, Albrecht B, Heinemann U (1994) Developmental changes in the number, size, and orientation of GFAP-positive cells in the CA1 region of rat hippocampus. Glia 12:180–195

Ongür D, Drevets WC, Price JL (1998) Glial reduction in the subgenual prefrontal cortex in mood disorders. Proc Natl Acad Sci U S A 95(22):13290-13295

Orr SP, Metzger LJ, Lasko NB, Macklin ML, Peri T, Pitman RK (2000) De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. J Abnorm Psychol 109(2):290-8

Park JA, Choi KS, Kim SY, Kim KW (2003) Coordinated interaction of the vascular and nervous systems: from molecule- to cell-based approaches. Biochem Biophys Res Commun 311:247-253

Pavlov IP (1927) Conditioned Reflexes: an investigation of the physiological activity of the cerebral cortex. Oxford University. Disponível em: http://s-f-walker.org.uk/pubsebooks/pdfs/Conditioned-Reflexes-Pavlov.pdf

Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32:421-43

Petty MA, Lo EH (2002) Junctional complexes of the blood–brain barrier: permeability changes in neuroinflammation. Progress in Neurobiology 68:311-323

Phan KL, Britton JC, Taylor SF, Fig LM, Liberzon I (2006) Corticolimbic blood flow during nontraumatic emotional processing in posttraumatic stress disorder. Arch Gen Psychiatry 63(2):184-92

Pietrzak RH, Averill LA, Abdallah CG, Neumeister A, Krystal JH, Levy I, Harpaz-Rotem I (2015) Amygdala-hippocampal volume and the phenotypic heterogeneity of posttraumatic stress disorder: a cross-sectional study. JAMA Psychiatry 72(4):396-8

Pitman RK (1988) Post-traumatic stress disorder, conditioning and network theory. Psychiatr Ann 18,182–189

Popoli M, Yan Z, McEwen BS, Sanacora G (2011) The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci 30;13(1):22-37

Price RB, Nock MK, Charney DS, Mathew SJ (2009) Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. Biol Psychiatry 1;66(5):522-526

Radley JJ, Rocher AB, Janssen WG, Hof PR, McEwen BS, Morrison JH (2005) Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. Exp Neurol 196(1):199-203

Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, McEwen BS, Morrison JH (2004) Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. Neuroscience 125(1):1-6

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, Overholser JC, Roth BL, Stockmeier CA (1999) Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 45(9):1085-1098

Ramage AE, Litz BT, Resick PA, Woolsey MD, Dondanville KA, Young-McCaughan S, Borah AM, Borah EV, Peterson AL, Fox PT (2016) Regional cerebral glucose metabolism differentiates danger- and non-danger-based traumas in post-traumatic stress disorder. Soc Cogn Affect Neurosci 11(2):234-42

Rasmusson AM, Shi L, Duman R (2002) Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. Neuropsychopharmacology 27(2):133-42

Rauch SL, van der Kolk BA, Fisler RE, Alpert NM, Orr SP, Savage CR, Fischman AJ, Jenike MA, Pitman RK (1996) A symptom provocation study of posttraumatic stress disorder using positron emission tomography and script-driven imagery. Arch Gen Psychiatry 53(5):380-387

Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, Orr SP, Pitman RK (2000) Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol Psychiatry 1;47(9):769-76

Ravindran R, Rathinasamy SD, Samson J, Senthilvelan M (2005) Noise-stress-induced brain neurotransmitter changes and the effect of Ocimum sanctum (Linn) treatment in albino rats. J Pharmacol Sci 98(4):354-60

Reber SO (2012) Stress and animal models of inflammatory bowel disease--an update on the role of the hypothalamo-pituitary-adrenal axis. Psychoneuroendocrinology 37(1):1-19

Rilling JK, Winslow JT, O'Brien D, Gutman DA, Hoffman JM, Kilts CD (2001) Neural correlates of maternal separation in rhesus monkeys. Biol Psychiatry 49(2):146-57

Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA (2004) Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol Psychiatry 55:708–714

Roozendaal B, McGaugh JL (1996) Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. Neurobiol Learn Mem 65:1–8

Ryoke R, Yamada K, Ichitani Y (2014) Long-term effects of traumatic stress on subsequent contextual fear conditioning in rats. Physiol Behav 129:30–35

Sapolsky RM (2003) Stress and plasticity in the limbic system. Neurochem Res 28(11):1735-1742

Sapolsky RM, Krey LC, McEwen BS (1985) Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. J Neurosci 5(5):1222-7

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

Saur L, Baptista PP, de Senna PN, Paim MF, Nascimento PD, Ilha J, Bagatini PB, Achaval M, Xavier LL (2014) Physical exercise increases GFAP expression and induces morphological changes in hippocampal astrocytes. Brain Struct Funct 219:293–302

Schmahl C, Berne K, Krause A, Kleindienst N, Valerius G, Vermetten E, Bohus M (2009) Hippocampus and amygdala volumes in patients with borderline personality disorder with or without posttraumatic stress disorder. J Psychiatry Neurosci 34:289–295

Schönenberg M, Reichwald U, Domes G, Badke A, Hautzinger M (2005) Effects of peritraumatic ketamine medication on early and sustained posttraumatic stress symptoms in moderately injured accident victims. Psychopharmacology 182(3):420-5

Schönenberg M, Reichwald U, Domes G, Badke A, Hautzinger M (2008) Ketamine aggravates symptoms of acute stress disorder in a naturalistic sample of accident victims. J Psychopharmacol 22(5):493-7

Shekhar A, Truitt W, Rainnie D, Sajdyk T (2005) Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. Stress 8(4):209-19

Shin LM, Handwerger K (2009) Is posttraumatic stress disorder a stress-induced fear circuitry disorder? J Trauma Stress 22(5):409-15

Shin LM, McNally RJ, Kosslyn SM, Thompson WL, Rauch SL, Alpert NM, Metzger LJ, Lasko NB, Orr SP, Pitman RK (1999) Regional cerebral blood flow during script-driven imagery in childhood sexual abuse-related PTSD: A PET investigation. Am J Psychiatry 156(4):575-584

Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, Peters PM, Metzger LJ, Dougherty DD, Cannistraro PA, Alpert NM, Fischman AJ, Pitman RK (2004b) Regional cerebral blood flow in the amygdala and medial prefrontal cortex during

traumatic imagery in male and female Vietnam veterans with PTSD. Arch Gen Psychiatry 61(2):168-76

Shin LM, Rauch SL, Pitman RK (2006) Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. Ann N Y Acad Sci 1071:67-79

Shin LM, Shin PS, Heckers S, Krangel TS, Macklin ML, Orr SP, Lasko N, Segal E, Makris N, Richert K, Levering J, Schacter DL, Alpert NM, Fischman AJ, Pitman RK, Rauch SL (2004a) Hippocampal function in posttraumatic stress disorder. Hippocampus 14(3):292-300

Shin LM, Whalen PJ, Pitman RK, Bush G, Macklin ML, Lasko NB, Orr SP, McInerney SC, Rauch SL (2001) An fMRI study of anterior cingulate function in posttraumatic stress disorder. Biol Psychiatry 15;50(12):932-42

Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, Macklin ML, Lasko NB, Cavanagh SR, Krangel TS, Orr SP, Pitman RK, Whalen PJ, Rauch SL (2005) A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 62(3):273-81

Short KR, Maier SF (1993) Stressor controllability, social interaction, and benzodiazepine systems. Pharmacol Biochem Behav 45(4):827-835

