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HDAC2 expression in parvalbumin interneurons regulates synaptic plasticity in the mouse visual cortex

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ABSTRACT

An experience-dependent postnatal increase in GABAergic inhibition in the visual cortex is important for the closure of a critical period of enhanced synaptic plasticity. Although maturation of the subclass of parvalbumin (Pv)–expressing GABAergic interneurons is known to contribute to critical period closure, the role of epigenetics on cortical inhibition and synaptic plasticity has not been explored. The transcription regulator, histone deacetylase 2 (HDAC2), has been shown to modulate synaptic plasticity and learning processes in hippocampal excitatory neurons. We found that genetic deletion of HDAC2 specifically from Pv interneurons reduces inhibitory input in the visual cortex of adult mice and coincides with enhanced long-term depression that is more typical of young mice. These findings show that HDAC2 loss in Pv interneurons leads to a delayed closure of the critical period in the visual cortex and supports the hypothesis that HDAC2 is a key negative regulator of synaptic plasticity in the adult brain.

Introduction

The normal maturation of the visual cortex is dependent on environmental visual stimulus during a period of postnatal development of enhanced plasticity, termed the *critical period*. In rodents, the onset of the critical period in the visual cortex coincides with eye opening at 2 weeks of age, and the subsequent closure of the critical period occurs around the onset of puberty, at 5 weeks. During this period, cortical responses within the primary visual cortex (V1) can be altered by simple manipulation of sensory experience. Mechanisms by which this experience-dependent cortical plasticity can be altered require changes in N-methyl-D-aspartate receptor (NMDAR)–mediated synaptic responses, such as long-term potentiation and long-term depression (LTD) (Smith et al., 2009). The subsequent decrease in cortical plasticity that occurs during the closure of the critical period has been shown to be modulated by γ-aminobutyric acid (GABA)-ergic inhibitory interneurons.

The maturation of inhibitory interneurons in the cortex of rodents mirrors the timing of the critical period, slowly maturing during postnatal development until around 5 weeks of age. Reduced or ablated expression of the GABA synthesizing enzymes delays the closure of the critical period (Chattopadhyaya et al., 2007; Fagiolini and Hensch, 2000; Hensch et al., 1998; Morales et al., 2002), while conversely, an early onset in critical period plasticity can be induced by accelerating GABA circuit function (Di Cristo et al., 2007; Jiang et al., 2010; Kirkwood et al., 1995; Sugiyama et al., 2008). The largest class of interneurons is the parvalbumin (Pv)–expressing neurons, comprising up to 50% of the inhibitory cells in the mouse cortex (Gonchar et al., 2007; Morales et al., 2002). In rodents, the Pv-expressing cells also emerge and mature with a postnatal time course that follows the critical period in the visual cortex (del Río et al., 1994; Huang et al., 1999; Jiang et al., 2007; Kirkwood et al., 1997). Maturation of Pv-expressing cells is triggered by non cell autonomous factors, such as brain-derived neurotrophic factor (BDNF) and Otx2, and artificial elevation of these factors accelerates Pv cell maturation and prematurely closes the critical period (Huang et al., 1999; Jiang et al., 2007; Kirkwood et al., 1997; Sugiyama et al., 2008).

Molecular mechanisms of cortical plasticity require changes in gene expression. Indeed, the alteration of visual experience during the critical period in rodents has been shown to modify the expression of many genes (Jiang et al., 2007; Kirkwood et al., 1997; Majdan and Shatz, 2006). Activity-dependent changes in neuronal gene expression are mediated in part by posttranslational modification of histones. The exposure of dark-reared mice to light triggers the phosphorylation and acetylation of histones and is more pronounced in mice during the critical period than in adulthood (Jiang et al., 2010; Kirkwood et al., 1995; Putignano et al., 2007). Manipulation of

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histone acetylation by treatment of adult mice with histone deacetylase (HDAC) inhibitors promotes increased plasticity of the visual cortex and is able to rescue visual acuity deficits elicited during early life that are normally irreversible (Gervain et al., 2013; Putignano et al., 2007; Silingardi et al., 2010; Yang et al., 2012). The positive effects of HDAC inhibition on the critical period are not confined to the visual system and can also increase auditory perception in both adult mice and humans (Gervain et al., 2013; Yang et al., 2012).

