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BIOQUÍMICA**

**ENRIQUECIMENTO AMBIENTAL MODIFICA A MORFOLOGIA DOS
ASTRÓCITOS DO HIPOCAMPO E A RESPOSTA COMPORTAMENTAL NO
RECONHECIMENTO DE OBJETO EM CAMUNDONGOS**

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Mas se a Ciência provar o contrário,
se o calendário nos contrariar
mas se o destino insistir em nos separar,
dane-se, os astros, os autos, os signos,
os dogmas, os búzios, as bulas, anúncios,
tratados, ciganas, projetos, profetas,
sinopses, espelhos, conselhos,
se dane o evangelho e todos os orijás,
serás o meu amor, serás a minha paz.

Chico Buarque

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RESUMO

O termo neuroplasticidade se refere às mudanças funcionais ou anatômicas que ocorrem no sistema nervoso decorrentes de experiências. O enriquecimento ambiental (EA) é um dos modelos experimentais utilizados para estudar eventos relacionados à neuroplasticidade, pois aumenta a neurogênese, os níveis de neurotrofinas, a sobrevivência neuronal, a sinaptogênese, além de induzir cascatas de sinalização e uma mudança na arborização dendrítica dos neurônios de diversas regiões encefálicas. Nos últimos anos a importância dos astrócitos tem ganho destaque, principalmente no que tange a sua capacidade de modular sinapses e na participação ativa em eventos plásticos no encéfalo, essas mudanças estão relacionadas a alterações funcionais e morfológicas. O EA é um modelo interessante para avaliar performances comportamentais pois é um modelo que ocasiona mudanças na capacidade de armazenar e acessar novas informações. Sendo assim, acreditamos que o EA é capaz de gerar efeitos plásticos nos astrócitos do Stratum Radiatum da região CA1 e alterar as respostas comportamentais a tarefa de reconhecimento de objetos. Após oito semanas de EA iniciado logo após o desmame, camundongos CF-1 albinos não apresentam diferenças significativas no número de astrócitos GFAP-ir e na densidade óptica para GFAP no Stratum Radiatum. Entretanto ocorreu um aumento no número de processos originários do soma, aumento no tamanho destes processos no eixo lateral, paralelo as colaterais de Schaffer e no número de intersecções aos círculos concêntricos de Scholl na mesma região. Os astrócitos adquirem um formato estrelado, este achado pode estar relacionado ao aumento da densidade sináptica nesta região e corroboram com a idéia de que modificações astrocitárias são parte ativa dos processos plásticos que ocorrem no encéfalo. Nossos resultados mostraram também uma mudança comportamental na resposta a tarefa de reconhecimento de objetos. Os animais expostos ao EA despendem menos tempo explorando os objetos, tanto familiar como não familiar e apresentam igual capacidade de discriminar os objetos. Estes resultados demonstram um comportamento mais propício a sobrevivência da espécie em animais expostos ao EA, o que inclui uma rápida exploração e um possível aumento na capacidade de aprender sobre o ambiente.

Palavras- chaves: Enriquecimento ambiental, astrócitos, GFAP, etologia, reconhecimento de objetos, camundongos

ABSTRACT

Environmental enrichment (EE) induces plastic changes in the brain, including morphological changes in hippocampal neurons, with increases in synaptic and spine densities. In recent years, the evidence for a role of astrocytes in regulating synaptic transmission and plasticity has increased, and it is likely that morphological and functional changes in astrocytes play an important role in brain plasticity. In others hand EE is used to investigate behavioral modifications associated with gene-environmental interaction. Our study was designed to evaluate changes in astrocytes induced by EE in the hippocampus, focusing on astrocytic density and on morphological changes in astrocytic processes and the performance in object recognition task (ORT) for evaluate animals ability to learn about their environment. After 8 weeks of EE starting at weaning, CF-1 mice presented no significant changes in astrocyte number or in the density of glial fibrillary acidic protein immunoreactivity (GFAP-ir) in the Stratum Radiatum. However, in the same region occur significant increase in the ramification of astrocytic processes, as well as by an increase in the number and length of primary processes extending in a parallel orientation to CA1 nerve fibers. This led astrocytes to acquire a more stellate morphology, a fact which could be related to the increase in hippocampal synaptic density observed in previous studies. These findings corroborate the idea that structural changes in astrocytic networks are an integral part of plasticity processes occurring in the brain. In other hand, our results indicate that EE decreased the time the animals spent exploring familiar and unfamiliar objects and total time spent exploring both objects, without affecting the capacity of discrimination of objects. These findings indicate a more propitious behavior for species survival in animals subjected to EE, including rapid exploration and learning about the environment.

Key words: environmental enrichment, astrocytes, GFAP, object recognition task, ethologic, mice

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INTRODUÇÃO

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LISTA DE ABREVIATURAS

ADN- ácido desoxirribonucléico

ATP- adenosina 5'- trifosfato

BDNF – fator neurotrófico derivado do encéfalo

CA1- corno de Ammon 1

EA - enriquecimento ambiental

GFAP- proteína glial fibrilar ácida

TrkB- receptor tirosina cinase B

SNC – sistema nervoso central

I. INTRODUÇÃO

O termo neuroplasticidade se refere às mudanças funcionais e anatômicas que ocorrem no sistema nervoso central (SNC) decorrentes de novas experiências (SPOLIDORO et al., 2008). Na literatura são descritos diversos modelos que promovem modificações plásticas do SNC, tais como, exercício físico, estresse crônico ou agudo, epilepsia, enriquecimento ambiental, entre outros. Esses modelos apresentam diversas particularidades e o SNC responde da maneira mais propícia a cada um destes estímulos, demonstrando assim sua capacidade plástica de se moldar às diferentes situações as quais é exposto (SPOLIDORO et al., 2008).

I.1. Enriquecimento ambiental

O protocolo de enriquecimento ambiental (EA) consiste na introdução de variedades criativas nos recintos a fim de contribuir com o bem-estar dos animais cativos. Existem diversos modelos de EA para roedores, com variações no tempo de enriquecimento, idade inicial de exposição ao EA, espécies e cepas utilizadas, entre outras. Entretanto, é importante ressaltar que o tipo de enriquecimento utilizado deve ser apropriado à espécie em questão (NITHIANANTHARAJAH & HANNAN, 2006).

O EA é um modelo experimental utilizado para estudar eventos relacionados à neuroplasticidade em diferentes regiões encefálicas, de diversas espécies de animais criados em laboratório (ratos, camundongos, gerbilos, entre outros), em

et al., 2006), além de uma mudança na arborização dendrítica dos neurônios de diversas regiões encefálicas (FAHERTY et al., 2003). Estas mudanças ocorrem em diversas regiões corticais, sendo que se destacam de forma particular os efeitos observados sobre o hipocampo de animais expostos ao EA (MOHAMMED et al., 2002).

Estudos recentes demonstraram que o EA provoca uma facilitação na transcrição do ADN e um subsequente aumento na síntese de proteínas no SNC (FISCHER et al., 2007, PIZZORUSSO et al., 2007).

Uma característica importante para otimização do modelo de EA, com o intuito de promover um aumento na formação de mapas espaciais e um aumento na plasticidade hipocampal dos animais submetidos a este protocolo é a troca dos objetos com os quais os animais interagem e a troca de posição desses objetos dentro do ambiente (NITHIANANTHARAJAH & HANNAN, 2006).

Sendo assim, as mudanças na capacidade de armazenar e acessar novas informações tornam o EA um modelo interessante para avaliar desempenhos comportamentais (TANG et al., 2001, AMARAL et al., 2008). Ademais, outro fator que faz com que o EA seja um interessante modelo de estudo para análises de tarefas comportamentais, é a diferença de resposta entre as espécies, ou mesmo cepa de roedores empregadas neste modelo, nas distintas tarefas (NITHIANANTHARAJAH & HANNAN, 2006). Nos últimos anos a discussão sobre interação gene-ambiente vem merecendo grande destaque em trabalhos envolvendo o EA (ABRAMOV et al., 2008).

Os protocolos de EA exacerbam as características comportamentais inerentes a cada espécie animal e suas diferentes cepas. Sendo assim, a

discrepância nos resultados obtidos em tarefas comportamentais, pode ser ocasionada pelo fato de o EA acentuar as características comportamentais intrínsecas a cada espécie animal e suas diferentes cepas (ABRAMOV et al., 2008).

Dentre os resultados contraditórios, comuns em estudos que utilizam o EA, podemos ressaltar as diferentes respostas entre espécies ou cepas a tarefas comportamentais, tais como: o labirinto aquático (ABRAMOV et al., 2008), o campo aberto (AMARAL et al 2008), o reconhecimento de objetos (RENNER, 1987, BRUEL-JUNGERMAN et al., 2005) entre outras.



Figura 2 : Foto das gaiolas utilizadas em nosso experimento. A esquerda gaiola do EA e a direita grupo controle (Amaral et al., 2008)

Quanto à expressão proteica, também encontramos algumas discrepâncias nos dados encontrados na literatura, por exemplo, o fator neurotrófico derivado do encéfalo (BDNF) e seu receptor tirosina cinase B (TrkB). Alguns estudos demonstram um aumento na expressão de BDNF (ICKES et al., 2000, ROSSI et al., 2006) enquanto outros não demonstram variação (BINDU et al., 2007, HICKS et al., 2002) na mesma região (hipocampo). Esta discrepância talvez seja explicada por diferentes fatores, tais como, espécie animal, ou cepa de animal utilizada, idade do animal, tipo de enriquecimento utilizado, tempo de manutenção do EA, entre outros.

Devido a estes fatos, o enriquecimento ambiental tornou-se um modelo utilizado em laboratórios com o intuito de melhor compreender as interações gene-ambiente, comportamentais, fisiológicas, bioquímicas e morfológicas no SNC dos diferentes animais utilizados para pesquisa científica. Além da grande gama de roedores (ratos, camundongos, gerbilos) de diferentes cepas utilizadas em laboratório como modelos para estes estudos, alguns zoológicos introduziram o EA, como forma de proporcionar uma melhor qualidade de vida aos animais em cativeiro e monitorar níveis de estresse, comportamentos estereotipados e outros malefícios causados pela vida em cativeiro (BURNS et al., 2009).

I. 2. Astrócitos

Em 1859 Virchow descreveu a porção não neuronal do SNC como “nerve glue”, ou um tecido conectivo do SNC, que ficou posteriormente conhecido como glia. As células gliais foram classificadas em microglia e macroglia, sendo a segunda dividida em astrócitos e oligodendrócitos.

Com o aumento de estudos envolvendo os astrócitos e suas funções, demonstrou-se que os astrócitos apresentam diversas funções no SNC (VOLTERRA & MELDOLESI, 2005). Nas últimas décadas foram encontrados novos papéis fisiológicos para estas células e uma gama de relações destas com os neurônios, tais como, síntese e captação de neurotransmissores, auxílio na sinaptogênese, entre outras (ARAQUE et al., 1999).

A participação dos astrócitos nas sinapses e seus diversos papéis fisiológicos envolvidos na funcionalidade sináptica levaram a Araque e colaboradores (1999) a cunhar o termo sinapse tripartita para designar essa gama de relações. Os astrócitos protoplasmáticos apresentam uma estrutura bastante intrincada e esta se encontra diretamente ligada a sua funcionalidade, qual seja, liberação e captação de neurotransmissores. Portanto é provável que modificações na estrutura destas células, provoque mudanças em suas funções (ARAQUE et al., 1999).

A proteína fibrilar glial ácida (GFAP) é uma proteína do citoesqueleto de astrócitos que contribui para a definição da forma astrocítica destas células. Esta proteína é expressa em astrócitos maduros e sua detecção ainda pode ser utilizada como um dos principais marcadores astrocitários no SNC (GARCIA-SEGURA & MCCARTHY, 2004). Mudanças na expressão ou fosforilação desta proteína podem acarretar modificações na morfologia astrocitária o que nos indica uma possível mudança na funcionalidade astrocitária (GOMES et al., 1999; LAPING et al., 1994).

Na região encefálica que abordaremos em nosso estudo (Stratum Radiatum da região CA1 do hipocampo) a grande maioria dos astrócitos de roedores adultos

joventes apresenta um formato fusiforme, e seguem uma orientação paralela aos dendritos apicais dos neurônios piramidais de CA1. Esta região, também apresenta em menor número alguns astrócitos esféricos e outros astrócitos com formato mais alongados (BUSHONG et al., 2002).

As alterações astrocitárias geralmente ocorrem em paralelo com as mudanças neuronais (THEODOSIS et al., 2008). Após o EA, ocorre um aumento da arborização dendrítica na região CA1 do hipocampo (FAHERTY et al., 2003) e aumento na sinaptogênese no mesmo local (MOSER et al., 1994). Contudo, que seja de nosso conhecimento, não foram documentadas, até o presente momento, alterações morfológicas em astrócitos, nesta região, após exposição aos protocolos de EA.

Até a década de 90, a dificuldade na observação de mudanças na expressão de GFAP (mais especificamente na morfologia astrocitária), frente a diferentes protocolos experimentais, era intimamente relacionada à análise observacional puramente qualitativa. Esta análise dependia basicamente da qualidade das técnicas imunoistoquímicas empregadas e da experiência prévia do observador.

Recentemente, com a padronização das técnicas imunoistoquímicas e os avanços das técnicas de análise de imagens, mais especificamente as medidas de densidade óptica, análise planar e os círculos concêntricos de Scholl, permitiram uma análise semi-quantitativa e quantitativa, com maior precisão e acurácia dos diferentes tipos celulares do SNC, entre estes, os astrócitos GFAP positivos (Martinez et al., 2006, XAVIER et al., 2005).

I. 3. Hipocampo

O hipocampo é uma estrutura allocortical, que possui três camadas, a camada de células piramidais, a camada plexiforme e a camada molecular (Amaral & Witter, 1989).

A camada piramidal concentra os corpos das células piramidais, enquanto que as outras camadas são caracterizadas pelo neuropilo, que é uma densa rede de axônios, dendritos e com grande quantidade de astrócitos, onde ocorre a maioria das sinapses (Amaral & Witter, 1989).

O hipocampo constitui, em associação com outras regiões do encéfalo, um complexo chamado formação hipocampal, que compreende seis regiões citoarquitônicas distintas. Estas regiões estão conectadas por meio de um circuito conhecido como via trissináptica (Amaral & Witter, 1989).

A via trissináptica (Figura 2) inicia-se no córtex entorrinal e termina nas células piramidais de CA1. Os axônios dos neurônios piramidais do córtex entorrinal formam um feixe nervoso, a via perforante, que faz sinapse com os dendritos das células granulares do giro denteado. Por sua vez, estas células emitem axônios denominados fibras musgosas, para as células piramidais de CA3. Essa via é finalizada com sinapses de axônios amielínicos, designados de Colaterais de Schaffer, no Stratum Radiatum da região CA1. Esta via tem sido demonstrada por estudos anatômicos e eletrofisiológicos. Este tipo de circuitária, excitatório, unidirecional e não recíproca é extremamente atípica em conexões cortico-corticais.