Shvil E, Rusch HL, Sullivan GM, Neria Y (2013) Neural, psychophysiological, and behavioral markers of fear processing in PTSD: a review of the literature. Curr Psychiatry Rep 15(5):358

Siegmund A, Wotjak CT (2007) A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitized fear. J Psychiatr Res 41:848–860

Sofuoglu M, Rosenheck R, Petrakis I (2014) Pharmacological treatment of comorbid PTSD and substance use disorder: recent progress. Addict Behav 39(2):428-33

Sousa N, Almeida OF (2012) Disconnection and reconnection: the morphological basis of (mal)adaptation to stress. Trends Neurosci 35(12):742-51

Steimer T (2002) The biology of fear- and anxiety-related behaviors. Dialogues Clin Neurosci 4(3):231-49

Stocker RP, Cieply MA, Paul B, Khan H, Henry L, Kontos AP, Germain A (2014) Combat-related blast exposure and traumatic brain injury influence brain glucose metabolism during REM sleep in military veterans. Neuroimage 99:207-14

Sussman D, Pang EW, Jetly R, Dunkley BT, Taylor MJ (2016) Neuroanatomical features in soldiers with post-traumatic stress disorder. BMC Neurosci 31;17:13

Takuma K, Baba A, Matsuda T (2004) Astrocyte apoptosis: implications for neuroprotection. Prog Neurobiol 72(2):111-127

Theodosis DT, Poulain DA, Oliet SH (2008) Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. Physiol Rev 88:983-1008

Tsigos C, Chrousos GP (2002) Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res 53(4):865-871

Tulogdi A, Sörös P, Tóth M, Nagy R, Biró L, Aliczki M, Klausz B, Mikics E, Haller J (2012) Temporal changes in c-Fos activation patterns induced by conditioned fear. Brain Res Bull 88:359–370

Tynan RJ, Beynon SB, Hinwood M, Johnson SJ, Nilsson M, Woods JJ, Walker FR (2013) Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. Acta Neuropathol 126:75–91

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001) Control of synapse number by glia. Science 291:657-61

van der Kolk B, Greenberg M, Boyd H, Krystal J (1985) Inescapable shock, neurotransmitters, and addiction to trauma: toward a psychobiology of post traumatic stress. Biol Psychiatry 20(3):314-325

Veer IM, Oei NY, van Buchem MA, Spinhoven P, Elzinga BM, Rombouts SA (2015) Evidence for smaller right amygdala volumes in posttraumatic stress disorder following childhood trauma. Psychiatry Res 30;233(3):436-42

Verkhratsky A, Butt A (2007) Glial Neurobiology. Wiley, England

Viola GG, Rodrigues L, Américo JC, Hansel G, Vargas RS, Biasibetti R, Swarowsky A, Gonc¸alves CA, Xavier LL, Achaval M, Souza DO, Amaral OB (2009) Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice. Brain Res 1274:47–54

Virdee K, Cumming P, Caprioli D, Jupp B, Rominger A, Aigbirhio FI, Fryer TD, Riss PJ, Dalley JW (2012) Applications of positron emission tomography in animal models of neurological and neuropsychiatric disorders. Neurosci Biobehav Rev 36(4):1188-216

Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci 1;22(15):6810-8

Wang W, Liu Y, Zheng H, Wang HN, Jin X, Chen YC, Zheng LN, Luo XX, Tan QR (2008) A modified single-prolonged stress model for post-traumatic stress disorder. Neurosci Lett 441:237–241

Watanabe Y, Gould E, McEwen BS (1992) Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res 21;588(2):341-345

Watson JB, Rayner R (2000) Conditioned emotional reactions. 1920. Am Psychol 55(3):313-317

Weniger G, Lange C, Sachsse U, Irle E (2008) Amygdala and hippocampal volumes and cognition in adult survivors of childhood abuse with dissociative disorders. Acta Psychiatr Scand 118:281–290

Wessa M, Flor H (2007) Failure of extinction of fear responses in posttraumatic stress disorder: evidence from second-order conditioning. Am J Psychiatry 164(11):1684-92

Williams LM, Kemp AH, Felmingham K, Barton M, Olivieri G, Peduto A, Gordon E, Bryant RA (2006) Trauma modulates amygdala and medial prefrontal responses to consciously attended fear. Neuroimage 15;29(2):347-57

Wilson CB, Ebenezer PJ, McLaughlin LD, Francis J (2014) Predator exposure/psychosocial stress animal model of post-traumatic stress disorder modulates neurotransmitters in the rat hippocampus and prefrontal cortex. PLoS One 14;9(2):e89104

Winter H, Irle E (2004) Hippocampal volume in adult burn patients with and without posttraumatic stress disorder. Am J Psychiatry 161(12):2194-200

Woolley CS, Gould E, McEwen BS (1990) Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res 29;531(1-2):225-231

Xia L, Zhai M, Wang L, Miao D, Zhu X, Wang W (2013) FGF2 blocks PTSD symptoms via an astrocyte-based mechanism. Behav Brain Res 256:472–480

Yang P, Wu MT, Hsu CC, Ker JH (2004) Evidence of early neurobiological alternations in adolescents with posttraumatic stress disorder: a functional MRI study. Neurosci Lett 3;370(1):13-8

Yehuda R (2002) Post-traumatic stress disorder. N Engl J Med 10;346(2):108-14

Yehuda R, Antelman SM (1993) Criteria for rationally evaluating animal models of posttraumatic stress disorder. Biol Psychiatry 1;33(7):479-486

Yehuda R, Harvey PD, Golier JA, Newmark RE, Bowie CR, Wohltmann JJ, Grossman RA, Schmeidler J, Hazlett EA, Buchsbaum MS (2009) Changes in relative glucose metabolic rate following cortisol administration in aging veterans with posttraumatic stress disorder: an FDG-PET neuroimaging study. J Neuropsychiatry Clin Neurosci 21(2):132-43

Zarate CA Jr, Brutsche NE, Ibrahim L, Franco-Chaves J, Diazgranados N, Cravchik A, Selter J, Marquardt CA, Liberty V, Luckenbaugh DA (2012) Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. Biol Psychiatry 1;71(11):939-946

Zeng MC, Niciu MJ, Luckenbaugh DA, Ionescu DF, Mathews DC, Richards EM, Franco-Chaves J, Brutsche NE, Zarate CA Jr (2013) Acute stress symptoms do not worsen in posttraumatic stress disorder and abuse with a single subanesthetic dose of ketamine. Biol Psychiatry 15;73(12)

Zhang LM, Zhou WW, Ji YJ, Li Y, Zhao N, Chen HX, Xue R, Mei XG, Zhang YZ, Wang HL, Li YF (2015) Anxiolytic effects of ketamine in animal models of posttraumatic stress disorder. Psychopharmacology 232(4):663-72

Zhang W, Hetzel A, Shah B, Atchley D, Blume SR, Padival MA, Rosenkranz JA (2014) Greater physiological and behavioral effects of interrupted stress pattern compared to daily restraint stress in rats. PLoS One 9:e102247

Zhu Y, Du R, Zhu Y, Shen Y, Zhang K, Chen Y, Song F, Wu S, Zhang H, Tian M (2016) PET Mapping of Neurofunctional Changes in a Post-traumatic Stress Disorder Model. J Nucl Med Mar 16. pii: jnumed.116.173443

Zimmerman JM, Maren S (2010) NMDA receptor antagonism in the basolateral but not central amygdala blocks the extinction of Pavlovian fear conditioning in rats. Eur J Neurosci 31(9):1664-70

Anexos

ANEXO A – Carta de aprovação da comissão de ética para o uso de animais

ANEXO B – Artigos publicados no período de doutoramento

ANEXO B1 – Artigo publicado “An evaluation of aversive memory and hippocampal oxidative status in streptozotocin-induced diabetic rats treated with resveratrol”. Neuroscience Letters, 2016.

