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ORIGINAL ARTICLE

Vorinostat (SAHA) May Exert Its Antidepressant-Like Effects Through the Modulation of Oxidative Stress Pathways

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Abstract

Suberoylanilide hydroxamic acid (SAHA/Vorinostat), a potent inhibitor of histone deacetylases (HDACs), is known to possess antidepressant properties. However, the exact mechanisms underlying this activity are unknown. In this study, we evaluated the effect of SAHA on the expression of GluN2A, GluN2B (NMDA receptor subunits), (p-)AMPK, and ΔFos proteins which are an integral part of the signal transduction pathways in the brain and also involved in the pathophysiology of depression as well as the mechanism of antidepressant action. We also measured the concentration of malondialdehyde (MDA - a product of lipid peroxidation). The study was carried out in the prefrontal cortex (PFC) and hippocampus (Hp), brain regions implicated in depression. Although SAHA induced changes in the expression of all the proteins and MDA concentration, the effects differed depending on the drug dose, time, and brain structure involved. SAHA reduced MDA concentration and significantly increased p-AMPK protein expression, indicating it may prevent oxidative stress. SAHA also increased the levels of HDAC3 and NMDA subunits (GluN2A and GluN2B), implying it is neuroprotective and may play a crucial role in synaptic plasticity. Moreover, ΔFosB and FosB levels were significantly elevated, suggesting that SAHA may modulate learning and memory processes. Overall, the data indicate that the Hp might play a pivotal role in the mechanism of action of SAHA, hinting at novel mechanisms it play in the antidepressant and neuroprotective effects of SAHA.

Keywords SAHA · Vorinostat · HDAC inhibitor · Cellular mechanisms

Introduction

A growing body of evidence suggests that epigenetics may be crucial in the pathophysiology of depressive disorders (Misztak et al. 2018; Chmielewska et al. 2019; Talarowska 2020). Epigenetic mechanisms regulate chromatin structure and function and mediate changes in gene expression. Histone acetylation, controlled reversibly by histone acetyltransferases (HATs) and histone deacetylases (HDACs), enhances gene transcription (Bartova et al. 2008). In mammals, two families of HADCs have been identified: the zinc (Zn^{2+})-dependent [classes: I (HDAC 1; 2; 3; 8), II a (HDAC 4; 5; 7; 9), II b (HDAC6; 10), and IV (HDAC 11)] and the nicotinamide adenine dinucleotide (NAD^{+})-dependent [HDAC class III (SIRT 1–7)]. HDAC classes I–III are widely expressed in the brain (Graff and Tsai 2013).

Numerous studies have shown that some HDAC I and II inhibitors reduce the neurological symptoms of neuropsychiatric diseases by increasing acetylation of histones (especially H3 and H4). On the other hand, reduced histone acetylation levels have been documented in several stress-induced models of depression (Covington et al. 2009; Fuchikami et al. 2009). Social defeat stress resulted in a significant decrease in H3ac and H2Bac levels in high responder rats but an increase in H3ac in low responder rats. Acetylation of histone H4 decreased following defeat with no individual variation (Hollis et al. 2011). Furthermore, Tsankova et al. (2006) found that acetylation of H3, HDAC 4, and 5 are disrupted following chronic social defeat in mice. More importantly, sodium butyrate, which inhibits most HDACs, except class IIb and

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III, reversed anxiety behavior in the Light-Dark Box test and increased the acetylation of H3 in male and female mice. When administered in combination with fluoxetine, sodium butyrate enhanced the therapeutic effect of the former (Schroeder et al. 2007). Another compound from the HDAC inhibitors (HDACi) group – valproate (VPA) has been extensively studied in animal models of depression. When microinjected into the ventrolateral orbital cortex, it induced antidepressant-like effects in the forced swim test (FST) in rats (Zhao et al. 2013). Moreover, in rats subjected to chronic unpredictable stress (CUS), VPA increased BDNF level and reduced corticosterone concentration (Qiu et al. 2014). More specifically, HDACi-MS-275, which strongly inhibits HDAC1 and HDAC 3, enhanced the antidepressant activity of fluoxetine, similar to sodium butyrate (Covington et al. 2011; Resende et al. 2013). Convington et al. (2009) also showed that MS-275 decreased HDAC2 expression in the nucleus accumbens and simultaneously increased H3 acetylation, level of CREB, co-REST, and up-regulated some genes (*slc17a*, *nrn1*, *rab3b*, *TNFRSF1A*, *sin3b*). Interestingly, these effects were similar to those observed after the administration of fluoxetine or SAHA (Covington et al. 2009).

SAHA is a class I and II HDACi. Recent studies have indicated that SAHA exhibits antidepressant activity, attenuates depression-like symptoms, and restores molecular abnormalities in animal models of depression. Treatment with SAHA was found to reduce depression-like behavior and normalize epigenetic changes in the Hp during ethanol withdrawal (Chen et al. 2019). Unlike conventional antidepressants, the chronic systemic administration of SAHA partially reversed the depression-like behavior of *Crtc1*$^{-/-}$ mice (animals lacking CREB-regulated transcription co-activator 1; CRTC1), as shown by the decreased immobility time in the FST and increased acetylation of histone H3 or H4 in the Hp and PFC, respectively (Meylan et al. 2016). What is more, chronic administration of SAHA was found to increase the acetylation of H3K9 (acetylation of H3 on lysine 9) and H4K12 (acetylation of H4 on lysine 12) in the cortex, ventral striatum, and Hp, with enhanced synaptic function and plasticity (Schroeder et al. 2013).

The exact mechanism of the antidepressant-like activity of SAHA is unknown. However, some data indicate that its action might be related to the modulation of oxidative stress and inflammatory pathways (Kv et al. 2018) and the modulation of glutamatergic transmission (Fujita et al. 2012). Based on the preceding data, our goal was to evaluate further the contribution of SAHA's antioxidative properties in its antidepressant-like activity. We measured the concentration of malondialdehyde (MDA), an end product of lipid peroxidation, and a sensor of cellular injury due to the oxidative stress process in cells and tissues. The concentration of MDA was measured as thiobarbituric acid reactive substances—TBARS.

In addition, we measured the expression of several proteins involved in the mechanisms of the pathophysiology and treatment of depression, such as the GluN2A and GluN2B subunits of NMDAR, AMPK, and FosB, as well as the level of HDCA3 protein. Brain regions studied included the prefrontal cortex (PFC) and hippocampus (Hp), two regions for which structural and functional abnormalities have been observed in patients suffering from major depression (Drevets et al. 2008).

Materials and Methods

Animals

Six-week-old male C57BL/6J mice (24–28 g) were purchased from the animal facility of the Maj Institute of Pharmacology, PAS (Krakow, Poland). The animals were kept in groups of ten in standard plastic cages with food and water freely available, under controlled conditions (12-h light/dark cycle, room temperature of 22 ± 2 °C, and humidity 55 ± 10%). Mice were randomly assigned to the experimental groups.

