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Neuroanatomical Profile of Antimaniac Effects of Histone Deacetylases Inhibitors

Camila O. Arent · Samira S. Valvassori · Gabriel R. Fries · Laura Stertz · Camila L. Ferreira · Jéssica Lopes-Borges · Edemilson Mariot · Roger B. Varela · Felipe Ornell · Flávio Kapczinski · Monica L. Andersen · João Quevedo

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Abstract An increasing number of studies have evaluated the potential therapeutic relevance of histone deacetylases (HDAC) inhibitors in mood disorder including bipolar disorder (BD). It has been suggested that the anterior limbic, which controls impulsivity and psychosis, is dysfunctional in BD. The present studies aims to evaluate the effects of microinjection of HDAC inhibitors in the ventricle, amygdala, striatum, prefrontal, and hippocampus on m-amphetamine-induced manic-like behavior in rats. Rats were given a single intracerebral (in the ventricle, amygdala, striatum, prefrontal, or hippocampus) injection of artificial cerebrospinal fluid, sodium butyrate (SB), or valproate (VPA) followed by an intraperitoneal injection of saline or m-AMPH 2 h before the open-field task. The activity of HDAC was evaluated in amygdala, striatum, prefrontal, and hippocampus of animals. The microinjection of SB and VPA in the ventricle, amygdala, striatum, and prefrontal, but not in hippocampus blocked the hyperactivity induced by m-AMPH. In addition, SB and VPA inhibited the HDAC activity; however, this effect varied depending on the experimental procedure and the brain structure evaluated. Our results suggest that the antimanic effects of SB and VPA, HDAC inhibitors, are related to the amygdala, striatum, and prefrontal, but not the hippocampus. More studies are needed to clarify the therapeutic effects of the HDAC inhibitor in BD and thereby develop new drugs.

Keywords Histone deacetylases · Bipolar disorder · Methamphetamine · Valproate · Butyrate

C. O. Arent · S. S. Valvassori · C. L. Ferreira · J. Lopes-Borges · E. Mariot · R. B. Varela · F. Ornell · J. Quevedo
Laboratory of Neurosciences and National Institute for Translational Medicine (INCT-TM), Postgraduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina,
88806-000 Criciúma, SC, Brazil

G. R. Fries · L. Stertz · F. Kapczinski
Bipolar Disorders Program, Laboratory of Molecular Psychiatry and National Institute for Translational Medicine (INCT-TM),
Federal University of Rio Grande do Sul,
90035-003 Porto Alegre, RS, Brazil

M. L. Andersen
Department of Psychobiology,
Universidade Federal de São Paulo,
04024-002 São Paulo, SP, Brazil

J. Quevedo (✉)
Laboratório de Neurociências, PPGCS, UNASAU,
Universidade do Extremo Sul Catarinense,
88806-000 Criciúma, SC, Brazil
e-mail: quevedo@unesc.net

Introduction

Histones play an important role in the regulation of gene expression; these proteins package and order the DNA into units called nucleosomes [1, 2]. The acetylation of histones by histone acetyltransferases promotes access to DNA and docking sites for transcriptional factors, permitting genetic transcription. Unlike, histone deacetylases (HDACs) are enzymes that catalyze the removal of acetyl group from lysine residues of histones, leading to gene expression silencing [3].

Previous studies have demonstrated that HDACs inhibitors, as valproate (VPA) and sodium butyrate (SB), control epigenetic programming associated with regulation of cognition and behavior (see [4]). An increasing number of studies have evaluated the potential therapeutic relevance of HDAC inhibitors in mood disorder, as bipolar disorder (BD) [4, 5].