ANEXO B2 – Artigo publicado “Astrocyte morphology after ischemic and hemorrhagic experimental stroke has no influence on the different recovery patterns”, Behavioural Brain Research, 278:257-261; 2015.

ANEXO B3 – Artigo publicado “Physical training improves non-spatial memory, locomotor skills and the blood brain barrier in diabetic rats”, Brain Research, 1618:75-82; 2015.

ANEXO B4 – Artigo publicado “Antidepressant Effects of Ketamine Are Not Related to 18F-FDG Metabolism or Tyrosine Hydroxylase Immunoreactivity in the Ventral Tegmental Area of Wistar Rats”, Neurochemical Research, 40:1153-1164; 2015.

ANEXO B5 – Artigo publicado “Physical exercise increases GFAP expression and induces morphological changes in hippocampal astrocytes”, Brain Structure & Function, 219:293-302; 2014.

ANEXO B6 – Artigo publicado “Sunitinib Improves Some Clinical Aspects and Reverts DMBA-Induced Hyperplasic Lesions in Hamster Buccal Pouch”, ISRN Otolaryngology, 2014:859621; 2014.

ANEXO B7 – Artigo publicado “Effect of prior exercise training and myocardial infarction-induced heart failure on the neuronal and glial densities and the GFAP-immunoreactivity in the posterodorsal medial amygdala of rats”, Histology and Histopathology, 29:1423-1435; 2014

ANEXO B8 – Artigo publicado “Resveratrol prevents akinesia and restores neuronal tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta of diabetic rats”, Brain Research, 1592:101-112; 2014.

ANEXO B9 – Artigo publicado “Sexual Dimorphism in the Human Vocal Fold Innervation”, Journal of Voice, 27:267-272; 2013.

ANEXO B10 – Artigo publicado “Physical exercise down-regulated locomotor side effects induced by haloperidol treatment in Wistar rats”, Pharmacology, Biochemistry and Behavior, 104:113-118; 2013.

ANEXO B11 – Artigo publicado “Behavior outcome after ischemic and hemorrhagic stroke, with similar brain damage, in rats”, Behavioural Brain Research, 244:82-89; 2013

Anexo A

Carta de Aprovação da Comissão de Ética
para o Uso de Animais

CEUA/PUCRS 13/00350

Ofício 95/13 - CEUA

Porto Alegre, 11 de novembro de 2013.

Prezado Sr(a). Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou a solicitação para alteração de modelo animal(de camundongos para ratos), datada de 22 de outubro, referente ao Protocolo de Pesquisa, registro CEUA 13/00350, intitulado **"Análise comportamental e histofisiológica dos efeitos da Cetamina em um modelo experimental e estresse pós-traumático".**

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está **autorizada**, a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Atenciosamente,

Prof. Dr. João Batista Blessmann Weber
Coordenador da CEUA/PUCRS

Ilmo. Sr.
Prof. Dr. Léder Leal Xavier
FABIO
Nesta Universidade

Anexo B

Artigos Publicados no período de doutoramento

Neuroscience Letters 636 (2017) 184–189

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

An evaluation of aversive memory and hippocampal oxidative status in streptozotocin-induced diabetic rats treated with resveratrol

Pamela Brambilla Bagatini[a,b,*], Léder Leal Xavier[c], Karine Bertoldi[d], Felipe Moysés[d], Gisele Lovatel[d], Laura Tartari Neves[c], Sílvia Barbosa[a], Lisiani Saur[c], Priscylla Nunes de Senna[b], André Arigony Souto[e], Ionara Rodrigues Siqueira[d], Matilde Achaval[a,b,*]

[a] Laboratório de Histofisiologia Comparada, Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Avenida Sarmento Leite, 500, 90040-060, Porto Alegre, RS, Brazil
[b] Programa de Pós-Graduação Ciências Biológicas: Neurociências, Universidade Federal do Rio Grande do Sul, Avenida Sarmento Leite, 500, 90040-060, Porto Alegre, RS, Brazil
[c] Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900, Porto Alegre, RS, Brazil
[d] Programa de Pós-Graduação em Ciências Biológicas: Fisiologia, Universidade Federal do Rio Grande do Sul, Avenida Sarmento Leite, 500, 90040-060, Porto Alegre, RS, Brazil
[e] Laboratório de Química de Produtos Naturais, Departamento de Química Pura, Faculdade de Química, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900, Porto Alegre, RS, Brazil

HIGHLIGHTS

- Diabetic rats exhibited normal freezing response in contextual fear conditioning.
- Hippocampal oxidative status was unaltered in diabetic rats.
- Resveratrol oral treatment had no significant effects in healthy or diabetic rats.

ARTICLE INFO

Article history:
Received 2 July 2016
Received in revised form
17 September 2016
Accepted 29 October 2016
Available online 31 October 2016

Keywords:
Diabetes
Resveratrol
Aversive memory
Free radical content
Antioxidant activity

ABSTRACT

The present study evaluated the effects of streptozotocin (STZ)-induced diabetes on aversive memory, free radical content and enzymatic antioxidant activity in the hippocampus of adult *Wistar* rats submitted to oral treatment with resveratrol. Animals were divided into eight groups: non-diabetic rats treated with saline (ND SAL), non-diabetic rats treated with resveratrol at a dose 5 mg/kg (ND RSV 5), non-diabetic rats treated with resveratrol at a dose 10 mg/kg (ND RSV 10), non-diabetic rats treated with resveratrol at a dose 20 mg/kg (ND RSV 20), diabetic rats treated with saline (D SAL), diabetic rats treated with resveratrol at a dose 5 mg/kg (D RSV 5), diabetic rats treated with resveratrol at a dose 10 mg/kg (D RSV 10) and diabetic rats treated with resveratrol at a dose 20 mg/kg (D RSV 20). The animals received oral gavage for 35 days. The contextual fear conditioning task was performed to evaluate aversive-based learning and memory. The oxidative status was evaluated in the hippocampus, by measuring the free radical content – using a 2′,7′-dichlorofluorescein diacetate probe – and enzymatic antioxidant activities, such as superoxide dismutase and glutathione peroxidase. Our main behavioral results demonstrated that rats from the D RSV 10 and D RSV 20 groups showed an increase in freezing behavior when compared, respectively, to the ND RSV 10 ($p < 0.01$) and ND RSV 20 ($p < 0.05$). Oxidative stress parameters remained unchanged in the hippocampus of all the experimental groups. In contrast to previous experimental findings, our study was unable to detect either cognitive impairments or oxidative stress in the hippocampus

* Corresponding authors at: Laboratório de Histofisiologia Comparada, Departamento de Ciências Morfológicas, Universidade Federal do Rio Grande do Sul (UFRGS), Avenida Sarmento Leite 500, Instituto de Ciências Básicas da Saúde, Sala 312, CEP 90050-170, Porto Alegre, RS, Brazil.
E-mail addresses: pamela.bagatini@yahoo.com.br, pamela@hemocord.com.br (P.B. Bagatini), machaval@pq.cnpq.br (M. Achaval).

http://dx.doi.org/10.1016/j.neulet.2016.10.050

Behavioural Brain Research 278 (2015) 257–261

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Astrocyte morphology after ischemic and hemorrhagic experimental stroke has no influence on the different recovery patterns

Régis Gemerasca Mestriner[a,b,c,d,*], Lisiani Saur[d], Pamela Brambilla Bagatini[d], Pedro Porto Alegre Baptista[d], Sabrina Pereira Vaz[c,d], Kelly Ferreira[c,d], Susane Alves Machado[c,d], Léder Leal Xavier[d], Carlos Alexandre Netto[a,b]

[a] Programa de Pós-Graduação em Ciências Biológicas: Fisiologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Faculdade de Enfermagem, Nutrição e Fisioterapia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[d] Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

HIGHLIGHTS

- Long-term astrocyte morphology has no influence on the different recovery patterns of stroke.
- Ischemic and hemorrhagic stroke subtypes have similar long-term astrocyte morphology in perilesional sensorimotor cortex and dorsolateral striatum.
- Long-term GFAP immunoreactivity profile is similar between ischemic and hemorrhagic stroke.

