Visual Experience Dependent Regulation of Neuronal Structure and Function by Histone Deacetylase 1 in Developing *Xenopus* Tectum *In Vivo*

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ABSTRACT: Histone deacetylase 1 (HDAC1) is thought to play pivotal roles in neurogenesis and neurodegeneration. However, the role of HDAC1 in neuronal growth and structural plasticity in the developing brain *in vivo* remains unclear. Here, we show that in the optic tectum of *Xenopus laevis*, HDAC1 knockdown dramatically decreased the frequency of AMPAR-mediated synaptic currents and increased the frequency of GABAAR-mediated currents, whereas HDAC1 overexpression significantly decreased the frequency of GABAAR-mediated synaptic currents. Both HDAC1 knockdown and overexpression adversely affected dendritic arbor growth and visual experience-dependent structural plasticity. Furthermore, HDAC1 knockdown decreased BDNF expression via a mechanism that involves acetylation of specific histone H4 residues at lysine K5. In particular, the deficits in dendritic growth and visually guided avoidance behavior in HDAC1-knockdown tadpoles could be rescued by acute tectal infusion of BDNF. These results establish a relationship between HDAC1 expression, histone H4 modification and BDNF signaling in the visual-experience dependent regulation of dendritic growth, structural plasticity and function in intact animals *in vivo*. © 2016 Wiley Periodicals, Inc. Develop Neurobiol 00: 000–000, 2016

Keywords: **histone deacetylase; BDNF; structural plasticity; *Xenopus laevis*; visual experience**

INTRODUCTION

Histone deacetylases (HDACs) are predominantly localized to the nucleus and play pivotal roles in neurogenesis, learning and brain diseases by regulating gene transcription (Haberland et al., 2009). The HDAC family consists of four classes (class I, II(A) IIb and IV), which are highly conserved from invertebrates to mammals (Haberland et al., 2009). Several studies have shown that application of broad-spectrum HDAC inhibitors can ameliorate deficits in learning and memory (Fischer et al., 2007; Morris et al., 2010; Hanson et al., 2013; Intlekofer et al., 2013). Epigenetic modification of histones by acetylation or methylation has been shown to participate in synaptic plasticity and learning memory (Guan et al., 2009; Calfa et al., 2012; Cortes-Mendoza et al., 2013). Neuronal dendrites are the main sites for the formation of neuronal connections, receiving synaptic inputs and

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integrating synaptic currents to generate action potentials. Dendritic development and structural plasticity strongly influence neuronal firing activity and circuit function (Cline, 2001). Characterizing the specific roles of HDAC family members in neuronal structural development and plasticity will help in understanding the role of epigenetic modulation in learning and memory in the central nervous system (CNS).

HDAC1 has been shown to be involved in multiple neural function via a nuclear localization signal (NLS) that allows it to function as a transcriptional repressor (de Ruijter et al., 2003; Haberland et al., 2009). Deletion of both HDAC1 and HDAC2 causes a robust facilitation of excitatory synapse formation during early synaptic development (Akhtar et al., 2009). In contrast, acute overexpression of HDAC1 regulates extinction of fear memory in the adult brain (Bahari-Javan et al., 2012) and inhibitory synaptic transmission in cultured hippocampal slices (Hanson et al., 2013). Although HDAC1 has been shown to play a role in neural proliferation/differentiation (Yamaguchi et al., 2005; Cunliffe and Casaccia-Bonnefil, 2006; Brunmeir et al., 2009; Ye et al., 2009; Dovey et al., 2010; Conway et al., 2012; Jacob et al., 2014; Tao et al., 2015), learning and memory (Bahari-Javan et al., 2012), neuronal survival and death (Kim et al., 2008; Kim et al., 2010; Bardai et al., 2012) and neurological diseases (Abel and Zukin, 2008; Chen et al., 2011; Jia et al., 2012; Rudenko and Tsai, 2014), there is limited evidence for HDAC1 in neuronal dendritic growth and structural plasticity. Recent work has shown that visual avoidance behavior can be used to measure the function of the visual circuit (Dong et al., 2009; Shen et al., 2011, 2014); however, the role of HDAC1 in the behavior and underlying cellular mechanisms remain unclear.

Experience-dependent establishment and refinement of neural circuits depend on both excitatory and inhibitory input activity (Sanes et al., 1992; Cline, 2001; Shen et al., 2009). Brain derived neurotrophic factor (BDNF) exposure increases excitatory neurotransmitter release (Lohof et al., 1993; Huang et al., 1999; Schuman, 1999; Du and Poo, 2004), synaptic maturation (Shen et al., 2006) and neuronal arbor growth (McAllister et al., 1995; Mertz et al., 2000; Alsina et al., 2001; Danzer et al., 2002; Marshak et al., 2007). Numerous studies have demonstrated essential roles for HDAC1 in embryogenesis (Dovey et al., 2010) and cell proliferation (Tao et al., 2015). By using HDAC inhibitors, HDAC activity has been shown to be required for BDNF to increase dendritic spine density and excitatory transmitter release in cultured hippocampal slices (Calfa et al., 2012). However, relatively little is known regarding the roles of HDAC1 and BDNF signaling in neuronal dendritic development and experience-dependent structural plasticity in the developing brain *in vivo*.

In the present study, we examined the role of HDAC1 in excitatory and inhibitory synaptic transmission and experience-dependent structural plasticity by using knockdown or overexpression of HDAC1 in *Xenopus* optic tectal neurons *in vivo*. Our findings describe a new role for HDAC1 in the regulation of dendritic growth and structural plasticity in the developing brain *in vivo*.

MATERIALS AND METHODS

Animals and Rearing

All animal procedures were performed according to the requirements of the "Regulation for the Use of Experimental Animals in Zhejiang Province." This study has been approved by the local ethics committee of the Hangzhou Normal University. Tadpoles were obtained by the mating of adult *albino Xenopus* injected with human chorionic gonadotropin (HCG) and raised on a 12 hr dark/12 hr light cycle in Steinberg's solution in a 20°C incubator. Tadpoles were anesthetized in 0.02% MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate, Sigma-Aldrich) for experimental manipulations.

DNA Constructs, Morpholinos, and Transfection

The tecta of tadpoles at stage 48 were dissected out and homogenized in ice-cold Trizol buffer. Total RNA was extracted according to the manufacturer's instructions (Axyprep Multisource RNA Miniprep Kit). First-strand cDNA was synthesized and amplified by PCR (Bio-Rad). Primers were designed for HDAC1 of *Xenopus laevis* (accession No. NM_001085927.1) with the following sequences: forward 5′-CCCAAGCTTATGGCGCTGAGT CAAGGAAC-3′ and reverse 5′-GCTCTAGAT TTAGCCT GTGCTGGGATAG-3′. The PCR products were amplified and separated using 1% agarose gels. The HDAC1-GFP plasmid was constructed by subcloning HDAC1 cDNA into a BICS2-EGFP vector, which is a dual CMV promoter plasmid expressing enhanced green fluorescent protein (eGFP). It was transfected into the tectum and used to visualize the morphology of tectal neurons. Control tadpoles were transfected with BICS2-EGFP.

To knock down the endogenous HDAC1 expression, we used a translation-blocking morpholino (MO) against the *Xenopus* HDAC1A to block HDAC1A and HDAC1B (HDAC1-MO, TCAGCGCCATTTTCCTTCCGCGTCT) and control MO (Ctrl-MO, CCTCTTACCTCAGTTA-CAATTTATA) were also purchased from Gene Tools (GeneTools, Philomath, OR). All morpholinos were tagged with Lissamine for fluorescent visualization. For the visualization of neuronal dendrites, morpholinos were co-transfected with BICS2-EGFP.

To transfect tectal cells, stage 45 tadpoles were anesthetized in 0.02% MS-222 and injected with the plasmids (1.5 μg/μL) or the morpholinos (10 μM) into the ventricle of the midbrain. For whole-brain electroporation, custom-made platinum electrodes were placed on the skin above the tectum, and current pulses with ±electric fields were applied to the midbrain. The current parameters used were as described previously (Shen et al., 2014). The electroporated tadpoles were screened by a fluorescent microscopy for further experiments.

