Journal of Molecular Neuroscience (2018) 65:301–311
https://doi.org/10.1007/s12031-018-1101-7

In Vivo and In Vitro Neuronal Plasticity Modulation by Epigenetic Regulators

Melisa C. Monteleone[1] · María Eugenia Pallarés[2] · Silvia C. Billi[1] · Marta C. Antonelli[2] · Marcela A. Brocco[1]

Received: 9 February 2018 / Accepted: 12 June 2018 / Published online: 22 June 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Prenatal stress (PS) induces molecular changes that alter neural connectivity, increasing the risk for neuropsychiatric disorders. Here we analyzed —in the hippocampus of adult rats exposed to PS— the epigenetic signature mediating the PS-induced neuroplasticity changes. Furthermore, using cultured hippocampal neurons, we investigated the effects on neuroplasticity of an epigenetic modulator. PS induced significant modifications in the mRNA levels of stress-related transcription factor MEF2A, SUV39H1 histone methyltransferase, and TET1 hydroxylase, indicating that PS modifies gene expression through chromatin remodeling. In *in vitro* analysis, histone acetylation inhibition with apicidin increased filopodium density, suggesting that the external regulation of acetylation levels might modulate neuronal morphology. These results offer a way to enhance neural connectivity that could be considered to revert PS effects.

Keywords Rat · MEF2A · Hydroxymethylation · Histone deacetylase inhibitors · Primary hippocampal neurons · GPM6A

Abbreviations

5-hmC	5-Hydroxymethylcytosine
acH4	Acetylated histone 4
DIV	Days in vitro
GPM6A	Glycoprotein of membrane 6A
HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitor
PS	Prenatal stress
TET	Ten-eleven translocation protein

Introduction

In a severe stressful situation, the levels of circulating glucocorticoids rise. If such a stressful situation occurs during pregnancy, glucocorticoids pass through the placenta and affect fetus normal development. During prenatal stress (PS), glucocorticoids reach the brain and modify gene expression triggering cellular, physiological, and behavioral changes (Van den Hove et al. 2006; Baier et al. 2012). In particular, hippocampal neurons from offspring of stressed mothers are negatively affected by PS (Mychasiuk et al. 2012) showing reduced neurogenesis (Lemaire et al. 2000), decreased dendritic tree

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12031-018-1101-7) contains supplementary material, which is available to authorized users.

✉ Marcela A. Brocco
mbrocco@iib.unsam.edu.ar

Melisa C. Monteleone
mmonteleone@iibintech.com.ar

María Eugenia Pallarés
mpallares@fmed.uba.ar

Silvia C. Billi
sbilli@iibintech.com.ar

Marta C. Antonelli
mca@fmed.uba.ar

[1] Instituto de Investigaciones Biotecnológicas - Instituto Tecnológico de Chascomús (IIB-INTECH), Universidad Nacional de San Martín, Consejo Nacional de Investigaciones Científicas y Técnicas (UNSAM-CONICET), Av. 25 de Mayo y Francia, CP: 1650, San Martín, Buenos Aires, Argentina

[2] Instituto de Biología Celular y Neurociencias "Prof. Eduardo De Robertis," Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, 3°Piso, C1121ABG Buenos Aires, Argentina

size (Hosseini-Sharifabad and Hadinedoushan 2007), and diminished synaptic density (Hayashi et al. 1998). In addition, there is a change in the expression of many genes linked to neuronal plasticity such as neurotrophic factors, neurotransmitter receptors, and synaptic proteins (Fumagalli et al. 2004, 2005; Van den Hove et al. 2006; Revest et al. 2010; Baier et al. 2012; Pallarés et al. 2013b; Monteleone et al. 2014; Adrover et al. 2015). Retrospective studies on children born from stressed mothers show as attention-deficit hyperactivity disorder (ADHD) and sleep disturbance during infancy (Beydoun and Saftlas 2008). For that reason, PS represents one of the main risk factors for affective disorders (Van den Bergh et al. 2005; Glover 2011; Baier et al. 2012).

In response to environmental stimuli, stress-induced genetic and epigenetic changes steer neuronal remodeling, a type of neuronal plasticity. Plasticity involves changes in gene expression and in the neuron's structure, function, and organization that underlie many key brain processes. The stress-induced structural alterations result in functional disconnections that may underlie the neuropsychiatric disease pathophysiology but might also include clues for treatment. Neural connectivity is modulated by plasticity-related genes whose expression is controlled by different transcription factors. Among them, the serum response factor (SRF) and the Yin Yang 1 (YY1) factor have been related to chronic stress. SRF promotes resilience to chronic social stress (Vialou et al. 2010) and YY1 participates in anxiety rescue mechanisms (Sotnikov et al. 2014). In addition, the myocyte enhancer factor 2A (MEF2A) is related to plasticity-related gene expression (Flavell et al. 2008). Moreover, some of the stress-induced molecular changes rely on the interaction between the glucocorticoid receptor (GR) and the MEF2A (Speksnijder et al. 2012). Despite this, none of these factors have been previously studied in the PS context.

Growing evidence indicates that stress-induced persistent effects are mediated by epigenetic factors. However, the precise molecules and responsible mechanisms remain undiscovered. Changes in DNA methylation status, histone modifications, and non-coding regulatory RNAs have been observed in response to prenatal stress. Moreover, current trends propose the epigenetic machinery as a molecule source to diagnose and treat stress-related disorders. Among the epigenetic modifications of chromatin, we gave attention to DNA demethylation because it permits a dynamic methylome regulation. 5-Methylcytosine is oxidized into 5-hydroxymethylcytosine (5-hmC) by the TET (ten-eleven translocation) dioxygenases (Tahiliani et al. 2009). The TET protein family consists of three members, TET1, TET2, and TET3, which catalyze a similar reaction (Ito et al. 2010). Most studies on TET function in the nervous system focused on TET1 and they showed its involvement in memory consolidation (Zhang et al. 2013; Kaas et al. 2013), cognition, and neurogenesis (Rudenko and Tsai 2014). Notably, 5-hmC is enriched in brain where it accounts for approximately 40% of modified cytosines in neurons (Kriaucionis and Heintz 2009). Moreover, 5-hmC content increases in the brain with postnatal age and in response to neuronal activity (Ficz et al. 2011; Szulwach et al. 2011; Guo et al. 2012; Hahn et al. 2013). The 5-hmC discovery, considered the "sixth base," revolutionized the epigenetic regulation and it has been proposed as a biosensor (Dao et al. 2014). However, little is known about 5-hmC, the TET enzymes, and their connection with PS or with affective disorders.

Changes in gene expression induced by stress occur also through histone post-translational modification (e.g., acetylation, deacetylation, and methylation). There are many enzymes mediating these reactions. In this work, we focused on particular histone-modifying enzymes (SUV39H1, the histone methyltransferase suppressor of variegation 39H1, and the histone deacetylases HDAC2 and HDAC3) as they were reported to both influence cognitive function and modulate stress-associated behavioral adaptations (Suri et al. 2014). SUV39H1 inhibition increases synaptic density in the hippocampus, improving memory and learning (Snigdha et al. 2016). Acetylation occurs at histone lysine residues through histone acetyl-transferases (HATs). HAT activity is antagonized by histone deacetylases (HDACs). Histone acetylation typically associates with a transcriptionally active state of chromatin (Shahbazian and Grunstein 2007). The balance between HAT and HDAC activities is tightly regulated since it controls chromatin dynamic state and transcription factor's accessibility to gene promoters (Shahbazian and Grunstein 2007). According to their homology with yeast enzymes, HDACs are grouped in several classes (Shahbazian and Grunstein 2007). Class I includes HDAC2 and HDAC3. HDAC2 negatively affects neuronal plasticity (Guan et al. 2009) and is reduced in chronic social defeat stressed mice and in depressed patients (Covington et al. 2009). HDAC3 deletion or inhibition enhances long-term memory formation (McQuown et al. 2011). Each HDAC class can be inhibited by a heterogeneous group of agents that hinder the HDAC function (Khan et al. 2008) and cause histone hyperacetylation, thereby changing gene expression. Particularly, class I HDAC inhibition has neurophysiological consequences, including reversal of contextual memory deficits in a mouse model of Alzheimer's disease (Kilgore et al. 2010). Interestingly, class I HDAC inhibitors exhibit antidepressant-like activity (Covington et al. 2009). Therefore, targeting HDACs with selective inhibitors may provide a novel approach for treating mood disorders.