ARTICLE INFO

Article history:
Received 26 July 2014
Received in revised form
29 September 2014
Accepted 2 October 2014
Available online 12 October 2014

Keywords:
Ischemic stroke
Hemorrhagic stroke
Glial fibrillary acidic protein
Astrocytes
Reactive astrogliosis

ABSTRACT

Stroke, broadly subdivided into ischemic and hemorrhagic subtypes, is a serious health-care problem worldwide. Previous studies have suggested ischemic and hemorrhagic stroke could present different functional recovery patterns. However, little attention has been given to this neurobiological finding. Coincidently, astrocyte morphology could be related to improved sensorimotor recovery after skilled reaching training and modulated by physical exercise and environmental enrichment. Therefore, it is possible that astrocyte morphology might be linked to differential recovery patterns between ischemic and hemorrhagic stroke. Thus, we decided to compare long-term GFAP-positive astrocyte morphology after ischemic (IS, n = 5), hemorrhagic (HS, n = 5) and sham (S, n = 5) stroke groups (induced by endothelin-1, collagenase type IV-S and salina, respectively). Our results showed ischemic and hemorrhagic stroke subtypes induced similar long-term GFAP-positive astrocyte plasticity ($P > 0.05$) for all evaluated measures (regional and cellular optical density; astrocytic primary processes ramification and length; density of GFAP positive astrocytes) in perilesional sensorimotor cortex and striatum. These interesting negative results discourage similar studies focused on long-term plasticity of GFAP-positive astrocyte morphology and recovery comparison of stroke subtypes.

Stroke, broadly subdivided into ischemic and hemorrhagic subtypes, is a serious health-care problem worldwide [1]. Some clinical studies have shown that by the time of hospital discharge hemorrhagic stroke presents a better functional improvement compared to ischemic stroke [2]. Moreover, ischemic stroke patients showed a longer functional recovery time window than those with the hemorrhagic etiology [3]. Yet, these recovery differences are not completely understood due to stroke heterogeneity [4]. Animal models aid researchers to control some factors and provide an unbiased analysis of stroke subtypes. We have recently published a study, using two controlled lesion "site and size" rat models, showing the spontaneous recovery pattern is better in hemorrhagic than in ischemic stroke [5]. Unfortunately, the neurobiological explanations for this finding remain poorly understood.

Coincidentally, our research group has also shown astrocyte morphology could be related to improved sensorimotor recovery after a rehabilitation protocol [6] and modulated by physical

* Corresponding author at: Faculdade de Enfermagem, Nutrição e Fisioterapia, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, Prédio 12/8° andar, Porto Alegre CEP 90619-900, RS, Brazil. Tel.: +55 51 33203646; fax: +55 51 33203646.
E-mail address: regis.mestriner@pucrs.br (R.G. Mestriner).

http://dx.doi.org/10.1016/j.bbr.2014.10.005
0166-4328/

BRAIN RESEARCH 1618 (2015) 75–82

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Physical training improves non-spatial memory, locomotor skills and the blood brain barrier in diabetic rats

Priscylla Nunes de Senna[a,b], Léder Leal Xavier[b], Pamela Brambilla Bagatini[a], Lisiani Saur[b], Fabiana Galland[c], Caroline Zanotto[c], Caren Bernardi[d], Patrícia Nardin[c], Carlos Alberto Gonçalves[a,c], Matilde Achaval[a,*]

[a]Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b]Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c]Departamento de Bioquímica, Instituto de Ciências Básica da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[d]Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

ARTICLE INFO

Article history:
Accepted 22 May 2015
Available online 29 May 2015

Keywords:
Diabetes
Exercise
Claudin-5
Aquaporin-4
Blood–brain barrier
Non-spatial memory

ABSTRACT

Type 1 diabetes mellitus (T1DM) progressively affects cognitive domains, increases blood–brain barrier (BBB) permeability and promotes neurovascular impairment in specific brain areas. Physical exercise, on the other hand, has beneficial effects on brain functions, improving learning and memory. This study investigated the effects of treadmill training on cognitive and motor behavior, and on the expression of proteins related to BBB integrity, such as claudin-5 and aquaporin-4 (AQP4) in the hippocampus and striatum in diabetic rats. For this study, 60 Wistar rats were divided into four groups (n=15 per group): non-trained control (NTC), trained control (TC), non-trained diabetic (NTD), trained diabetic (TD). After diabetic induction of 30 days by streptozotocin injection, the exercise groups were submitted to 5 weeks of running training. After that, all groups were assessed in a novel object-recognition task (NOR) and the rotarod test. Additionally, claudin-5 and AQP4 levels were measured using biochemical assays. The results showed that exercise enhanced NOR task performance and rotarod ability in the TC and TD animals. Diabetes produced a decrease in claudin-5 expression in the hippocampus and striatum and reduced AQP4 in the hippocampus. Exercise preserved the claudin-5 content in the striatum of TD rats, but not in the hippocampus. The reduction of AQP4 levels produced by diabetes was not reversed by exercise. We conclude that exercise improves short-term memory retention, enhances motor performance in diabetic rats and affects important

*Correspondence to: Laboratório de Histofisiologia Comparada, Departamento de Ciências Morfológicas, ICBS, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Sarmento Leite 500, CEP 90050-170 Porto Alegre, RS, Brazil.
E-mail address: machaval@pq.cnpq.br (M. Achaval).

http://dx.doi.org/10.1016/j.brainres.2015.05.026

Neurochem Res (2015) 40:1153–1164
DOI 10.1007/s11064-015-1576-3

ORIGINAL PAPER

Antidepressant Effects of Ketamine Are Not Related to ^{18}F-FDG Metabolism or Tyrosine Hydroxylase Immunoreactivity in the Ventral Tegmental Area of Wistar Rats

Pedro Porto Alegre Baptista[1] · Lisiani Saur[1] · Pamela Bambrilla Bagatini[1] · Samuel Greggio[2] · Gianina Teribele Venturin[2] · Sabrina Pereira Vaz[1] · Kelly dos Reis Ferreira[1] · Juliana Silva Junqueira[1] · Diogo Rizzato Lara[1] · Jaderson Costa DaCosta[2] · Cristina Maria Moriguchi Jeckel[2] · Régis Gemerasca Mestriner[1] · Léder Leal Xavier[1,2]

Received: 24 November 2014 / Revised: 16 March 2015 / Accepted: 6 April 2015 / Published online: 17 April 2015