Weaver and colleagues [6] have reported that lack of maternal care altered the offspring epigenome at glucocorticoid receptor (GR) gene promoter in the hippocampus. In the same study, HDAC inhibitor administration in the offspring reverses the group differences in GR expression, histone acetylation, DNA methylation, and hypothalamic–pituitary–adrenal response to stress. Other study found that co-treated with SB and estradiol benzoate resulted in a significant decrease in immobility behavior in FST, a measure for depression-like behavior [7]. In a rat model of cocaine-induced conditioned place preference, SB treatment facilitates extinction of drug-seeking behavior [8]. In addition, chronic microinjection of VPA into the nucleus accumbens attenuates amphetamine-induced locomotor activity [9]. Finally, previous studies of our research group have demonstrated that intraperitoneal VPA administration reverse and prevent d-AMPH-induced manic-like behaviors [10–12].

BD is one of the most severe psychiatric disorders, which is associated with morbidity and mortality and psychiatric comorbidity [13, 14]. However, despite the severity of the disorder, its pathophysiology remains largely unknown. The main symptoms of BD are neurovegetative abnormalities, impulsivity, and psychosis, suggesting that anterior limbic brain networks controlling these behaviors are dysfunctional. It is well known that the amygdala modulates the limbic system, controlling an iterative circuit, prefrontal–striatal–thalamic, which control complex socio-emotional behaviors [15, 16].

Considering (1) the lack of knowledge about the BD pathophysiology and (2) the growing body of data of the therapeutic effects of HDAC inhibitors in mood disorders, the present study aims to evaluate the effects of microinjection of HDAC inhibitors in the ventricle, amygdala, striatum, prefrontal, and hippocampus on m-amphetamine-induced manic-like behavior in rats.

Experimental Methods

Animals

Adult male Wistar rats (250–300 g) were obtained from the Central Animal House of Universidade do Extremo Sul Catarinense. They were caged in groups of five with free access to food and water, and were maintained on a 12-h light–dark cycle (lights on at 7:00 am), at the temperature of 23°C±1°C. These conditions were maintained constant throughout the experiments.

All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of the Ethics Committee of Animals Use from Universidade do Extremo Sul Catarinense (Protocol 49/2009).

Surgical Procedure

Animals were intraperitoneally anesthetized with ketamine (80 mg/kg) and xylasine (10 mg/kg). In a stereotaxic apparatus, the skin of the rat skull was removed for placement of guide cannula (27 gauge 9 mm) aimed 1 mm above lateral ventricle, basolateral nuclear complex nuclear of the amygdala, ventral striatum/nucleus accumbens, medial prefrontal cortex, and CA1 hippocampus region, according to Paxinos and Watson [17].

In the ventricle, a single canulla was implanted, however, in the amygdala, striatum, prefrontal, and hippocampus the cannula were bilaterally implanted in the animals at coordinates: ventricle—posterior 0.9 mm, lateral 1.5 mm, and ventral 2.6 mm; amygdala—anterior 4.2 mm, lateral 3.0 mm, and ventral 1.3 mm; striatum—anteroposterior 1.6 mm, lateral 1.8 mm, and ventral 7.5 mm; prefrontal—3.2 mm anteroposterior, lateral 0.6 mm, and ventral 1.5 mm; and hippocampus—anteroposterior 4.2 mm, lateral 3 mm, and ventral 2 mm.

Drugs and Infusion Procedures

The drugs used in this study were sodium butyrate (SB—Sigma, St Louis, MO, USA), sodium valproate (VPA—Sanofi-Aventis Pharmaceutic, São Paulo, Brazil), and methamphetamine 1 mg/kg (m-AMPH—Sigma, St Louis, MO, USA). SB (10 mM) and VPA (300 μg) were dissolved in artificial cerebrospinal fluid (ACSF) and injected directly into the ventricle, amygdala, striatum, prefrontal, and hippocampus in a volume of 0.5 μl. m-AMPH (0.25 mg/kg) was dissolved in saline (NaCl 0.9%) and injected intraperitoneally (i.p.) in a volume of 1 mL/kg. The doses of SB and VPA were based on previous studies of Wang et al. [18] and Kim et al. [9], respectively. Controls groups received ACSF intracerebral and saline intraperitoneal.