Previous work of our group showed that chronic stress alters the expression of several genes in the hippocampus (Alfonso et al. 2004, 2006). Among those genes is *gpm6a* that codifies the transmembrane protein GPM6A expressed mainly in the neuronal surface. GPM6A participates in neurite outgrowth, filopodium formation, and synaptogenesis (Alfonso et al. 2005; Fuchsova et al. 2009; Brocco et al.

2010; Scorticati et al. 2011; Formoso et al. 2016). In humans, altered hippocampal *GPM6A* mRNA levels have been reported in postmortem brain of depressed suicides (Fuchsova et al. 2015). Furthermore, polymorphisms in the *GPM6A* gene sequence have been associated with pathological conditions such as schizophrenia (Boks et al. 2008), bipolar disorders (Greenwood et al. 2012), and claustrophobia (El-Kordi et al. 2013). In addition, increased *GPM6A* mRNA levels resulting from the de novo duplication of the *GPM6A* gene have been reported in a patient with learning disability and behavioral anomalies (Gregor et al. 2014). Later, using a prenatal stress model in rats, we found, in the brain of stressed adult offspring, altered mRNA levels of different neural plasticity-related genes (Pallarés et al. 2013a, b; Monteleone et al. 2014; Adrover et al. 2015; Baier et al. 2015) including *gpm6a* (Monteleone et al. 2014). In addition, we demonstrated that changes in *gpm6a* expression are mediated by changes in the methylation status of certain cytosines within the *gpm6a* gene and through the microRNA-133b (Monteleone et al. 2014). Altogether, these findings point out that the gene *gpm6a* responds to environmental cues (e.g., stress) and suggest the protein GPM6A might participate in the neuronal plasticity changes elicited during stress response.

Overall, the goal of this study was to broaden the current knowledge about PS effects on the epigenetic machinery, which ultimately affect the expression of neuroplasticity genes. We also analyzed in an in vitro approach the impact on neuronal plasticity of an external modification to the epigenetic machinery. Thus, we investigated the effect of apicidin, a class I HDAC inhibitor (HDACi), on neuronal morphology in primary hippocampal neurons in culture. Moreover, we analyzed apicidin effect on the stress-modulated and plasticity-related neuronal glycoprotein GPM6A.

Materials and Methods

Experimental Design for Prenatal Stress

Pregnant Wistar dams were randomly assigned to either the control or the PS group. Control rats ($n = 6$) were left undisturbed in the home cage, while PS dams ($n = 6$) were subjected to a restraint stress procedure on the last week of gestation. No other subjects were present in the experimental room during the stress exposure. Prenatal stress protocol was in accordance with the guidelines laid down by the Committee for the Care and Use of Animals for Experimentation (CICUAI-University of Buenos Aires no. 121/2013). On postnatal day 60 (PND 60, adult), animals were euthanized by decapitation. The hippocampus was surgically removed and immediately homogenized in TRIzol® Reagent (Life Technologies, Rockville, NY, USA).

RNA Isolation and cDNA Synthesis

Total RNA was isolated with DirectZol RNA Miniprep (Zymo Research, Irvine, CA, USA) following manufacturer's instructions. Complementary DNA was synthesized by retrotranscription using oligo-dT and SuperScript® II Reverse Transcriptase enzyme (Life Technologies) according to the manufacturer's instructions.

Quantitative Reverse Transcription-Polymerase Chain Reaction

All qPCRs were carried out in a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). mRNA quantification was achieved using Kapa SYBR fast qPCR kit (KAPA Biosystems, Woburn, USA) measured in triplicates. The real-time PCR was analyzed by using the linear standard curve method. For datum normalization, we measured mRNA levels of two reference genes: *cyclophilin-a* and tyrosine 3-monooxygenase/tryptophan5-monooxygenase activation protein, zeta polypeptide (*ywhaz*). A primer sequence list has been provided in Supplementary Information file.

Dot Blot Assay

Genomic DNA was denatured by heat and manually spotted on nitrocellulose membranes. As loading control, membranes were stained with 4% methylene blue solution. The membranes were then incubated with antibody anti 5-hmC (Zymo Research, Irvine, CA USA) and with HRP-conjugated anti-rabbit secondary antibody (Sigma). Antigen-antibody complexes were detected according to enhanced chemiluminescence (ECL) Western blotting protocol SuperSignal West Pico chemiluminescent substrate (Thermo Scientific Pierce, Waltham, MA, USA).

Primary Neuron Hippocampal Cell Culture

Animals used were Sprague Dawley rats maintained at Facultad de Ciencias Veterinarias (Buenos Aires, Argentina). All animal procedures carried out in this study were in accordance with the guidelines laid down by the Committee for the Care and Use of Animals for Experimentation (CICUAE-UNSAM no. 04/2012). Hippocampal neuron primary cultures were established from 19-day-old fetal Sprague Dawley rat hippocampi as described previously (Brocco et al. 2010).

HDAC Inhibitor Treatment

Cultures (7 days in vitro, DIV) of primary hippocampal neurons (2×10^6) were treated for 48 h with HDACi apicidin (500 nM) diluted in conditioned media. Incubation time and concentration were obtained from Marinova et al. (2011).

Stock inhibitor solutions were prepared in dimethyl sulfoxide (DMSO); thus, DMSO reagent was used as vehicle control.

Western Blot

Antibodies: polyclonal anti-acetylated histone 4 (Millipore) and polyclonal serum anti-GPM6A intracytoplasmic C terminus (Aviva Systems Biology, 1/500). Antigen-antibody complexes were detected using an Odyssey clx infrared imaging system. Immunoblots were quantified by densitometric analyses using ImageJ software (http://www.macbiophotonics.ca/imagej) and normalized against total proteins as described elsewhere (Romero-calvo et al. 2010; Eaton et al. 2013). Briefly, total proteins (i.e., all the proteins transferred in each lane together with the protein of interest) were stained with Ponceau-S and the signal intensity of the entire loading lane was used as a loading control.

Immunofluorescence and Image Analysis

Neurons were fixed and stained for GPM6A (MBL) and the F-actin marker phalloidin (Invitrogen). Fluorescent images were acquired with a Nikon Eclipse 80i microscope (Plan APO 60X oil 1.4 NA, 0.13 mmWD, objective) equipped with CoolLEDpE excitation system. Filopodium density was quantified as previously described (Alfonso et al. 2005). At least three coverslips from three independent experiments were used and 45 neurites (three neurites per neuron) per group were analyzed. Each experiment was scored blind. Sholl analysis was performed using the Sholl Analysis Plugin from the ImageJ software.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism statistical package Version 5.00 (GraphPad, San Diego, CA) and IS (Infostat software, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina). Experimental differences were assessed by Student's *t* test or by its non-parametric equivalent Mann–Whitney test when data did not meet the assumptions of normality. The Pearson correlation coefficient was calculated and used on normalized mRNA expression levels for the control and PS group separately to examine correlations. Results were reported as mean ± SEM. For all tests,$^{*}p < 0.05$ was considered statistically significant.

The artworks were created with ImageJ, Photoshop, and Adobe Illustrator.

For additional details, see the Supplementary Information file.

Results

PS Epigenetic Signature on Adult Offspring Hippocampus

It is clear the dynamic nature of DNA methylation in response to environmental factors; however, the PS effect on the cytosine demethylation process remains largely unknown. Thus, we analyzed the expression of the methylcytosine dioxygenases TET1, TET2, and TET3. *tet1* mRNA levels were significantly decreased in PS animals compared with control ones while no changes were detected for *tet2* mRNA (Fig. 1a). *tet3* mRNA yielded such low amplification rates that it was not possible to carry out a reliable quantification. Since PS changed *tet1* expression, we next evaluated 5-hmC levels. Hippocampal genomic DNA samples from control animals showed higher 5-hmC-associated intensity than samples from stressed animals (Fig. 1b). Altogether, these results suggest that PS alters the methylation/ hydroxymethylation dynamics.

