FACULDADE DE BIOCIÊNCIAS
PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR
MESTRADO

MANOEL OSÓRIO ALBUQUERQUE FILHO

ASSOCIAÇÃO ENTRE EXPOSIÇÃOAO ESTRESSE PRECOCE E A COGNIÇÃO NA VIDA ADULTA: VULNERABILIDADE, RESILIÊNCIA E EPIGENÉTICA

Porto Alegre
2016

MANOEL OSÓRIO ALBUQUERQUE FILHO

ASSOCIAÇÃO ENTRE EXPOSIÇÃO AO ESTRESSE PRECOCE E A COGNIÇÃO NA VIDA ADULTA: VULNERABILIDADE, RESILIÊNCIA E EPIGENÉTICA

Dissertação apresentada como requisito para obtenção do grau de Mestre pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociência da Pontifícia Universidade católica do Rio Grande do Sul – PUCRS

Orientadora: Profª. Drª. Nadja Schröder

Porto Alegre, RS

2016

RESUMO

Estudos recentes têm mostrado que o estresse no período de desenvolvimento do SNC pode ter consequências variáveis sobre a cognição, alterando a forma como o indivíduo responde frente a situações semelhantes a que é exposto na vida adulta, aumentando a susceptibilidade ou resiliência de uma forma dependente tanto do *background* genético do indivíduo como da forma como ele é exposto ao estressor na infância e na vida adulta. A técnica da privação materna, que busca mimetizar a exposição ao estresse na infância por meio da separação da mãe de sua prole no período considerado chave para o desenvolvimento do SNC, foi utilizada neste estudo com a finalidade de se investigar a variabilidade de resposta ao estresse na vida adulta em animais separados da mãe, bem como para se investigar possíveis mecanismos epigenéticos envolvidos no comprometimento da memória de reconhecimento dos animais adultos e níveis de BDNF no hipocampo, proteína esta chave para o processo de consolidação da memória. Epigenética é um termo que se refere a um tipo de memória que envolve mudança estrutural e funcional na cromatina, incluindo a expressão de genes na resposta a um estímulo ambiental, porém sem promover mudanças na sequência de DNA. Para investigar, neste estudo, os efeitos da modulação epigenética sobre a memória, foi realizada a medida da atividade da enzima histona deacetilase (HDAC), dos níveis de acetilação da Histona H3 e dos níveis da proteína BDNF, marcadores estes que dão ideia sobre a atividade de expressão do gene, e comparados os resultados com aqueles obtidos em tarefas de memória de reconhecimento. Investigamos também as propriedades potenciais do Butirato de Sódio, conhecido inibidor da HDAC, em reverter através dos mecanismos epigenéticos os déficits de memória nos animais de pior desempenho cognitivo. Verificamos que a atividade da HDAC foi maior em ratos submetidos à privação materna com pior desempenho cognitivo e que o uso do Butirato não só reverteu os níveis de acetilação de histonas H3 como ainda influenciou a melhora da cognição nos animais separados que haviam tido desempenho cognitivo inferior no primeiro teste de reconhecimento, restaurando sua memória. Esta descoberta reforça a ideia de que o aumento da atividade da HDAC reduz a acetilação da Histona H3 levando a uma redução na transcrição de BDNF e consequentemente a um déficit de memória. Além disso, observamos uma correlação positiva entre níveis de BDNF e o desempenho de memória em ratos separados da mãe. Juntos os resultados indicam o uso de inibidores da HDAC como um possível agente terapêutico no tratamento de déficits cognitivos. Entretanto, mais estudos que reforcem o entendimento das alterações persistentes observadas em adultos e induzidas por eventos estressores precoces, bem como dos mecanismos envolvidos no desenvolvimento da resiliência, são necessários.

Palavras-chave: BDNF, HDAC, estresse precoce, prejuízo cognitivo, memória reconhecimento, Butirato de Sódio

ABSTRACT

Exposure to stressful events early in life may have permanent deleterious consequences on nervous system function and increase the susceptibility to cognitive impairment later in life. Maternal deprivation, commonly used as a source of early life stress, impairs memory in adult rats and reduces hippocampal brain-derived neurotrophic factor (BDNF) levels in a very heterogeneous way among individuals. The aim of the present study was to investigate the possible epigenetic modulation underlying recognition memory impairment and reduced BDNF levels in the hippocampus of adult maternal deprived rats. We also evaluated the potential ameliorating properties of the histone deacetylase (HDAC) inhibitor, sodium butyrate, on memory deficits and BDNF changes associated with maternal deprivation. For this study, maternal deprived animals were categorized as 'inferior learners' and 'superior learners' according to their performance in novel object recognition memory task. Results indicated that HDAC activity was higher in individuals submitted to maternal deprivation with the worst cognitive performance (inferior learners). Acute administration of sodium butyrate increased histone H3 acetylation and BDNF levels, and restored recognition memory in maternally deprived animals with the worst cognitive performance. Moreover, we also showed that there is a positive correlation between BDNF levels and memory performance. Taken together, the results indicated that HDAC inhibitors could be considered as a possible therapeutic agent to improve cognitive performance in inferior learners individuals. Further studies need to be conducted for a better understanding of the mechanisms associated with persistent alterations observed in adult life induced by early stressful events and those leading to resilience.

Keywords: post-natal stress, object recognition, sodium butyrate, histone acetylation, BDNF, rats.

AGRADECIMENTOS

Agradecimento especial à minha esposa Júlia Henrich Stefenon, cuja cobrança e apoio para que retornasse à academia foi parte primordial para a realização deste mestrado. É de quem vem o maior apoio e sobre quem recaem os maiores méritos por toda esta trajetória.

À minha mãe Carmen Inez Weiller Albuquerque, meu pai Manoel Osório Albuquerque "*in memoriam"* e meus irmãos Ubirajara Albuquerque Neto "*in memoriam" e* Omar Marx Weiller Albuquerque que durante toda minha vida sempre foram fonte de inspiração, exemplos de dedicação e de quem sempre tive o reflexo da importância da atualização constante.

Minha Orientadora prof. Drª Nadja Schröder pela oportunidade dada quando do aceite de orientação, que foi mola propulsora para que chegasse até aqui, oportunizando a mim não apenas um crescimento pessoal e profissional como também permitindo uma experiência de mestrado que deve ser norteadora às próximas etapas acadêmicas.

Aos amigos, à equipe do laboratório e da iniciação científica e em especial à Betânia Souza de Freitas e prof. Drª Maria Noêmia Martins de Lima que sempre que necessário estiveram dispostos a estender a mão pra ajudar no que fora preciso e sem os quais tudo teria sido mais difícil.

Aos programas e bolsas de fomento à pesquisa que garantem estímulo à realização da pós-graduação no país.

SUMÁRIO

1 INTRODUÇÃO

1.1 Efeitos do Estresse sobre o Sistema Neuroendócrino e Modelagens Animais

O estresse é tido como um processo de real ou perceptível ameaça ao equilíbrio homeostático (Smith 2006; Guest *et al*., 2013). A resposta ao estresse representa um sistema adaptativo que auxilia o organismo a reconhecer e responder a situações de mudanças ambientais, que embora necessária à sobrevivência, dependendo da intensidade e duração do estresse, pode ter consequências benéficas, inócuas ou nocivas à saúde, estando associado, segundo estudos, a um maior risco de aterosclerose, hipertensão arterial, atrofia dos neurônios hipocampais, imunossupressão e câncer (Campbell, 2003; Bisaz e Sullivan, 2012; Babenko *et al*, 2015).

Para que a homeostase seja mantida quando da presença de estímulos nocivos (estressores) cabe ao organismo a ativação de uma complexa gama de respostas, envolvendo o sistema endócrino, nervoso e imune (Smith 2006), num processo de resposta ao estresse, mediada pelo eixo Hipotálamo-Pituitário-Adrenal (HPA) que é responsável pela ativação de uma reação do tipo “luta ou fuga” perante a situação de perigo potencial (Guest *et al*., 2013).

A atividade do eixo HPA é componente essencial ao indivíduo na resposta ao estímulo estressor, decorrendo da hiperatividade deste a liberação do Fator Liberador de Corticotrofina (CRF, do inglês *corticotropin releasing factor*), pelo núcleo Paraventricular (PVN) do Hipotálamo (Figura 1). Este, por sua vez, estimula a liberação do Hormônio Adenocorticotrófico (ACTH), da Pituitária anterior na corrente

sanguínea, o qual estimula a liberação de glicocorticóides, como o cortisol, no córtex da adrenal, que prontamente entram no cérebro participando, então, da modulação dos processos de aprendizagem e memória em animais e humanos (Heim *et al.*, 2004; Aisa *et al.*, 2007; Pfau e Russo, 2015), desempenhando efeito ou positivo ou negativo dependendo da ligação dos corticosteróides sobre receptores mineralocorticóides ou glicocorticóides, bem como do tempo em que se dá esta interação dentre as várias fases de processamento da informação, se durante a aquisição, consolidação e/ou retenção da memória e da duração deste estímulo, se agudo ou crônico (de Kloet *et al.*, 1999).

O estresse tem sido reconhecido como o principal fator precipitante em transtornos de humor e seus efeitos sobre o Sistema Nervoso Central (SNC) parecem ter direta correlação com a duração e a intensidade da experiência adversa. Assim, os mesmos mediadores envolvidos na série de mecanismos adaptativos aos efeitos agudos do estresse, que tornam o indivíduo resiliente emocional e cognitivamente às mesmas condições de estresse em exposições futuras, como já demonstrado em primatas e roedores, podem estar envolvidos, também, na superatividade do eixo HPA observada quando da exposição prolongada e repetida a condições estressantes, que resulta nos efeitos prejudiciais sobre os mesmos mecanismos, reduzindo a plasticidade neuronal e prejudicando a memória dependente do hipocampo (Calabrese *et al*, 2009; Pfau e Russo, 2015).

O hipocampo surge como um alvo preferencial da ação de hormônios do estresse por causa de sua abundante expressão de receptores de glicocorticóides (McEwen, 1999) e, ainda, pelo seu envolvimento na regulação do eixo HPA e na função cognitiva, de modo que experiências estressantes nos períodos iniciais de vida podem produzir efeitos duradouros no desenvolvimento normal do cérebro,

promovendo modificações adaptativas em resposta ao estresse agudo ou crônico, que trazem consequências funcionais e estruturais ao funcionamento normal do hipocampo e desencadeiam alteração na capacidade de responder a estímulos estressantes em períodos posteriores como já observado em populações humanas e roedores homogêneos de laboratório (Hulshof *et al.* 2011; McEwen *et al, 2012*).

Em indivíduos saudáveis, já ficou demonstrado que tanto o estresse crônico como a presença de glicocorticóides circulantes, induzidos experimentalmente, leva a prejuízos cognitivos reversíveis na memória (Ohl *et. al*, 2000). E que as experiências adversas precoces podem agir programando mudanças em circuitos neurais em resposta ao estresse, causando disfunção do sistema HPA e levando a aumento de ansiedade e perturbação de cognição na idade adulta, que pode variar ao longo da vida e diferir, em natureza temporal, promovendo mudanças tanto adaptativas quanto mal adaptativas (Aisa *et al.,* 2007).

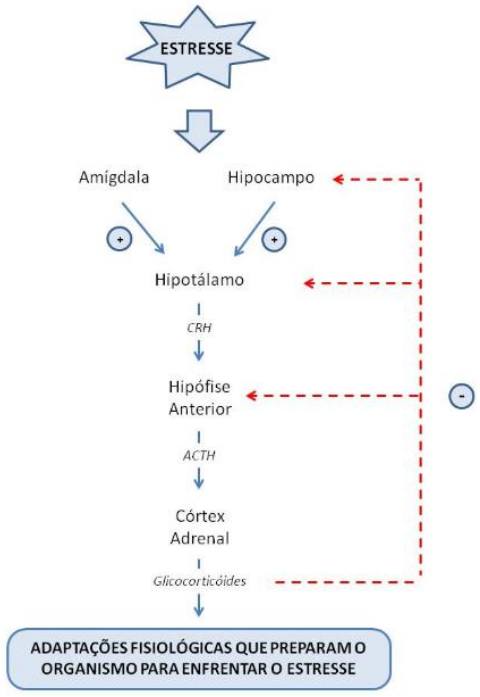

Figura 1: Adaptações fisiológicas para enfrentamento do estresse.
Legenda: ACTH: hormônio adrenocorticotrófico; CRH: hormônio liberador de corticotrofina; HPA: Eixo Hipotálamo-Pituitária-Adrenal**.** O sinal "+" representa ativação. O sinal "-" representa inibição. As linhas pontilhadas ilustram o sistema de retroalimentação negativa de regulação do eixo HPA. Figura da autoria da Dra. Maria Noêmia Martins de Lima.

Os modelos animais representam uma ferramenta importante para o estudo dos mecanismos subjacentes aos fenômenos supracitados, uma vez que permitem a utilização de técnicas que são inviáveis em estudos envolvendo seres humanos (Kaffman e Meaney, 2007; Levine, 1967).

Um dos primeiros paradigmas experimentais descritos em roedores foi realizado por Levine (1957), quando o mesmo propôs a manipulação física diária dos filhotes de sua matriz durante curtos períodos de tempo (3-15 min) nas duas a três primeiras semanas de vida, comportamento que se viu estimular o cuidado da mãe com sua prole, induz respostas neuroendócrinas agudas dos filhotes (Schmidt, Wang e Meijer, 2011).

Já o método da separação materna é uma técnica realizada em animais que mimetiza repetidas exposições ao estresse em períodos precoces da vida dos filhotes, por meio de sua separação do convívio com a mãe, resultando em elevada reatividade ao estresse na vida adulta (Lehmann e Feldon, 2000; Ploj *et al.*, 2003).

Este período pós-natal precoce, que compreende as duas primeiras semanas de vida (entre o 3º e 14º dia pós-natal em ratos), também conhecido como “período hiporresponsivo ao estresse”, garante baixos níveis de corticóides estáveis que parecem ser ideais para o desenvolvimento neuronal (Daskalaskis *et al.*, 2014). É um período no qual o animal apresenta alta vulnerabilidade a injúrias e eventos adversos, de forma que repetidas exposições ao estresse, como a causada pela separação materna, promovem ativação do eixo HPA, tornando-o sensível ao estresse e desencadeando modificações neurobiológicas e comportamentais que tendem a permanecer por toda a vida (de Lima *et al.*, 2011, Pinheiro *et al.*, 2012, Wang *et al.*, 2014).

Em roedores e primatas não humanos, já está bem demonstrado que o estresse durante o período de desenvolvimento do SNC resulta em efeitos neurobiológicos e comportamentais de longo prazo na função hipocampal, que pode culminar em inibição da neurogênese no giro denteado (Hui *et al., 2011;* Lajud *et. al.*, 2012), redução na expressão de níveis do fator neurotrófico derivado do cérebro (BDNF, sigla do inglês *brain-derived neurotrophic factor*) e desregulação do eixo HPA, culminando em déficits na consolidação da memória e em diferentes paradigmas comportamentais (Lubin *et al*., 2008; Hui *et. al.,* 2011; Suri *et al*., 2013; Marco *et al, 2015*).

1.2 *Relação entre exposição ao estresse durante o período de desenvolvimento do sistema nervoso central (SNC) e performance cognitiva na vida adulta.*

Aprendizado e memória são caracterizados como processos de aquisição, consolidação e evocação de informações. A aquisição é a fase onde se dá a exposição dos sistemas sensoriais às novas informações, a qual é seguida quase que concomitantemente do processo de consolidação onde uma série de processos bioquímicos ocorre no hipocampo e outras estruturas cerebrais em duas janelas de tempo distintas, uma logo após o treinamento onde se dá a expressão de fatores de transcrição, e, a segunda, entre três a seis horas após o treino, onde a expressão de proteínas estruturais promove o remodelamento sináptico requerido na formação da memória de longo prazo (LTM). E a evocação é o momento onde se dá a recordação da memória, na qual ocorre a reativação de redes sinápticas modificadas pelo processo de consolidação da memória (Igaz *et al.*, 2002; McGaugh, 2000; Izquierdo e Medina, 1997).

Estes são tidos como processos multissistemas que podem sofrer influência tanto de fatores genéticos, que envolvem polimorfismos em genes como da APOE, APOC1, em receptores e transportadores de serotonina e dopamina e em proteínas neurotróficas, tais como BDNF e enzima conversora de angiotensina (ECA) bem como de interações ambientais, as quais vão determinar diferentes suscetibilidades ao déficit de memória (de Andrade *et al.*, 2011).

Memória não é um processo passivo e indiscriminado de retenção de conhecimento, mas um processo vital que nos permite interagir e reconhecer nosso ambiente e prepararmo-nos cognitiva, emocional e fisiologicamente a situações semelhantes que vierem a ocorrer no futuro (McEwen e Sapolsky, 1995; Reul, 2014).

Os primeiros relatos sugerindo a interferência de fatores estressores sobre a expressão fenotípica da capacidade cognitiva data do início do século 20, quando Freud (1918) propôs que as neuroses adultas eram decorrentes de experiências infantis, o que foi inicialmente testado em roedores por Weininger, em 1950 (Macrì *et al.*, 2008).

Estudo realizado por McEwen (2012), em modelos animais, relata a importância do cuidado materno sobre a responsividade dos filhotes ao estresse, segundo o qual, pequenos períodos de manipulação modulam de forma positiva o comportamento dos animais, aumentando, assim, sua capacidade exploratória ambiental e diminuindo a reatividade emocional e resposta do eixo HPA. Já animais expostos a períodos longos de separação materna e condições de estresse crônico o observado seria um aumento da reação emocional e reatividade do eixo HPA com menor capacidade de exploração de novos ambientes.