Electrophysiology

All recordings were performed at room temperature (20–22°C). For recordings of miniature synaptic currents, tecta were dissected and perfused with external solution containing (in m*M*: 115 NaCl, 2 KCl, 3 $CaCl_2$, 1.5 $MgCl_2$, 5 HEPES, 10 glucose, 0.01 glycine, and 0.1 n*M* TTX, pH 7.2, osmolality 255 mOsm). Miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) were recorded by holding the membrane potential at −60 mV and 0 mV, respectively, using a K+-based intracellular solution (in m*M*: 110 K-gluconate, 8 KCl, 5 NaCl, 1.5 $MgCl_2$, 20 HEPES, 0.5 EGT(A) 2 ATP, and 0.3 GTP). Recording micropipettes were pulled from borosilicate glass capillaries and had resistances in the range of 7 to 9 MΩ. Liquid junction potential was adjusted during recording. Signals were filtered at 2 kHz with a MultiClamp 700A amplifier (Molecular Devices, Palo Alto, CA). Data were sampled at 10 kHz and analyzed using ClampFit 10 (Molecular Devices) or MiniAnalysis (Synaptosoft).

Immunohistochemistry

Tadpoles were anesthetized in 0.02% MS-222 and then fixed in 4% paraformaldehyde (PFA, pH 7.4) at 4°C overnight. Tadpoles were rinsed with 0.1 *M* PB and submerged in 30% sucrose overnight for dehydration. On the second day, animals were embedded in optimal cutting temperature (OCT) media and then cut into 20 μm cryostat sections with a microtome (Microm, HM550 VP). Sections were rinsed with 0.1 *M* PB, permeabilized with 0.3% Triton X-100, and blocked in 5% donkey serum for 1 hr before being incubated with primary antibody (rabbit anti-HDAC1, 1:200, Abcam, ab33278) at 4°C overnight. Sections were rinsed with PB and incubated with FITC-conjugated secondary antibody for 1 hr at room temperature. After sections were counterstained with DAPI, mounted, and sealed, immunofluorescent images were collected using a Zeiss LSM 710 confocal microscope.

In Vivo Time-Lapse Imaging and Analysis

To transfect single neurons *in vivo*, sharp needles were inserted into the tectal brain and one to three drops of plasmids or morpholinos were locally injected to the brain tissue. The tadpoles labeled with single neurons were screened for *in vivo* imaging. To image single neurons, tadpoles were anesthetized and mounted under glass coverslips in a custom-made Sylgard. Fluorescent neurons were imaged using a confocal microscope. Total dendritic branch length (TDBL) and branch tip numbers were analyzed using Imaris 7.4.2 with Filament Tracer (Bitplane, Zurich, Switzerland) (Shen et al., 2009).

Visual Stimulation

For all other experiments, animals were placed in a 22°C incubator with a 12 hr dark/12 hr light cycle. For visual stimulation, single neurons were first imaged 48 to 72 hr after electroporation. After animals were placed in a dark chamber for 4 hr, the same neurons were imaged for the second time and animals were placed into a chamber with five lines of green light-emitting diodes turning on and off sequentially (1 Hz). After 4 hr of enhanced visual stimulation, the same neurons were imaged for the third time (Shen et al., 2009).

Western Blotting

The dissected optic tecta or murine cerebral cortices were homogenized in radioimmunoprecipitation assay (RIPA) buffer with a protease inhibitor cocktail (1:100, Sigma Aldrich) at 4°C. Protein homogenates were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked in 1% nonfat milk or rabbit serum for 1 hr with TBS buffer containing 0.1% Tween-20 (Sigma Aldrich) (TBST) and incubated with primary antibodies overnight at 4°C. Antibody specificity was evaluated using anti-HDAC1 (1:2000, abcam), anti-GAPDH (1:8000, Millipore), anti-H2BK5Ac (1:2000, Abcam, ab40886), anti-H3K9Ac (1:2000, Abcam, ab10812), anti-H4K5Ac (1:2000, Abcam, ab51997), anti-H4K8Ac (1:2000, Abcam, ab45166), anti-H4K12Ac (1:2000, Abcam, ab46983), and anti-BDNF (1:500, Sheep, Abcam, ab75040), which were diluted in 1% nonfat milk or 0.1% rabbit serum. Blots were rinsed with TBST and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000, CWBIO) for 1 hr at room temperature. Bands were visualized using ECL chemiluminescence reagents (1:1, Pierce).

Visual Avoidance Assay

The visual avoidance behavior was measured using a custom-made equipment according to previous studies (Dong et al., 2009; Shen et al., 2011; McKeown et al., 2013). Single tadpoles were placed in a 9 × 8 × 3 cm (length × width × height) chamber filled with ∼1.5 cm Steinberg's rearing solution. Visual stimuli, namely moving spots, were presented on the bottom of the chamber with a back-projection screen using a microprojector (3M, MPro110). The tadpoles were illuminated by infrared LEDs, and the videos were recorded with a digital camera. Visual stimuli were generated and presented using

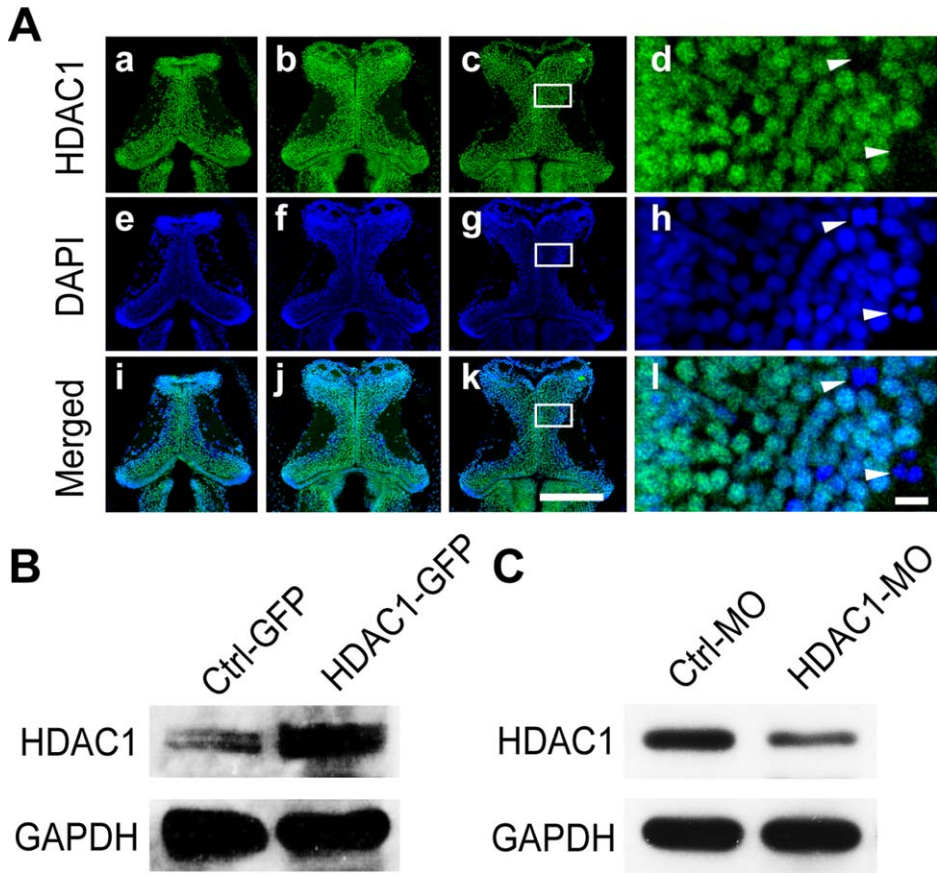

Figure 1 HDAC1 is widely localized throughout the *Xenopus* tectum. (A) Three representative immunofluorescent images showing HDAC1 immunoreactivity within a whole tectal brain at stage 47. Scale bar: 100 μm. A higher-magnification image of the tectum is shown right (d, h, l). Arrows indicate HDAC1-immuno negative cell nuclei. Scale bar: 10 μm. (B) A full-length HDAC1 sequence was obtained from a *Xenopus laevis* cDNA library and inserted into the BICS2-GFP construct (HDAC1-GFP). HDAC1-GFP was transfected into 293T cells, and HDAC1 expression was evaluated by immunostaining with an anti-HDAC1 antibody. Intensity ratios of HDAC1 to GAPDH: Ctrl-GFP: 0.39 ± 0.07; HDAC1-GFP: 1.09 ± 0.02; $N = 3$. $p < 0.01$. (C) Ctrl-MO and HDAC1-MO were transfected into the optic tectum, as indicated. The brains were homogenized and immunostained with anti-HDAC1 antibody. Intensity ratios of HDAC1 to GAPDH: Ctrl-MO: 0.51 ± 0.13; HDAC1-MO: 0.24 ± 0.06; $N = 3$. $p < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