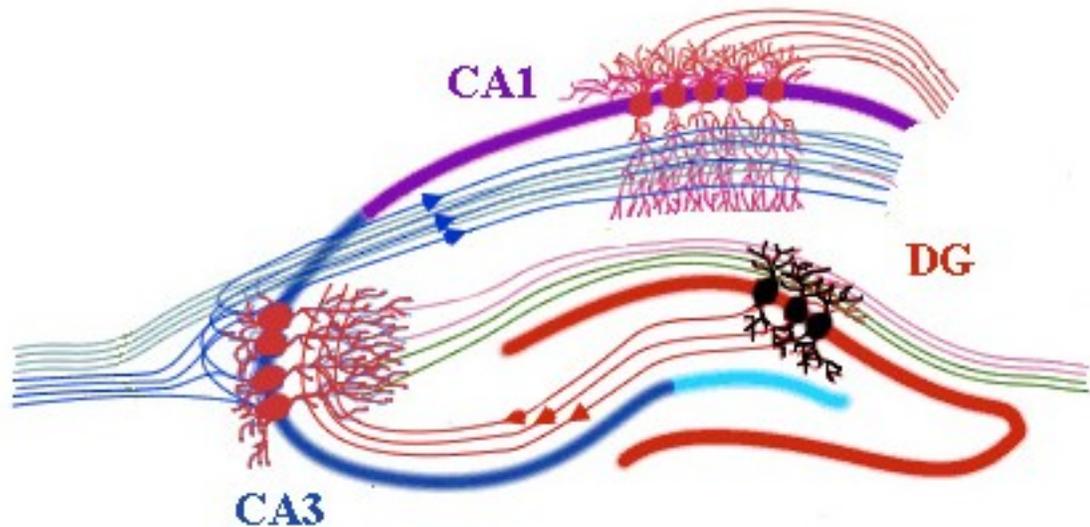


Figura 3: Desenhos esquemáticos do hipocampo, mostrando as regiões hipocâmpais e a circuitária hipocâmpal. Destacando: a via perforante em verde e rosa, fazendo sinapse com neurônios do giro denteado (em preto), as fibras musgosas em vermelho, fazendo sinapse com neurônios da região CA3 em vermelho e as Colaterais de Schaffer, em azul que saem de CA3 fazendo contato com dendritos localizados no Stratum Radiatum da região CA1

O hipocampo é uma região encefálica que exerce um papel fundamental em processos de formação, consolidação e evocação da memória (ROSSATO et al., 2007, FARIOLI-VECCHIOLI et al., 2008). O EA modifica a fisiologia das sinapses no hipocampo, além de aumentar a neurogênese (MOSER et al., 1994) e a arborização dendrítica na região CA1 (FAHERTY et al., 2003).

I. 4. Enriquecimento ambiental e comportamento

Cada vez mais na literatura relacionada a comportamento animal, é ressaltada a importância de se levar em conta perspectivas etológicas, envolvidas

nas tarefas comportamentais, a serem realizadas por animais de laboratório (BRANCHI & RICCERI, 2004).

Animais, em geral, apresentam a característica de explorar o ambiente a que são expostos, obtendo assim informações para melhor se relacionar com este ambiente (LIMA & DILL, 1990). A capacidade de responder a mudanças no ambiente é importante para a manutenção da vida animal, tanto na natureza quanto em cativeiro (laboratório, zoológico). Animais de laboratório apresentam uma série de comportamentos distintos aos encontrados em seus congêneres de vida selvagem, entre os quais podemos destacar comportamentos estereotipados (PRICE, 1999). Estes comportamentos são atenuados em animais expostos ao EA, possibilitando que estes animais tenham um comportamento mais propício a espécie (BURNS et al., 2009).

Em seu estágio juvenil grande parte dos animais necessita adquirir o máximo de informações sobre o ambiente em que vivem, criando assim ferramentas fisiológicas, morfológicas e comportamentais para as fases seguintes da vida, em muitas espécies, caracterizada por migração e formação de colônia (STAMPS et al, 2007). O conhecimento do ambiente é necessário para aumentar as chances de sobrevivência e de sucesso reprodutivo (LIMA & DILL, 1990). Camundongos selvagens necessitam, portanto, encontrar comida e parceiros sexuais para manutenção da espécie, ao mesmo tempo em que é necessário evitar camundongos rivais pelos recursos e predadores, sendo, necessário um bom conhecimento sobre o ambiente em que vivem (LIMA & DILL, 1990).

Animais domésticos apresentam encéfalos de 8 a 33% menores (principalmente no telencéfalo) quando comparados com seus congêneres

selvagens (KRUSKA, 1988). Esta diminuição é atribuída às diferenças genéticas geradas pela seleção artificial para escolha de características comportamentais, como por exemplo, docilidade (PRICE, 1999). O EA possibilita um aumento do telencéfalo, tornando-o mais parecido com o de um animal selvagem, provocando uma melhora nas habilidades comportamentais e diminuindo os comportamentos estereotipados em diversas espécies cativas (BURNS et al., 2009).

O enriquecimento ambiental propicia uma melhora no desempenho das funções cognitivas avaliadas em animais testados em diferentes tarefas comportamentais (RAMPON et al., 2000, TANG et al., 2001), além de reduzir o déficit de memória decorrente do envelhecimento (BENNETT et al., 2006). Entretanto, no que tange a trabalhos que avaliam a atividade exploratória, a literatura apresenta resultados discrepantes (CHAPILLON et al., 1999 e AMARAL et al., 2008), o que corrobora com a idéia que o EA exacerba as características comportamentais inerentes a cada espécie animal e suas diferentes cepas.

Diversos trabalhos utilizam à tarefa de reconhecimento de objetos pela sua capacidade de avaliar comportamentos naturais de roedores, tais como, a preferência por explorar objetos novos em detrimento de objetos familiares e a capacidade de recordar sobre o ambiente a que são expostos (BEVINS & BESHEER, 2006, COSTA et al., 2008).

Sendo assim, sugerimos que o EA é capaz de gerar efeitos plásticos nos astrócitos do Stratum Radiatum da região CA1 e alterar as respostas comportamentais a tarefa de reconhecimento de objetos.

II. OBJETIVOS

Objetivo geral

Avaliar parâmetros neuroplásticos gerados pelo EA em camundongos jovens.

Objetivos específicos

Avaliar uma possível modificação na forma dos astrócitos do Stratum Radiatum da região CA1 hipocampal.

Avaliar uma possível variação na densidade astrócitaria e no imunorreatividade a GFAP em astrócitos da mesma região.

Avaliar uma possível mudanças no imunoconteúdo de BDNF e seu receptor TrkB.

Avaliar uma possível mudanças comportamentais na tarefa de reconhecimento de objetos.

III. ARTIGOS CIENTÍFICOS

III. 1. CAPÍTULO 1 - VIOLA, G.G.; RODRIGUES, L.; AMERICO, J.C.; HANSEL, G.; VARGAS, R.S.; BIASIBETTI, R.; SWAROWSKY, A.; GONCALVES, C.A.; XAVIER, L.L.; ACHAVAL, M.; SOUZA, D.O.; AMARAL, O.B. Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice. Artigo publicado no periódico Brain Research, 1274: 47-54, 2009.

available at www.sciencedirect.comwww.elsevier.com/locate/brainres**BRAIN
RESEARCH**

Research Report

Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice

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ABSTRACT

Environmental enrichment is known to induce plastic changes in the brain, including morphological changes in hippocampal neurons, with increases in synaptic and spine densities. In recent years, the evidence for a role of astrocytes in regulating synaptic transmission and plasticity has increased, and it is likely that morphological and functional changes in astrocytes play an important role in brain plasticity. Our study was designed to evaluate changes in astrocytes induced by environmental enrichment in the CA1 region of the hippocampus, focusing on astrocytic density and on morphological changes in astrocytic processes. After 8 weeks of environmental enrichment starting at weaning, male CF-1 mice presented no significant changes in astrocyte number or in the density of glial fibrillary acidic protein (GFAP) immunoreactivity in the stratum radiatum. However, they did present changes in astrocytic morphology in the same region, as expressed by a significant increase in the ramification of astrocytic processes measured by the Sholl concentric circles method, as well as by an increase in the number and length of primary processes extending in a parallel orientation to CA1 nerve fibers. This led astrocytes to acquire a more stellate morphology, a fact which could be related to the increase in hippocampal synaptic density observed in previous studies. These findings corroborate the idea that structural changes in astrocytic networks are an integral part of plasticity processes occurring in the brain.

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1. Introduction

Neuroplasticity refers to the changes that occur in the functional and anatomical organization of the brain as a result of experience (Spolidoro et al., 2009). Environmental enrichment (EE) is an experimental model that allows the study of neuroplasticity by providing increases in physical activity, learning experiences, somatosensory and visual inputs and social interaction among animals in their home environment (van Praag et al., 2000; Mohammed et al., 2002). EE promotes plasticity and neuronal protection through increased levels of neurotrophins (Ickes et al., 2000; Rossi et al., 2006), changes in cell proliferation (Kempermann et al., 1997; Steiner et al., 2004), induction of signaling cascades (Li et al., 2006), and enhanced dendritic branching and synaptogenesis (Moser et al., 1994; Rampon et al., 2000b). It also leads to chromatin remodeling and histone acetylation, which reg-

ulate the susceptibility of DNA to transcription and therefore control protein formation (Fischer et al., 2007). These changes lead to alterations in neuronal morphology and physiology, such as altered synaptic responses and increased synaptic number and size (Moser et al., 1994; Rampon et al., 2000b).

Astrocytic and neuronal changes in response to various stimuli generally occur in parallel (Theodosios et al., 2008), and increases in astrocytic volume and number have been reported after EE in cortical regions (Szeligo and Leblond, 1977; Sirevaag and Greenough, 1991). In the visual cortex, exposure to a complex environment also increases the contact between astrocytic processes and synapses (Jones and Greenough, 1996). This enhanced morphological synergism may be a mechanism by which astrocytes can better regulate the synaptic microenvironment in response to increased neural activity (Jones and Greenough, 1996; Dong and Greenough, 2004). However, most of the work demonstrating changes in

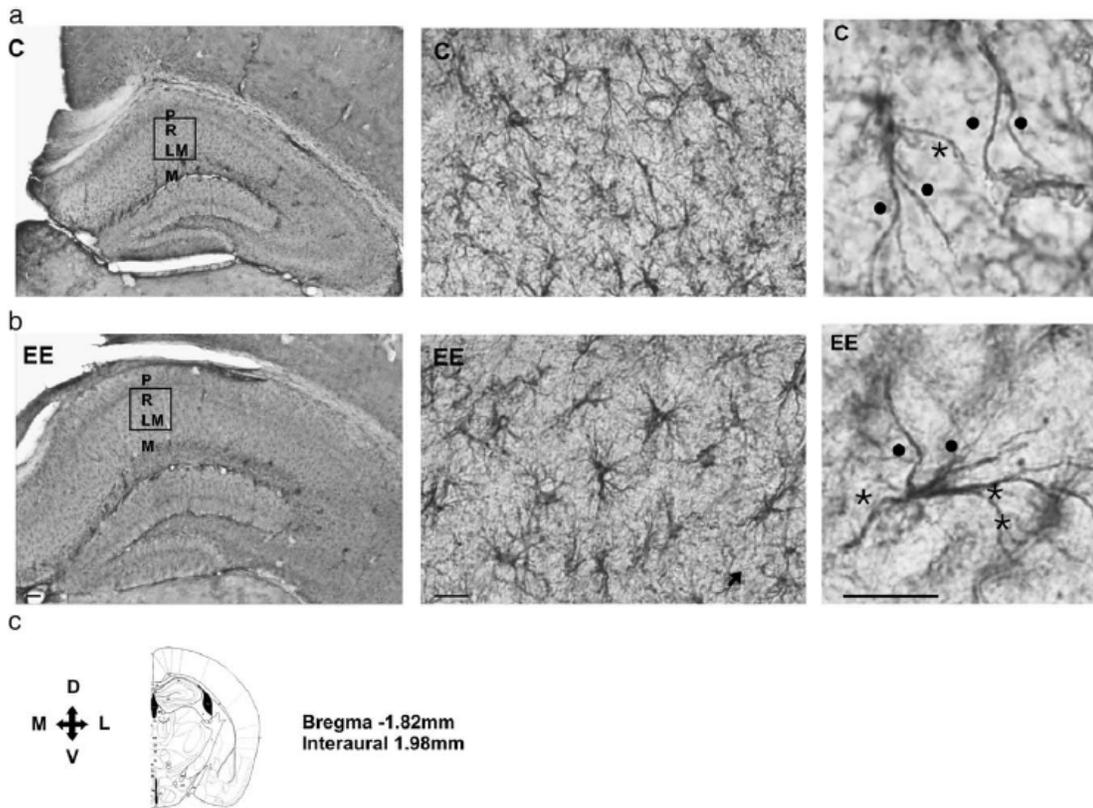


Fig. 1 – Digitized images of GFAP immunohistochemistry in the CA1 region of the hippocampus in animals weaned in control and enriched environments. In the stratum radiatum of control animals (a, top line), astrocytes tended to display a fusiform shape, while astrocytes in animals which underwent enrichment (b, bottom line) had more stellate shapes. In the right image, primary processes extending in the central (dots) and lateral (asterisks) quadrants of astrocytes are shown; lateral processes tended to be more numerous and longer in animals undergoing enrichment. (c) The orientation and location of the images are shown on a schematic drawing of the brain slice (adapted from Franklin and Paxinos, 1997). P, stratum pyramidale; R, stratum radiatum; LM, stratum lacunosum moleculare; M, stratum moleculare of the dentate gyrus; Scale bars: left, 100 μ m; center, 50 μ m; right, 10 μ m.

astrocytic morphology after EE has been performed in the neocortex. Data on whether such changes also occur in the hippocampus are scarcer and more controversial, with studies demonstrating either an increase (Briones et al., 2006) or no change (Sirevaag et al., 1991) in astrocytic surface density. Moreover, no data on astrocytic morphology was provided in either one of these studies.

Protoplasmic astrocytes play many important roles in the brain, including guiding synapse development (Ullian et al., 2001), regulating extracellular concentrations of various molecules (Vernadakis, 1996; Theodosis et al., 2008), supporting neuronal energetic metabolism (Simpson et al., 2007) and modulating neuronal and synaptic function (Perea and Araque, 2005; Santello and Volterra, 2009; Fiacco et al., 2009). Astrocytic networks establish astrocyte-specific anatomical domains in the hippocampus (Bushong et al., 2002) and are susceptible to plasticity, controlled by mediators secreted by neurons and other cell types in the brain including other astrocytes, microglia and endothelial cells (Theodosis et al., 2008). Since EE promotes synaptic changes in neurons (Foster et al., 1996; Rampon et al., 2000a), modulation of astrocytic shape is probably involved in the effects of enrichment, as plastic changes in synapses involve the interaction between new spines and astrocytic processes (Ullian et al., 2001; Haber et al., 2006).

EE has been shown to increase synaptic and spine density in the hippocampus (Moser et al., 1994; Rampon et al., 2000a), with morphological changes observed in neurons in CA1 and in the dentate gyrus (Faherty et al., 2003). The hippocampus is involved in several important functions including learning and memory (Squire et al., 2004), which have been widely shown to be positively modulated by environmental enrichment (Kempermann et al., 1997; Tang et al., 2001; Bruehl-Jungerman et al., 2005). It also contains numerous astrocytes that are located predominantly in Ammon's horn, dentate gyrus and subiculum (Catalani et al., 2002). In the presence of long-term potentiation (LTP) in the dentate gyrus, astrocytes around potentiated synapses have been shown to increase the ramification of their processes to accompany neural plasticity (Wenzel et al., 1991).

However, even though astrocytic plasticity is probably involved in the effects of EE in the hippocampus, there is little data on whether EE can induce changes in hippocampal

astrocytic networks (Sirevaag et al., 1991; Briones et al., 2006). We have therefore decided to study whether EE induces morphologic changes in astrocytes in the mouse hippocampus, focusing on the stratum radiatum of CA1, a region where synapses between CA3 Schaffer collaterals and CA1 neurons are found (Megias et al., 2001), and where astrocytic calcium currents have been shown to be induced by neuronal activity (Porter and McCarthy, 1996). For this means, we performed immunohistochemical analysis of GFAP in hippocampal slices obtained from animals weaned under enriched or control conditions, followed by quantitative analysis of astrocytic density and morphology.

2. Results

Male CF1 albino mice were weaned at P21 and housed under environmental enrichment or control conditions for 8 weeks. After this period, animals were sacrificed and their brains were prepared for immunohistochemical analysis using an anti-GFAP antibody. The stratum radiatum of the CA1 region was analyzed in these sections with the use of an automated software in order to evaluate the number of astrocytes, regional GFAP optical density (OD) and GFAP OD in individual astrocytes. Astrocytic morphology was evaluated in the same region by analyzing the degree of astrocytic process ramification with the Sholl concentric circles method, as well as the number, orientation and length of astrocytic primary processes.

Astrocytic cell bodies and processes were identified in the CA1 stratum radiatum as shown in Fig. 1. Those observed in the control group usually had rod or fusiform shapes, while stellate shapes were observed less often. In the EE group, on the other hand, qualitative analysis showed an increase in astrocytic branching, with more astrocytes acquiring stellate shapes.

