OXFORD

International Journal of Neuropsychopharmacology, (2016) 19(9): 1–11

doi:10.1093/ijnp/pyw040
Advance Access Publication: May 3, 2016
Research Article

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

Received: March 6, 2016; **Revised:** April 18, 2016; **Accepted:** April 27, 2016

Introduction

The mechanisms underlying the therapeutic action of electroconvulsive 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 (1:10 000, 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.

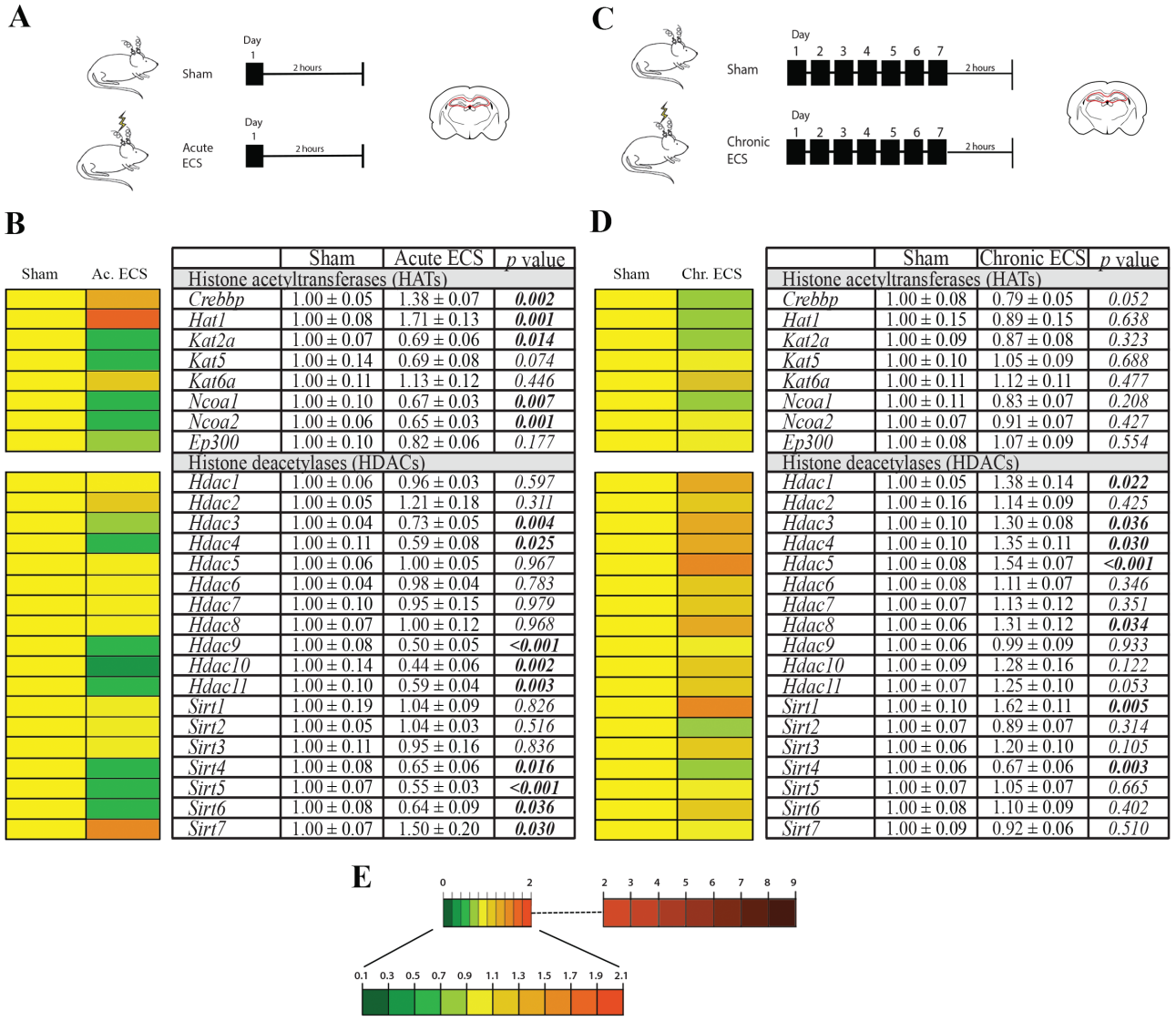
Acute and Chronic ECS Alter the Expression of HMTs and KDMs in the Adult Rat Hippocampus

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).