MATLAB 2012a (The MathWorks, Psychophysics Toolbox). Randomly positioned moving spots of 0.4 cm in diameter were presented for 60 s. Visual avoidance behavior was scored as a change in swim trajectory during the first ten encounters of the moving spots by the tadpoles (plotted as avoidance index).

Statistics

Paired data were tested with Student's *t*-test. Multiple group data were tested with an ANOVA followed by *post hoc* Tukey's test unless noted. Data are represented as the mean $\pm$ SEM. Experiments and analysis were performed blind to the experimental condition unless otherwise noted.

RESULTS

Localization of HDAC1 in the Developing *Xenopus* Tectum and Manipulation of HDAC1 Expression

The HDAC1 orthologues in *homo sapiens* and *Xenopus laevis* share 90.46% protein sequence homology (Tao et al., 2015). We found that HDAC1 was ubiquitously expressed throughout the cell layer and predominantly localized within the cell nuclei of the *Xenopus* tectum at stage 47 [Fig. 1(A)]. Only few cells showed low levels of HDAC1 expression [Fig. 1(A)—d, Ah, and Al].

To avoid the circuit-wide effects of HDAC inhibitors, we constructed an overexpression plasmid, HDAC1-GFP, and a knockdown morpholino, HDAC1-MO. For HDAC1-GFP, we subcloned HDAC1b from a *Xenopus laevis* cDNA library into a bidirectional double-promoter-driven construct. To confirm the efficiency of the HDAC1 overexpression, Ctrl-GFP or HDAC1-GFP was transfected into cultured 293T cells. We found that HDAC1 expression level was 2.8-fold higher in HDAC1-GFP transfected cells than in Ctrl-GFP cells [Fig. 1(B)]. HDAC1-MO tagged with Lissamine was synthesized according to the cDNA sequence of HDAC1b in *Xenopus laevis*. To determine the efficiency of HDAC1 knockdown, Ctrl-MO or HDAC1-MO was electroporated into tectal cells. Tadpoles with high electroporation rates were selected and homogenized for Western blotting. The level of HDAC1 expression was significantly decreased in HDAC1-MO brains compared to Ctrl-MO brains [Fig. 1(C)]. Taken together, these tools allow us to overexpress or knock down the HDAC1 in cells to study the roles of HDAC1 in dendritic growth, structural plasticity and function *in vivo*.

HDAC1 Regulates Excitatory and Inhibitory Synaptic Transmission

To test whether HDAC1 affects glutamatergic or GABAergic synaptic transmission, we transfected tectal neurons with HDAC1-GFP or HDAC1-MO to overexpress or knock down HDAC1 expression by whole-brain electroporation (WBE). Recordings were performed 3 days after electroporation. We first recorded AMPAR-mediated mEPSCs from neurons transfected with Ctrl-GFP, Ctrl-MO, HDAC1-GFP, or HDAC1-MO by holding the membrane potential at −60 mV [Fig. 2(A–C)]. The inter-event interval (IEI) of mEPSCs in HDAC1-knockdown neurons was significantly greater than that in control neurons [Fig. 2(D,E)], while the amplitudes of mEPSCs were not altered [Fig. 2(F,G)]. Furthermore, we recorded GABAAR-mediated mIPSCs from neurons transfected with Ctrl-GFP, Ctrl-MO, HDAC1-GFP or HDAC1-MO by holding the membrane potential at 0 mV [Fig. 2(H–J)]. The IEI of mIPSCs in HDAC1-GFP neurons was significantly increased compared to Ctrl-GFP neurons [Fig. 2(K,L)]. In contrast to the mEPSCs, the IEI of mIPSCs was dramatically decreased in HDAC1-knockdown neurons compared to that in control neurons [Fig. 2(D,E)]. These data indicate that HDAC1 affects both glutamatergic and GABAergic synaptic transmission.

HDAC1 Activity Alters Dendritic Arbor Growth

To test whether HDAC1 affects the growth of the neuronal dendritic arbor, we used HDAC1-MO and Ctrl-GFP to knock down HDAC1 or HDAC1-GFP to overexpress HDAC1. A single neuron in each optic tectum was selected and imaged once per day for three consecutive days *in vivo* [Fig. 3(A)]. We first transfected tectal neurons with Ctrl-GFP and observed that the dendritic arbor was considerably extended over three days [Fig. 3(A,B)]. Time-series imaging and three-dimensional dendritic reconstruction showed that total dendritic branch length (TDBL) and total branch tip number (TBTN) were significantly increased at 72 hr compared to the first day of imaging neurons [Fig. 3(B,C)]. In contrast, transfection with either HDAC1-GFP or HDAC1-MO and Ctrl-GFP abolished the increase in the dendritic arbor over 3 days. Furthermore, HDAC1-GFP expression dramatically decreased the TBTN [Fig. 3(C)]. To test HDAC1-GFP or HDAC1-MO transfection will result in cell death, we performed TUNEL experiments as described before (Gao et al., 2016). We found that either knockdown or overexpression of HDAC1 did not induce cell apoptosis in the tectal cells (Supporting Information Fig. 1). Taken together, these results indicate that both overexpression and knockdown of HDAC1 disrupt the growth of the dendritic arbor but not leading to cell death in the developing tectal brain.

HDAC1 Regulates Experience-Dependent Structural Plasticity

Many studies have shown that dendritic arbor growth and branch additions in optic tectal neurons are greatly enhanced by visual stimulation (Haas et al., 2006; Shen et al., 2009). To test whether HDAC1 regulates visual experience-dependent structural plasticity, we used a protocol in which tadpoles are exposed to a 4 hr period of darkness followed by a 4 hr period of enhanced visual stimulation to determine the effect of visual experience on HDAC1-GFP-expressing and HDAC1-MO-expressing neurons [Fig. 4(A)]. We found that Ctrl-GFP-expressing neurons showed dramatically increased TDBL and TBTN in response to 4 hr of visual stimulation, whereas HDAC1-GFP-expressing and HDAC1-MO-expressing neurons failed to increase dendritic length after visual stimulation [Fig. 4(B,C)]. The dendritic growth rate was significantly increased in Ctrl-GFP neurons after 4 hr of visual stimulation. However, TDBL and

