RESEARCH ARTICLE

Acute and Chronic Electroconvulsive Seizures (ECS) Differentially Regulate the Expression of Epigenetic Machinery in the Adult Rat Hippocampus

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Abstract

Background: Electroconvulsive seizure treatment is a fast-acting antidepressant therapy that evokes rapid transcriptional, neurogenic, and behavioral changes. Epigenetic mechanisms contribute to altered gene regulation, which underlies the neurogenic and behavioral effects of electroconvulsive seizure. We hypothesized that electroconvulsive seizure may modulate the expression of epigenetic machinery, thus establishing potential alterations in the epigenetic landscape.

Methods: We examined the influence of acute and chronic electroconvulsive seizure on the gene expression of histone modifiers, namely histone acetyltransferases, histone deacetylases, histone methyltransferases, and histone (lysine) demethylases as well as DNA modifying enzymes, including DNA methyltransferases, DNA demethylases, and methyl-CpG-binding proteins in the hippocampi of adult male Wistar rats using quantitative real time-PCR analysis. Further, we examined the influence of acute and chronic electroconvulsive seizure on global and residue-specific histone acetylation and methylation levels within the hippocampus, a brain region implicated in the cellular and behavioral effects of electroconvulsive seizure.

Results: Acute and chronic electroconvulsive seizure induced a primarily unique, and in certain cases bidirectional, regulation of histone and DNA modifiers, and methyl-CpG-binding proteins, with an overlapping pattern of gene regulation restricted to *Sirt4*, *Mll3*, *Jmjd3*, *Gadd45b*, *Tet2*, and *Tet3*. Global histone acetylation and methylation levels were predominantly unchanged, with the exception of a significant decline in H3K9 acetylation in the hippocampus following chronic electroconvulsive seizure.

Conclusions: Electroconvulsive seizure treatment evokes the transcriptional regulation of several histone and DNA modifiers, and methyl-CpG-binding proteins within the hippocampus, with a predominantly distinct pattern of regulation induced by acute and chronic electroconvulsive seizure.

Keywords: histone acetyltransferase, histone deacetylase, histone methyltransferase, histone demethylase, DNA methyltransferase, DNA demethylase, methyl-CpG-binding proteins

Introduction

The mechanisms underlying the therapeutic action of electro-convulsive seizure (ECS) therapy, a highly effective, rapid-action antidepressant treatment, remain poorly understood. Studies in rodent models indicate that ECS evokes specific molecular and cellular changes, hypothesized to mechanistically contribute to the effects of ECS on mood. ECS evokes robust regulation of gene expression, including immediate-early genes, synaptic plasticity-associated genes (Dyrvig et al., 2014), transcription factors (Cole et al., 1990), neurotrophic and angiogenic factors (Newton et al., 2003), signaling pathway genes (Kodama et al., 2005), and antiapoptotic factors (Kosten et al., 2008). ECS also results in cellular changes, including enhanced hippocampal progenitor turnover, survival, and maturation (Madsen et al., 2000; Malberg et al., 2000), increased axonal sprouting (Vaidya et al., 1999), dendritic plasticity (Zhao et al., 2012), and endothelial cell proliferation (Hellsten et al., 2004). ECS-dependent gene regulation is implicated in mediating the ECS-evoked cytoarchitectural and behavioral changes. Dynamic chromatin modifications that emerge in a time-dependent and promoter-specific fashion are thought to be critical for both the transient and long-term transcriptional regulation following ECS. While epigenetic mechanisms have been implicated in ECS-evoked gene regulation (de Jong et al., 2014), thus far, few studies have addressed whether components of the epigenetic machinery themselves serve as transcriptional targets of ECS.

Epigenetic mechanisms involve: (1) regulation of transcription by modifications of histone proteins catalyzed by 2 classes of enzymes namely writers, histone acetyltransferases (HATs) and histone methyltransferases (HMTs), and erasers, histone deacetylases (HDACs) and histone demethylases; (2) modification of DNA via the addition or removal of methyl groups on cytosine residues by DNA methyltransferases (DNMTs) and DNA demethylases, respectively; and (3) noncoding RNA-based epigenetic regulation that influences chromatin structure and the targeting of epigenetic regulatory complexes to specific gene loci (Jakovcevski and Akbarian, 2012). Epigenetic mechanisms play a critical role in the coupling of neuronal activity to gene regulation, which contributes to the facilitation of synaptic and structural plasticity (Sweatt, 2013). Several studies have examined the consequences of ECS on histone and DNA modifications at candidate gene loci, primarily focusing on growth factor (Tsankova et al., 2004), immediate-early genes, and transcription factor genes (Dyrvig et al., 2012). However, only a few reports have directly examined whether ECS or neuronal activity can influence the expression of epigenetic enzymatic machinery itself. ECS treatment can perturb the expression of *Hdac2* (Park et al., 2014b), *Sirt1* (Chung et al., 2013), *Jmjd3* (Link et al., 2015), *Gadd45b* (Ma et al., 2009; Jun et al., 2015), and *Dnmt1* and *Dnmt3a* (Dyrvig et al., 2015) and is associated with alterations in the global methylation profile, primarily within the hippocampus (Guo et al., 2011a). Further, neuronal activity regulates the gene expression of the Ten-eleven translocation (TET) proteins, *Tet1* and *Tet3*, which catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (Kaas et al., 2013; Yu et al., 2015). Here we have performed an extensive analysis of the influence of ECS on the gene expression of histone and DNA modifiers of the eraser and writer classes as well as methyl-CpG-binding proteins within the hippocampus, a brain region implicated in the cellular and behavioral effects of ECS. Our findings indicate that acute and chronic ECS evoke a predominantly distinct, and in specific cases bidirectional, pattern of regulation of histone and DNA modifying enzymes, as well as methyl-CpG-binding proteins, with only a few specific examples of overlapping regulation.

Methods

Animals

Adult male Wistar rats (3 months, 250–300 g) bred in the Tata Institute of Fundamental Research (TIFR) animal house facility were used for all experiments. Animals were group housed with 3 to 4 animals per cage and maintained on a 12-hour normal light-dark cycle (lights on: 7 AM, lights off: 7 PM) with ad libitum access to food and water. All experimental procedures were in accordance with the guidelines of the Committee for the Supervision and Care of Experimental Animals, Government of India and were approved by the TIFR Institutional Animal Ethics committee.

ECS Paradigm

Animals received ECS through ear-clip electrodes (ECS unit, UGO Basile, Comerio, Italy) (frequency: 100 pulses/s; pulse width: 0.9 milliseconds; pulse duration: 0.5 seconds; current: 70 mA). Sham-treated controls received the application of ear-clip electrodes without electrical stimulation. For acute ECS, animals were subjected to a single ECS or sham treatment 2 hours prior to sacrifice (n = 6/group). For chronic ECS, animals received ECS or sham treatment once daily for 7 consecutive days and were sacrificed 2 hours post the final ECS treatment (n = 10/group). Animals were rapidly decapitated and hippocampi were dissected, frozen in liquid nitrogen, and stored at -70°C until further use.

Quantitative Real-Time PCR (qPCR)

Hippocampal RNA was reverse transcribed (High capacity cDNA Reverse Transcription Kit, Applied Biosystems), and the synthesized cDNA was subjected to qPCR (Bio-Rad, Hercules, CA) with primers specific to the genes of interest (supplementary Table 1). qPCR analysis was performed to assess the expression of epigenetic machinery, namely: histone acetyl transferases (HATs): cyclic-AMP response element binding (CREB) binding protein (*Crebbp*), histone acetyltransferase 1 (*Hat1*), K(lysine) acetyltransferase 2a (*Kat2a*), K(lysine) acetyltransferase 5 (*Kat5*), K(lysine) acetyltransferase 6a (*Kat6a*), nuclear receptor coactivator 1 (*Ncoa1*), nuclear receptor coactivator 2 (*Ncoa2*), E1A binding protein 300 (*Ep300*); HDACs: histone deacetylases 1 through 11 (*Hdac1-Hdac11*); sirtuins: sirtuin 1 through 7 (*Sirt1-Sirt7*); HMTs: ASH1 (Absent, small, or homeotic)-like (Drosophila) (*Ash1l*), ASH2 (Absent, small, or homeotic)-like (Drosophila) (*Ash2l*), euchromatic histone-lysine N-methyltransferase 1 and 2 (*Ehmt1* and *Ehmt2*), enhancer of zeste homologue 1 and 2 (*Ezh1* and *Ezh2*), mixed lineage leukemia 1 through 3 (*Mll1-Mll3*), PR domain containing 2 (*Prdm2*), SET domain, bifurcated 1 (*Setdb1*), SET and MYND domain containing 3 (*Smyd3*), suppressor of variegation 3–9 homolog 1 (*Suv39h1*), suppressor of variegation 4–20 homolog 1 (*Suv420h1*); histone lysine demethylases (KDMs): jumonji domain containing 3 (*Jmjd3*), lysine(K)-specific demethylase 1A and 2A (*Kdm1a* and *Kdm2a*), PHD finger protein 8 (*Phf8*); DNMTs: DNA (cytosine-5)-methyltransferase 1, 3a, and 3b (*Dnmt1*, *Dnmt3a*, and *Dnmt3b*); DNA demethylases: growth arrest and DNA-damage-inducible protein alpha and beta (*Gadd45a* and *Gadd45b*), TET methylcytosine dioxygenase 1–3 (*Tet1-Tet3*); methyl-CpG-binding proteins: methyl-CpG-binding domain

protein 1 to 4 (*Mbd1-Mbd4*), and methyl-CpG-binding protein 2 (*Mecp2*). qPCR results were internally normalized to the endogenous housekeeping gene, hypoxanthine-guanine phosphoribosyltransferase (*Hprt*), which was unaltered across the sham and ECS groups. Data analysis was performed using the $\Delta\Delta$Ct method as described previously (Bookout and Mangelsdorf, 2003) and is represented as the fold change ± SEM.