	Sham	Acute ECS	*p* value
Histone acetyltransferases (HATs)			
Crebbp	1.00 ± 0.05	1.38 ± 0.07	***0.002***
Hat1	1.00 ± 0.08	1.71 ± 0.13	***0.001***
Kat2a	1.00 ± 0.07	0.69 ± 0.06	***0.014***
Kat5	1.00 ± 0.14	0.69 ± 0.08	*0.074*
Kat6a	1.00 ± 0.11	1.13 ± 0.12	*0.446*
Ncoa1	1.00 ± 0.10	0.67 ± 0.03	***0.007***
Ncoa2	1.00 ± 0.06	0.65 ± 0.03	***0.001***
Ep300	1.00 ± 0.10	0.82 ± 0.06	*0.177*
Histone deacetylases (HDACs)			
Hdac1	1.00 ± 0.06	0.96 ± 0.03	*0.597*
Hdac2	1.00 ± 0.05	1.21 ± 0.18	*0.311*
Hdac3	1.00 ± 0.04	0.73 ± 0.05	***0.004***
Hdac4	1.00 ± 0.11	0.59 ± 0.08	***0.025***
Hdac5	1.00 ± 0.06	1.00 ± 0.05	*0.967*
Hdac6	1.00 ± 0.04	0.98 ± 0.04	*0.783*
Hdac7	1.00 ± 0.10	0.95 ± 0.15	*0.979*
Hdac8	1.00 ± 0.07	1.00 ± 0.12	*0.968*
Hdac9	1.00 ± 0.08	0.50 ± 0.05	***<0.001***
Hdac10	1.00 ± 0.14	0.44 ± 0.06	***0.002***
Hdac11	1.00 ± 0.10	0.59 ± 0.04	***0.003***
Sirt1	1.00 ± 0.19	1.04 ± 0.09	*0.826*
Sirt2	1.00 ± 0.05	1.04 ± 0.03	*0.516*
Sirt3	1.00 ± 0.11	0.95 ± 0.16	*0.836*
Sirt4	1.00 ± 0.08	0.65 ± 0.06	***0.016***
Sirt5	1.00 ± 0.07	0.55 ± 0.03	***<0.001***
Sirt6	1.00 ± 0.08	0.64 ± 0.09	***0.036***
Sirt7	1.00 ± 0.07	1.50 ± 0.20	***0.030***