Estudos que utilizam a exposição ao estresse no período inicial de desenvolvimento têm demonstrado que este promove alterações comportamentais e

funcionais que tendem a permanecer ao longo da vida (Plotsky *et al.*, 2005), interferindo sobre os processos de aquisição, consolidação e evocação de memórias relacionadas ao medo, alterando a habilidade com que o organismo responde, lida e se adapta a situações estressantes (Aisa *et al.* 2007).

Em nível molecular, já foi observado nos filhotes adultos separados na infância elevados níveis de mRNA do CRF no núcleo paraventricular e elevados níveis plasmáticos de ACTH e corticosterona (Huot *et al.*, 2002; Leckman *et al.*, 2002) que foi demonstrado afetar aprendizado espacial de forma modesta, porém estatisticamente significante durante período de aquisição de memória na tarefa do labirinto aquático de Morris (Huot *et al.*, 2002).

Em nosso grupo de pesquisa já se demonstrou que a exposição a eventos precoces de estresse provocados pela separação materna durante 180 minutos diários entre o 1° e 14° dia pós-natal podem ter consequências prejudiciais permanentes sobre função do Sistema Nervoso com aumento de suscetibilidade a condições psiquiátricas na vida adulta e com prejuízos na memória evidenciados pelos testes comportamentais de campo aberto e de reconhecimento do objeto novo, além de causar redução nos níveis de BDNF hipocampal (de Lima *et al.*, 2011a; Pinheiro *et al.*, *2014)*

Resultados que são corroborados por estudos em ratos adultos submetidos a períodos de separação materna que revelaram, também, múltiplas e persistentes mudanças no SNC na vida adulta que desencadeiam alterações comportamentais marcantes incluindo aumento de ansiedade, anedonia, interrupção do sono, diminuição do apetite (Heim *et al.,* 2004), além de déficits cognitivos no aprendizado observados no teste de reconhecimento de objeto novo (Hulshof *et al.*, 2011; Wang *et al.*, 2011; Baudin *et al*, 2012).

Entretanto a literatura ainda aponta algumas contradições sobre estes resultados, já tendo sido demonstrado que repetidas separações podem afetar parâmetros neuroendócrinos e fisiológicos impactando não só na vulnerabilidade como também na resiliência quando de desafios subsequentes (Schmidt, Wang e Meijer, 2011).

Estudos desenvolvidos em roedores durante o período hiporresponsivo ao estresse (1º ao 14º dia pós-natal) já identificaram desde modificações a nível molecular, com redução de corticosterona global (sem diferenças em níveis de CRF no núcleo paraventricular) e reduzida atividade do eixo HPA (com menor reatividade a situações estressantes) (Slotten *et al.*, 2006), até modificações fenotípicas, com consequente aumento de peso corporal, hiperatividade locomotora no ambiente novo, elevado aprendizado espacial dependente do hipocampo e melhoria na ansiedade em animais adultos jovens, observado em testes como o de campo aberto, labirinto em cruz elevado e de transição claro/escuro (Suri *et al.*, 2014; Zhang *et al.*, 2014) que caracterizam os efeitos positivos da separação materna relacionados ao aumento da resiliência.

Há atualmente o entendimento de que estudos de separação materna realizados sobre mesma metodologia analítica variam a cerca da porcentagem de animais que sofrem com os efeitos negativos da separação materna (Hulsoff *et al.*, 2011) de modo que alguns autores classificam o impacto da separação materna como sendo responsável não por uma deterioração generalizada das funções cognitivas, mas pela amplificação das diferenças individuais na capacidade de aprendizagem (Oitz *et al.,* 2000).

Está, portanto, bem estabelecido que a exposição ao estresse durante o período de desenvolvimento do SNC pode desencadear múltiplos efeitos sobre as

funções cognitivas e o comportamento de acordo com: 1) *background* genético do indivíduo; 2) condições em que o indivíduo foi exposto ao estresse durante o período de desenvolvimento e 3) condições em que o indivíduo é exposto ao estresse na vida adulta. E, dependendo destes contextos, as adversidades podem desencadear tanto um efeito deletério sobre a cognição (aumento da vulnerabilidade) quanto um aumento da capacidade do indivíduo de se adaptar a situações de estresse na vida adulta (aumento da resiliência), consequentemente melhorando seu desempenho em determinados desafios cognitivos (Daskalakis *et al*., 2014; Suri *et al*., 2013; Pryce *et al*., 2003).

1.3 Mecanismos epigenéticos envolvidos na regulação dos efeitos da exposição ao estresse durante o período de desenvolvimento do SNC sobre a performance cognitiva na vida adulta.

O termo epigenética refere-se a um tipo de memória celular dinâmica, que envolve mudanças na estrutura e no funcionamento da cromatina, em resposta a estímulos ambientais sem que ocorram alterações na sequência do DNA podendo ativar ou reprimir a atividade do gene dependendo do sítio ou tipo de modificação (Cowansage *et al.*, 2010).

A cromatina é formada pela cadeia de DNA genômico enovelada ao redor de octâmeros formados por proteínas denominadas de histonas (H2A/H2B/H3/H4), estrutura esta denominada nucleossomo (Jarome e Lubin, 2013). As histonas são alvo de vários tipos de modificações pós-traducionais que alteram acessibilidade e o recrutamento da maquinaria transcriscional ao DNA determinando se a transcrição gênica pode ocorrer. Entre estes mecanismos epigenéticos que controlam a

expressão gênica estão a acetilação de resíduos de lisina das histonas H3 (que **favorecem** a transcrição gênica) e a metilação de resíduos de lisina das histonas H3 (que **desfavorecem** a transcrição gênica). As enzimas que promovem a acetilação das histonas são conhecidas como histonas acetiltransferases (HATs) e as que promovem a sua deacetilação como histonas deacetilases (HDACs) (Figura 2). As enzimas que promovem a metilação das histonas são conhecidas como histona metiltransferases (KMTs) e as que promovem a sua demetilação como histonas demetilases (KDMs) (Bonasio *et al*., 2010; Huan *et al.*, 2014).

Ambos os grupos de enzimas têm sido estudadas como possíveis alvos para o desenvolvimento de fármacos para o tratamento de déficits cognitivos que acompanham transtornos neuropsiquiátricos (Fischer *et al*., 2010; Peter e Akbarian, 2011).

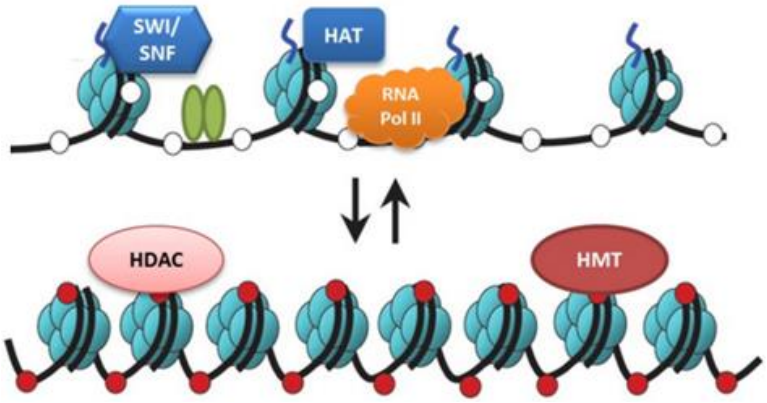

Figura 2: Mecanismo epigenético de remodelamento da cromatina.
Legenda: SWI/SNF: Complexo de proteínas capaz de remodelamento do nucleossoma; HAT: Histona Acetiltransferase; RNA pol II: RNA polimerase II; HDAC: Histona Deacetilase; HMT: Histona metiltransferase; As flechas representam um mecanismo passível de modulação epigenética que pode favorecer ou desfavorecer a transcrição gênica. Adaptada de Luong P, 2009

A inibição da HDAC já demonstrou efeitos positivos de neuroproteção tanto em modelos in vivo quanto in vitro de doenças cerebrais, por meio da indução da expressão de múltiplos alvos que agem com efeitos neuroprotetores (Chuang *et al*, 2009). E o Butirato de Sódio, inibidor das HDACs, quando utilizado em estudos

anteriores, mostrou melhorar a memória em modelos animais que apresentaram déficits cognitivos relacionados a previa submissão à separação materna e estresse crônico leve, tendo demonstrado influenciar a melhora nos níveis de BDNF, revertendo prejuízos de memória no teste de reconhecimento de objetos (Valvassori *et al.,* 2014*;* Penney e Tsai, 2014)

Durante o processo de formação e armazenamento das memórias de longa duração se faz necessário que haja transcrição e síntese de proteínas *in vivo*, um processo limitado a períodos críticos logo após o período de aprendizado demonstrando haver uma linha de tempo necessária de eventos de expressão gênica (Peixoto e Abel, 2013).

Dentre estas proteínas, o BDNF, que é um membro da família dos fatores neurotróficos, tem recebido atenção especial dado o seu envolvimento no crescimento, diferenciação e sobrevivência de populações neuronais durante o desenvolvimento e seu envolvimento na regulação estrutural e funcional de diferentes circuitos neuronais ao longo da vida (Ortiz *et al.*, 2014).

O BDNF além de estar envolvido na formação de diferentes tipos de memória ainda se mostra crítico para manter o armazenamento duradouro de informação no hipocampo, amígdala e córtex insular muitas horas após o aprendizado (Bekinschtein *et al.*, 2014)

Alguns estudos sugerem, ainda, que a regulação epigenética da expressão de BDNF possa estar relacionada com os déficits cognitivos observados nos indivíduos adultos que foram expostos ao estresse durante o período de desenvolvimento do SNC (Lubin *et al.,* 2008; Jarome e Lubin, 2013; Suri *et al*., 2013).

Adultos que foram desmamados precocemente também apresentaram maior ansiedade e responsividade ao estresse, efeitos estes relacionados com diminuição

da síntese de BDNF no hipocampo (Kikusui *et al.*, 2009). Da mesma forma que ratos adultos que receberam pouca atenção da mãe aumentaram comportamento relacionado com ansiedade que era acompanhado de níveis anormais de BDNF no hipocampo e amígdala (Macrì *et al.*, 2009) ou animais expostos a mães abusivamente estressadas por 30 minutos diários, que apresentavam mudanças duradouras na expressão de neurotrofinas, com redução nos níveis de mRNA de BDNF no córtex pré-frontal quando atingiam a idade adulta devido ao processo de metilação do gene de BDNF em promotores específicos (Calabrese *et al.* 2009).

Há uma crescente literatura vinculando a regulação epigenética, especialmente do gene BDNF, com a plasticidade e função cognitiva do cérebro e como a regulação epigenética desempenha processo ativo na regulação da capacidade de um animal responder e formar memórias do seu ambiente e experiências, então modificações epigenéticas realizadas no início do desenvolvimento podem ter a capacidade de subsequentemente afetar a cognição (Roth e Sweatt, 2011).

Schaaf e colaboradores (2001) em estudo realizado associando desempenho de memória e expressão gênica de BDNF em ratos senescentes separados maternalmente em períodos precoces de vida evidenciou que níveis aumentados de expressão de BDNF em animais que realizaram tarefa de aprendizado espacial não estavam ligados a prejuízo na cognição enquanto animais que não apresentavam alterações na expressão de BDNF após a tarefa de aprendizado mostraram déficit na performance cognitiva.

Como os efeitos da exposição ao estresse durante o período de desenvolvimento do SNC são muito heterogêneos entre os indivíduos, se propôs, com este trabalho, investigar, se uma melhor performance cognitiva poderia estar

relacionada com um maior nível de acetilação das histonas e se estes efeitos correlacionam-se com os níveis de BDNF no hipocampo, região chave para o aprendizado e a memória. E, ainda, se uso do butirato de sódio, um conhecido inibidor das HDACs, pode melhorar a performance cognitiva nos indivíduos que apresentaram déficits.

2 JUSTIFICATIVA

O estudo da relação entre a exposição ao estresse durante o período de desenvolvimento do SNC e a performance cognitiva na vida adulta pode contribuir para a identificação de mecanismos moleculares através dos quais a exposição ao estresse possa estar contribuindo para um melhor ou pior desempenho cognitivo na fase adulta (aumento da “resiliência” ou aumento da “vulnerabilidade”). Além disso, a identificação de mecanismos moleculares envolvidos nesta dinâmica pode contribuir para o desenvolvimento de fármacos que possam ser utilizados para a atenuação dos déficits cognitivos naqueles indivíduos mais susceptíveis ao estresse e que apresentam um pior desempenho cognitivo na vida adulta.

3 OBJETIVOS

3.1 Objetivo Geral

Investigar, se uma melhor performance cognitiva poderia estar relacionada com um maior nível de acetilação das histonas e se estes efeitos correlacionam-se com os níveis de BDNF no hipocampo, região cerebral chave para o aprendizado e

a memória, em indivíduos que foram expostos ao estresse durante o período de desenvolvimento do SNC e o uso de um inibidor das HDACs como um possível agente terapêutico para melhorar a performance cognitiva nos indivíduos que apresentarem déficits.

3.2 Objetivos Específicos

- Avaliar se existe a variabilidade dos efeitos da exposição ao estresse durante o período de desenvolvimento do SNC (através do método de privação materna) sobre a memória de reconhecimento em indivíduos adultos.
- Avaliar a influência da exposição ao estresse durante o período de desenvolvimento do SNC (através do método de privação materna) sobre os níveis de BDNF no hipocampo dos indivíduos adultos durante a fase de consolidação da memória de reconhecimento.
- Avaliar a influência da exposição ao estresse durante o período de desenvolvimento do SNC (através do método de privação materna) sobre os níveis de acetilação das histonas H3 no hipocampo dos indivíduos adultos durante a fase de consolidação da memória de reconhecimento.
- Avaliar a influência da exposição ao estresse durante o período de desenvolvimento do SNC (através do método de privação materna) sobre a atividade das HDACs no hipocampo dos indivíduos adultos durante a fase de consolidação da memória de reconhecimento.
- Avaliar o efeito da administração intraperitoneal aguda de Butirato de Sódio (um inibidor das HDACs) sobre a atividade das HDACs, os níveis de

acetilação das histonas H3 e de BDNF no hipocampo dos indivíduos adultos durante a fase de consolidação da memória de reconhecimento.

- Avaliar o efeito da administração intraperitoneal aguda de Butirato de sódio sobre a memória de reconhecimento (através do teste de retenção de memória de longa duração).
- Avaliar a possível correlação entre o desempenho no teste de memória e os níveis de BDNF no hipocampo.

4 ARTIGO

Highlights

- Early-life stress affects memory in a very heterogeneous way among individuals.
- HDAC activity was higher in hippocampus of individuals submitted to maternal deprivation with the worst cognitive performance.
- HDAC inhibition increases BDNF levels in hippocampus and restores memory deficits in individuals submitted to maternal deprivation with the worst cognitive performance.
- There is a correlation between memory and BDNF levels in hippocampus of maternal deprived individuals.
- HDAC inhibition offers a possible therapeutic target to improve cognitive performance in memory-impaired individuals exposed to maternal deprivation.

Neurobiology of Learning and Memory

Original paper

Association between early life stress exposure and cognition in adult life: vulnerability, resilience and epigenetic

Manoel Osório Albuquerque Filho • Betânia Souza de Freitas • Rebeca Carvalho Lacerda Garcia • Pedro Castilhos de Freitas Crivelaro • Nadja Schröder • Maria Noêmia Martins de Lima

Neurobiology and Developmental Biology Laboratory, Faculty of Biosciences, Pontifical Catholic University, 90619-900 Porto Alegre, RS, Brazil

Corresponding author
Maria Noêmia Martins de Lima
Faculty of Biosciences
Pontifical Catholic University
Av. Ipiranga, 6681 Predio 12D
90619-900, Porto Alegre - RS
Brazil
Tel.: +55 - 51 33534743 Fax: +55 51 33203612
E-mail address: maria.lima@pucrs.br

Abstract

Exposure to stressful events early in life may have permanent deleterious consequences on nervous system function and increase the susceptibility to cognitive impairment later in life. Maternal deprivation, commonly used as a source of early life stress, impairs memory in adult rats and reduces hippocampal brain-derived neurotrophic factor (BDNF) levels in a very heterogeneous way among individuals. The aim of the present study was to investigate the possible epigenetic modulation underlying recognition memory impairment and reduced BDNF levels in the hippocampus of adult maternal deprived rats. We also evaluated the potential ameliorating properties of the histone deacetylase (HDAC) inhibitor, sodium butyrate, on memory deficits and BDNF changes associated with maternal deprivation. For this study, maternal deprived animals were categorized as 'inferior learners' and 'superior learners' according to their performance in novel object recognition memory task. Results indicated that HDAC activity was higher in individuals submitted to maternal deprivation with the worst cognitive performance (inferior learners). Acute administration of sodium butyrate increased histone H3 acetylation and BDNF levels, and restored recognition memory in maternally deprived animals with the worst cognitive performance. Moreover, we also showed that there is a positive correlation between BDNF levels and memory performance. Taken together, the results indicated that HDAC inhibitors could be considered as a possible therapeutic agent to improve cognitive performance in inferior learners individuals. Further studies need to be conducted for a better understanding of the mechanisms associated with persistent alterations observed in adult life induced by early stressful events and those leading to resilience.

Keywords: post-natal stress, object recognition, sodium butyrate, histone acetylation, BDNF, rats.