Abstract Major depressive disorder (MDD) is an important health problem that is often associated to stress. One of the main brain regions related to MDD is the ventral tegmental area (VTA), a dopaminergic center, part of the reward and motivation circuitry. Recent studies show that changes to VTA dopaminergic neurons are associated with depression and treatment. Ketamine has recently shown a fast, potent antidepressant effect in acute, sub-anesthetic doses. Thus, our aims were to elucidate if ketamine would be able to revert depression-like behaviors induced by a chronic unpredictable stress (CUS) protocol and if it could cause alterations to metabolism and tyrosine hydroxylase (TH)-immunoreactivity in VTA. For this, 48 Wistar rats were divided into four groups: control + saline (CTRL + SAL), control + ketamine (CTRL + KET), CUS + saline (CUS + SAL), CUS + ketamine (CUS + KET). The CUS groups underwent 28 days of CUS protocol. Saline or ketamine (10 mg/kg) was administered intraperitonially once on day 28. The behavior was assessed by the sucrose preference test, the open field test, and the forced swim test. Glucose brain metabolism was assessed and quantified with microPET. TH-immunoreactivity was assessed by estimating neuronal density and regional and cellular optical densities. A decrease in sucrose intake in the CUS groups and an increase in immobility was rapidly reverted by ketamine ($p < 0.05$). No difference was observed in the open field test. There was no alteration to VTA metabolism and TH-immunoreaction. These results suggest that the depressive-like behavior induced by CUS and the antidepressant effects of ketamine are unrelated to changes in neuronal metabolism or dopamine production in VTA.

Keywords Depression · Ketamine · MicroPET · Tyrosine hydroxylase · Ventral tegmental area

✉ Pedro Porto Alegre Baptista
pedroposa@gmail.com; pedro.baptista@acad.pucrs.br

[1] Laboratório de Biologia Celular e Tecidual e Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Ciências Morfofisiológicas, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Avenida Ipiranga, 6681, Prédio 12, Sala 104, Porto Alegre, RS CEP 90619-900, Brazil

[2] Centro de Pesquisa Pré-Clínica e Centro de Produção de Radiofármacos, Instituto do Cérebro do Rio Grande do Sul – INSCER-PUCRS, Porto Alegre, Brazil

Introduction

Major depressive disorder (MDD) is a psychiatric illness affecting 300–350 million people worldwide that baffles doctors and researchers because of its complexity. Despite scientific efforts, the cause or origin of MDD remains unknown [17, 27, 64, 66]. As there is no apparent precise organic alteration, it is classified as a mood disorder characterized by chronic feelings of sadness, hopelessness, and self-worthlessness, often leading to suicidal thoughts and behaviors [3, 62]. In the attempt to understand the condition, researchers are currently investigating many brain regions, such as the prefrontal cortex, amygdala, and cingulate cortex. However, the core symptoms of MDD, anhedonia and lack of motivation, are typically associated with the reward and motivation circuitry, specifically, with an important dopaminergic center, the ventral tegmental area (VTA) [8, 48, 66].

The VTA, located in the ventral part of the mesencephalon, consists mainly of dopaminergic neurons (60–65 %) [48]. It is believed to be the initial region in the reward and motivation system and, interestingly enough, it

Brain Struct Funct (2014) 219:293–302
DOI 10.1007/s00429-012-0500-8

ORIGINAL ARTICLE

Physical exercise increases GFAP expression and induces morphological changes in hippocampal astrocytes

Lisiani Saur · Pedro Porto Alegre Baptista · Priscylla Nunes de Senna · Mariana Fontoura Paim · Patricia do Nascimento · Jocemar Ilha · Pamela Brambilla Bagatini · Matilde Achaval · Léder Leal Xavier

Received: 6 August 2012 / Accepted: 20 December 2012 / Published online: 4 January 2013

Abstract Physical exercise has an important influence on brain plasticity, which affects the neuron–glia interaction. Astrocytes are susceptible to plasticity, and induce and stabilize synapses, regulate the concentration of various molecules, and support neuronal energy metabolism. The aim of our study was to investigate whether physical exercise is capable of altering the morphology, density and expression of glial fibrillary acidic protein (GFAP) in astrocytes from the CA1 region of rat hippocampus. Thirteen male rats were divided in two groups: sedentary ($n = 6$) and exercise ($n = 7$). The animals in the exercise group were submitted to a protocol of daily physical exercise on a treadmill for four consecutive weeks. GFAP immunoreactivity was evaluated using optical densitometry and the morphological analyses were an adaptation of Sholl's concentric circles method. Our results show that physical exercise is capable of increasing the density of GFAP-positive astrocytes as well as the regional and cellular GFAP expression. In addition, physical exercise altered astrocytic morphology as shown by the increase observed in the degree of ramification in the lateral quadrants and in the length of the longest astrocytic processes in the central quadrants. Our data demonstrate important changes in astrocytes promoted by physical exercise, supporting the idea that these cells are involved in regulating neural activity and plasticity.

Keywords Physical exercise · Astrocytes · GFAP · Hippocampus · Sholl

L. Saur · P. P. A. Baptista · M. F. Paim · J. Ilha · L. L. Xavier (✉)
Departamento de Ciências Morfofisiológicas, Laboratório de Biologia Celular e Tecidual, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Avenida Ipiranga, 6681, Prédio 12C, Sala 104, Porto Alegre, RS CEP 90619-900, Brazil
e-mail: llxavier@pucrs.br

P. N. de Senna · P. d. Nascimento · P. B. Bagatini · M. Achaval
Departamento de Ciências Morfológicas, Laboratório de Histofisiologia Comparada, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul., Avenida Sarmento Leite, 500, Porto Alegre, RS 90040-060, Brazil

Introduction

There is a considerable evidence to show that physical exercise has a positive effect on brain function in both humans (Hillman et al. 2008; Laurin et al. 2001) and animals (Albeck et al. 2006; Farmer et al. 2004; Stranahan et al. 2010). Physical exercise improves cognitive functions (Kashihara et al. 2009; Kramer et al. 2006) and memory (Alaei et al. 2008; de Senna et al. 2011), reduces anxiety and depression (Martinsen 2008), and has protective properties on a wide variety of neurological diseases, such as Parkinson's disease (Smith and Zigmond 2003), Alzheimer's disease (Mirochnic et al. 2009) and ischemic stroke (Stummer et al. 1994).

Studies designed to shed light on the neurobiological bases of these benefits have demonstrated that physical exercise is involved in cerebral plasticity. Exercise can induce neurogenesis (Kim et al. 2003; van Praag et al. 1999a, b, 2005) and increase the release of neurotrophic factors such as: brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), fibroblast growth factor (FGF) and their miRNAs (Berchtold et al. 2010; Gómez-Pinilla et al. 1997; Neeper et al. 1996). Physical exercise can also induce long-term potentiation (LTP) (van Praag et al. 1999a)

Hindawi Publishing Corporation
ISRN Otolaryngology
Volume 2014, Article ID 859621, 7 pages
http://dx.doi.org/10.1155/2014/859621

Research Article

Sunitinib Improves Some Clinical Aspects and Reverts DMBA-Induced Hyperplasic Lesions in Hamster Buccal Pouch

Fernanda Lopes de Souza, Mariana Oliveira, Marianne Brochado Nunes, Lucas Horstmann Serafim, Alan Arrieira Azambuja, Luisa Maria G. de M. Braga, Lisiani Saur, Maria Antonieta Lopes de Souza, and Léder Leal Xavier

Laboratório de Biologia Celular e Tecidual, Faculdade de Biociências, PUCRS, Avenida Ipiranga 6681, Prédio 12, Sala 104, 90619-900 Porto Alegre, RS, Brazil