Next, we studied histone methyltransferase *suv39h1*. Figure 1a shows that *suv39h1* expression was significantly higher in PS offspring compared to control animals. This suggests that some of the PS-induced changes in gene expression might occur through an increase in histone trimethylation. To explore whether PS could also alter other histone-modifying enzyme expression, we measured the histone deacetylase *hdac2* and *hdac3* mRNA levels. Figure 1c shows that, compared to control rats, none of these factors showed significant changes in the PS offspring.

Finally, we looked for factors linking changes in the environment (i.e., PS) and genome output. Thus, we analyzed the expression of transcription factors SRF, YY1, and MEF2A, previously associated with stress. No changes were detected in *srf* or *yy1* mRNA levels. In contrast, we found higher *mef2a* mRNA levels in stressed animals than in control ones (Fig. 2a). These findings suggest that MEF2A, through its target's regulation, could mediate prenatal stress effects. Interestingly, it has been depicted a MEF2 recognition element in the *Gpm6a* promoter (Gu et al. 2017). Since *gpm6a* is also upregulated by PS (Monteleone et al. 2014), a correlation analysis between *gpm6a* and *mef2a* levels was carried out. Although no correlation was found in control animals (Fig. 2c), a significant positive correlation between *mef2a* and *gpm6a* levels was observed in the prenatally stressed animals (Fig. 2c). These results suggest that PS can affect coordination of gene expression.

Neuron Morphology Can Be Modulated with the Class I HDAC Inhibitor Apicidin

HDAC inhibitors may promote neuroprotection in a wide range of neurodegenerative conditions through their neurotrophic, neuroprotective, and anti-inflammatory properties

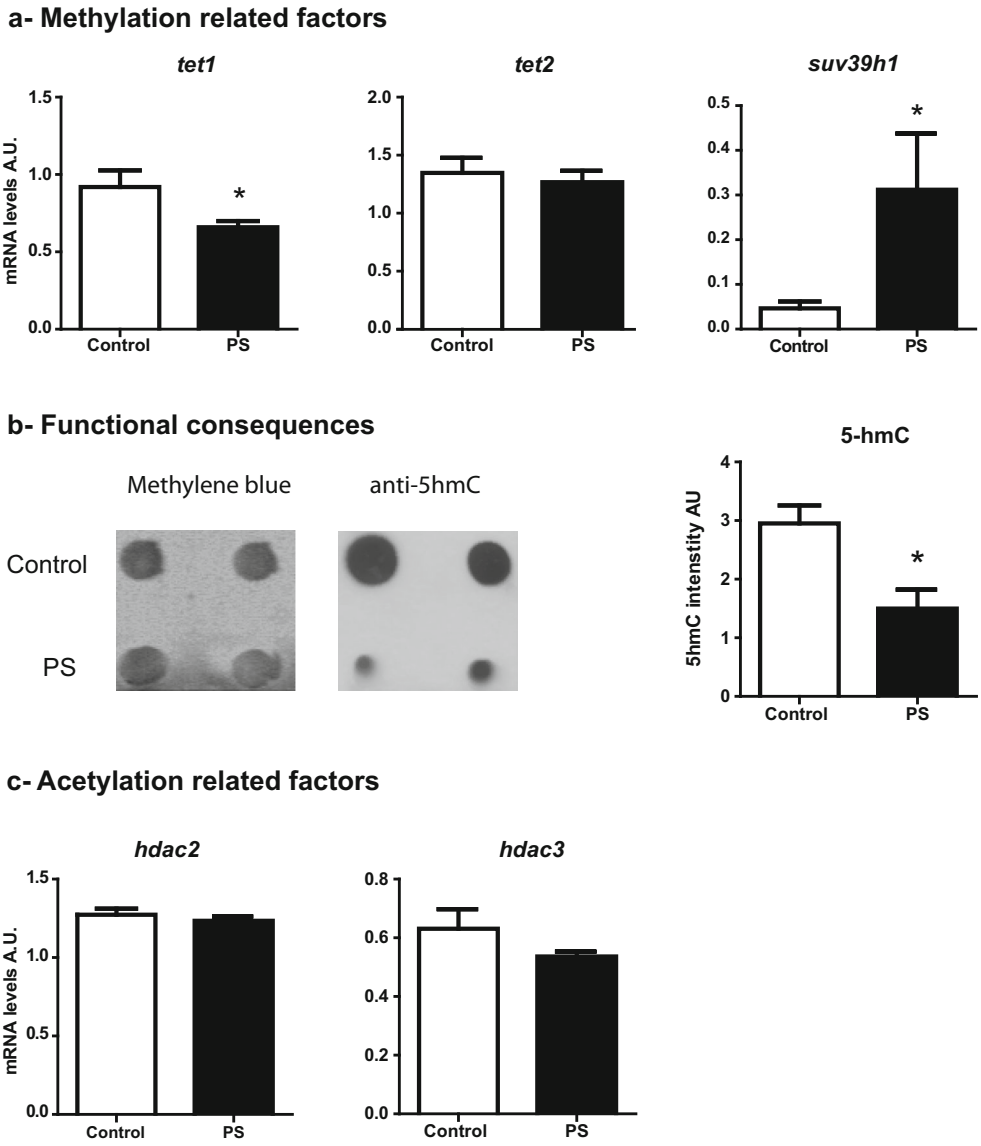

Fig. 1 PS and chromatin remodelers. mRNA levels in the hippocampus of control and PS animals were quantified by RT-qPCR. **a** Chromatin methylation-related factors: *tet1*, *tet2*, and *suv39h1*. **b** Dot blot assay with anti-5-hmC antibody showing representative samples of genomic DNA (200 ng/dot) spotted on a nitrocellulose membrane. Left, methylene blue staining shows loading control. For negative controls, water was spotted onto the membrane. Right panel, dot blot semi-quantification (*$p < 0.05$ Mann–Whitney test, $n = 5$). **c** Acetylation factors: *hdac2* and *hdac3* ($p > 0.05$. Mann–Whitney test, $n = 6$ per group)

(Chuang et al. 2009). Apicidin, a class I-specific HDAC inhibitor (Khan et al. 2008), can elicit neuroprotection (Marinova et al. 2011). Therefore, we treated hippocampal neurons in culture with the HDACi. First, acetylated histone 4 (acH4) levels were evaluated by Western blot (Fig. 3a). Since HDACi treatment may affect the levels of proteins commonly used for normalization such as tubulin or GAPDH (Glozak et al. 2005; Campo 2017), for quantification, total proteins were used to normalize Western blots (Romero-Calvo et al. 2010; Eaton et al. 2013; Campo 2017) (Fig. 3b). In comparison with control (DMSO treated) neurons, apicidin treatment significantly increased acH4 levels. Since apicidin treatment was effective in remodeling the acetylation pattern, we evaluated its effects on neuron morphology (i.e., dendritic complexity) through Sholl analysis (Fig. 3c–e). No differences in the number of dendrite intersections neither with concentric circles (Fig. 3c) nor in the total number of intersections (Fig. 3d) were observed between treatments. In contrast, analysis in the proximal region (distance from soma < 25 μm, dashed line on Fig. 3c) revealed an increase in the mean number of dendrite intersections in apicidin-treated neurons compared with control cells (Fig. 3e). Also, filopodium density was analyzed in the proximal region. While control cells displayed the typical filopodium density of untreated neurons, apicidin-treated cells (Fig. 3e) showed a significant increase in filopodium number (Fig. 3f, see arrowheads in magnifications).