1. **Introduction**

It is well established that exposure to stress during central nervous system (CNS) development can trigger multiple effects on cognitive function and behavior in adulthood in accordance to: 1) the individual's genetic background; 2) conditions under which the individual was exposed to stress during the development and 3) conditions under which the individual is exposed to stress in adulthood - a concept known as three-hit paradigm. So depending on the context, adversity can trigger both a deleterious effect on cognition (increased vulnerability) or an increase in the individual's ability to adapt to stress in adulthood (increased resilience) and consequently have a better performance in certain cognitive challenges (Daskalakis et al., 2013; Suri et al., 2013; Hulshof et al., 2011; Pryce et al., 2003).

Animal models represent an important tool for the study of the mechanisms underlying the mentioned phenomena since they allow the use of techniques that are unfeasible in studies involving humans. In the last decades, it has been consistently shown that exposure to stress during CNS development, using maternal deprivation paradigms, may induce increased anxiety and stress hormone levels; changes in neurogenesis and apoptosis rates; decrease in neurotrophic factors and enzymes responsible for transducing signals important for learning and cognition in adults (Kaffman and Meaney, 2007). In fact, previous studies conducted by our laboratory have repeatedly demonstrated that exposure to stress during CNS development, using the maternal deprivation paradigm, induce cognitive impairments in adult life that is accompanied by a decrease in the levels of brain-derived neurotrophic factor (BDNF) and an increase in IL-10 (anti-inflammatory cytokine) and TNF-α (pro-inflammatory cytokine) in key brain regions for learning and memory (Pinheiro et al., 2015; 2012; Garcia et al., 2013; de Lima et al., 2011). Studies also revealed that epigenetic regulation of gene transcription could

be involved in the modulation of BDNF expression and memory consolidation in different behavioral paradigms (Suri et al., 2013; Cowansage et al., 2010; Lubin et al., 2008).

It has been suggested that the epigenetic regulation of the expression of BDNF may be linked to the cognitive deficits observed in adults who were exposed to stress during CNS development (Suri et al., 2013; Jarome and Lubin, 2013; Lubin et al. 2008). The epigenetic term refers to a type of memory that involves permanent changes in the chromatin structure and action, including gene expression in response to the environment without changes occurring in the DNA sequence. Chromatin is formed by the genomic DNA chain coiled around octamers formed by proteins called histones (H2A / H2B / H3 / H4), which are subject to several types of post-translational modifications that alter gene expression. Among such modifications are the acetylation of lysine residues of histone H3 (favoring gene transcription) and methylation of lysine residues of histone H3 (which disadvantage gene transcription). Enzymes that promote acetylation of histones are known as histone acetyltransferases (HATs) and promoting its deacetylation as histone deacetylases (HDACs) (Bonasio et al., 2010). Both groups of enzymes have been studied as possible targets for drug development for the treatment of cognitive impairment accompanying neuropsychiatric disorders (Peter and Akbarian, 2011; Fischer et al., 2010).

As the effects of exposure to stress during CNS development are very heterogeneous among individuals, the aim of the present study was to investigate, for the first time, if a better cognitive performance could be related to a higher level of histone H3 acetylation and if these effects could be associated to BDNF levels in hippocampus (a key brain region for learning and memory). We also investigated the use of a HDAC inhibitor (sodium butyrate, NaBu) as a possible therapeutic agent to improve cognitive performance in inferior learners individuals.

2. Materials and Methods

2.1. Experimental Design

The animals were submitted to maternal deprivation from post-natal day 1-14. When they reached adulthood, these animals were submitted to object recognition task in order to identify who were the individuals submitted to maternal deprivation with worse and better cognitive performance, categorizing them in two groups in relation to controls: inferior learners (IL) and superior learners (SL). One month later the same animals were submitted to a new training session of the object recognition task (in order to induce a new memory consolidation phase). Immediately after training, these animals received an intraperitoneal injection of vehicle (control group) or NaBu, a HDACs inhibitor (Blank et al., 2015; Silva et al., 2012; Reolon et al., 2011). These animals were then divided into 2 other groups: 1) those who were euthanized 3 hours after the training session (for biochemical analysis), and 2) those who were submitted to the long-term memory retention test of object recognition task. The purpose of this subdivision was to verify if NaBu alters HDAC activity, histone H3 acetylation and BDNF levels during memory consolidation phase (subgroup 1) and improves cognitive performance (subgroup 2) in controls and maternally deprived animals. Figure 1 summarizes the experimental design.

2.2. Animals

Pregnant Wistar rats were obtained from the Centro de Modelos Biológicos Experimentais (CeMBE/PUCRS), Pontifical Catholic University, Porto Alegre, Brazil. After

birth, each litter was adjusted within 48 h to eight rat pups and to contain offspring of both genders (males varying from three to five and females varying from three to five in each litter in order to obtain a final number of eight pups). Each pup was kept with its mother in a plastic cage with sawdust bedding at room temperature of 21±1 °C and a 12/12-h light/dark cycle. At the age of 3 weeks, pups were weaned and the males selected and raised in groups of three to five in individually ventilated cages with sawdust bedding. After weaning animals were given standardized pellet food and tap water ad libitum. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and the recommendations on animal use of the Brazilian Guidelines for the Care and Use of Animals in Research and Teaching (DBCA, published by CONCEA, MCTI), and approved by the Ethics Committee for the Use of Animals of Pontifical Catholic University (CEUA 14/00397). All efforts were made to minimize the number of animals used and their suffering.

2.3. Maternal Deprivation

Maternal deprivation was performed based on previous reports (Pinheiro et al. 2015; 2013; Garcia et al. 2013; de Lima et al. 2011; Levine 1967). Rat pups were exposed to one of the following maternal rearing conditions from post-natal day 1-14: *(1)* non-deprived (ND), animals were exposed to a daily 15 min period in which the dam was removed and the litter was weighed or *(2)* deprived (D), animals were exposed to a daily 180 minutes period in which the dam was removed and the litter was weighed. During the separation period, rat pups of each litter were maintained together in a plastic cage with standard bedding material in an adjacent room to its dams on an incubator at the temperature of 35°C to avoid hypothermia. After the separation period, pups were returned to the nest and rolled in home cage bedding material, and the dam was returned. In rats, the mother is routinely off the litter

for periods of 20-25 minutes (Jans and Woodside 1990). Thus, only the group exposed to a 180 minutes period of separation (deprived), but not the group exposed to a 15 minutes period of separation (non-deprived), results in a deprivation of maternal care.

2.4. Open-field Behavior

Open-field behavior was measured as previously described (Pinheiro et al. 2015; 2013; Garcia et al. 2013; de Lima et al. 2011). The open field was a 40 X 45 X 60 cm arena surrounded by 50 cm high walls, made of plywood with a frontal glass wall. The floor of the arena was divided into 12 equal squares by black lines. Animals were placed in the rear left corner and left to explore the field freely for 5 min. Latency to start locomotion, number of line crossings and rearings, and number of fecal pellets were measured during the experimental sessions by an investigator unaware of treatment group of the animals.

2.5. Novel Object Recognition Task

The object recognition task was performed as previously described (Pinheiro et al. 2015; 2013; Garcia et al. 2013; de Lima et al. 2011). Recognition memory was assessed 24 hours after open-field behavior evaluations in the same apparatus except that the arena floor was covered with sawdust [from bedding material] during the recognition memory training and test trials. The open field exploration was thus used as a context habituation trial for the recognition memory task.

2.5.1. Novel Object Recognition Task I (NOR I)

NOR I was performed in order to categorize the animals in inferior learners (IL) and superior learners (SL). On the first day, rats were given one 5-minutes training trial in which they were exposed to two identical objects: A1 and A2 (Duplo Lego toys). The objects were positioned in two adjacent corners, 9 cm from the walls. On the long-term memory (LTM) testing trial (24 hours after the training session), rats were allowed to explore the open field for 5 minutes in the presence of two objects: a copy of the familiar object A and a novel object B. These were placed in the same locations as in the training session. All objects presented similar textures, colors, and sizes, but distinctive shapes. Between trials the objects were washed with 10% ethanol solution. Object exploration was videotaped and analyzed by an experimenter blind to group treatment assignments using two stopwatches to record the time spent exploring the objects during the experimental sessions by an investigator. Exploration was defined as sniffing or touching the object with the nose. Sitting on the object was not considered as exploration. A recognition index calculated for each animal was expressed by the ratio $T_N/(T_F+T_N)$ [T_F= time spent exploring the familiar object; T_N= time spent exploring the novel object]. Animals that during training and testing trials added up to less than 10 seconds of total exploration time of objects were excluded in order to avoid confusing low exploratory activity with low memory retention rates.

2.5.2. Novel Object Recognition Task II (NOR II)

One month later the same animals were submitted to a new training session of the object recognition task (NOR II) in order to investigate the effects of NaBu on HDAC activity, histone H3 acetylation and BDNF levels during memory consolidation phase (subgroup 1) and on cognitive performance (subgroup 2).

NOR II was conducted as NOR I except that a different set of objects (i.e., objects that have never been presented to these animals) was used a in the training and memory retention trials.

2.5.3. Criteria for Categorizing SL and IL in the NOR Task

The animals of D group were divided into 2 subgroups: **1)** superior learners (SL) - those who achieved a significantly higher recognition memory index than the ND group (control) in NOR I LTM retention test; **2)** inferior learners (IL) - those who obtained a significantly lower recognition memory index than ND group in NOR I LTM retention test. The categorizing protocol was adapted from the study conducted by Gerstein and colleagues (2013).

2.6. Treatment

The animals received a single intraperitoneal injection of saline solution (NaCl 0.9 g%, control group) or NaBu (Sigma, St. Louis, MO, USA) dissolved in saline solution at the dose of 1.0 g/kg of body weight immediately after the training session of the NOR II. The dose of NaBu was selected based on previous studies (Silva et al., 2012; Reolon et al., 2011; Dash et al., 2009). Drugs were prepared freshly before each experiment.

2.7. Sample Preparation for Biochemical Analysis

Three hours after NOR II training or twenty four hours after NOR II LTM testing animals were killed by decapitation and the hippocampus from rats randomly selected from each experimental group were quickly dissected and stored at −80 °C for posterior analyses.

2.8. HDAC Activity

HDAC activity in hippocampus was measured by sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (product code # 17-374, Upstate Biotechnology Inc., Billerica, MA, USA) as previously described (Réus et al., 2013). The assay is a simple two-step procedure performed in a microtiter plate. In the first step, samples are incubated with the HDAC assay substrate, allowing deacetylation of the substrate. Next, the activator solution releases p-nitroanilide from the deacetylated substrate or standard. Briefly, microtiter plates (96-well flat-bottom) were coated with 10 µL of HDAC assay buffer or HDAC assay buffer containing 4µM of Trichostatin A (an inhibitor of HDAC classes I and II) into each well. Next, 20µl of test sample, or 20µl of HeLa nuclear extract (positive control), or 20µl of water (negative control), or standard control at different concentrations (i.e., 0.01575, 0.0315, 0.0635, 0.125, 0.25, 0.5 and 1 mM) were added into the wells. The HDAC extract was prepared using a CelLytic NuCLEAR Extraction Kit (product code # NXTRACT, Sigma, St. Louis, MO, USA). Then, 10µl of the 4 mM HDAC assay substrate was added and the plates were mixed thoroughly and incubated for 60 minutes at 37°C. Next, 20µl of the diluted activator solution was added to each well and the plates were mixed thoroughly. After incubation at room temperature for 10-20 minutes, the HDAC activity was determined (absorbance set at 405 nm). The standard curve demonstrates a direct relationship between optical density and deacetylated substrate concentration. Total protein was measured by Bradford's method using bovine serum albumin as standard (Bradford et al., 1976).

2.9. Histone H3 Total Acetylation

Histone H3 total acetylation in hippocampus was measured by sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (product code # ab115124, Abcam, Bristol, UK) as previously described (Kocic et al., 2014). Briefly, microtiter plates (96-well flat-bottom) were coated with 50 µL of antibody buffer. Next, 1 µg of test sample was added into the sample wells and 1 µL of standard control at the different concentrations, into the standard control wells (i.e., 1.5, 3, 6, 12, 25, 50, and 100 ng/µl). The histone extract was prepared using a histone extraction kit (product code # ab113476, Abcam, Bristol, UK). The plates were then mixed and covered with Parafilm M and incubated for 1-2 hours at room temperature. After, the wells were washed three times with 150 µL of wash buffer. Next, 50 µL of diluted detection antibody was added to each well and incubate for 60 minutes at room temperature on an orbital shaker (100 rpm). Then, wells were washed six times with 150 µL of wash buffer. After, 100 µL of color developer was added into the wells and the plates were incubated for 2-10 minutes at room temperature away from light. After addition of 50 µL of stop solution in each well, the amount of histone H3 acetylation was determined (absorbance set at 450 nm). The standard curve demonstrates a direct relationship between optical density and acetyl histone H3 concentration. Total protein was measured by Bradford's method using bovine serum albumin as standard (Bradford et al., 1976).

2.10. BDNF Measurements

BDNF levels in brain tissue were measured by sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (product code # CYT306, Millipore,

Darmstadt, Alemanha) as previously described (Pinheiro et al., 2015). Briefly, microtiter plates (96-well flat-bottom) were coated for 24 hours at 4 °C with the samples diluted 1:3 in sample diluent and standard curve ranging from 15.63 to 500 pg/ml of BNDF. Plates were then washed four times with wash buffer followed by the addition of biotinylated mouse anti-human BNDF monoclonal antibody (diluted 1:1000 in sample diluent), which was incubated for 3 hours at room temperature. After washing, a second incubation with streptavidin-horseradish peroxidase conjugate solution (diluted 1:1000) for 1h at room temperature was carried out. After addition of substrate and stop solution, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve demonstrates a direct relationship between optical density and BDNF concentration. Total protein was measured by Bradford's method using bovine serum albumin as standard (Bradford et al., 1976).

2.11. Statistical Analysis

Data for open field behavior, recognition indexes and total time spent exploring the familiar and novel objects in training and retention test trials are expressed as mean ± SEM and were analyzed by one way analysis of variance (ANOVA), followed by LSD *post hoc* test, when necessary, in order to categorize animals according to their memory performance in inferior learners and superior learners in comparison to the control group (NOR I). Biochemical data are expressed as mean ± SEM. Biochemical data and NOR II recognition indexes were analyzed by two-way analysis of variance (two-way ANOVA) was performed, with recognition memory performance (non-deprived, control group, or superior learner or inferior learner) and treatment (vehicle or sodium butyrate) as fixed factors. Further comparisons were performed by one-way ANOVA followed by LSD *post hoc* test. One-tailed Pearson's correlation was performed between recognition indexes in LTM retention session

and BDNF levels in the hippocampus. For all cases, *P* values less than 0.05 were considered statistically significant (Pinheiro et al., 2015).

3 Results

3.1 Open-field Behavior

In order to control for possible effects of neonatal maternal deprivation on general sensorimotor functions, we analyzed open field behavior in adult rats (Figure 2). One-way ANOVA showed no statistically significant differences among the groups in the latency to start locomotion ($F_{(2,145)} = 0.983$, $p = 0.377$, Fig 1A), in the number of crossings ($F_{(2,145)} = 0.802$, $p = 0.451$, Fig 1B), in the number of rearings ($F_{(2,145)} = 0.496$, $p = 0.610$, Fig 1C), and in the number of fecal pellets produced during session ($F_{(2,145)} = 0.904$, $p = 0.407$, Fig 1D). These results suggest that maternal deprivation does not affect locomotion, exploration, or anxiety.

3.2 Novel Object Recognition Memory

The effects of maternal deprivation on NOR I are shown in Figure 3. Descriptive statistics showed that ND group had a LTM retention test recognition index of 0.678 ± 0.00689. Based on that, animals of D group were divided into 2 subgroups: **1)** superior learners (SL) - those who had a LTM retention test index > 0.678 (n = 28); **2)** inferior learners (IL) - those who had a LTM retention test index < 0.678 (n = 63). One way analysis of variance (ANOVA), revealed a significant difference among the groups in LTM retention test ($F_{(2, 145)} = 70.082$, $p < 0.001$), but not in training session ($F_{(2, 145)} = 0.556$, $p = 0.574$). Subsequent analysis using LSD *post hoc* test confirmed that animals that were separated from

their mothers and were categorized as IL showed lower recognition indexes in LTM retention test when compared to the ND group ($p < 0.001$), indicating that maternal deprivation impairs long-term recognition memory. However, a subset of animals that were separated from their mothers, those categorized as SL showed statistically significant higher recognition indexes in LTM retention test when compared to the ND group and to the IL group (both *p's* < 0.001). All experimental groups showed statistical differences between recognition indexes in training and LTM retention test, indicating that all groups preferred the novel object ($p < 0.001$ for ND, SL and IL groups; T-Test). Statistical comparisons of total time exploring objects during the training ($F_{(2, 145)} = 2.521$, $p = 0.084$) or LTM retention ($F_{(2, 145)} = 0.384$, $p = 0.682$) sessions showed no significant differences among the groups (Table 1).