Correspondence should be addressed to Léder Leal Xavier; llxavier@pucrs.br

Received 5 December 2013; Accepted 6 January 2014; Published 13 February 2014

Academic Editors: S. Y. Kwon, D. Thurnher, and S. C. Winter

Oral squamous cell carcinoma (OSCC) is a public health problem. The hamster buccal pouch model is ideal for analyzing the development of OSCC. This research analysed the effects of sunitinib (tyrosine kinase inhibitor) in precancerous lesions induced by 7,12-dimethylbenz(a)anthracene (DMBA) in this model. Thirty-four male hamsters, divided into six groups: control—C ($n = 7$), acetone—A ($n = 12$), carbamide peroxide—CP ($n = 5$), acetone and CP—A+CP ($n = 8$), 1% DMBA in acetone and CP—DA+CP ($n = 6$), and 1% DMBA in acetone and CP and 4-week treatment with sunitinib—DA+CP+S ($n = 7$). The aspects evaluated were anatomopathological features (peribuccal area, paws, nose, and fur), histological sections of the hamster buccal pouches (qualitatively analyzed), epithelium thickness, and the rete ridge density (estimated). Sunitinib was unable to attenuate the decrease in weight gain induced by DMBA; no increase in volume was detected in the pouch and/or ulceration, observed in 43% of the animals in the DA+CP group. DA+CP groups presented a significant increase in rete ridge density compared to the control groups ($P < 0.01$) which was reverted by sunitinib in the DA+CP+S group. Sunitinib seems to have important benefits in early stage carcinogenesis and may be useful in chemoprevention.

1. Introduction

Oral squamous cell carcinoma is a global public health problem with about 300,000 new cases diagnosed per year representing 5% of all cancers for men and 2% for women [1], two-thirds of which are from developing countries [2].

Squamous cell carcinoma of the upper aerodigestive tract has a high risk of primary-treatment failure and death. If cured, patients are often disfigured or cannot speak and/or swallow [2]. Some patients will be at risk for malnutrition, infection [3], severe depression, or suicide. Globally, with few exceptions, survival rates have not improved for decades [1, 4–7].

Oral squamous cell carcinoma (OSCC) is caused by DNA mutation, often spontaneous but increased by the exposure to a range of mutagens [8]—one of them being chemical. The changes in the DNA can progress from a normal keratinocyte to a premalignant or a potentially malignant keratinocyte that is characterized by the ability to proliferate in a less-controlled way than normal. The cells become autonomous and cancer results (characterized by invasion through the epithelial basement membrane) [9].

In the initial phase of OSCC, cells may proliferate in a process known as hyperplasia. From hyperplasia, cells can progress to mild dysplasia; then to moderate dysplasia, and later to severe dysplasia; the last phase would be OSCC [1].

Animal tumor models that closely mimic human oral cancers are very important in elucidating the mechanism of neoplastic transformation and so providing leads to effective chemoprevention. The hamster buccal pouch (HBP) carcinogenesis model is the most well-characterized system for analyzing the development of OSCC [5]. The HBP is covered by a thin layer of stratified squamous epithelium that is very similar to the floor of the mouth and the ventral surface of the tongue in humans, which is the most common site of human OSCC [10].

HISTOLOGY AND HISTOPATHOLOGY

Cellular and Molecular Biology

Effect of prior exercise training and myocardial infarction-induced heart failure on the neuronal and glial densities and the GFAP-immunoreactivity in the posterodorsal medial amygdala of rats

Ana Paula S. Salazar[1], Edson Quagliotto[2], Jadson Alves[2], Fernando A. Oliveira[2], Lisiani Saur[3], Léder L. Xavier[3], Aline S. Pagnussat[1] and Alberto Rasia-Filho[1,2]

[1]PPG-CR, Federal University of Health Sciences of Porto Alegre, Brazil, [2]Laboratory of Physiology, Department of Basic Sciences, Federal University of Health Sciences of Porto Alegre, Brazil and [3]Laboratory of Cellular and Tissue Biology, Faculty of Biosciences, PUCRS, Porto Alegre, Brazil

Offprint requests to: Prof. Alberto A. Rasia-Filho. Federal University of Health Sciences of Porto Alegre, Dept. Basic Sciences / Physiology, Sarmento Leite 245, Porto Alegre RS 90170-050, Brazil. e-mail: rasiafilho@yahoo.com or: aarf@ufcspa.edu.br

Summary. Exercise training has neuroprotective effects whereas myocardial infarction (MI) and heart failure (HF) can cause neuronal death and reactive gliosis in the whole amygdala. The posterodorsal medial amygdala (MePD) is involved with cardiovascular reflexes and the central control of sympathetic/parasympathetic responses. Our aim was to study the effects of prior exercise training and of MI-induced HF on the neuronal and glial densities and the glial fibrillary acidic protein-immunoreactivity (GFAP-ir) in the MePD of adult male rats. Animals (n=5/group) were: control, sedentary submitted to a sham MI (Sed Sham), sedentary submitted to MI/HF (Sed HF), trained on a treadmill and submitted to a sham MI (T Sham) or trained on a treadmill and submitted to MI/HF (T HF). The number of neurons and glial cells in the MePD was estimated using the optical fractionator and the GFAP-ir was quantified by optical densitometry. In the respective groups, treadmill training improved physical performance and MI damaged near 40% of the left ventricle. There was a hemispheric lateralization effect on the density of neurons (higher in the right MePD), but no significant difference in either the neuronal or the glial densities due to experimental condition. Regional GFAP-ir results revealed that the Sed HF group had a higher expression in the left MePD compared to the control and the Sed Sham rats ($p<0.01$). The present data did not evidence the effects of training or MI/HF in the MePD cellular density, but indicate a possible local restructuring of astrocytic cytoskeleton after MI/HF in rats. **Histol Histopathol 29, 1423-1435 (2014)**

Key words: Extended amygdala, Optical fractionator, Cellular density, Astrocytic cytoskeleton

BRAIN RESEARCH 1592 (2014) 101–113

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Resveratrol prevents akinesia and restores neuronal tyrosine hydroxylase immunoreactivity in the substantia *nigra pars* compacta of diabetic rats

Pamela Brambilla Bagatini[a,b], *Léder Leal Xavier*[c], *Laura Tartari Neves*[c], *Lisiani Saur*[c], *Sílvia Barbosa*[a], *Pedro Porto Alegre Baptista*[c], *Otávio Américo Augustin*[a], *Priscylla Nunes de Senna*[a,b], *Régis Gemerasca Mestriner*[c], *André Arigony Souto*[d], *Matilde Achaval*[a,b,*]

[a]Laboratório de Histofisiologia Comparada, Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Avenida Sarmento Leite, 500, 90040-060 Porto Alegre, RS, Brazil
[b]Programa de Pós-Graduação em Neurociências, Universidade Federal do Rio Grande do Sul, Avenida Sarmento Leite, 500, 90040-060 Porto Alegre, RS, Brazil
[c]Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil
[d]Laboratório de Química de Produtos Naturais, Departamento de Química Pura, Faculdade de Química, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Accepted 5 October 2014
Available online 22 October 2014

Keywords:
Diabetes
Resveratrol
Akinesia
Immunoreactivity
Tyrosine hydroxylase
Substantia nigra pars compacta