At the time of infusion, an infusion cannula was fitted into the guide cannula and drugs or vehicle infusions were performed using a microsyringe attached to the cannulae by a polyethylene tube. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula and was aimed at the ventricle, amygdala, striatum, prefrontal, and hippocampus. The cannula were linked to a microsyringe by polyethylene tubing, and the infusions were carried out over 30 s first on one side, then on the other; the infusion cannula remained in place 15 s after the infusion, to minimize backflow.

Rats were given a single intracerebral (in the ventricle, amygdala, striatum, prefrontal, or hippocampus) injection of ACSF, SB, or VPA followed by an i.p. injection of saline or m-AMPH 2 h before the behavioral test, totaling six

experimental groups (ACSF+saline, SB+saline, VPA +saline, ACSF+m-AMPH, SB+m-AMPH, VPA+m-AMPH; n=12 per group).

Note Postmortem verification of cannula placements was performed as described in previous papers [19]. Brains were verified by histological examination, in 33% of animals in each group. Only behavioral data from these animals were analyzed. In all analyzed animals the cannula was correctly placed.

Locomotor Activity

Locomotor activity was assessed using the open-field task as previously described [10, 11]. This task was performed in a 40–60-cm open field surrounded by 50 cm high walls, made of brown plywood, with the floor divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear rectangle, and left free to explore the arena for 5 min. Crossings of the black lines (locomotor activity/horizontal activity) and rearings (exploratory activity/vertical activity) were counted.

Biochemical Analysis

After locomotor activity, rats were killed by decapitation and the brain transferred within 1 min to ice-cold isolation buffer (0.23 M mannitol, 0.07 M sucrose, 10 mM Tris–HCl, and 1 m MEDTA, pH 7.4). The amygdala, striatum, prefrontal, and hippocampus (n=8 animals per group) were dissected in ice-cold buffer in a Petri dish.

Nuclear Extraction

The tissue samples were submitted to a nuclear extraction protocol, according to a Nuclear Extraction kit (Chemicon, USA). Briefly, the tissues and lymphocytes were homogenized in cytoplasmic lysis buffer containing dithiothreitol (DTT) and protease inhibitors. The suspension was kept in ice for 15 min and was later centrifuged in 250×g for 5 min at 4°C. The supernatant was discarded and the pellet was resuspended in two volumes of cold cytoplasmic lysis buffer. The suspension was homogenized using a small gauge needle syringe and centrifuged in 8,000×g for 20 min at 4°C. The resulting pellet contained the nuclear portion of the cellular lysate. The pellet was resuspended in a nuclear extraction buffer containing DTT and protease inhibitors, and the suspension was homogenized with a small gauge needle syringe. The resulting sample was kept in slow agitation for 30–60 min in an orbital shaker at 4°C. Later, the nuclear suspension was centrifuged in 16,000×g for 5 min at 4°C and the nuclear extract-containing supernatant was transferred to a new tube and stored at −80°C until further analysis.

HDAC Activity

The nuclear extracts were submitted to a HDAC activity assay with the use of HDAC Assay kit (Fluorometric Detection), according to the manufacturer's instructions (Upstate, USA). Briefly, 5 μL of nuclear extracts were mixed with 5 μL of HDAC Assay Buffer and 5 μL of HDAC Assay Substrate in a 384-well plate and incubated at 30°C for 45 min. Concomitantly, a standard curve was performed with serial dilutions of deacetylated substrate and positive and negative controls were added to the plate. After, 10 μL of activator solution were added to the wells and the plate was incubated at room temperature for 15 min. The fluorescence reading was performed in a fluorescence plate reader, with 360 nm for excitation and 460 nm for emission. The calculation of the HDAC activity was performed based on the standard curve, and the values are presented as micromolars per microgram of protein. Total protein was measured by the modified Lowry's method [20] using bovine serum albumin as a standard.