All of the procedures used in this study were in accordance with the ethical standards laid down by the respective Polish and EU regulations (Directive No. 86/609/EEC) and were approved by the 2nd Local Ethical Committee at the Maj Institute of Pharmacology, PAS. All the experimental procedures were carried out with a minimization of animal suffering using as few animals as possible.

Drug Administration

SAHA (suberoylanilide hydroxamic acid/Vorinostat; Sequoia Research Products Ltd., UK) was dissolved in 10% aqueous DMSO solution and immediately administered intraperitoneally (i.p.) to mice at a dose of 25 or 50 mg/kg. Doses were selected based on previous studies investigating the antidepressant effect of SAHA *in vivo* (Fujita et al. 2012; Meylan et al. 2016; Kv et al. 2018). Drug administration was either acute (single injection) or chronic (14 days, once daily). The control groups received a 10% aqueous DMSO solution in a volume of 10 ml/kg (Table 1). Mice were divided into appropriate groups ($n = 5$).

Tissues' Preparation

At the end of the experimental period, mice were sacrificed by decapitation, and brain structures (PFC, Hp) collected, rapidly frozen, and stored at − 80 °C for further studies. The PFC was taken by cutting the anterior part of the forebrain at the level of bregma 1.54 mm (Paxinos and Franklin 2001). For all biochemical analyses, frozen brain structures

Table 1 Characteristics of experimental groups: doses and period of SAHA administration/time of tissue isolation

	Doses of SAHA	
	25 mg/kg	50 mg/kg
	Number of mice per group	
Time after acute injection		
30 min	5	5
60 min	5	5
90 min	5	5
120 min	5	5
Time after chronic injection		
24 h	5	-
Control		
Control groups received 10% aqueous DMSO solution in a volume of 10 ml/kg		

(PFC, Hp) were homogenized in a 2% aqueous SDS solution on wet ice. Then, total protein concentration was measured using the Pierce BCA protein assay kit (Thermo Fisher Scientific, USA), following which homogenates were portioned and used in specific assays as described afterwards**.**

Western Blot Analysis

Western blot analysis was conducted as described previously (Pochwat et al. 2018). Fifty micrograms of homogenate (from each tissue sample) were mixed with a 2% aqueous SDS solution and sample buffer (2× Tris-Glycine Sample Buffer, Invitrogen, USA), and denatured for 10 min at 95 °C. Samples were then centrifuged for 5 min at 10,000 rpm at 4 °C and proteins fractionated on polyacrylamide gel (SDS-PAGE), and proteins were transferred to nitrocellulose membrane (0.45-µm pore; Bio-Rad, USA). To block non-specific signals, membranes were incubated in 1% blocking solution (BM Chemiluminescence Western Blotting Kit Mouse/Rabbit Roche, Germany). After blocking, membranes were incubated overnight at 4 °C with the following primary antibodies: polyclonal anti-NMDAR2A rabbit antibody (Abcam, cat. ab14596; 1:1000), polyclonal anti-NMDAR2B rabbit antibody (Abcam, cat. ab65783; 1:1000), monoclonal anti-HDAC3 mouse IgG2a antibody (Cell Signaling, cat. 7G6C5; 1:1000), monoclonal anti-Delta FosB rabbit IgG antibody (Cell Signaling, cat. D3S8R; 1:1000), polyclonal anti-p-AMPKα1/2 (Thr172) rabbit antibody (Santa Cruz Biotechnology, cat.sc-33524; 1:200), and polyclonal anti-AMPKα1/2 (H-300) rabbit antibody (Santa Cruz Biotechnology, cat. sc-25792; 1:200). Next, membranes were washed 3 times in TBST (Tris-buffered saline with Tween) for 10 min, followed by incubation with the appropriate secondary antibody anti-mouse IgG-peroxidase-conjugated or anti-rabbit IgG-peroxidase-conjugated antibodies (from BM Chemiluminescence Western Blotting Kit Mouse/Rabbit Roche,Germany; 1:7000) for 1 h at room temperature. Membranes were then washed 3 times (5 min. each) in TBST and incubated in detection reagent (BM Chemiluminescence Western Blotting Kit Mouse/Rabbit Roche, Germany). The proteins present on the membranes were visualized using a Fuji-Las 1000 system, and concentration measured using a Fuji Image Gauge v.4.0 software. To check for transfer and loading, β-actin (detected with mouse monoclonal anti-β-actin antibody; Sigma Aldrich, Germany; 1:10,000) was detected on each blot. Final results are presented as the ratio of a particular protein's optical density to the optical density of β-actin present in the same sample. Data on the graph are expressed as % of change vs. control.

Measurement of the Malondialdehyde Concentration

Malondialdehyde (MDA) is an end product of lipid peroxidation and a sensor of cellular injury due to the oxidative stress process in cells and tissues. The concentration of MDA in Hp and PFC tissues was measured using a TBARS assay kit (Cayman Chemical, USA; cat.10009055) and calculated according to the manufacturer's protocol. TBARS is one of the oldest and most widely used assays for measuring MDA, a reactive aldehyde produced by lipid peroxidation of polyunsaturated fatty acids. MDA forms (under high temperature 90–100 °C) an adduct with two thiobarbituric acid molecules to produce species that can be measured fluorometrically at an excitation wavelength of 530 nm and an emission wavelength of 550 nm. The final results were normalized to the total protein concentration in the sample.

Statistical Analysis

The data are presented as means ± SEM. Statistical analysis of the data was performed using GraphPad PRISM 7.0a. One-way ANOVA, followed by Newman-Keuls post hoc comparison test, were used for the analysis. A $p < 0.05$ was considered statistically significant.