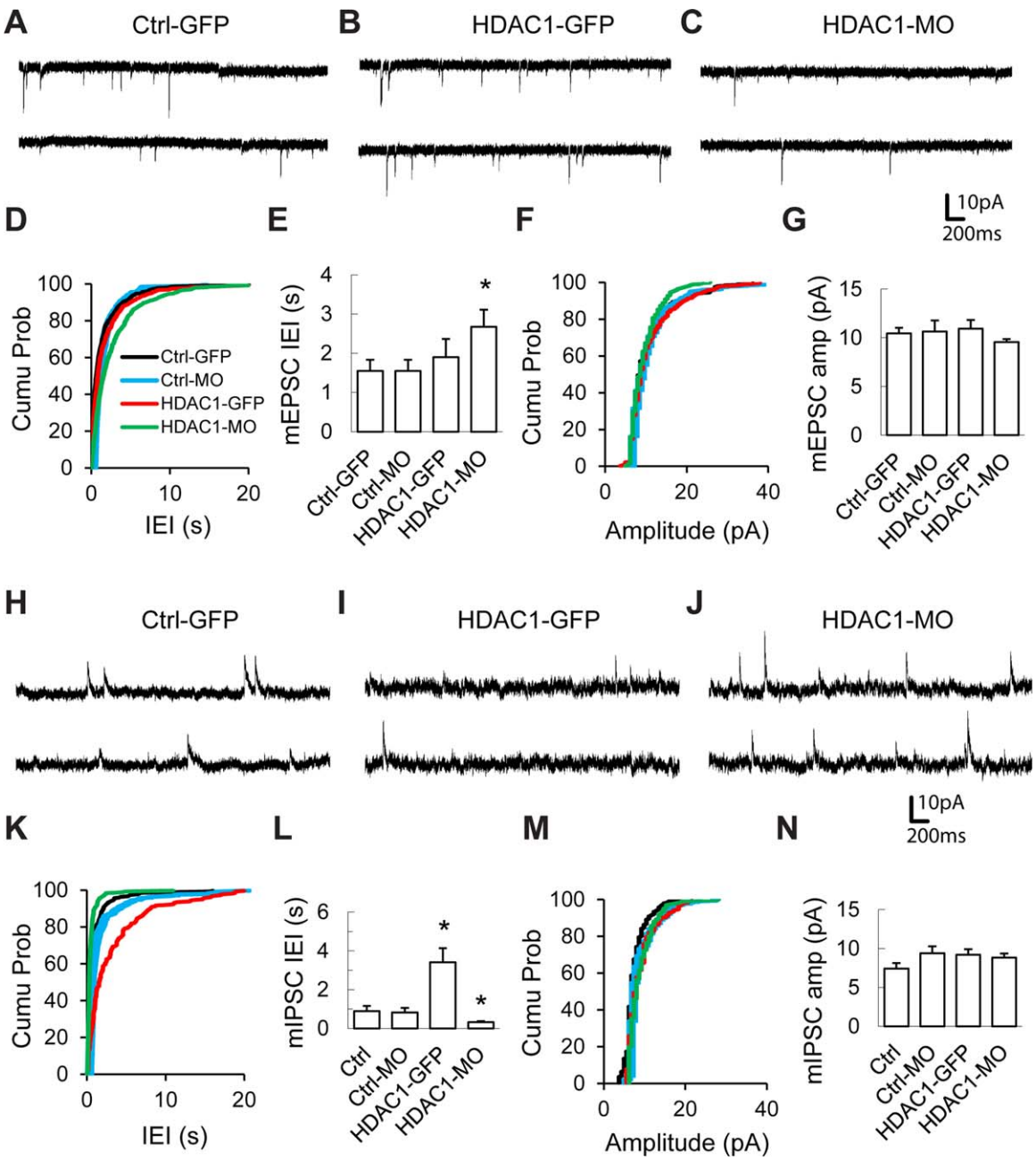

Figure 2 Manipulation of HDAC1 expression alters excitatory and inhibitory synaptic transmission. (A and B) Representative traces of mEPSCs in neurons transfected with Ctrl-GFP (A) HDAC1-GFP (B) or HDAC1-MO (C). (D and E) HDAC1-MO transfection significantly increases the inter-event interval (IEI) of mEPSCs. (F and G) Amplitudes of mEPSCs do not differ among neurons transfected with Ctrl-GFP, HDAC1-GFP or HDAC1-MO. $N = 28$, 10, 15, and 18 for Ctrl-GFP, Ctrl-MO, HDAC1-GFP and HDAC1-MO, respectively; $p > 0.05$. (H–J) Representative traces of mIPSCs in neurons transfected with Ctrl-GFP (H), HDAC1-GFP (I), or HDAC1-MO (J). (K) Cumulative probability plot showing significant shifts in the distribution of the IEI of mIPSCs in HDAC1-GFP and HDAC1-MO-transfected neurons compared to Ctrl-GFP-transfected cells (Kolmogorov-Smirnov test, $^*p < 0.05$). (L) HDAC1-GFP overexpression increases IEI of mIPSCs, whereas HDAC1 knockdown decreases the IEI of mIPSCs. Mann-Whitney test, $^*p < 0.05$. (M and N) Cumulative probability plot and average amplitudes of mIPSCs showing that there are no differences among Ctrl-GFP, HDAC1-GFP and HDAC1-MO transfected cells. $p > 0.05$. $N = 28$, 10, 15, and 18 for Ctrl-GFP, Ctrl-MO, HDAC1-GFP, and HDAC1-MO, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