Statistical Analysis

Statistical analyses were performed using SPSS 16.0 for Windows. Behavioral data (number of crossing and rearings) and HDAC activities were fitted in a standard distribution curve and were therefore subjected to parametric analyses. For the comparisons between the groups, one-way analysis of variance (ANOVA) test was performed, followed by the Tukey post-hoc test when ANOVA was significant. p Values <0.05 two-tailed were considered statistically significant.

Results

Firstly, in the present study we showed that administration of m-AMPH increased crossings and rearings in ACSF-treated rats in all experimental procedures, however, m-AMPH does not alter the activity of HDACs in any of the brain structures evaluated.

As shown in Fig. 1a, intracerebroventricular (ICV) administration of VPA and SB blocks m-AMPH-induced increased crossing and rearing. In Fig. 1b, we observed that SB and VPA ICV administration in saline and m-AMPH groups inhibited the HDAC activity in the prefrontal and striatum. In hippocampus, the ICV administration of VPA and SB in the saline groups inhibited HDAC activity; and administration of SB, but not VPA, in m-AMPH-administered rats also decreased the activity of this enzyme. In amygdala there were no significant results.

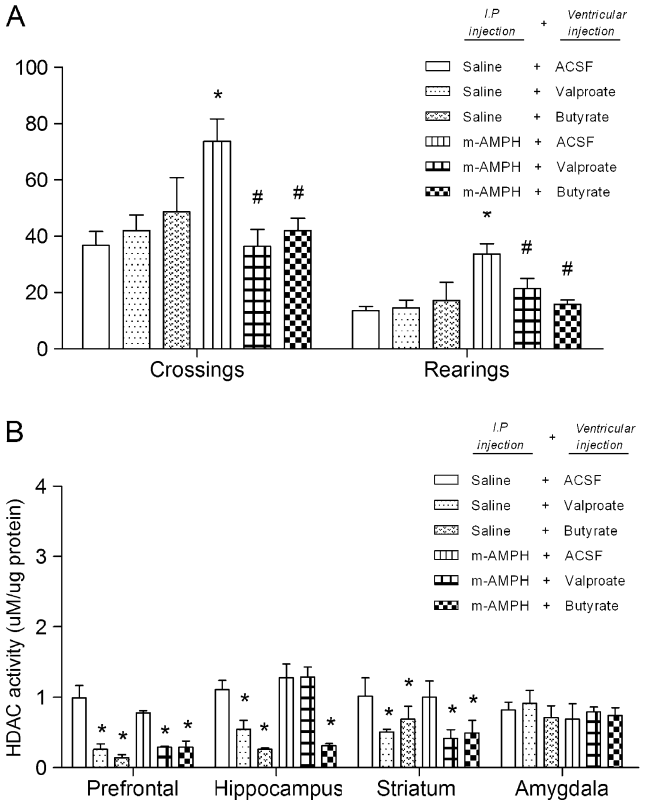

Fig. 1 Microinjection of SB, VPA, or ACSF in the ventricle followed by an i.p. injection of saline or m-AMPH. Open-field task (**a**), $n=12$ animals per group, intracerebroventricular administration of VPA and SB blocks m-AMPH-induced increased crossing and rearing. HDAC activity (**b**), $n=8$ animals per group, HDAC activity varied according with the drug administered and brain structure analyzed. *Asterisk* different from saline+ACSF group, $p<0.001$. *Number sign* different from m-AMPH+ACSF, $p<0.001$