Quantitative results for number of astrocytes (Fig. 2a), regional GFAP OD (Fig. 2b) and GFAP OD within individual astrocytes (Fig. 2c) are shown in Fig. 2. There was no significant difference in any of these parameters between the enriched and control groups (unpaired t test, $p=0.98$ for astrocyte number, 0.79 for regional OD and 0.74 for astrocytic

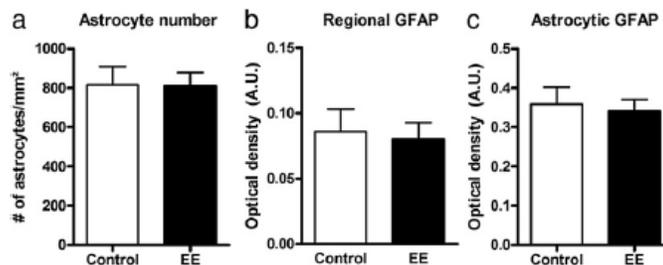


Fig. 2 – Effects of environmental enrichment on astrocyte number and GFAP density in the stratum radiatum of CA1. (a) number of astrocytes per mm² in sections from control (white bar) and EE (black bar) animals (unpaired t test, $p=0.98$); (b) regional GFAP optical density measurements in sections from control and EE animals (unpaired t test, $p=0.79$); (c) astrocytic GFAP optical density measurements in astrocytes within sections from control and EE animals (unpaired t test, $p=0.74$). All values are expressed as mean \pm standard error ($n=5-6$). A.U., arbitrary units.

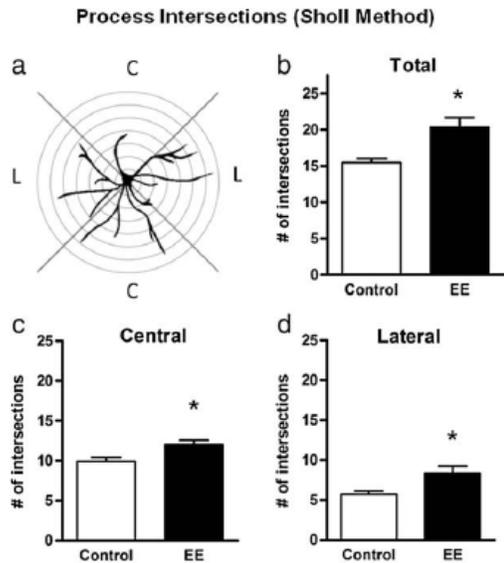


Fig. 3 – Effects of environmental enrichment on astrocytic ramification in the stratum radiatum of CA1. (a) Schematic representation of the Sholl method used for estimating the degree of astrocytic ramification. Seven virtual circles with 3.91 μm intervals were drawn around each astrocyte, and the number of intersections of astrocytic processes with virtual circles was quantified in the lateral (L) and central (C) quadrants around astrocytes (image adapted from www.antanitus.com, with permission). (b) Mean number of intersections of astrocytic processes with concentric circles in control (white bar) and EE (black bar) animals. An increased number of intersections was observed in EE animals (unpaired t test, $p=0.01$). This increase is observed in both central processes (c, unpaired t test, $p=0.02$) and lateral processes (d, unpaired t test, $p=0.03$). All values are expressed as mean \pm standard error ($n=5-6$). *, $p < 0.05$.

OD), demonstrating that the number of astrocytes and GFAP density were not affected by EE.

The degree of astrocyte process ramification was measured through an adaptation of the Sholl concentric circles method (Sholl, 1953; Dall'Oglio et al., 2008), as shown in Fig. 3a. Environmental enrichment induced an increase in astrocyte ramification (Fig. 3b), as measured by counting the intersections of astrocytic processes with the concentric circles ($p=0.0116$). This increase was observed both in processes oriented perpendicularly to CA3–CA1 projections (“central” processes, Fig. 3c, $p=0.0339$), which is the usual orientation of astrocytic processes in this region (Nixdorf-Bergweiler et al., 1994), and in those oriented parallel to these fibers (“lateral” processes, Fig. 3d, $p=0.0212$); however, it was more prominent in lateral processes (46.6% increase vs. 21.6% in central processes).

Since the above-mentioned increase in the number of intersections could be due either to an increase in the number of primary processes, to an increase in the ramification of each primary process, or to an increase in process length, we proceeded to count the number of primary astrocytic processes in both groups. As shown in Fig. 4, EE did not induce significant differences in the total number of primary processes (Fig. 4a, $p=0.18$) or in the number of central processes (i.e. those oriented perpendicularly to CA3–CA1 axons; Fig. 4b, $p=0.22$). However, the number of lateral primary processes (those oriented in parallel to axons) was significantly increased by enrichment (Fig. 4c, $p=0.038$), a finding which was counterbalanced by the slight and non-significant decrease in the number of central processes shown in Fig. 4b.

Finally, we measured the length of the longest central and lateral primary processes of each astrocyte included in the analysis, to examine whether astrocytic process size could also be involved in the increased ramification observed with the Sholl method. As shown in Fig. 5, central processes were distinctly longer than lateral ones in the control group, and their length was not significantly affected by EE (although there was a non-significant trend for decreased length in EE animals; Fig. 5a, $p=0.053$). On the other hand, the longest

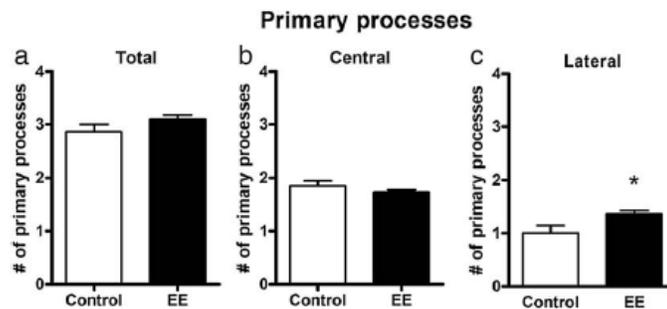


Fig. 4 – Effects of environmental enrichment on the mean number of primary processes of astrocytes in the stratum radiatum of CA1. Graphs show the number of primary processes per astrocyte in control (white bar) and EE (black bar) animals. No significant change is seen in the total number of processes per astrocyte (a, unpaired t test, $p=0.18$) or in the number of central processes (b, $p=0.22$). However, a significant increase in the number of lateral processes is observed (c, $p=0.04$). All values are expressed as mean \pm standard error ($n=5-6$). *, $p < 0.05$.

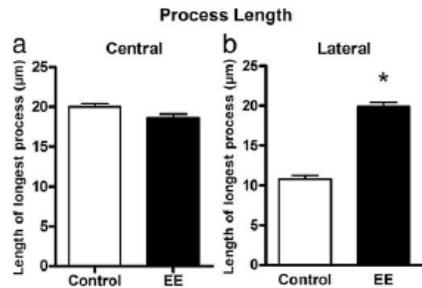


Fig. 5 – Effects of environmental enrichment on the length of astrocytic processes in the stratum radiatum of CA1. Graphs show the length of the longest processes in each pair of quadrants (central or lateral) in control (white bar) and EE (black bar) animals. The length of central processes shows a non-significant trend for decrease (a, $p=0.053$). On the other hand, a significant increase in the length of the longest lateral processes is observed (b, $p<0.001$). All values are expressed as mean \pm standard error ($n=5-6$). *, $p<0.05$.

lateral processes in the EE group were markedly increased in length when compared with those in the control group (Fig. 5b, $p<0.001$), becoming about as long as the central processes in these animals. The greater number and increased length of lateral primary processes observed in the EE group, therefore, probably account at least partially for the greater number of intersections observed with the Sholl method in the lateral quadrants of astrocytes, as well as for the more stellate morphology observed qualitatively in Fig. 1.

3. Discussion

Evidence shows that synaptic organization in the hippocampus is influenced by environmental experience, both during initial postnatal development and after adulthood (van Praag et al., 2000). Plastic changes induced by EE include morphological alterations in hippocampal neurons, both in CA1 and in the dentate gyrus (Faherty et al., 2003). In our study, we describe changes in astrocytic morphology in the stratum radiatum of CA1, which could be functionally related to the synaptogenesis induced by EE in this region (Moser et al., 1994; Rampon et al., 2000b), as changes in astrocytic processes have been shown to correlate with alterations in hippocampal synapses induced both by physiologic (Klintonova et al., 1995) and pathologic conditions (Hawrylak et al., 1993) *in vivo*, as well as with LTP induction in slices (Wenzel et al. 1991).

The changes in astrocytic morphology described in our study seem to reflect both an increase in astrocytic branching and a change in the orientation of astrocytic processes. The number and length of primary processes were increased by EE in the lateral axis of astrocytes, but not in the central axis (which is their usual orientation in this region), leading astrocytes to change from their usually fusiform morphology (Nixdorf-Bergweiler et al., 1994; Bushong et al. 2002) to a more stellate shape. Additionally, both central and lateral processes in EE animals were found to form a greater number of intersections with concentric circles when analyzed by the

Sholl method. Since the number and length of primary processes in the central axis did not appear to increase significantly, this suggests that at least in this axis, an increased branching of individual processes probably occurs in response to EE.

Such enrichment-induced changes are somewhat analogous to those observed in the stratum radiatum over the course of postnatal development. Between P16 and P30, astrocyte cell bodies become smaller and develop longer processes with thin and ramified branches, a fact which has been proposed to be related to maturation of synapses between hippocampal neurons (Catalani et al., 2002). It is possible that post-weaning EE can make such changes more extensive, by stimulating further plasticity in the hippocampus during later postnatal development. However, unlike what happens in normal development (Catalani et al., 2002), the enrichment-induced morphological changes in our study were not accompanied by an increase in the number of astrocytes.

Unlike what has been described in cortical regions after EE (Zeligo and Leblond, 1977; Sirevaag and Greenough, 1991), we found no increase in the number of astrocytes or GFAP immunoreactivity in the hippocampus after EE, a fact which is in agreement with the results of Sirevaag et al. (1991). On the other hand, an increase in total GFAP-reactive surface with EE has been recently reported (Briones et al., 2006). The variation among these results might be due to differences in the enrichment protocols — for example, Briones et al.'s study started enrichment at 3–4 months of age, as opposed to after weaning as in ours. Our results also seem to be somewhat at odds with studies showing evidence of gliogenesis in the hippocampus, as shown by increases in BrdU-labeled GFAP positive cells in the subgranular zone (Steiner et al., 2004) and an increase in nestin-expressing astrocytes in CA1 (Kronenberg et al., 2007). It is possible that these precursor cells are either short-lived or too few in number to cause a significant increase in the total number of astrocytes; it is also possible that protocol differences could be involved in this apparent discrepancy between studies, as the animals used in these studies were also slightly older than ours. Finally, a study of EE in which animals were sacrificed at old age reported a decrease in the number of astrocytes in the hippocampus of animals undergoing enrichment (Soffié et al., 1999); however, those results could be related to reactive astrocytes responding to neuronal injury, and cannot be directly compared to our data.

Previous studies demonstrate that plastic changes in astrocytes are controlled by products secreted by neurons and other cell types in the brain including astrocytes, microglial and endothelial cells (Stevens, 2008). Astrocytes in the stratum radiatum of CA1 have also been shown to respond to the stimulation of Schaffer collaterals with increases in the intracellular calcium concentration (Porter and McCarthy, 1996), an event which could potentially be involved in the initial induction of plastic changes. Signaling molecules which could play a role in inducing astrocytic morphological responses include neurotransmitters such as glutamate and ATP, nitric oxide (De Seranno et al., 2004), ephrins (Nestor et al., 2007) and growth factors such as brain-derived neurotrophic factor (BDNF) (Ohira et al., 2007), which could

presumably set in motion plastic cascades culminating in changes in the astrocytic cytoskeleton. BDNF in particular seems a likely candidate for such a role, as it has been shown both to induce increases in astrocytic ramification in brain slices (Ohira et al., 2007) and to have its levels increased by EE in the hippocampus (Ickes et al., 2000; Gobbo and O'Mara, 2004). Conversely, astrocytic morphological changes could also lead to modifications in hippocampal synapses through the release of mediators such as glutamate, ATP, adenosine, D-serine, and others (Volterra and Meldolesi, 2005; Fiacco et al., 2009), as well as through changes in the uptake of neurotransmitters in the synaptic cleft (Theodosis et al., 2008).

In recent years, knowledge about reciprocal signaling between astrocytes and synaptic terminals has dramatically increased, leading to the notion of a 'tripartite synapse' which includes pre- and post-synaptic terminals and astrocytic processes (Araque et al., 1999; Perea and Araque, 2005; Santello and Volterra, 2009). This has led to an increasing recognition of the role of glial cells as active elements in synaptic transmission. Our results demonstrate morphological changes in astrocytes in the stratum radiatum of CA1 after environmental enrichment, a finding which is probably relevant for the interaction between astrocytes and synaptic terminals and related to hippocampal synaptic plasticity. These results emphasize the need for studies on biochemical and physiological changes in astrocytes induced by environmental enrichment and other plastic events in the brain.

4. Experimental procedures

4.1. Animals

Male albino CF1 mice were obtained from State Foundation for Health Science Research (FEPPS, Porto Alegre/RS, Brasil). All experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) Recommendations for Animal Care.

4.2. Housing conditions

Mice were weaned on P21 and assigned to enriched or standard housing conditions immediately after weaning. All animals were kept in a temperature-controlled colony room with food and water available *ad libitum*, and maintained on a 12-h light/dark cycle (lights on at 7:00 A.M.). Over the course of the study, animals were kept in either standard or enriched housing conditions for 8 weeks. Standard housing consisted of a 27 cm × 16 cm × 12 cm acrylic box with sawdust containing groups of 5 mice. Enriched housing consisted of a 38 cm × 32 cm × 16 cm acrylic box connected to a 28 cm × 21 cm × 50 cm three-story metal cage with sawdust at the bottom, housing 10 mice at a time. The enrichment housing apparatus contained two running wheels and a variety of objects, including toys, tunnels, hiding places and nesting material, which were changed three times per week, taking care not to repeat objects during the same week. The metal cage also allowed full view of the colony room, as well as of other enrichment cages. The same enrichment protocol has been previously shown to

induce long-lasting behavioral alterations in this strain of mice (Amaral et al., 2008).

4.3. Immunohistochemistry

For the immunohistochemical study, animals (5 in the control group, 6 in the EE group) were deeply anesthetized with sodium thiopental (50 mg/kg, i.p.) and injected with 400 IU of heparin. They were then transcardially perfused through the left cardiac ventricle using a peristaltic pump (10 mL/min) with 50 mL of saline solution followed by 50 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4; PB). The brains were removed and post-fixed in the same fixative solution at room temperature for 4 h, then cryoprotected in a 30% sucrose solution in PB at 4 °C until they sank, and then frozen in isopentane and liquid nitrogen.

Coronal sections (50 µm) were obtained using a cryostat (Leica, Germany). Sections were collected in phosphate buffer saline solution (PBS, pH=7.4) and processed for GFAP immunohistochemistry following the unlabeled antibody peroxidase-antiperoxidase (PAP) procedure (Stemberger, 1979). Free-floating sections were pretreated with 10% methanol in 3% H₂O₂ for 30 min, then carefully washed and blocked with 2% bovine serum albumin (BSA) in PBS containing 0.4% Triton X-100 (PBS-Tx, Sigma Chemical Co., USA) for 30 min. They were then incubated with polyclonal GFAP antiserum raised in rabbit (Dako, UK) diluted 1:200 in PBS-Tx containing 2% BSA for 48 h at 4 °C. After being washed several times with PBS-Tx, sections were incubated in PAP-conjugated anti-rabbit IgG (Amersham, UK) diluted 1:50 in PBS-Tx at room temperature for 2 h. The reaction was developed by incubating the sections in a histochemical medium that contained 0.06% 3,3'-diaminobenzidine (DAB, Sigma Chemical Co., USA) dissolved in PBS for 10 min and in the same solution containing 1 µL of 3% H₂O₂ per mL of DAB medium for an additional 10 min. Finally, sections were rinsed in PBS, dehydrated in ethanol, cleared with xylene and covered with Permunt and coverslips. A few sections were prepared omitting the primary antibody by replacing it with PBS as a control to rule out unspecific binding. The brains in both experimental groups were fixed and post-fixed for the same time in identical solutions, processed at the same time, and incubated at the same immunostaining medium for the same period of time, in order to minimize differences in the staining of astrocytes and in background levels.