We next aimed at investigating whether HDAC inhibition using NaBu, would attenuate the memory-impairing effects of maternal deprivation on IL (Figure 4). One way analysis of variance (ANOVA), revealed a significant difference among the groups in LTM retention test ($F_{(3, 34)} = 4.309$, $p = 0.011$), but not in training session ($F_{(3, 34)} = 0.239$, $p = 0.869$). Subsequent analysis using LSD *post hoc* test revealed that animals that were separated from their mothers, categorized as IL, and treated with vehicle showed lower recognition indexes in LTM retention test when compared to the ND group ($p = 0.007$), confirming that maternal deprivation impairs long-term recognition memory in a subset of animals. However, animals that were separated from their mothers, categorized as IL, and treated with NaBu showed no significant differences in recognition indexes in LTM retention test when compared to the ND group ($p = 0.592$) indicating that NaBu was able to restore recognition memory in IL animals. In addition, the IL group treated with NaBu showed a significantly higher recognition index than vehicle-treated IL group ($p = 0.020$). Only IL vehicle-treated animals that were separated from their mothers showed no statistical differences between recognition indexes in training and LTM retention test, indicating that this group had no

preference for the novel object (p = 0.092; T-Test). Conversely, the IL animals that received NaBu showed statistical differences between recognition indexes in training and LTM retention test, corroborating the idea that NaBu was able to restore the preference for the novel object ($p < 0.001$; T-Test).

Statistical comparisons of total time exploring objects during the training ($F_{(3, 34)} = 1.172$, $p = 0.335$) or LTM retention ($F_{(3, 34)} = 0.239$, $p = 0.869$) sessions showed no significant differences among the groups (Table 1). Similarly, statistical comparisons of total time exploring objects during the training ($F_{(5, 75)} = 1.113$, $p = 0.361$) session showed no significant differences among the groups of animals that were euthanized 3 hours after training session for biochemical analyses (Table 1).

3.3 HDAC Activity

Figure 5 exhibits the results for HDAC activity in the hippocampus of rats submitted to neonatal maternal deprivation and adult acute treatment with NaBu. Two way ANOVA revealed a significant main effect of memory performance ($F_{(2, 19)} = 5.826$, $p < 0.011$). Further statistical comparisons of HDAC activity in the hippocampus, using one way ANOVA, has indicated a significant difference among the groups ($F_{(5, 19)} = 3.111$, $p < 0.032$), and LSD *post hoc* comparisons indicated that animals that were separated from their mothers and were categorized as IL and received saline showed higher HDAC activity 3 hours after recognition memory training when compared to the ND group that received saline (p = 0.004), suggesting that higher HDAC activity could be related to recognition memory deficits seen in maternal deprived individuals with worse learning performance (IL). No significant differences were observed in the comparison of NaBu-treated animals with their respective control groups, when HDAC activity was measured 3 h after NaBu administration.

3.4 H3 Total Acetylation

Figure 6 shows the results for H3 total acetylation measurements in the hippocampus of rats submitted to neonatal maternal deprivation and adult acute treatment with NaBu immediately after training (NOR II). Two way ANOVA revealed a significant main effect of treatment ($F_{(1, 24)} = 63.54$, $p < 0.0001$). Statistical comparison of H3 total acetylation levels in the hippocampus of animals euthanized 3 h after NOR II training, using one-way ANOVA, has indicated a significant difference among the groups ($F_{(5, 24)} = 14.899$, $p < 0.001$). Further LSD *post hoc* comparisons indicated that NaBu treatment induced a significant increase in H3 total acetylation levels in all three groups that received NaBu (ND, SL and IL) when compared to their respective control groups ($p = 0.003$, $p < 0.001$ and $p = 0.015$, respectively), suggesting that NaBu single administration produced an effective inhibition of HDAC (the enzyme responsible for deacetylation of H3 lysine residues).

3.5 BDNF Measurements

Figure 7 presents BDNF levels in the hippocampus of rats submitted to neonatal maternal deprivation and adult acute treatment with NaBu that were euthanized 3 hours after training (7a) or 24 hours after LTM retention test (7b). Two-way analysis of variance showed statistically significant main effects of memory performance ($F_{(2, 23)} = 8.29$, $p = 0.002$) and treatment ($F_{(1, 23)} = 4.69$, $p = 0.041$). Moreover, a significant interaction between memory performance and treatment was observed ($F_{(2, 23)} = 4.247$, $p = 0.027$). Further comparisons of BDNF levels in the hippocampus, using one-way ANOVA, revealed a significant difference among the groups ($F_{(5, 23)} = 6.273$, $p = 0.001$) 3 hours after training. LSD *post hoc*

comparisons have shown that NaBu acute treatment induced a significant increase in BDNF levels in individuals submitted to maternal deprivation when compared to ND-vehicle group ($p = 0.006$ and $p = 0.011$, respectively for D-SL and D-IF).

In contrast, statistical comparison of BDNF levels in the hippocampus has revealed no significant difference among the groups ($F_{(3, 14)} = 1.436$, $p = 0.274$) 24 hours after training. Although BDNF levels 24 after training did not differ among the groups, interestingly, there is a positive correlation between recognition index and BDNF levels in hippocampus (Pearson's test coefficient $r = 0.511$, $p = 0.036$, Figure 8).

4 Discussion

Exposure to stressful events early in life may have permanent deleterious consequences on nervous system function and increase the susceptibility to cognition impairments later in life (Aisa et al., 2009; 2007; Choy et al., 2008). Consistently with our previous findings (Pinheiro et al. 2015; 2013; Garcia et al. 2013; de Lima et al. 2011), here we show that maternal deprivation, which is commonly used as a source of early life stress, impairs recognition memory in most adult rats. Memory deficits reported here cannot be attributed to general alterations in exploratory or sensory motor behavior, since no differences were found among the groups in the open-field test or in the time spent exploring objects during training or testing sessions in the novel object recognition task.

Previous reports showed that maternal deprivation could trigger both a deleterious effect on cognition (increased vulnerability) or an increase in the individual's ability to adapt to stress in adulthood (increased resilience) and consequently have a better performance in certain cognitive challenges (Suri et al., 2014; Makena et al., 2012; Rüedi-Bettschen et al., 2005; Oitzl et al., 2000). Interestingly, here we showed, for the first time, that maternal deprivation impairs recognition memory a very heterogeneous way among individuals. A

subset of animals submitted to maternal separation did not display any memory impairment, performing even better than controls (Figure 3).

A classical study performed by Levine (1967) demonstrated that short periods of maternal deprivation during the postnatal period induced a reduction in stress response in adult life. Meaney and colleagues (1991) indicated that this effect could be associated to an increase in the efficiency of endocrine response to stress, preventing excessive exposure to the highly catabolic adrenal steroids. The subtle manipulation early in life appears to protect the animal from potentially damaging effects of these steroids, ensuring the anatomical integrity of brain structures involved in cognitive functioning later in life. The same research group also suggested that the increased resilience effect was mediated by an increment in maternal care after the separation period (Meaney, 2001). In a recent review, Bock and colleagues (2014) expanded the three-hit paradigm (see Introduction) proposing the view that an individual predisposition of stress responsive peripheral and central nervous systems is not only a genetic predisposition (for example, polymorphisms or gender) but acts in close cooperation with epigenetic predispositions.

We had hypothesized that the maternal deprivation-induced memory impairment could be related to altered epigenetic patterns of BDNF transcription since our previous studies suggested that there is a correlation between decreased BDNF levels in hippocampus and impaired cognitive performance (Pinheiro et al. 2015; 2013; de Lima et al. 2011). Histone acetylation is a form of chromatin modification involved in the transcriptional regulation underlying memory formation (Lopez-Atalaya & Barco, 2014; Penney & Tsai, 2014) and also shown to be involved in the modulation of BDNF transcription (Cortés-Mendoza et al., 2013). Here, we investigated whether recognition memory impairment induced by maternal deprivation is related to altered histone acetylation in the hippocampus, and if it could be related to BDNF levels in this key brain region for memory and cognition. The present results

show that HDAC activity is increased in the IL group, compared to controls and to SL (Figure 5). In agreement, Levine and colleagues (2012) demonstrated, in an elegant study, that the expression of class I and II HDACs and the acetylation of histone H3 and histone H4 proteins were altered in hippocampus and other brains regions during postnatal development of stress susceptible mice exposed to early life stress when compared to stress resilient ones. Although in the present study, we did not perform separated measures of class I and II HDACs activities as Levine and colleagues (2012), the increased activity of HDAC that we observed could represent a possible explanation for the differences seen between SL and IL in recognition memory index.

Intriguingly, animals that received NaBu did not present a decrement in HDAC activity when compared to their respective controls that received vehicle. This fact may be due to the period in which the analysis was performed (3 hours after the NaBu injection). However, our results showing that all NaBu-treated groups presented higher H3 acetylation levels, suggest that HDAC inhibition was achieved, but perhaps undetectably at 3h after NaBu administration.

In fact, acute administration of the HDAC inhibitor, NaBu, immediately after training, increased H3 acetylation levels 3 hours later in all groups of animals, including the IL group, confirming its HDAC's inhibitory effect. Thus, it is possible that, by inhibiting HDAC and allowing H3 acetylation to increase in the group of IL (that had increased HDAC activity), NaBu was able to restore memory in the group of IL animals subjected to LTM retention test. Those animals were probably more susceptible to the effects of maternal separation than the SL group.

NaBu, shown to display HDAC inhibitor properties, improves memory in different tasks such as recognition memory (Valvassori et al., 2014), contextual fear conditioning (Zhong et al., 2014) and inhibitory avoidance (Blank et al., 2015a). It also improves BDNF

transcription probably via epigenetic mechanisms that include the acetylation of lysine residues of histone H3 (promoting gene transcription) in behavioral paradigms of memory impairment (Singh et al., 2015; Barichello et al., 2015; Valvassori et al., 2014). In accordance, a recent study performed by Blank and colleagues (2015b) showed that blocking TrkB (a BDNF receptor) in the dorsal hippocampus after learning or retrieval impairs retention of memory for inhibitory avoidance. More importantly, the impairing effect of TrkB antagonism on consolidation was completely prevented by the NaBu. Here we show that administration of NaBu increased BDNF levels in the hippocampus of maternally deprived rats, irrespectively of their memory performance group, as both IL and SL treated with NaBu displayed increased BDNF 3 hours after training. It is well established that recognition memory consolidation requires protein synthesis in a time-dependent manner and that the hippocampus plays a key role in this process (Furini et al., 2015). Studies using protein synthesis inhibitors have shown that protein synthesis is required during a limited post-training window for consolidation of this type of memory. For example, inhibition of hippocampal protein synthesis 6 hours after training did not blocked consolidation of long-term recognition memory, whereas inhibition 3 hours after training did (Rossato et al., 2007). In addition, although when BDNF levels were measured 24 hours later, no significant differences among the groups were observed, a positive correlation between BDNF levels in the hippocampus and memory performance was found. Taken together these results suggest that HDAC inhibition may regulate BDNF expression during consolidation, and in turn, BDNF increases in IL may also underlie, at least in part, NaBu memory improving effects.

5 Conclusions

In summary, here we show, for the first time, that maternal deprivation induces persistent memory deficits in a very heterogeneous way among individuals. The present results show that HDAC activity was higher in individuals submitted to maternal deprivation with the worst cognitive performance. This finding supports the idea that increased HDAC activity reduces the histone H3 acetylation which may lead to a decrease in BDNF transcription and consequently to a memory deficit. In addition, we also showed that there is a correlation between higher BDNF levels and better memory performance regardless of being subjected to maternal separation or pharmacological treatment and that NaBu (a HDAC inhibitor) restored memory in the IL group. Taken together, the results indicated that HDAC inhibitors could be considered as a possible therapeutic agent to improve cognitive performance in IL individuals. Further studies need to be conducted for a better understanding of the mechanisms associated with persistent alterations observed in adult life induced by early stressful events and those leading to resilience.

Acknowledgements

N.S. is Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) research fellow. Funding for this study was provided by MCTI/CNPq/Universal 14/2014 (grant 448332/2014-0). R.C.L.G. is supported by a CNPq/PIBIC scholarship and P.C.de F.C. is supported by a BPA/PUCRS scholarship.

Role of the funding source

The funding source had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Conflict of interests

The authors declare that they have no conflict of interest.

References

Aisa, B., Tordera, R., Lasheras, B., Del Río, J., & Ramírez, M.J. (2007). Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. Psychoneuroendocrinology 32, 256-266. http://dx.doi.org/10.1016/j.psyneuen.2006.12.013

Aisa, B., Elizalde, N., Tordera, R., Lasheras, B., Del Río, J., & Ramírez, M.J. (2009). Effects of neonatal stress on markers of synaptic plasticity in the hippocampus: implications for spatial memory. Hippocampus 19, 1222-1231. http://dx.doi.org/10.1002/hipo.20586.

Barichello, T., Generoso, J.S., Simões, L.R., Faller, C.J., Ceretta, R.A., Petronilho, F., Lopes-Borges, J., Valvassori, S.S., & Quevedo, J. (2015). Sodium Butyrate Prevents Memory Impairment by Re-establishing BDNF and GDNF Expression in Experimental Pneumococcal Meningitis. Mol Neurobiol. 52 (1), 734-740. http://dx.doi.org/10.1007/s12035-014-8914-3.

Blank, M., Werenicz, A., Velho, L.A., Pinto, D.F., Fedi, A.C., Lopes, M.W., Peres, T.V., Leal, R.B., Dornelles, A.S., & Roesler, R. (2015a). Enhancement of memory consolidation by the histone deacetylase inhibitor sodium butyrate in aged rats. Neurosci Lett, 594, 76-81. http://dx.doi.org/10.1016/j.neulet.2015.03.059.

Blank, M., Petry, F.S., Lichtenfels, M., Valiati, F.E., Dornelles, A.S., & Roesler, R. (2015b). TrkB blockade in the hippocampus after training or retrieval impairs memory: protection from consolidation impairment by histone deacetylase inhibition. J Neural Transm [in press].

Bock, J., Rether, K., Gröger, N., Xie, L., & Braun, K. (2014). Perinatal programming of emotional brain circuits: an integrative view from systems to molecules. Front Neurosci, 8, 1-16. http://dx.doi.org/10.3389/fnins.2014.00011.

Bonasio, R., Tu, S., & Reiberg, D. (2010). Molecular signals of epigenetic states. Science, 330, 612-616. http://dx.doi.org/10.1126/science.1191078.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem, 72, 248-254.

Cowansage, K.K., LeDoux, J.E., & Monfils, M.H. (2010). Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity. Curr Mol Pharmacol, 3, 12-29. http://dx.doi.org/10.2174/1874467211003010012

Choy, K.H.C., Visser, Y., Nichols, N.R., & Van den Buuse, M. (2008). Combined neonatal stress and young-adult glucocorticoid stimulation in rats reduce BDNF expression in hippocampus: Effects on learning and memory. Hippocampus 18, 655-667. http://dx.doi.org/10.1002/hipo.20425.

Cortés-Mendoza, J., Díaz de León-Guerrero, S., Pedraza-Alva, G., & Pérez-Martínez, L. (2013). Shaping synaptic plasticity: the role of activity-mediated epigenetic regulation on gene transcription. Int J Dev Neurosci, 31(6), 359-69. http://dx.doi.org/10.1016/j.ijdevneu.2013.04.003. Epub 2013 May 9.

Daskalakis, N.P., Bagot, R.C., Parker, K.J., Vinkers, C.H., & de Kloet, E.R. (2013). The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology, 38, 1858-1873. http://dx.doi.org/10.1016/j.psyneuen.2013.06.008.

Dash, P.K., Orsi, S.A. & Moore, A.N. (2009). Histone deactlylase inhibition combined with behavioral therapy enhances learning and memory following traumatic brain injury. Neuroscience, 16,: 1-8. http://dx.doi.org/10.1016/j.neuroscience.2009.06.028.

de Lima, M.N., Presti-Torres, J., Vedana, G., Alcalde, L.A., Stertz, L., Fries, G.R., Roesler, R., Andersen, M.L., Quevedo, J., Kapczinski, F., & Schröder, N. (2011). Early life stress decreases hippocampal BDNF content and exacerbates recognition memory deficits induced by repeated D-amphetamine exposure. Behav Brain Res, 224, 100-106. http://dx.doi.org/10.1016/j.bbr.2011.05.022.

Fischer, A., Sananbenesi, F., Mungenast, A., & Tsai, L.H. (2010). Targeting the correct HDAC(s) to treat cognitive disorders. Trends Pharmacol Sci, 31, 605-317. http://dx.doi.org/10.1016/j.tips.2010.09.003.

Furini, C.R., Myskiw, J. de C., Schmidt, B.E., Zinn, C.G., Peixoto, P.B., Pereira, L.D., & Izquierdo, I. (2015).The relationship between protein synthesis and protein degradation in object recognition memory. Behav Brain Res, 294, 17-24. http://dx.doi.org/10.1016/j.bbr.2015.07.038.

Garcia, V.A., Hirotsu, C., Matos, G., Alvarenga, T., Pires, G.N., Kapczinski, F., Schröder, N., Tufik, S., & Andersen, M.L. (2013). Modafinil ameliorates cognitive deficits induced by maternal separation and sleep deprivation. Behav Brain Res, 253, 274-279. http://dx.doi.org/10.1016/j.bbr.2013.07.029.

Gerstein, H., Hullinger, R., Lindstrom, M.J., & Burger, C. (2013). A behavioral paradigm to evaluate hippocampal performance in aged rodents for pharmacological and genetic target validation. PLoS One, 8, e62360. http://dx.doi.org/10.1371/journal.pone.0062360.

Hulshof, H.J., Novati, A., Sgoifo, A., Luiten, P.G.M., den-Boer, J.A., & Meerlo, P. (2011). Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats. Behav Brain Res, 216, 552-560. http://dx.doi.org/10.1016/j.bbr.2010.08.038.

Jans, J.E., & Woodside, B.C. (1990). Nest temperature: effects on maternal behavior, pup development, and interactions with handling. Dev Psychobiol, 23, 519-534.

