UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

AVALIAÇÃO DO EFEITO DO ENRIQUECIMENTO AMBIENTAL NO COMPORTAMENTO E RESPOSTAS A ANTIDEPRESSIVOS DE CAMUNDONGOS CF1.

MARTA LORENA SPECK DA SILVA

Porto Alegre, 2018.

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RESUMO

O enriquecimento ambiental (EA) exerce efeitos benéficos nos desempenhos cognitivo e emocional, ramificação dendrítica, densidade sináptica, neurogênese, modulação de sistemas neurotróficos e neurotransmissores em roedores. No entanto, a influência do EA nas respostas farmacológicas e comportamentais em modelos animais de transtornos psiquiátricos ainda não foi totalmente estabelecida. Neste contexto, o objetivo deste estudo foi avaliar a influência da exposição ao EA no comportamento de camundongos em testes de campo aberto (CA) e natação forçada (TNF), bem como a resposta a antidepressivos (fluoxetina 30 mg / kg e bupropiona 30 mg / kg, vo). Camundongos CF1 foram expostos a uma condição de alojamento com EA em diferentes estágios de desenvolvimento: do acasalamento ao dia pós-natal (PND) 55 (EA ao longo da vida), do acasalamento ao PND21 (EA perinatal) e do PND21 ao PND55 (EA pós-desmame). No PND55 os filhotes machos foram avaliados no CA e TNF. A expressão gênica do BDNF no hipocampo foi determinada por RT-qPCR. Os camundongos expostos ao EA perinatal permaneceram mais tempo na zona periférica do CA e realizaram menos *grooming* do que os camundongos alojados sob condições padrão, e esses efeitos foram independentes do tratamento medicamentoso. O EA pós-desmame e ao longo da vida aumentou o comportamento de *grooming*. Bupropiona reduziu *grooming* em todos os grupos, exceto em EA perinatal. Por sua vez, a fluoxetina diminuiu o *grooming* apenas no grupo enriquecido pós-desmame. Nenhuma das condições de alojamento de EA alterou o tempo de imobilidade no TNF, o que indica que o EA não teve efeito antidepressivo. No entanto, todas as condições de alojamento enriquecidas aboliram o efeito anti-imobilidade da bupropiona. Nenhum dos protocolos de EA afetou a expressão do hipocampo de BDNF. A principal conclusão é que o comportamento do camundongo no CA é sensível a alterações no ambiente de habitação e depende do estágio de desenvolvimento de exposição. A bupropiona e a fluoxetina produziram respostas divergentes dependendo da condição do alojamento, o que sugere que a EA modula as vias de neurotransmissão monoaminérgicas.

Palavras-chave: Enriquecimento ambiental, enriquecimento perinatal, enriquecimento pós-desmame, bupropiona, fluoxetina, natação forçada, campo aberto.

ABSTRACT

It has been described that environmental enrichment (EE) exerts beneficial effects on cognitive and emotional performances, dendritic branching, synaptic density, neurogenesis and modulation of neurotrophic systems and neurotransmitters in rodents. However, the influence of EE on pharmacological and behavioral responses in animal models of psychiatric disorders has not been fully established yet. In this context, the aim of this study was to evaluate the influence of exposure to environmental enrichment on mice behavior in the open field (OF) and forced swimming (FST) tests, as well as the response to antidepressant drugs (fluoxetine 30 mg/kg and bupropion 30 mg/kg, p.o.). CF1 mice were exposed to an enriched housing condition at different developmental stages: from mating to postnatal day (PND) 55 (lifelong enrichment), from mating to PND21 (perinatal enrichment) and from PND21 to PND55 (post-weaning enrichment). At PND55 the male offspring were evaluated in the OFT and FST. BDNF gene expression in the hippocampus was determined through RT-qPCR. Mice exposed to perinatal enrichment remained longer in the peripheral zone of the OFT and performed fewer grooming than mice housed under standard condition, and these effects were independent of drug treatment. Post-weaning and lifelong enrichment increased grooming behavior. Bupropion reduced grooming in all groups except in perinatal enriched one. In turn, fluoxetine decreased grooming only in post-weaning enriched group. None of the enriched housing conditions altered the immobility time in the FST, which indicates that EE had no antidepressant-like effect. However, all enriched housing conditions abolished the anti-immobility effect of bupropion. None of the EE protocols affected BDNF hippocampal expression. The main conclusion is that mice behavior in the open field is sensitive to alterations in the housing environment and depends on the developmental stage of exposure. Bupropion and fluoxetine yielded divergent responses depending on the housing condition, which suggests that EE modulates monoaminergic neurotransmission pathways.

Keywords: Environmental enrichment, perinatal enrichment, post-weaning enrichment, bupropion, fluoxetine, forced swimming, open field.

ABREVIATURAS E SIGLAS

EE: environmental enrichment

FST: Forced Swimming Test

OFT: Open Field Test

BDNF: Brain-derived neurotrophic factor

EA: Enriquecimento ambiental

GD: gestational day

PND: postnatal

TNF: Teste de natação forçada

LTP: Potenciação de longa duração (do inglês *Long Term Potentiation*)

NE: No enrichment

LE: Lifelong enrichment

PE: Perinatal enrichment

PWE: Post-weaning enrichment

FLU: Fluoxetina

BUP: Bupropiona

CeMBE: Centro de Modelos Biológicos Experimentais, Pontifícia Universidade Católica

CEUA: Comitê de Ética no Uso de Animais

PPGCF: Programa de Pós-Graduação em Ciências Farmacêuticas

UFRGS: Universidade Federal do Rio Grande do Sul

SERT: Serotonin meuronal transporter

DAT: Dopaminergic neuronal transporters

NAT: Noradrenaline neuronal transporters

SUMÁRIO

INTRODUÇÃO E OBJETIVOS

O cérebro dos mamíferos é originado por programas genéticos e epigenéticos que asseguram que a maioria das células e áreas estruturais esteja em vigor ao nascimento. A estimulação sensorial, cognitiva e motora através da influência mútua com o meio ambiente desde o nascimento até a senescência desempenha um papel fundamental nos circuitos neurais necessários para a função normal do cérebro. (Kaas, 2015).

As implicações neurocomportamentais das interações entre indivíduo e meio ambiente ocorrem desde a concepção e continuam sendo importantes ao longo da vida. Durante o desenvolvimento embrionário e fetal, a ativação dos genes é a principal força motriz direcionando os processos maturacionais do sistema nervoso central (Caporali *et al,* 2014).

Além do impacto do controle genético, os estímulos ambientais influenciam as estruturas de desenvolvimento, de modo que o ambiente experimentado pela mãe (grávida) exerce efeitos no ambiente intrauterino e pode alterar a organogênese fetal (Caporali *et al*, 2014).

Durante a fase pós-natal, também ocorrem processos que moldam o cérebro (Mandolesi, 2017). Esse período é altamente plástico e as experiências iniciais podem afetar o curso do desenvolvimento, sendo demonstrada a associação entre alterações de expressão de neurotrofinas, como o BDNF (*brain derived neurotrofic factor*) e alterações do ambiente social inato, como a introdução de enriquecimento ambiental. (Branchi *et al,* 2011).