4.4. Estimation of GFAP density and astrocyte number

The intensity of GFAP immunoreactivity was measured by semi-quantitative densitometric analysis (Ferraz et al., 2003; Xavier et al., 2005; Martinez et al., 2006) using a Nikon Eclipse E-600 microscope (500×, Japan) coupled to a Nikon DXM 1200C CCD camera and to NIS Elements AR 2.30 software. Digitized images obtained from the selected areas were converted to an 8-bit gray scale (256 gray levels). All lighting conditions and magnifications were kept constant during the capture process. Picture elements (pixels) employed to measure optical density were obtained from region of interest (ROI) squares with 5827.78 µm² overlaid on the gray scale image, with background correction. Obvious blood vessels and other artifacts were avoided. 15 images were analyzed per stratum

of hippocampus from each animal (3 fields per section). Background correction was performed according to the formula previously described in Xavier et al. (2005). This and all other analyses were performed in the stratum radiatum of the CA1 region, within an area of the hippocampus extending between 1.58 mm and 1.74 mm posteriorly and between 1.74 mm to 2.22 mm laterally from bregma (Franklin and Paxinos, 1997).

The number of GFAP-immunoreactive astrocytes per mm² in the stratum radiatum of CA1 was also estimated, using the same software employed to measure optical density. A ROI square of 5827.78 μm² was overlaid on the analyzed regions, and GFAP-reactive astrocytes located inside this square or intersected by the lower and/or right edges of the square were counted. Astrocytes intersected by the upper and/or left edges of the square were not counted. Fifteen fields were analyzed in each animal. The intensity of GFAP immunoreactivity within astrocytes was estimated using the same software, but with ROI squares measuring 10.37 μm², which were overlaid in the astrocytic cell bodies located within the larger squares used to measure regional optical density.

4.5. Morphological analysis of astrocytes

Morphologic analysis was performed on 15 astrocytes in each animal (3 sections, each including a single focal plane, with 5 astrocytes analyzed per section). Astrocytes within the same ROI used for estimating the number of astrocytes were used for morphological analysis, taking care to avoid those which were superimposed upon other astrocytes or blood vessels. Morphological analysis was performed by two separate observers who were blind to the experimental groups, and results for both observers were averaged in the final results.

For a general analysis of astrocytic ramification, we used an adaptation of Sholl's concentric circles technique (Sholl, 1953; Dall'Oglio et al., 2008). Briefly, seven virtual circles with 3.91 μm intervals were drawn around each astrocyte, and the number of intersections of astrocytic processes with each virtual circle was quantified in both the lateral (i.e. right/left) and central (i.e. superior/inferior) quadrants around astrocytes. Primary process quantification was performed by counting the processes extending directly from the soma in both the lateral and central quadrants of astrocytes in the same sections. The longest primary process in each quadrant was measured by tracing the process with an automated measurement tool included in the NIS Elements AR 2.30 package.

4.6. Statistical analysis

Data for all variables is expressed as mean ± standard error. An unpaired (Student's) t test was used for all comparisons between groups, and a p value of less than 0.05 was considered to indicate statistical significance.

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REFERENCES

- Amaral, O.B., Vargas, R.S., Hansel, G., Izquierdo, I., Souza, D.O., 2008. Duration of environmental enrichment influences the magnitude and persistence of its behavioral effects on mice. *Physiol. Behav.* 93, 388–394.
- Araque, A., Parpura, V., Sanzgiri, R.P., Haydon, P.G., 1999. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215.
- Briones, T.L., Woods, J., Wadowska, M., Rogozinska, M., Nguyen, M., 2006. Astrocytic changes in the hippocampus and functional recovery after cerebral ischemia are facilitated by rehabilitation training. *Behav. Brain Res.* 171, 17–25.
- Bruel-Jungerman, E., Laroche, S., Rampon, C., 2005. New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur. J. Neurosci.* 21, 513–521.
- Bushong, E.A., Martone, M.E., Jones, Y.Z., Ellisman, M.H., 2002. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* 22, 183–192.
- Catalani, A., Sabbatini, M., Consoli, C., Cinque, C., Tomassoni, D., Azmitia, E., Angelucci, L., Amenta, F., 2002. Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus. *Mech. Ageing Dev.* 123, 481–490.
- Dall'Oglio, A., Gehlen, G., Achaval, M., Rasia-Filho, A.A., 2008. Dendritic branching features of Golgi-impregnated neurons from the "ventral" medial amygdala subnuclei of adult male and female rats. *Neurosci. Lett.* 439, 287–292.
- De Seranno, S., Estrella, C., Loyens, A., Comea, A., Ojeda, S.R., Beauvillain, J.C., Prevot, V., 2004. Vascular endothelial cells promote acute plasticity in ependymogial cells of the neuroendocrine brain. *J. Neurosci.* 24, 10353–10363.
- Dong, W.K., Greenough, W.T., 2004. Plasticity of nonneuronal brain tissue: roles in developmental disorders. *Ment. Retard. Dev. Disabil. Res. Rev.* 10, 85–90.
- Faherty, C.J., Kerley, D., Smeyne, R.J., 2003. A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment. *Brain Res. Dev. Brain Res.* 141, 55–61.
- Ferraz, A.C., Xavier, L.L., Hernandez, S., Sulzbach, M., Viola, G.G., Anselmo-Franci, J.A., Achaval, M., da Cunha, C., 2003. Failure of estrogen to protect the substantia nigra pars compacta of female rats from lesion induced by 6-hydroxydopamine. *Brain Res.* 986, 200–205.
- Fiacco, T.A., Agulhon, C., McCarthy, K.D., 2009. Sorting out astrocyte physiology from pharmacology. *Annu. Rev. Pharmacol. Toxicol.* 49, 151–174.
- Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., Tsai, L.H., 2007. Recovery of learning and memory is associated with chromatin remodelling. *Nature* 447, 178–182.
- Foster, T.C., Gagne, J., Massicotte, G., 1996. Mechanism of altered synaptic strength due to experience: relation to long-term potentiation. *Brain Res.* 736, 243–250.
- Franklin, K.B.J., Paxinos, G., 1997. *The Mouse Brain in Stereotaxic Coordinates*. Academic Press, San Diego.
- Gobbo, O.L., O'Mara, S.M., 2004. Impact of enriched-environment housing on brain derived neurotrophic factor and on cognitive performance after a transient global ischemia. *Behav. Brain Res.* 152, 231–241.
- Haber, M., Zhou, L., Murai, K.K., 2006. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. *J. Neurosci.* 26, 8881–8891.

- Hawrylak, N., Chang, F.L., Greenough, W.T., 1993. Astrocytic and synaptic response to kindling in hippocampal subfield CA1. II. Synaptogenesis and astrocytic process increases to in vivo kindling. *Brain Res.* 19, 309–316.
- Ickes, B.R., Pham, T.M., Sanders, L.A., Albeck, D.S., Mohammed, A.H., Granholm, A.C., 2000. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp. Neurol.* 164, 45–52.
- Jones, T.A., Greenough, W.T., 1996. Ultrastructural evidence for increased contact between astrocytes and synapses in rats reared in a complex environment. *Neurobiol. Learn. Mem.* 65, 48–56.
- Kempermann, G., Kuhn, H.G., Gage, F.H., 1997. More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386, 493–495.
- Klintonova, A., Levy, W.B., Desmond, N.L., 1995. Astrocytic volume fluctuates in the hippocampal CA1 region across the estrous cycle. *Brain Res.* 690, 269–274.
- Kronenberg, G., Wang, L.P., Geraerts, M., Babu, H., Synowitz, M., Vicens, P., Lutsch, G., Glass, R., Yamaguchi, M., Baekelandt, V., Debysers, Z., Kettenmann, H., Kempermann, G., 2007. Local origin and activity-dependent generation of nestin-expressing protoplasmic astrocytes in CA1. *Brain Struct. Funct.* 212, 19–35.
- Li, S., Tian, X., Hartley, D.M., Feig, L.A., 2006. The environment versus genetics in controlling the contribution of MAP kinases to synaptic plasticity. *Curr. Biol.* 16, 2303–2313.
- Martinez, F.G., Hermel, E.E., Xavier, L.L., Viola, G.G., Riboldi, J., Rasia-Filho, A.A., Achaval, M., 2006. Gonadal hormone regulation of glial fibrillary acidic protein immunoreactivity in the medial amygdala subnuclei across the estrous cycle and in castrated and treated female rats. *Brain Res.* 1108, 117–126.
- Megias, M., Emri, Z.S., Freund, T.F., Gulyás, A.I., 2001. Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience* 102, 527–540.
- Mohammed, A.H., Zhu, S.W., Darmopil, S., Hjerling-Leffler, J., Emfors, P., Winblad, B., Diamond, M.C., Eriksson, P.S., Bogdanovic, N., 2002. Environmental enrichment and the brain. *Prog. Brain Res.* 138, 109–133.
- Moser, M.B., Trommald, M., Andersen, P., 1994. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc. Natl. Acad. Sci. U. S. A.* 91, 12673–12675.
- Nestor, M.W., Mok, L.P., Tulapurkar, M.E., Thompson, S.M., 2007. Plasticity of neuron–glial interactions mediated by astrocytic EphA2s. *J. Neurosci.* 27, 12817–12828.
- Nixdorf-Bergweiler, B.E., Albrecht, D., Heinemann, U., 1994. Developmental changes in the number, size, and orientation of GFAP-positive cells in the CA1 region of rat hippocampus. *Glia* 12, 180–195.
- Ohira, K., Funatsu, N., Homma, K.J., Sahara, Y., Hayashi, M., Kaneko, T., Nakamura, S., 2007. Truncated TrkB-T1 regulates the morphology of neocortical layer I astrocytes in adult rat brain slices. *Eur. J. Neurosci.* 25, 406–416.
- Perea, G., Araque, A., 2005. Synaptic information processing by astrocytes. *J. Physiol. (Paris)* 99, 92–97.
- Porter, J.T., McCarthy, K.D., 1996. Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. *J. Neurosci.* 16, 5073–5081.
- Rampon, C., Jiang, C.H., Dong, H., Tang, Y.P., Lockhart, D.J., Schultz, P.G., Tsien, J.Z., Hu, Y., 2000a. Effects of environmental enrichment on gene expression in the brain. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12880–12884.
- Rampon, C., Tang, Y.P., Goodhouse, J., Shimizu, E., Kyin, M., Tsien, J.Z., 2000b. Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nat. Neurosci.* 3, 238–244.
- Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., Fabbri, M.E., Tessarollo, L., Maffei, L., Berardi, N., Caleo, M., 2006. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur. J. Neurosci.* 24, 1850–1856.
- Santello, M., Volterra, A., 2009. Synaptic modulation by astrocytes via Ca²⁺-dependent glutamate release. *Neuroscience* 158, 253–259.
- Sholl, D.A., 1953. Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat.* 87, 387–406.
- Simpson, I.A., Carruthers, A., Vannucci, S.J., 2007. Supply and demand in cerebral energy metabolism: the role of nutrient transporters. *J. Cereb. Blood Flow Metab.* 27, 1766–1791.
- Sirevaag, A.M., Greenough, W.T., 1991. Plasticity of GFAP-immunoreactive astrocyte size and number in visual cortex of rats reared in complex environments. *Brain Res.* 540, 273–278.
- Sirevaag, A.M., Black, J.E., Greenough, W.T., 1991. Astrocyte hypertrophy in the dentate gyrus of young male rats reflects variation of individual stress rather than group environmental complexity manipulations. *Exp. Neurol.* 111, 74–79.
- Soffié, M., Hahn, K., Terao, E., Eclancher, F., 1999. Behavioural and glial changes in old rats following environmental enrichment. *Behav. Brain Res.* 101, 37–49.
- Spolidoro, M., Sale, A., Berardi, N., Maffei, L., 2009. Plasticity in the adult brain: lessons from the visual system. *Exp. Brain Res.* 192, 335–341.
- Squire, L.R., Stark, C.E.L., Clark, R.E., 2004. The medial temporal lobe. *Annu. Rev. Neurosci.* 27, 279–306.
- Steiner, B., Kronenberg, G., Jessberger, S., Brandt, M.D., Reuter, K., Kempermann, G., 2004. Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. *Glia* 46, 41–52.
- Sternberger, L.A., 1979. The unlabeled antibody (PAP) method, introduction. *J. Histochem. Cytochem.* 27, 1657.
- Stevens, B., 2008. Neuron–astrocyte signaling in the development and plasticity of neural circuits. *Neurosignals* 16, 278–288.
- Szeligo, F., Leblond, C.P., 1977. Response of the three main types of glial cells of cortex and corpus callosum in rats handled during suckling or exposed to enriched, control and impoverished environments following weaning. *J. Comp. Neurol.* 172, 247–263.
- Tang, Y.P., Wang, H., Feng, R., Kyin, M., Tsien, J.Z., 2001. Differential effects of enrichment on learning and memory function in NR2B transgenic mice. *Neuropharmacology* 41, 779–790.
- Theodosis, D.T., Poulain, D.A., Olié, S.H.R., 2008. Activity-dependent structural and functional plasticity of astrocyte–neuron interactions. *Physiol. Rev.* 88, 983–1008.
- Ullian, E.M., Sapperstein, S.K., Christopherson, K.S., Barres, B.A., 2001. Control of synapse number by glia. *Science* 291, 657–661.
- van Praag, H., Kempermann, G., Gage, F.H., 2000. Neural consequences of environmental enrichment. *Nat. Rev. Neurosci.* 1, 191–198.
- Vernadakis, A., 1996. Glia–neuron intercommunications and synaptic plasticity. *Prog. Neurobiol.* 49, 185–214.
- Volterra, A., Meldolesi, J., 2005. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* 6, 626–640.
- Wenzel, J., Lammert, G., Meyer, U., Krug, M., 1991. The influence of long-term potentiation on the spatial relationship between astrocyte processes and potentiated synapses in the dentate gyrus neuropil of rat brain. *Brain Res.* 560, 122–131.
- Xavier, L.L., Viola, G.G., Ferraz, A.C., da Cunha, C., Deonizio, J.M., Netto, C.A., Achaval, M., 2005. A simple and fast densitometric method for the analysis of tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and in the ventral tegmental area. *Brain Res. Brain Res. Protoc.* 16, 58–64.

III. ARTIGOS CIENTÍFICOS

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Influence of environmental enrichment on an object recognition task in CF1 mice

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Running head: Environmental enrichment in CF1 mice.

Abbreviations: BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; EE, environmental enrichment; TrkB, tyrosine kinase receptor.

Abstract

Environmental enrichment (EE) is an experimental model for studying neuroplasticity. EE is used to investigate behavioral modifications associated with gene-environmental interaction. The object recognition task (ORT) evaluates animals' ability to learn about their environment, which depends on their innate instinct. By using young CF1 mice, the present study evaluated the effect of 8 weeks of EE on the ORT. Our results indicate that EE decreased the time the animals spent exploring familiar and unfamiliar objects and total time spent exploring both objects, without affecting the capacity of discrimination of objects. These findings indicate a more propitious behavior for species survival in animals subjected to EE, including rapid exploration and learning about the environment.

Key Words: Environmental enrichment; mice strain; BDNF; TrkB; object recognition task; behavior, ethology

1. Introduction

Domesticated animals, such as rats (*Rattus norvegicus*) and cats (*Felis catus*), present brains 8–33% smaller than their wild congeners (when corrected for body size), with the greatest reduction usually seen in the forebrain [1]. These findings have been attributed to genetic changes resulting from artificial selection for traits such as docility. Conversely, animals maintained in enriched environments tend to have larger brain structures, increased neurogenesis, higher learning ability and less stereotyped behaviors than those that developed in standard conditions [1].

Environmental enrichment (EE), an experimental model that allows the study of neuroplasticity, increases physical activity, learning experiences, visual inputs and social interactions [2,3]. EE promotes neuroplasticity in the hippocampus and cerebral cortex due to an increase in the levels of neurotrophins [4,5], changes in cell proliferation [6,7], changes in astrocyte shape [8], and increase in dendritic branching and synaptogenesis [9,10]. EE also causes chromatin remodeling and histone acetylation, which regulates DNA activity and therefore the protein synthesis [11,12].