Results

Effect of SAHA (25 mg/kg) on p-AMPK Protein Level in the PFC and Hp

Acute (25 mg/kg, 50 mg/kg) and chronic (25 mg/kg) administration of SAHA did not affect total AMPK protein levels in the PFC and Hp (Fig. 1b, d, f, g). Instead, increased level of p-AMPK protein was observed after chronic treatment (25 mg/kg) and 90 and 120 min after acute (25 mg/kg)

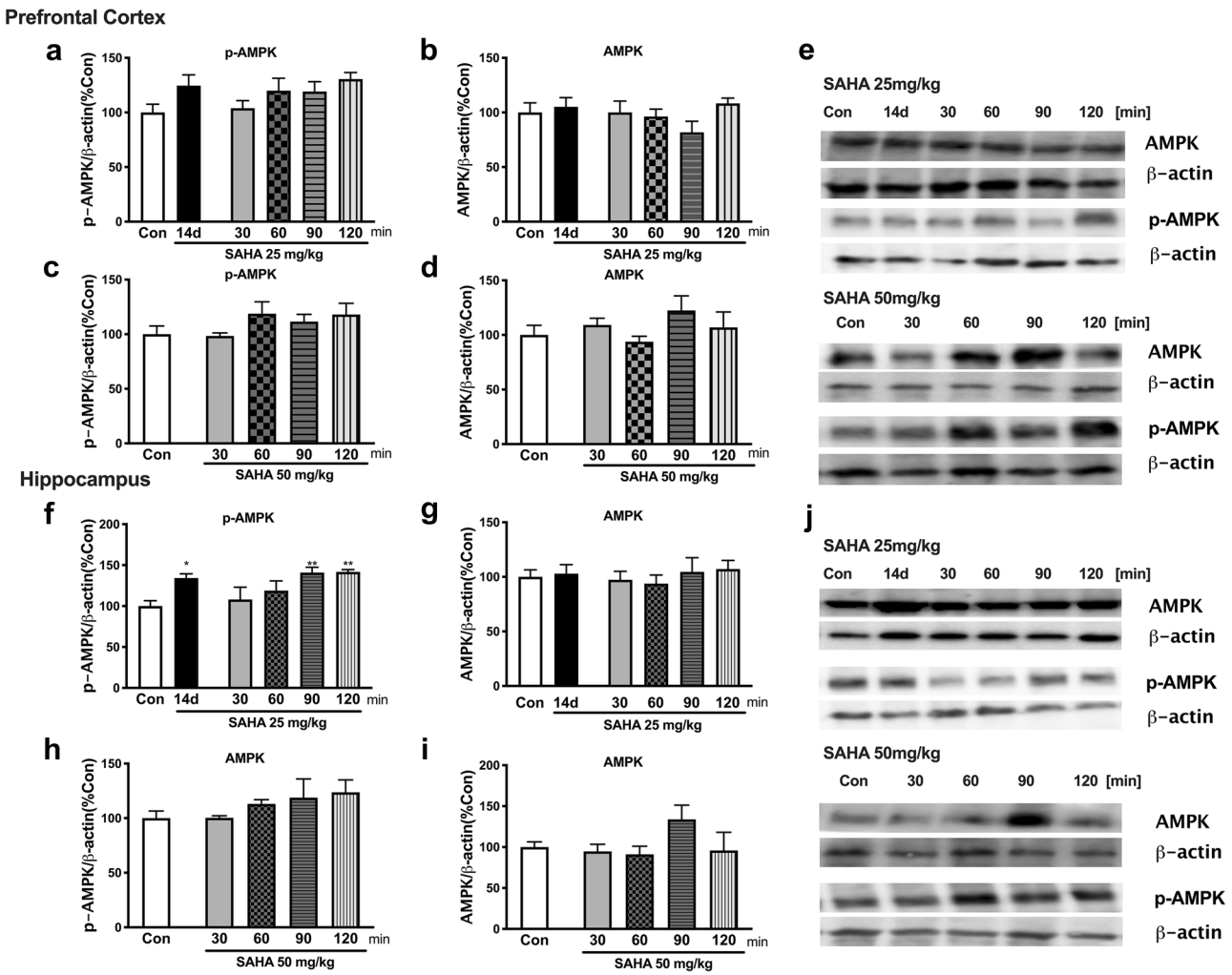

Fig. 1 AMPK and p-AMPKα1/2 (Thr 172) protein levels (bar chart) in the prefrontal cortex and hippocampus with corresponding Western blot representations: **a/f** p-AMPKα1/2 (Thr 172) protein level after SAHA (25 mg/kg i.p.) administration; **b/g** AMPK protein level after 25 mg/kg i.p. SAHA administration; **c/h** p-AMPKα1/2(Thr 172) protein level after 50 mg/kg i.p. SAHA administration; **d/i** AMPK protein level after 50 mg/kg i.p. **e/j** Western blot representation for analyzed proteins (**e/j**). Con -control; 14d–14 days of SAHA administration; 30 -SAHA 30 min; 60 -SAHA 60 min; 90 -SAHA 90 min; 120 -SAHA 120 min. All data were analyzed by one-way ANOVA and Newman-Keuls multiple comparisons test. Values are expressed as mean ± S.E.M % of controls; $*p < 0.05$; $**p < 0.001$; vs control

treatment in the Hp of mice (($F(3,240) = 4.899$; $p < 0.05$; $F(3,614) = 4.899$; $p < 0.001$; $F(3,678) = 4.899$; $p < 0.001$; respectively (Fig. 1f)). No change in p-AMPK protein level was observed in the PFC (Fig. 1a,c).

Effect of SAHA on FosB and ΔFosB Protein Levels in the PFC and Hp

Both acute (60, 90, and 120 min) and chronic treatments with SAHA (25 mg/kg) increased the level of ΔFosB ($F(3,362) = 8.737$; $p < 0.05$; $F(3,516) = 8.737$; $p < 0.001$; $F(5,290) = 8.737$; $p < 0.0001$; $F(5,115) = 8.737$; $p < 0.0001$, respectively) in the PFC of mice (Fig. 2a). Chronic ($F(3,978) = 7.901$; $p < 0.001$) and acute administration at a dose of 25 mg/kg for 60, 90, and 120 min ($F(3,336) = 7.901$; $p < 0,05$; $F(5,488) = 7.901$; $p < 0.0001$; $F(4,236) = 7.901$; $p < 0.001$, respectively) significantly increased ΔFosB protein level in the Hp (Fig. 2f).

In the PFC, SAHA (50 mg/kg) increased ΔFosB protein level only after 30 and 60 min of treatment ($F(3,287) = 6.779$; $p < 0,05$; $F(4,681) = 6.779$; $p < 0,0001$, respectively) (Fig. 2c). After 120 min, ΔFosB protein level returned to basal level (Fig. 2c). Acute treatment (50 mg/kg) also increased ΔFosB protein level in the Hp after 60, 90, and 120 min ($F(3,675) = 5.543$; $p < 0.001$; $F(3,307) = 5.543$; $p < 0.05$; $F(3,382) = 5.543$; $p < 0.05$; respectively) but this increase was not noticeable after 30 min (Fig. 2f).