ABSTRACT

This study evaluated the effects of resveratrol on locomotor behaviors, neuronal and glial densities, and tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta of rats with streptozotocin-induced diabetes. Animals were divided into four groups: non-diabetic rats treated with saline (SAL), non-diabetic rats treated with resveratrol (RSV), diabetic rats treated with saline (DM) and diabetic rats treated with resveratrol (DM+RSV). The animals received oral gavage with resveratrol (20 mg/kg) for 35 days. The open field test and the bar test were performed to evaluate bradykinesia and akinesia, respectively. The Nissl-stained neuronal and glial densities and the dopaminergic neuronal density were estimated using planar morphometry. Tyrosine hydroxylase immunoreactivity was evaluated using regional and cellular optical densitometry. In relation to the locomotor behaviors, it was observed that the DM group developed akinesia, which was attenuated by resveratrol in the DM+RSV group, while the DM and DM+RSV groups showed bradykinesia. Our main morpho-physiological results demonstrated: a decrease in the cellular tyrosine hydroxylase immunoreactivity in the DM group, which was attenuated by resveratrol in

*Corresponding author at: Laboratório de Histofisiologia Comparada, Departamento de Ciências Morfológicas, Universidade Federal do Rio Grande do Sul (UFRGS), Avenida Sarmento Leite 500, Instituto de Ciências Básicas da Saúde, Sala 312, CEP 90050-170, Porto Alegre, RS, Brazil.
E-mail addresses: pamela.bagatini@yahoo.com.br (P.B. Bagatini), lbxavier@pucrs.br (L.L. Xavier), laura.tartari@hotmail.com (L.T. Neves), lisi_saur@yahoo.com.br (L. Saur), slgentil@hotmail.com (S. Barbosa), pedropoa@gmail.com (P.P.A. Baptista), otavio_aa@yahoo.com.br (O.A. Augustin), priscyllasenna@hotmail.com (P.N. de Senna), regis.mestriner@pucrs.br (R.G. Mestriner), arigony@pucrs.br (A.A. Souto), machaval@pq.cnpq.br (M. Achaval).

http://dx.doi.org/10.1016/j.brainres.2014.10.007
0006-8993/

Sexual Dimorphism in the Human Vocal Fold Innervation

*,†,‡Deivis de Campos, †Joel Henrique Ellwanger, *Patrícia Severo do Nascimento, †Helen Tais da Rosa, §Lisiani Saur, *,‡,‖Geraldo Pereira Jotz, and §Léder Leal Xavier, *†‡‖*Porto Alegre, †Santa Cruz do Sul, Rio Grande do Sul, Brazil*

Summary: This study investigated the sexual dimorphism in the recurrent laryngeal nerve (RLN) and thyroarytenoid (TA) muscle, which control the vocal fold. The RLN and TA were bilaterally studied in human specimens obtained from necropsies (seven men and seven women). Analysis of the morphometric parameters showed that the RLN of the men were significantly larger, as shown by the intraperineural area (42.5%) ($P = 0.006$), total number of fibers (38.0%) ($P = 0.0002$), axonal area (34.3%) ($P = 0.0001$), axonal diameter (19.0%) ($P = 0.0001$), and the area of the nerve occupied by myelinated fibers (34.9%) ($P = 0.001$). By contrast, in women, our results showed that the area of the RLN occupied by endoneurial connective tissue was larger (5.7%) ($P = 0.001$). Estimation of the fiber area and shape coefficient showed that the histologic organization of TA is similar in men and women. These results may contribute toward enhancing our understanding about the voice neurobiology.

Key Words: Sexual dimorphism–Recurrent laryngeal nerve–Thyroarytenoid muscle–Voice.

INTRODUCTION

Several studies have demonstrated the presence of sexual dimorphism in the organization of the nervous system in different groups of vertebrates, such as amphibians,[1] reptiles,[2,3] birds,[4] and mammals.[5–9] Similarly, numerous studies with animals have shown sexual dimorphism in different regions of the nervous system involved in vocalization/vocal control.[4,10,11] However, little is known about this aspect in humans.

Although some authors have reported the existence of sexual dimorphism in the neural structures involved in vocal control at the level of the central nervous system in humans,[12] to our knowledge, there is no study in the current literature that shows the presence or absence of sexual dimorphism in structures related to the peripheral nervous system and muscles related to vocalization, especially in the recurrent laryngeal nerve (RLN) and thyroarytenoid (TA) muscle.

In addition, classically, the variability between the voices of men and women has been explained by the differences in the mass of the vocal folds.[13] This sexual dimorphism is attributable to increased testosterone at puberty in males, which stimulates growth in the laryngeal cartilages.[14] In the 20th century, the dominant model of sexual differentiation stated that genetic sex (*XX* vs *XY*) causes differentiation of the gonads, which then secrete gonadal hormones that act directly on tissues to induce sex differences in function. This serial model of sexual differentiation was simple, unifying, and seductive. Recent evidence, however, indicates that the linear model is incorrect and that sex differences arise in response to diverse sex-specific signals originating from inherent differences in the genome and involves cellular mechanisms that are specific to individual tissues or brain regions.[15]

Likewise, studies of songbirds and rodents suggest that male and female brain cells are also intrinsically different because of the sex differences in the expression of sex chromosome genes within the cells.[16] As those differences in gene expression and alterations in brain structures are often responsible for important changes in different body characteristics, such as innervation and morphology, our hypothesis is that sexual dimorphism may be present in the RLN and TA of subjects of different genders. This study aims to investigate this matter.

MATERIALS AND METHODS

Specimens

All the nerves and muscles analyzed were obtained from necropsies of 14 Caucasian subjects who had died suddenly (seven men [age = 71.14 ± 8.07 years] and seven women [age = 75.71 ± 7.83 years]) (mean ± standard deviation), from the Department of Forensic Medicine. It should be pointed out that although our analysis was performed in older individuals because of the difficulty in obtaining younger specimens, this limitation was also found in a previous study, in which the subjects with average age of 70 years for men and 75 years for women were analyzed.[17] Furthermore, in the present study, the men and women were in the same age group. This study was approved by the Ethics Committee of the *Universidade Federal do Rio Grande do Sul*, Rio Grande do Sul, Brazil.

Dissection

The dissection of the RLN was performed according to Jotz et al.[18] The TA muscle was bilaterally removed (~10 mm) from the larynx, and the middle region of the TA was chosen for our study. This choice was based on a previous study,[19] which showed that this region presents more and better defined

Accepted for publication December 17, 2012.
This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
From the *Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil; †Laboratório de Histologia e Patologia, Departamento de Biologia e Farmácia, Universidade de Santa Cruz do Sul, Santa Cruz do Sul, Rio Grande do Sul, Brazil; ‡Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil; §Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil; and the ‖Departamento de Ciências Básicas da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil.
Address correspondence and reprint requests to Léder Leal Xavier, Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6681, Porto Alegre 90619-900, Rio Grande do Sul, Brazil. E-mail: lleavier@pucrs.br
Journal of Voice, Vol. 27, No. 3, pp. 267-272
0892-1997/$36.00

http://dx.doi.org/10.1016/j.jvoice.2012.12.008

Pharmacology, Biochemistry and Behavior 104 (2013) 113–118

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Physical exercise down-regulated locomotor side effects induced by haloperidol treatment in Wistar rats

Pedro Porto Alegre Baptista [a,*], Priscylla Nunes de Senna [b], Mariana Fontoura Paim [a], Lisiani Saur [a], Martina Blank [c], Patricia do Nascimento [b], Jocemar Ilha [b], Mônica Ryff Moreira Vianna [c], Régis Gemerasca Mestriner [a,d], Matilde Achaval [b], Léder Leal Xavier [a]

[a] Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Laboratório de Histofisiologia Comparada, Departamento de Ciências Morfológicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Laboratório de Biologia e Desenvolvimento do Sistema Nervoso, Departamento de Ciências Morfofisiológicas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil
[d] Faculdade de Enfermagem, Nutrição e Fisioterapia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 30 August 2012
Received in revised form 30 November 2012
Accepted 28 December 2012
Available online 2 January 2013