In the natural world, the juvenile stage is preparatory for the next stages of life: dispersal, habitat selection, settlement and residency in a new habitat. Prior to leaving their birth habitat, the juveniles need to be equipped with physiological, morphological, and behavioral tools [13]. The wild and laboratory animals need to learn about their environment in order to enlarge and select the behavioral repertory, which is specie-specific. The literature does not present a consensus about the cognitive and behavioral changes promoted by EE in distinct animals and strains in

diverse behavioral tasks [14,15]. Therefore, the ethological perspective is important for selection of behavioral tasks used for evaluating alterations occasioned by EE [16].

The object recognition task (ORT) has been widely used to evaluate the effects of pharmacological and genetic interventions on memory processes [17-19]. This task deals with the natural motivation of the animals to explore novelty (as new/unfamiliar objects), an innate instinct that drives animals to learn about their environment (discrimination ratio). In the natural world, mice need to display rapid exploration and knowledge on the environment. This behavior is important for species preservation, such as mating or eating, and minimizing associated risks, such as exposure to predators or rivals [20]; this ethological perspective is evaluated by the ORT paradigm. The hippocampus seems to present a central role in this task, for processes involved both in memory and in environmental interactions [21-23].

The hippocampus exerts a vital role in learning and memory processes [24,25]. EE can modify synaptic physiology in hippocampal neurons and influence hippocampal neurogenesis [26]. Spatial and nonspatial memories present deficits in animals following hippocampal lesions [27], but exposure of animals to EE induces spontaneous recovery after these lesions [28].

Enriched housing stimulates the production of specific neurotrophic factors that promote cell proliferation and/or survival of newborn hippocampal neurons [5]. One of the candidates mediating the effects of EE on hippocampal neurogenesis is BDNF (acting via TrkB receptors) [5]; however, there is no consensus whether BDNF and TrkB hippocampal levels are influenced by EE. In fact, several articles show an increase in hippocampal BDNF levels in animals exposed to EE (4, 5, 29, 30) whereas other studies show no changes in the BDNF and TrkB levels in the hippocampus (31, 32, 33).

Our study evaluated changes in behavioral parameters and in BDNF and TrkB immunocontent in CF1 mice submitted to 8 weeks of EE. A first goal of this study was to

evaluate the influence of EE on the behavioral response in the ORT, and a second goal was to associate the behavioral findings to hippocampal BDNF and TrKB immuncontent.

2. Material and Methods:

2.1 Animals

Male albino CF1 mice were obtained from State Foundation for Health Science Research (FEPPS, Porto Alegre, RS, Brasil). All experimental procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and Brazilian Society for Neuroscience and Behavior (SBNeC) Recommendations for Animal Care and approved by the ethical committee from Universidade Federal do Rio Grande do Sul. All efforts were made to minimize the number of animals and their suffering.

2.2 Housing conditions

Animals (n=60) were weaned at 21 days and assigned randomly to standard or enriched housing immediately after weaning for 60 days. All animals were kept in a temperature-controlled colony room with food and water available *ad libitum*, and maintained on a 12-h light/dark cycle (light on at 7:00 A.M.). Standard housing consisted of a 27 cm x 16 cm x 12 cm acrylic box with sawdust containing groups of 5 mice. Enriched housing consisted of a 38 cm x 32 cm x 16 cm acrylic box connected to a 28 cm x 21 cm x 50 cm three-story metal cage with sawdust, housing 10 mice at a time. The enrichment housing apparatus contained two running wheels and a variety of objects, including wood and plastic objects, tunnels, hiding places and nesting material, where the mice were keep out of luminosity, the natural behavior of wild mice. The EE model presented the possibility of changing in the objects and/or their positions in the enriched housing, which might provide additional cognitive stimulation regarding the formation of spatial map [34, 35].

2.3 Object recognition task

The ORT was performed in an apparatus consisting of a painted wood small chamber: 25 cm x 25 cm x 40 cm. Before the experimental sessions, the animals were habituated to the experimental room for 60 minutes in dim light conditions. A light bulb was switched on during the experimental sessions. The light intensity was equal in the different parts of the apparatus. The objects were placed equidistant from two corners, 12 cm apart the wall. Mice were placed individually into the chamber. In the adaptation sessions, the mice explored the apparatus during 10 min, with no object. In training sessions, performed 24 h later, 2 similar objects were utilized, not familiar to the mice. In test sessions, performed 90 min later, the two objects, a familiar and a novel, were presented. The objects employed were two glass bottles presenting the same texture and size, but with different shapes and colors (white and amber). The objects were not known to have ethological significance for the mice. Discrimination ratio was expressed by the ratio $TN/(TN+TF)$, (TN time spent exploring the novel object; TF time spent exploring familiar object), both in the training and test sessions. Between the sessions the objects were cleaned with 70% ethanol solution. Exploration was defined by directing the nose to the object at a distance less than 2 cm and/or touching the object with the nose or forepaws. The time of exploration was measured by 3 blinded observers, with the use of chronometers. Animals that explored the objects less than 3 sec in a session were excluded from the study [according to 19].

2.4 Immunoblotting

After the behavioral experiments, mice were killed by cervical displacement; the whole hippocampus was dissected out and immediately homogenized in 5% SDS with protease inhibitors cocktail. The protein content was determined by using Bicinchoninic acid assay and bovine serum albumin (BSA) as standard. Hippocampal homogenates (80 µg protein/ sample) were separated in SDS-PAGE (12%) and transferred to nitrocellulose membranes. Membranes

were blocked with 5% BSA for 2 h. After blocking, membranes were incubated for 24 h at 4 °C with rabbit anti-TrkB antibody (1:1000), mouse anti-BDNF antibody (1:500) or mouse anti-actin antibody (1:1000) overnight, and followed by incubation with secondary antibodies anti-rabbit (1:3000) and anti-mouse (1:2000) for 2 h at room temperature and developed with ECL kit. The densitometric analyses were performed using public domain NIH Image Program (developed at the U.S. National Institutes of Health and available on the internet at <http://rsb.info.nih.gov/ni-image/>). As an additional control of the protein loading, membranes were stained with the Ponceau S stain.

2.5 Statistical evaluation

For the behavioral parameters, the statistical differences were analyzed using parametric analysis (Two-way ANOVA) followed by Bonferroni post hoc test. For immunoblotting parameters, the multiple comparisons between groups were analyzed by using the parametric analysis of the t unpaired test. Statistically significant differences were considered when $P < 0.05$.

3 Results

During the study, 3 animals of control group and 8 animals of EE group were excluded because they explored the objects less than 3 seconds in a session (see Methods).

The time spent in the exploration of both objects decreased in the test session compared to training session in the control group ($n=27$) [89.5 ± 24.9 s and 54.5 ± 21.5 ; $F(1,94) = 44.84$ $p < 0.001$] and in the EE group ($n=22$) [30.5 ± 17.3 s and 13.5 ± 8.0 , $F(1,94) = 44.84$ $p < 0.001$]; these findings indicate habituation to the ORT in both groups. Control group spent more time exploring both objects compared to EE group in training [89.5 ± 24.9 s and 30.5 ± 17.3 s $F(1,94) = 165.4$ $p < 0.001$] and test [54.5 ± 21.5 and 13.5 ± 8.0 $F(1,94) = 165.4$ $p < 0.001$] sessions, which could indicate that EE decreased the levels of curiosity and interest for the objects (both

objects were not ethologically relevant and were unfamiliar to animals). In fact the EE group presented more rapid exploration and equal capacity of learning about the environment, which is a more propitious behaviors for species survival. Interaction between housing conditions (EE or control) and session type (training or test) presented $F(1,94) = 5.321, p = 0.0232$ (Fig. 1).

The discrimination ratio was similar in control and EE groups and increased in test sessions compared to training sessions [control: 0.51 ± 0.04 vs. 0.63 ± 0.07 ; $F(1,94) = 50.45, p < 0.001$; EE: 0.50 ± 0.09 vs. 0.60 ± 0.1 ; $F(1,94) = 50.45, p < 0.001$] (Fig. 2), indicating that the animals learned about the environment.

The time spent exploring the familiar object was shorter than the time spent exploring the unfamiliar object in both groups ($F(1,94) = 18.33, p < 0.001$), indicating a capacity of object discrimination in control [20.48 ± 9.46 and $34.04 \pm 13.63, F(1,94) = 18.33, p < 0.001$], and EE [5.53 ± 3.78 and $7.55 \pm 4.36, F(1,94) = 18.33, p < 0.001$] groups. However, the time spent in the exploration of either familiar or unfamiliar objects by the control group was higher than by the EE group, both in the unfamiliar [34.04 ± 13.63 and $7.55 \pm 4.36, F(1,94) = 129.7, p < 0.001$] and familiar [20.48 ± 9.46 and $5.53 \pm 3.78, F(1,94) = 129.7, p < 0.001$] objects (Fig 3). This latter result could indicate that EE in fact decreased the levels of curiosity and interest for the objects and/or that the EE group habituates faster than control group; at any event, a lower time required for exploration of objects is a more propitious behavior to survival of mice. Interaction between housing conditions (EE or control) and object exploration time (familiar or unfamiliar) presented a statistical significance ($F(1,94) = 10.05, p = 0.002$; see Fig 3).

The BDNF and TrkB immunocontent in the whole hippocampus presented no statistical difference between groups [BDNF: $F(5, 4) = 1.033, p = 0.73$; [TrkB: $F(4, 5) = 1.912, p = 0.94$].

4 Discussion

In this study, we showed that EE strongly decreased the time that the mice spent exploring the objects, both in training and test sessions, without affecting the discrimination ratio of the objects. This could indicate that EE caused a lessening of the interest to objects utilized in ORT (objects were not ethologically relevant and were unfamiliar to animals), without affecting the ORT paradigm. Our results show that both groups presented a decrease in the time spent exploring the familiar object in test session and these findings indicate that the animals learned about the environment. EE group spent less time in both objects in both sessions, which indicates a more rapid exploration, minimizing associated costs, in spite the equal performance of both groups in the capacity of discrimination. These animals presented no changes in the hippocampal immunocontent of BDNF and TrkB proteins.

Although the literature presents few studies about behavioral effects of EE in CF1 albino mice, we previously demonstrated that EE decreases the exploration in the second day of exposure to an open field arena [15]; this behavioral change suggests a more propitious behavior in mice exposed to EE, such as decrease in time exposed to predators, and/or improved memory of the exposure to the open field apparatus in the first day [15].

The ORT evaluates natural behavior of rodents, such as approaching and exploring novel objects rather than familiar objects. Thus this task deals with the natural motivation of the animals to explore novelty, an innate instinct that animals use to recognize their environment [18]. Here, the EE group expended less time exploring the objects, which could indicate reduction of motivation, curiosity and/or interest for objects, probably because these animals previously experienced more stimulating environmental conditions (learning, social and physical), which make the novelties not so appealing. Renner (1987) demonstrated an increase in the time spent exploring the objects, but their protocols employed different material and methods

than the ones used in our study, as, for instance, hemioctogonal arena, familiar and unfamiliar objects in both training and test sessions, and the species of animals studied (rat strain (Berkeley S1)) [36]. However, in the study by Bruel-Jugerman et al (2005), employing Sprague Dawley rats, the authors have demonstrated a slight reduction in the time spent in the objects by the EE group in training sessions [37]. Together with our present results, we believe that the discrepancy among these findings is possibly related to different factors, including different animals or strain employed, as well as different EE protocols and particularities of the ORT used.

However, here the discrimination ratio was not affected by EE, which could be considered that the memory was actually improved, since EE mice could correctly discriminate using less time exploring the objects. In fact the ethology of the mice indicates the necessity of rapid exploration and knowledge about the environment for species maintenance, such as mating or food, and minimizing associated costs, such as exposure to predators or rivals [20]; thus, these findings could indicate an increase in behavioral ability of CF-1 albino mice exposed to EE.

In the literature there is a consensus that several behavioral differences among the strains and other genetic and epigenetic variables are more pronounced after EE [14], possibly associated to gene–environment interactions. Concerning EE, there is no consensus about its behavioral effects on distinct behavioral paradigms, such as open field [15], water maze [14] and others. Thus, the behavioral response of laboratory animals submitted to EE is more similar to the ethological responses presented by wild animals, and the EE may attenuate artificial selection for traits; this fact is one of the likely responsible for the conflicting findings among different species.

A recent study suggests that EE effects might be mediated, at least in part, by chromatin remodeling and histone acetylation [11], indicating an involvement of DNA transcription and subsequent increase in protein formation. The literature reports different genetic models for

evaluating environment condition [27], including BDNF heterozygous [38], demonstrating gene-environment interactions. However, in our study we did not find any changes in the immunoccontent of the hippocampal proteins BDNF and TrkB. This result does not exclude that other neurotrophins could be involved on synaptic plasticity and behavioral modulation by EE exposure in the specific mouse strain (male albino CF1 mice) used here.

Previous studies showed distinct results concerning the neurotrophins levels in the hippocampus of animals maintained in the enrichment housing, with some studies reporting increase in BDNF-trkB [4, 5, 30] whereas others, including ours, did not find any changes [31, 32, 33]. The divergence among these results might be due to differences in the enrichment protocols and distinct animals or strain employed; for example, Bindou et al 2007, which also did not find changes in BDNF levels, used EE for 10 days (6 hours day) in Wistar rats after lesion of ventral subiculum [27], whereas Ickes et al 2000, reporting changes, used Sprague-Dawley rats maintained for 12 months in EE condiction. The discrepancy in these findings demonstrates the importance of more studies for the understanding of the variation of response in neurotrophins and their receptors, as BDNF and TrkB, in distinct animal species or strains exposed to different protocols of EE.

In summary, the present study demonstrated equal discrimination ratio by control and EE groups. On the other hand, the EE group presented a decrease in the time spent exploring both objects evaluated together, and in the time expended exploring familiar and unfamiliar objects when evaluated separately. These results could indicate that the EE mice present diminished levels of motivation, curiosity and/or interest in exploring the objects utilized in ORT (objects were not ethologically relevant and were unfamiliar to animals). In an ethologic perspective, the EE group presented a more propitious behavior for species survival, including rapid exploration and knowledge about the environment. In the view of these findings, further studies in different

animal models or strains, including wild animals and/or variations of EE protocols, are important for understanding potential gene–environment interactions leading to different behavioral profiles.

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References

- [1]. Burns JG, Saravanan A, Rodd FH: Rearing environment affects the brain size of guppies. *Ethology* 2009; 115: 122–133
- [2]. van Praag H, Kempermann G, Gage FH: Neural consequences of environmental enrichment. *Nat Rev Neurosci* 2000;1:191-198.
- [3]. Mohammed AH, Zhu SW, Darmopil S, Hjerling-Leffler J, Ernfors P, Winblad B, Diamond MC, Eriksson PS, Bogdanovic N: Environmental enrichment and the brain. *Prog Brain Res* 2002;138:109-133.
- [4]. Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC: Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp Neurol* 2000;164:45-52.
- [5]. Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N, Caleo M: Brain-derived neurotrophic factor (bDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur J Neurosci* 2006;24:1850-1856.
- [6]. Kempermann G, Kuhn HG, Gage FH: More hippocampal neurons in adult mice living in an enriched environment. *Nature* 1997;386:493-495.
- [7]. Steiner B, Kronenberg G, Jessberger S, Brandt MD, Reuter K, Kempermann G: Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. *Glia* 2004;46:41-52.
- [8]. Viola GG, Rodrigues L, Americo JC, Hansel G, Vargas RS, Biasibetti R, Swarowsky A, Goncalves CA, Xavier LL, Achaval M, Souza DO, Amaral OB: Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice. *Brain Res* 2009

- [9]. Moser MB, Trommald M, Andersen P: An increase in dendritic spine density on hippocampal cal pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc Natl Acad Sci U S A* 1994;91:12673-12675.
- [10]. Rampon C, Tang YP, Goodhouse J, Shimizu E, Kiyin M, Tsien JZ: Enrichment induces structural changes and recovery from nonspatial memory deficits in cal nmdar1-knockout mice. *Nat Neurosci* 2000;3:238-244.
- [11]. Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH: Recovery of learning and memory is associated with chromatin remodelling. *Nature* 2007;447:178-182.
- [12]. Pizzorusso T, Berardi N, Maffei L: A richness that cures. *Neuron* 2007;54:508-510.
- [13]. Stamps JA, Swaisgood, RR: Someplace like home: experience, habitat selection and conservation biology, *Applied Animal Behavior Science* 2007; 102: 392–409.
- [14]. Abramov U, Raud S, Innos J, Lasner H, Kurrikoff K, Turna T, Puussaar T, Okva K, Matsui T, Vasar E: Different housing conditions alter the behavioural phenotype of cck(2) receptor-deficient mice. *Behav Brain Res* 2008;193:108-116.
- [15]. Amaral OB, Vargas RS, Hansel G, Izquierdo I, Souza DO: Duration of environmental enrichment influences the magnitude and persistence of its behavioral effects on mice. *Physiol Behav* 2008;93:388-394.
- [16]. Branchi I, Ricceri L: Refining learning and memory assessment in laboratory rodents. An ethological perspective. *Ann Ist Super Sanita* 2004;40:231-236.
- [17]. Bertaina-Anglade V, Enjuanes E, Morillon D, Drieu la Rochelle C: The object recognition task in rats and mice: A simple and rapid model in safety pharmacology to detect amnesic properties of a new chemical entity. *J Pharmacol Toxicol Methods* 2006;54:99-105.