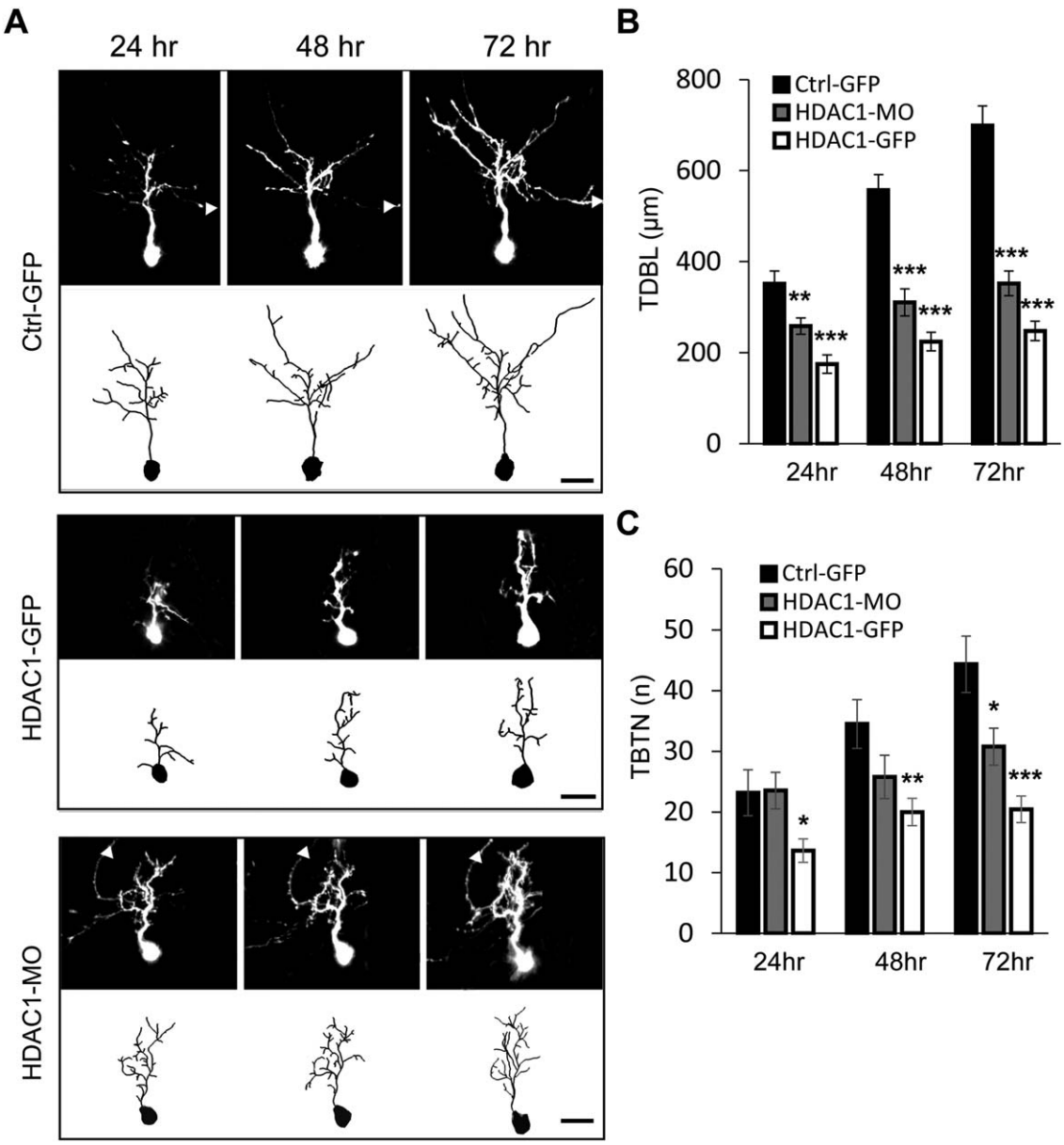

Figure 3 Manipulation of HDAC1 expression affects dendritic growth. (A) Representative images of transfected neurons and dendritic arbor reconstructions of time-lapse images taken at 0 hr, 24 hr, and 48 hr; Ctrl-GFP (top), HDAC1-GFP (middle), and HDAC1-MO (bottom). Scale bar: 30 μm. (B) At 72 hr, total dendritic branch length (TDBL) was significantly decreased in neurons transfected with HDAC1-GFP or HDAC1-MO. (C) At 72 hr, total branch tip number (TBTN) was dramatically decreased in HDAC1-GFP neurons and significantly decreased in HDAC1-MO neurons. $*p < 0.05$, $***p < 0.001$, Mann-Whitney test. $N = 12$ for each group.

TBTN were not altered in HDAC1-GFP or HDAC1-MO transfected neurons in response to visual stimulation [Fig. 4(D)]. These results indicate that expression of HDAC1-GFP or HDAC1-MO abolishes experience-dependent dendritic growth in tectal neurons *in vivo*.

HDAC1 Knockdown Selectively Decreases BDNF Expression Through Histone Modification at H4K5

To test whether BDNF expression was regulated by HDACs, we used a broad spectrum HDACs inhibitor,

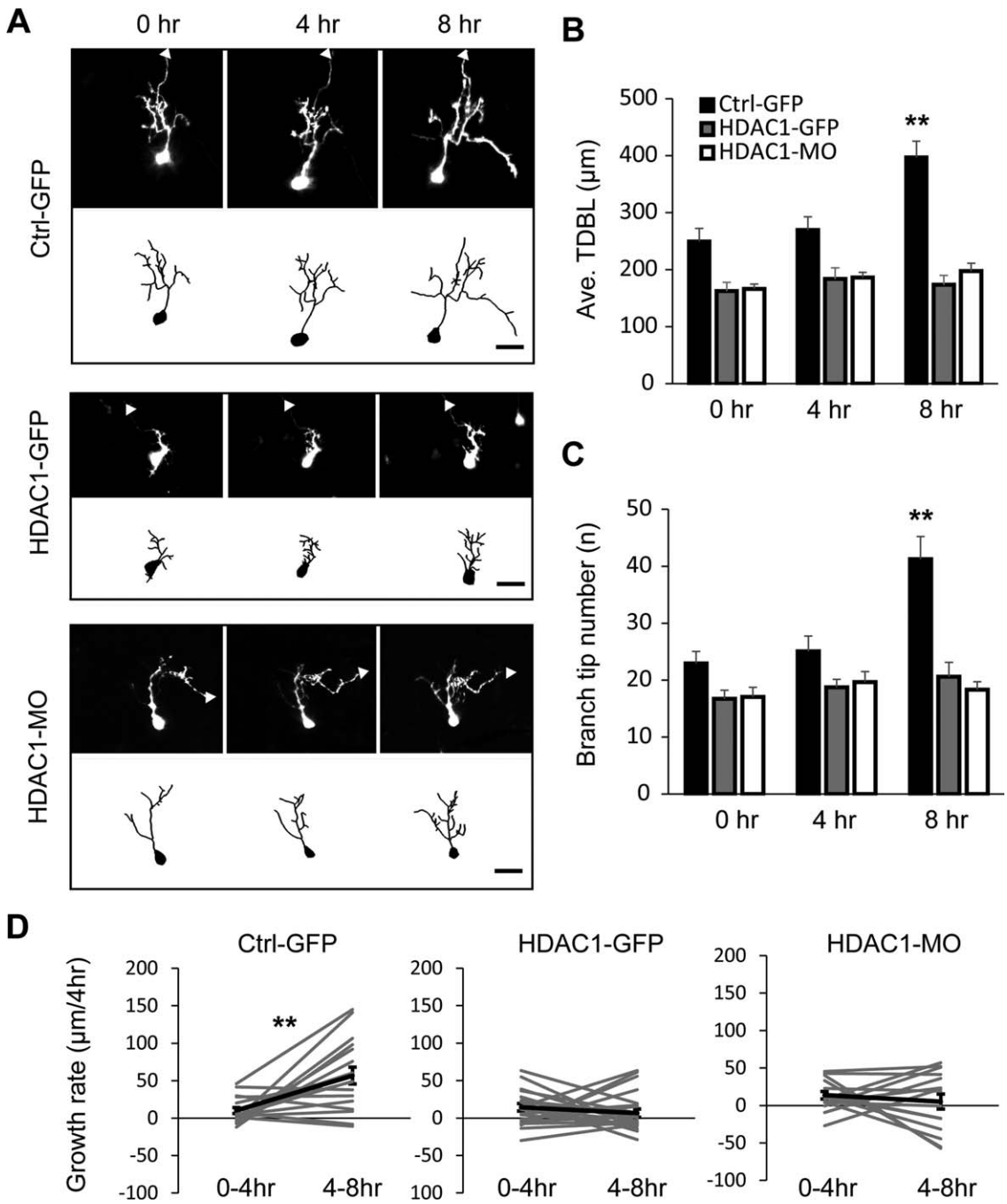

Figure 4 Overexpression or knockdown of HDAC1 affects experience-dependent structural plasticity in tectal neurons. (A) Representative dendritic arbor reconstructions of time-lapse images taken before treatment (0 hr), after 4 hr of dark (4 hr), and after 4 hr of enhanced visual stimulation (8 hr). Scale bar: 30 μm. (B and C) The increases in TDBL and TBTN in response to enhanced visual stimulation were prevented in neurons transfected with HDAC1-GFP or HDAC1-MO. (D) The growth rates of HDAC1-GFP and HDAC1-MO neurons were dramatically decreased compared to Ctrl-GFP neurons. $*p < 0.05$, $**p < 0.01$, Mann-Whitney test. $N = 17$ for each group.

TSA, to incubate tadpoles for 48 hours. Tectal brains were immunoblotted with an anti-BDNF antibody. We found that mature BDNF expression was significantly decreased in TSA-treated tadpoles compared to control tadpoles [Fig. 5(A)]. To further investigate the downstream signaling underlying