Hyperacetylation induced remarkable morphological changes in neurons and the neuronal glycoprotein GPM6A participates in filopodium and spine formation. Therefore, in our in vitro model, we assayed GPM6A levels to explore apicidin impact on this plasticity-related protein. Western blot showed that apicidin treatment significantly increased GPM6A levels compared to control cells (Fig. 4a, b). This augmentation was further demonstrated by

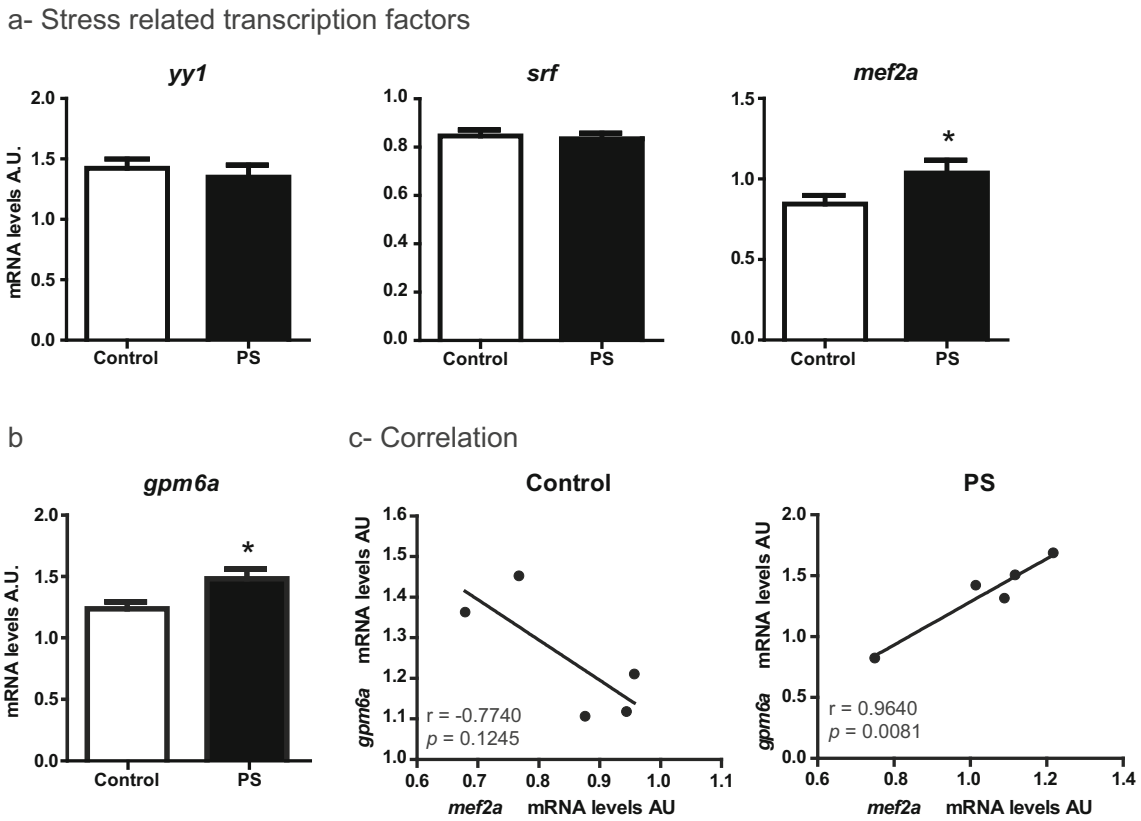

Fig. 2 Stress-related transcription factors in the hippocampus of PS animals. **a** *yy1*, *srf*, and *mef2a* mRNA levels from control and PS samples were quantified by RT-qPCR (*$p < 0.05$, Student *t* test, $n = 5$). **b** *gpm6a* mRNA levels here again quantified. **c** Correlation between *mef2a* and *gpm6a* mRNA levels in control and PS animals

immunofluorescence intensity analysis. GPM6A signal was higher in apicidin-treated cells compared with control cells, both in single cell and in average analysis (Fig. 4c, d).

Overall, these results indicate that apicidin treatment, through changes in the acetylation pattern, alters neuronal morphology probably by modulating the expression of plasticity genes such as the one coding GPM6A.

Discussion

The epigenetic signature of prenatal stress can alter adaptation to stressful situations and thereby set the hallmarks for the development —in humans— of affective disorders later in life. Using a model of prenatal stress in rats, we investigated epigenetic changes occurring in the hippocampus because stress impairs various hippocampal-dependent memory tasks and alters ensuing synaptic plasticity and firing properties of hippocampal neurons. We observed an altered expression of *suv39h1* and *tet1*, two chromatin remodeling genes. We also found a significant increase in *mef2a* mRNA levels after PS exposure that could mediate changes in plasticity-related gene expression. Finally, we moved to an in vitro model and found that neuronal plasticity can be manipulated through changes in histone acetylation status.

We showed that PS affected TET1-mediated chromatin demethylation. Recently, Feng et al. (2017) reported that TET1 but not TET2 or TET3 is downregulated in mice exposed to chronic social defeat stress (Feng et al. 2017). Likewise, we did not observe changes in TET2 expression. Remarkably, decreased levels of *tet1* correlated with a global decrease in 5-hmC levels. Since in mammal brain 5-hmC is enriched in genes with synapse-related functions (Khare et al. 2012), the observed decrease may underlie an altered synaptic plasticity on individuals exposed to stress. Notably, genomic DNA from leukocytes of patients with major depression (Tseng et al. 2014) and from postmortem brain of patients with Alzheimer's disease (Condliffe et al. 2014) shows lower levels on 5-hmC than healthy individuals. Similarly, in TET1 knockout mice, the loss of TET1 (that should reduce 5-hmC content) impairs learning and memory (Rudenko et al. 2013). On the contrary, 5-hmC accumulation in the adult brain promotes rapid behavioral adaptation (Li et al. 2014). Thus, the reduced expression of *tet1* depicted here may explain, in part, the learning deficits induced by prenatal stress (Lemaire et al. 2000). Moreover, 5-hmC levels inversely correlate with methyl-CpG-binding protein 2 dosage (MeCP2) (Szulwach et al. 2011). In agreement, we have previously reported that PS upregulates *mecp2* levels (Monteleone et al. 2014).