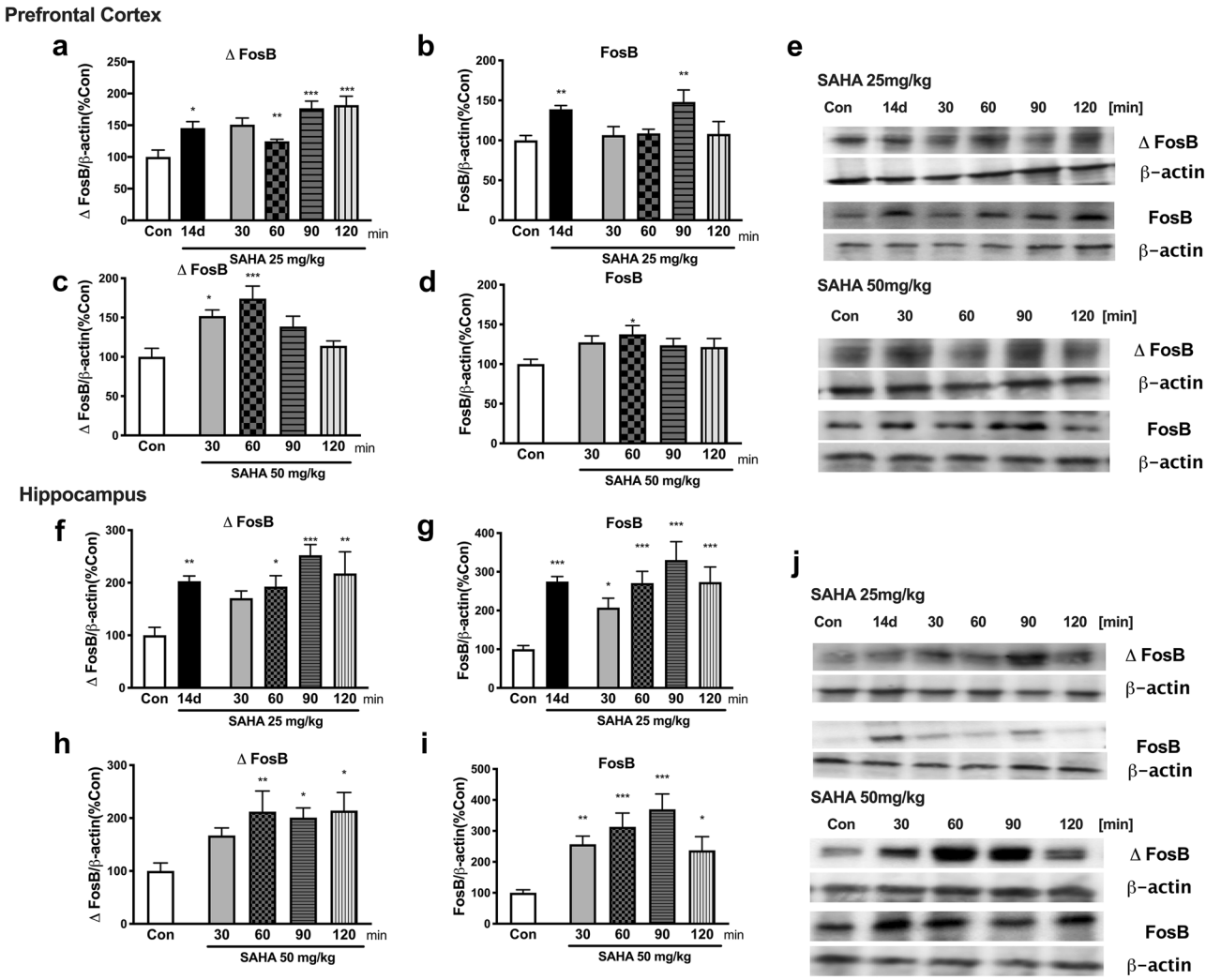

Fig. 2 FosB and ΔFosB protein levels in the prefrontal cortex and hippocampus with corresponding Western blot representations: **a/f** ΔFosB protein level after 25 mg/kg i.p. SAHA administration; **b/g** FosB protein level after 25 mg/kg i.p. SAHA administration **c/h**) ΔFosB protein level after 50 mg/kg i.p. SAHA administration; **d/i** FOSB protein level after 50 mg/kg i.p. SAHA administration; Western Blot representation for analyzed proteins (**e/j**). Con -control; 14d–14 days of SAHA administration; 30 -SAHA 30 min; 60 -SAHA 60 min; 90 -SAHA 90 min; 120 -SAHA 120 min. All data were analyzed by one-way ANOVA and Newman-Keuls multiple comparisons test. Values are expressed as mean ± S.E.M % of controls; *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$ vs control

FosB protein level was significantly elevated after chronic administration of SAHA (25 mg/kg) ($F(3,519) = 5.048$; $p < 0.001$) and acute (25 mg/kg) administration after 90 min ($F(4,041) = 5.048$; $p < 0.001$) in the PFC. At 50 mg/kg, FosB protein level was significantly elevated after 60 min in the PFC ($F(3,295) = 3.361$; $p < 0.05$) (Fig. 2b, d). In the Hp, SAHA (25 mg/kg) (chronic = $F(5,467) = 12.780$; $p < 0.0001$) and acute (30 min = $F(3,126) = 12.780$; $p < 0.05$; 60 min = $F(4,978) = 12.780$; $p < 0.0001$; 90 min = $F(6,712) = 12.780$; $p < 0.0001$; 120 min = $F(5,048) = 12.780$; $p < 0.0001$) significantly increased FosB protein level (Fig. 2f). Similarly, in the PFC, acute treatment (50 mg/kg) significantly increased FosB protein level after 60 min (Fig. 2d), while in the Hp acute treatment (50 mg/kg) increased FosB protein level after 30, 60, 90, and 120 min (30 min = $F(3,701) = 12.750$; $p < 0.001$; 60 min = $F(5,035) = 12.750$; $p < 0.0001$; 90 min = $F(6,364) = 12.750$; $p < 0.0001$; 120 min = $F(3,237) = 12.750$; $p < 0.05$) (Fig. 2i).

Effect of SAHA on GluN2A and GluN2B Protein Levels in the Hp and PFC

SAHA (25 and 50 mg/kg) did not alter GluN2A and GluN2B protein levels in the PFC (Fig. 3a–d). In the Hp, GluN2A protein level was significantly increased after 30 min of acute treatment of SAHA at the dose of 25 mg/kg ($F(4,049) = 3.545$; $p < 0.001$) and after 90 and 120 min of