Enriquecimento ambiental (EA) consiste na exposição dos animais a ambientes ricos em estimulação sensorial (Chamove *et al*,1989; Zimmermann *et al*, 2001; Clemenson, 2015) com objetivo principal de dar ao animal em cativeiro condições que estimulem seu comportamento natural. (Frajblat *et al*, 2008).

O EA pode ser dividido em enriquecimento físico e enriquecimento social (Stewart e Bayne, 2004; Johansson e Ohlsson, 1996): o enriquecimento físico envolve modificações estruturais, incluindo aumento de espaço e inclusão de funcionalidades que permitam aos animais algum controle sobre seu ambiente, como exercício e exploração. Exemplos de elementos de enriquecimento físico: cama reforçada com materiais naturais (papel e cama à base de fibras), túneis,

objetos de madeira para roer, cordas, balanços, rodas de corrida, bolas, rampas, escadas e outros brinquedos de tamanho adequado. O enriquecimento social, por outro lado, refere-se à habitação de animais em grupos sociais, sempre que possível. O ideal sempre é uma combinação de ambos os elementos de enriquecimento.

Estudos comportamentais, celulares e moleculares revelaram resultados significativos de ambientes enriquecidos em roedores e outras espécies e forneceram novos *insights* sobre mecanismos de plasticidade dependentes da experiência vivida, incluindo neurogênese de adultos e plasticidade sináptica (Lometti *et al*, 2010; Garthe *et al*, 2016; Nithianantharajah *et al*, 2006).

Essas mudanças de plasticidade incluem: aumento da neurogênese e sobrevivência celular (Kempermann *et al*, 2010; Van Praag *et al*, 2000) e aumento da regulação de fatores de crescimento, incluindo o BDNF (Ickes *et al*, 2000).

BDNF é uma neurotrofina envolvida na diferenciação e crescimento neural que não só modifica a atividade sináptica durante o desenvolvimento do cérebro, mas também ocorre na idade adulta (Vasquez Sanroman *et al*, 2013).

Sabe-se que as neurotrofinas, como BDNF, influenciam o desenvolvimento do cérebro, a plasticidade neuronal (formação de sinapses, crescimento axonal e remodelação de circuitos), bem como mecanismos plásticos envolvidos na aprendizagem, memória e na resposta ao estresse ou lesão (Gelfo *et al,* 2011). Elas estão relacionadas a mecanismos de plasticidade de longo prazo que apoiam a restauração de redes cerebrais alteradas em distúrbios, como autismo (Kondo *et al*, 2008), esquizofrenia (Mcomish *et al*, 2008), lesão cerebral (Segovia *et al*, 2009), Alzheimer (Mandolesi *et al*, 2008).

O EA estimula a neurogênese no cérebro do roedor, especialmente no hipocampo, uma estrutura límbica importante para a formação da memória e processamento emocional, que é afetada em doença de distúrbios do humor como a depressão. (Kempermann *et al.,* 1997). O EA ajudou a melhorar os efeitos prejudiciais da depressão colaborando na redução dos efeitos negativo, (Seong, 2018), bem como demonstrou que pode prevenir os comportamentos de depressão e ansiedade induzidos pelo isolamento social. (Grippo, 2014).

Sabe-se que a depressão induz um amplo espectro de prejuízos comportamentais, alterações estruturais, bem como perturbações eletrofisiológicas

no cérebro. Essas alterações podem ser restauradas com uma exposição de curto prazo ao ambiente enriquecido e os estímulos ambientais positivos podem ter efeitos duradouros nos prejuízos comportamentais, alterações estruturais e plasticidade sináptica (Mahati, 2016). Enquanto os estímulos ambientais negativos, como o estresse crônico, experiências temerárias e trauma causam alterações degenerativas no cérebro, os ambientes positivamente estimulantes provocam mudanças restaurativas por reorganização neural e ligação em áreas cerebrais danificadas para facilitar a recuperação da depressão e condições associadas (Mahati, 2016).

No entanto, a influência do EA nas respostas farmacológicas e comportamentais em modelos animais de doenças psiquiátricas ainda não está totalmente estabelecida.

Desta forma, o objetivo deste estudo foi avaliar o efeito de um programa de enriquecimento ambiental nos períodos pré-natal e pós-desmame inicial em respostas comportamentais e farmacológicas nos modelos de campo aberto (CA) e natação forçada (TNF), em camundongos, assim como os seus efeitos na expressão de BDNF hipocampal.

Este trabalho foi orientado pela Profa. Dr. Stela Maris Kuze Rates e estão apresentados em forma de artigo cientifico e será submetido ao periódico *Behavioural Brain Research*

Artigo a ser submetido ao periódico *Behavioural Brain Research*

ENVIRONMENTAL ENRICHMENT AFFECTS BEHAVIORAL AND PHARMACOLOGICAL RESPONSE TO ANTIDEPRESSANTS IN CF1 MICE

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ABSTRACT

It has been described that environmental enrichment (EE) exerts beneficial effects on cognitive and emotional performances, dendritic branching, synaptic density, neurogenesis and modulation of neurotrophic systems and neurotransmitters in rodents. However, the influence of EE on pharmacological and behavioral responses in animal models of psychiatric disorders has not been fully established yet. In this context, the aim of this study was to evaluate the influence of exposure to environmental enrichment on mice behavior in the open field (OF) and forced swimming (FST) tests, as well as the response to antidepressant drugs (fluoxetine 30 mg/kg and bupropion 30 mg/kg, p.o.). CF1 mice were exposed to an enriched housing condition at different developmental stages: from mating to postnatal day (PND) 55 (lifelong enrichment), from mating to PND21 (perinatal enrichment) and from PND21 to PND55 (post-weaning enrichment). At PND55 the male offspring were evaluated in the OFT and FST. BDNF gene expression in the hippocampus was determined through RT-qPCR. Mice exposed to perinatal enrichment remained longer in the peripheral zone of the OFT and performed fewer grooming than mice housed under standard condition, and these effects were independent of drug treatment. Post-weaning and lifelong enrichment increased grooming behavior. Bupropion reduced grooming in all groups except in perinatal enriched one. In turn, fluoxetine decreased grooming only in post-weaning enriched group. None of the enriched housing conditions altered the immobility time in the FST, which indicates that EE had no antidepressant-like effect. However, all enriched housing conditions abolished the anti-immobility effect of bupropion. None of the EE protocols affected BDNF hippocampal expression. The main conclusion is that mice behavior in the open field is sensitive to alterations in the housing environment and depends on the developmental stage of exposure. Bupropion and fluoxetine yielded divergent responses depending on the housing condition, which suggests that EE modulates monoaminergic neurotransmission pathways.

Keywords: Environmental enrichment, perinatal enrichment, post-weaning enrichment, bupropion, fluoxetine, forced swimming, open field.