Jarome, T.J., & Lubin, F.D. (2013). Histone lysine methylation: critical regulator of memory and behavior. Rev Neurosci, 24, 375-387. http://dx.doi.org/10.1515/revneuro-2013-0008.

Kaffman, A., & Meaney, M.J. (2007). Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. J Child Psychol Psychiatry, 48, 224-244.

Kocic, G., Cukuranovic, J., Stoimenov, T.J., Cukuranovic, R., Djordjevic, V., Bogdanovic, D., & Stefanovic, V. (2014). Global and specific histone acetylation pattern in patients with Balkan endemic nephropathy, a worldwide disease. Ren Fail 36, 1078-1082. http://dx.doi.org/10.3109/0886022X.2014.917562.

Lubin, F.D., Roth, T.L., & Sweatt, D. (2008). Epigenetic regulation of bdnf gene transcription in the consolidation of fear memory. J Neurosci, 28, 10576-10586. http://dx.doi.org/10.1523/JNEUROSCI.1786-08.2008.

Levine, A., Worrell, T.R., Zimnisky, R., & Schmauss, C. (2012). Early life stress triggers sustained changes in histone deacetylase expression and histone H4 modifications that alter responsiveness to adolescent antidepressant treatment. Neurobiol Dis, 45 (1), 488-498. http://dx.doi.org/10.1016/j.nbd.2011.09.005.

Levine, S. (1967). Maternal and environmental influences on the adrenocortical response to stress in weanling rats. Science, 156, 258-260.

Lopez-Atalaya, J.P., & Barco, A. (2014). Can changes in histone acetylation contribute to memory formation? Trends Genet, 30(12), 529-539. http://dx.doi.org/10.1016/j.tig.2014.09.003.

Makena, N., Bugarith, K., & Russel, V. (2012). Maternal separation enhances object location memory and prevents exercise-induced MAPK/ERK signalling in adult Sprague–Dawley rats. Metab Brain Dis 27 (3), 377-385. http://dx.doi.org/10.1007/s11011-012-9298-6.

Meaney, M.J., Mitchell, J.B., Aitken, D.H., Bhatnagar, S., Bodnoff, S.R., Iny, L.J., & Sarrieau, A. (1991). The effects of neonatal handling on the development of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. Psychoneuroendocrinology, 16 (1-3), 85-103.

Meaney, M.J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci, 24, 1161-1192. http://dx.doi.org/10.1146/annurev.neuro.24.1.1161

Oitzl, M.S., Workel, J.O., Fluttert, M., Frösch, F., & De Kloet. E.R. (2000). Maternal deprivation affects behaviour from youth to senescence: amplification of individual differences in spatial learning and memory in senescent Brown Norway rats. Eur J Neurosci. 12, 3771-3780. http://dx.doi.org/10.1046/j.1460-9568.2000.00231.x

Penney, J., & Tsai, L.H. (2014). Histone deacetylases in memory and cognition. Sci Signal, 7 (355), re12. http://dx.doi.org/10.1126/scisignal.aaa0069.

Peter, C.J., & Akbarian, S. (2011). Balancing histone methylation activities in psychiatric disorders. Trends Mol Med, 17, 372-379. http://dx.doi.org/10.1016/j.molmed.2011.02.003

Pinheiro, R.M., de Lima, M.N., Fries, G.R., Garcia, V.A., Presti-Torres, J., Hallmenschlager, L.H., Alcalde, L.A., Roesler, R., Andersen, M.L., Quevedo, J., Kapczinski, F., & Schröder, N. (2012). Early life stress exacerbates cognitive dysfunction induced by d-

amphetamine: amelioration by valproic acid. J Neural Transm, 119, 627-637. http://dx.doi.org/10.1007/s00702-011-0754-y.

Pinheiro, R.M., de Lima, M.N., Portal, B.C., Busato, S.B., Falavigna, L., Ferreira, R.D., Paz, A.C., de Aguiar, B.W., Kapczinski, F., & Schröder, N. (2015). Long-lasting recognition memory impairment and alterations in brain levels of cytokines and BDNF induced by maternal deprivation: effects of valproic acid and topiramate. J Neural Transm, 122, 709-719. http://dx.doi.org/10.1007/s00702-014-1303-2.

Pryce, C.R., Bettschen, D., Nanz-Bahr, N.I., & Feldon, J. (2003). Comparison of the effects of early handling and early deprivation on conditioned stimulus, context, and spatial learning and memory in adult rats. Behav Neurosci, 117, 883-893.

Reolon, G.K., Maurmann, N., Werenicz, A., Garcia, V.A., Schröder, N., Wood, M.A., & Roesler, R. (2011). Posttraining systemic administration of the histone deacetylase inhibitor sodium butyrate ameliorates aging-related memory decline in rats. Behav Brain Res, 221, 329-332. http://dx.doi.org/10.1016/j.bbr.2011.03.033.

Réus, G.Z., Abelaira, H.M., dos Santos, M.A., Carlessi, A.S., Tomaz, D.B., Neotti, M.V., Liranço, J.L., Gubert, C., Barth, M., Kapczinski, F., & Quevedo, J. (2013). Ketamine and imipramine in the nucleus accumbens regulate histone deacetylation induced by maternal deprivation and are critical for associated behaviors. Behav Brain Res, 256, 451-456. http://dx.doi.org/10.1016/j.bbr.2013.08.041.

Rossato, J.I., Bevilaqua, L.R.M., Myskiw, J.C., Medina, J.H., Izquierdo, I., & Cammarota, M. (2007). On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory, Learn Mem, 14, 36-46. http://dx.doi.org/10.1101/lm.422607

Rüedi-Bettschen, D., Pederson, E.M., Feldon, J., & Pryce, C.R. (2005). Early deprivation under specific conditions leads to reduced interest in reward in adulthood in Wistar rats. Behav. Brain Res. 156, 297-310. http://dx.doi.org/10.1016/j.bbr.2004.06.001

Singh, P., Konar, A., Kumar, A., Srivas, S., & Thakur, M.K. (2015). Hippocampal chromatin-modifying enzymes are pivotal for scopolamine-induced synaptic plasticity gene expression changes and memory impairment. J Neurochem 134 (4), 642-651. http://dx.doi.org/10.1111/jnc.13171.

Silva, P.F., Garcia, V.A., Dornelles, A.da S., Silva, V.K., Maurmann, N., Portal, B.C., Ferreira, R.D., Piazza, F.C., Roesler, R., & Schröder. N. (2012). Memory impairment induced by brain iron overload is accompanied by reduced H3K9 acetylation and ameliorated by sodium butyrate. Neuroscience, 200, 42-49. http://dx.doi.org/10.1016/j.neuroscience.2011.10.038.

Suri, D., Veenit, V., Sarkar, A., Thiagarajan, D., Kumar, A., Nestler, E.J., Galande, S., & Vaidya, V.A. (2013). Early Stress Evokes Age-Dependent Biphasic Changes in Hipocampal Neurogenesis, Bdnf Expression, and Cognition. Biol Physhiatry, 73, 658-666. http://dx.doi.org/10.1016/j.biopsych.2012.10.023.

Valvassori, S.S., Varela, R.B., Arent, C.O., Dal-Pont, G.C., Bobsin, T.S., Budni, J., Reus, G.Z., & Quevedo, J. (2014). Sodium butyrate functions as an antidepressant and improves cognition with enhanced neurotrophic expression in models of maternal deprivation and chronic mild stress. Curr Neurovasc Res 11(4), 359-366. http://dx.doi.org/10.2174/1567202611666140829162158

Zhong, T., Qing, Q.J., Yang, Y., Zou, W.Y., Ye, Z., Yan, J.Q., & Guo, Q.L. (2014). Repression of contexual fear memory induced by isoflurane is accompanied by reduction in histone acetylation and rescued by sodium butyrate. Br J Anaesth. 113 (4), 634-643. http://dx.doi.org/10.1093/bja/aeu184.

Table 1. Mean ± S.E.M. total time (in seconds) exploring both objects in the training or long-term (LTM) retention test of novel object recognition task I and II (NOR I and II) in rats submitted to neonatal maternal deprivation (D) or not (ND) and to acute saline (vehicle) or sodium butyrate (1g/Kg) administration immediately after training. No significant differences among the groups were observed.

Novel Object Recognition Task I (NOR I)

Total time spent exploring objects during training session

Group	*Mean (s)*	*S.E.M.*	*N*
ND	62.9198	±1.89477	60
D-SL	62.1511	±2.47883	28
D-IL	68.0857	±1.91676	60

Total time spent exploring objects during LTM retention test

Group	*Mean (s)*	*S.E.M.*	*N*
ND	61.7000	±3.16528	60
D-SL	57.3039	±2.76666	28
D-IL	59.9855	±2.86917	60

Novel Object Recognition Task I (NOR II)

Euthanasia 3 hours after training

Total time spent exploring objects during training session

Group	*Mean (s)*	*S.E.M.*	*N*
ND-Vehicle	40.1993	±3.31765	14
ND-Sodium butyrate (1g/Kg)	35.9629	±3.85978	14
D-SL-Vehicle	41.2633	±5.83485	12
D-SL-Sodium butyrate (1g/Kg)	39.8131	±2.24375	13
D-IL-Vehicle	34.4979	±3.69357	14
D-IL-Sodium butyrate (1g/Kg)	46.3029	±4.65002	14

Euthanasia 24 hours after LTM retention test

Total time spent exploring objects during training session

Group	*Mean (s)*	*S.E.M.*	*N*
ND-Vehicle	32.4100	±5.33661	8
ND-Sodium butyrate (1g/Kg)	39.7010	±4.02335	10
D-IL-Vehicle	44.0140	±5.48839	10
D-IL-Sodium butyrate (1g/Kg)	43.9210	±4.32588	10

Total time spent exploring objects during LTM retention test

Group	*Mean (s)*	*S.E.M.*	*N*
ND-Vehicle	42.5513	±5.69381	8
ND-Sodium butyrate (1g/Kg)	39.7430	±4.90473	10
D-IL-Vehicle	49.4120	±4.84454	10
D-IL-Sodium butyrate (1g/Kg)	35.4820	±2.71457	10

Figure captions

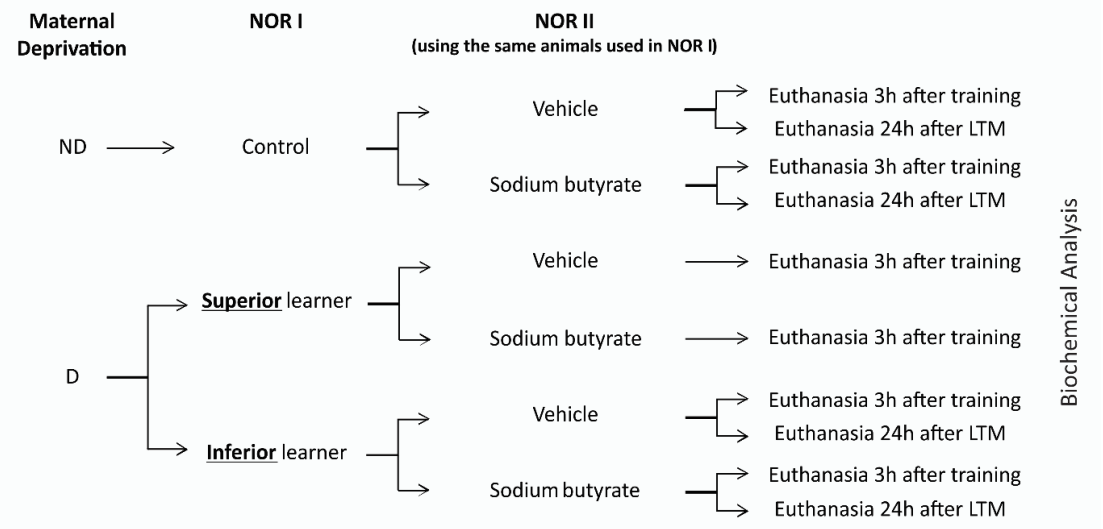

Fig. 1 Experimental design - Animals were submitted to maternal deprivation (D) or not (ND) from post-natal day 1-14. When they reached adulthood, these animals were submitted to object recognition task (NOR I) in order to identify who are the individuals with worse (IL – inferior learners) and better (SL – superior learners) cognitive performance in comparison to controls. After 1 month, the same animals were submitted to a new training session on the object recognition task (NOR II) in order to induce a new memory consolidation phase. Immediately after training, these animals received an intraperitoneal injection of vehicle (control group) or sodium butyrate (1g/Kg). These animals were then divided in other 2 groups: **1)** those who were euthanized 3 h after the training session (for biochemical analysis) and **2)** those submitted to the long-term memory (LTM) retention test of novel object recognition task and euthanized 24 h later.

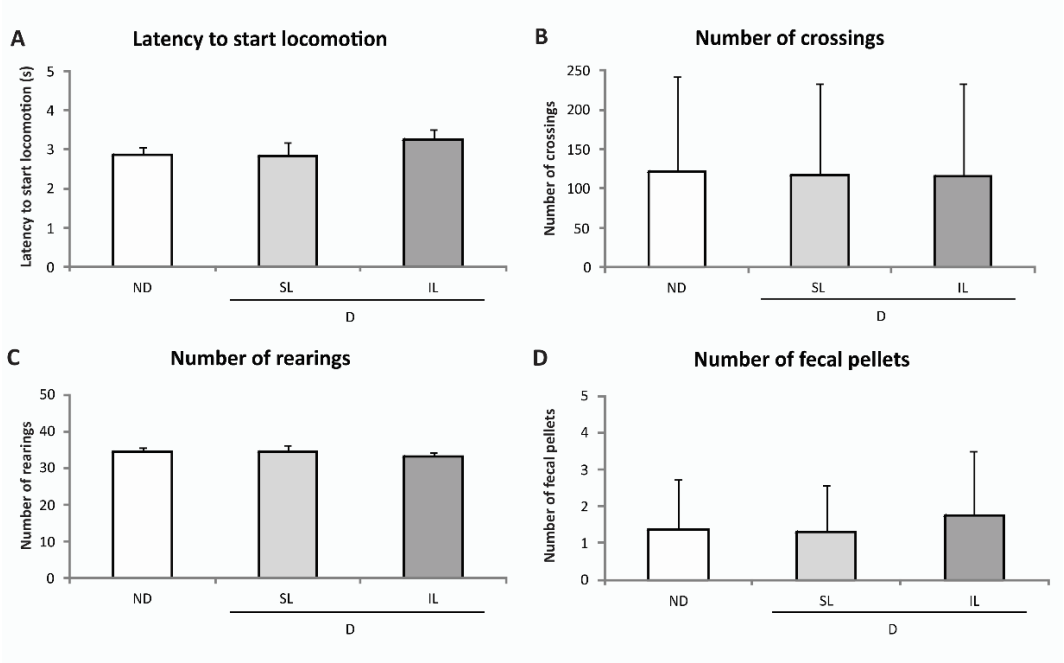

Fig. 2 Effects of maternal deprivation on open-field behavior. **(A)** Latency to start locomotion (s), **(B)** number of crossings, **(C)** number of rearings and **(D)** number of fecal pellets were evaluated in an open-field apparatus in adult life. The open-field behavior measurement was used as a habituation session for the novel object recognition task I (NOR I). Data are expressed as mean ± SEM. No significant differences among the groups were observed.

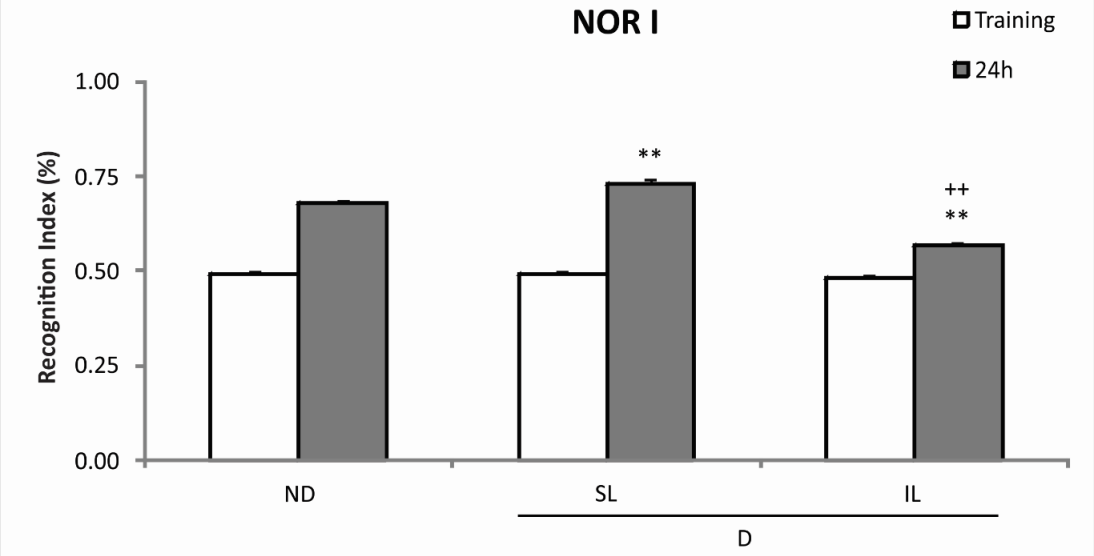

Fig. 3 Effects of maternal deprivation on novel object recognition task. Long-term memory (LTM) retention test were performed 24 hours after training in NOR I. The proportion of the total exploration time that the animal spent investigating the novel object was the "Recognition Index" expressed by the ratio $T_N/(T_F + T_N)$, T_F = time spent exploring the familiar object and T_N = time spent exploring the novel object. Data expressed as mean ± SEM, *n* =28-60 per group (NOR I). Groups of animals submitted (Deprived, D) or not (non-deprived, ND) to maternal deprivation in the neonatal period were subdivided into SL (superior learners) and IL (inferior learners) according to their performance in LTM retention test of NOR I (see item 2.5.3. Criteria for Categorizing SL and IL in the NOR Task). Differences between ND (control) group and all other groups are indicated as: $^{**}p < 0.001$ (LSD *post hoc* test); between D-SL group from other groups as: $^{++}p < 0.001$ (LSD *post hoc* test).