Histone deacetylation, therefore, plays an important role in mediating enhanced cortical plasticity of the visual system; however, the importance of cell-type specific histone deacetylation and its regulation by individual HDACs on cortical plasticity has not been explored. Previous studies show that HDAC2 acts as an epigenetic blockade for learning and memory processes through its interaction at the promoters of synaptic plasticity genes causing a reduction in the expression of these genes (Gräff et al., 2012, 2014; Guan et al., 2009). The aim of this study was to investigate the role of HDAC2-mediated gene regulation of Pv-expressing cells on synaptic plasticity of the visual cortex. We report that the loss of HDAC2 expression specifically in Pv-positive interneurons leads to a reduction in inhibitory synaptic strength and an increase in NMDAR-mediated long-term plasticity in adult mice and more closely reflects that of responses normally observed in young mice associated with enhanced cortical plasticity.

Methods

Animals

HDAC2 conditional knockout mice (HDAC2f/f;Pv-cre) were generated by crossing HDAC2 floxed mice (HDAC2f/f) (Guan et al., 2009) with the transgenic Pv promoter-driven Cre line (Hippenmeyer et al., 2005). All experiments were performed using male mice at either 3 weeks or 8 weeks old in a FVB × C57/BL6 background, and each experiment was performed using littermate age-matched controls. The age and number of mice used for each experiment have been indicated in the appropriate figure legends. All animal work was approved by the Committee for Animal Care of the Division of Comparative Medicine at the Massachusetts Institute of Technology.

Immunohistochemistry

Eight-week-old male mice were perfused with 10% formaldehyde under deep anesthesia, and brains were post-fixed overnight in 10% formaldehyde. Brains were sectioned at 40 μm using a vibratome (Leica, Nussloch, Germany). Sections were permeabilized and blocked in phosphate-buffered saline (PBS) containing 0.3 % Triton X-100 and 10 % normal donkey serum at room temperature for 1 hour. Sections were incubated overnight at 4 °C in primary antibody diluted 1:200 in PBS with 0.3 % Triton X-100 and 10 % normal donkey serum. Primary antibodies used were anti-HDAC2 (Abcam; #12169) and anti-Pv (Swant, 235). Primary antibodies were visualized with Alexa Fluor 488, and Alexa Fluor 647 antibodies (Jackson ImmunoResearch Laboratories) and Hoechst 33342, all diluted 1:500 in PBS and incubated at room temperature for 90 minutes. Sections were mounted on slides with Fluoromount G (Electron Microscopy Sciences) overnight at room temperature and stored at 4 °C. Images were acquired using an LSM 710 Zeiss confocal microscope and analyzed using ImageJ 1.46a software.

Western blot

Whole cell lysates of the V1 visual cortex were prepared using tissue from 8-week-old male mice. Tissue was homogenized in 1-mL RIPA buffer (50 mM Tris HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% Sodium Dodecyl Sulfate (SDS) with a hand homogenizer (Sigma), incubated on ice for 15 minutes, and rotated at 4 °C for 30 minutes. Cell debris were isolated and discarded by centrifugation at 14,000 rpm for 10 minutes. Lysates were loaded on 10 % acrylamide gels and protein transferred from acrylamide gels to polyvinyl difluoride (PVDF) membranes (Invitrogen) at 100 V for 90 minutes. Membranes were blocked using bovine serum albumin (5 % w/v) diluted in TBS:Tween. Membranes were incubated in primary antibodies overnight at 4 °C and secondary antibodies at room temperature for 90 minutes. Western blots were imaged using the Odyssey Imaging System (LI-COR Biosciences) and analyzed with ImageJ 1.46a software.