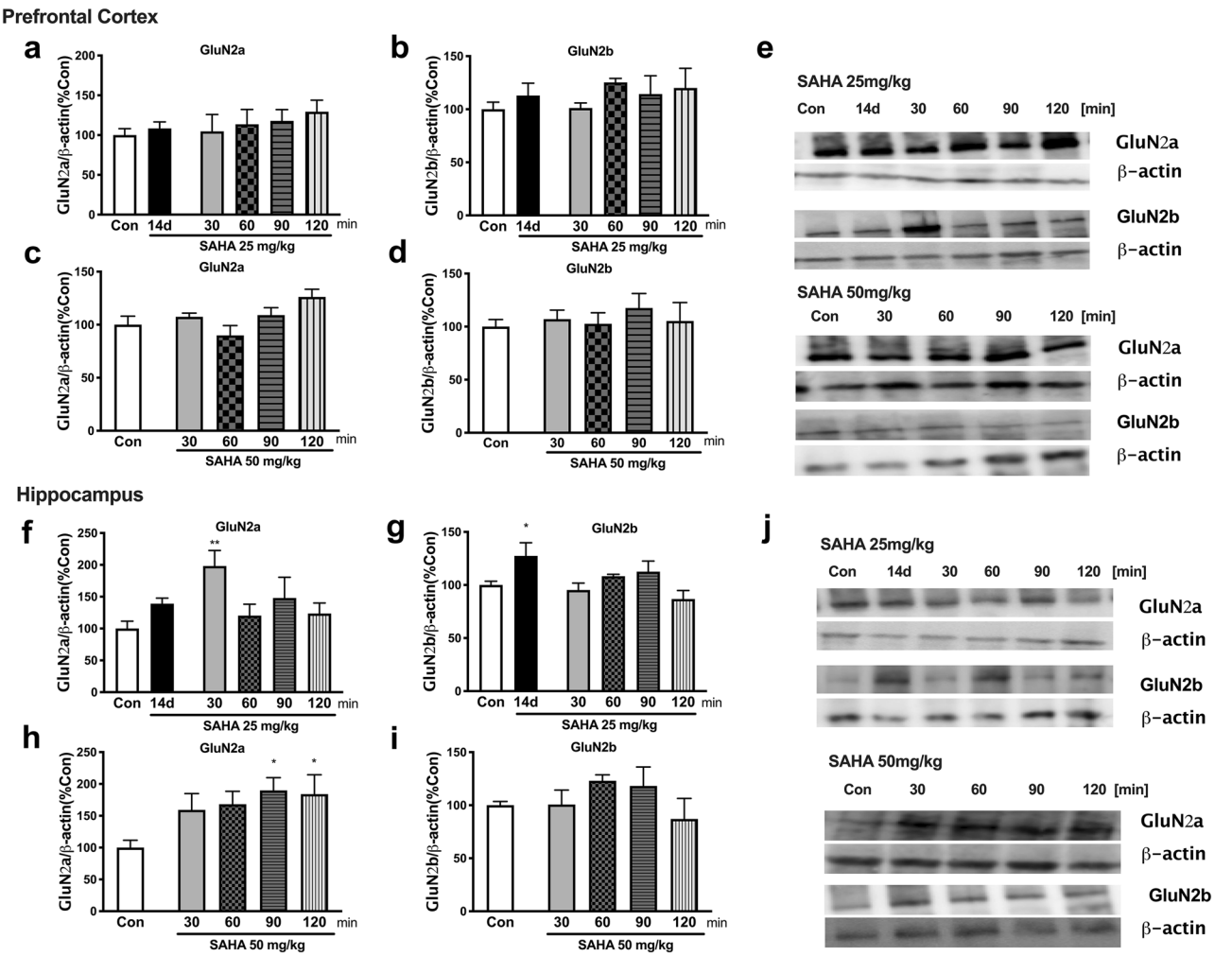

Fig. 3 GluN2a and GluN2b protein levels in the prefrontal cortex and hippocampus with corresponding Western blot representations: **a/f** GluN2a protein level after 25 mg/kg i.p. SAHA administration; **b/g** GluN2b protein level after 25 mg/kg i.p. SAHA administration; **c/h** GluN2a protein level after 50 mg/kg i.p. SAHA administration; **d/i** GluN2b protein level after 50 mg/kg i.p. SAHA administration. Western Blot representation for analyzed proteins (**e/j**). Con - control; 14d–14 days of SAHA administration; 30 -SAHA 30 min; 60 -SAHA 60 min; 90 -SAHA 90 min; 120 -SAHA 120 min. All data were analyzed by one-way ANOVA and Newman-Keuls multiple comparisons test. Values are expressed as mean ± S.E.M % of controls; $^{*}p < 0.05$; $^{**}p < 0.001$ vs control

SAHA treatment at the dose of 50 mg/kg ($F(3,360) = 4.276$; $p < 0,05$; $F(3,138) = 4.276$; $p < 0.05$, respectively) (Fig. 3f, h). GluN2B protein level was only altered by chronic treatment (25 mg/kg) ($F(2,918) = 3.444$; $p < 0.05$) in the Hp (Fig. 3g).

Effect of SAHA on HDAC3 Protein Levels in the Hp and PFC

Chronic administration of SAHA (25 mg/kg) increased HDAC3 protein level in the PFC ($F(4,403) = 4.795$; $p < 0.001$) (Fig. 4a). Increases in HDAC3 protein levels were also seen in the PFC after acute administration (30 and 120 min) ($F(3,000) = 4.795$; $p < 0.05$; $F(2,945) = 4.795$; $p < 0.05$, respectively) (Fig. 4a). At a dose 50 mg/kg, SAHA significantly increased HDAC3 protein level after 60, 90, and 120 min ($F(4,800) = 7.256$; $p < 0.0001$; $F(3,591) = 7.256$; $p < 0.001$; $F(3,518) = 7.256$; $p < 0.001$, respectively) (Fig. 4b) in the PFC. In the Hp, increased HDAC3 protein level was also observed after chronic (25 mg/kg) ($F(6,008) = 7.586$; $p < 0.0001$) (Fig. 4d) and acute (50 mg/kg) administration of SAHA after 90 and 120 min ($F(5,977) = 11.020$; $p < 0.0001$; $F(4,558) = 11.020$; $p < 0.0001$, respectively) (Fig. 4e).