1. Introduction

Sensory, cognitive and motor stimulation delivered by the environment plays a key role in the neural circuits necessary for normal brain function from birth to senescence [1].

Prenatal environmental characteristics may exert critical influences on the fetus and on the young and mature organism into which it develops. These processes in which early environmental factors induce long-term effects are termed “early programming” [2]. The term implies that the organism is endowed with basic plasticity, which enables it to be influenced by the environment in early stages, and to develop into a mature organism with the qualifications and systems that are necessary for survival and reproduction [2].

The neurobehavioral implications of the interactions between the individual and the environment occur from conception and continue to be important throughout life. During embryonic and fetal development, the activation of gene strings is the main driving force directing the maturational processes of the central nervous system [3]. However, in addition to the impact of genetic control, environmental stimuli influence developmental structures, and the environment experienced by the (pregnant) mother alters the intrauterine environment and fetal organogenesis [3].

The term enriched environment (EE) is often used to describe an environmental manipulation administered to rodents. EE is often characterized as a large environment with toys, tunnels, bedding and running wheels and designed to provide social, physical and sensory stimulation [4]. It is considered a condition that provides major stimulation in comparison to a standard environment [5].

EE has been shown to exert beneficial effects on many behaviors such as motor, cognitive and emotional performance, dendritic branching, synaptic density, neurogenesis, modulation of neurotrophic systems and neurotransmitters [6]. In $BDNF^{+/-}$ mice, LTP, LTD and recognition memory are impaired, whereas EE enhanced LTP and recognition memory in both wildtype and $BDNF^{+/-}$ mice [7]. These effects were accompanied by elevations in the expression of mature BDNF. In fact, EE resulted in restoration of LTP, learning, and BDNF expression in $BDNF^{+/-}$ mice to levels seen in non-enriched wildtype animals [7].

EE stimulates neurogenesis in the rodent brain, especially in the hippocampus, a limbic structure important for memory formation and emotional processing, which is affected in Alzheimer's disease and mood disorders [8]. EE has been shown to improve the detrimental effects of depression by helping to reduce negative effects [9], and to prevent depression and anxiety behaviors induced by social isolation [10].

Depression induces a wide spectrum of behavioral deficits, structural alterations as well as electrophysiological perturbations in rodent brains. These alterations can be restored with a short-term exposure to enriched environment [11]. Furthermore, positive environmental stimuli can have lasting effects on behavioral deficits, structural alterations and synaptic plasticity [11]. The environment influences and interacts with several physiological effectors. While negative environmental stimuli such as chronic stress, fearful experiences and trauma cause degenerative changes in the brain, positively stimulating environments elicit restorative changes by neural reorganization and rewiring in damaged brain areas to facilitate recovery from depression and associated conditions [11].

However, the influence of EE on pharmacological and behavioral responses in animal models of psychiatric disorders has not been fully established yet. In this context, the aim of this study was to evaluate the influence of exposure to environmental enrichment on mice behavior in the open field (OF) and forced swimming (FST) tests, as well as the response to antidepressant drugs. We also investigated the influence of EE on BDNF gene expression in mice hippocampi.

2. MATERIALS AND METHODS

2.1. Animals

Female and male CF1 mice (25-35 g) from CeMBE / PUCRS (Centro de Modelos Biológicos Experimentais, Pontifícia Universidade Católica do Rio Grande do Sul) were used. Dams were exposed (N=15) or not (N=15) to an environmental enrichment program (EE) from mating until offspring weaning as described below. Then, the offspring (male only) were randomly allocated to receive or not the EE. The animals were kept at 23 to 25 °C under a light/dark cycle of 12h (lights on between 7:00 a.m. and 7:00 p.m.), in a ventilated cage system (39 cm x 20 cm x 17

cm), housed 4 per cage. They received food (irradiated Nuvilab® CR-1, Nuvital) and filtered water ad libitum.

Three days before the behavioral tests (postnatal day (PND) 55), the offspring were transported to the vivarium of the College of Pharmacy,UFRGS, in an air-conditioned car. Mice were housed in plastic cages (17 × 28 × 13 cm), 4 per cage, and kept under a light/dark cycle of 12 h (lights on between 7:00 a.m. and 7:00 pm), constant temperature of 23 ± 1 °C, with free access to standard Nuvilab® certified rodent diet and water. During this period, mice were housed in their previous conditions (cages), under enrichment or not.

All experiments were approved by Animal Care Local Ethical Committee (CEUA-UFRGS; protocol nº 31882).

2.2 Enrichment program

The environmental enrichment program started immediately after mating, with the introduction of the enrichment objects in the dams' cages. Different groups of dams (genitors) were exposed or not to environmental enrichment (EE) from mating until offspring weaning. At PND 21, puppies were weaned, sexed and grouped in 4 animals per cage, where they remained (under EE or not) until behavioral tests (PND 58). In order to avoid habituation, the enrichment elements (models 1 to 5, Figure 1) were changed every 7 days.

The enrichment objects presented the following characteristics: absorbent but not dehydrating, inedible, durable and disposable, free of toxins or other contaminants. The materials were purchased from specialized companies: aspen wood, disposable paper house, cellulose cylinder and shaving bedding (Souralit®, Spain), relax (Granja SA®, Brazil), mouse house (Tecniplast®, Italy).

Model1 Model 2 Model 3 Model 4 Model 5

Figure 1: Enrichment models.

2.3 Drugs and treatments

The following drugs were used: bupropion 30 mg/kg (Eurofarma®, Brazil) and fluoxetine 30 mg/kg (Galena®, São Paulo, Brazil). The drugs were administered at PND 58 (1 mL/100 g animal body weight, p.o.) 60 minutes before behavioral experiments (open field followed by forced swimming test)**.** The doses were chosen according to other studies by our group [12,13 and 14]. All drugs were solubilized in NaCl 0.9% plus 1% polysorbate 80 at the following final concentrations: fluoxetine 3 mg/mL, bupropion 3 mg/mL. The control (vehicle) group received NaCl 0.9% solution plus 1% polysorbate 80 (1 mL/100 g animal body weight).

2.4 Experimental Design

After weaning, puppies were balanced to comprise four housing condition groups, as follows: NE - mice housed under standard condition all their life (genitor and descendants under no enrichment); LE - mice housed under enriched condition all their life (from genitor mating until PND 58); PE - mice housed under enriched conditions during perinatal period (from genitor mating until weaning – PND21); PWE - mice housed under enriched conditions from weaning until PND 58 (post-weaning enrichment).

On PND 58, mice (n=10-12) from each housing condition were randomly allocated to receive different treatments (fluoxetine - FLU, bupropion - BUP or vehicle - VHC) one hour before behavioral testing (open field followed by forced swimming). Immediately after swimming, mice were euthanized and their

hippocampi were removed to carry out BDNF quantification. The BDNF was measured only in vehicle treated mice.

Figure 2 shows a timeline with the key events of the study.