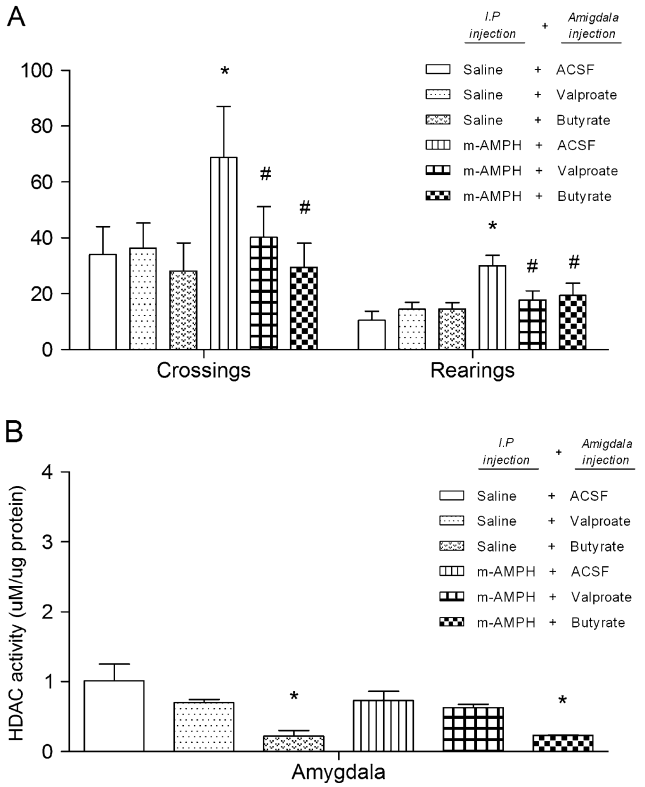

Fig. 2 Microinjection of SB, VPA, or ACSF in the amygdala, followed by an i.p. injection of saline or m-AMPH. Open-field task (**a**), $n=12$ animals per group, microinjection of SB and VPA in the amygdala blocked the hyperactivity induced by m-AMPH. HDAC activity (**b**), $n=8$ animals per group, the microinjection of SB in the amygdala in rat saline and m-AMPH treated decreased the HDAC activity. *Asterisk* different from saline+ACSF group, $p<0.001$. *Number sign* different from m-AMPH+ACSF, $p<0.001$

In Fig. 2a, the microinjection of SB and VPA in the amygdala blocked the hyperactivity induced by m-AMPH. In the same procedure, the microinjection of SB in the amygdala in rat saline and m-AMPH treated decreased the HDAC activity in the amygdala (Fig. 2b). However, microinjection of VPA in the amygdala did not alter the activity of HDAC in this brain structure.

The Fig. 3a shows that microinjection of SB and VPA in the striatum blocked the m-AMPH-induced hyperactivity. In addition, striatal microinjection of SB and VPA in the saline and m-AMPH groups decreased HDAC activity in the striatum (Fig. 3b).

According to Fig. 4a, microinjection of SB and VPA in the prefrontal also blocked the hyperactivity induced by m-AMPH. The SB and VPA microinjection in the prefrontal in saline and m-AMPH groups inhibit the HDAC activity in this brain structure (Fig. 4b).

As shown in Fig. 5a, microinjection of SB and VPA did not alter the hyperactivity induced by m-AMPH. However, the hippocampal microinjection of SB and VPA inhibited the HDAC activity in hippocampus of rat saline and m-AMPH treated.

Discussion

It has been reported in the literature that epigenetics mediates diverse environmental aspects involved in the pathophysiology of major psychotic disorders [21]. Recently, it was demonstrated that in major depressive disorder patients the expression of HDAC2 and -5 mRNA was increased in a depressive state, and in BD, the expression of HDAC4 mRNA was increased also in a depressive state, suggesting that changes in transcriptional regulation caused by the altered expression of HDACs is associated with the pathophysiology of mood disorders [22]. In an analysis of the National Brain Databank microarray collection, was observed that HDAC1 expression is increased in the prefrontal cortex of schizophrenia subjects [23].