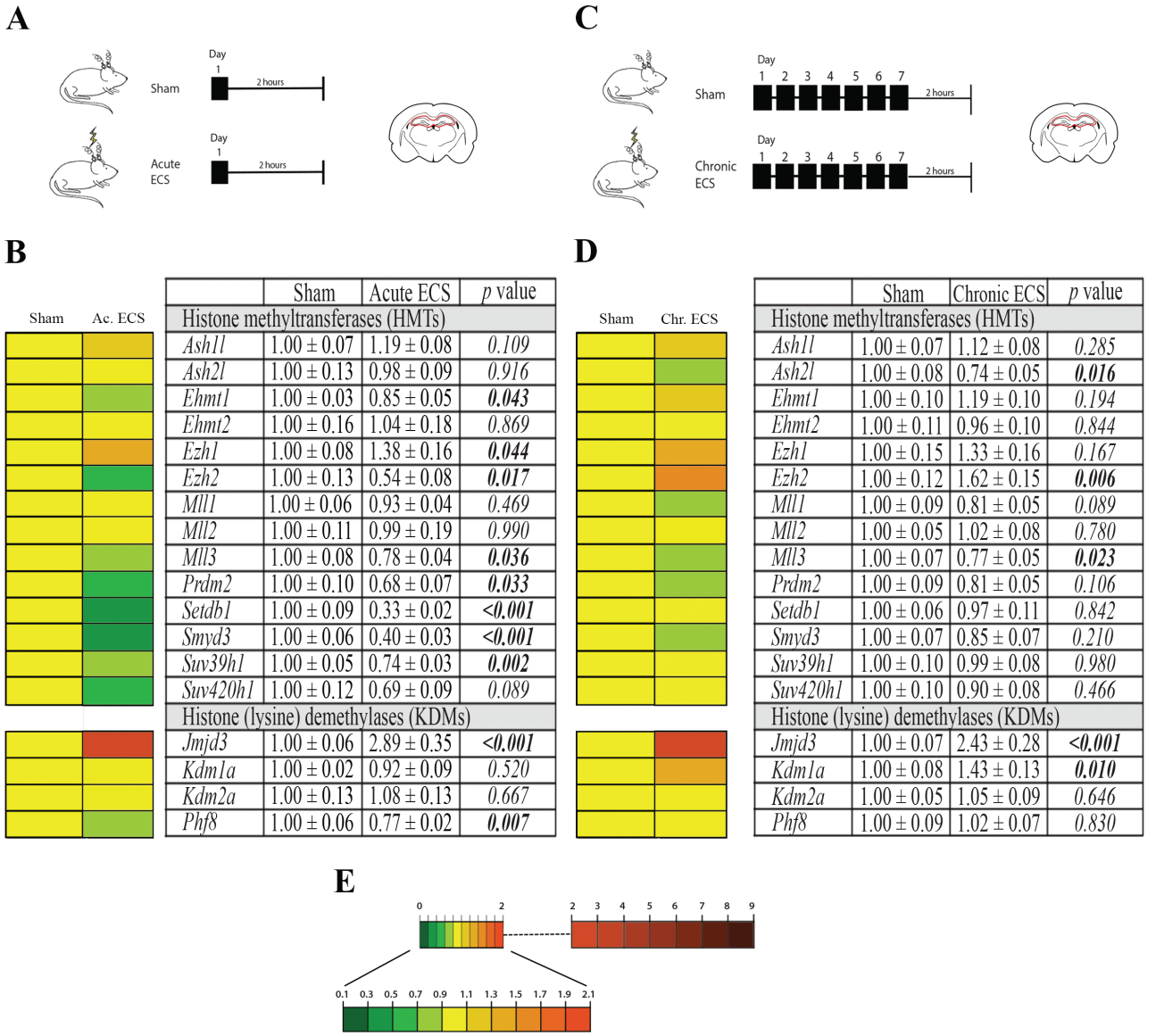
	Sham	Chronic ECS	*p* value
Histone acetyltransferases (HATs)			
Crebbp	1.00 ± 0.08	0.79 ± 0.05	*0.052*
Hat1	1.00 ± 0.15	0.89 ± 0.15	*0.638*
Kat2a	1.00 ± 0.09	0.87 ± 0.08	*0.323*
Kat5	1.00 ± 0.10	1.05 ± 0.09	*0.688*
Kat6a	1.00 ± 0.11	1.12 ± 0.11	*0.477*
Ncoa1	1.00 ± 0.11	0.83 ± 0.07	*0.208*
Ncoa2	1.00 ± 0.07	0.91 ± 0.07	*0.427*
Ep300	1.00 ± 0.08	1.07 ± 0.09	*0.554*
Histone deacetylases (HDACs)			
Hdac1	1.00 ± 0.05	1.38 ± 0.14	***0.022***
Hdac2	1.00 ± 0.16	1.14 ± 0.09	*0.425*
Hdac3	1.00 ± 0.10	1.30 ± 0.08	***0.036***
Hdac4	1.00 ± 0.10	1.35 ± 0.11	***0.030***
Hdac5	1.00 ± 0.08	1.54 ± 0.07	***<0.001***
Hdac6	1.00 ± 0.08	1.11 ± 0.07	*0.346*
Hdac7	1.00 ± 0.07	1.13 ± 0.12	*0.351*
Hdac8	1.00 ± 0.06	1.31 ± 0.12	***0.034***
Hdac9	1.00 ± 0.06	0.99 ± 0.09	*0.933*
Hdac10	1.00 ± 0.09	1.28 ± 0.16	*0.122*
Hdac11	1.00 ± 0.07	1.25 ± 0.10	*0.053*
Sirt1	1.00 ± 0.10	1.62 ± 0.11	***0.005***
Sirt2	1.00 ± 0.07	0.89 ± 0.07	*0.314*
Sirt3	1.00 ± 0.06	1.20 ± 0.10	*0.105*
Sirt4	1.00 ± 0.06	0.67 ± 0.06	***0.003***
Sirt5	1.00 ± 0.07	1.05 ± 0.07	*0.665*
Sirt6	1.00 ± 0.08	1.10 ± 0.09	*0.402*
Sirt7	1.00 ± 0.09	0.92 ± 0.06	*0.510*