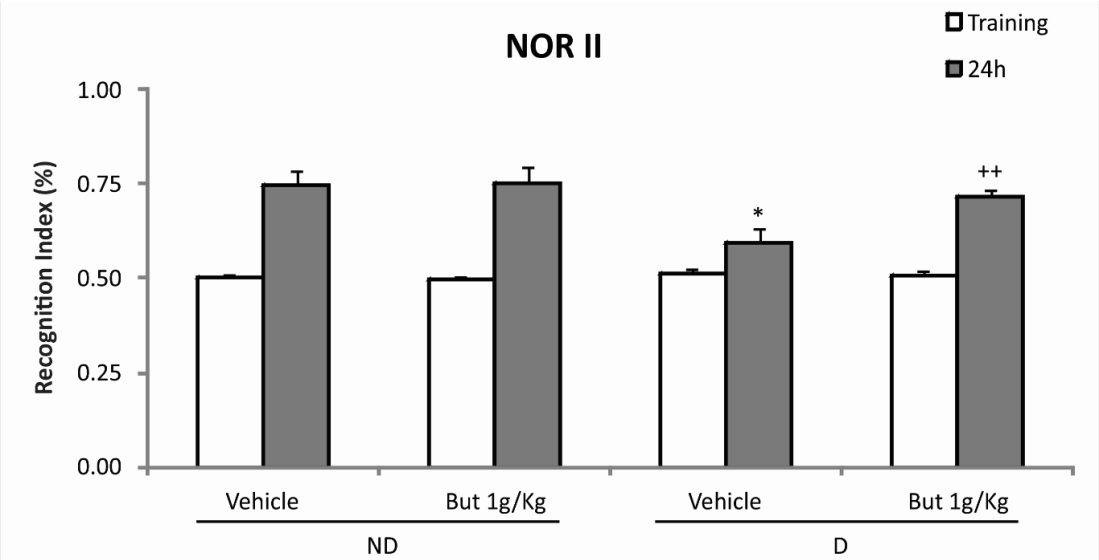

Fig. 4 Effects of NaBu on novel object recognition task. Long-term memory (LTM) retention test were performed 24 hours after training in NOR II. The proportion of the total exploration time that the animal spent investigating the novel object was the "Recognition Index" expressed by the ratio $T_N/(T_F + T_N)$, T_F = time spent exploring the familiar object and T_N = time spent exploring the novel object. Data expressed as mean ± SEM, n = 8-10 per group (NOR II). Groups of animals submitted (Deprived, D) and categorized as inferior learners (D-IL) or not (non-deprived, ND) to maternal deprivation in the neonatal period received vehicle or sodium butyrate (NaB, 1g/Kg/ip) immediately after training of NOR II. Differences between ND-Vehicle (control) group and all other groups are indicated as: $^{**}p < 0.01$ (LSD *post hoc* test); between D-IL-Vehicle group from other groups as: $^{++}p < 0.01$ (LSD *post hoc* test).

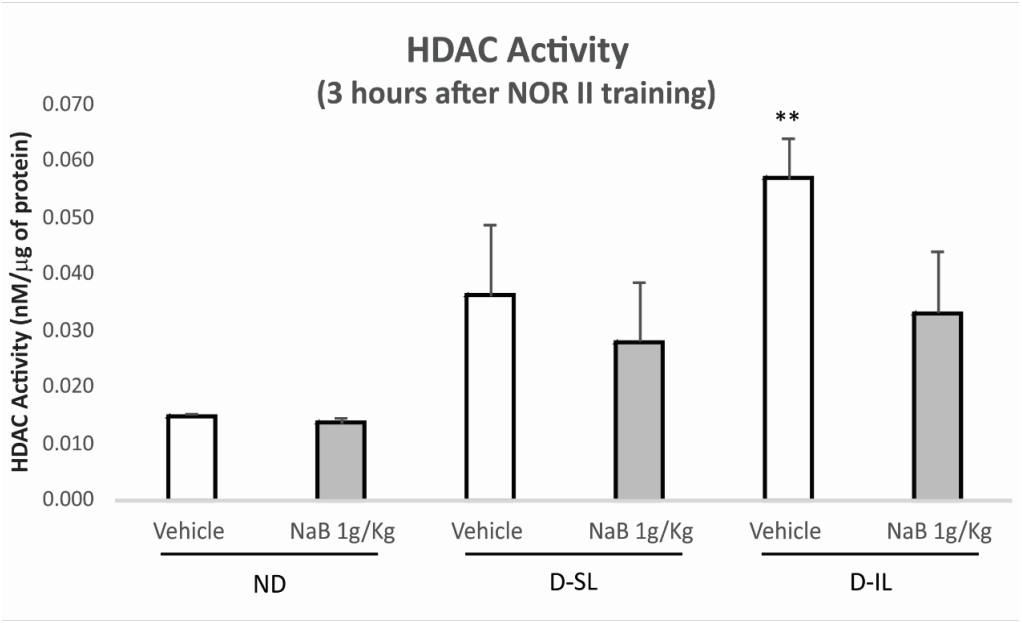

Fig. 5 Effects of maternal deprivation and sodium butyrate acute treatment (1g/Kg, ip) on histone deacetylase activity (HDAC) in hippocampus 3 hours after the training session of novel object recognition task II (NOR II). Data are expressed as mean ± SEM nM of deacetylated substrate/μg of protein, N = 4-5 animals per group. Differences between ND-Vehicle *versus* other groups are indicated as: $^{**}p < 0.01$ (LSD *post hoc* test).

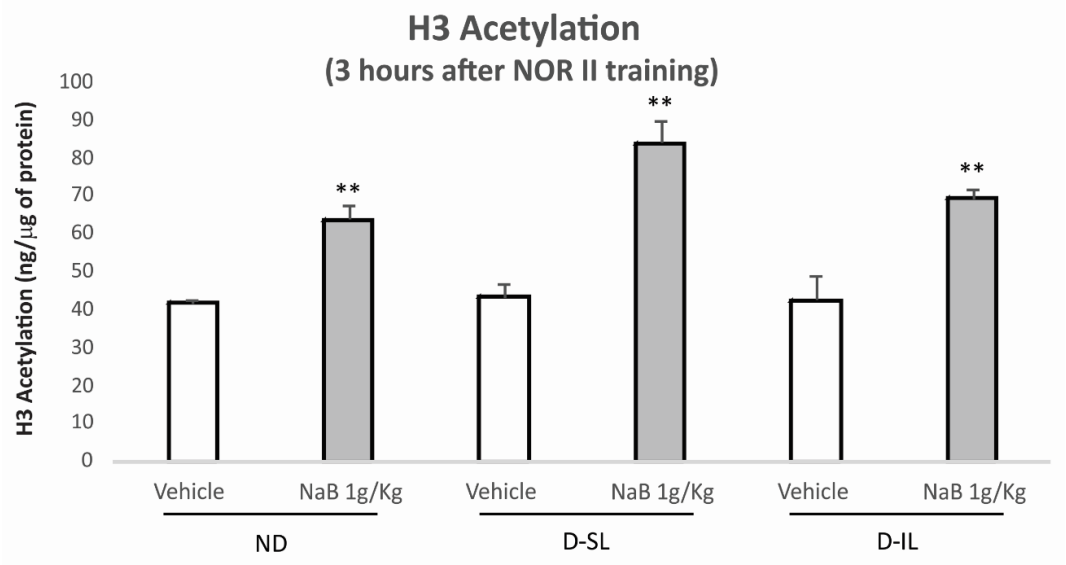

Fig. 6 Effects of maternal deprivation and sodium butyrate acute treatment (1g/Kg, ip) on H3 total acetylation in hippocampus 3 hours after the training session of novel object recognition task II (NOR II). Data are expressed as mean ± SEM ng of histone H3 acetylated/μg of protein, N = 4–6 animals per group. Differences between ND-Vehicle *versus* other groups are indicated as: $^{**}p < 0.01$ (LSD *post hoc* test).

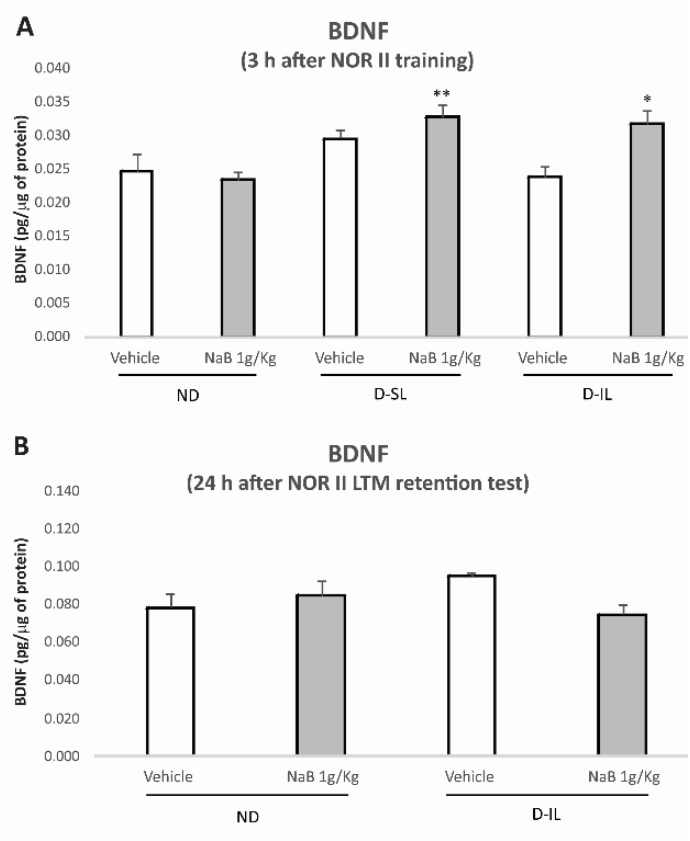

Fig. 7 Effects of maternal deprivation and sodium butyrate acute treatment (1g/Kg/ip) on brain-derived neurotrophic factor (BDNF) levels in hippocampus 3 hours after the training session (a) and 24 hours after LTM retention test (b) of novel object recognition task II (NOR II). Data are expressed as mean ± SEM pg of BDNF/μg of protein, N = 4-7 (3 h after training) and N = 3-6 (24h after training) animals per group. Differences between MD-IL-Vehicle *versus* other groups are indicated as: *$p < 0.05$ and **$p < 0.01$ (LSD *post hoc* test).

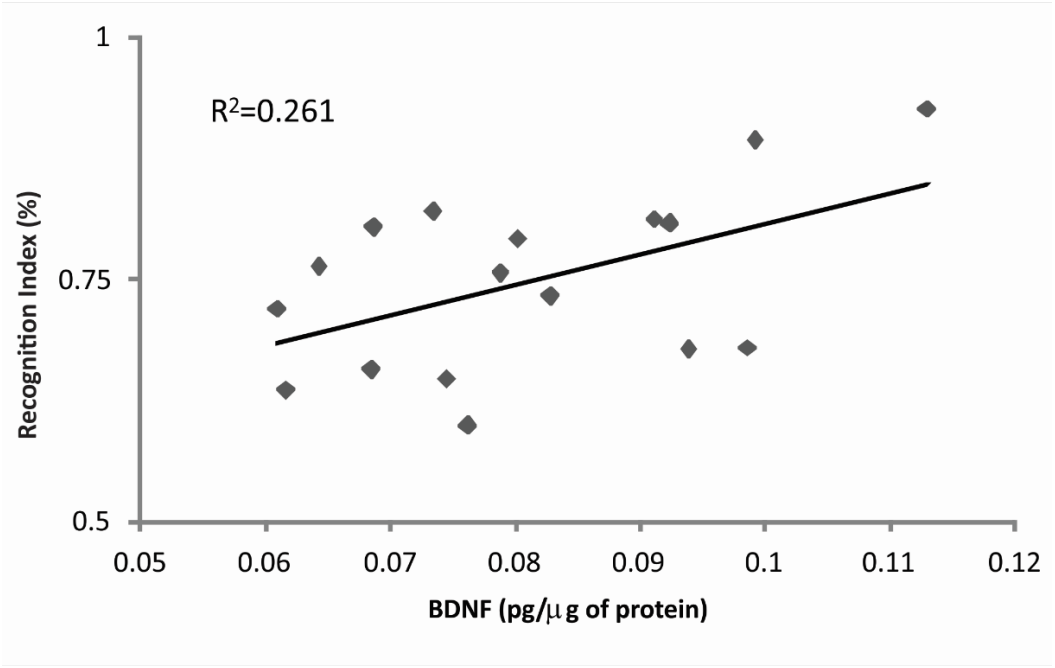

Fig. 8 Analyses of correlation between recognition index in LTM retention test and BDNF levels in the hippocampus 24 hours after LTM retention test of novel object recognition task II (NOR II). Each diamond represents an individual animal. Correlation between hippocampal BDNF levels and LTM recognition indexes (Pearson's test coefficient r = 0.511, p = 0. 036).

5 CONSIDERAÇÕES FINAIS

O primeiro dos parâmetros avaliados no estudo foi o parâmetro comportamental, no qual os roedores, ao atingirem a idade adulta, foram avaliados por meio do teste de Campo Aberto a fim de identificar o efeito da separação materna sobre a atividade locomotora, exploratória geral e ansiedade. Os resultados mostram não haver diferença significativa na locomoção, exploração e ansiedade causada pela separação materna, resultado este que corrobora com alguns achados da literatura (Shalev e Kafkafi, 2002; Marco *et al.*, 2013; Mela *et al.*, 2015).

Quanto ao índice de reconhecimento e retenção de memória, após 24 horas do teste de campo aberto, os animais foram, então, submetidos a testes de Reconhecimento do Objeto Novo I com a finalidade de se identificar nos animais do grupo Separado aqueles que tiveram melhor ou pior capacidade de aprendizado.

Os resultados dos testes de Memória de Reconhecimento I demonstraram que a separação materna teve efeito variável sobre a memória dos animais, havendo animais que tiveram desempenho de aprendizagem superior e inferior quando comparados aos animais do grupo Handling.

Os animais do grupo Handling foram considerados como animais controle no estudo e como padrão de boa memória. Resultados obtidos pelo nosso grupo de pesquisa, bem como em outros estudos de memória espacial já demostraram que Handling Neonatal tem efeitos positivos sobre o desempenho em testes de memória, tais como Labirinto Aquático de Morris (Garoflos *et al.*, 2005*;* Stamatakis *et al*., 2008), Labirinto de Braço Radial (Valee *et al.*, 1999), e Reconhecimento do Objeto Novo (Fenoglio *et. al.*, 2005), sugerindo, inclusive, que tais efeitos sejam mais evidentes em animais que atingem o envelhecimento, período no qual a

manipulação tende a atenuar o declínio de aprendizagem e memória que normalmente ocorrem (Raineki *et al*., 2014).

Já com relação à Separação Materna, como mostra a literatura, pode influenciar os animais tanto prejudicando no desempenho cognitivo, como visto na tarefa de Reconhecimento do Objeto Novo (Aisa *et al.*, 2007; Benetti *et al.*, 2009; Solas *et al.*, 2010; de Lima *et al*., 2011; Hulshof *et al.*, 2011; Wang *et al.*, 2011; Baudin *et al*, 2012; Wang *et al.*, 2014) e Labirinto Aquático de Morris (Hui *et al.*, 2011, Aisa *et al.*, 2007; Wang *et al.*, 2014), quanto melhorando ou mantendo capacidade cognitiva similar ao grupo controle, como visto nas tarefas de Labirinto de Cruz Elevado (Rüedi-Bettschen *et al.*, 2005), Labirinto Aquático de Morris (Oitz *et al.*, 2000, Suri *et al.*, 2014) e Reconhecimento do Objeto Novo (Makena *et al.*, 2012) demonstrando que as repetidas separações afetam parâmetros neuroendócrinos e fisiológicos e impactam não só levando a vulnerabilidade como também resiliência quando de desafios subsequentes (Schmidt, Wang e Meijer, 2011).

Portanto, se procurou, neste trabalho, não só evidenciar esta variabilidade como correlacioná-la a uma proteína já bem descrita e tida como imprescindível no processo de formação e consolidação da memória, no caso o BDNF.

Com os animais divididos entre os grupos Handling e Separados Superior e Inferior os mesmos foram submetidos novamente à tarefa de reconhecimento de objeto (Reconhecimento do Objeto Novo II) e desta vez, ao tratamento com Veículo e Butirato de Sódio a fim de analisar a influência do Butirato e sua capacidade em reestabelecer o aprendizado nos animais de pior desempenho.