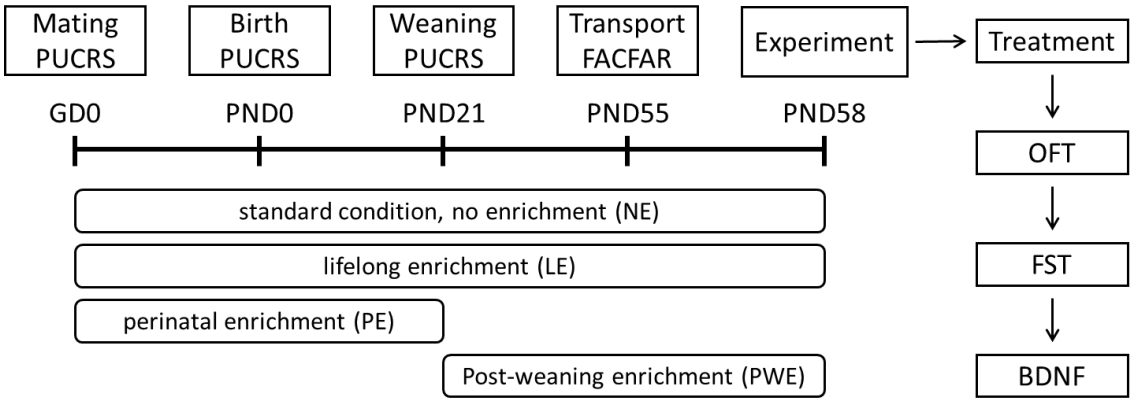

Figure 2: Timeline of the experimental design. GD: gestational day; PND: postnatal day; OFT: open field test; FST: forced swimming test; PUCRS: Pontifícia Universidade Católica do Rio Grande do Sul; FACFAR: Faculdade de Farmácia (College of Pharmacy) – UFRGS.

2.5 Open Field Test (OFT)

The apparatus used for the OFT consisted of a transparent acrylic box, measuring 40 x 30 x 30 cm, with the soil divided into 24 quadrants. The animals were placed in the center of the apparatus, with the face facing the wall. Parameters recorded were: number of crossings; number of rearings (episodes in which the animals raise their body by leaning only on the hind legs); number of groomings (self-cleaning behaviors) and time remained in the apparatus periphery. The parameters were recorded for 15 minutes by observers blind to the experimental groups. The apparatus was cleaned with 30% ethanol between each animal. The entire protocol was performed in a penumbra environment.

2.6 Forced Swimming Test (FST)

Immediately after the open field test, the animals were assessed on FST. FST was performed as described by Porsolt et al. (1977) [15] with small modifications previously validated in our laboratory conditions [12]. The animals were forced to swim individually in a cylinder 10 cm in diameter and 13 cm in height

with water at 22 ± 1 °C, and the duration of immobility behavior was recorded for 6 min by observers blind to treatments. The animals were considered as immobile when they remained floating or making only the movements necessary to keep their heads above the water. After swimming, the animals were immediately euthanized and their hippocampi were removed to evaluate the expression of BDNF.

2.7 BDNF Expression

Real-time quantitative PCR was performed following a protocol previously established [16]. Briefly, mice were euthanized by rapid decapitation and brains were removed. The hippocampi were dissected and frozen at –80 °C. Total RNA was isolated from tissues (pool of four hippocampi constituted one sample) using Trizol reagent (Life Technologies, Carlsbad, CA, USA), in accordance with the manufacturer's instructions, and RNA concentration was determined using a Nano-Drop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). One µg of total RNA was reverse transcribed into first-strand cDNA with the M-MLV Reverse transcriptase (Invitrogen) in a total reaction volume of 20 µL. All SYBR Green I-based real-time PCR mixtures were performed using the GoTaq® qPCR Master Mix (Promega) following the manufacturer's recommendation, with 1µL cDNA to a 25 µL final volume reaction mix using the Rotor-Gene Q (Qiagen). The reaction conditions were 95 °C for 1 min and 40 cycles of 10 s at 95 °C, 15 s at 60 °C, and 20 s at 72 °C. For the relative quantification (ΔΔCT) of BDNF, real-time PCR reactions were performed in triplicate using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control. The ΔCT values were calculated by subtracting the mean CT value of GAPDH from the mean CT value of target genes, and the ΔΔCT values were further calculated by subtracting the ΔCT value of naïve mice (not exposed to environmental enrichment and behavioral tests) from the ΔCT value of each group. All data were expressed as relative change in mRNA expression level. The following primers were used: 5′ GATGCCGCAAACATGTCTATGA-3′ (forward) and 5′ -TAATACTGTC ACACACGCTCAGCTC-3′ (reverse) for BDNF; 5′ -GGCAAATTCAACGGCACAGT-3′ (forward) and 5′ -AGATGGTG ATGGGCTTCCC-3′ (reverse) for GAPDH.

2.8 Statistical Analysis

The data were analyzed by one-way ANOVA (BDNF) or two-way ANOVA (OFT and FST) followed by Student-Newman-Keuls test when applicable. Results are expressed as mean ± S.E.M

. The level for statistical significance was set as $p \leq 0.05$. Statistical procedures were performed using the Sigma Stat software 3.5.

3 RESULTS

3.1 Open field

Figure 3 depicts the results from the open field test.

Grooming behavior (Figure 3A)

ANOVA revealed significant main effects of housing condition and treatment on grooming behavior, as well as a significant interaction between both factors [$F_{\text{housing condition (3,134)}} = 38.391$; $P<0.001$; $F_{\text{treatment (2,134)}} = 12.776$; $P<0.001$; $F_{\text{interaction (6,134)}} = 5.312$; $P<0.001$]. Mice housed all their life (LE) and post weaning (PWE) under enriched condition had a higher number of groomings than those housed under standard condition (NE) ($P<0.001$), whereas mice housed under enriched condition during perinatal period (PE) presented lower grooming number ($P<0.05$). Bupropion (BUP) induced a significant decrease on grooming number in all housing conditions (NE $P<0.005$; LE $P<0.001$; PWE $P<0.005$) except on PE enriched housing ($P=0.707$). Fluoxetine (FLU) did not affect grooming when compared with vehicle (VHC) in any housing condition (LE $P=0.053$ and PE $P=0.828$), except on PWE enriched housing, where it reduced grooming ($P<0.001$).

*Periphery time (*Figure 3B)

ANOVA revealed a significant main effect of housing condition only [$F_{\text{housing condition (3,134)}} = 9.818$; $P <0.001$; $F_{\text{treatment (2,134)}} = 1.417$; $P=0.246$; $F_{\text{interaction (6,134)}} = 1.172$; $P=0.326$]. Mice housed under enriched conditions during the perinatal period (PE) spent more time in the periphery than those housed all their life under standard

condition (NE) (P<0.01). Treatment with BUP or FLU did not alter this behavior in any housing condition.