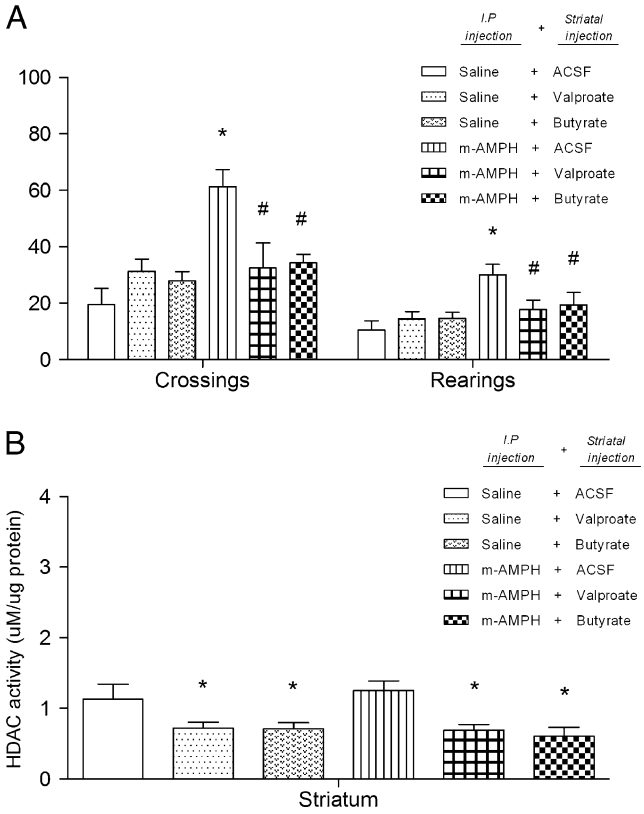

Fig. 3 Microinjection of SB, VPA, or ACSF in the striatum, followed by an i.p. injection of saline or m-AMPH. Open-field task (**a**), $n=12$ animals per group, microinjection of SB and VPA in the striatum blocked the m-AMPH-induced hyperactivity. HDAC activity (**b**), $n=8$ animals per group, striatal microinjection of SB and VPA in the saline and m-AMPH groups decreased HDAC activity. *Astersik* different from saline+ACSF group, $p<0.001$. *Number sign* different from m-AMPH +ACSF, $p<0.001$

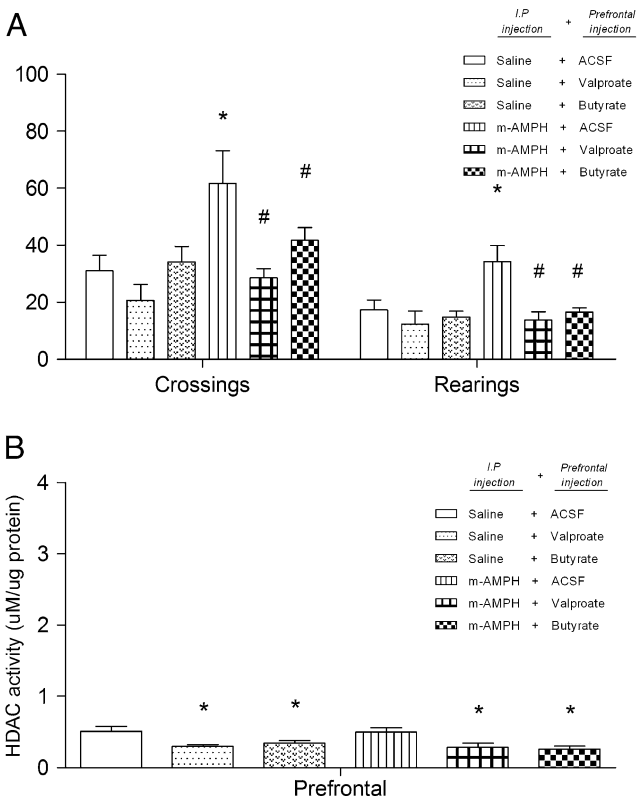

Fig. 4 Microinjection of SB, VPA, or ACSF in the prefrontal, followed by an i.p. injection of saline or m-AMPH. Open-field task (**a**), $n=12$ animals per group, microinjection of SB and VPA in the prefrontal also blocked the hyperactivity induced by m-AMPH. HDAC activity (**b**), $n=8$ animals per group. The SB and VPA microinjection in the prefrontal in saline and m-AMPH groups inhibit the HDAC activity. *Asterisk* different from saline+ACSF group, $p<0.001$. *Number sign* different from m-AMPH+ACSF, $p<0.001$