Field potential recording

Coronal slices (400 μm thick) of mice visual cortical slices were prepared in ice-cold dissection buffer (in mM: 211 sucrose, 3.3 KCl, 1.3 NaH_2PO_4, 0.5 $CaCl_2$, 10 $MgCl_2$, 26 $NaHCO_3$, and 11 glucose) that is oxygenated with 95% O_2 and 5% CO_2 (Kirkwood et al., 1997). After dissection using a Leica VT1000S vibratome (Leica, Nussloch, Germany), slices were moved into recovery chamber with 95 % O_2 and 5 % CO_2–saturated artificial cerebrospinal fluid consisting of (mM) 124 NaCl, 3.3 KCl, 1.3 NaH_2PO_4, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 26 $NaHCO_3$, and 11 glucose for 1 hour at 28–30 °C. To stimulate layer IV pyramidal neurons, tungsten bipolar electrode (200 μm diameter; FHC, Bowdoinham, ME, USA) was used, and glass microelectrode filled with artificial cerebrospinal fluid was placed in layer II/III to record extracellular field potential. White matter (WM) to layer IV recording was made with the same paradigm. LTD was induced by single-pulse low-frequency stimulation (SP-LFS) (900 pulses at 1 Hz).

Whole-cell patch-clamp recording

Coronal brain slices (250 μm thick) were used for whole-cell patch-clamp recordings. Miniature inhibitory postsynaptic potential was measured from layer II/III pyramidal neurons using recording pipettes (3–5 MΩ) filled with internal solution containing (mM) 145 CsCl, 5 NaCl, 10 HEPES-CsOH, 10 EGTA, 4 MgATP, and 0.3 Na_2GTP in the presence of tetrodotoxin (1 μM), 2-amino-5-phosphonovaleric acid (100 μM), and 6-cyano-7-nitroquinoxaline-2,3-dione (200 μM). For compound inhibitory postsynaptic currents (IPSCs) and excitatory postsynaptic currents (EPSCs), synaptic responses were evoked with a bipolar stimulating electrode with 0.2 milliseconds of current pulse delivery at layer IV of the visual cortical slices. Layer II/III cells were held at 0 mV for IPSCs and −60 mV for EPSCs with recording pipettes filled with internal solution containing (mM) 130 Cs-gluconate, 2 $MgCl_2$, 2 $CaCl_2$, 10 EGTA, 10 Hepes, 2 Na-ATP, and 10 QX-314 (Gianfranceschi et al., 2003; Morales et al., 2002). A MultiClamp 700B amplifier and a Digidata 1440A A-D converter (Axon Instruments, Union City, CA, USA) were used for data acquisition, and data were analyzed with pClamp10 (Axon Instruments).

Results

Conditional deletion of HDAC2 in Pv-expressing interneurons

To address the effects of the loss of HDAC2 on Pv inhibitory neurons, HDAC2 conditional knockout mice (HDAC2f/f;Pv-cre) were generated by crossing HDAC2 floxed mice (HDAC2f/f) (Guan et al., 2009), with the transgenic Pv promoter-driven Cre line (Hippenmeyer et al., 2005). HDAC2 was first verified as being strongly expressed in Pv-positive interneurons by co-immunostaining brain slices for HDAC2 and Pv from HDAC2f/f wild-type