Rearing behavior (Figure 3C)

ANOVA revealed significant main effects of housing condition and treatment on rearing behavior, as well as a significant interaction between both factors [$F_{\text{housing condition (3,134)}}$ = 10.347; p <0.001; $F_{\text{treatment (2,134)}}$ = 19.333; P<0.001; $F_{\text{interaction (6,134)}}$ = 4.054; P <0.001]. FLU treatment decreased the number of rearing of mice housed under standard (NE, P<0.001) and perinatal enriched conditions (PE, P<0.001), when compared with its respective VHC groups. Mice housed under enriched condition all their life (LE) and treated with BUP or FLU presented higher number of rearing, when compared to the respective group treated with VHC (P<0.001 and P<0.05, respectively). BUP and FLU did not affect rearing behavior of mice housed under enriched condition post-weaning (PWE) (P=0.586 and P=0.357, respectively).

*Crossings (*Figure 3D)

ANOVA revealed significant main effects of housing condition and treatment on number of crossings, as well as a significant interaction between both factors [$F_{\text{housing condition (3,134)}}$ = 5.174; P<0.01; $F_{\text{treatment (2,134)}}$ = 17.371; P<0.001; $F_{\text{interaction (6,134)}}$ = 11.550; P<0.001]. Mice housed under enriched condition during perinatal period (PE) and treated with vehicle had a higher number of crossings than those housed under standard condition (NE) (P<0.05). Treatment with BUP increased the number of crossings only in mice housed under standard (NE, P<0.001) and enriched conditions all their life (LE, P< 0.001). At these housing conditions, FLU had no effect (P=0.892). When mice were housed under enriched condition during the perinatal period (PE), FLU decreased crossings in relation to its respective VHC group (P<0.001), and BUP had no significant effect (P=0.119). When mice were housed under post-weaning enriched condition (PWE), FLU increased crossings in relation to its respective VHC group (P< 0.05), and BUP had no significant effect (P=0.807).

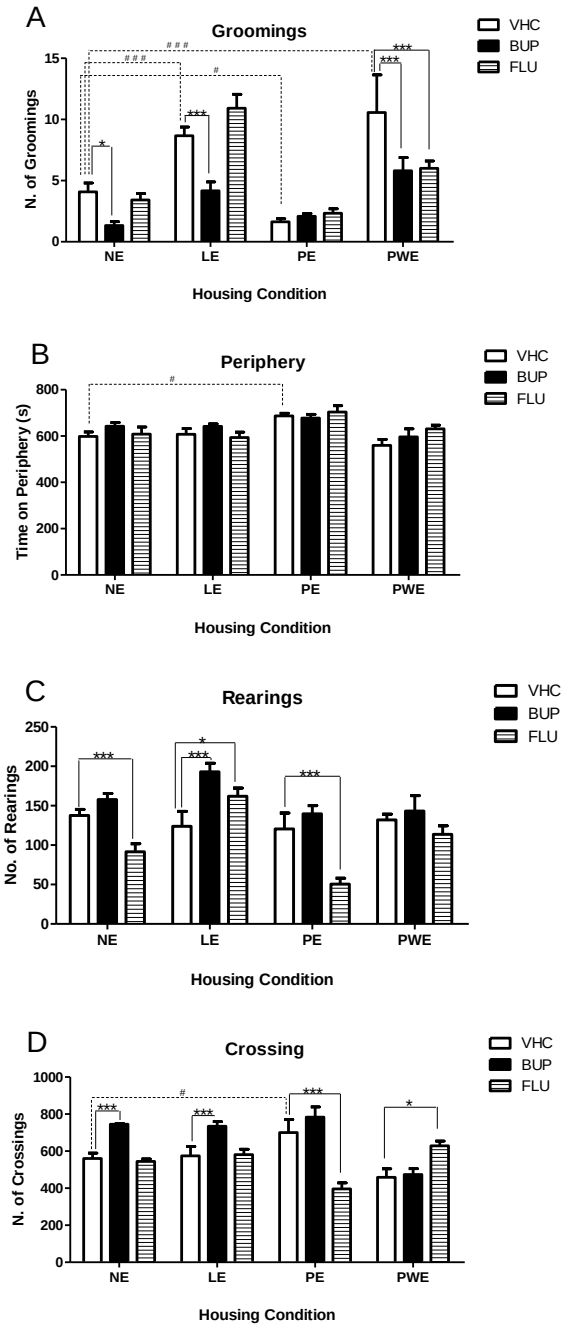

Figure 3: Effects of mice exposure to environmental enrichment and response to antidepressant drugs in the open field test (OFT). Housing conditions: standard (no enrichment) (NE), lifelong enrichment (LE), perinatal enrichment (PE) and post-weaning enrichment (PWE). Mice (n=7-12 mice/group) were treated by gavage with fluoxetine (FLU) 30 mg/kg, bupropion (BUP) 30 mg/kg or vehicle (VHC: NaCl 0.9% plus 1% polysorbate 80 1 mL/100 g) and exposed to OFT 1 h after drug administration. Results are expressed as mean ± S.E.M. Two-way ANOVA followed by Student-Newman-Keuls test: *p≤0.05, ***p≤0.001, compared to NE; $^{\#}$p≤0.05 , $^{\#\#\#}$ p≤0.001, compared to VHC.

3.2 Forced swimming test

The results from forced swimming test are depicted in figure 4.

ANOVA revealed significant main effects of treatment and housing condition, but no interaction between factors [$F_{housing\ condition\ (3,134)}$ = 4.766; P <0.004; $F_{treatment\ (2,134)}$ = 11.604; P<0.001; $F_{interaction\ (6,134)}$ = 1.196; P=0.313]. PWE immobility time is lower than LE (P<0.05) and PE (P=0.001) immobility times. BUP treatment was effective in reducing immobility time of mice housed under standard condition (NE, P<0.001) only. FLU was not effective in any housing condition. Mice housed under enrichment during perinatal period (PE) and treated with BUP presented higher immobility time than those housed under standard condition and treated with BUP (P<0.05). In addition, the immobility time of mice enriched post-weaning (PWE) and treated with BUP was not different from NE-BUP treated group (P=0.351), but was lower than respective PE (P<0.05) and LE (P<0.05) groups.

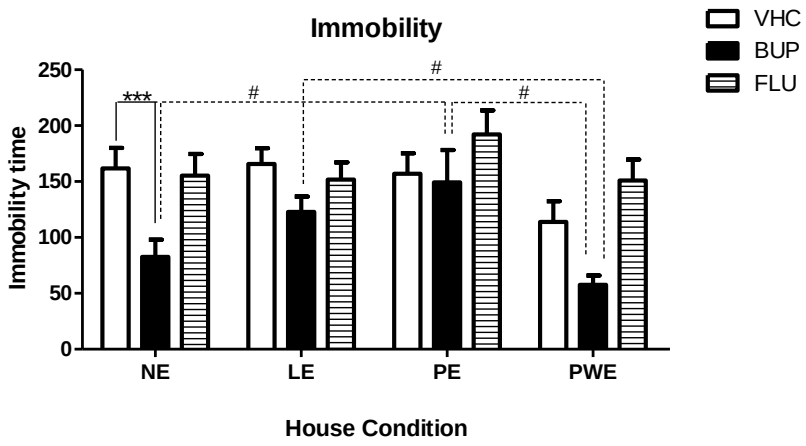

Figure 4: Effects of mice exposure to environmental enrichment and response to antidepressant drugs in the forced swimming test (FST). Housing condition: standard (no enrichment) (NE), lifelong enrichment (LE), perinatal enrichment (PE) and post-weaning enrichment (PWE). Mice (n=7-12 mice/group) were treated by gavage with fluoxetine (FLU) 30 mg/kg, bupropion (BUP) 30 mg/kg or vehicle (VHC: NaCl 0.9% plus 1% polysorbate 80 1 mL/100 g) and exposed to OFT 1 h after drug administration. Results are expressed as mean ± S.E.M. Two-way ANOVA followed by Student-Newman-Keuls test: #p≤0.05, ***p≤0.001.