- [18]. Heldt SA, Stanek L, Chhatwal JP, Ressler KJ: Hippocampus-specific deletion of *bdnf* in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry* 2007;12:656-670.
- [19]. Costa MS, Botton PH, Mioranza S, Souza DO, Porciuncula LO: Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tyrosine kinase receptor (*trkb*) content in mice. *Neuroscience* 2008;153:1071-1078.
- [20]. Lima, S. L. & Dill, L. M. 1990: Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68, 619—640.
- [21]. Buffalo EA, Bellgowan PS, Martin A: Distinct roles for medial temporal lobe structures in memory for objects and their locations. *Learn Mem* 2006;13:638-643.
- [22]. Rossato JI, Bevilaqua LR, Myskiw JC, Medina JH, Izquierdo I, Cammarota M: On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learn Mem* 2007;14:36-46.
- [23]. Squire LR, Zola-Morgan M, Clark RE: Recognition memory and the medial temporal lobe: A new perspective. *Nat Rev Neurosci* 2007;8:872-883.
- [24]. Amaral DG, Witter MP: The three-dimensional organization of the hippocampal formation: A review of anatomical data. *Neuroscience* 1995;31:571-591.
- [25]. Farioli-Vecchioli S, Saraulli D, Costanzi M, Pacioni S, Cina I, Aceti M, Micheli L, Bacci A, Cestari V, Tirone F: The timing of differentiation of adult hippocampal neurons is crucial for spatial memory. *PLoS Biol* 2008;6:e246.
- [26]. van Praag H, Kempermann G, Gage FH: Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 1999;2:266-270.

- [27]. Rampon C, Jiang CH, Dong H, Tang YP, Lockhart DJ, Schultz PG, Tsien JZ, Hu Y: Effects of environmental enrichment on gene expression in the brain. *Proc Natl Acad Sci U S A* 2000;97:12880-12884.
- [28]. Briones TL, Woods J, Wadowska M, Rogozinska M, Nguyen M: Astrocytic changes in the hippocampus and functional recovery after cerebral ischemia are facilitated by rehabilitation training. *Behav Brain Res* 2006;171:17-25.
- [29]. Roskoden T, Otten U, Schwegler H: Early postnatal corticosterone administration regulates neurotrophins and their receptors in septum and hippocampus of the rat. *Exp Brain Res* 2004;154:183-191.
- [30]. Pham TM, Soderstrom S, Winblad B, Mohammed AH: Effects of environmental enrichment on cognitive function and hippocampal ngf in the non-handled rats. *Behav Brain Res* 1999;103:63-70.
- [31]. Bindu B, Alladi PA, Mansooralikhan BM, Srikumar BN, Raju TR, Kutty BM: Short-term exposure to an enriched environment enhances dendritic branching but not brain-derived neurotrophic factor expression in the hippocampus of rats with ventral subicular lesions. *Neuroscience* 2007;144:412-423.
- [32]. Turner CA, Lewis MH: Environmental enrichment: Effects on stereotyped behavior and neurotrophin levels. *Physiol Behav* 2003;80:259-266.
- [33]. Hicks RR, Zhang L, Atkinson A, Stevenon M, Veneracion M, Seroogy KB: Environmental enrichment attenuates cognitive deficits, but does not alter neurotrophin gene expression in the hippocampus following lateral fluid percussion brain injury. *Neuroscience* 2002;112:631-637.

- [34]. Tang YP, Wang H, Feng R, Kyin M, Tsien JZ: Differential effects of enrichment on learning and memory function in nr2b transgenic mice. *Neuropharmacology* 2001;41:779-790.
- [35]. Nithianantharajah J, Hannan AJ: Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat Rev Neurosci* 2006;7:697-709.
- [36]. Renner MJ: Experience dependent changes in exploratory behaviour in the adult rats: overall activity levels and interactions with objects. *J Comp Psychol* 101, 94-100, 1987
- [37]. Bruel-Jungerman E, Laroche S, Rampon C: New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur J Neurosci* 2005;21:513-521.
- [38]. Chourbaji S, Brandwein C, Vogt MA, Dormann C, Hellweg R, Gass P: Nature vs. Nurture: Can enrichment rescue the behavioural phenotype of bdnf heterozygous mice? *Behav Brain Res* 2008;192:254-258.

Figure Legends

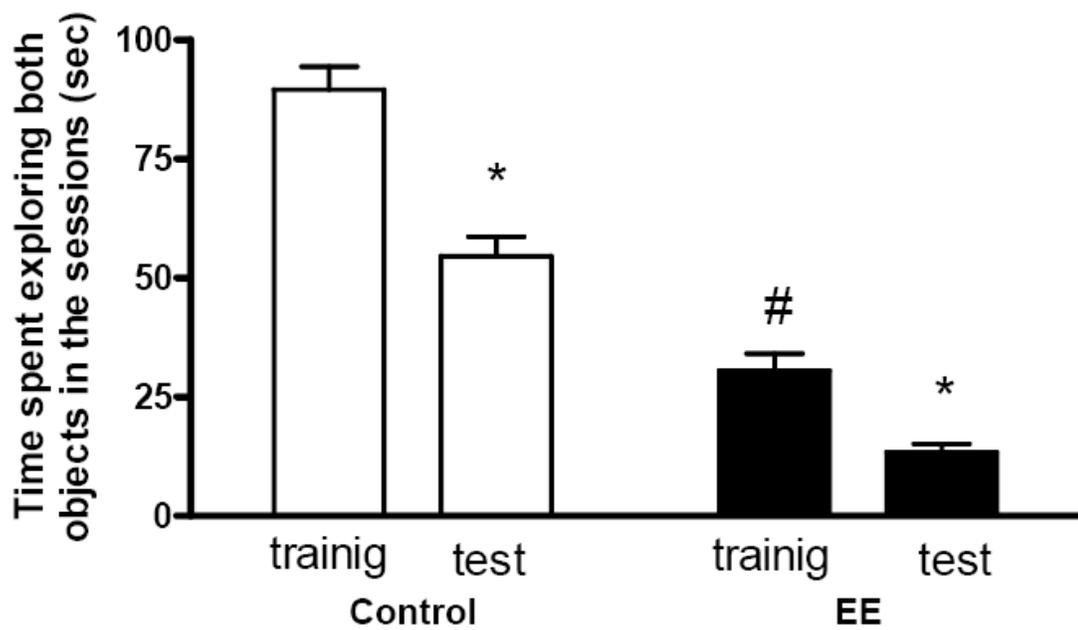
Fig. 1: Total time (in seconds) recorded for the objects exploration in the training and test sessions, for control and EE groups of mice (n 27–22 animals in each group). Results are presented as means \pm S.E.M. of the seconds spent in both objects during 10 min. The test session was performed 90 min after the training session. * $P \leq 0.01$ indicates significant difference for the

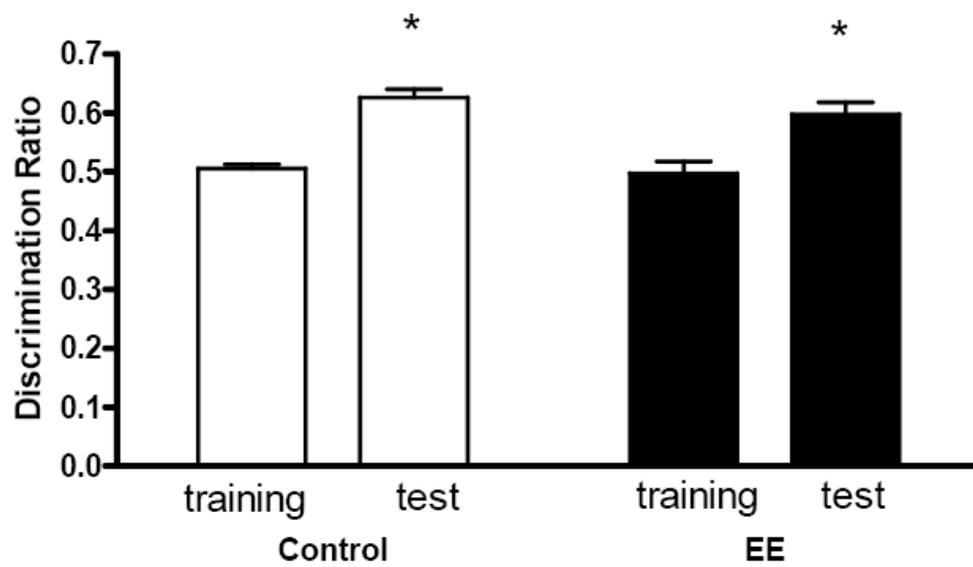
time spent in both objects between training and test sessions. # $P \leq 0.001$ indicates significant difference for the time spent in both objects between control and EE groups (two-way ANOVA followed by Bonferroni post hoc test).

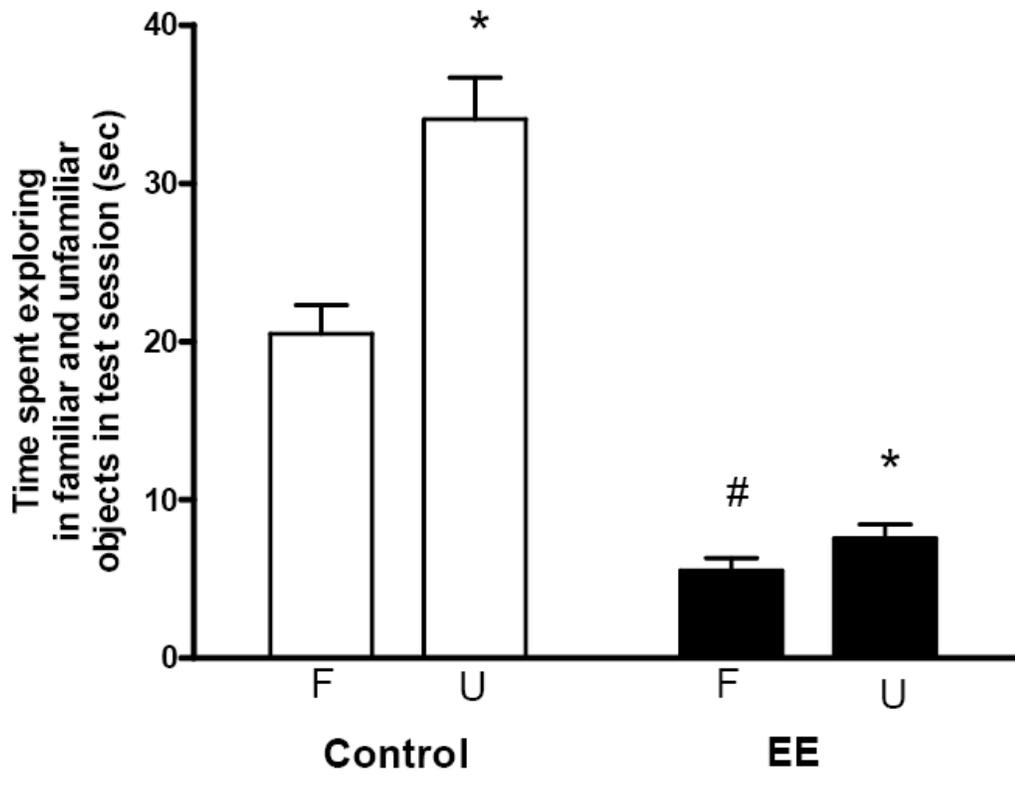
Fig. 2: Discrimination ratio for the objects in the training and test sessions for control and EE groups of mice: (n = 27–22 animals in each group). Results are presented as means \pm S.E.M. of the discrimination ratio. The test session was performed 90 min after the training session. * $P \leq 0.001$ indicates difference from the discrimination ratio between training and test session (two-way ANOVA followed by Bonferroni post hoc test).

Fig. 3: Time spent in familiar and unfamiliar objects (in seconds) in test sessions, for control and EE groups of mice (n 27–22 animals in each group). Results are presented as means \pm S.E.M. of the time spent in objects during 10 min. The test session was performed 90 min after the training session. * $P \leq 0.01$ indicates significant difference for the time spent between familiar and unfamiliar objects in test sessions (two-way ANOVA followed by Bonferroni post hoc test). # $P \leq 0.001$ indicates significant difference for the time spent in both familiar and unfamiliar objects between control and EE groups (two-way ANOVA followed by Bonferroni post hoc test).

Labels: F familiar object, U unfamiliar object.







IV. DISCUSSÃO

As alterações na plasticidade sináptica promovida por um ambiente enriquecido estão relacionadas a um aumento na entrada de estímulos sensoriomotores e visuais, além de ampliação das relações intraespecíficas e da possibilidade de realização de exercícios físicos (NITHIANANTHARAJAH & HANNAN, 2006). Esta neuroplasticidade pode ser caracterizada por mudanças nas conexões entre os neurônios (SPOLIDORO et al, 2008), na arborização dendrítica de neurônios hipocâmpais (FAHERTY et al., 2003) e por alteração na quantidade e no tamanho das sinapses hipocâmpais (MOSER et al., 1994). Estas alterações podem estar envolvidas nas diversas mudanças comportamentais, geradas pela modificação do ambiente. (RAMPON et al., 2000, NITHIANANTHARAJAH & HANNAN, 2006).

Em nosso estudo, observamos que o EA promove neuroplasticidade em camundongos CF1 albinos jovens, alterando a morfologia de astrócitos do Stratum Radiatum da região CA1 do hipocampo. A análise comportamental utilizando a tarefa de reconhecimento de objetos revela que os animais submetidos ao EA diminuíram o tempo despendido em objetos etologicamente irrelevantes. Assim, demonstramos mudanças morfológicas e funcionais, que ocorrem no sistema nervoso, em decorrência de uma exposição a um ambiente com uma quantidade maior de estímulos sensoriomotores, o que conseqüentemente propicia uma ampliação nas possibilidades de aquisição de uma gama maior de experiências.

Nossos resultados apontam para mudanças na forma dos astrócitos do Stratum Radiatum da região CA1, em animais mantidos em ambiente enriquecido

por oito semanas. Observamos que os astrócitos passaram do formato fusiforme normalmente encontrado nesta região (BUSHONG et al., 2002) para um formato estrelado. Estes achados demonstram a capacidade plástica astrocitária e reforçam a importância destas células nas respostas plásticas do SNC, em animais mantidos no ambiente enriquecido, corroborando com a hipótese de que mudanças astrocíticas e neuronais são eventos que acontecem em paralelo (THEODOSIS et al., 2008), visto que, Faherty e colaboradores (2003) demonstraram que neurônios do Stratum Pyramidal da região CA1 aumentam a arborização dendrítica pós EA.

As modificações morfológicas astrocíticas descritas em nosso trabalho são caracterizadas por uma gama de fatores: aumento na quantidade de processos originários do soma e aumento no comprimento desses processos no eixo lateral, ou seja, paralelos as Colaterais de Schaffer. Além disso, tanto no eixo lateral quanto no eixo central, ocorreu um aumento no número de intersecções com os círculos concêntricos, ou seja, um aumento na área ocupada pelos astrócitos. Esses resultados demonstram a importância dos astrócitos para os eventos neuroplásticos, ocasionados por um ambiente com maior quantidade de estímulos, para os animais cativos, bem como a capacidade astrocitária de responder a um aumento de estímulos sensoriais e de informações geradas por este protocolo.