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



	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***

	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*

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).

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

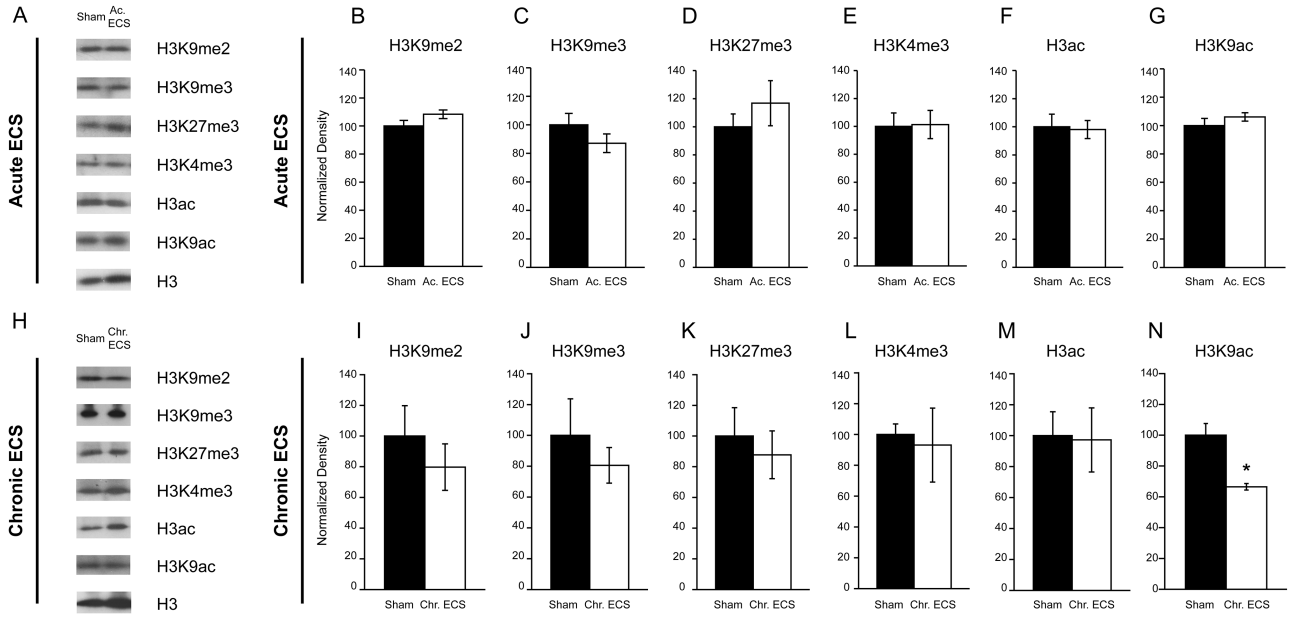


Figure 3. Influence of acute and chronic electroconvulsive seizure (ECS) on global and residue-specific levels of histone methylation and acetylation in the rat hippocampus. Hippocampal levels of residue-specific histone methylation (H3K9me2, H3K9me3, H3K27Me3, H3K4me3), and global, as well as residue-specific, 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).