3.3 BDNF expression

Figure 5 depicts the results from BDNF expression.

There was no significant effect of housing enrichment on hippocampal BDNF mRNAm expression [$F_{(3, 11)} = 0.676$; P=0.591].

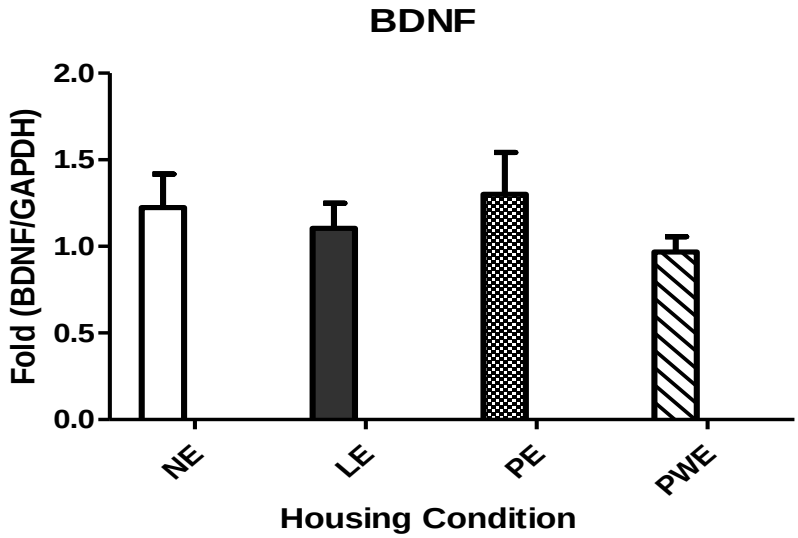

Figure 5: Effects of mice exposure to environmental enrichment on hippocampal BDNF expression. Housing condition: standard (no enrichment) (NE), lifelong enrichment (LE), perinatal enrichment (PE) and post-weaning enrichment (PWE). Results are expressed as mean ± SEM (n=3; pool of 4 mice each sample). One-way ANOVA.

4. DISCUSSION

Our results have shown that environmental enrichment (EE) presented at different developmental stages altered mice behavior as compared to standard housing and affected the response to the antidepressants bupropion and fluoxetine in the open field and forced swimming tests.

The forced swim test (FST) is widely used to evaluate the antidepressant-like activity of compounds and is sensitive to stimuli that cause depressive-like behavior in rodents [17]. The immobility behavior observed during the test has been considered to represent behavioral despair. In addition, some studies suggest that the FST impairs rats' performance on cognitive tests [18].

The open field test (OFT) was originally described for the study of emotionality in rats (Hall, 1934 *apud* [19]). Nowadays, it is also useful to assess habituation to novelty, and environmental enrichment has proved to be a reliable way to enhance open-field test habituation [20]. Several open field apparatus versions are available, differing in shape (circular, square or rectangular), lighting and presence of objects inside the arena. Rats or mice are placed in the center or close to the walls of the apparatus and several parameters of exploratory behavior/spontaneous locomotion and emotionality can be observed: horizontal locomotion (crossing), vertical activity (rearing), grooming (protracted washing of the coat), fecal bolus and thigmotaxis. The procedure usually involves forced confrontation of a rodent with the situation in which rodents spontaneously prefer the periphery of the apparatus to the central parts of the open field. Indeed, mice and rats tend to walk close to the walls, a behavior called thigmotaxis. An increase of time spent in the central part (with consequent reduction in periphery time) as well as of the ratio central/total locomotion or a decrease of the latency to enter the central part are indicatives of anxiolysis [19].

Bupropion is effective for treating nicotine dependence and depression [21]. In general, it stimulates the dopaminergic and noradrenergic systems by inhibiting dopamine and noradrenaline neuronal transporters – DAT and NAT - with greater affinity for the former [21]. In turn, fluoxetine is an antidepressant (also used for treating panic and generalized anxiety disorders) that selectively acts on neuronal serotonin transporter (SERT) and is classically classified as a selective serotonin reuptake inhibitor [21]. In addition to its action at SERT, fluoxetine may also

antagonize the 5-HT_{2C} receptor, which causes a slight intensification of the release of noradrenaline and dopamine, an effect that may be correlated with a mild euphoria reported by patients after receiving an acute administration of this drug [21].

Data reported herein indicate that perinatal enrichment resulted in anxiety-like behavior since PE mice remained longer in the peripheral zone of the open field than NE mice, and this effect was independent of drug treatment. This finding is in line with Connors and coworkers [22], who demonstrated that rats underwent perinatal EE presented anxiety-like behavior in the elevated plus maze and light-dark tests and postulated that exposure of dams to EE shapes maternal care by increasing naturalistic periods of separation. According to these authors, the altered parental investment induced by environmental enhancement (i.e. novelty and physical space) appears to mediate the anxiety response patterns of offspring [22].

Perinatal enrichment also increased horizontal locomotion (crossings) in vehicle-treated animals, suggesting a stimulant effect of perinatal EE. Surprisingly, this effect was blocked by fluoxetine, which decreased crossings in PE animals while increased this parameter in mice exposed to post-weaning (PWE) EE. As expected, bupropion increased horizontal activity in mice not submitted to any enrichment program (NE). This drug also increased crossings in mice exposed to lifelong environmental enrichment (LE), but had no effect in PE and PWE groups, which indicates that perinatal and post-weaning EE impair the stimulant properties of this drug. These results may be related to DAT function since Darna and coworkers [23] demonstrated that post-weaning enrichment decreases DAT activity in the medial prefrontal cortex of rats [23]. Furthermore, Del Arco and coworkers [24] demonstrated that EE during adult life reduces the function of D1 dopamine receptors in the rat prefrontal cortex.

None enrichment protocol affected untreated mice (VEH) vertical activity (rearing). Nevertheless, the antidepressants once again had divergent effects depending on the timing of environmental enrichment. Fluoxetine reduced rearing in NE and PE mice and did not affect it in PWE, whereas it increased this behavior in LE mice. In contrast, bupropion only affected rearing behavior in mice exposed to lifelong EE (LE), which displayed increased vertical exploration when treated with either bupropion or fluoxetine.