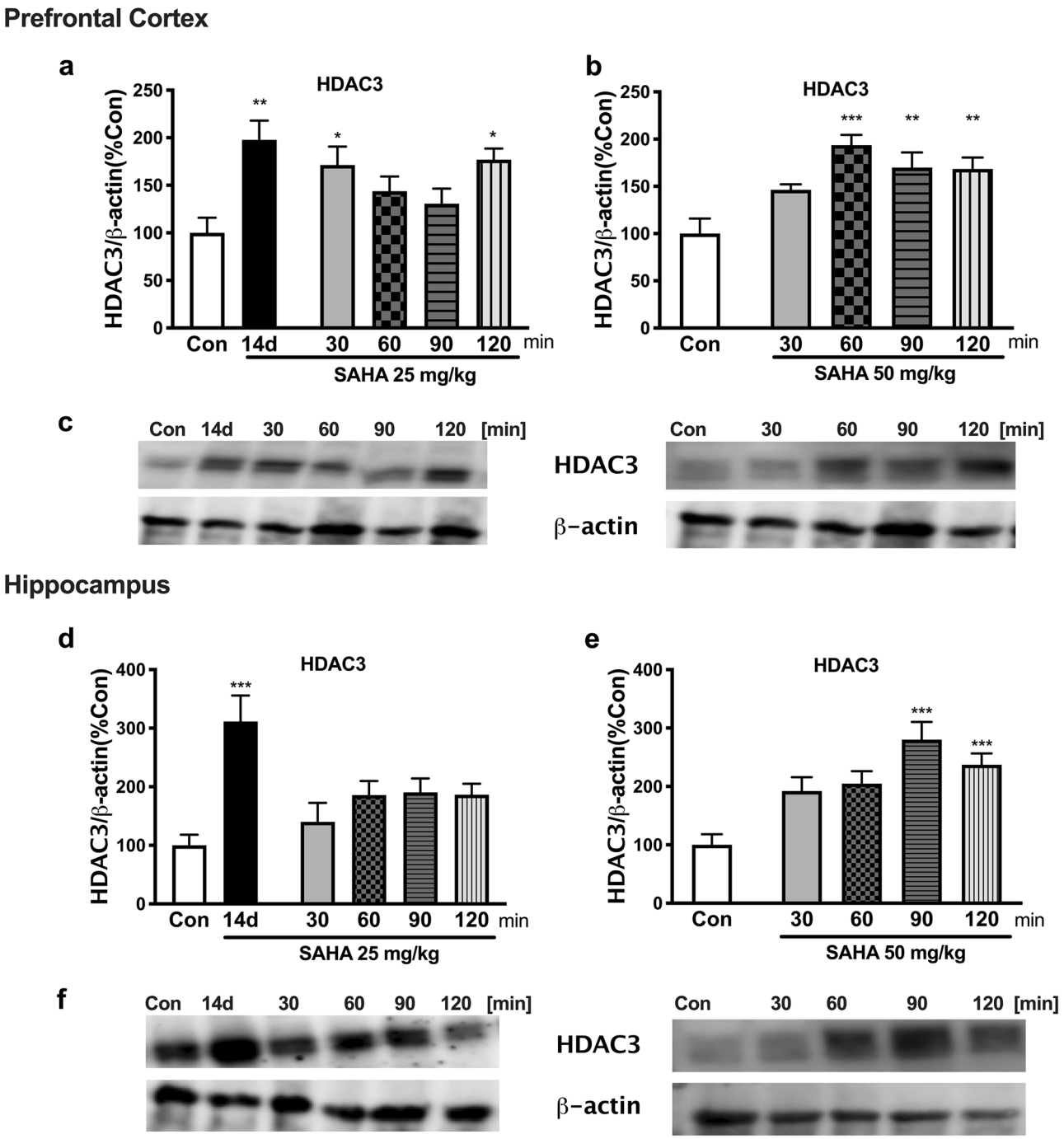

Fig. 4 HDAC3 protein levels in the prefrontal cortex and hippocampus with corresponding Western blot representations: **a** HDAC3 protein level after 25 mg/kg i.p. SAHA administration in the prefrontal cortex; **b** HDAC3 protein level 50 mg/kg i.p. SAHA administration in the prefrontal cortex; **d** HDAC3 protein level after 25 mg/kg i.p. SAHA administration in the hippocampus; **e** HDAC3 protein level 50 mg/kg i.p. SAHA administration in the hippocampus. Western blot representation for analyzed proteins (**c/f**). Con -control; 14d–14 days of SAHA administration; 30 -SAHA 30 min; 60 -SAHA 60 min; 90 -SAHA 90 min; 120 -SAHA 120 min All data were analyzed by one-way ANOVA and Newman-Keuls multiple comparisons test. Values are expressed as mean ± S.E.M % of controls; $*p < 0.05$; $**p < 0.001$; $***p < 0.0001$ vs control

Effect of SAHA on MDA (TBARS) Concentration in the Hp and PFC

Acute administration of SAHA (25 mg/kg) significantly reduced MDA concentration after 90 and 120 min in the PFC ($F(4,269) = 5.928$; $p < 0.001$; $F(4,183) = 5.928$; $p < 0.001$). A similar effect was seen after chronic administration ($F(3,287) = 5.928$; $p < 0.05$) (Fig. 5a). Acute administration of 50 mg/kg reduced MDA concentration after 60 and 120 min ($F(2,893) = 3.559$; $p < 0.05$; $F(2,908) = 3.559$; $p < 0.05$, respectively) in the PFC (Fig. 5b). In the Hp, acute administration of SAHA (25 mg/kg) significantly reduced MDA concentration only after 120 min ($F(2,763) = 2.367$; $p < 0.05$) (Fig. 5c). However, the 50 mg/kg dose decreased MDA concentration after 90 ($F(2,937) = 6.577$; $p < 0.05$) (Fig. 5d).

Discussion

SAHA has been shown to possess pro-oxidative stress properties (Samuni et al. 2014; Leone et al. 2015; Zhou et al. 2019). In addition to the induction of oxidative stress and production of reactive oxygen species (ROS), which might be useful in cancer therapy, SAHA also possesses protective properties against oxidative stress and cell damage (Petruccelli et al. 2011; Singh et al. 2011; Masadeh et al. 2017; You and Park 2014; Wang et al. 2015; Zhao et al. 2015). Oxidative stress defined as an imbalance between antioxidants and pro-oxidants processes in favor of the latter, resulting in the excessive production of free radicals (ROS). Free radicals at low physiological concentrations may function as signaling molecules, play important roles