Western-Blot Analysis

Hippocampal tissue lysates from the acute (n=6 for sham, n=7 for acute ECS) and chronic (n=4 for sham, n=3 for chronic ECS) ECS experiments were separated by SDS polyacrylamide gel electrophoresis and proteins were transferred to polyvinyldifluoride membranes (GE Healthcare). Following blocking, blots were incubated with rabbit anti-H3 acetylation (H3ac, 1:1000; Abcam), rabbit anti-H3K9 acetylation (H3K9ac, 1:1000; Abcam), rabbit anti-H3K4 trimethylation (H3K4me3, 1:1000; Millipore Bioscience Research Reagents), mouse anti-H3K9 dimethylation (H3K9me2, 1:1000; Abcam), rabbit anti-H3K9 trimethylation (H3K9me3, 1:1000; Abcam), rabbit anti-H3K27 trimethylation (H3K27me3, 1:1000; Abcam), or rabbit anti-H3 (H3, 1:1000; Abcam) antibodies. Blots were also probed with rabbit anti-beta-III-tubulin (1:5000; Covance) antibody as the loading control. Immunoblots were incubated with horseradish peroxidase-conjugated donkey anti-rabbit or sheep anti-mouse secondary antibodies (1:10000, GE Healthcare) and were visualized with an ECL substrate (GE Healthcare). Normalization of blots for histone modifications was done to the total amount of histone H3. Densitometric analysis was performed using Image J 1.48 (NIH).

Statistical Analysis

Statistical analysis was performed using the unpaired Student's *t* test with significance determined at $P<.05$ (Prism, Graphpad Software Inc.). We have not applied multiple testing corrections to our qPCR data analysis and have reported uncorrected exact *P* values in all figures. In the absence of corrections applied for multiple testing and significance set at $P<.05$, if all null hypotheses were true there would be a 5% chance of obtaining uncorrected $P<.05$.

Results

Acute and Chronic ECS Differentially Regulate the Expression of HATs and HDACs in the Adult Rat Hippocampus

We examined whether acute and chronic ECS (Figure 1A-E) altered the hippocampal expression of HATs and HDACs that catalyze the addition or removal of acetyl groups from histone tails, respectively. Acute ECS significantly enhanced the expression of *Crebbp* and *Hat1* and reduced the expression of *Kat2a*, *Ncoa1*, and *Ncoa2* (Figure 1B). Acute ECS also evoked a significant decline in the expression of the class I HDAC, *Hdac3*, the class IIa HDACs, *Hdac4* and *Hdac9*, the class IIb HDAC, *Hdac10* and the class IV HDAC, *Hdac11* (Figure 1B). Acute ECS also altered the expression of specific members of the Sirtuin family of NAD-dependent HDACs that deacetylate both histone and nonhistone proteins. Acute ECS downregulated the expression of the sirtuins *Sirt4*, *Sirt5*, and *Sirt6*, and enhanced *Sirt7* gene expression (Figure 1B).

We next addressed whether chronic ECS (Figure 1C) had similar or distinct effects on the hippocampal expression of HATs and HDACs. Strikingly, chronic ECS did not alter the expression of any of the HATs examined (Figure 1D), including those HATs (*Crebbp*, *Hat1*, *Kat2a*, *Ncoa1*, and *Ncoa2*) that were regulated by acute ECS (Figure 1B). We did note a trend ($P=.052$) towards a decline in *Crebbp* expression following chronic ECS. Chronic ECS evoked an upregulation of several HDACs, including the class I HDACs *Hdac1*, *Hdac3*, and *Hdac8* and the class IIa HDACs *Hdac4* and *Hdac5* (Figure 1D). We observed a trend ($P=.053$) towards an upregulation in the expression of the class IV HDAC *Hdac11*. Chronic ECS enhanced *Sirt1* and decreased *Sirt4* gene expression (Figure 1D). Acute and chronic ECS evoked strikingly distinct patterns of regulation of both HATs and HDACs, with specific HDACs showing an opposing pattern of regulation and the only overlap restricted to the reduction in *Sirt4* expression.

Acute and Chronic ECS Alter the Expression of HMTs and KDMs in the Adult Rat Hippocampus

We next profiled the influence of acute and chronic ECS on the expression of the histone methylation machinery, namely HMTs and KDMs (Figure 2A-E). Acute ECS predominantly evoked a decline in the expression of several HMTs, including *Ehmt1*, *Ezh2*, *Mll3*, *Prdm2*, *Setdb1*, *Smyd3*, and *Suv39h1* (Figure 2B). The exception to this pattern of regulation was *Ezh1*, which exhibited significant upregulation following acute ECS (Figure 2B). Amongst the KDMs examined, we noted a robust 2.8-fold upregulation of *Jmjd3* mRNA and a decline in *Phf8* expression following acute ECS (Figure 2B).

Several HMTs that were regulated by acute ECS (*Ehmt1*, *Ezh1*, *Prdm2*, *Setdb1*, *Smyd3*, and *Suv39h1*) did not show any change following chronic ECS (Figure 2D), with the exception of *Ezh2* that showed a contrasting upregulation after chronic ECS. Chronic ECS also evoked a decline in *Ash2l* and *Mll3* mRNA expression (Figure 2D). Similar to the acute ECS regulation of KDMs, chronic ECS evoked a robust and significant 2.4-fold induction of *Jmjd3* mRNA levels (Figure 2D). *Kdm1a* expression was enhanced following chronic ECS, whereas the acute ECS-evoked decline in *Phf8* was lost following chronic ECS (Figure 2D). Acute and chronic ECS evoke largely distinct changes in the hippocampal expression of HMTs and KDMs, with a common pattern noted for *Jmjd3* and *Mll3* expression and an opposing regulation of *Ezh2* expression.

Influence of Acute and Chronic ECS on Global and Residue-Specific Histone Acetylation and Methylation Marks