The effect of environmental enrichment on grooming behavior was also dependent on the life period. Mice exposed to LE or just PWE made more grooming when compared to those housed under standard condition all their life (NE). When mice underwent perinatal enrichment (PE) they performed fewer grooming. Bupropion reduced grooming behavior in all groups except PE. Gomez and coworkers (2017) demonstrated that an acute administration of bupropion induces a dose-dependent reduction in grooming behavior in mice, an effect independent on whether animals were group- or single-housed, and suggest that this drug exhibits anxiogenic-like properties in social encounters between adolescent mice, especially when a transition in housing conditions has been experienced during this period [25]. On the other hand, it has been reported that dopamine transporter (DAT)-deficient mice, which have elevated levels of extra-neuronal dopamine, exhibit more stereotyped and predictable syntactic grooming sequences than their wild-type counterparts, and systemic administration of dopamine D1 receptor agonists amplifies complex behavioral super-stereotypy, leading to excessive production of self-grooming chains in rodents [24].

Although anxiety levels influence grooming, the locomotion-enhancing effects of bupropion, as measured by increased number of crossings, may at least partially explain the reduction in grooming behavior induced by bupropion in NE and LE groups, but not in PWE animals, in which bupropion reduced grooming without affecting crossings. In addition, it is noteworthy that untreated (VHC) PE mice decreased grooming while increased horizontal locomotion when compared with VHC-NE mice, which suggests a motor stimulant or anxiety-like effects of perinatal environmental enrichment.

Taken together, these findings indicate that grooming increase induced by LE or PWE in the current study may be due to an impairment on dopaminergic and/or noradrenergic neurotransmission and that inhibition of grooming observed in mice underwent perinatal enrichment (PE) does not depend on DAT and/or NAT functioning.

Fluoxetine, in turn, significantly reduced grooming behavior only in mice housed under enrichment after weaning (PWE), which indicates that the increasing grooming behavior induced by this housing condition may be at least in part due to an impairment of serotoninergic neurotransmission. On the other hand, the effect of

fluoxetine on grooming may be also due to the concomitant increase in the number of crossings.

Self-grooming is a complex innate behavior with an evolutionary conserved sequencing pattern and is one of the most frequently performed behavioral activities in rodents. Studies on rodent models of neuropsychiatric disorders — including models of autism spectrum disorder and obsessive-compulsive disorder — have assessed self-grooming phenotypes and suggested that rodent self-grooming may be a useful measure of repetitive behavior in such models [26]. This corroborates studies demonstrating that serotonergic drugs that are effective in treating some symptoms of clinical obsessive-compulsive disorder are also successful in reducing aberrant self-grooming phenotypes in mutant mice [26]. Based on this, we suggest that exposure to post-weaning EE may have induced a repetitive behavior, which was responsive to fluoxetine. On the other hand, the OFT is regarded as a rodent model of state anxiety and may not model features of anxiety disorders. It is sensitive to the anxiolytic-like effects of classical benzodiazepines and 5-HT_{1A} receptor agonists, but not to the effects of selective serotonin reuptake inhibitors, which have a different spectrum of therapeutic efficacy in anxiety disorders such as panic attacks, generalized anxiety disorder or obsessive-compulsive disorder [19]. In addition, several authors consider grooming behavior as a stress response [27], as well as a measure of rodents' habituation to novelty [20]. Rojas-Carvaja and coworkers [20] found that environmental enrichment enhanced short-term and long-term open-field test habituation in rats, and increased grooming, particularly body licking, suggesting that the appearance of more complex and longer grooming sequences is part of a de-arousal inhibition system subserving novelty habituation, and it does not mean anxiety.

None of the enrichment protocols altered the immobility time of the animals in the FST, which suggests that these EE protocols have no antidepressant-like effect. In addition, all enriched housing conditions abolished the anti-immobility effect of bupropion. These results corroborate with perinatal enrichment (PE) effects on crossing behavior and indicate this protocol impairs responses mediated by dopaminergic and noradrenergic neurotransmission. Of note, post-weaning EE (PWE) significantly reduced immobility time when compared with LE and PE protocols and showed a tendency to diminish it when compared with non-

enrichment (NE). This finding suggests a latent antidepressant-like effect of post-weaning environmental enrichment, which deserves further investigation [28].

Fluoxetine did not show anti-immobility effect in any group, confirming earlier work demonstrating that the sensitivity of the forced swimming test to this antidepressant is low [29], and showing that enrichment does not potentiate serotonergic responses in this model. This is in agreement with a study by Sequeria-Cordero and coworkers (2014) [30], where rats with low immobility in the FST showed significantly higher accumbal 5-HT levels than animals with high immobility, whereas no neurochemical differences were observed between enriched and standard animals. Possamai and coworkers [28] found results somewhat different. They found that repeated treatment with fluoxetine and imipramine or housing enrichment counteract the high immobility in repeated FST; enrichment changed the effects of antidepressants depending on the type, and the dose of a substance. Furthermore, Possamai and coworkers [28] postulated that the effects of antidepressants and enrichment on repeated FST are neurogenesis-independent. This assumption is in line with our findings regarding the effect of EE on hippocampal expression of BDNF, where we did not observe any differences regarding housing condition.

A recent hypothesis regarding the action of SSRIs posits that this drug class may not affect mood per se but enhance neural plasticity, rendering the individual more susceptible to the environment [31]. Treatment would thus improve symptoms in a favorable environment and worsen the prognosis in a stressful environment, as claimed by human and animal studies [31-32]. Our study corroborates and extends this idea by showing that even the response to a single acute administration of fluoxetine depends on the environmental conditions and varies according to the timing of housing manipulations.

The environmental enrichment program used in this study did not affect the hippocampal expression of BDNF. This was unexpected, as several data from the literature indicate that EE increases neurogenesis and BDNF levels in the hippocampus of rodents in animal models of psychiatric or cognitive disorders [33]. Enriched housing was found to increase hippocampal BDNF mRNA levels in healthy mice and autism spectrum disorder-like phenotypes [34]. The hippocampal BDNF level increased in both heterozygous $BDNF^{+/-}$ and wildtype mice following EE [35].

Environmental enrichment of Wistar rats from weaning to young-adulthood led to stronger dorsal hippocampal BDNF response and higher serum BDNF levels, while rats from standard laboratory condition showed higher amygdala BDNF response [36].

Interestingly, Borsoi et al. (2014) [18] reported a significant negative correlation between frontal BDNF levels and immobility behavior in the classical FST in rats, suggesting a protective role of BDNF against behavioral despair. Accordingly, a correlational analysis by Sequeira-Cordero and coworkers [30] suggests that immobility in the FST, probably reflecting despair, is related to prefrontal cortical BDNF, and that individual differences in the FST could be associated with differential temporal dynamics of gene expression and neurotransmitter activity. Therefore, the lack of the EE effect on BDNF mRNA observed in our study is coherent with its absence of effect on immobility behavior in mice exposed to a single forced swimming session. Anyway, further experiments might verify whether individual differences in depression-like behavior can be associated to this apparent lack of EE influence on BDNF mRNA hippocampal levels. In addition, future analyses of BDNF protein levels should be performed in order to investigate posttranscriptional effects.