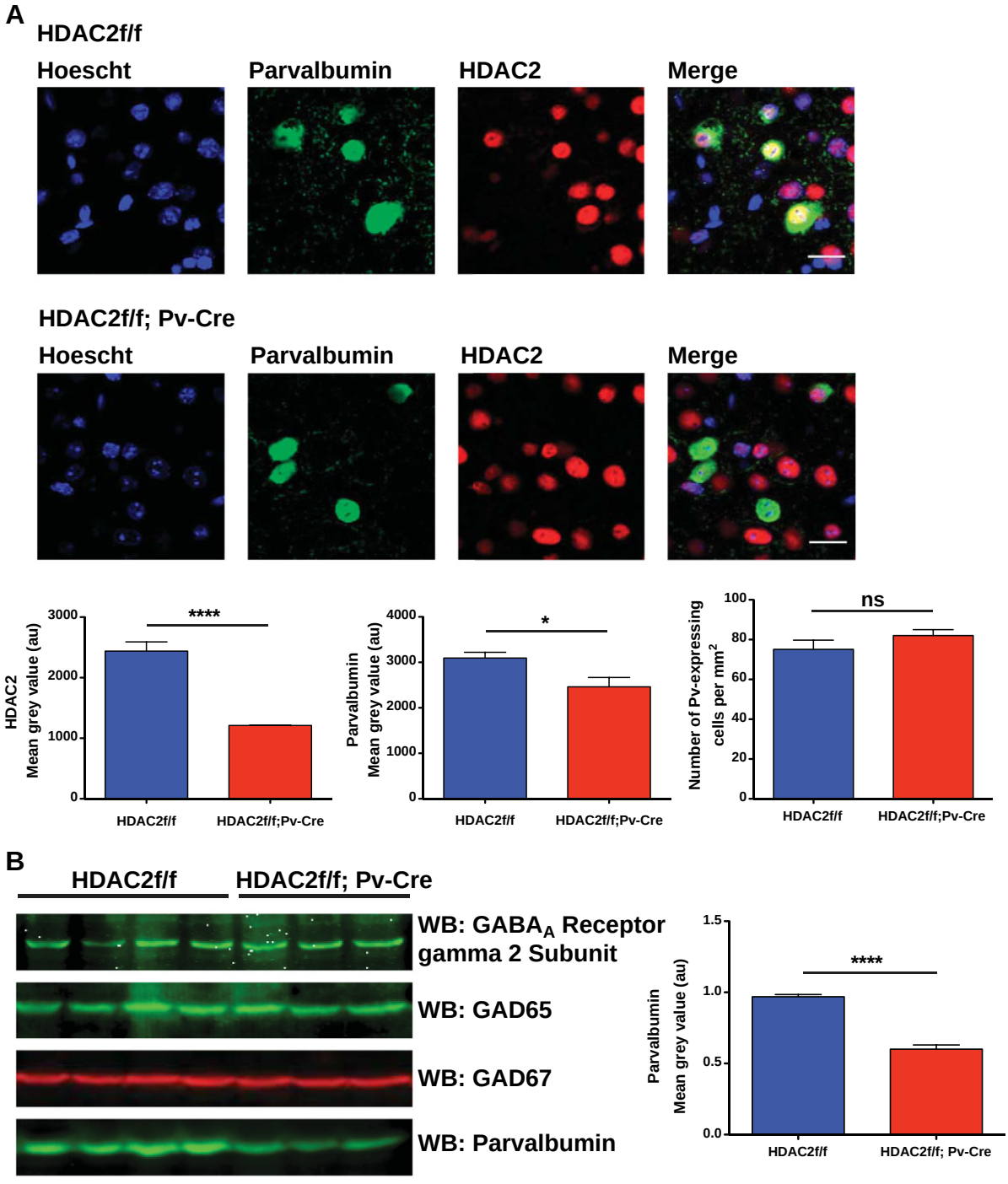

Fig. 1. HDAC2 conditional knockout from Pv-expressing interneurons. (A) Coronal brain slices of HDAC2f/f and HDAC2f/f;Pv-cre male mice at 8 weeks old were stained using Hoescht (blue), anti-Pv (green), and anti-HDAC2 (red), and images captured of the V1 visual cortex using a confocal microscope. Scale bar, 20 μm. Mean gray value quantification of HDAC2 and Pv immunofluorescence was compared between HDAC2f/f and HDAC2f/f;Pv-cre mice in the visual cortex. Two-tailed *t* test; $P < .0001$, HDAC2; $P < .05$, Pv; n = 5 mice (HDAC2f/f), 5 mice (HDAC2f/f;Pv-cre). Cell number quantification of Pv expressing cells in the V1 visual cortex. Two-tailed *t* test; $P > .05$; n = 5 mice (HDAC2f/f), 7 mice (HDAC2f/f; Pv-cre). (B) Western blot analysis of $GABA_A$ receptor gamma 2 subunit, GAD65, GAD67, and Pv protein levels using whole cell lysates of the V1 visual cortex from HDAC2f/f and HDAC2f/f;Pv-cre mice. Mean gray value quantification of Pv protein levels. Two-tailed *t* test; $P < .0001$; n = 4 mice (HDAC2f/f), 3 mice (HDAC2f/f;Pv-cre).

mice (Fig. 1A). The loss of HDAC2 in Pv inhibitory neurons of the V1 region of the visual cortex was confirmed by co-immunostaining of brain slices from HDAC2f/f;Pv-cre mice (Fig. 1A). Loss of HDAC2 expression leads to a decrease in expression of Pv, as quantified by immunostaining and Western blot analysis of tissue from the V1 region of the visual cortex, with no loss in the total number of Pv-expressing cells (Fig. 1A and B). The loss of HDAC2 expression in Pv neurons had no effect on the expression of glutamate decarboxylase 1 (GAD1/GAD67) or GAD2 (GAD65) in the visual cortex as determined by Western blot analysis (Fig. 1B).