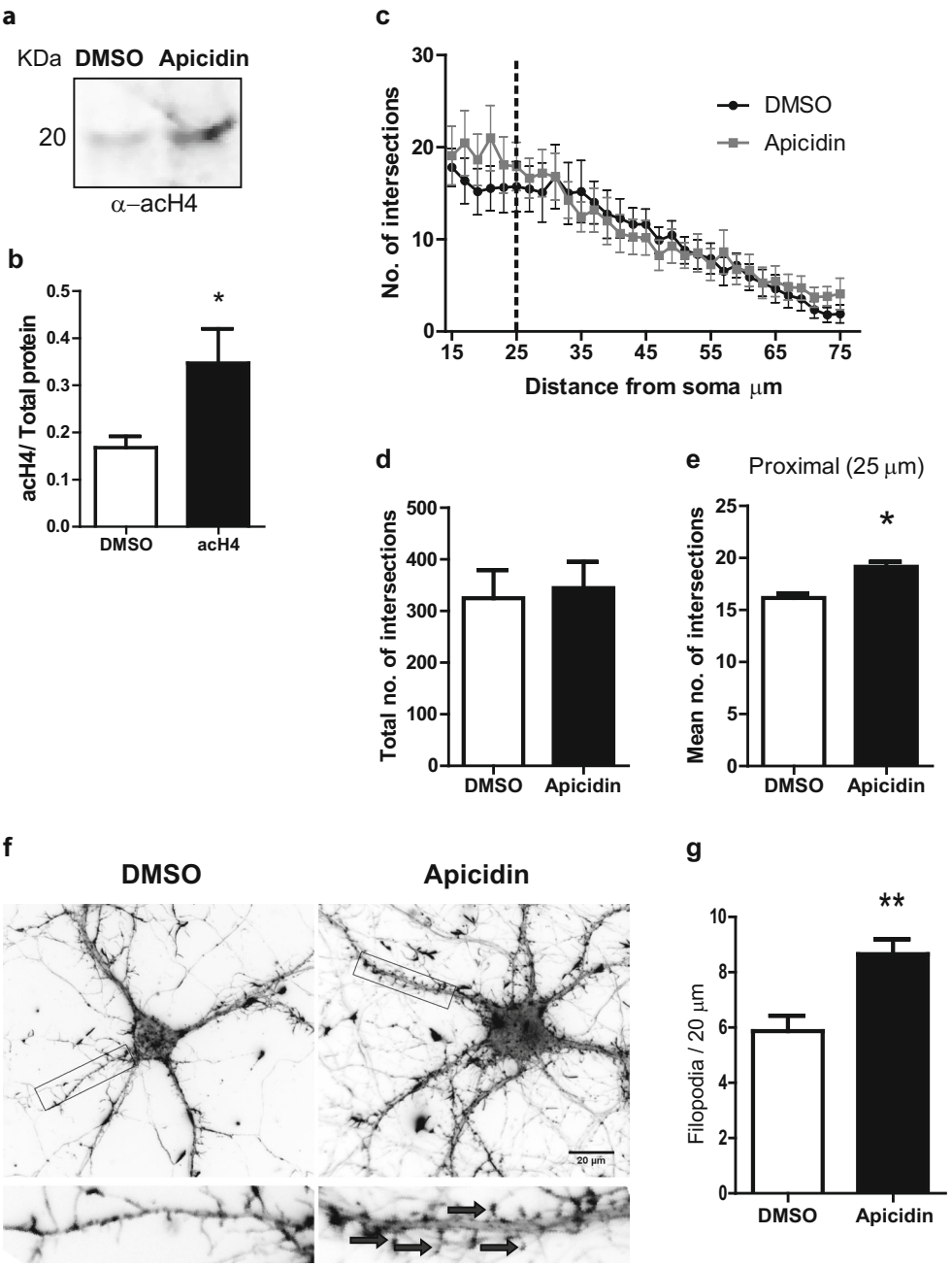

Fig. 3 Apicidin treatment changes neuron morphology. Hippocampal neurons (7 DIV) treated for 48 h with apicidin or with the vehicle DMSO as control. **a** Western blot stained with anti-acetylated histone 4 (acH4). **b** Western blot semi-quantification. The signal intensity of Ponceau-S staining was used as loading control and for normalization purposes (*$p <$ 0.05, Student *t* test, $n = 2$ independent experiments). **c–g** Effect of apicidin treatment on neuron morphology. **c–e** Sholl analysis ($n = 2$ independent experiments, 10 cells per treatment). **c** Graph plotting the number of intersections in relation to the distance from the center of the soma of hippocampal neurons. **d** Total number of intersections, as index of complexity. **e** Quantification of number of intersections in the proximal zone, 25 μm from the soma of the neuron (dashed line in C), *$p < 0.05$; Student *t* test. **f**, **g** Filopodium number (arrows in neurite magnifications) was quantified on phalloidin-stained neurons. **$p < 0.0025$; Student *t* test, $n = 20$ cells/condition

Therefore, our results suggest that PS alteration of the methylation patterns could set the landscape that leads to neurological disorders later in life. A crosstalk between DNA and histone methylation has been reported as changes in 5-hmC levels negatively correlate with H3K27me3- and H3K9me3-marked genomic regions (Wen et al. 2014). In coincidence, we have showed a PS-induced increased expression of the methyltransferase SUV39H1 that trimethylates the histone 3, suggesting changes in this post-translational modification. SUV39H1 inhibition improves synaptic plasticity (Snigdha et al. 2016), so the altered neuroplasticity characteristic of stressed animals may be given by the increased *suv39h1* expression. Furthermore, there is an interplay between histone methylation and acetylation (Marinova et al. 2011).

Acetylation/deacetylation is the most widely investigated histone modification; thus, we evaluated the deacetylating enzyme expression. PS did not alter *hdac2* nor *hdac3* mRNA levels in the adult offspring hippocampus. Using a similar stress procedure, Dong et al. (2015) reported no changes in *hdac* expression (Dong et al. 2015). It is then possible that prenatal stress induces a transient change in HDAC mRNA levels, undetectable in the adult individuals, but sufficient to cause persistent changes in the expression of HDAC target genes. To add complexity, reversible acetylation can also occur on non-histone proteins (Glozak et

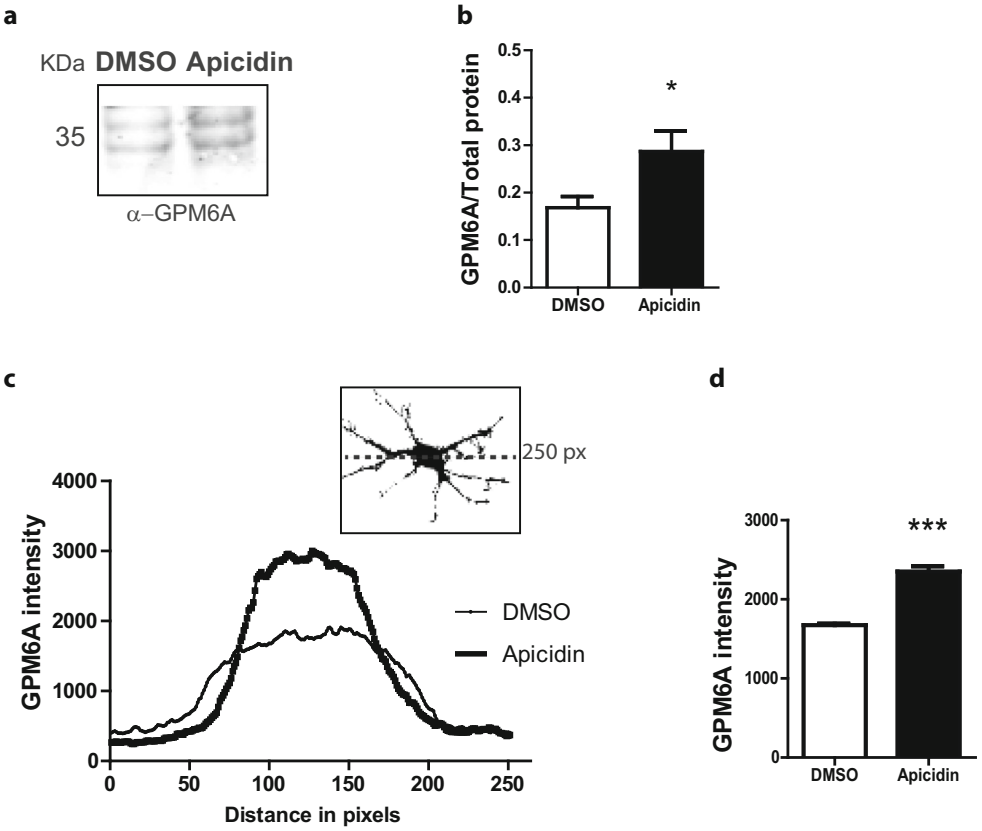

Fig. 4 Apicidin treatments enhance M6a expression. Hippocampal neurons (7 DIV) treated for 48 h with apicidin or with the vehicle DMSO as control. **a** Western blot stained with anti-GPM6A antibodies. GPM6A appears as a double band of approximately 35 kDa. **b** Western blot semi-quantification of apicidin effect on GPM6A levels. The signal intensity of Ponceau-S staining was used as loading control and for normalization purposes. $*p < 0.05$, Student *t* test, $n = 2$ independent experiments. **c** Plot profile for GPM6A intensity obtained by measurement along a 250-pixel line drawn across cell soma (inset). **d** GPM6A intensity average intensity $***p < 0.001$; Student *t* test, $n = 2$ independent experiments

al. 2005; Eom and Kook 2014). Among them, there are cellular proteins (such as tubulin) and transcription factors including MEF2A that can be acetylated as a mechanism to regulate its transcriptional activity (Shalizi et al. 2006). Moreover, MEF2A responds to upstream signaling pathways (e.g., the glucocorticoid receptor activation, see below) and associates indirectly through a repressor complex with class I HDACs serving as a key intermediary in the transmission of extracellular signals to the genome (McKinsey et al. 2002).