5. CONCLUSION

We conclude that mice behavior in the open field and forced swim tests are sensitive to alterations in the housing environment and depend on the developmental stage of exposure. Compared to standard non-enriched environment as our control condition, perinatal enrichment produced a different set of behavioral and pharmacological alterations than exposure to EE only after weaning, which probably reflects alterations in maternal care mediating the effects of the former and direct effects to the offspring in the latter case. When EE was extended throughout life, yet another profile of behavioral alterations and response to antidepressant drugs was observed, mainly in the open field test. Further studies are necessary to elucidate the mechanisms underlying such differential outcomes, as BDNF levels in the hippocampus were not altered by our protocol. Considering that bupropion and fluoxetine yielded divergent responses depending on the housing condition and reversed some parameters to levels close to vehicle-treated standard-housed animals, modulation of monoaminergic neurotransmission pathways is likely to be involved.

A major implication of our study is that differences in housing conditions, such as the introduction of objects to the animal cage, mostly done to improve animal welfare, may result in altered behavioral profiles and response to psychotropic drugs. Although many institutional review committees and regulatory authorities worldwide are increasingly requiring animal facilities to implement environmental enrichment protocols, researchers must be aware that transitioning from one housing condition to another may affect experimental results, particularly when dealing with anxiety and stress-sensitive measures. Caution should be taken when interpreting research data and comparing the literature, as housing condition varies widely across laboratories and is an important variable that may influence animal behavior and confound experimental results.

The data reported in our study should also raise awareness to the reporting of methodological details in the biomedical literature. Although guidelines to improve the reporting of preclinical studies have been published [37], compliance is not mandatory in most journals and most published articles provide incomplete information on methods, including housing conditions, environmental enrichment

and even type of facility/sanitary status [38, 39]. The observations reported in our study reinforce the call for more transparency and adherence to reporting guidelines, to ultimately improve reproducibility in the biomedical sciences and reduce the risk of bias.

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CONSIDERAÇÕES FINAIS

O paradigma experimental do enriquecimento ambiental (EA) foi descrito pela primeira vez em um contexto neurocientífico por Donald Hebb, quando comparou ratos que podiam vagar livremente em sua casa com aqueles que haviam sido deixados em gaiolas de laboratório e contribuiu com características importantes em relação ao enriquecimento como um ambiente com novidade e complexidade aprimoradas em relação às condições padrão.

O EA é um assunto que, nos últimos anos, atraiu muita atenção científica e mediática devido a sua ligação com o bem-estar dos animais, é um campo crescente e é uma prática na produção de animais que tem se mostrado com efeitos positivos sobre o bem-estar. O uso de animais adaptáveis e saudáveis contribui para a qualidade dos dados científicos e as agências reguladoras nos Estados Unidos, Europa bem como no Brasil estão cada vez mais preocupadas com estas questões.

Em uma revisão geral sobre EA na literatura nota-se que não existe uma definição consensual de EA, e pouco se sabe ainda sobre a influência que tais modificações de criação podem ter nos parâmetros biológicos, elevando as preocupações da comunidade cientifica. A preocupação crescente com o bem-estar, bem como o uso incorreto e exacerbado de EA pode vir a interferir na reprodução de trabalhos científicos. Deste modo todos os trabalhos que possam contribuir para elucidar melhor os efeitos do EA são de grande interesse.

Uma implicação importante de nosso estudo é que as diferenças nas condições de moradia, como a introdução de objetos na gaiola dos animais, principalmente para melhorar o bem-estar animal, podem resultar em perfis comportamentais alterados e resposta a drogas psicotrópicas. Embora muitos comitês institucionais de revisão e autoridades regulatórias em todo o mundo estejam exigindo cada vez mais que as instalações de animais implementem protocolos de enriquecimento ambiental, os pesquisadores devem estar cientes de que a transição de uma condição habitacional para outra pode afetar os resultados experimentais, particularmente quando se trata de ansiedade e medidas sensíveis ao estresse. Deve-se ter cautela ao interpretar dados de pesquisa e comparar a literatura, já que a condição de moradia varia amplamente entre os laboratórios e é

uma variável importante que pode influenciar o comportamento animal e confundir os resultados experimentais.

Os dados relatados em nosso estudo também devem aumentar a conscientização para o relato de detalhes metodológicos na literatura biomédica. Embora tenham sido publicadas diretrizes para melhorar o relato de estudos pré-clínicos, o cumprimento não é obrigatório na maioria dos periódicos e a maioria dos artigos publicados fornece informações incompletas sobre métodos, incluindo condições de moradia, enriquecimento ambiental e até mesmo tipo de instalação estado sanitário. As observações relatadas em nosso estudo reforçam o apelo por mais transparência e aderência às diretrizes de relato, para finalmente melhorar a reprodutibilidade nas ciências biomédicas e reduzir o risco de viés.

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ANEXOS

Anexo 1: Carta de aprovação da Comissão de Ética para Uso de Animais-CEUA

Anexo 1: Guide for authors, rev. Behavioural Brain Research

CEUA
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PRÓ-REITORIA DE PESQUISA

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CARTA DE APROVAÇÃO/ADENDO

Processo Nº: 31882

TÍTULO: AVALIAÇÃO DO EFEITO DO ENRIQUECIMENTO AMBIENTAL NO COMPORTAMENTO DE CAMUNDONGOS NO TESTE DA NATAÇÃO FORÇADA E RESPOSTA A ANTIDEPRESSIVOS.

Pesquisador Responsável:
STELA MARIS KUZE RATES

Comissão De Ética No Uso De Animais aprovou o Adendo ao Projeto **31882** em reunião realizada em 08/05/2017 - Sala 330 do Anexo 1 da Reitoria - Campus Centro - Porto Alegre - RS, em seus aspectos éticos e metodológicos, para a ampliação no número de animais no presente projeto, ficando autorizado a obtenção de 96 camundongos machos CF1 de 25 a 30 gramas do CEMBE-PUC, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa. Este documento revoga a Carta de Aprovação emitida anteriormente.

Porto Alegre, 22 de maio de 2017.

Marcelo Meller Alievi

Coordenador da CEUA/UFRGS

BEHAVIOURAL BRAIN RESEARCH

AUTHOR INFORMATION PACK

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DESCRIPTION

Behavioural Brain Research is an international, interdisciplinary journal dedicated to the publication of articles in the field of **behavioural neuroscience**, broadly defined. Contributions from the entire range of disciplines that comprise the **neurosciences**, **behavioural sciences** or **cognitive sciences** are appropriate, as long as the goal is to delineate the neural mechanisms underlying behaviour. Thus, studies may range from neurophysiological, neuroanatomical, neurochemical or neuropharmacological analysis of brain-behaviour relations, including the use of molecular genetic or behavioural genetic approaches, to studies that involve the use of brain imaging techniques, to neuroethological studies. Reports of original research, of major methodological advances, or of novel conceptual approaches are all encouraged. The journal will also consider critical reviews on selected topics.

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[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003).
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