Keywords:
Exercise
Antipsychotics
Dopamine
Parkinsonism
Akinesia
Gait

ABSTRACT

Extra-pyramidal symptoms (EPS) such as akinesia, dystonia, gait alteration and tremors are observed when dopamine D2-receptors are blocked by pharmacological agents such as haloperidol. These alterations produce a Parkinson disease-like state (PLS). Physical exercise has been proven to improve gait and locomotor symptoms in Parkinson's disease; we sought to elucidate the effects of physical exercise on PLS induced by chronic administration of haloperidol in rats. We used 48 rats distributed into four groups: Control, Exercise, Haloperidol, and Hal+Exe. All the animals received a daily injection of saline or haloperidol for 30 days, and the exercise groups underwent a daily 30-minute exercise protocol for 20 days. The animals were subjected to the ink-paw test, bar test and open-field test throughout the training period. The haloperidol-induced akinesia increased throughout the days of injections, but exercise was shown to alleviate it. The assessment showed shortened stride length and increased stance width with the use of haloperidol, which were significantly alleviated by exercise. These results indicate that exercise could be an interesting approach towards reducing unwanted EPS caused by haloperidol.

1. Introduction

Extrapyramidal symptoms (EPS) are a collection of motor side-effects that can arise with the use of dopamine D2-receptor blockers. Drugs of this nature are widely used in the treatment of psychotic illnesses such as schizophrenia and bipolarity (Inada et al., 2002). Haloperidol is an example of a dopamine antagonist and, although it belongs to the first generation of antipsychotics drugs (APD), it is still the reference treatment for schizophrenia (McCue et al., 2006). EPS presents a very specific set of motor deficits such as tremors, akinesia, dystonia and gait alterations (Lieberman et al., 2005; Miyamoto et al., 2005), which greatly resemble the motor characteristics observed in Parkinson's Disease (PD) patients and animal models (Amende et al., 2005; Guillot et al., 2008; Kurz et al., 2007). For this reason, APD is said to cause Parkinsonism (Peluso et al., 2012) or, as it will be referred to in this study, a Parkinson's-like state (PLS).

Physical exercise is widely prescribed to PD patients in an attempt to improve motor control and enhance life quality (Uitti, 2012). Treadmill training, in particular, has been shown to greatly improve the gait quality of PD patients (Herman et al., 2008) and in PD animal models (Pothakos et al., 2009). On the other hand, very little has been written about gait alterations in PLS induced by APD, with some studies merely mentioning the presence of a gait deficit in this state (Hansen et al., 1997; Lieberman et al., 2005). Additionally, previous studies have shown that physical exercise has some beneficial effects on EPS induced by haloperidol in rats (Teixeira et al., 2011).

Given that APD induces PLS, generating important gait alterations that are not completely understood, and that physical exercise has a beneficial effect on EPS (Herman et al., 2008; Uitti, 2012), the main goals of this study were to improve the knowledge about the motor gait deficit induced by D2 blockers and to investigate the effects of physical exercise in PLS induced by haloperidol.

2. Materials and methods

2.1. Animals

For this study, 48 male Wistar rats, three months old and weighing 200–300 g were obtained from the Institute of Basic Health Sciences (ICBS) — UFRGS. They were maintained in a controlled environment and housed in groups of five with food and water ad libitum, in a 12:12 h dark:light schedule. The animals were allocated into four groups (twelve each): 1 — Saline and Sedentary (Control), 2 — Saline and

* Corresponding author at: Departamento de Ciências Morfofisiológicas, Faculdade de Biociências, PUCRS, Avenida Ipiranga, 6681, Prédio 12 Sala 144, CEP 90619-900, Porto Alegre, RS, Brazil. Tel.: +55 51 33203545.
E-mail address: pedroporto@gmail.com (P.P.A. Baptista).

0091-3057/$ – see front matter
http://dx.doi.org/10.1016/j.pbb.2012.12.030

Behavioural Brain Research 244 (2013) 82–89

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Behavior outcome after ischemic and hemorrhagic stroke, with similar brain damage, in rats

Régis Gemerasca Mestriner[a,b,c,d,*], Patrícia Maidana Miguel[b], Pamela Brambilla Bagatini[d], Lisiani Saur[d], Lígia Simões Braga Boisserand[b], Pedro Porto Alegre Baptista[d], Léder Leal Xavier[d], Carlos Alexandre Netto[a,b]

[a] Programa de Pós-Graduação em Ciências Biológicas: Fisiologia, Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brazil
[b] Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre-RS, Brazil
[c] Faculdade de Enfermagem, Nutrição e Fisioterapia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre-RS, Brazil
[d] Laboratório de Biologia Celular e Tecidual, Departamento de Ciências Morfofisiológicas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre-RS, Brazil

HIGHLIGHTS

- ET-1 and collagenase could produce comparable ischemic and hemorrhagic injuries.
- Ischemic and hemorrhagic rats showed similar spontaneous locomotor activity.
- Ischemic and hemorrhagic rats showed similar forelimb asymmetry profile.
- Hemorrhagic stroke showed better performance in skilled walking then ischemic form.

ARTICLE INFO

Article history:
Received 18 December 2012
Received in revised form 29 January 2013
Accepted 1 February 2013
Available online 10 February 2013

Key words:
Stroke
Endothelin-1
Collagenase type VI-S
Recovery
Stereology
Anymaze

ABSTRACT

Stroke causes disability and mortality worldwide and is divided into ischemic and hemorrhagic subtypes. Although clinical trials suggest distinct recovery profiles for ischemic and hemorrhagic events, this is not conclusive due to stroke heterogeneity. The aim of this study was to produce similar brain damage, using experimental models of ischemic (IS) and hemorrhagic (HS) stroke and evaluate the motor spontaneous recovery profile. We used 31 Wistar rats divided into the following groups: Sham (n = 7), ischemic (IS) (n = 12) or hemorrhagic (HS) (n = 12). Brain ischemia or hemorrhage was induced by endotelin-1 (ET-1) and collagenase type IV-S (collagenase) microinjections, respectively. All groups were evaluated in the open field, cylinder and ladder walk behavioral tests at distinct time points as from baseline to 30 days post-surgery (30 PS). Histological and morphometric analyses were used to assess the volume of lost tissue and lesion length. Present results reveal that both forms of experimental stroke had a comparable long-term pattern of damage, since no differences were found in volume of tissue lost or lesion size 30 days after surgery. However, behavioral data showed that hemorrhagic rats were less impaired at skilled walking than ischemic ones at 15 and 30 days post-surgery. We suggest that experimentally comparable stroke design is useful because it reduces heterogeneity and facilitates the assessment of neurobiological differences related to stroke subtypes; and that spontaneous skilled walking recovery differs between experimental ischemic and hemorrhagic insults.

1. Introduction

Stroke is an important public health problem and is broadly subdivided into ischemic and hemorrhagic subtypes [1]. Although considerable development has been made in acute stroke care, current data on functional recovery according to stroke subtypes are not conclusive [2]. Clinical observations have shown that the hemorrhagic form presents greater functional impairment than ischemic stroke at hospital admission, but shows greater functional improvement at discharge [3]. On the other hand, ischemic

* Corresponding author at: Faculdade de Enfermagem, Nutrição e Fisioterapia, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, Prédio 12/8/andar, Porto Alegre, RS, CEP: 90619-900, Brazil. Tel.: +55 51 33203646.
E-mail addresses: regis.mestriner@pucrs.br, regis.mestriner@gmail.com (R.G. Mestriner).

0166-4328/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2013.02.001