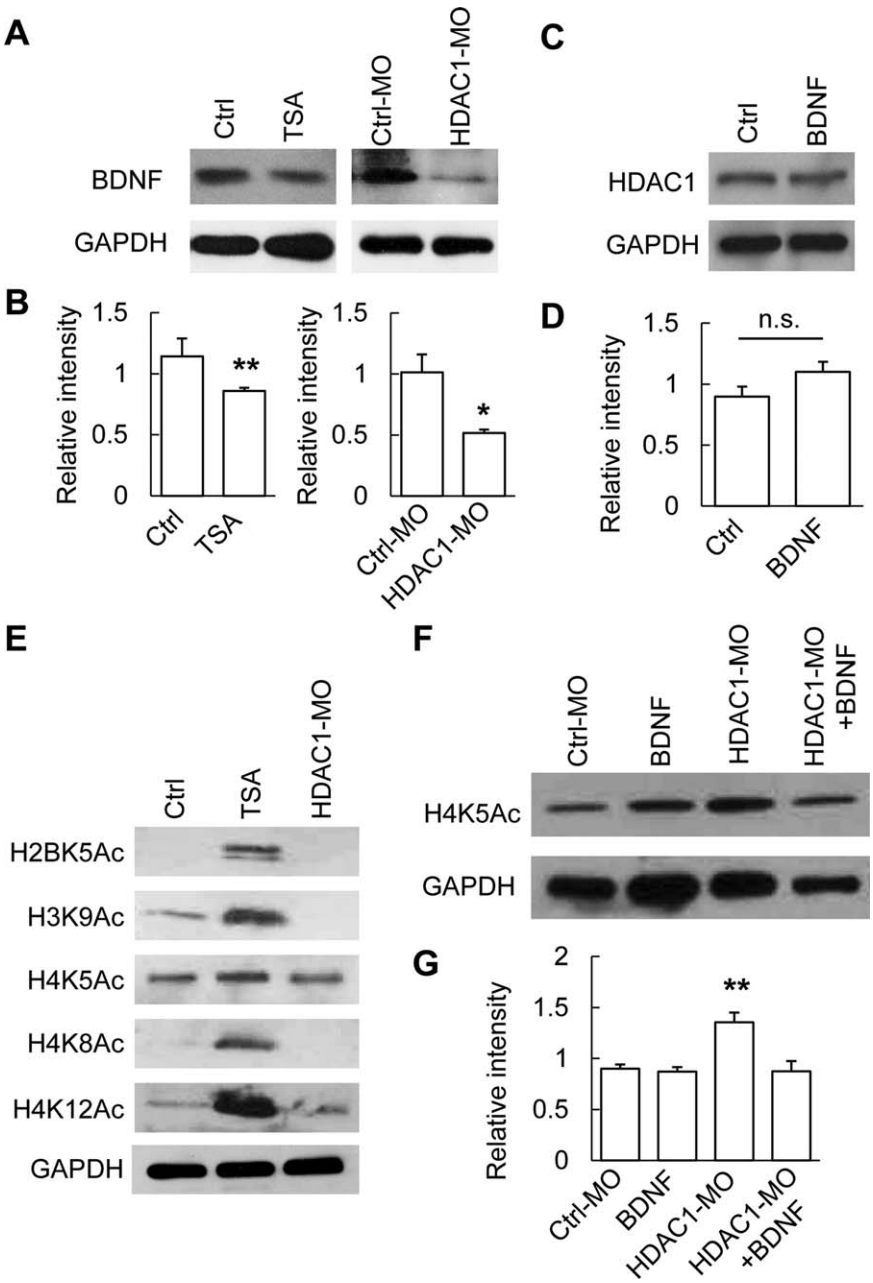

Figure 5 HDAC1 knockdown downregulates BDNF expression and increases histone H4 acetylation at K5. (A) Tectal brains were exposed to TSA (50 n*M*) for 2 days or transfected with Ctrl-MO or HDAC1-MO for 3 days as indicated. Brains were homogenized and blotted with an anti-BDNF antibody. (B) Summary of data showing that the relative intensity of BDNF to GAPDH was significantly decreased in TSA-treated or HDAC1-MO brains compared to Ctrl or Ctrl-MO brains. $N = 3$ for each group, $*p < 0.05$, $**p < 0.01$. (C and D) Tadpoles were injected with BDNF (200 ng/μL) for 20 hr and blotted with anti-HDAC1 antibody. HDAC1 expression was not changed after BDNF treatment. (E) Homogenized tecta were blotted with antibodies of anti-H2BK5Ac, anti-H3K9Ac, anti-H4K5Ac, anti-H4K8Ac, and anti-H4K12Ac respectively. (F) Tectal brains injected with BDNF, or transfected with HDAC1-MO, or transfected with HDAC1-MO and injected with BDNF were blotted with an anti-H4K5Ac antibody. (E) Summary of data showing that the relative intensity of H4K5Ac to GAPDH was significantly increased in HDAC1-MO brains compared to Ctrl-MO brains. $N = 3$ for each group, $*p < 0.05$.

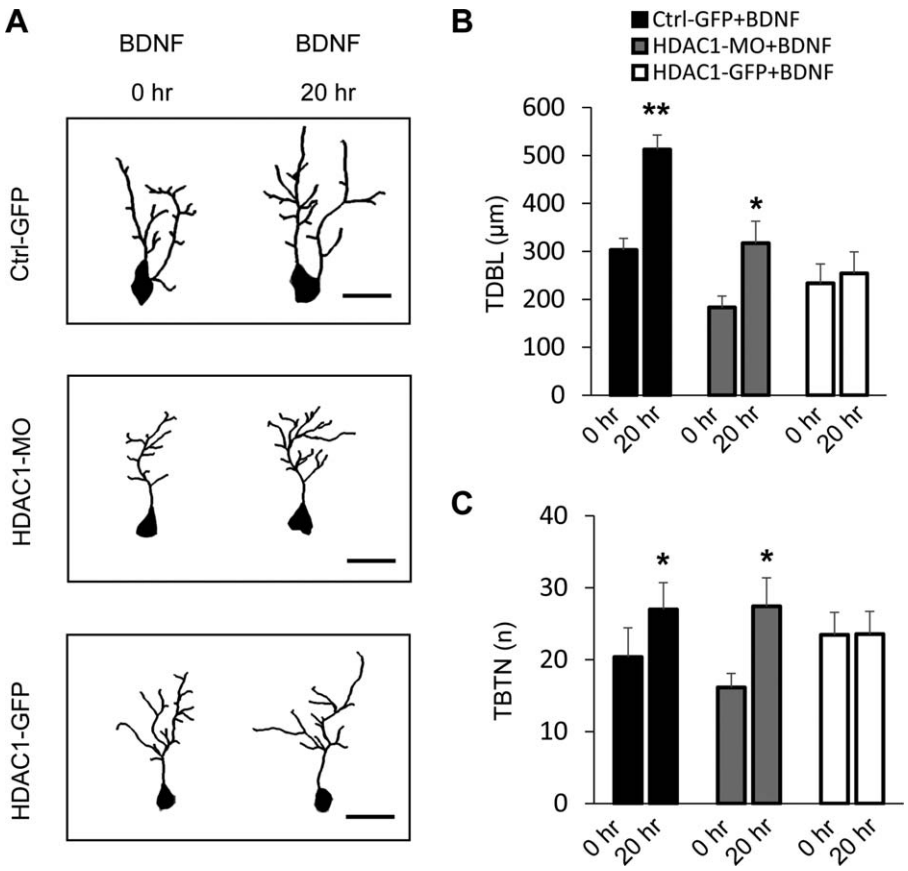

Figure 6 Loss of dendritic growth is rescued by BDNF treatment. (A) Tectal brains were electroporated with Ctrl-GFP, HDAC1-GFP, or HDAC1-MO, as indicated. After 1 to 2 days, single neurons were selected and imaged for the first time. Tectal brains were injected with BDNF (200 ng/μL) and incubated in Steinberg's solution for 20 hr. The same neurons were imaged again after 20 hr. Scale bar: 30 μm. Axons are denoted by white arrowheads on the original figures above. (B) Summary of data showing that the loss of TDBL in HDAC1-MO-transfected neurons was rescued by BDNF treatment. (C) Quantification of data showing that the TBTN was rescued by BDNF application. $*p < 0.05$, $**p < 0.01$. $N = 8$, 9, and 7 for Ctrl-GFP, HDAC1-GFP, and HDAC1-MO.

HDAC1-dependent neuronal structural plasticity, we examined the BDNF expression after HDAC1 downregulation. Our whole brain electroporation resulted in MO transfection in ~50% of tectal cells at stage 45 tadpoles. After 72 hr, tecta electroporated with Ctrl-MO or HDAC1-MO were dissected out and homogenized for BDNF staining [Fig. 5(A)]. We found that BDNF expression was significantly decreased in HDAC1-MO-transfected brains compared to Ctrl-MO brains [Fig. 5(B)] but not in HDAC1 overexpression brains (Ctrl-GFP: 1.02 ± 0.02, $N = 5$; HDAC1-GFP: 0.99 ± 0.02, $N = 5$. $p > 0.05$). In particular, BDNF treatment did not change the expression of HDAC1 [Fig. 5(C,D)]. These data imply that HDAC1 knockdown may sculpt neuronal structure by specifically decreasing BDNF expression in the developing tectum.