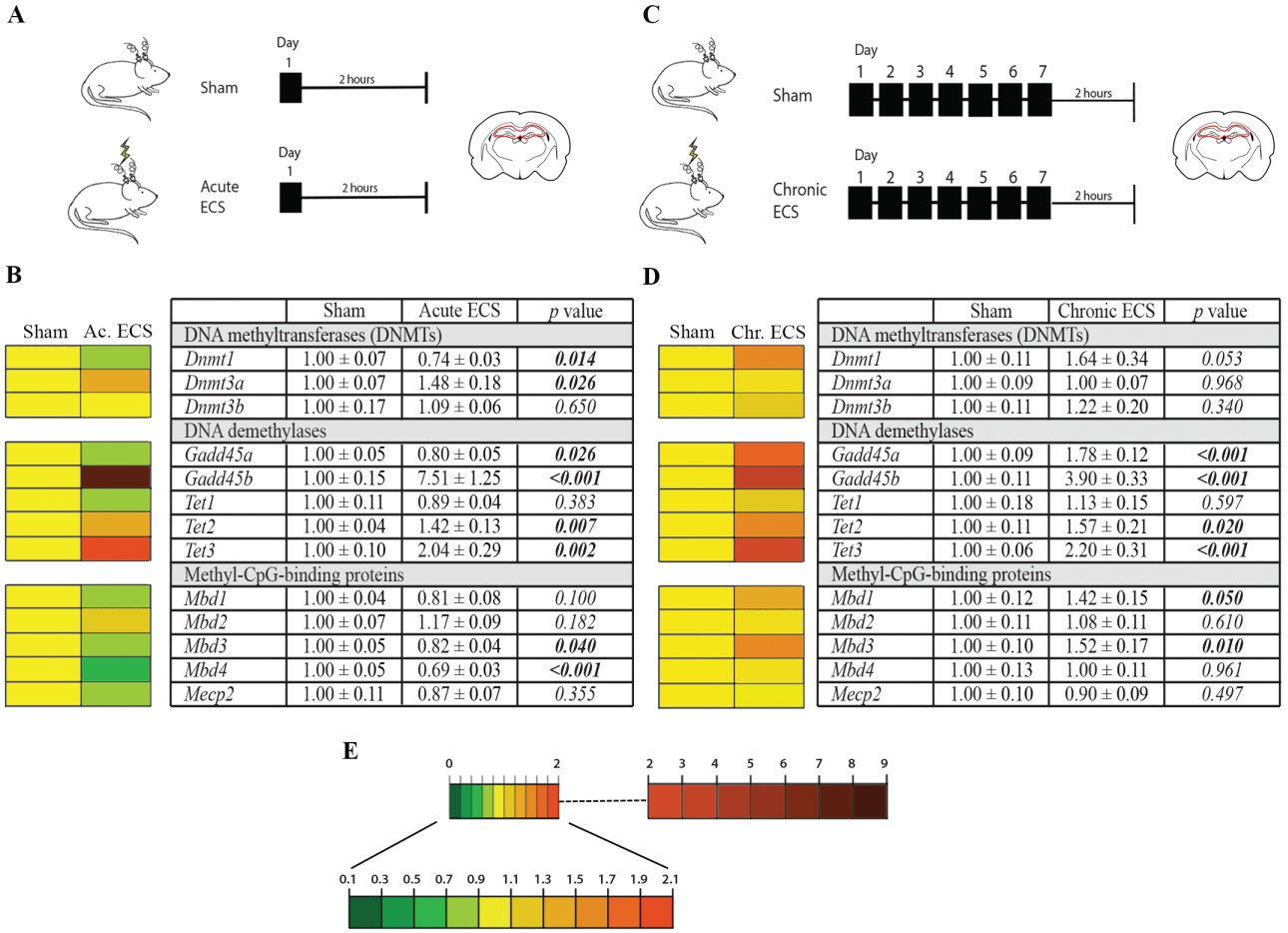
DNA modifiers, as well as methyl-CpG-binding proteins, and highlight their distinctive pattern of regulation by both acute and chronic ECS.