In the present study, our data demonstrated that microinjection of SB and VPA in the ventricle, amygdala, striatum, and prefrontal block the d-AMPH-induced hyperlocomotion. Nevertheless, the microinjection of HDAC inhibitors in the hippocampus did not alter the d-AMPH-induced manic-like effects. Taken together, our results indicate that the antimanic effects of HDAC inhibitors are related to the amygdala, striatum, and prefrontal, but not the hippocampus.

The mechanisms of antimanic effects of HDAC inhibitors are still unknown. However, a previous study has demonstrated that trichostatin A (TSA)—a HDAC inhibitor—completely blocked the cocaine-induced behavioral sensitization [24]. Wu and colleagues [25] showed that HDA inhibitors up-regulate astrocyte glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor gene transcription, protecting dopaminergic neurons. Moreover, pretreatment with VPA, SB, or TSA caused a robust decrease in lipopolysaccharide-induced pro-inflammatory responses and protected DA neurons from damage in mesencephalic neuron-glia cultures [26]. In addition, administration of phenylbutyrate significantly attenuated MPTP-induced depletion of striatal dopamine and loss of tyrosine hydroxylase-positive neurons in the substantia nigra; suggesting that administration of phenylbutyrate may be a useful approach for the treatment of neurodegenerative diseases [27]. In a positron emission tomography study, VPA has been shown to decrease [18F]-dopa uptake constants in the striatum of patients with mania, suggesting that VPA treatment decreases aromatic amino acid decarboxylase activity, which should decrease the rate of dopamine synthesis [28].

However, no concrete studies exist that prove that the BD or the administration of AMPH change HDAC; nevertheless, studies show that administration of HDAC inhibitors improves both, manic symptoms in humans and