We sought to address whether the transcriptional changes in histone modifiers observed following acute and chronic ECS were associated with alterations of global and residue-specific histone acetylation and methylation marks. Immunoblot analysis revealed that acute ECS did not influence residue-specific histone methylation (H3K9me2, H3K9me3, H3K27me3, and H3K4me3) (Figure 3A-E) levels and did not alter global or residue-specific histone acetylation (pan H3ac, H3K9ac) levels (Figure 3A,F,G). Chronic ECS did not influence levels of residue-specific histone methylation marks (H3K9me2, H3K9me3, H3K27me3, and H3K4me3) (Figure 3H-L). While global histone acetylation (pan H3ac) did not differ between sham and chronic ECS groups (Figure 3H,M), we noted a significant decline in hippocampal levels of residue-specific histone acetylation (H3K9ac) following chronic ECS (Figure 3H,N).
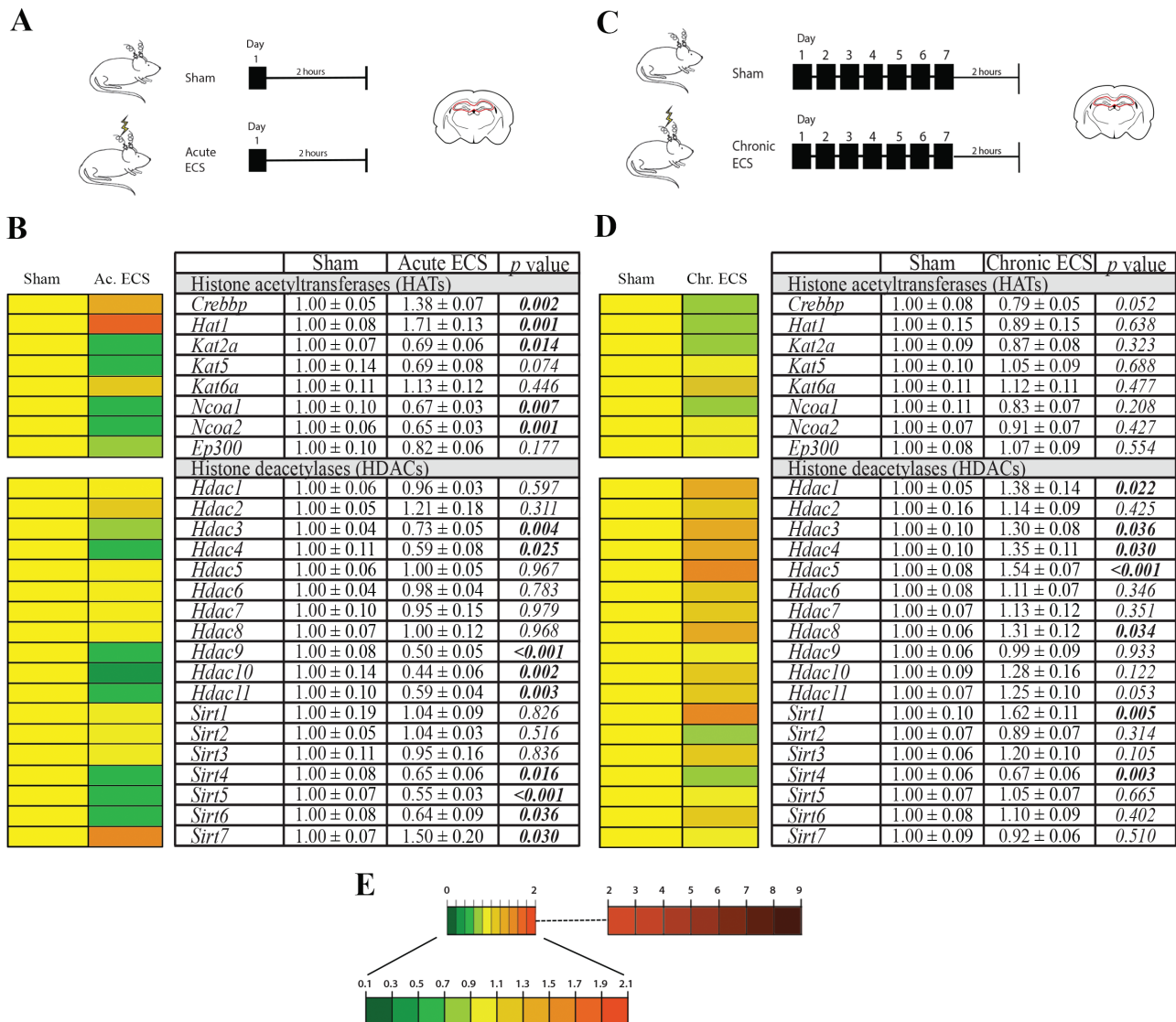
Figure 1. Influence of acute and chronic electroconvulsive seizure (ECS) on the expression of histone acetyl transferases (HATs) and histone deacetylases (HDACs) in the rat hippocampus. Shown is the experimental design for acute ECS (Ac. ECS, A) and chronic ECS (Chr. ECS, C) treatment. Represented are normalized gene expression levels in the hippocampus for specific HATs and HDACs following acute ECS (B) and chronic ECS (D) treatment compared with their respective sham groups. Heat maps indicate the magnitude of regulation, with upregulated genes shown in red and downregulated genes shown in green (key, E). Quantitative real-time PCR (qPCR) analysis results indicate differential regulation of several of the HATs and HDACs profiled following acute or chronic ECS treatment. The pattern of gene regulation shows a predominantly distinct, and in specific cases opposing, regulation following acute vs chronic ECS. The data are represented as fold change ± SEM with significance determined at *P < .05, Student's *t* test (acute ECS: n = 6/group, chronic ECS: n = 10/group).

Acute and Chronic ECS Alter the Expression of DNMTs, DNA Demethylases, and Methyl-CpG-Binding Proteins in the Adult Rat Hippocampus

We next assessed the influence of acute and chronic ECS on the transcriptional regulation of DNMTs and DNA demethylases that regulate the addition/maintenance and removal of methyl residues at cytosine nucleotides in the genome, respectively (Figure 4A-E). We also examined effects on the expression of methyl-CpG-binding proteins that specifically bind methylated DNA and serve as recruiting hubs for chromatin remodeling machinery (Hashimoto et al., 2010). Acute ECS significantly reduced the hippocampal expression of *Dnmt1* and enhanced *Dnmt3a* expression (Figure 4B). The DNA demethylases *Gadd45a* and *Gadd45b* exhibited an opposing pattern, with a decline in *Gadd45a* mRNA levels and a robust 7.5-fold induction in *Gadd45b*

expression following acute ECS. Further, the Tet enzymes *Tet2* and *Tet3* were upregulated following acute ECS. Amongst the methyl-CpG-binding proteins, *Mbd3* and *Mbd4* were downregulated following acute ECS.

The effects of chronic ECS on DNA methylation machinery showed both distinct and overlapping patterns of regulation to those observed with acute ECS. While none of the DNMTs profiled exhibited a significant regulation following chronic ECS, we noted a trend (P = .053) towards an increase in *Dnmt1* mRNA levels following chronic ECS. Similar to acute ECS, chronic ECS also evoked significant increases in *Gadd45b*, *Tet2*, and *Tet3* expression. The exception to this pattern was the upregulation of *Gadd45a* expression following chronic ECS, contrasting with the decline noted following acute ECS. Chronic ECS induced a significant increase in hippocampal *Mbd1* and *Mbd3* mRNA levels. Taken together, these results indicate that while acute and
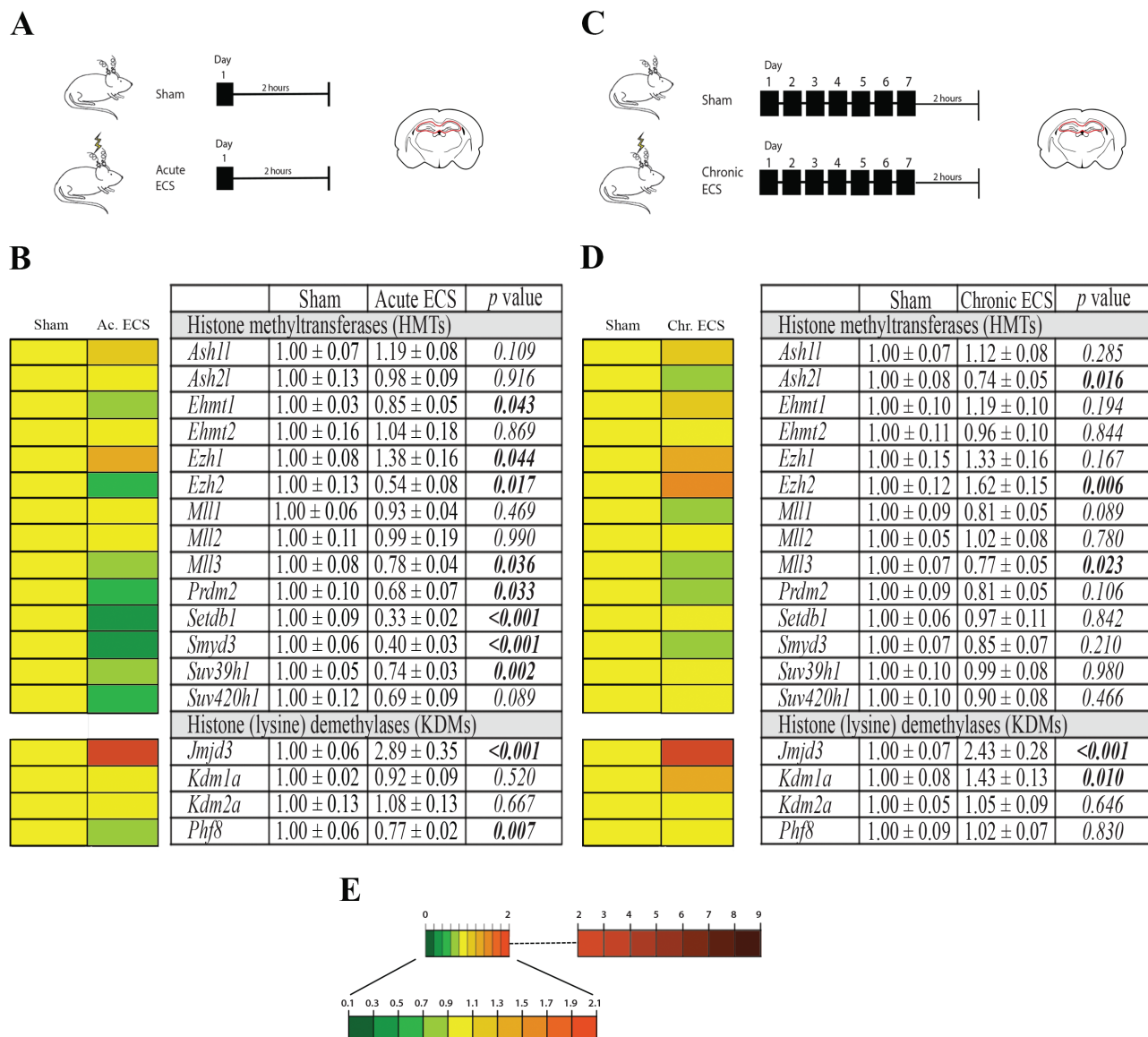
Figure 2. Influence of acute and chronic electroconvulsive seizure (ECS) on the expression of histone methyltransferases (HMTs) and histone (lysine) demethylases (KDMs) in the rat hippocampus. Shown is the experimental design for acute ECS (Ac. ECS, A) and chronic ECS (Chr. ECS, C) treatment. Shown are normalized gene expression levels in the hippocampus for specific HMTs and KDMs relative to sham following acute ECS (B) and chronic ECS (D) treatment. Heat maps indicate the magnitude of regulation, with upregulated genes shown in red and downregulated genes shown in green (key, E). qPCR analysis results revealed differential regulation of several HMTs and KDMs in response to acute or chronic ECS. The data are represented as fold change ± SEM with significance determined at * $P < .05$, Student's t test (acute ECS: n = 6/group, chronic ECS: n = 10/group).