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



	Sham	Acute ECS	*p* value
DNA methyltransferases (DNMTs)			
Dnmt1	1.00 ± 0.07	0.74 ± 0.03	***0.014***
Dnmt3a	1.00 ± 0.07	1.48 ± 0.18	***0.026***
Dnmt3b	1.00 ± 0.17	1.09 ± 0.06	*0.650*
DNA demethylases			
Gadd45a	1.00 ± 0.05	0.80 ± 0.05	***0.026***
Gadd45b	1.00 ± 0.15	7.51 ± 1.25	***<0.001***
Tet1	1.00 ± 0.11	0.89 ± 0.04	*0.383*
Tet2	1.00 ± 0.04	1.42 ± 0.13	***0.007***
Tet3	1.00 ± 0.10	2.04 ± 0.29	***0.002***
Methyl-CpG-binding proteins			
Mbd1	1.00 ± 0.04	0.81 ± 0.08	*0.100*
Mbd2	1.00 ± 0.07	1.17 ± 0.09	*0.182*
Mbd3	1.00 ± 0.05	0.82 ± 0.04	***0.040***
Mbd4	1.00 ± 0.05	0.69 ± 0.03	***<0.001***
Mecp2	1.00 ± 0.11	0.87 ± 0.07	*0.355*

	Sham	Chronic ECS	*p* value
DNA methyltransferases (DNMTs)			
Dnmt1	1.00 ± 0.11	1.64 ± 0.34	*0.053*
Dnmt3a	1.00 ± 0.09	1.00 ± 0.07	*0.968*
Dnmt3b	1.00 ± 0.11	1.22 ± 0.20	*0.340*
DNA demethylases			
Gadd45a	1.00 ± 0.09	1.78 ± 0.12	***<0.001***
Gadd45b	1.00 ± 0.11	3.90 ± 0.33	***<0.001***
Tet1	1.00 ± 0.18	1.13 ± 0.15	*0.597*
Tet2	1.00 ± 0.11	1.57 ± 0.21	***0.020***
Tet3	1.00 ± 0.06	2.20 ± 0.31	***<0.001***
Methyl-CpG-binding proteins			
Mbd1	1.00 ± 0.12	1.42 ± 0.15	***0.050***
Mbd2	1.00 ± 0.11	1.08 ± 0.11	*0.610*
Mbd3	1.00 ± 0.10	1.52 ± 0.17	***0.010***
Mbd4	1.00 ± 0.13	1.00 ± 0.11	*0.961*
Mecp2	1.00 ± 0.10	0.90 ± 0.09	*0.497*

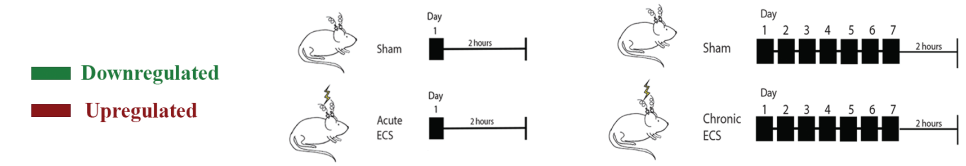
Figure 4. Influence of acute and chronic electroconvulsive seizure (ECS) on the expression of DNA methylation machinery in the rat hippocampus. Shown is the experimental design for acute ECS (Ac. ECS, A) and chronic ECS (Chr. ECS, C) treatment. Shown are normalized gene expression levels in the hippocampus 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* *Hdac10* *Hdac11* *Sirt4* *Sirt5* *Sirt6* *Sirt7*	*Hdac1* *Hdac3* *Hdac4* *Hdac5* *Hdac8* *Sirt1* *Sirt4*
Histone methyltransferases (HMTs)	*Ehmt1* *Ezh1* *Ezh2* *Mll3* *Prdm2* *Setdb1* *Smyd3* *Suv39h1*	*Ash2l* *Ezh2* *Mll3*
Histone (lysine) demethylases (KDMs)	*Jmjd3* *Phf8*	*Jmjd3* *Kdm1a*
DNA methyl transferases (DNMTs)	*Dnmt1* *Dnmt3a*	
DNA demethylases	*Gadd45a* *Gadd45b* *Tet2* *Tet3*	*Gadd45a* *Gadd45b* *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

We acknowledge Dr. Shital Suryavanshi and Ishira Nanavaty for technical assistance.

This work was supported by a TIFR intramural grant (V.A.V.) and support from the Department of Biotechnology, Centre of Excellence in Epigenetics, IISER (V.A.V. and S.G., BT/01/COE/09/07). The authors have no conflict of interest to declare.

Statement of Interest

None.

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