Os resultados do teste de Reconhecimento do Objeto Novo II mostram que não houve diferença significativa de aprendizado quando comparados os animais do grupo Handling tratados ou com Veículo ou com Butirato de Sódio, de modo que o

Butirato de Sódio não foi capaz de melhorar ainda mais a capacidade de aprendizado nos animais cujo desempenho se encontrava normal. Já quando comparados animais do grupo Handling aos do grupo Separado Inferior, que tiveram pior desempenho de memória no teste de Reconhecimento do Objeto Novo I, o que se viu foi a confirmação do efeito prejudicial da Separação Materna sobre o aprendizado destes animais e, que os animais que foram tratados com Butirato de Sódio apresentaram melhora na capacidade de aprendizado quando comparados aos animais tratados com Veículo, demonstrando que o Butirato de Sódio reverteu o déficit de memória no grupo de animais que inicialmente apresentaram déficit cognitivo, tornando sua capacidade de aprendizado e memória comparável àquela dos animais pertencentes ao grupo não separado.

Estudos anteriores já haviam demonstrado a capacidade do Butirato de Sódio em melhorar a memória em modelos animais que apresentavam déficits cognitivos relacionados à separação materna e/ou estresse crônico leve precoce, revertendo prejuízos de memória no teste de Reconhecimento de Objeto Novo, num mecanismo diretamente relacionado com o aumentando nos níveis de neurotrofinas (Yasuda *et al.*, 2009; Valvassori *et al.,* 2014*;* Penney e Tsai, 2014).

A fim de identificar esta correlação e elucidar os aspectos bioquímicos envolvidos no processo de aprendizado e as diferenças entre os grupos Handling e Separado, bem como a resposta desencadeada pelo uso do Butirato de Sódio nos animais, foi realizada a eutanásia de parte dos animais de cada um dos grupos 3 horas após a administração aguda do Veículo e/ou Butirato de Sódio, a fim de avaliar a etapa na qual se dá início ao processo de síntese de proteínas necessárias à consolidação da memória. Foram medidos o nível de Acetilação das Histonas H3, a atividade da HDAC e o nível de BDNF no hipocampo dos animais eutanaziados 3

horas após a administração de Butirato de Sódio. Além disso, os níveis de BDNF também foram medidos nos animais que foram submetidos ao teste de retenção de memória de longa duração.

Nos últimos anos tem sido pontuada a importância da epigenética no funcionamento cerebral, incluído aí os processos de aprendizado e memória. Como os neurônios não se dividem nem podem ser substituídos, estudos sugerem que a regulação epigenética de proteínas necessárias ao processo de aprendizado e memória esteja relacionada com os déficits cognitivos observados nos indivíduos adultos que foram expostos ao estresse durante o período de desenvolvimento do SNC (Lubin *et al.,* 2008; Molfese, 2011; Sultan e Day, 2011; Jarome e Lubin, 2013; Suri *et al*., 2013).

Com relação ao nível de Acetilação das Histonas H3 viu-se que todos os grupos (não separado, separado inferior e separado superior) tiveram um aumento significativo induzido por Butirato de Sódio quando comparado com seus respectivos controles, o que foi obtido por administração de dose única. Isto comprova a efetiva inibição do Butirato de Sódio sobre HDAC, e efeito esperado sobre a acetilação da Histona H3.

Quando analisados os resultados da Atividade da HDAC de animais não Separados e Separados Inferiores que receberam injeção de salina, vê-se que os animais Separados Inferior apresentaram níveis de atividade da HDAC significativamente maiores se comparado ao grupo não separado, o que sugere que a atividade da HDAC esteja relacionada com o déficit de memória de reconhecimento visto em animais separados da mãe com pior desempenho cognitivo, pois estando aumentada impede a chegada da polimerase ao DNA e consequente transcrição de proteínas necessárias à consolidação da memória. Já

os animais separados da mãe com desempenho superior, não apresentaram diferenças na atividade da HDAC em relação grupo controle.

E, a análise do nível de BDNF demonstra uma correlação positiva entre o maior nível de BDNF no hipocampo e a melhor resposta cognitiva observada no teste da tarefa de reconhecimento realizado 24 horas após injeção aguda do Butirato de Sódio. A administração de Butirato de Sódio fez com que os níveis de BDNF no hipocampo dos animais privados maternalmente, independente do grupo de desempenho, apresentassem aumento 3 horas após o tratamento, sugerindo que a inibição da HDAC leva ao aumento da acetilação das histonas H3 que culmina com aumento da expressão de BDNF, neurotrofina esta de suma importância para a promoção da LTP, necessária ao processo de consolidação da memória de longo prazo.

Os resultados reforçam achados anteriores com relação à eficácia do Butirato de Sódio, enquanto inibidor as HDAC's, em induzir a expressão de um subconjunto de genes associados a efeitos neuroprotetores sobre a neurodegeneração e distúrbios da cognição, dentre os quais o BDNF (Abel e Zukin, 2008; Wu *et al.*, 2008; Chuang *et al.*, 2009; Yasuda *et al.*, 2009; Wang *et al*, 2014) o que torna o uso do inibidor da HDAC um alvo promissor sobre o controle de padrões de transcrição de genes que estabelecem e estabilizam processos comportamentais e cognitivos (Covington *et al.*, 2009).

O remodelamento da cromatina por meio da acetilação da histona tem importante papel na regulação da transcrição de gene BDNF (Martinowich et al., 2003; Aid et al., 2007), já tendo sido visto em estudos anteriores que o uso de inibidores da HDAC, tal como o Butirato de Sódio, tem efeito benéfico na recuperação da memória de longo prazo e na plasticidade sináptica (Fischer *et al.*,

2007; Guan *et al.*, 2009; Zeng *et al.*, 2011). Inibidores da HDAC não tem capacidade de modificar a expressão de BDNF em ratos não estressados atuando apenas sobre aqueles com estresse crônico. Um estudo anterior indicou que no estresse crônico o que se observa é uma redução persistente na acetilação da histona H3 e BDNF no hipocampo o qual é revertido após tratamento com Butirato de Sódio, auxiliando, assim, no processo de consolidação da memória de longo prazo (Huan *et al*, 2014).

Como já foi demonstrado em estudos anteriores, o BDNF, além de estar envolvido na formação de diferentes tipos de memória, também se mostra crítico para manter o armazenamento duradouro de informação no hipocampo, amígdala e córtex insular muitas horas após o aprendizado (Bekinschtein *et al.*, 2014). E a expressão anormal desta neurotrofina durante o desenvolvimento do cérebro causa perturbação funcional do sistema nervoso central, levando a deficiência na aprendizagem, memória e cognição, bem como desordens neuropsiquiátricas na vida adulta (Kikusui *et al.*, 2009; Miki *et al.*, 2013).

Estudos com ratos expostos ao estresse durante períodos precoces de vida evidenciam reduções na expressão de neurotrofinas (Kuma *et al.*, 2004; Duman e Monteggia, 2006) as quais estão intimamente ligadas a anomalias comportamentais (Weickert *et al.*, 2003; Karpova *et al.*, 2009). Curiosamente, no presente estudo, entretanto, não observamos diferenças significativas nos níveis de BDNF provocadas pela separação materna, 24 h após o treino do Reconhecimento do Objeto Novo II.

Como o BDNF têm efeitos demonstrados sobre a plasticidade e função cognitiva do cérebro, vários estudos realizados dão conta que experiências negativas na infância atuam sobre a regulação epigenética desta neurotrofina causando reduções na expressão de mRNA de BDNF que tendem a permanecer

constantes ao longo da vida e acabam influenciando a forma como o indivíduo responde e forma memórias do ambiente, alterando sua sensibilidade ao estresse bem como a capacidade cognitiva (Branchi *et al.*, 2004; Fumagalli *et al.*, 2007; Lippmann *et al.*, 2007; Francis *et al.*, 2009; Roth e Sweatt, 2011; Babenko, Kovalchuk e Mentz, 2015), processo este que foi demonstrado ser reversível a partir do uso de drogas inibidoras das HDACs.

6 CONCLUSÃO

O estresse precoce, como o mimetizado pelo método da Separação Materna pode afetar de diferentes formas a cognição na vida adulta, determinando tanto a vulnerabilidade como a resiliência nos indivíduos submetidos à condição estressora numa resposta que varia conforme a genética do indivíduo bem como de condições ambientais. Devido a apontamentos de muitos estudos a cerca desta variabilidade e sua correlação com níveis de BDNF se procurou investigar neste trabalho a relação entre BDNF, sua regulação epigenética no hipocampo, uma região chave do cérebro para o processamento do aprendizado e a memória em animais adultos. O verificado foi uma correlação entre melhor desempenho cognitivo e maiores níveis de BDNF, estando o pior desempenho cognitivo associado a aumento da atividade da HDAC e baixos níveis de acetilação de histonas H3, resultados estes revertidos pela utilização de Butirato de Sódio, um conhecido inibidor das HDACs e que coloca os inibidores da HDAC como um possível alvo terapêuticos em busca da melhora da performance cognitiva em indivíduos com déficits de aprendizado. Necessitando ainda de mais estudos que permitam um melhor entendimento dos mecanismos envolvidos no desenvolvimento de resiliência.

7 REFERÊNCIAS BIBLIOGRÁFICAS

Abel T e Zukin RS. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. Current Opinion in Pharmacology. ***Neurosci***. 8:57–64, 2008.

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T. Mouse and rat BDNF gene structure and expression revisited. ***J Neurosci Res***.85:525–535, 2007.

Aisa B, Tordera R, Lasheras B, Del Rio J, Ramirez MJ. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. ***Psychoneuroendocrinol***. (32): 256-266, 2007.

Babenko O, Kovalchuk I, Mentz GAS. Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. ***Neurosci and Biobehav. Rev***. 48: 70–91, 2015.

Baudin A, Blot K, Verney C, Estevez L, Santamaria J, Gressens P, Giros B, Otani S, Daugé V, Naudon L. Maternal deprivation induces deficits in temporal memory and cognitive flexibility and exaggerates synaptic plasticity in the rat medial prefrontal córtex. ***Neurobiol Learn Mem***. 98:207-214, 2012.

Bekinschtein P, Cammarota M, Medina JH. BDNF and memory processing. ***Neuropharmacol***. 76: 677-683, 2014.

Benetti F, Mello PB, Bonini JS, Monteiro S, Cammarota M, Izquierdo I. Early postnatal maternal deprivation in rats induces memory deficits in adult life that can be reversed by donepezil and galantamine. ***Int J of Dev Neurosci***. 27:59–64, 2009. [PubMed: 18948184]

Benoit JD, Rakic P, Frick KM. Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. ***Behav Brain Res***. 281: 1–8, 2015.

Bisaz R e Sullivan RM. Developmental Neurobiology of the Rat Attachment System and Its Modulation by Stress. **Behav. Sci.** 2, 79-102, 2012.

Bonasio R, Tu S, Reiberg D. Molecular signals of epigenetic states. ***Sci***. 330(6004):612-6, 2010.

Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. ***Anal Biochem***. 72:218-254, 1976.

Branchi I, Francia N, Alleva E. Epigenetic control of neurobehavioural plasticity: The role of neurotrophins. ***Behav Pharmacol***. 15:353– 362, 2004.

Calabrese F, Molteni R, Racagni G e Riva MA. Neuronal plasticity: A link between stress and mood disorders. ***Psychoneuroendocrinol***. 34S, S208—S216, 2009.

Campbell T, Lin S, Devries C, Lambert K. Coping strategies in male and female rats exposed to multiple stressors. ***Physiol Behav***. 78(3): 495-504, 2003.

Chuang DM, Leng Y, Marinova Z, Kim HJ and Chiu CT. Multiple roles of HDAC inhibition in neurodegenerative conditions. ***Trends in Neurosci***. Vol.32 No.11, 2009.

Covington HE, Maze I, LaPlant QC, Vialou VF, Yoshinori ON, Berton O, Fass DM, Renthal W, Rush III AJ , Wu EY, Ghose S, Krishnan V, Russo SJ, Tamminga C, Haggarty SJ, Nestler EJ . ANTIDEPRESSANT ACTIONS OF HDAC INHIBITORS. ***J Neurosci***. 16; 29(37): 11451–11460, 2009.

Cowansage KK, LeDoux JE, Monfils MH. Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity. ***Curr Mol Pharmacol***. 3(1):12-29, 2010.

Daskalakis NP, Enthoven L, Schoonheere E, De_kloet ER and Oitzl MS. Immediate effects of maternal deprivation on the (re)activity of the HPA axis differ in CD1 and C57Bl/6J mouse pups. ***Front. Endocrinol***. 5:147, 2014.

Dash PK, Orsi SA e Moore AN. Histone deactlylase inhibition combined with behavioral therapy enhances learning and memory following traumatic brain injury. ***Neurosci***. (163):1-8, 2009.

de Andrade FM, da Silva VK, Schuch JB. A influência genética sobre a memória humana: uma revisão. ***Braz J Biosci***. 9(4):520-532, 2011.

de Kloet ER, Oitzl MS e Joëls M. Stress and cognition: are corticosteroids good or bad guys? ***Trends Neurosci***. 22:422-426, 1999.

de Lima MN, Presti-Torres J, Vedana G, Alcalde LA, Stertz L, Fries GR, Roesler R, Andersen ML, Quevedo J, Kapczinski F, Schröder N. Early life stress decreases hippocampal BDNF content and exacerbates recognition memory deficits induced by repeated D-amphetamine exposure. ***Behav Brain Res***. 224(1):100-6, 2011a.

de Lima MN, Presti-Torres J, Dornelles A, Scalco FS, Roesler R, Garcia VA, Schröder N. Modulatory influence of dopamine receptors on consolidation of object recognition memory. ***Neurobiol Learn Mem***. 95(3):305-10, 2011b.

Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. ***Biol Psychiatry***. 59:1116–27, 2006.

Fenoglio KA, Brunson KL, Avishai-Eliner S, Stone BA, Kapadia BJ, Baram TZ. Enduring handlingevoked enhancement of hippocampal memory function and glucocorticoid receptor expression involves activation of the corticotropin-releasing factor Type 1 receptor. ***Endocrinol***. 146:4090–4096, 2005. [PubMed: 15932935]

Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH. Recovery of learning and memory is associated with chromatin remodelling. ***Nature***. 447:178–182, 2007.

Fischer A, Sananbenesi F, Mungenast A, Tsai LH. Targeting the correct HDAC(s) to treat cognitive disorders.***Trends Pharmacol Sci***. 31(12):605-17, 2010.

Flinn MV, Nepomnaschy PA, Muehlenbein MP, Ponzi D. Evolutionary functions of early social modulation of hypothalamic-pituitary-adrenal axis development in humans. ***Neurosci. Biobehav. Rev****.* 35: 1611-1629, 2011.

Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH. 2007. Recovery of learning and memory is associated with chromatin remodelling. Nature 447:178–182

Francis YI, Fa` M, Ashraf H, Zhang H, Staniszewski A, Latchman DS, Arancio O. Dysregulation of histone acetylation in the APP/PS1 mouse model of Alzheimer's disease. ***J Alzheimers Dis***. 18:131–139, 2009.

Fumagalli F, Molteni R, Racagni G, Riva MA. Stress during development: Impact on neuroplasticity and relevance to psychopathology. ***Prog Neurobiol***. 81:197–217, 2007.

Garcia VA, Hirotsu C, Matos G, Alvarenga T, Pires GN, Kapczinski F, Schröder N, Tufik S, Andersen ML. Modafinil ameliorates cognitive deficits induced by maternal separation and sleep deprivation. ***Behav Brain Res***. 253:274-9, 2013.

Garoflos E, Panagiotaropoulos T, Pondiki S, Stamatakis A, Philippidis E, Stylianopoulou F. Cellular mechanisms underlying the effects of an early experience on cognitive abilities and affective states. ***An of Gen Psych***. 4:8, 2005. [PubMed: 15876359]

Gerstein H, Hullinger R, Lindstrom MJ, Burger C. A behavioral paradigm to evaluate hippocampal performance in aged rodents for pharmacological and genetic target validation. ***PLoS One***. 8(5):e62360, 2013.

Guest FL, de-Souza DM, Rahmoune H, Bahn S, Guest PC. The effects of stress on hypothalamic-pituitary-adrenal (HPA) axis function in subjects with schizophrenia. ***Rev Psiq Clín****.* 40(1): 20-7, 2013.

Heim C, Plotsky PM, Nemeroff CB. Importance of Studying the Contributions of Early Adverse Experience to Neurobiological Findings in Depression. ***Neuropsychopharmacol***. 29:641-648, 2004.

Huan A, Sung YB, Chung SY, Kwon MS. Possible additional antidepressant-like mechanism of sodium butyrate: Targeting the hippocampus. ***Neuropharmacol***. 81: 292e302, 2014.

Hui J, Zhang Z, Liu S, Xi G, Zhang X, Teng G, Chan KC, Wu EX, Nie B, Shan B, Li L, Reynolds GP. Hippocampal neurochemistry is involved in the behavioural effects of neonatal maternal separation and their reversal by post-weaning environmental enrichment: A magnetic resonance study. ***Behav Brain Res***. (217):122-7, 2011.

Hulshof HJ, Novati A, Sgoifo A, Luiten PGM, den-Boer JA, Meerlo P. Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats. ***Behav Brain Res***. 216:552-60, 2011.

Huot RL, Plotsky PM, Lenox RH, McNamara RK. Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. ***Brain Res***. 950: 52–63, 2002.

Igaz LM, Vianna MR, Medina JH, Izquierdo I. Two times periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. J Neurosci. 22:6781-9, 2002.

Izquierdo I, Medina JH. Memory formation: The sequence of biochemical eventsin the hippocampus and its connection to activity in other brain structures. Neurobiol. of Learning and Memory. 68:287-316, 1997.

Jarome TJ, Lubin FD. Histone lysine methylation: critical regulator of memory and behavior. ***Rev Neurosci***. 24(4):375-87, 2013.

Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. ***J Child Psychol Psychiatry***. 48(3-4):224-44, 2007.