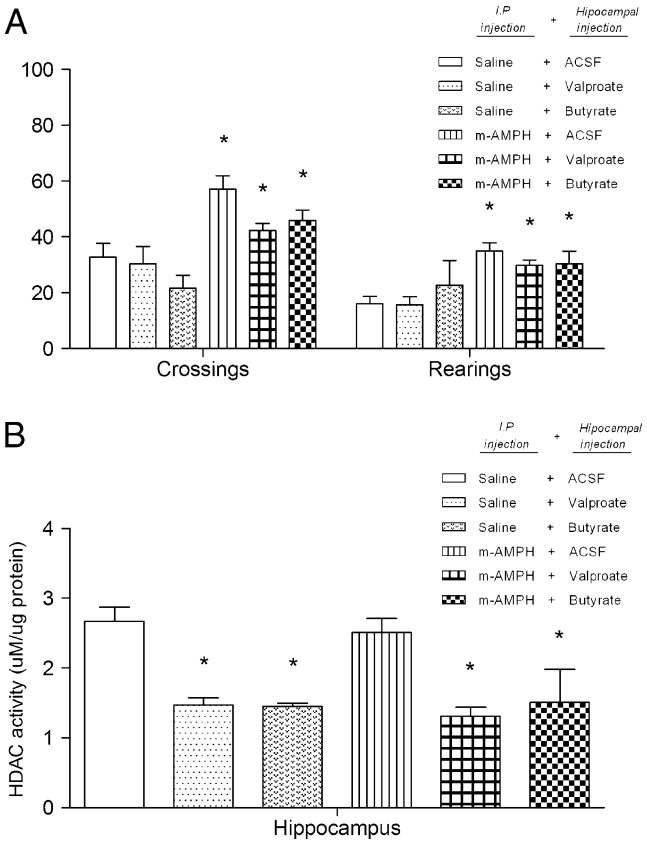

Fig. 5 Microinjection of SB, VPA, or ACSF in the hippocampus, followed by an i.p. injection of saline or m-AMPH. Open-field task (**a**), $n=12$ animals per group, microinjection of SB and VPA did not alter the hyperactivity induced by m-AMPH. HDAC activity (**b**), $n=8$ animals per group, the hippocampal microinjection of SB and VPA inhibited the HDAC activity. *Asterisk* different from saline+ACSF group, $p<0.001$. *Number sign* different from m-AMPH+ACSF, $p<0.001$

manic-like symptoms induced by psychostimulants in rats [10, 23]. In addition, we suggest that a pathway underlying, yet unknown, HDAC inhibitors may have antimanic effects.

It has been suggested that anterior limbic, which controls impulsivity and psychosis, is dysfunctional in BD. It is well known that the amygdala modulates the limbic system, controlling an iterative circuit, prefrontal–striatal–thalamic, which control complex socioemotional behaviors [15, 16].

Recently, a functional magnetic resonance imaging (fMRI) study have suggested that stable amygdala over-activation across prolonged vigils may interfere with sustained attention and exacerbate attentional deficits in bipolar patients. In the same study, differential striatal and thalamic deactivation in BD is interpreted as a loss of amygdala (emotional brain) modulation by the ventrolateral prefrontal–subcortical circuit, which interferes with attentional maintenance [29]. In another fMRI study, bipolar manic patient exhibit blunted brain response to emotional cues throughout the ventrolateral prefrontal emotional arousal network; this emotional network alteration may contribute to the mood dysregulation of bipolar disorder [30]. Narita and colleagues [31] have also demonstrated that the BD patients showed gray matter volume reduction in the prefrontal cortex. Moreover, subjects with BD exhibit reduced gray matter volume in the dorsolateral prefrontal cortex, left nucleus accumbens, and left amygdala, but not in the hippocampus [32]. Despite the deficits in attention and memory in bipolar patients, most of the structural brain imaging studies did not find changes in hippocampal volume in BD [33].

Here we suggested that the decrease in HDAC activity can be related to the antimanic effects of HDAC inhibitors. However, the change in the activity of HDACs—induced by VPA and SB—does not prove the existence of differences in chromatin remodeling, because there are other epigenetic mechanisms that can alter the DNA conformation as: methylation/demethylation and ubiquinatination/deubiquinatination (see [34]). In addition, in the amygdala VPA did not inhibit the enzyme activity and still continued to have antimanic effect. This can be explained by the fact that VPA has other molecular targets. VPA has been found to enhance GABA activity within the brain by inhibiting its degradation, stimulating its synthesis and release, and directly enhancing its postsynaptic effects [35]. VPA also acts on the protein kinase C—as inhibitor—decreasing the release of neurotransmitters [36]. Another important fact to be reviewed is that when SB and VPA were administered in the ventricle, HDAC activity was not inhibited in the amygdala. Nevertheless, HDAC inhibitors continued to have antimanic effect. This may be by fact that in other brain structures HDAC activity was inhibited, thus compensating the lack of effect in the amygdala.

The ICV administration of VPA and SB blocks m-AMPH-induced increased crossing and rearing, meantime administration of SB and VPA ICV did not alter the HDAC activity in the amygdala—unlike other brain regions. This can be explained partly because regions of the brain may respond differently [37], and here we evaluated HDAC activity of different brain regions that in part correspond to different cell types. Moreover, within a homogeneous population of cells, there is heterogeneity in terms of physiological and metabolic characteristics [38–40].

In summary, our results suggest that the antimanic effects of SB and VPA, HDAC inhibitors, are related to the amygdala, striatum, and prefrontal, but not the hippocampus. More studies are needed to clarify the therapeutic effects of the HDAC inhibitor in BD and thereby develop new drugs.

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