Panel B:

	Sham	Acute ECS	*p* value
Histone methyltransferases (HMTs)			
Ash1l	1.00 ± 0.07	1.19 ± 0.08	*0.109*
Ash2l	1.00 ± 0.13	0.98 ± 0.09	*0.916*
Ehmt1	1.00 ± 0.03	0.85 ± 0.05	***0.043***
Ehmt2	1.00 ± 0.16	1.04 ± 0.18	*0.869*
Ezh1	1.00 ± 0.08	1.38 ± 0.16	***0.044***
Ezh2	1.00 ± 0.13	0.54 ± 0.08	***0.017***
Mll1	1.00 ± 0.06	0.93 ± 0.04	*0.469*
Mll2	1.00 ± 0.11	0.99 ± 0.19	*0.990*
Mll3	1.00 ± 0.08	0.78 ± 0.04	***0.036***
Prdm2	1.00 ± 0.10	0.68 ± 0.07	***0.033***
Setdb1	1.00 ± 0.09	0.33 ± 0.02	***<0.001***
Smyd3	1.00 ± 0.06	0.40 ± 0.03	***<0.001***
Suv39h1	1.00 ± 0.05	0.74 ± 0.03	***0.002***
Suv420h1	1.00 ± 0.12	0.69 ± 0.09	*0.089*
Histone (lysine) demethylases (KDMs)			
Jmjd3	1.00 ± 0.06	2.89 ± 0.35	***<0.001***
Kdm1a	1.00 ± 0.02	0.92 ± 0.09	*0.520*
Kdm2a	1.00 ± 0.13	1.08 ± 0.13	*0.667*
Phf8	1.00 ± 0.06	0.77 ± 0.02	***0.007***

Panel D:

	Sham	Chronic ECS	*p* value
Histone methyltransferases (HMTs)			
Ash1l	1.00 ± 0.07	1.12 ± 0.08	*0.285*
Ash2l	1.00 ± 0.08	0.74 ± 0.05	***0.016***
Ehmt1	1.00 ± 0.10	1.19 ± 0.10	*0.194*
Ehmt2	1.00 ± 0.11	0.96 ± 0.10	*0.844*
Ezh1	1.00 ± 0.15	1.33 ± 0.16	*0.167*
Ezh2	1.00 ± 0.12	1.62 ± 0.15	***0.006***
Mll1	1.00 ± 0.09	0.81 ± 0.05	*0.089*
Mll2	1.00 ± 0.05	1.02 ± 0.08	*0.780*
Mll3	1.00 ± 0.07	0.77 ± 0.05	***0.023***
Prdm2	1.00 ± 0.09	0.81 ± 0.05	*0.106*
Setdb1	1.00 ± 0.06	0.97 ± 0.11	*0.842*
Smyd3	1.00 ± 0.07	0.85 ± 0.07	*0.210*
Suv39h1	1.00 ± 0.10	0.99 ± 0.08	*0.980*
Suv420h1	1.00 ± 0.10	0.90 ± 0.08	*0.466*
Histone (lysine) demethylases (KDMs)			
Jmjd3	1.00 ± 0.07	2.43 ± 0.28	***<0.001***
Kdm1a	1.00 ± 0.08	1.43 ± 0.13	***0.010***
Kdm2a	1.00 ± 0.05	1.05 ± 0.09	*0.646*
Phf8	1.00 ± 0.09	1.02 ± 0.07	*0.830*

chronic ECS evoke distinct effects on the expression of DNMTs and methyl-CpG-binding proteins, a similar pattern of regulation was noted in specific DNA demethylases with significant increases in *Gadd45b*, *Tet2*, and *Tet3*.

For all the gene expression analysis performed with qPCR, we have reported the uncorrected, exact *P* values in the figures. We have not performed corrections for multiple testing on the data, hence even if all null hypotheses were true there would still be a 5% chance across all genes profiled of obtaining uncorrected *P* < .05.

Discussion

The major novel finding of our study is that acute and chronic ECS led to discrete and largely nonoverlapping changes in the hippocampal gene expression of several HATs, HDACs, HMTs,

KDMs, DNMTs, DNA demethylases, and methyl-CpG-binding proteins (Figure 5). ECS has been hypothesized to couple transient neuronal activity to transcriptional regulation, possibly through epigenetic mechanisms, thus establishing a substrate to mediate long-lasting effects on synaptic and structural plasticity (de Jong et al., 2014). An intriguing possibility is that ECS may transcriptionally target epigenetic enzymatic machinery itself, hence leading to potential changes in chromatin remodeling, an altered chromatin landscape, and persistent effects on gene regulation. Previous reports suggest that the expression of specific histone and DNA modifiers (Park et al., 2014b; Dyrvig et al., 2015) and their recruitment to immediate-early gene and neurotrophic factor loci (Tsankova et al., 2004; Dyrvig et al., 2012) may be altered by ECS. To the best of our knowledge, our results provide the first comprehensive profiling of the effects of ECS on the transcriptional regulation of several classes of histone and
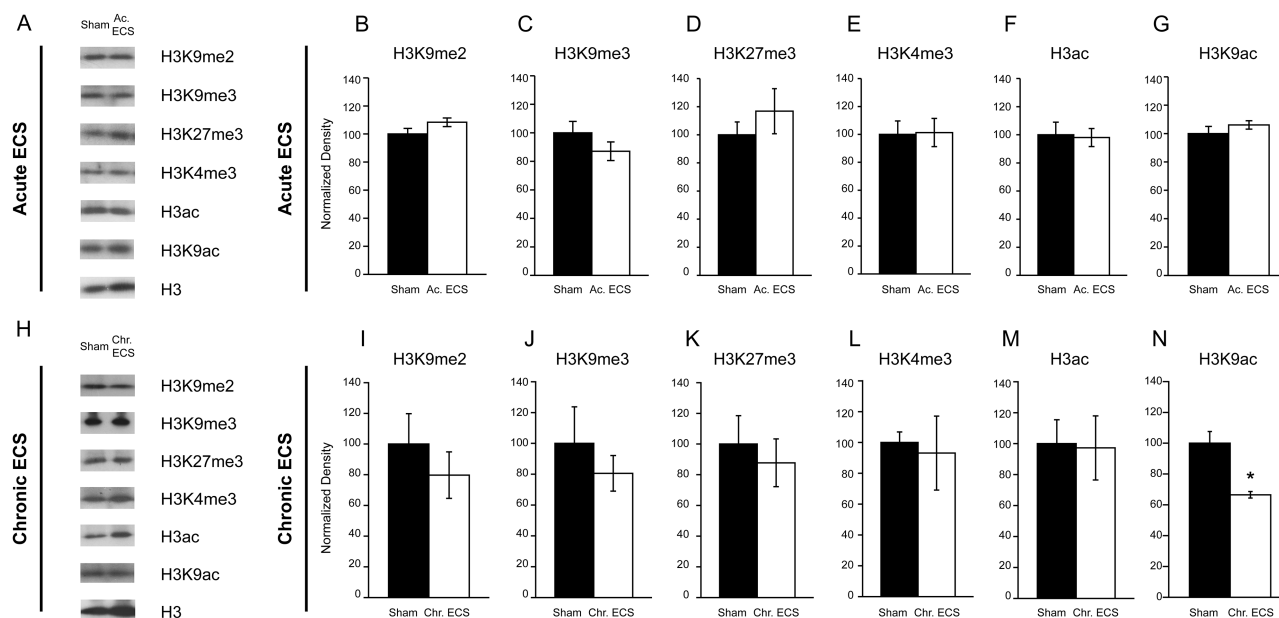
Figure 3. Influence of acute and chronic electroconvulsive seizure (ECS) on global and residue-specific levels of histone methylation and acetylation in the rat hippocampus. Hippocampal levels of residue-specific histone methylation (H3K9me2, H3K9me3, H3K27Me3, H3K4me3), and global, as well as residue-specific, histone acetylation (H3ac, H3K9ac) were determined by western-blot analysis in sham and ECS-treated animals following acute (A-G) and chronic ECS (H-N). The levels of the H3K9me2, H3K9me3, H3K27me3, H3K4me3, H3ac, and H3K9ac were normalized to H3 levels. Shown are representative images from acute (A) and chronic (H) ECS animals with their respective sham controls. Quantitative densitometric analysis is represented as percent of the sham controls. The results revealed no significant influence of acute or chronic ECS on levels of H3K9me2 (acute ECS: A, B; and chronic ECS: H, I), H3K9me3 (acute ECS: A, C; and chronic ECS: H, J), H3K27me3 (acute ECS: A, D; and chronic ECS: H, K), H3K4me3 (acute ECS: A, E; and chronic ECS: H, L) and H3ac (acute ECS: A, F; and chronic ECS: H, M). While acute ECS did not alter levels of H3K9ac (A, G), a significant decline in hippocampal H3K9ac levels was observed following chronic ECS treatment (H, N). Significance was determined at *$P < .05$, Student's t test, acute ECS experiment: (sham, n = 6, acute ECS, n = 7) chronic ECS experiment: (sham, n = 4, chronic ECS, n = 3).