In the stress context, glucocorticoid excess in early life can permanently alter tissue glucocorticoid signaling. Speksnijder et al. (2012) showed that the GR pharmacological activation by dexamethasone treatment regulates MEF2A transcriptional activity (Speksnijder et al. 2012), which in turn regulates the expression of genes that control neuronal plasticity and dendritic remodeling (Flavell et al. 2008; Speksnijder et al. 2012). Now, we found a significant increase in *mef2a* mRNA levels after PS exposure. Therefore, we propose that, similarly to pharmacological activation, GR activation induced by PS may affect MEF2A transcriptional activity altering the transcription of genes involved in neuronal plasticity. MEF2A regulates the transcription of many genes that function at the synapse, including *bdnf* (Flavell et al. 2008). In addition, bioinformatics tools DECODE and JASPAR CORE (Mathelier et al. 2014) predict MEF2A binding sites upstream of the transcription start site of rat *gpm6a* gene (see Supplementary Information). At the time this manuscript was in preparation, using a luciferase assay, Gu et al. (2017) reported that *gpm6a* is a MEF2C direct target (Gu et al. 2017). Moreover, Lanz et al. (2013) showed that *Mef2a* knockdown in murine primary cortical neurons severely affects *Gpm6a* expression (Lanz et al. 2013). MEF2A might contribute to the PS-induced upregulation of the neuronal plasticity genes such as *gpm6a*. Interestingly, only in stressed animals, there was a correlation between *mef2a* and *gpm6a* levels; thus, MEF2A and GPM6A might participate in a pathway exclusively activated in stressed animals. Hence, we postulate that PS may dysregulate the coordination of gene expression, which has been reported as one of the mechanisms leading to mood disorders (Gaiteri et al. 2014). Accordingly, an uncoordinated expression of *GPM6A* and its associated neuroplasticity genes has been reported in the brain of depressed suicides (Fuchsova et al. 2015).

Altogether, these results suggest that PS triggers epigenetic modifications that could have an impact later in life. Among the reported stress effects, changes in neuronal morphology are one of the most studied (Hayashi et al. 1998; Lemaire et al. 2000; Hosseini-Sharifabad and Hadinedoushan 2007; Mychasiuk et al. 2012). It is in this field that HDAC inhibitors (in particular for class I HDACs) are being studied given their neuroprotective and mood stabilizer effects. For this reason, using an in vitro model, we tested the effect of apicidin, a class I HDAC inhibitor, on neuronal morphology. Inhibitor treatment increased filopodium. Apicidin also increased the levels

of GPM6A, a plasticity-related protein, suggesting that GPM6A might mediate the effect of HDAC inhibition on neuronal morphology remodeling. Similarly, HDAC5 inhibition upregulates GPM6A levels and promotes neurite development (Gu et al. 2017).

In vitro treatment of neuronal cells with corticosterone induces morphological (Alfarez et al. 2009) and gene expression (Revest et al. 2010) changes very similar to those found in the hippocampus of stressed animals. Since chronic stress effects on *gpm6a* expression can be reverted with antidepressant treatment (Alfonso et al. 2004, 2006), GPM6A represents a neuroprotective protein to induce via HDAC inhibition.

In conclusion, we studied global effects of prenatal stress on transcription and chromatin remodeling factors in the hippocampus. Previously, we have shown that PS induces changes in DNMT3a and in methylated DNA-binding proteins (Monteleone et al. 2014) and now, that induced changes in *tet1* expression and in the 5-hmC content in the genome. That is PS affects the DNA methylation, although the extent of these changes remains not well understood. PS induced changes in *mef2a*, which may function as an intermediary between PS and the plasticity gene expression. Stress and glucocorticoid administration alter gene expression and ultimately affect structural plasticity. These results offer potential candidates, MEF2A and 5-hmC, already proposed as an environmental sensor (Dao et al. 2014), to the discovery of new stress biomarkers. Neural connectivity alterations are difficult to reverse with typical antidepressants (Duman 2014); therefore, the search for new targets to modulate neural plasticity is a promising field for therapeutic intervention. Our in vitro experiments revealed that neuroplasticity can be recovered through an epigenetic modulator such as a class I HDAC inhibitor. This highlights the therapeutic potential of HDAC inhibitors to counteract adverse stress effects and to alleviate mood-related disorders.

Acknowledgements The authors thank Dr. Vanina Campo for the HDAC inhibitor and technical advice about their use. MCM is a fellow from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina (CONICET). SCB is a technician and MEP, MCA, and MAB are researchers from CONICET.

Author Contributions Conceived and designed the experiments: MCM, MEP, MCA, and MAB. Designed, performed, and supervised prenatal stress protocol: MEP and MCA. Performed bench experiments: MCM and SCB. Analyzed the data: MCM, SCB, MEP, and MAB. Wrote the paper: MCM and MAB. Made manuscript revisions: MEP and SCB.

Funding This work was supported by the National Agency for Scientific and Technological Promotion (ANPCyT, PICT 0807-2011 and 1093-2014) and by the UNSAM (Grants 2014-2015).

Compliance with Ethical Standards

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

References

Adrover E, Pallarés ME, Baier CJ, Monteleone MC, Giuliani FA, Waagepetersen HS, Brocco MA, Cabrera R, Sonnewald U, Schousboe A, Antonelli MC (2015) Glutamate neurotransmission is affected in prenatally stressed offspring. Neurochem Int 88:73–87. https://doi.org/10.1016/j.neuint.2015.05.005

Alfarez DN, De Simoni A, Velzing EH et al (2009) Corticosterone reduces dendritic complexity in developing hippocampal CA1 neurons. Hippocampus 19:828–836. https://doi.org/10.1002/hipo.20566

Alfonso J, Pollevick GD, van der Hart MG, Flugge G, Fuchs E, Frasch ACC (2004) Identification of genes regulated by chronic psychosocial stress and antidepressant treatment in the hippocampus. Eur J Neurosci 19:659–666. https://doi.org/10.1111/j.1460-9568.2004.03178.x

Alfonso J, Fernández ME, Cooper B et al (2005) The stress-regulated protein M6a is a key modulator for neurite outgrowth and filopodium/spine formation. Proc Natl Acad Sci U S A 102:17196–17201. https://doi.org/10.1073/pnas.0504262102

Alfonso J, Frick LR, Silberman DM, Palumbo ML, Genaro AM, Frasch AC (2006) Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. Biol Psychiatry 59:244–251. https://doi.org/10.1016/j.biopsych.2005.06.036

Baier CJ, Katunar MR, Adrover E, Pallarés ME, Antonelli MC (2012) Gestational restraint stress and the developing dopaminergic system: an overview. Neurotox Res 22:16–32. https://doi.org/10.1007/s12640-011-9305-4

Baier CJ, Pallarés ME, Adrover E, Monteleone MC, Brocco MA, Barrantes FJ, Antonelli MC (2015) Prenatal restraint stress decreases the expression of alpha-7 nicotinic receptor in the brain of adult rat offspring. Stress 3890:1–11. https://doi.org/10.3109/10253890.2015.1022148

Beydoun H, Saftlas AF (2008) Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. Paediatr Perinat Epidemiol 22:438–466. https://doi.org/10.1111/j.1365-3016.2008.00951.x

Boks MPM, Hoogendoorn M, Jungerius BJ, Bakker SC, Sommer IE, Sinke RJ, Ophoff RA, Kahn RS (2008) Do mood symptoms subdivide the schizophrenia phenotype? Association of the GMP6A gene with a depression subgroup. Am J Med Genet B Neuropsychiatr Genet 147:707–711. https://doi.org/10.1002/ajmg.b.30667

Brocco MA, Fernández ME, Frasch ACC (2010) Filopodial protrusions induced by glycoprotein M6a exhibit high motility and aids synapse formation. Eur J Neurosci 31:195–202. https://doi.org/10.1111/j.1460-9568.2009.07064.x

Campo VA (2017) Comparative effects of histone deacetylases inhibitors and resveratrol on Trypanosoma cruzi replication, differentiation, infectivity and gene expression. Int J Parasitol Drugs Drug Resist 7:23–33. https://doi.org/10.1016/j.ijpddr.2016.12.003

Chuang DM, Leng Y, Marinova Z, Kim HJ, Chiu CT (2009) Multiple roles of HDAC inhibitors in neurodegenerative conditions. Trends Neurosci 32:591–601. https://doi.org/10.1016/j.tins.2009.06.002.Multiple

Condliffe D, Wong A, Troakes C, Proitsi P, Patel Y, Chouliaras L, Fernandes C, Cooper J, Lovestone S, Schalkwyk L, Mill J, Lunnon K (2014) Cross-region reduction in 5-hydroxymethylcytosine in Alzheimer's disease brain. Neurobiol Aging 35:1850–1854. https://doi.org/10.1016/j.neurobiolaging.2014.02.002

Covington HE, Maze I, LaPlant QC et al (2009) Antidepressant actions of histone deacetylase inhibitors. J Neurosci 29:11451–11460. https://doi.org/10.1523/JNEUROSCI.1758-09.2009