We next examined the acetylation levels of histones H2B, H3 and H4 in the presence of a general HDAC inhibitor, TSA (50 n*M*). We detected a global increase in the acetylation status of H2BK5, H3K9, H4K5, H4K8 and H4K12. To determine the substrate of histone acetylation for HDAC1, tecta were downregulated by HDAC1-MO and immunostained with these antibodies. We only detected a slight increase in the acetylation of H4K5 and H4K12 in HDAC1-MO transfected brains [Fig. 5(E)]. We further tested the BDNF effect on elevated H4K5 acetylation in HDAC1-MO transfected brains [Fig. 5(F)]. We found that the acetylation at H4K5 was not altered in BDNF exposed tadpoles, whereas HDAC1 knockdown significantly increased the acetylation at K5, which was suppressed by BDNF exposure for 20 hr [Fig. 5(G)]. These data suggest that HDAC1 may regulate BDNF

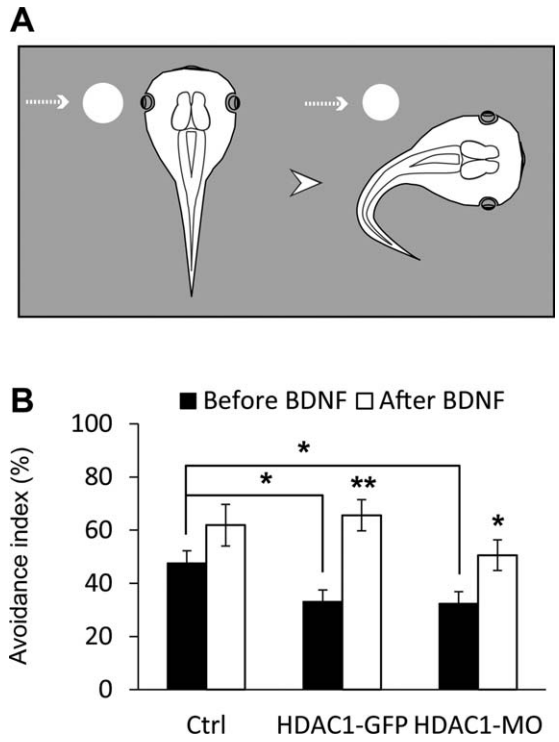

Figure 7 Deficit of visual avoidance behavior in HDAC1 overexpression or knockdown tadpoles is rescued by BDNF treatment. (A) Cartoon showing the tadpole avoiding the approaching spot. Tadpoles were transfected with HDAC1-GFP or HDAC1-MO, and the higher electroporation rate of transfection was selected for the first test. The same tadpoles were injected with BDNF (200 ng/μL), and the avoidance behavior was examined again. (B) Summary of data showing that the decrease in avoidance behavior in HDAC1-GFP or HDAC1-MO tadpoles was rescued by BDNF treatment. $*p < 0.05$, $**p < 0.01$. $N = 17$ for each group.

expression by changing specific residues in histone acetylation.

HDAC1-MO-Induced Deficits in Neuronal Structure are Rescued by BDNF Treatment

To determine whether HDAC1-knockdown-induced changes in neuronal structure could be restored by BDNF application, we transfected tadpoles at stage 45 with Ctrl-GFP, HDAC1-MO, or HDAC1-GFP. Single neurons were imaged, and tadpoles were immediately injected with BDNF as described previously (200 ng/μL) (Alsina et al., 2001). After 20 hr, the same neurons were imaged for a second time [Fig. 6(A)]. We found that both TDBL and TBTN were significantly increased in BDNF-treated Ctrl-GFP neurons. The TDBL and TBTN of HDAC1-MO-transfected neurons were rescued by BDNF treatment. However, the TDBL and TBTN of HDAC1-GFP-transfected neurons remained unchanged after BDNF treatment [Fig. 6(B)]. These data suggest that HDAC1 may regulate the development of neuronal structure through BDNF signaling.

HDAC1-Mediated Loss of Visual Avoidance Behavior is Recovered by BDNF Treatment

We electroporated tadpoles at stage 45 with HDAC1-MO and measured visual avoidance behavior after 72 hr by projecting moving spots of 0.4 cm in diameter to the tadpoles as reported previously (Shen et al., 2011). Visual avoidance behavior was evaluated based on the number of times that tadpoles swam away when encountering the moving spots [Fig. 7(A)]. We found that the avoidance index was significantly decreased in HDAC1-GFP and HDAC1-MO tadpoles compared to control tadpoles [Fig. 7(B)]. To examine whether BDNF treatment could rescue the behavioral deficit, the HDAC1-GFP or HDAC1-MO transfected tadpoles were injected with recombinant BDNF (200 ng/μl), and the avoidance behavior was measured again after 20 hr. We found that the deficits in swimming behavior of HDAC1-GFP or HDAC1-MO tadpoles were rescued when tadpoles were injected with BDNF [Fig. 7(B)].

DISCUSSION

The fundamental findings of this study demonstrate that HDAC1 is essential for neuronal development and visual experience-dependent structural plasticity in the developing tectum of *Xenopus laevis* tadpoles. In particular, HDAC1 knockdown downregulates BDNF expression and selectively increases histone H4 acetylation at K5. Importantly, the deficits in neuronal structural development and visually guided avoidance behavior in HDAC1-MO-transfected tadpoles are rescued by recombinant BDNF treatment.

It is generally accepted that HDAC family members are involved in the regulation of synaptic transmission and neuronal plasticity. Previous studies have shown that HDAC2 knockdown could regulate the balance of excitation to inhibition by enhancing excitatory synaptic transmission and reducing inhibitory synaptic transmission in cultured hippocampal neurons (Hanson et al., 2013). HDAC2 negatively regulates learning and memory (Guan et al., 2009) by controlling the balance of excitation *versus* inhibition (Hanson et al., 2013). However, the role of HDAC1

in neuronal synaptic transmission and dendritic arbor growth is still unclear. Our data suggest that knockdown of HDAC1 decreases the frequency of glutamatergic synaptic transmission and increases the frequency of GABAAR-mediated synaptic transmission. Studies in cultured hippocampal slices have shown that knockdown of HDAC2 but not HDAC1 significantly alters mEPSC amplitude (Hanson et al., 2013). This apparent discrepancy may arise due to differences in animal models, *in vivo* methods and experimental procedures. Furthermore, HDAC activity may play differential roles during brain development. During early brain development, decreases in the activities of both HDAC1 and HDAC2 facilitate the maturation of excitatory synapses, whereas in mature neurons, HDAC2 only controls synapse maturation (Akhtar et al., 2009). We speculate that HDAC1 facilitates the maturation of tectal neurons during early brain development.

A fundamental process for neural circuit development is maintaining the balance of excitation to inhibition, which is required for the spatial and temporal control of circuit information transfer (Eichler and Meier, 2008). BDNF not only potentiates glutamatergic synapse maturation but also promotes the maturation of GABAergic synapses in the CNS (Huang et al., 1999; Baldelli et al., 2005). HDAC1 knockdown dramatically increases the frequency of mIPSCs, suggesting that BDNF may not directly exert its function on GABAergic transmission. Alternatively, decreased BDNF expression in neurons with HDAC1 knockdown results in decrease in glutamatergic transmission, which in turn may promote the strength of feedforward inhibition on the transfected neuron. Almost two-thirds of the neurons in the optic tectum are excitatory neurons. Separate measurements of excitatory and inhibitory neurons may be necessary for further understanding the primary and secondary effects of HDAC1 and BDNF in inhibitory synapse maturation.