DNA modifiers, as well as methyl-CpG-binding proteins, and highlight their distinctive pattern of regulation by both acute and chronic ECS.

ECS-Mediated Regulation of HATs and HDACs

Acute and chronic ECS induce differential effects on HATs, with altered hippocampal expression of several HATs following a single ECS (upregulation: *Hat1*, *Crebbp*; downregulation: *Kat2a*, *Ncoa1*, *Ncoa2*) and no regulation noted following repeated seizures. Amongst the HATs upregulated by acute ECS are *Hat1* and *Crebbp*, which are linked to the regulation of adult neurogenesis (Lim et al., 2006; Lopez-Atalaya et al., 2011), and to the regulation of brain-derived growth factor (*Bdnf*) gene expression (Tian et al., 2009) synaptic plasticity (Wood, 2005; Barrett et al., 2011) and cognitive function (Korzus et al., 2004; Chen et al., 2010). Acute ECS also resulted in a decline in expression of *Ncoa1* and *Ncoa2*, which are transcriptional coregulators of steroid receptors (Tetel and Acharya, 2013) and play a critical role in regulating central stress responsivity (Patchev et al., 2007; Lachize et al., 2009). It is interesting that the regulation of HAT expression noted following a single seizure is lost upon repeated seizure administration, suggesting a possible desensitization of the transcriptional regulation of HATs when exposed to repeated bouts of neuronal activity.

The gene regulation of HDACs by acute vs chronic ECS was distinct, with a decline in several HDACs following acute ECS and an opposing upregulation after chronic ECS. Chronic ECS robustly upregulated several HDACs (*Hdac1*, *3*, *4*, *5*, and *8*), that are known to catalyze the removal of acetyl moieties from H3K9 and may mediate the global hypo-acetylation of H3K9 following repeated seizures (Bhaskara et al., 2010; Dovey et al., 2010; Fu

et al., 2014). The H3K9ac mark is highly correlated with active promoters and enhancers and is associated with transcriptionally active chromatin (Karmodiya et al., 2012). A global reduction of H3K9 acetylation marks within the hippocampus points towards major changes that arise in the epigenetic landscape following chronic ECS. Amongst the HDACs regulated, HDAC3, which is required for neural stem cell division (Jiang and Hsieh, 2014), exhibited a decline in expression following acute and an increase following chronic ECS. It is tempting to speculate that such a shift may contribute to the greater induction of neural stem cell turnover (~5 fold) observed with chronic ECS as compared with that noted with acute ECS (~1.7 fold) (Segi-Nishida et al., 2008). One important factor to consider is that ECS treatment robustly enhances neuronal progenitor proliferation and increases newborn neurons, which could also contribute to the nature of regulation of epigenetic machinery observed following ECS in our study, although progenitors and immature neurons represent a relatively small percentage of the total number of hippocampal cells.

HDAC inhibition, either stand-alone or in combination with pharmacological antidepressants, ameliorates depressive behavior in animal models of depression (Tsankova et al., 2006; Covington et al., 2009, 2011; Han et al., 2014; Sarkar et al., 2014). It is important to note that all our studies were performed in animals that did not have either a history of stress and did not exhibit depressive-like behavior. The nature of regulation of epigenetic machinery by ECS may indeed differ in animal models of depressive-like behavior, which more closely mimic the clinical scenario. The fact that chronic ECS enhances the hippocampal expression of several HDACs may appear counterintuitive, given that HDAC inhibition is linked to improved mood-related behavior. However, HDAC activity is also required for the effects of
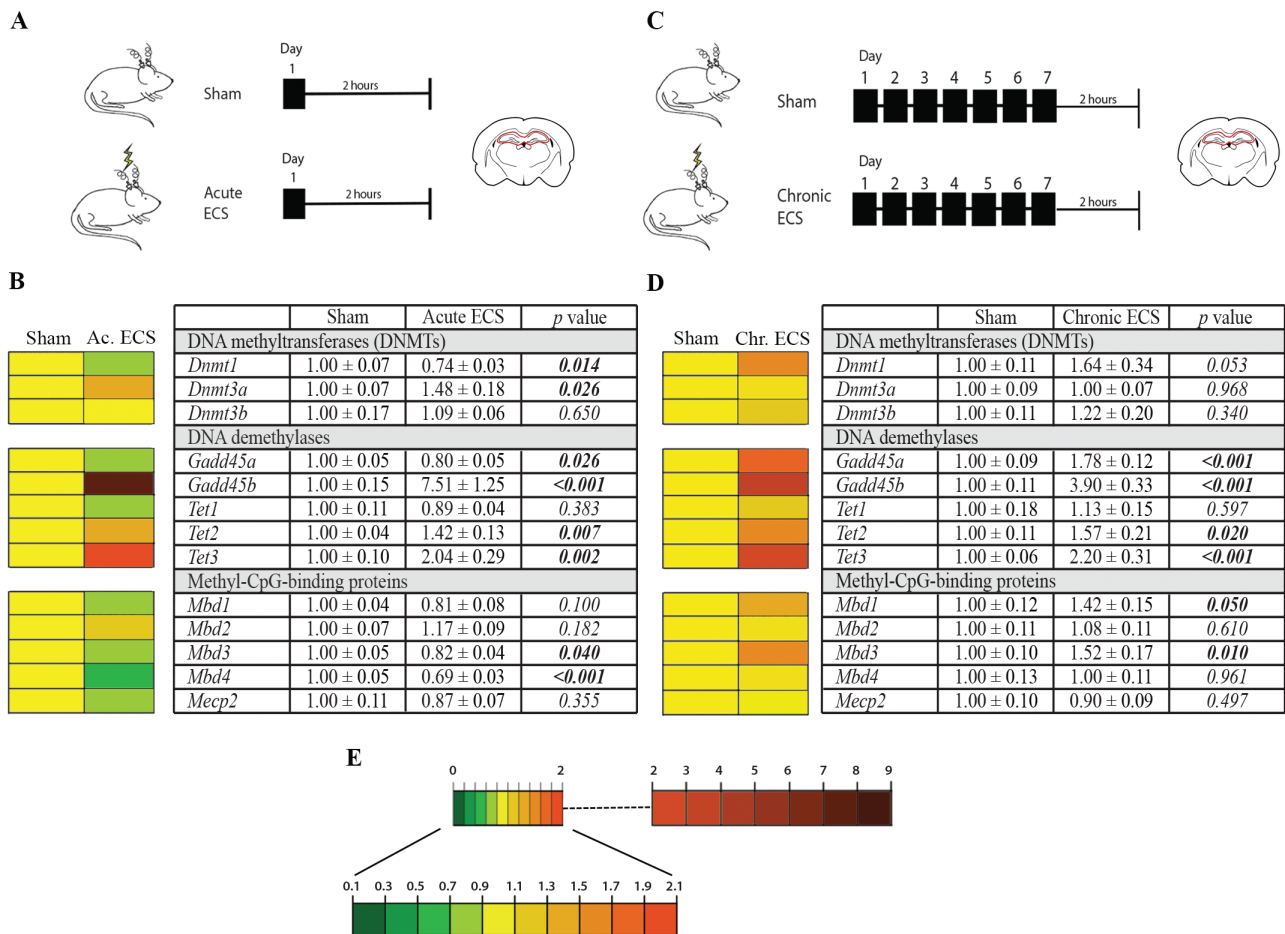
Figure 4. Influence of acute and chronic electroconvulsive seizure (ECS) on the expression of DNA methylation machinery in the rat hippocampus. Shown is the experimental design for acute ECS (Ac. ECS, A) and chronic ECS (Chr. ECS, C) treatment. Shown are normalized gene expression levels in the hippocampus for specific DNMTs, DNA demethylases, and methyl-CpG-binding proteins relative to sham groups following acute ECS (B) and chronic ECS (D) treatment. Heat maps indicate the magnitude of regulation, with upregulated genes shown in red and downregulated genes shown in green (key, E). Quantitative real-time PCR (qPCR) analysis revealed differential regulation of the hippocampal expression of several DNMTs, DNA demethylases, and methyl-CpG-binding proteins in response to acute or chronic ECS. The pattern of regulation includes distinct, overlapping as well as opposing changes in specific DNMTs, DNA demethylases, and methyl-CpG-binding proteins. The data are represented as fold change ± SEM with significance determined at * $P < .05$, Student's t test (acute ECS: n = 6/group, chronic ECS: n = 10/group).