Entretanto, em nosso estudo, não encontramos um aumento no número de astrócitos GFAP-imunorreativos, tampouco um aumento na imunoreatividade astrocitária a GFAP ou imunoreatividade regional a GFAP no Stratum Radiatum como um todo. Esses resultados indicam que nossos animais apresentam plasticidade astrocitária independente do aumento na gênese de astrócitos.

Demonstramos também, que após oito semanas de EA, as modificações da forma astrocitária não estão necessariamente associadas a um aumento na imunorreatividade a GFAP por astrócito.

Uma vez que nosso estudo encontrou uma modificação na morfologia astrocitária no Stratum Radiatum de CA1 de animais expostos ao EA, ocorrendo a mudança da morfologia fusiforme, normalmente encontrada em astrócitos desta região (BUSHONG et al., 2002) para o formato estrelado, sugerimos que esta mudança pode estar associada a modificações da arborização dendrítica (FAHERTY et al., 2003) e a alterações do micro ambiente sináptico (MOSER et al 1994) provocadas pelo EA. De fato é bem documentado que as respostas plásticas de astrócitos e neurônios a diversos estímulos ocorrem em paralelo (THEODOSIS et al., 2008).

Modificações plásticas em astrócitos podem ser controladas por produtos secretados por neurônios, microglia, células endoteliais ou pelos próprios astrócitos (STEVENS, 2008). Astrócitos do Stratum Radiatum da região CA1 modificam a concentração intracelular de cálcio em resposta à estimulação das colaterais de Schaffer (PORTER E MCCARTHY, 1996). Portanto, a modificação na forma astrocitária que ocorre pós EA parece ser um evento chave no que se refere às modificações plásticas ocasionadas por esse modelo nesta região, na medida em que nela também ocorrem mudanças na sinaptogênese (RAMPON et al., 2000) e na arborização dendrítica (FAHERTY et al., 2003), sendo necessários mais estudos para compreensão de quais as moléculas mediadoras estão envolvidas nas modificações geradas pelo enriquecimento ambiental.

Diferentes moléculas estão relacionadas às modificações na morfologia astrocitária, dentre as quais o glutamato, o ATP e o óxido nítrico (DE SERANNO et al., 2004). Além dessas moléculas, cabe salientar que Ohira e colaboradores (2007) reportaram a participação do BDNF em cascatas de sinalização que culminam em mudanças no citoesqueleto astrocitário. Nossos resultados mostram mudança no citoesqueleto astrocitário do Stratum Radiatum da CA1 hipocampal e sem variação no imunoconteúdo de BDNF no hipocampo, o que nos leva a crer que outras moléculas e suas cascatas de sinalização sejam responsáveis por essas mudanças morfológicas que ocorrem em astrócitos de camundongos CF1 albinos, ou ainda que um aumento anterior nos níveis e ou na expressão de BDNF possa estar relacionado com as mudanças morfológicas observadas em astrócitos do Stratum Radiatum da CA1 após oito semanas de enriquecimento.

A plasticidade astrocitária pode estar relacionada à liberação de mediadores sinápticos pelos próprios astrócitos, tais como o glutamato, o ATP, a adenosina e a D-serina (VOLTERRA & MELDOLESI, 2005; FIACCO et al., 2009), além de um aumento na captação de neurotransmissores da fenda sináptica (THEODOSIS et al., 2008). Portanto, nossos resultados que envolvem mudanças morfológicas nos astrócitos do Stratum Radiatum na região CA1 do hipocampo parecem ser importantes para o entendimento fisiológico das modificações causadas pelo enriquecimento ambiental, visto que estas acompanham os efeitos plásticos observados tanto em neurônios (FAHERTY et al., 2003) quanto nas sinapses (MOSER et al 1994, RAMPON et al., 2000) e devem estar ligados a mudanças na funcionalidade destas células em animais mantidos em um ambiente enriquecido.

No desenvolvimento pós-natal, entre o 16º e 30º dia de vida, ocorrem diversos processos plásticos no SNC, dentre os quais, o aumento no comprimento dos processos astrocitários do Stratum Radiatum de CA1, nas ramificações desses processos e na gênese astrocítica nesta região (CATALANI et al., 2002). Dessa forma, a maturação sináptica deste período é acompanhada por mudanças morfológicas astrocitárias. Em nosso trabalho ocorreu fato semelhante nos animais expostos ao EA, observamos: um aumento na quantidade de processos provenientes do soma astrocitário; aumento no tamanho dos processos acompanhado de uma maior ramificação. Entretanto, como o aumento no número de astrócitos nesta região, não foi observado sugerimos que os eventos plásticos que envolvem astrócitos do Stratum Radiatum, da região CA1 hipocampal, em camundongos CF1 expostos ao EA decorrem de modificações morfológicas, bioquímicas e fisiológicas dos astrócitos presentes no tecido e é independente da gênese de novas células.

No que diz respeito à morfologia astrocitária nossos resultados reforçam a necessidade de ampliar os estudos que levam em conta aspectos bioquímicos, fisiológicos e morfológicos dos astrócitos, em diferentes eventos que exacerbem a plasticidade sináptica no SNC. A sinalização recíproca entre astrócitos e terminais pré e pós sinápticos tem sido bastante estudada, com modificações importantes sobre o conceito das funções astrocitárias. Estes estudos contribuíram para a origem do termo *sinapse tripartita* para designar este conjunto de relações (ARAQUE et al., 1999; PEREA & ARAQUE, 2005; SANTELLO & VOLTERRA, 2009). Este novo conceito coloca os astrócitos como elementos ativos na sinapse e nossos dados corroboram para esta hipótese, demonstrando a capacidade

plástica astrocitária em animais submetidos a um modelo que gera mudanças comportamentais (NITHIANANTHARAJAH & HANNAN, 2006), mudanças na arborização dendrítica (FAHERTY et al., 2003), alterações do micro ambiente sináptico (MOSEY et al., 1994).

Briones e colaboradores (2006) encontraram um aumento na superfície ocupada por prolongamentos GFAP imunoreativos em hipocampus tanto de ratos expostos ao EA como em ratos expostos ao EA após sofrerem isquemia, nossos dados mostram um número igual de astrócitos GFAP positivos e na quantificação da imunoreatividade a GFAP por astrócito, contudo observamos um aumento no número de processos e no tamanho desses processos em camundongos expostos ao EA. Comparações entre o nosso estudo e o estudo pré-citado devem levar em conta variações no protocolo de EA. Nosso estudo utiliza camundongos CF1 de 21 dias, por oito semanas enquanto Briones utiliza ratos Wistar com 3-4 meses, por 14 dias.

Dentre os grupos de moléculas alvo para entendimento da neuroplasticidade induzida pelo EA, encontramos o grupo das neurotrofinas (ROSSI et al, 2006). Dentre as atividades destas moléculas, as ações do BDNF através de seu receptor TrkB, merecem destaque, uma vez que diversos estudos demonstraram um aumento dos níveis de BDNF no hipocampo (ICKES et al 2000, ROSSI et al 2006). Entretanto, outros trabalhos não apresentam variações no nível dessa neurotrofina (BINDU et al 2007, HICKS et al., 2002) esses resultados controversos podem ser explicados pela utilização de diferentes animais ou cepa de animais, diferentes protocolos de EA, além da variação de idade de início do EA e tempo de manutenção neste. Em nosso estudo utilizando camundongos CF1 albinos de 21

dias, mantidos no EA por oito semanas o imunoconteúdo de BDNF e de TrkB no hipocampo em animais expostos ao enriquecimento ambiental não foram diferentes dos animais controle. A análise de outras neurotrofinas, ou de BDNF e TrkB em tempos menores de exposição ao EA, nos parece importante para entendermos adequadamente a seqüência de acontecimentos que ocasionam as mudanças morfológicas e comportamentais encontradas em nosso estudo.

Estudos recentes (FISCHER et al., 2007, PIZZORUSSO et al., 2007) demonstraram que o EA provoca um aumento no rearranjo da cromatina e na acetilação das histonas, gerando uma facilitação na transcrição do ADN e um subsequente aumento na síntese de proteínas no SNC. Em nosso trabalho não encontramos aumento nos níveis de BDNF e TrkB hipocampais e tampouco aumento na imunorreatividade a GFAP. Estes resultados podem estar relacionados ao tempo de exposição ao EA, ou ao fato de nossos animais podem ter aumentado à expressão de outras proteínas, não analisadas em nosso estudo.

O enriquecimento ambiental vem sendo amplamente utilizado com a função de reverter danos causados ao SNC, por diferentes injúrias. Por exemplo, Cao et al., 2008 demonstram que o EA é capaz de recuperar a capacidade de realização de potenciação de longa duração no hipocampo, além de reverter alguns déficits cognitivos, em ratos Wistar intoxicados por chumbo durante a gestação e lactação e mantidos no EA a partir do desmame até os 56 dias de vida. O EA está envolvido na recuperação da capacidade cognitiva em ratos expostos a hipóxia isquemia (PEREIRA et al., 2008) e tem envolvimento na resposta encefálica a danos causados pela isquemia cerebral (BRIONES et al., 2006). OEA também é capaz de reverter déficits cognitivos ocasionados por dois modelos experimentais

da doença de Alzheimer, tanto em camundongos transgênicos APP, quanto em APP/PS1 (JANKOWSKY et al., 2005). Nosso estudo, corroborando com diversos outros, mostra que o ambiente enriquecido por si só também é capaz de promover neuroplasticidade e reforça a necessidade de melhor compreender os eventos que possibilitam mudanças de respostas bioquímicas, fisiológicas, morfológicas e comportamentais em animais mantidos em ambiente enriquecido (RAMPON et al., 2000, NITHIANANTHARAJAH & HANNAN, 2006).

A tarefa de reconhecimento de objetos avalia comportamentos naturais de roedores, tais como, a preferência pela exploração de objetos novos em detrimento de objetos familiares e a capacidade destes animais de aprender e recordar sobre o ambiente ao qual são expostos (BEVINS & BESHEER, 2006), sendo o hipocampo uma das regiões encefálicas envolvidas para a realização desta tarefa (ROSSATO et al., 2007). Em nossos resultados observamos que os animais do grupo EA exploram os objetos etologicamente insignificantes, por menos tempo. Esses resultados podem indicar uma redução no interesse destes animais pelos objetos utilizados na tarefa, uma das explicações possíveis é que por viverem em um ambiente que lhes propicia uma maior estimulação estes animais apresentam uma habituação mais rápida a ambientes menos interessantes, diminuindo assim o tempo de exploração destes ambientes (AMARAL et al., 2008).

A literatura apresenta poucos estudos comportamentais com camundongo CF1 albinos mantidos em um ambiente enriquecido. Nosso grupo demonstrou previamente uma diminuição na exploração na tarefa de campo aberto no segundo dia de exposição (AMARAL et al., 2008), indicando uma melhora na

capacidade de habituação ao aparato do campo aberto. Estes dados podem representar um comportamento mais propício a espécie, pois na natureza representariam uma diminuição no tempo de exposição a predadores ou rivais. Estes dados reforçam a importância de sempre se levar em conta às perspectivas etológicas envolvidas nas tarefas comportamentais a serem realizadas pelos animais (BRANCHI & RICCERI, 2004).

Em nosso estudo demonstramos que animais expostos ao EA despendem menos tempo explorando objetos não relevantes etologicamente, sejam estes familiares ou não-familiares aos animais quando comparados ao grupo controle, tanto na sessão treino, quanto na sessão teste. Esta diminuição na exploração dos objetos não alterou a capacidade de discriminação dos objetos, o que nos indica que este animal pode apresentar uma melhor capacidade de recordar sobre os objetos, pois os exploram por menos tempo e os discriminam com igual eficiência.

Os animais expostos ao EA apresentam repertório comportamental mais parecido com o de animais selvagens e uma diminuição dos comportamentos estereotipados causados pelo cativeiro (BURNS et al., 2009), nossos dados corroboram com esta teoria, visto que os animais expostos ao EA despendem menos tempo explorando os objetos presentes na arena, o que possivelmente acarretaria um menor tempo de exposição a predadores e rivais em camundongos selvagens.

Os animais do grupo controle e do grupo EA apresentam uma diminuição no tempo despendido nos dois objetos na sessão teste, o que nos indica uma habituação à arena onde é realizada a tarefa e aos objetos, o que corrobora com a idéia de que os animais são capazes de aprender sobre as situações a que são

expostos (ROSSATO et al., 2007). A menor exploração dos objetos pelos animais mantidos no ambiente enriquecido pode indicar uma habituação à arena e aos objetos desde a sessão de treino, pois a literatura mostra que camundongos CF1 albinos se habitua mais rapidamente na tarefa de campo aberto, diminuindo assim a exploração no segundo dia de exposição à arena utilizada nesta tarefa (AMARAL et al., 2008).

As variações comportamentais entre cepas de animais são mais pronunciadas após o EA (ABRAMOV et al., 2008). Este fato pode estar relacionado com a discrepância dos nossos dados com os de Renner (1987), que demonstrou um aumento no tempo de exploração dos objetos; e Bruel-Jugerman e colaboradores (2005) que demonstram uma diminuição não significativa na exploração dos objetos após EA, visto que estes trabalhos utilizaram ratos de diferentes cepas mantidos em ambiente enriquecido, enquanto o nosso foi realizado com camundongos CF1 albinos.

Nossos dados corroboram com a idéia de que animais mantidos no EA apresentam um comportamento mais próximo a de seus congêneres selvagens (BURNS et al., 2009), qual seja, um camundongo necessita para sua sobrevivência, pouca exposição a rivais ou predadores, contrabalanceada por um possível acesso a comida e a parceiros sexuais (LIMA & DILL, 1990), para que esse balanço ocorra satisfatoriamente os animais devem apresentar um rápido aprendizado sobre o ambiente no qual vive.

Abramov e colaboradores (2008) demonstram diferenças no comportamento de duas cepas de camundongos, a C57Bl/6 (B6, Scanbur) e a 129S6/SvEv/Tac (129, Taconic), em diversas tarefas comportamentais, além de demonstrar uma

correlação na resposta comportamental da cepa empregada com a caixa moradia utilizada, ocorrendo uma exacerbação do comportamento decorrente do enriquecimento ambiental. Nossos resultados demonstram uma diminuição no tempo despendido na exploração de objetos familiares por camundongos CF1 com uma interação entre a caixa moradia e a sessão avaliada (treino, teste). Esses resultados corroboram com a premissa de que estes animais preferem explorar objetos novos em detrimento de objetos familiares além de ter a capacidade de aprender sobre o ambiente a que são expostos (BEVINS & BESHEER, 2006). Além disso, nossos resultados demonstraram que animais expostos ao EA têm exacerbada a característica de despende pouco tempo na exploração de objetos etologicamente irrelevantes para espécie.

Briones e colaboradores (2006) demonstraram uma reversão pelo EA nos danos causados pela isquemia, acompanhado de uma recuperação na capacidade de aquisição na tarefa de labirinto aquático além de uma correlação entre o tempo no quadrante alvo e o número de processos GFAP imunorreativos, enquanto os ratos expostos apenas ao EA não apresentam variação no que tange a correlação entre tempo no quadrante alvo e o número de processos GFAP imunorreativos. Nossos resultados indicam um aumento no número de processos originários do soma, do tamanho dos processos e na área ocupada pelos astrócitos, além de uma diminuição no tempo despendido na exploração de objetos etologicamente irrelevantes aos animais.

O BDNF pode estar envolvido em cascatas de sinalização que culminam em mudanças no citoesqueleto astrocitário no córtex cerebral (Ohira et al.,2007). Nossos resultados não apresentam variação no imunoconteúdo de BDNF no

hipocampo, por outro lado, demonstramos uma mudança no citoesqueleto de astrócitos do Stratum Radiatum da CA1, estes resultados nos levam a crer que outras moléculas e suas cascatas de sinalização sejam responsáveis por essas mudanças morfológicas no hipocampo pós EA, ou ainda que, um aumento anterior nos níveis e ou na expressão de BDNF e/ou TrkB no hipocampo possa estar relacionado com as mudanças morfológicas observadas nos astrócitos dos animais expostos a oito semanas de enriquecimento.