As an important transcriptional repressor, HDAC1 is reported to be involved in many biological processes. Studies in developing zebrafish retina suggest that HDAC1 suppresses neurogenesis and cell cycle exit through the Wnt and Notch signaling pathways (Yamaguchi et al., 2005). Our previous studies demonstrate that HDAC1 blocks the experience-dependent increase in the proliferation of radial glial cells in the developing *Xenopus* tectum (Tao et al., 2015). Others have shown that HDAC1 and HDAC2 play essential roles in cell proliferation and stem cell self-renewal by performing a double knockout of *Hdac1/2* in embryonic stem cells (Jamaladdin et al., 2014). We examined whether HDAC1 regulates experience-dependent structural plasticity in the developing brain. Experience-dependent dendritic growth is well established in the *Xenopus* tectum by *in vivo* time-lapse imaging (Aizenman et al., 2003; Shen et al., 2009). A relatively brief (4 hr) exposure of tadpoles to enhanced visual stimulation results in a significant increase in the rate of dendritic arbor growth (Sin et al., 2002). Strikingly, our study reveals that either knockdown or overexpression of HDAC1 in tectal neurons disrupts the neuronal structural development and experience-dependent structural plasticity as well. A variety of factors have been reported to be correlated with the visual experience-dependent structural plasticity, such as AMPAR-, GABAAR-, and NMDAR-mediated synaptic transmission (Sin et al., 2002; Haas et al., 2006; Ewald et al., 2008; Shen et al., 2009). The functional consequences of the disruption in the balance of excitation and inhibition result in multiple deficits in receptive field mapping and visually guided avoidance behavior (Tao and Poo, 2005; Shen et al., 2011; Zhang et al., 2011; Dong and Aizenman, 2012; Khakhalin et al., 2014). Combined with the data showing that HDAC1 regulates excitatory and inhibitory synaptic transmission, we propose that HDAC1 may modulate neuronal growth and structural plasticity by changing neuronal transmission.

Previous studies have shown that overexpression of HDAC1 can induce neurotoxicity, which would interfere with dendritic growth and structural plasticity. However, HDAC1 may play opposing roles in controlling neuronal survival or death under different experimental conditions. HDAC1 nuclear export induced by pathological conditions promotes axonal damage and degeneration (Kim and Casaccia, 2010; Kim et al., 2010). HDAC1 may function as a modulator of neuronal death via interaction with HDAC3 but not with HDRP (Bardai et al., 2012). On the contrary, increased HDAC1 function protects neurons from neurotoxicity by p25/Cdk5 in cultured neurons and ischemic mice (Kim et al., 2008). Our data indicate that overexpression or knockdown of HDAC1 in the developing tectum results in deficits of dendritic growth and experience-dependent structural plasticity. However, cell apoptosis is not induced after three days of HDAC1 overexpression or knockdown *in vivo* (Supporting Information Fig. 1), indicating that HDAC1 regulates neuronal structure and function but not leads to neuronal death.

BDNF is known to regulate multiple biological processes in synaptic maturation, dendritic growth and neuronal plasticity (Wong and Ghosh, 2002; Du and Poo, 2004; Ji et al., 2010; Schwartz et al., 2011). It is generally accepted that BDNF potentiates

glutamatergic synaptic transmission by increasing mEPSC frequency and synapse number (Tyler and Pozzo-Miller, 2001; Tyler and Pozzo-Miller, 2003; Du and Poo, 2004; Shen et al., 2006; Calfa et al., 2012). BDNF-TrkB signaling has been shown to be under epigenetic control (Martinowich et al., 2003; Tsankova et al., 2006; Guan et al., 2009). Although the effects of HDAC inhibitors on cell functions have been extensively studied, there is limited knowledge of the specific role of each HDAC in neural structure and function. Previous studies failed to detect the changes in BDNF mRNA following treatment with TSA, a broad spectrum HDAC inhibitor, in cultured hippocampal neurons (Akhtar et al., 2009). Our data suggest that reduced BDNF expression by TSA treatment or HDAC1 knockdown decreases glutamatergic synaptic transmission, which is required for neuronal development and experience-dependent dendritic arbor growth. BDNF-dependent dendritic growth may also depend on the strength of glutamatergic synaptic inputs and the maturation of neurons (Singh et al., 2006). We thus speculate that reduced excitatory synaptic transmission may disrupt the balance of excitation to inhibition, which adversely increases inhibitory synaptic transmission by increasing the frequency of mIPSCs in HDAC1-knockdown neurons (Fig. 2). Overexpression of HDAC1 in the optic tectum does not result in changes of BDNF expression, suggesting that HDAC1 may modulate dendritic arbor growth through differential HDAC1 targets other than BDNF signaling pathway (Zupkovitz et al., 2006; Nambiar et al., 2007; Akhtar et al., 2009)

Specific downregulation of histone acetylation at H4K12 is associated to memory impairment in aging mice brain (Peleg et al., 2010). Loss of HDAC1 results in elevated acetylation in H3K56 in embryonic stem cells (Dovey et al., 2010), suggesting that H3K56 is the substrate for HDAC1. Our findings indicate that *Xenopus* tecta display low acetylation at H4K8 and H2BK5, whereas a HDAC inhibitor TSA induces a global increase in histone acetylation. BDNF-induced H3 acetylation at K9 and K14 is occluded by a broad spectrum HDAC inhibitor, TSA (Calfa et al., 2012). HDAC1 knockdown data support the idea that specific acetylation modification at H4K5 and H4K12 might be engaged in the BDNF-dependent structural plasticity. Although we cannot exclude that other histone modifications may also contribute to the effect, H4K5 acetylation seems to be of particular importance for HDAC1-mediated gene transcription. BDNF is one of the most important transcriptional factor which is regulated by chromatin modifications (Bredy et al., 2007; Tsankova et al., 2007). On the other hand, BDNF can influence chromatin remodeling by *S*-nitrosylation of HDAC2 (Nott et al., 2008). Long-term effects of BDNF on neuronal morphology and function are prevented by HDAC inhibition, suggesting a divergent transcriptional regulation between HDAC activity and BDNF expression (Segal and Greenberg, 1996). These data support the hypothesis that plasticity-related gene expression requires specific lysine residues on histones for corresponding transcriptional programs (Fischer et al., 2007; Bousiges et al., 2013).

BDNF is also a general modulator of GABAergic synaptic transmission. BDNF can induce the increase in the frequency of GABAergic mIPSC activity (Palizvan et al., 2004; Baldelli et al., 2005) or the numbers of inhibitory synaptic terminals (Marty et al., 2000; Aguado et al., 2003; Henneberger et al., 2005). BDNF could also directly act on cells to exert its neurotrophic effect to increase dendritic branching and synaptic transmission (Lom et al., 2002). However, we found that the frequency of mIPSCs is increased in HDAC1-knockdown neurons. The weakening of excitatory synaptic transmission may perturb the homeostatic regulation of developing neuronal networks, which in turn potentiate the strength of inhibitory transmission. More work is still necessary to clarify the direct relationship between the BDNF effect and the homeostatic regulation in developing brain.

The optic tectum is a critical visual processing center for visually guided avoidance behavior (Dong et al., 2009; Schwartz et al., 2011; Shen et al., 2011, 2014; Khakhalin et al., 2014). Perturbation in either excitatory or inhibitory transmission impairs visually guided behavior, suggesting that deficits in circuit function can disrupt behaviors. BDNF may potentiate cellular function that are not affected by HDAC1 overexpression, which promotes visually-guided avoidance behavior. Importantly, HDAC1-knockdown-induced deficits in dendritic growth were rescued by acute BDNF application. These data reveal that BDNF treatment may potentiate synaptic contacts and synaptic transmission to regain the circuit connectivity and function after the loss of dendritic growth and deficits in neural transmission caused by HDAC1 knockdown, and these circuit function is required for retinotectal visual responses (Ruthazer and Aizenman, 2010).

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: H.R., Y.T., and W.S. Acquisition of data: H.R., Y.T., J.G., X.Q., X.G., Z.G., L.Z., and W.S. Analysis and interpretation of data: H.R., J.G., X.Q., Y.T., and W.S. Drafting of the manuscript: W.S. Statistical analysis: H.R. and W.S.

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