Dao T, Cheng R, Revelo M et al (2014) Hydroxymethylation as a novel environmental biosensor. Curr Environ Health Rep 1:1–10. https://doi.org/10.1016/j.biotechadv.2011.08.021.Secreted

Dong E, Dzitoyeva SG, Matrisciano F, Tueting P, Grayson DR, Guidotti A (2015) Brain-derived neurotrophic factor epigenetic modifications associated with schizophrenia-like phenotype induced by prenatal stress in mice. Biol Psychiatry 77:589–596. https://doi.org/10.1016/j.biopsych.2014.08.012

Duman RS (2014) Neurobiology of stress, depression, and rapid acting antidepressants: remodeling synaptic connections. Depress Anxiety 31:291–296. https://doi.org/10.1002/da.22227.Neurobiology

Eaton SL, Roche SL, Llavero Hurtado M, Oldknow KJ, Farquharson C, Gillingwater TH, Wishart TM (2013) Total protein analysis as a reliable loading control for quantitative fluorescent western blotting. PLoS One 8:1–9. https://doi.org/10.1371/journal.pone.0072457

El-Kordi A, Kästner A, Grube S et al (2013) A single gene defect causing claustrophobia. Transl Psychiatry 3:e254. https://doi.org/10.1038/tp.2013.28

Eom GH, Kook H (2014) Pharmacology & therapeutics posttranslational modifications of histone deacetylases: implications for cardiovascular diseases. Pharmacol Ther 143:168–180. https://doi.org/10.1016/j.pharmthera.2014.02.012

Feng J, Pena CJ, Purushothaman I, Engmann O, Walker D, Brown AN, Issler O, Doyle M, Harrigan E, Mouzon E, Vialou V, Shen L, Dawlaty MM, Jaenisch R, Nestler EJ (2017) Tet1 in nucleus accumbens opposes depression- and anxiety-like behaviors. Neuropsychopharmacology 42:1657–1669. https://doi.org/10.1038/npp.2017.6

Ficz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, Marques CJ, Andrews S, Reik W (2011) Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature 473:398–402. https://doi.org/10.1038/nature10008

Flavell SW, Kim TK, Gray JM, Harmin DA, Hemberg M, Hong EJ, Markenscoff-Papadimitriou E, Bear DM, Greenberg ME (2008) Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. Neuron 60:1022–1038. https://doi.org/10.1016/j.neuron.2008.11.029

Formoso K, Garcia MD, Frasch AC, Scorticati C (2016) Evidence for a role of glycoprotein M6a in dendritic spine formation and synaptogenesis. Mol Cell Neurosci 77:95–104. https://doi.org/10.1016/j.mcn.2016.10.005

Fuchsova B, Fernández ME, Alfonso J, Frasch AC (2009) Cysteine residues in the large extracellular loop (EC2) are essential for the function of the stress-regulated glycoprotein M6a. J Biol Chem 284:32075–32088. https://doi.org/10.1074/jbc.M109.012377

Fuchsova B, Alvarez-Juliá A, Rizavi HS et al (2015) Altered expression of neuroplasticity-related genes in the brain of depressed suicides. Neuroscience 299:1–17. https://doi.org/10.1016/j.neuroscience.2015.04.057

Fumagalli F, Bedogni F, Perez J, Racagni G, Riva MA (2004) Corticostriatal brain-derived neurotrophic factor dysregulation in adult rats following prenatal stress. Eur J Neurosci 20:1348–1354. https://doi.org/10.1111/j.1460-9568.2004.03592.x

Fumagalli F, Bedogni F, Slotkin TA et al (2005) Prenatal stress elicits regionally selective changes in basal FGF-2 gene expression in adulthood and alters the adult response to acute or chronic stress. Neurobiol Dis 20:731–737. https://doi.org/10.1016/j.nbd.2005.05.005

Gaiteri C, Ding Y, French B, Tseng GC, Sibille E (2014) Beyond modules and hubs: the potential of gene coexpression networks for investigating molecular mechanisms of complex brain disorders. Genes Brain Behav 13:13–24. https://doi.org/10.1111/gbb.12106

Glover V (2011) Annual research review: prenatal stress and the origins of psychopathology: an evolutionary perspective. J Child Psychol Psychiatry 52:356–367. https://doi.org/10.1111/j.1469-7610.2011.02371.x

Glozak MA, Sengupta N, Zhang X, Seto E (2005) Acetylation and deacetylation of non-histone proteins. Gene 363:15–23. https://doi.org/10.1016/j.gene.2005.09.010

Greenwood TA, Akiskal HS, Akiskal KK, Kelsoe JR (2012) Genome-wide association study of temperament in bipolar disorder reveals significant associations with three novel loci. Biol Psychiatry 72:303–310. https://doi.org/10.1016/j.biopsych.2012.01.018

Gregor A, Donders R, Reis A et al (2014) Altered GPM6A/M6 dosage impairs cognition and causes phenotypes responsive to cholesterol in human and Drosophila. https://doi.org/10.1002/humu.22697.This

Gu X, Fu C, Lin L, Liu S, Su X, Li A, Wu Q, Jia C, Zhang P, Chen L, Zhu X, Wang X (2017) miR-124 and miR-9 mediated downregulation of HDAC5 promotes neurite development through activating MEF2C-GPM6A pathway. J Cell Physiol 233:1–38. https://doi.org/10.1002/jcp.25927

Guan J-S, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJF, Zhou Y, Wang X, Mazitschek R, Bradner JE, DePinho RA, Jaenisch R, Tsai LH (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60. https://doi.org/10.1038/nature07925

Guo JU, Ma DK, Mo H et al (2012) Neuronal activity modifies DNA methylation landscape in the adult brain. Nat Neurosci 14:1345–1351. https://doi.org/10.1038/nn.2900.Neuronal

Hahn MA, Qiu R, Wu X et al (2013) Dynamics of 5-hydroxymethylcytosine and chromatin marks in mammalian neurogenesis. Cell Rep 3:291–300. https://doi.org/10.1016/j.celrep.2013.01.011.Dynamics

Hayashi A, Nagaoka M, Yamada K, Ichitani Y, Miake Y, Okado N (1998) Maternal stress induces synaptic loss and developmental disabilities of offspring. Int J Dev Neurosci 16:209–216. https://doi.org/10.1016/S0736-5748(98)00028-8

Hosseini-Sharifabad M, Hadinedoushan H (2007) Prenatal stress induces learning deficits and is associated with a decrease in granules and CA3 cell dendritic tree size in rat hippocampus. Anat Sci Int 82:211–217. https://doi.org/10.1111/j.1447-073X.2007.00186.x

Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y (2010) Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 466:1129–1133. https://doi.org/10.1038/nature09303

Kaas GA, Zhong C, Eason DE, Ross DL, Vachhani RV, Ming GL, King JR, Song H, Sweatt JD (2013) TET1 controls CNS 5-methylcytosine hydroxylation, active DNA demethylation, gene transcription, and memory formation. Neuron 79:1086–1093. https://doi.org/10.1016/j.neuron.2013.08.032

Khan N, Jeffers M, Kumar S, Hackett C, Boldog F, Khramtsov N, Qian X, Mills E, Berghs SC, Carey N, Finn PW, Collins LS, Tumber A, Ritchie JW, Jensen PB, Lichenstein HS, Sehested M (2008) Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors. Biochem J 409:581–589

Khare T, Pai S, Koncevicius K, Pal M, Kriukiene E, Liutkeviciute Z, Irimia M, Jia P, Ptak C, Xia M, Tice R, Tochigi M, Moréra S, Nazarians A, Belsham D, Wong AHC, Blencowe BJ, Wang SC, Kapranov P, Kustra R, Labrie V, Klimasauskas S, Petronis A (2012) 5-hmC in the brain is abundant in synaptic genes and shows differences at the exon-intron boundary. Nat Struct Mol Biol 19:1037–1043. https://doi.org/10.1038/nsmb.2372

Kilgore M, Miller CA, Fass DM, Hennig KM, Haggarty SJ, Sweatt JD, Rumbaugh G (2010) Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology 35:870–880. https://doi.org/10.1038/npp.2009.197

Kriaucionis S, Heintz N (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324:929–930. https://doi.org/10.1126/science.1169786