Em seu estágio juvenil os animais necessitam adquirir o máximo de informações sobre o ambiente no qual vivem, esta aquisição de informações influencia diretamente na capacidade do animal em ampliar seus repertórios comportamentais, fisiológicos e morfológicos necessários para a vida (STAMPS et al, 2007). O modelo de EA é utilizado com o intuito de promover um aumento na formação de mapas espaciais, além de proporcionar um aumento na interação social, dos estímulos visuais e sensoriais, possibilitando assim, um aumento na plasticidade hipocampal dos animais (NITHIANANTHARAJAH & HANNAN, 2006). Nossos resultados demonstram que camundongos jovens expostos ao EA apresentam modificações comportamentais. Ocorre diminuição no tempo despendido por estes animais na exploração de objetos etologicamente irrelevantes. Acreditamos que estes resultados indicam um repertório comportamental mais parecido com o de animais selvagens e portanto mais propício à manutenção da espécie. Por outro lado, animais expostos ao EA apresentam modificações morfológicas astrocitárias, caracterizadas por um aumento na quantidade de processos originários do soma e um aumento no

comprimento desses processos, além de um aumento da área ocupada pelos astrócitos do Stratum Radiatum.

Assim sendo, consideramos que estas alterações neuroplásticas reafirmam o EA como um modelo a ser utilizado para melhor compreender modificações causadas por um aumento na possibilidade de aquisição de estímulos visuais e sensoriais, na necessidade de interpretar estes estímulos, além da possibilidade de realização de atividade física e de um aumento no bem-estar, em animais de laboratório.

V Conclusões gerais

Nossos resultados permitem concluir que:

O protocolo de enriquecimento ambiental utilizado neste trabalho, que consistiu em expor os camundongos CF1 albinos por oito semanas é um modelo que nos permitiu estudar modificações na plasticidade no SNC, tanto no que diz respeito a alterações morfológicas em astrócitos como no que diz respeito a alterações comportamentais.

O protocolo de enriquecimento ambiental por nós utilizado, permitiu demonstrar alterações na morfologia dos astrócitos do Stratum Radiatum na região CA1 do hipocampo. Essas alterações são independentes da variação no número de astrócitos e da imunorreatividade a GFAP por astrócito e no neuropilo. Estes resultados podem estar ligados a alterações em neurônios do Stratum Pyramidal e nas sinapses que ocorrem no Stratum Radiatum, em animais expostos ao EA.

Os animais expostos ao protocolo de enriquecimento ambiental por nós utilizado, não apresentaram alterações no imunoconteúdo de BDNF e TrkB no hipocampo. A análise do imunoconteúdo de BDNF e TrkB no hipocampo em diferentes períodos de EA seria interessante para avaliar a importância do BDNF e TrkB nas mudanças plásticas geradas pelo EA.

Os animais expostos ao protocolo de enriquecimento ambiental por nós utilizados, apresentaram a diminuição do tempo despendido na exploração de objetos, indicando uma aproximação do comportamento de camundongos na natureza por parte dos animais expostos ao EA.

Os animais do grupo controle e do grupo exposto ao enriquecimento ambiental, apresentam igual capacidade de discriminar um objeto familiar de um objeto não familiar, apesar de os animais expostos ao EA despendem menos tempo nos objetos. Possivelmente este resultado indica uma aumento no que se refere a memória e aprendizado, pois mesmo explorando menos os animais apresentam igual capacidade de discriminação.

VI PERSPECTIVAS

Avaliar se as alterações morfológicas em astrócitos da CA1 são acompanhadas por alterações funcionais destes.

Avaliar se as alterações morfológicas em astrócitos ocorrem em outras regiões de neuropilo hipocampal (CA3 e giro denteado).

Avaliar alterações neuroplásticas ocasionadas em outras regiões do SNC, tais como córtex visual e cerebelo.

Comparar diferentes respostas comportamentais em outra cepa de camundongo.

Analisar o comportamento destes animais em outras tarefas comportamentais, tais como, labirinto em cruz elevado e exposição a predador, entre outras.

VII REFERÊNCIAS BIBLIOGRÁFICAS

Abramov, U.; Puussaar, T.; Raud, S.; Kurrikoff, K.; Vasar, E. Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment, *Neurosci. Lett.* 443: 223–227, 2008.

Amaral, D.G.; Witter, M.P. The three-dimensional organization of the hippocampal formation: A review of anatomical data. *Neuroscience* 31:571-591, 1989.

Amaral, O.B.; Vargas, R.S.; Hansel, G.; Izquierdo, I.; Souza, D.O. Duration of environmental enrichment influences the magnitude and persistence of its behavioral effects on mice. *Physiol. Behav.* 93: 388–394, 2008.

Araque, A.; Parpura, V.; Sanzgiri, R.P.; Haydon, P.G. Tripartite synapses: glia, the unacknowledged partner. *Trends. Neurosci.* 22: 208–215, 1999.

Arnaiz, S.L.; D'Amico, G.; Paglia, N.; Arismendi, M.; Basso, N; del Rosario Lores Arnaiz, M. Enriched environment, nitric oxide production and synaptic plasticity prevent the aging-dependent impairment of spatial cognition, *Mol. Aspects Med.* 25: 91–101, 2004.

Bevins, R.A.; Besheer, J. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory.' *Nat. Protoc.* 1: 1306–1311, 2006.

Bennett, J.C.; McRae, P.A.; Levy, L.J.; Frick, K.M. Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice, *Neurobiol. Learn. Mem.* 85: 139–152, 2006.

Bindu, B.; Alladi, P.A.; Mansooralikhan, B.M.; Srikumar, B.N.; Raju, T.R.; Kutty, B.M. Short-term exposure to an enriched environment enhances dendritic branching but not brain-derived neurotrophic factor expression in the hippocampus of rats with ventral subicular lesions. *Neuroscience* 144: 412-423, 2007.

Branchi, I.; Ricceri, L. Refining learning and memory assessment in laboratory rodents. An ethological perspective. *Ann. Ist. Super Sanita* 40:231-236, 2004.

Briones, T.L.; Woods, J.; Wadowska, M.; Rogozinska, M.; Nguyen, M. Astrocytic changes in the hippocampus and functional recovery after cerebral ischemia are facilitated by rehabilitation training. *Behav. Brain Res.* 171: 17–25, 2006..

Bruel-Jungerman, E.; Laroche, S.; Rampon, C. New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur. J. Neurosci.* 21: 513–521, 2005.

Burns, J.G.; Saravanan, A.; Rodd, F.H. Rearing environment affects the brain size of guppies. *Ethology* 115: 122–133, 2009.

Bushong, E.A.; Martone, M.E.; Jones, Y.Z.; Ellisman, M.H. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* 22: 183–192, 2002.

Cao, X.; Huang, S.; Ruan, D. Enriched environment restores impaired hippocampal long-term potentiation and water maze performance induced by developmental lead exposure in rats. *Dev. Psychobiol.* 50(3):307-13: 2008.

Catalani, A.; Sabbatini, M.; Consoli, C.; Cinque, C.; Tomassoni, D.; Azmitia, E.; Angelucci, L.; Amenta, F. Glial fibrillary acidic protein immunoreactive

astrocytes in developing rat hippocampus. *Mech. Ageing Dev.* 123: 481–490, 2002.

Chapillon, P.; Manneché, C.; Belzung, C.; Caston, J. Rearing environmental enrichment in two inbred strains of mice: 1. Effects on emotional reactivity. *Behav Genet.* 29(1):41-6, 1999.

Costa MS, Botton PH, Mioranza S, Souza DO, Porciuncula LO: Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tyrosine kinase receptor (trkb) content in mice. *Neuroscience* 153:1071-1078, 2008.

De Seranno, S. ; Estrella, C. ; Loyens, A. ; Cornea, A. ; Ojeda, S.R. ; Beauvillain, J.C. ; Prevot, V. Vascular endothelial cells promote acute plasticity in ependymogial cells of the neuroendocrine brain. *J. Neurosci.* 24: 10353–10363, 2004.

Faherty, C.J.; Kerley, D.; Smeyne, R.J. A Golgi–Cox morphological analysis of neuronal changes induced by environmental enrichment. *Brain Res. Dev. Brain Res.* 141: 55–61, 2003.

Farioli-Vecchioli, S.; Saraulli, D.; Costanzi, M.; Pacioni, S.; Cina, I.; Aceti, M.; Micheli, L.; Bacci, A.; Cestari, V.; Tirone, F. The timing of differentiation of adult hippocampal neurons is crucial for spatial memory. *PLoS Biol*;6:e246, 2008.

Fiacco, T.A. ; Agulhon, C. ; McCarthy, K.D. Sorting out astrocyte physiology from pharmacology. *Annu. Rev. Pharmacol. Toxicol.* 49: 151–174, 2009.

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

Garcia-Segura, L.M.; McCarthy, M.M. Minireview: role of glia in neuroendocrine function. *Endocrinology* 145: 1082–1086, 2004.

Gomes, F.C.; Paulin, D.; Moura Neto, V., Glial fibrillary acidic protein (GFAP): modulation by growth factors and its implication in astrocyte differentiation. *Braz. J. Med. Biol. Res.* 32: 619–631, 1999.

Hicks, R.R.; Zhang, L.; Atkinson, A.; Stevenon, M.; Veneracion, M.; Seroogy, K.B. Environmental enrichment attenuates cognitive deficits, but does not alter neurotrophin gene expression in the hippocampus following lateral fluid percussion brain injury. *Neuroscience* 112:631-637, 2002.

Ickes, B.R.; Pham, T.M.; Sanders, L.A.; Albeck, D.S.; Mohammed, A.H.; Granholm, A.C. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp. Neurol.* 164: 45–52, 2000.

Kempermann, G.; Kuhn, H.G.; Gage, F.H. More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386: 493-495, 1997.

Kronenberg, G.; Wang, L.P.; Geraerts, M.; Babu, H.; Synowitz, M.; Vicens, P.; Lutsch, G.; Glass, R.; Yamaguchi, M.; Baekelandt, V.; Debyser, Z.; Kettenmann, H.; Kempermann, G. Local origin and activity-dependent generation of nestin-expressing protoplasmic astrocytes in CA1. *Brain Struct Funct.* 212(1):19-35, 2007.

Kruska, D. Mammalian domestication and its effect on brain structure and behavior. In: *Intelligence and Evolutionary Biology* (Jerison, H. J. & Jerison, I., eds). Springer-Verlag, Berlin, 211—250, 1988.

Jankowsky, J.L.; Melnikova, T.; Fadale, D.J.; Xu, G.M.; Slunt, H.H., Gonzales, V.; Younkin, L.H.; Younkin, S.G.; Borchelt, D.R.; Savonenko, A.V. Environmental

enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *Neurosci.* 25(21):5217-24, 2005.

Laping, N.J.; Teter, B.; Nichols, N.R.; Rozowsky, I.; Finch, C.E.. Glial fibrillary acidic protein: regulation by hormones, cytokines and growth factors. *Brain Pathol.* 1: 259–275, 1994.

Lima, S. L.; Dill, L. M. Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* 68: 619—640, 1990.

Li, S.; Tian, X.; Hartley, D.M.; Feig, L.A. The environment versus genetics in controlling the contribution of MAP kinases to synaptic plasticity. *Curr. Biol.* 16: 2303–2313, 2006.

Martinez, F.G.; Hermel, E.E.; Xavier, L.L.; Viola, G.G.; Riboldi, J.; Rasia-Filho, A.A.; Achaval, M. Gonadal hormone regulation of glial fibrillary acidic protein immunoreactivity in the medial amygdala subnuclei across the estrous cycle and in castrated and treated female rats. *Brain Res.* 1108: 117–126, 2006.

Mohammed, A.H.; Zhu, S.W.; Darmopil, S.; Hjerling-Leffler, J.; Ernfors, P.; Winblad, B.; Diamond, M.C.; Eriksson, P.S.; Bogdanovic, N. Environmental enrichment and the brain. *Prog. Brain Res.* 138: 109–133, 2002.

Moser, M.B.; Trommald, M.; Andersen, P. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc. Natl. Acad. Sci. U. S. A.* 91, 12673–12675, 1994.

Nithianantharajah, J.; Hannan, A.J. Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat. Rev. Neurosci.* 7:697-709, 2006;.

Ohira, K.; Funatsu, N.; Homma, K.J.; Sahara, Y.; Hayashi, M.; Kaneko, T.; Nakamura, S. Truncated TrkB-T1 regulates the morphology of neocortical layer I astrocytes in adult rat brain slices. *Eur. J. Neurosci.* 25: 406–416, 2007.

Perea, G.; Araque, A. Synaptic information processing by astrocytes. *J. Physiol. (Paris)* 99: 92–97, 2005.

Pereira, L.O.; Strapasson, A.C.; Nabinger, P.M.; Achaval, M.; Netto, C..A. Early enriched housing results in partial recovery of memory deficits in female, but not in male, rats after neonatal hypoxia-ischemia. *Brain Res.* 1218:257-66, 2008

Pizzorusso, T.; Berardi, N.; Maffei, L. A richness that cures. *Neuron*; 54:508-510, 2007.

Porter, J.T.; McCarthy, K.D. Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. *J. Neurosci.* 16: 5073–5081, 1996.

Price, E. O. Behavioral development in animals undergoing domestication. *Appl. Anim. Behav. Sci.* 65: 245—271, 1999.

Rampon, C.; Jiang, C.H.; Dong, H.; Tang, Y.P.; Lockhart, D.J.; Schultz, P.G.; Tsien, J.Z.; Hu, Y. Effects of environmental enrichment on gene expression in the brain. *Proc. Natl. Acad. Sci. U. S. A.* 97: 12880–12884, 2000a.

Rampon, C., Tang, Y.P., Goodhouse, J., Shimizu, E., Kyin, M., Tsien, J.Z., Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nat. Neurosci.* 3: 238–244, 2000b.

Renner, M.J. Experience dependent changes in exploratory behaviour in the adult rats: overall activity levels and interactions with objects. *J Comp Psychol* 101: 94-100, 1987.

Rossato, J.I.; Bevilaqua, L.R.; Myskiw, J.C., Medina, J.H.; Izquierdo, I.; Cammarota, M. On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learn. Mem.*14: 36-46, 2007.

Rossi, C.; Angelucci, A.; Costantin, L.; Braschi, C.; Mazzantini, M.; Babbini, F.; Fabbri, M.E.; Tessarollo, L.; Maffei, L.; Berardi, N.; Caleo, M. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *Eur. J. Neurosci.* 24: 1850–1856, 2006.

Sale, A.; Cenni, M.C.; Ciucci, F.; Putignano, E.; Chierzi, S.; Maffei, L. Maternal enrichment during pregnancy accelerates retinal development of the fetus. *PLoS ONE* 2:e1160, 2007.

Santello, M.; Volterra, A. Synaptic modulation by astrocytes via Ca²⁺-dependent glutamate release. *Neuroscience* 158: 253–259, 2009.

Spolidoro, M.; Sale, A.; Berardi, N.; Maffei, L. Plasticity in the adult brain: lessons from the visual system. *Exp. Brain Res.* 192: 335–341, 2009.

Stamps, J.A.; Swaisgood, R.R. Someplace like home: experience, habitat selection and conservation biology, *Applied Animal Behavior Science* 102: 392–409, 2007.

Stevens, B. Neuron–astrocyte signaling in the development and plasticity of neural circuits. *Neurosignals* 16: 278–288, 2008.

Tang, Y.P.; Wang, H.; Feng, R.; Kyin, M.; Tsien, J.Z. Differential effects of enrichment on learning and memory function in nr2b transgenic mice. *Neuropharmacology* 41: 779-790, 2001.

Theodosios, D.T.; Poulain, D.A.; Oliet, S.H.R. Activity-dependent structural and functional plasticity of astrocyte–neuron interactions. *Physiol. Rev.* 88: 983–1008, 2008.

van Praag, H.; Kempermann, G.; Gage, F.H. Neural consequences of environmental enrichment. *Nat. Rev. Neurosci.* 1:191-198, 2000.

Volterra, A.; Meldolesi, J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* 6: 626–640, 2005.

Xavier, L.L.; Viola, G.G.; Ferraz, A.C.; da Cunha, C.; Deonizio, J.M.; Netto, C.A.; Achaval, M. A simple and fast densitometric method for the analysis of tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and in the ventral tegmental area. *Brain Res. Protoc.* 16: 58–64, 2005.