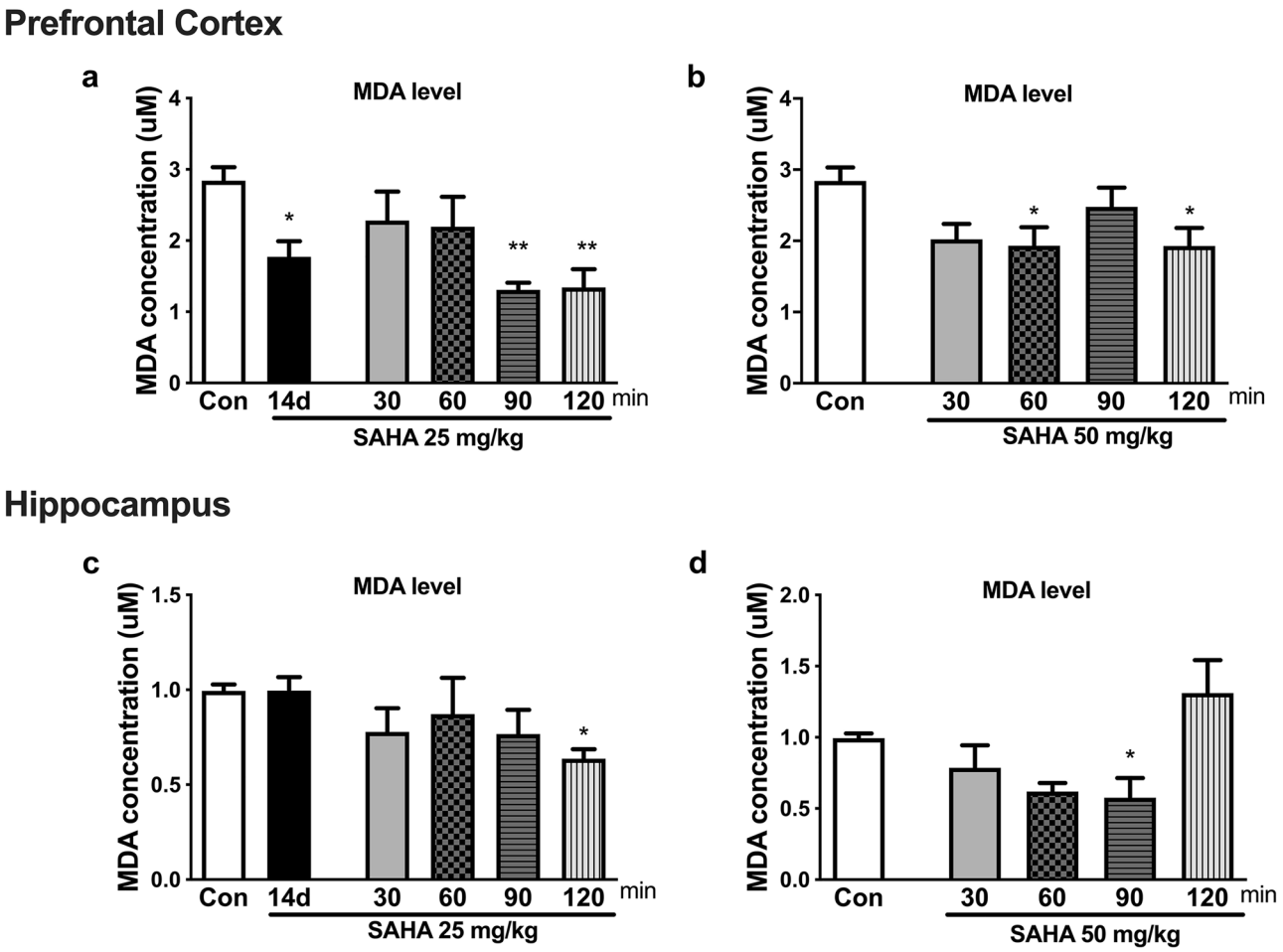

Fig. 5 MDA concentration in the prefrontal cortex and hippocampus: **a** MDA concentration after 25 mg/kg i.p. SAHA administration in the prefrontal cortex; **b** MDA concentration after 50 mg/kg i.p. SAHA administration in the prefrontal cortex; **c** MDA concentration after 25 mg/kg i.p. SAHA administration in the hippocampus; **d** MDA concentration after 50 mg/kg i.p. SAHA administration in the hippocampus. Con -control; 14d–14 days of SAHA administration; 30 -SAHA 30 min; 60 -SAHA 60 min; 90 -SAHA 90 min; 120 -SAHA 120 min. All data were analyzed by one-way ANOVA and Newman-Keuls multiple comparisons test. Values are expressed as mean ± S.E.M; *$p < 0.05$; **$p < 0.001$ vs control

in the immunological response and regulate various cell activities (e.g., mitosis). At elevated concentrations, free radicals may damage cell components, including proteins (enzymes, receptors), lipids, and DNA, which may eventually lead to apoptosis and cell death (Sies 1991; Halliwell 2011). Accordingly, oxidative stress may play a destructive role in the nervous system leading to the development of mental disorders like schizophrenia or depression or improving cancer therapy (Hybertson et al. 2011; Siwek et al. 2013; Menezo et al. 2016).

MDA is a well-established marker of oxidative damage of lipids in many human diseases (Grotto et al. 2009). According to a meta-analysis conducted by Jiménez-Fernández et al., the concentration of TBARS was increased in mania and depressed patients indicating that TBARS may be a state marker of bipolar disorder (Jimenez-Fernandez et al. 2020). Another meta-analysis confirmed that elevated MDA levels are characteristic for bipolar and depressed patients (Maes et al. 2019). Similar findings were revealed by Lv et al., who showed that MDA level was elevated in bipolar disorder but decreased after lithium treatment (Lv et al. 2019). Interestingly, antidepressant treatment also reduced MDA levels (Maes et al. 2019); increased levels of MDA and other oxidative stress enzymes have also been documented in animal models of depression (Chen et al. 2007; Tao et al. 2016; Abd El-Fattah et al. 2018). In our study, the chronic treatment of mice with SAHA decreased MDA concentration in the PFC. Although the acute administration of SAHA reduced MDA concentration in the Hp and PFC, the effect in the PFC was more pronounced, suggesting that the protective activity of SAHA against lipid peroxidation might be brain region-dependent. Incidentally, it took about 90 to 120 min for reductions in MDA concentration, indicating that a more extended period is required to develop SAHA's protective effects.

AMP-activated protein kinase (AMPK) is an enzyme that plays a crutial role in cellular energy metabolism (Hinchy et al. 2018). Decreased phosphorylation and inactivation of AMPK has been shown to be associated with depression-like behaviors in rats and mice exposed to chronic stress (Zhu et al. 2014)**.** AMPK can be activated by metabolic stress, including hypoxia, glucose deprivation, and oxidative stress (McCullough et al. 2005; Zhu et al. 2014). SAHA likely controls mitochondrial metabolism through the upregulation of p-AMPK with a concomitant decrease in ROS level, thereby averting the destructive effects of ROS and the activation of the apoptosis pathway (Okubo et al. 2020).

Our data indicated that p-APMK is only upregulated in the Hp and not in the PFC. However, because of the upward trend observed in the PFC, longer treatment times may be required to observe any significant upregulation of p-AMPK in the PFC. Nevertheless, these results confirm that the antidepressant effect of SAHA may be associated with its protective action and the reduction of oxidative stress observed in the course of depression (Kv et al. 2018).