BDNF on excitatory neurotransmission and spine density in the hippocampus (Calfa et al., 2012). This raises the possibility that the robust induction of several HDACs by chronic ECS may play a role in facilitating the cellular and molecular effects of BDNF, a trophic factor implicated in the regulation of hippocampal neurogenesis and mood-related behavior (Schmidt and Duman, 2007; Björkholm and Monteggia, 2016).

Acute and chronic ECS evoked a common decline in *Sirt4* mRNA levels. Acute ECS also regulated *Sirt5*, *Sirt6*, and *Sirt7* expression; chronic ECS did not exhibit these changes, but rather induced an upregulation of *Sirt1* expression. While few studies have directly examined the importance of the sirtuins Sirt 4 to 7 in the brain, several studies have focused on the critical role of Sirt1 in the regulation of cognitive function (Michán et al., 2010), hippocampal neurogenesis (Saharan et al., 2013), dendritic remodeling (Codocedo et al., 2012), synaptic plasticity (Gao et al., 2010), neuronal survival (Guo et al., 2011b), and mood-related behavior (Libert et al., 2011). A previous report indicates enhanced Sirt1 immunoreactivity observed in the mouse hippocampus following acute ECS (Chung et al., 2013). The discrepancy in our results and this report may arise due to differences in species used and the methods employed. Taken together, our

findings highlight the widespread regulation of several HATs and HDACs by acute ECS and indicate a strikingly differing pattern of regulation following repeated seizure administration.

ECS-Mediated Regulation of HMTs and KDMs

Acute and chronic ECS evoked a distinct pattern of gene regulation of HMTs, with a broader extent of gene regulation noted with acute ECS and a restricted pattern following chronic ECS (Figure 5). Interestingly, acute ECS evokes a decline in *Suv39h1*, the depletion of which has been previously reported to enhance BDNF-mediated neuritogenesis (Sen and Snyder, 2011). Acute and chronic ECS upregulated *Ezh1* and *Ezh2*, respectively, which regulate repressive H3K27 methylation (Margueron et al., 2008; Shen et al., 2008). Given that ECS increases PSD-95 expression (Dyrvig et al., 2014) and synaptogenesis (Chen et al., 2009), it is interesting that *Psd-95* gene transcription is regulated by both Ezh1 and Ezh2 (Henriquez et al., 2013). Ezh2 has previously been implicated in the positive regulation of adult hippocampal neurogenesis and cognitive function (Zhang et al., 2014), and our results suggest that enhanced *Ezh2* expression may contribute to the neurogenic effects of chronic ECS.
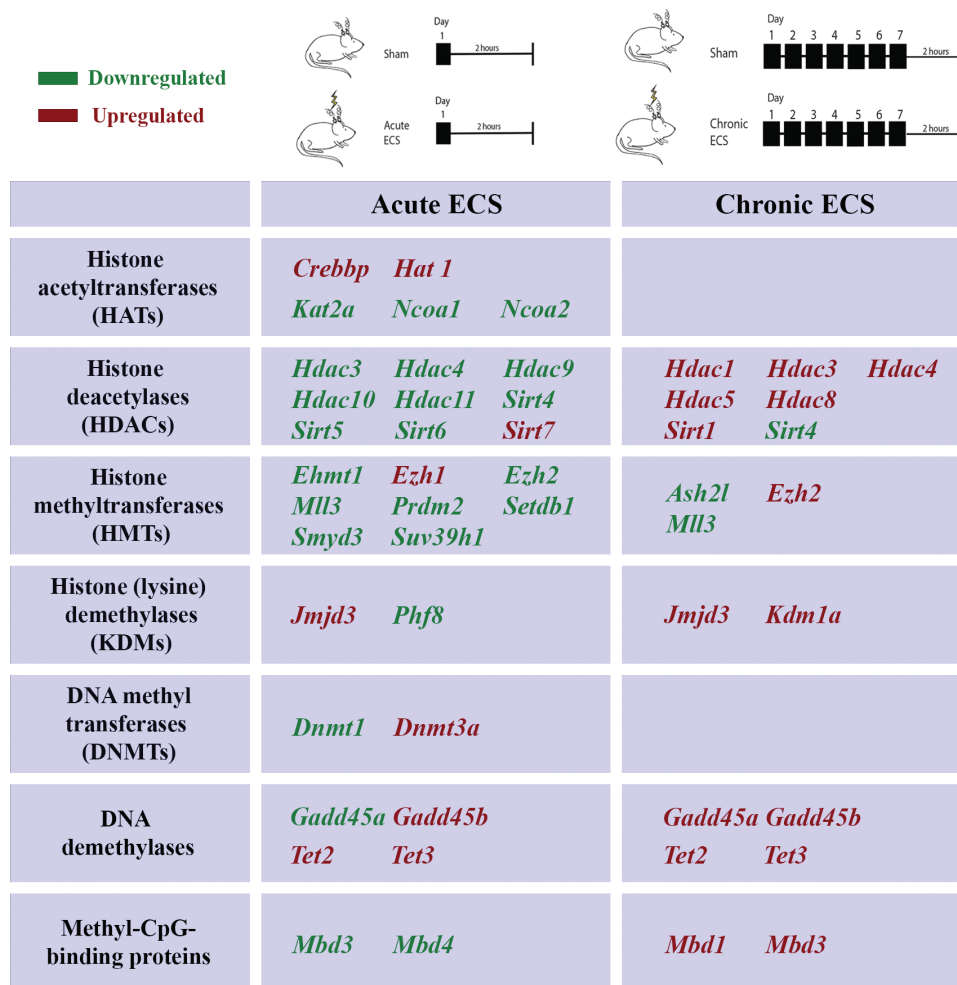
	Acute ECS			Chronic ECS		
Histone acetyltransferases (HATs)	*Crebbp*	*Hat 1*				
	Kat2a	*Ncoa1*	*Ncoa2*			
Histone deacetylases (HDACs)	*Hdac3*	*Hdac4*	*Hdac9*	*Hdac1*	*Hdac3*	*Hdac4*
	Hdac10	*Hdac11*	*Sirt4*	*Hdac5*	*Hdac8*	
	Sirt5	*Sirt6*	*Sirt7*	*Sirt1*	*Sirt4*	
Histone methyltransferases (HMTs)	*Ehmt1*	*Ezh1*	*Ezh2*	*Ash2l*	*Ezh2*	
	Mll3	*Prdm2*	*Setdb1*	*Mll3*		
	Smyd3	*Suv39h1*				
Histone (lysine) demethylases (KDMs)	*Jmjd3*	*Phf8*		*Jmjd3*	*Kdm1a*	
DNA methyl transferases (DNMTs)	*Dnmt1*	*Dnmt3a*				
DNA demethylases	*Gadd45a*	*Gadd45b*		*Gadd45a*	*Gadd45b*	
	Tet2	*Tet3*		*Tet2*	*Tet3*	
Methyl-CpG-binding proteins	*Mbd3*	*Mbd4*		*Mbd1*	*Mbd3*	

Figure 5. Transcriptional regulation of histone and DNA modifiers and methyl-CpG-binding proteins following acute and chronic electroconvulsive seizure (ECS). Shown is a summary of the transcriptional changes evoked in the hippocampal expression of both histone and DNA modifiers, as well as methyl-CpG-binding proteins, following acute or chronic ECS. We extensively profiled the effects of acute and chronic ECS on several histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone (lysine) demethylases (KDMs), DNA methyltransferases (DNMTs), DNA demethylases, and methyl-CpG-binding proteins. This figure highlights the predominantly distinct pattern of gene regulation of histone and DNA modifying enzymes evoked by acute vs chronic ECS. We also observed specific cases of opposing patterns of regulation (*Hdac3, Hdac4, Ezh2, Gadd45a, Mbd3*) following acute and chronic ECS. The genes that showed an overlapping pattern of regulation with both acute and chronic ECS were *Sirt4, Mll3, Jmjd3, Gadd45b, Tet2,* and *Tet3*. Red indicates upregulation and green indicates downregulation of mRNA levels.

In our profiling of the regulation of KDMs that catalyze the removal of histone methylation marks, we noted a robust induction of *Jmjd3* hippocampal expression following both acute (2.8-fold) and chronic (2.4-fold) ECS. Our results are in agreement with a previous report that indicates a significant induction of *Jmjd3* in the hippocampus 2 hours following acute ECS (Link et al., 2015). JMJD3 catalyzes the removal of methyl groups from H3K27me3, a repressive epigenetic mark, and has been shown to play a vital role in the regulation of adult neurogenesis, through the regulation of key neurogenic genes via interactions within both promoter and enhancer elements (Park et al., 2014a). Collectively, our results reveal a differing pattern of regulation of HMTs and KDMs in response to acute vs chronic ECS, with an overlap noted in the upregulation of catalytic components of the PRC2 complex and the demethylase, *Jmjd3*.