Karpova NN, Lindholm J, Pruunsild P, Timmusk T, Castrén E. Long-lasting behavioural and molecular alterations induced by early postnatal fluoxetine exposure are restored by chronic fluoxetine treatment in adult mice. ***Eur Neuropsychopharmacol***. 19:97-108, 2009.

Kikusui T, Ichikawa S, Mori Y. Maternal deprivation by early weaning increases corticosterone and decreases hippocampal BDNF and neurogenesis in mice. ***Psychoneuroendocrinol***. 34:762–772, 2009.

Kuma H, Miki T, Matsumoto Y, Gu H, Li H-P, Kusaka T, Satriotomo I, Okamoto H, Yokoyama T, Bedi KS, Onishi S, Suwaki H, Takeuchi Y. Early maternal deprivation induces alterations in brain-derived neurotrophic factor expression in the developing rat hippocampus. ***Neurosci Letters*** 372. 68–73, 2004.

Lajud N, Roque A, Cajero M, Gutiérrez-Ospina G, Torner L. Periodic maternal separation decreases hippocampal neurogenesis without affecting basal corticosterone during the stress hyporesponsive period, but alters HPA axis and coping behavior in adulthood. ***Psychoneuroendocrinol***. 37:410-20, 2012.

Leckman JF, Mayes LC, Cohen DJ. Primary maternal preoccupation revisited: circuits, genes, and the crucial role of early life experience. ***SA Psych Rev***. 5:4-12, 2002.

Lehmann, J., Feldon, J. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? ***Rev.Neurosci***. 11, 383-408, 2000.

Levine S. Infantile experience and resistance to physiological stress. ***Sci.*** N Y 126:405, 1957.
Levine S. Maternal and environmental influences on the adrenocortical response to stress in weanling rats. ***Sci***. 156(772):258-60, 1967.

Lippmann M, Bress A, Nemeroff CB, Plotsky PM, Monteggia LM. Long-term behavioural and molecular alterations associated with maternal separation in rats. ***Eur J Neurosci***. 25:3091–3098, 2007.

Lubin FD, Roth TL, Sweatt D. Epigenetic regulation of bdnf gene transcription in the consolidation of fear memory. ***J Neurosci***. 28(42):10576-86, 2008.

Macrì S, Chiarotti F, Würbel H. Maternal separation and maternal care act independently on the development of HPA responses in male rats. ***Behav Brain Res***. 191(2):227-34, 2008.

Macrì S, Laviola G, Leussis MP, Andersen SL. Abnormal behavioral and neurotrophic development in the younger sibling receiving less maternal care in a communal nursing paradigm in rats. ***Psychoneuroendocrinol***. 35:392–402, 2009.

Makena N, Bugarith K, Russel V. Maternal separation enhances object location memory and prevents exercise-induced MAPK/ERK signalling in adult Sprague–Dawley rats. ***Metab. Brain Dis.*** 27(3): 377-385, 2012.

Marco EM, Valero M, de la Serna O, Aisa B, Borcel E, Ramirez MJ, Viveros MP. Maternal deprivation effects on brain plasticity and recognition memory in adolescent male and female rats. ***Neuropharmacol***. (68) 223e231, 2013.

Marco E, Llorente R, López-Gallardo M, Mela V, Llorente-Berzal A, Prada C, Viveros MP. The maternal deprivation animal model revisited. **Neurosci. Ad Biobehav. Rev.** 51, 151-163, 2015.

Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun YE. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. ***Sci***. *302*:890 – 893, 2003.

McEwen BS, Sapolsky RM. Stress and cognitive function. ***Curr Opin Neurobiol***. 5:205-216, 1995.

McEwen BS. Stress and hippocampal plasticity. *Annu.* ***Rev. Neurosci***. 22:105-122, 1999.

McEwen BS. Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. ***Physiol Rev***. 87:873-904, 2007.

McEwen BS, Eiland L, Hunter RG e Miller MM. Stress and anxiety: Structural plasticity and epigenetic regulation as a consequence of stress. ***Neuropharmacol***. 62, 3e12, 2012.

McGaugh JL. Memory – A century of Consolidation. Sci. 287:248-51, 2000.

Mela V, Díaz F, Borcel E, Argente J, Chowen JÁ, Viveros MP. Long Term Hippocampal and Cortical Changes Induced by Maternal Deprivation and Neonatal Leptin Treatment in Male and Female Rats. ***PLOS ONE***. 10 (9). DOI:10.1371/journal.pone.0137283, 2015.

Miki T, Lee K-Y, Yokoyama T, Liu J-Q, Kusaka T, Suzuki S, Ohta K-I, Warita K, Jamal M, Ueki M, Yakura T, Hosomi N, Takeuchi Y. Differential effects of neonatal maternal separation on the expression of neurotrophic factors in rat brain. II: Regional differences in the cerebellum versus the cerebral cortex. ***Okajimas Folia Anat. Jpn***., 90(3): 53–58, 2013.

Molfese, D. L. Advancing neuroscience through epigenetics: Molecular mechanisms of learning and memory. ***Dev Neuropsychol***. 36, 810–827, 2011.

Ohl F, Michaelis T, Vollmann-Hons Dorf GK, Kirschbaum C, Fuchs E. Effect of chronic psycossocial stress and long term cortisol treatment on hippocampus-mediated memory and hippocampal volume: a pilot-study in tree shrews. ***Psychoneuroendocrinol***. 25(4):357-63, 2000.

Oitzl MS, Workel JO, Fluttert M, Frösch F, De Kloet ER. Maternal deprivation affects behaviour from youth to senescence: amplification of individual differences in spatial learning and memory in senescent Brown Norway rats. ***Eur J Neurosci***. 12:3771–80, 2000.

Ortiz JB, Mathewson CM, Hoffman AN, Hanavan PD, Terwilliger EF, Conrad CD. Hippocampal brain-derived neurotrophic factor mediates recovery from chronic stress-induced spatial reference memory déficits. ***Eur J Neurosci***. 1-12, 2014.

Papaioannou A, Gerozissis K, Prokopiou A, Bolaris S, Stylianopoulou F. Sex differences in the effects of neonatal handling on the animal's response to stress and the vulnerability for depressive behaviour. ***Behav Brain Res***. 129:131–139, 2002.

Peixoto L, Abel T. The role of histone acetylation in memory formation and cognitive impairments. ***Neuropsychopharmacol****.* 38,62-76, 2013.

Penney J e Tsai LH. Histone deacetylases in memory and cognition. ***Neurosci***. ***Rev***. Vol 7 Issue 355 re12, 2014.

Peter CJ, Akbarian S. Balancing Histone Methylation Activities in Psychiatric Disorders. ***Trends Mol Med***. 17(7):372-79, 2011.

Pinheiro RM, de Lima MN, Fries GR, Garcia VA, Presti-Torres J, Hallmenschlager LH, Alcalde LA, Roesler R, Andersen ML, Quevedo J, Kapczinski F, Schröder N. Early life stress exacerbates cognitive dysfunction induced by d-amphetamine: amelioration by valproic acid. ***J Neural Transm***. 119(6):627-37, 2012.

Pinheiro RMC, de Lima MNM, Portal BCD, Busato SB, Falavigna L, Ferreira RDP, Paz AC, de Aguiar BW, Kapczinski F, Schröder N. Long-lasting recognition memory impairment and alterations in brain levels of cytokines and BDNF induced by

maternal deprivation: effects of valproic acid and topiramate. ***J Neural* Transm**. 2014. No prelo

Pfau ML e Russo SJ. Peripheral and central mechanisms of stress resilience. ***Neurobiology of Stress 1*.** 66e79, 2015.

Ploj, K., Roman, E., Nylander, I. Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. ***Neurosci***. 121, 787-799, 2003.

Plotsky PM, Thrivikraman KV, Nemeroff CB, Caldji C, Sharma S, Meaney MJ. Long-term consequences of neonatal rearing on central corticotrophinreleasing factor systems in adult male rat offspring. ***Neuropsychopharmacol***. 30:2192–204, 2005.

Preethi J, Sing HK, Venkataraman JS, Rajan KE. Standardised extract of Bacopa monniera (CDRI-08) improves contextual fear memory by the diferentially regulating the activity of histone acetylation and protein phosphatases (PP1α, PP2A) in hippocampus. ***Cell Mol Neurobiol***. 34(4):577-89, 2014.

Pryce CR, Bettschen D, Nanz-Bahr NI, Feldon J. Comparison of the effects of early handling and early deprivation on conditioned stimulus, context, and spatial learning and memory in adult rats. ***Behav Neurosci***. 117(5):883-93, 2003.

Raineki C, Lucion AB, Weinberg J. Neonatal Handling: An Overview of the Positive and Negative Effects. **Dev. *Psychobiol*.** 56: 1613–1625, 2014.

Reul JMHM. Making memories of stressful events: a journey along epigenetic, gene transcription, and signaling pathways. ***Front Psychiatry***. 5:5, 2014.

Rüedi-Bettschen D, Pederson EM, Feldon J, Pryce CR. Early deprivation under specific conditions leads to reduced interest in reward in adulthood in Wistar rats. ***Behav. Brain Res***. 156:297-310, 2005.

Roth TL, Sweatt JD. Epigenetic marking of the BDNF gene by early-life adverse experiences. ***Horm Behav***. 59 (3): 315-320, 2011.

Ryu, H. et al. Histone deacetylase inhibitors prevent oxidative neuronal death independent of expanded polyglutamine repeats via an Sp1-dependent pathway. ***Proc. Natl. Acad. Sci***. U. S. A. 100, 4281–4286, 2003.

Sánchez MM, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. ***Dev Psychopathol***. 13:419–49, 2001.

Schaaf MJM, Workel JO, Lesscher HM, Vreugdenhil E, Oitzl MS, de Kloet ER. Correlation between hippocampal BDNF mRNA expression and memory performance in senescent rats. ***Brain Res***. 915: 227-233, 2001.

Schmidt MV, Wang XD, Meijer OC. Early life stress paradigms in rodents: potential animal models of depression? ***Psychopharmacol*.** 214:131–140, 2011.

Shalev U, Kafkafi N. Repeated maternal separation does not alter sucrose-reinforced and open-field behaviors. ***Pharmacol., Biochem. and Behav*.** (73): 115 – 122, 2002.

Silva PF, Garcia VA, Dornelles Ada S, Silva VK, Maurmann N, Portal BC, Ferreira RD, Piazza FC, Roesler R, Schröder N. Memory impairment induced by brain iron overload is accompanied by reduced H3K9 acetylation and ameliorated by sodium butyrate. ***Neurosci**.* 200: 42-49, 2012.

Slotten HA, Kalinichev M, Hagan JJ, Marsden CA, Fone KCF. Long-lasting changes in behavioural and neuroendocrine indices in the rat following neonatal maternal separation: gender-dependent effects. ***Brain Res***. 1097: 123–32, 2006.

Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues* ***Clin Neurosci***. 8(4): 383-395, 2006.

Solas M, Aisa B, Mugueta MC, DelRio J, Tordera RM, Rarmirez MJ. Interactions between age, stress and insulin on cognition: implications for Alzheimer's Disease. ***Neuropsychopharmacol***. 35:1664–1673, 2010. [PubMed: 20182419]

Stamatakis A, Toutountzi E, Fragioudaki K, Kouvelas ED, Stylianopoulou F, Mitsacos A. Selective effects of neonatal handling on rat brain N-methyl-D-aspartate receptors. ***Neurosci***. 164:1457–1467, 2009. [PubMed: 19778590]

Steckler T, Drinkenburg NHIM, Sahgal A, Aggleton JP. Recognition memory in the rat I: Concepts and classification. ***Progress in Neurobiol***, v. 54, p. 289 – 311, 1998.

Steckler T, Drinkenburg NHIM, Sahgal A, Aggleton JP. Recognition memory in rats-II. Neuroanatomical substrates. ***Progress in Neurobiol***. v. 54, p. 313-332, 1998.

Steckler T, Drinkenburg NHIM, Sahgal A, Aggleton JP. Recognition memory in rats-III. Neuroanatomical substrates. ***Progress in Neurobiol***. v. 54, p. 333-348, 1998.

Sultan, F. A., & Day, J. J. Epigenetic mechanisms in memory and synaptic function. ***Epigenomics***. 3, 157–181, 2011.

Suri D, Veenit V, Sarkar A, Thiagarajan D, Kumar A, Nestler EJ, Galande S, Vaidya VA. Early Stress Evokes Age-Dependent Biphasic Changes in Hipocampal Neurogenesis, Bdnf Expression, and Cognition. ***Biol Phys***. 73:658-66, 2013.

Suri D, Bhattacharya A, Vaidya VA. Early stress evokes temporally distinct consequences on the hippocampal transcriptome, anxiety and cognitive behaviour. *Intern J* ***Neuropsychopharmacol***. 17, 289-301, 2014.

Takase K, Yamamoto Y, Yagami T. Maternal deprivation in the middle of a stress hyporesponsive period decreases hippocampal calcineurin expression and causes abnormal social and cognitive behaviours in adult male Wistar rats: Relevance to negative symptoms of schizophrenia. ***Behav. Brain Res*.** (232) 306–315, 2012.

Vallee M, MacCari S, Dellu F, Simon H, LeMoal M, Mayo W. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and

cognitive performance: a longitudinal study in the rat. ***Eur J of Neurosci***. 11:2906–2916, 1999. [PubMed: 10457187]

Valvassori SS, Varela RB, Arent CO, Dal-Pont GC, Bobsin TS, Budni J, Reus GZ, Quevedo J. Sodium Butyrate Functions as an Antidepressant and Improves Cognition with Enhanced Neurotrophic Expression in Models of Maternal Deprivation and Chronic Mild Stress. ***Curr Neurovasc Res****.* 2014. No prelo

Wang L, Jiao J, Dulawa SC. Infant maternal separation impairs adult cognitive performance in BALB/cJ mice. ***Psychopharmacol***. 216:207-218, 2011.

Wang A, Nie W, Li H, Hou Y, Yu Z, Fan Q, Sun R. Epigenetic Upregulation of Corticotrophin-Releasing Hormone Mediates Postnatal Maternal Separation Induced Memory Deficiency. ***PLoS One***, 2014.

Weickert CS, Hyde TM, Lipska BK, Herman MM, Weinberger DR, Kleinman JE. Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. ***Mol Psychiatry***. 8:592–610, 2003.

Wu X, Chen PS, Dallas S, Wilson B, Block ML, Wang CC *et al*. Histone deacetylase inhibitors up-regulate astrocyte GDNF and BDNF gene transcription and protect dopaminergic neurons. ***Int J Neuropsychopharmacol***. 11: 1123–1134, 2008.

Yasuda S, Liang MH, Marinova Z, Yahyavi A, Chuang DM. The mood stabilizers lithium and valproate selectively activate the promoter IV of brain-derived neurotrophic factor in neurons. ***Mol. Psychiatry*****.** 14, 51–59, 2009.

Zeng Y, Tan M, Kohyama J, Sneddon M, Watson JB, SunYE, Xie CW. Epigenetic Enhancement of BDNF Signaling Rescues Synaptic Plasticity in Aging. ***The J of Neurosci***. 31(49):17800 –17810, 2011.

Zhang X, Wang B, Jin J, An S, Zeng Q, Duan Y, Yang L, Ma J, Cao X. Early deprivation reduced anxiety and enhanced memory in adult male rats. ***Brain Res Bull***. 108,44-50, 2014.

8 ANEXOS

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA, INOVAÇÃO E DESENVOLVIMENTO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Ofício 41/2014 - CEUA

Porto Alegre, 09 de junho de 2014.

Prezado Sr(a). Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou seu Protocolo de Pesquisa, registro CEUA 14/00397, intitulado **"Associação entre exposição ao estresse durante o período de desenvolvimento do sistema nervoso central e performance cognitiva na vida adulta: vulnerabilidade, resiliência e epigenética".**

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.

Nº de Animais	Espécie	Duração do Projeto
300	Rattus novergicus	03/2014 – 03/2015

Atenciosamente,

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

Ilma. Sra.
Profa. Dra. Maria Noêmia Martins de Lima
FABIO
Nesta Universidade

PUCRS
Campus Central
Av. Ipiranga, 6681 – P. 99 – Portal Tecnopuc – sala 1512
CEP: 90619-900 – Porto Alegre/RS
Fone: (51) 3353-6365
E-mail: ceua@pucrs.br

Assunto: Neurobiology of Learning and Memory: Submission Confirmation

Title: Association between early life stress exposure and cognition in adult life: vulnerability, resilience and epigenetic
Corresponding Author: Dr. Maria Lima
Authors: Manoel O Albuquerque-Filho; Betânia S de Freitas; Rebeca L Garcia; Pedro F Crivelaro; Nadja Schröder;
Article Type: Regular Article

Dear Dr. Lima,

This is to confirm that the above-mentioned manuscript has been received for consideration in Neurobiology of Learning and Memory.

You will be able to check on the progress of your manuscript by logging on to the Elsevier Editorial System for Neurobiology of Learning and Memory as an author:

http://ees.elsevier.com/ynlme/
Your username is: mnoemia@terra.com.br
If you need to retrieve password details, please go to: http://ees.elsevier.com/YNLME/automail_query.asp.

Your paper will be given a manuscript number shortly and you will soon receive an e-mail with this number for your reference.

Thank you for submitting your manuscript to Neurobiology of Learning and Memory. Should you have any questions, please feel free to contact our office.

Kind regards,

Ted Abel
Editor
Neurobiology of Learning and Memory
Elsevier
Email: nlm@elsevier.com

For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.