Lanz TA, Guilmette E, Gosink MM et al (2013) Transcriptomic analysis of genetically defined autism candidate genes reveals common mechanisms of action. Mol Autism 4:45. https://doi.org/10.1186/2040-2392-4-45

Lemaire V, Koehl M, Le Moal M, Abrous DN (2000) Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc Natl Acad Sci U S A 97: 11032–11037

Li X, Wei W, Zhao Q-Y, Widagdo J, Baker-Andresen D, Flavell CR, D'Alessio A, Zhang Y, Bredy TW (2014) Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. Proc Natl Acad Sci 111:7120–7125. https://doi.org/10.1073/pnas.1318906111

Marinova Z, Leng Y, Leeds P, Chuang DM (2011) Histone deacetylase inhibition alters histone methylation associated with heat shock protein 70 promoter modifications in astrocytes and neurons. Neuropharmacology 60:1109–1115. https://doi.org/10.1016/j.neuropharm.2010.09.022

Mathelier A, Zhao X, Zhang AW, Parcy F, Worsley-Hunt R, Arenillas DJ, Buchman S, Chen CY, Chou A, Ienasescu H, Lim J, Shyr C, Tan G, Zhou M, Lenhard B, Sandelin A, Wasserman WW (2014) JASPAR 2014: an extensively expanded and updated open-access database of transcription factor binding profiles. Nucleic Acids Res 42:D142–D147. https://doi.org/10.1093/nar/gkt997

McKinsey TA, Zhang CL, Olson EN (2002) MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends Biochem Sci 27:40–47. https://doi.org/10.1016/S0968-0004(01)02031-X

McQuown SC, Barrett RM, Matheos DP et al (2011) HDAC3 is a critical negative regulator of long-term memory formation. J Neurosci 31: 764–774. https://doi.org/10.1523/JNEUROSCI.5052-10.2011

Monteleone MC, Adrover E, Pallarés ME, Antonelli MC, Frasch AC, Brocco MA (2014) Prenatal stress changes the glycoprotein GPM6A gene expression and induces epigenetic changes in rat offspring brain. Epigenetics 9:152–160. https://doi.org/10.4161/epi.25925

Mychasiuk R, Gibb R, Kolb B (2012) Prenatal stress alters dendritic morphology and synaptic connectivity in the prefrontal cortex and hippocampus of developing offspring. Synapse 66:308–314. https://doi.org/10.1002/syn.21512

Pallarés ME, Adrover E, Baier CJ, Bourguignon NS, Monteleone MC, Brocco MA, González-Calvar SI, Antonelli MC (2013a) Prenatal maternal restraint stress exposure alters the reproductive hormone profile and testis development of the rat male offspring. Stress 16: 429–440. https://doi.org/10.3109/10253890.2012.761195

Pallarés ME, Baier CJ, Adrover E, Monteleone MC, Brocco MA, Antonelli MC (2013b) Age-dependent effects of prenatal stress on the corticolimbic dopaminergic system development in the rat male offspring. Neurochem Res 38:2323–2335. https://doi.org/10.1007/s11064-013-1143-8

Revest J-M, Kaouane N, Mondin M, le Roux A, Rougé-Pont F, Vallée M, Barik J, Tronche F, Desmedt A, Piazza PV (2010) The enhancement of stress-related memory by glucocorticoids depends on synapsin-Ia/Ib. Mol Psychiatry 15(1125):1140–1151. https://doi.org/10.1038/mp.2010.40

Romero-Calvo I, Ocón B, Martínez-Moya P et al (2010) Reversible Ponceau staining as a loading control alternative to actin in Western blots. Anal Biochem 401:318–320. https://doi.org/10.1016/j.ab.2010.02.036

Rudenko A, Tsai L (2014) Neuropharmacology epigenetic modifications in the nervous system and their impact upon cognitive impairments. Neuropharmacology 80:70–82. https://doi.org/10.1016/j.neuropharm.2014.01.043

Rudenko A, Dawlaty MM, Seo J, Cheng AW, Meng J, le T, Faull KF, Jaenisch R, Tsai LH (2013) Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. Neuron 79: 1109–1122. https://doi.org/10.1016/j.neuron.2013.08.003

Scorticati C, Formoso K, Frasch AC (2011) Neuronal glycoprotein M6a induces filopodia formation via association with cholesterol-rich lipid rafts. J Neurochem 119:521–531. https://doi.org/10.1111/j.1471-4159.2011.07252.x

Shahbazian MD, Grunstein M (2007) Functions of site-specific histone acetylation and deacetylation. Annu Rev Biochem 76:75–100. https://doi.org/10.1146/annurev.biochem.76.052705.162114

Shalizi A, Gaudilliere B, Yuan Z et al (2006) A calcium-regulated MEF2 Sumoylation switch controls postsynaptic differentiation. Science (80-) 311:1012–1017. https://doi.org/10.1126/science.1122513

Snigdha S, Prieto GA, Petrosyan A, Loertscher BM, Dieskau AP, Overman LE, Cotman CW (2016) H3K9me3 inhibition improves memory, promotes spine formation, and increases BDNF levels in the aged hippocampus. J Neurosci 36:3611–3622. https://doi.org/10.1523/JNEUROSCI.2693-15.2016

Sotnikov SV, Markt PO, Malik V, Chekmareva NY, Naik RR, Sah A, Singewald N, Holsboer F, Czibere L, Landgraf R (2014) Bidirectional rescue of extreme genetic predispositions to anxiety: impact of CRH receptor 1 as epigenetic plasticity gene in the amygdala. Transl Psychiatry 4:e359. https://doi.org/10.1038/tp.2013.127

Speksnijder N, Christensen KV, Didriksen M, de Kloet ER, Datson NA (2012) Glucocorticoid receptor and myocyte enhancer factor 2 cooperate to regulate the expression of c-JUN in a neuronal context. J Mol Neurosci 48:209–218. https://doi.org/10.1007/s12031-012-9809-2

Suri D, Bhattacharya A, Vaidya VA (2014) Early stress evokes temporally distinct consequences on the hippocampal transcriptome, anxiety and cognitive behaviour. Int J Neuropsychopharmacol 17:289–301. https://doi.org/10.1017/S1461145713001004

Szulwach KE, Li X, Li Y, Song CX, Wu H, Dai Q, Irier H, Upadhyay AK, Gearing M, Levey AI, Vasanthakumar A, Godley LA, Chang Q, Cheng X, He C, Jin P (2011) 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nat Neurosci 14: 1607–1616. https://doi.org/10.1016/j.biotechadv.2011.08.021.Secreted

Tahiliani M, Koh KP, Shen Y et al (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL Partner TET1. Science (80-) 324:930–935. https://doi.org/10.1126/science.1170116.Conversion

Tseng P-T, Lin P-Y, Lee Y et al (2014) Age-associated decrease in global DNA methylation in patients with major depression. Neuropsychiatr Dis Treat 10:2105–2114. https://doi.org/10.2147/NDT.S71997

Van den Bergh BRH, Mulder EJH, Mennes M, Glover V (2005) Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A review. Neurosci Biobehav Rev 29:237–258. https://doi.org/10.1016/j.neubiorev.2004.10.007

Van den Hove DLA, Steinbusch HWM, Scheepens A et al (2006) Prenatal stress and neonatal rat brain development. Neuroscience 137:145–155. https://doi.org/10.1016/j.neuroscience.2005.08.060

Vialou V, Robison AJ, Laplant QC et al (2010) DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. Nat Neurosci 13:745–752. https://doi.org/10.1038/nn.2551

Wen L, Li X, Yan L, Tan Y, Li R, Zhao Y, Wang Y, Xie J, Zhang Y, Song C, Yu M, Liu X, Zhu P, Li X, Hou Y, Guo H, Wu X, He C, Li R, Tang F, Qiao J (2014) Whole-genome analysis of 5-hydroxymethylcytosine and 5-methylcytosine at base resolution in the human brain. Genome Biol 15:R49. https://doi.org/10.1186/gb-2014-15-3-r49

Zhang R, Cui Q, Murai K et al (2013) Tet1 regulates adult hippocampal neurogenesis and cognition. Cell Stem Cell 13:237–245. https://doi.org/10.1016/j.stem.2013.05.006.Tet1