ECS-Mediated Regulation of Dnmts, DNA Demethylases, and Methyl-CpG-Binding Proteins

Acute ECS evoked a rapid decline in *Dnmt1* and an upregulation of *Dnmt3a* mRNA levels in the hippocampus. Our results

are consistent with a previous report that indicates a decline in *Dnmt1* and an increase in *Dnmt3a* mRNA levels 4 hours following acute ECS (Dyrvig et al., 2015). Given prior reports that neuronal activity evokes an altered DNA methylation landscape (Guo et al., 2011a), this supports the notion that acute ECS through its effects on both DNMT1, a maintenance methyltransferase, and DNMT3a, a de novo methyltransferase, may modulate gene expression through effects on DNA methylation. Interestingly, DNMT1 and DNMT3 have been implicated in the regulation of neurogenesis (Wu et al., 2010; Noguchi et al., 2015), synaptic plasticity, and cognitive performance (Miller et al., 2008; Feng et al., 2010).

The nature of gene regulation of DNA demethylases evoked by acute and chronic ECS was largely overlapping with an increased expression noted in *Gadd45b, Tet2,* and *Tet3* mRNA expression, with the exception of *Gadd45a* mRNA levels, which exhibited a bidirectional regulation. Gadd45b contributes to the neurogenic effects of ECS on quiescent neural progenitors (Ma et al., 2009; Jun et al., 2015) and has been speculated to target the promoters of specific bivalently poised neurogenic genes for

dynamic DNA demethylation (Ma et al., 2009). The TET family of dioxygenases catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, an epigenetic mark that is not only an intermediate in DNA demethylation but is an epigenetic signature which itself may modulate transcriptional regulation (Pastor et al., 2013). Both acute and chronic ECS robustly enhanced *Tet2* and *Tet3* mRNA levels within the hippocampus. Tet2 and Tet3 have been implicated in the regulation of neuronal survival (Mi et al., 2015), neuronal progenitor maintenance and differentiation (Li et al., 2014a), learning-evoked transcriptional regulation, and behavioral modifications (Li et al., 2014b). Our results provide impetus for future studies focused on examining the enrichment of 5-hydroxymethylcytosine levels in the hippocampus following ECS, both globally and in a gene-locus specific fashion.

Conclusion

Our results provide a comprehensive description of the transcriptional regulation of several histone and DNA modifying enzymes within the hippocampus following acute and chronic ECS. These results highlight the influence of ECS treatments on the gene expression of key epigenetic enzymatic machinery. Such a regulation of epigenetic modifiers may be critical in coupling transient changes in neuronal activity to long-lasting alterations within the epigenetic landscape and transcriptional alteration, thus contributing to the persistent cellular and behavioral effects that emerge post ECS treatment.

Acknowledgments

Statement of Interest

None.

References

Barrett RM, Malvaez M, Kramar E, Matheos DP, Arrizon A, Cabrera SM, Lynch G, Greene RW, Wood MA (2011) Hippocampal focal knockout of CBP affects specific histone modifications, long-term potentiation, and long-term memory. Neuropsychopharmacology 36:1545–1556.

Bhaskara S, Knutson SK, Jiang G, Chandrasekharan MB, Wilson AJ, Zheng S, Yenamandra A, Locke K, Yuan JL, Bonine-Summers AR, Wells CE, Kaiser JF, Washington MK, Zhao Z, Wagner FF, Sun ZW, Xia F, Holson EB, Khabele D, Hiebert SW (2010) Hdac3 is essential for the maintenance of chromatin structure and genome stability. Cancer Cell 18:436–447.

Björkholm C, Monteggia LM (2016) BDNF: a key transducer of antidepressant effects. Neuropharmacology 102:72–79.

Bookout AL, Mangelsdorf DJ (2003) Quantitative real-time PCR protocol for analysis of nuclear receptor signaling pathways. Nucl Recept Signal 1:e012.

Calfa G, Chapleau CA, Campbell S, Inoue T, Morse SJ, Lubin FD, Pozzo-Miller L (2012) HDAC activity is required for BDNF to

increase quantal neurotransmitter release and dendritic spine density in CA1 pyramidal neurons. Hippocampus 22:1493–1500.

Chen F, Madsen TM, Wegener G, Nyengaard JR (2009) Repeated electroconvulsive seizures increase the total number of synapses in adult male rat hippocampus. Eur Neuropsychopharmacol 19:329–338.

Chen G, Zou X, Watanabe H, van Deursen JM, Shen J (2010) CREB binding protein is required for both short-term and long-term memory formation. J Neurosci 30:13066–13077.

Chung S, Kim HJ, Yoon IS, Kim HJ, Choi SH, Kim YS, Shin KH (2013) Electroconvulsive shock increases SIRT1 immunoreactivity in the mouse hippocampus and hypothalamus. J ECT 29:93–100.

Codocedo JF, Allard C, Godoy JA, Varela-Nallar L, Inestrosa NC (2012) SIRT1 regulates dendritic development in hippocampal neurons. PLoS One 7:e47073.

Cole AJ, Abu-Shakra S, Saffen DW, Baraban JM, Worley PF (1990) Rapid rise in transcription factor mRNAs in rat brain after electroshock-induced seizures. J Neurochem 55:1920–1927.

Covington HE, Maze I, LaPlant QC, Vialou VF, Ohnishi YN, Berton O, Fass DM, Renthal W, Rush AJ, Wu EY, Ghose S, Krishnan V, Russo SJ, Tamminga C, Haggarty SJ, Nestler EJ (2009) Antidepressant actions of histone deacetylase inhibitors. J Neurosci 29:11451–11460.

Covington HE, Vialou VF, LaPlant Q, Ohnishi YN, Nestler EJ (2011) Hippocampal-dependent antidepressant-like activity of histone deacetylase inhibition. Neurosci Lett 493:122–126.

de Jong JOZ, Arts B, Boks MP, Sienaert P, van den Hove DL a, Kenis G, van Os J, Rutten BPF (2014) Epigenetic effects of electroconvulsive seizures. J ECT 30:152–159.

Dovey OM, Foster CT, Cowley SM (2010) Histone deacetylase 1 (HDAC1), but not HDAC2, controls embryonic stem cell differentiation. Proc Natl Acad Sci 107:8242–8247.

Dyrvig M, Hansen HH, Christiansen SH, Woldbye DPD, Mikkelsen JD, Lichota J (2012) Epigenetic regulation of Arc and c-Fos in the hippocampus after acute electroconvulsive stimulation in the rat. Brain Res Bull 88:507–513.

Dyrvig M, Christiansen SH, Woldbye DPD, Lichota J (2014) Temporal gene expression profile after acute electroconvulsive stimulation in the rat. Gene 539:8–14.

Dyrvig M, Gøtzsche CR, Woldbye DPD, Lichota J (2015) Epigenetic regulation of Dnmt3a and Arc gene expression after electroconvulsive stimulation in the rat. Mol Cell Neurosci 67:137–143.

Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, Silva AJ, Fan G (2010) Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 13:423–430.

Fu Y, Zhang P, Ge J, Cheng J, Dong W, Yuan H, Du Y, Yang M, Sun R, Jiang H (2014) Histone deacetylase 8 suppresses osteogenic differentiation of bone marrow stromal cells by inhibiting histone H3K9 acetylation and RUNX2 activity. Int J Biochem Cell Biol 54:68–77.

Gao J, Wang W-Y, Mao Y-W, Gräff J, Guan J-S, Pan L, Mak G, Kim D, Su SC, Tsai L-H (2010) A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 466:1105–1109.

Guo JU, Ma DK, Mo H, Ball MP, Jang M, Bonaguidi MA, Balazer JA, Eaves HL, Xie B, Ford E, Zhang K, Ming G, Gao Y, Song H (2011a) Neuronal activity modifies the DNA methylation landscape in the adult brain. Nat Neurosci 14:1345–1351.

Guo W, Qian L, Zhang J, Zhang W, Morrison A, Hayes P, Wilson S, Chen T, Zhao J (2011b) Sirt1 overexpression in neurons promotes neurite outgrowth and cell survival through inhibition of the mTOR signaling. J Neurosci Res 89:1723–1736.

Han A, Sung Y-B, Chung S-Y, Kwon M-S (2014) Possible additional antidepressant-like mechanism of sodium butyrate: targeting the hippocampus. Neuropharmacology 81:292–302.

Hashimoto H, Vertino PM, Cheng X (2010) Molecular coupling of DNA methylation and histone methylation. Epigenomics 2:657–669.

Hellsten J, Wennström M, Bengzon J, Mohapel P, Tingström A (2004) Electroconvulsive seizures induce endothelial cell proliferation in adult rat hippocampus. Biol Psychiatry 55:420–427.

Henriquez B, Bustos FJ, Aguilar R, Becerra A, Simon F, Montecino M, van Zundert B (2013) Ezh1 and Ezh2 differentially regulate PSD-95 gene transcription in developing hippocampal neurons. Mol Cell Neurosci 57:130–143.

Jakovcevski M, Akbarian S (2012) Epigenetic mechanisms in neurological disease. Nat Med 18:1194–1204.