The NMDA receptor is involved in learning and memory processes, as well as excitotoxicity. NMDARs contain mostly two GluN1 and GluN2 subunits (four types 2A–D). In the forebrain, subunits GluN2A and GluN2B are more prevalent than the other subunits (Hardingham 2019). The GluN2B subunit is mainly associated with LTP and LTD procession, learning, and memory storage, while GluN2A may be crucial for short-term spatial memory deficits (Shipton and Paulsen 2014). Fujita et al. (2012) showed that the administration of SAHA (i.p.) increased NR2B (GluN2B) protein and gene expression in the Hp of rats after 4hrs. In this study, rats were subjected to fear conditioning training for 2 days, followed by the 50 mg/kg SAHA administration on the 1st and 2nd days—24 h before the main test (Fujita et al. 2012). Compared with the study by Fujita et al. (2012), mice were injected with 25 or 50 mg/kg SAHA in our study. Treatment effect was evaluated at shorter time intervals (30 min, 60 min, 90 min, and 120 min) after acute treatment. We also subjected mice to chronic (14 days) SAHA treatment. Both low and high doses of SAHA increased the levels of GluN2A in the Hp. However upregulation was evident at the low dose after 30 min, while the high dose effect was observed 90 and 120 min after treatment. On the other hand, the GluN2B subunit protein level in the Hp only increased after chronic treatment. While the Fujita study showed the effect of SAHA in the fear conditioning test and the subsequent effect on GluN2B protein and gene expression, ours showed the effect of SAHA on the expression of GluN2B in naive mice. Our study, therefore, is complementary to that of Fujita et al. (2012) and indicates that the mechanism of action of SAHA may be dependent on the manner of administration (acute vs. chronic), animal species (rats vs. mice), and naive animals vs. animals subjected to a contextual fear conditioning (FC) paradigm.

The involvement of the NMDA receptor in learning and memory processes and excitotoxicity supports the evidence that SAHA can also improve learning and memory processes (Yang et al. 2013; Lin et al. 2014). The increased level of GluN2A after SAHA treatment may induce the influx of calcium and increase synaptic plasticity. The equally high expression of GluN2B after chronic SAHA treatment may cause an increased influx of calcium ions, which may influence the learning process. This data is reflective of p-AMPK levels. The increased expression of p-AMPK protein requires a decrease in ATP production or mitochondrial ROS generation. Also, the activation of CaMKKβ/influx of calcium ions upregulates the expression of p-AMPK (Mungai et al. 2011). The increased expression of the GluN2A and GluN2B subunits seen in our study may be related to the increased level of p-AMPK and the influx of calcium ions/CaMKKβ pathway activation, which enhances synaptic plasticity.

Table 2 Changes in the tested proteins and MDA/TBARS levels in the PFC after SAHA administration

	Treatment								
	SAHA (25 mg/kg)					SAHA (50 mg/kg)			
	Chronic	Acute				Acute			
	Time of isolation								
	24 h	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
AMPK	↔	↔	↔	↔	↔	↔	↔	↔	↔
p-AMPK	↔	↔	↔	↔	↔	↔	↔	↔	↔
GluN2A	↔	↔	↔	↔	↔	↔	↔	↔	↔
GluN2B	↔	↔	↔	↔	↔	↔	↔	↔	↔
FosB	↑	↔	-↔	↑	↔	↔	↑	↔	↔
ΔFosB	↑	↔	↑	↑	↑	↑	↑	↔	↔
HDAC3	↑	↑	↔	↔	↑	↔	↑	↑	↑
TBARS (MDA)	↓	↔	↔	↓	↓	↔	↓	↔	↓

↑ Increase, ↓ Decrease, ↔ No change

Chronic and acute treatment with SAHA at low and high doses induce elevated levels of ΔFosB in the Hp and PFC. ΔFosB, a spliced variant of the FosB gene, is expressed for several weeks or months compared with FosB and plays a role in the drug addiction process, Parkinson's disease, and mental disorders like depression and maybe a molecular switch for long-term adaptations in the nervous system (McCullough et al. 2005). Our data shows that SAHA caused region, time, and dose-dependent increases of ΔFosB protein. The level of FosB was also increased in the Hp and PFC. The increased levels of ΔFosB and FosB, especially in the Hp, may suggest that SAHA is involved in cell proliferation and differentiation, consistent with the expression of p-AMPK and GluN2A.

HDAC3 plays a significant role in modulating cell growth, apoptosis, and inflammatory processes, including the down-regulation of p53 function, amongst others (Guo et al. 2019). Inhibition of HDAC3 following cerebral ischemia/reperfusion injury of mice reduced the cerebral infarct volume and apoptosis, impaired oxidative stress, and enhanced autophagy (Demyanenko et al. 2020). HDAC3 downregulates p53 synthesis, and thus modulates apoptosis and cell growth, and acts as a repressor of transcription in the JAK/STAT pathway. On the other hand, HDAC3 is necessary for TNF-activity (Zhu et al. 2010). It inhibits p65 activity related to several biological processes like inflammation, apoptosis, cell growth, immunity, and many others (Zhu et al. 2010). Results from our study would indicate the repression of some genes necessary for oxidative stress or immune activity as a result of the increased levels of HDAC3 following treatment with SAHA.

Table 3 Changes in the tested proteins and MDA/TBARS levels in the Hp after SAHA administration

	Treatment								
	SAHA (25 mg/kg)					SAHA (50 mg/kg)			
	Chronic	Acute				Acute			
	Time of isolation								
	24 h	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
AMPK	↔	↔	↔	↔	↔	↔	↔	↔	↔
p-AMPK	↑	↔	↔	↑	↑	↔	↔	↔	↔
GluN2A	↔	↑	↔	↔	↔	↔	↔	↑	↑
GluN2B	↑	↔	↔	↔	↔	↔	↔	↔	↔
FosB	↑	↑	↑	↑	↑	↑	↑	↑	↑
ΔFosB	↑	↔	↑	↑	↑	↔	↑	↑	↑
HDAC3	↑	↔	↔	↔	↔	↑	↑	↑	↑
TBARS(MDA)	↔	↔	↔	↔	↓	↔	↔	↓	↔

↑ Increase, ↓ Decrease, ↔ No change

Conclusion

Several proteins involved in neuroprotective processes and synaptic plasticity were evaluated as possible targets for SAHA activity. Both acute and chronic SAHA treatment altered the levels of p-AMPK, GluN2A, and GluN2B (NMDA receptor subunits) or FosB proteins, and also MDA concentration. The alterations were predominantly in the Hp. Furthermore, a higher dose of SAHA (50 mg/kg) was more effective. Our data indicate that SAHA might exert its neuroprotective effects and prevent oxidative stress through the reduction of lipid peroxidation and increasing the levels of p-AMPK and HDAC3 proteins. SAHA also increased GluN2A and GluN2B protein levels, suggesting a crucial role for SAHA in synaptic plasticity. SAHA increased ΔFosB and FosB protein levels and may modulate learning and memory processes and synaptic plasticity, which are perturbed in depression (Tables 2 and 3).

Author Contributions PM designed and carried out all the experiments, analyzed the data, and wrote the first version of the manuscript; BS and GN prepared the last version of the manuscript; and MS-K performed the experiments, worked on the latest version of the manuscript.

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Compliance with Ethical Standard

Conflict of Interest The authors declare that they have no conflicts of interest.

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