Jiang Y, Hsieh J (2014) HDAC3 controls gap 2/mitosis progression in adult neural stem/progenitor cells by regulating CDK1 levels. Proc Natl Acad Sci U S A 111:13541–13546.

Jun H, Hussaini SMQ, Cho CH, Welby J, Jang M-H (2015) Gadd45b mediates electroconvulsive shock induced proliferation of hippocampal neural stem cells. Brain Stimul 8:1021–1024.

Kaas GA, Zhong C, Eason DE, Ross DL, Vachhani R V, Ming G-L, 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.

Karmodiya K, Krebs AR, Oulad-Abdelghani M, Kimura H, Tora L (2012) H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. BMC Genomics 13:424.

Kodama M, Russell DS, Duman RS (2005) Electroconvulsive seizures increase the expression of MAP kinase phosphatases in limbic regions of rat brain. Neuropsychopharmacology 30:360–371.

Korzus E, Rosenfeld MG, Mayford M (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 42:961–972.

Kosten TA, Galloway MP, Duman RS, Russell DS, D'Sa C (2008) Repeated unpredictable stress and antidepressants differentially regulate expression of the bcl-2 family of apoptotic genes in rat cortical, hippocampal, and limbic brain structures. Neuropsychopharmacology 33:1545–1558.

Lachize S, Apostolakis EM, van der Laan S, Tijssen AMI, Xu J, de Kloet ER, Meijer OC (2009) Steroid receptor coactivator-1 is necessary for regulation of corticotropin-releasing hormone by chronic stress and glucocorticoids. Proc Natl Acad Sci U S A 106:8038–8042.

Li T, Yang D, Li J, Tang Y, Yang J, Le W (2014a) Critical role of Tet3 in neural progenitor cell maintenance and terminal differentiation. Mol Neurobiol 51:142–154.

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

Libert S, Pointer K, Bell EL, Das A, Cohen DE, Asara JM, Kapur K, Bergmann S, Preisig M, Otowa T, Kendler KS, Chen X, Hettema JM, van den Oord EJ, Rubio JP, Guarente L (2011) SIRT1 activates MAO-A in the brain to mediate anxiety and exploratory drive. Cell 147:1459–1472.

Lim DA, Suárez-Fariñas M, Naef F, Hacker CR, Menn B, Takebayashi H, Magnasco M, Patil N, Alvarez-Buylla A (2006) In vivo transcriptional profile analysis reveals RNA splicing and chromatin remodeling as prominent processes for adult neurogenesis. Mol Cell Neurosci 31:131–148.

Link AS, Kurinna S, Havlicek S, Lehnert S, Reichel M, Kornhuber J, Winner B, Huth T, Zheng F, Werner S, Alzheimer C (2015) Kdm6b and Pmepa1 as targets of bioelectrically and behaviorally induced activin A signaling. Mol Neurobiol. DOI 10.1007/s12035-015-9363-3.

Lopez-Atalaya JP, Ciccarelli A, Viosca J, Valor LM, Jimenez-Minchan M, Canals S, Giustetto M, Barco A (2011) CBP is required for environmental enrichment-induced neurogenesis and cognitive enhancement. EMBO J 30:4287–4298.

Ma DK, Jang M-H, Guo JU, Kitabatake Y, Chang M -l., Pow-anpongkul N, Flavell RA, Lu B, Ming G -l., Song H (2009) Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science 323:1074–1077.

Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingström A (2000) Increased neurogenesis in a model of electroconvulsive therapy. Biol Psychiatry 47:1043–1049.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104–9110.

Margueron R, Li G, Sarma K, Blais A, Zavadil J, Woodcock CL, Dynlacht BD, Reinberg D (2008) Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. Mol Cell 32:503–518.

Mi Y, Gao X, Dai J, Ma Y, Xu L, Jin W (2015) A novel function of TET2 in CNS: sustaining neuronal survival. Int J Mol Sci 16:21846–21857.

Michán S, Li Y, Chou MM, Parrella E, Ge H, Long JM, Allard JS, Lewis K, Miller M, Xu W, Mervis RF, Chen J, Guerin KI, Smith LEH, McBurney MW, Sinclair DA, Baudry M, de Cabo R, Longo VD (2010) SIRT1 is essential for normal cognitive function and synaptic plasticity. J Neurosci 30:9695–9707.

Miller CA, Campbell SL, Sweatt JD (2008) DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. Neurobiol Learn Mem 89:599–603.

Newton SS, Collier EF, Hunsberger J, Adams D, Terwilliger R, Selvanayagam E, Duman RS (2003) Gene profile of electroconvulsive seizures: induction of neurotrophic and angiogenic factors. J Neurosci 23:10841–10851.

Noguchi H, Kimura A, Murao N, Matsuda T, Namihira M, Nakashima K (2015) Expression of DNMT1 in neural stem/precursor cells is critical for survival of newly generated neurons in the adult hippocampus. Neurosci Res 95:1–11.

Park DH, Hong SJ, Salinas RD, Liu SJ, Sun SW, Sgualdino J, Testa G, Matzuk MM, Iwamori N, Lim DA (2014a) Activation of neuronal gene expression by the JMJD3 demethylase is required for postnatal and adult brain neurogenesis. Cell Rep 8:1290–1299.

Park HG, Yu HS, Park S, Ahn YM, Kim YS, Kim SH (2014b) Repeated treatment with electroconvulsive seizures induces HDAC2 expression and down-regulation of NMDA receptor-related genes through histone deacetylation in the rat frontal cortex. Int J Neuropsychopharmacol 17:1487–1500.

Pastor WA, Aravind L, Rao A (2013) TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. Nat Rev Mol Cell Biol 14:341–356.

Patchev A V, Fischer D, Wolf SS, Herkenham M, Götz F, Gehin M, Chambon P, Patchev VK, Almeida OFX (2007) Insidious adrenocortical insufficiency underlies neuroendocrine dysregulation in TIF-2 deficient mice. FASEB J 21:231–238.

Saharan S, Jhaveri DJ, Bartlett PF (2013) SIRT1 regulates the neurogenic potential of neural precursors in the adult subventricular zone and hippocampus. J Neurosci Res 91:642–659.

Sarkar A, Chachra P, Kennedy P, Pena CJ, Desouza LA, Nestler EJ, Vaidya VA (2014) Hippocampal HDAC4 contributes to postnatal fluoxetine-evoked depression-like behavior. Neuropsychopharmacology 39:2221–2232.

Schmidt HD, Duman RS (2007) The role of neurotrophic factors in adult hippocampal neurogenesis, antidepressant treatments and animal models of depressive-like behavior. Behav Pharmacol 18:391–418.

Segi-Nishida E, Warner-Schmidt JL, Duman RS (2008) Electroconvulsive seizure and VEGF increase the proliferation of neural stem-like cells in rat hippocampus. Proc Natl Acad Sci U S A 105:11352–11357.

Sen N, Snyder SH (2011) Neurotrophin-mediated degradation of histone methyltransferase by S-nitrosylation cascade regulates neuronal differentiation. Proc Natl Acad Sci U S A 108:20178–20183.

Shen X, Liu Y, Hsu Y-J, Fujiwara Y, Kim J, Mao X, Yuan G-C, Orkin SH (2008) EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. Mol Cell 32:491–502.

Sweatt JD (2013) The emerging field of neuroepigenetics. Neuron 80:624–632.

Tetel MJ, Acharya KD (2013) Nuclear receptor coactivators: regulators of steroid action in brain and behaviour. J Neuroendocrinol 25:1209–1218.

Tian F, Hu X-Z, Wu X, Jiang H, Pan H, Marini AM, Lipsky RH (2009) Dynamic chromatin remodeling events in hippocampal neurons are associated with NMDA receptor-mediated activation of Bdnf gene promoter 1. J Neurochem 109:1375–1388.

Tsankova NM, Kumar A, Nestler EJ (2004) Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. J Neurosci 24:5603–5610.

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 9:519–525.

Vaidya VA, Siuciak JA, Du F, Duman RS (1999) Hippocampal mossy fiber sprouting induced by chronic electroconvulsive seizures. Neuroscience 89:157–166.

Wood MA (2005) Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. Learn Mem 12:111–119.

Wu H, Coskun V, Tao J, Xie W, Ge W, Yoshikawa K, Li E, Zhang Y, Sun YE (2010) Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. Science 329:444–448.

Yu H, Su Y, Shin J, Zhong C, Guo JU, Weng YL, Gao F, Geschwind DH, Coppola G, Ming GL, Song H (2015) Tet3 regulates synaptic transmission and homeostatic plasticity via DNA oxidation and repair. Nat Neurosci 18:836–843.

Zhang J, Ji F, Liu Y, Lei X, Li H, Ji G, Yuan Z, Jiao J (2014) Ezh2 regulates adult hippocampal neurogenesis and memory. J Neurosci 34:5184–5199.

Zhao C, Warner-Schmidt J, Duman RS, Gage FH (2012) Electroconvulsive seizure promotes spine maturation in newborn dentate granule cells in adult rat. Dev Neurobiol 72:937–942.