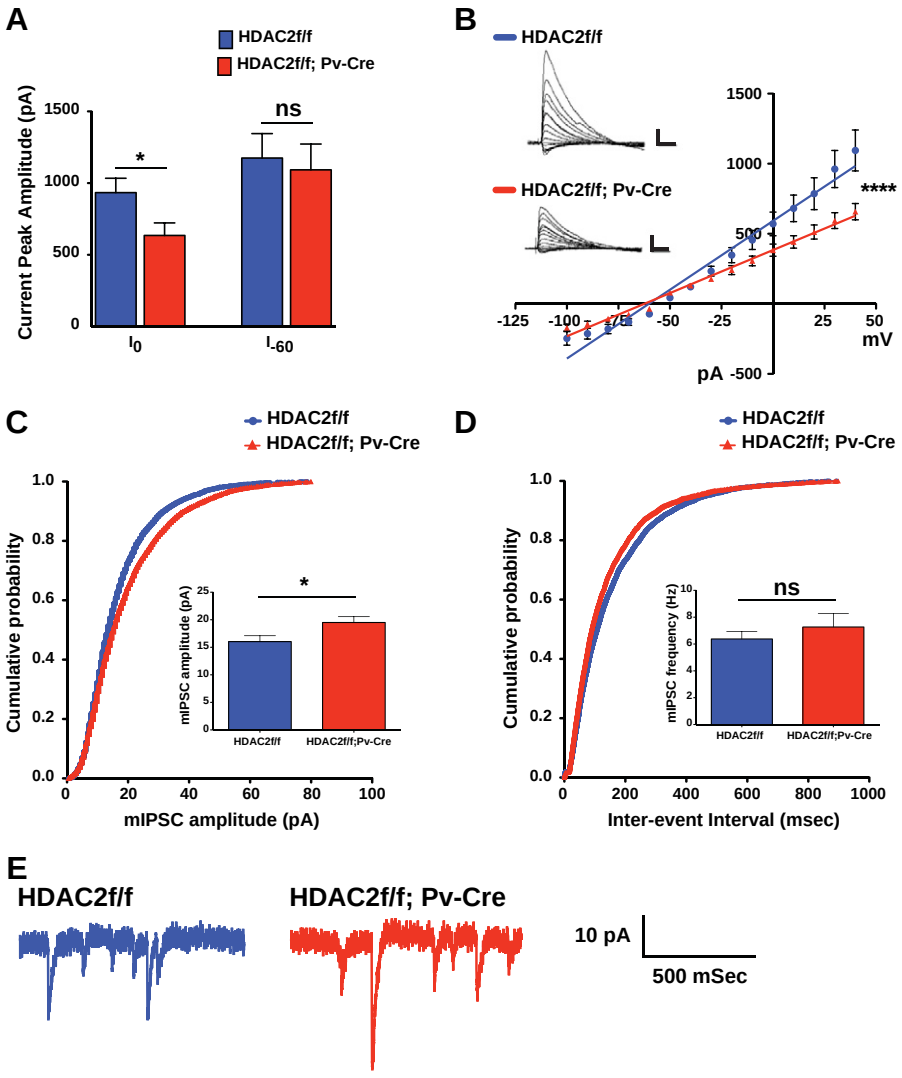

Fig. 2. Effects of HDAC2 conditional knockout in Pv-positive interneurons on IPSCs recorded in layer II/III pyramidal neurons of the visual cortex at 8 weeks old. (A) Average magnitude of maximal IPSCs and maximal EPSCs of pyramidal cells from the visual cortex of 8-week-old HDAC2f/f and HDAC2f/f;Pv-cre mice. Two-tailed *t* test; IPSC, $P < .05$; EPSC, $P > .05$; n = 16 cells, 4 mice (HDAC2f/f), 16 cells, 4 mice (HDAC2f/f;Pv-cre). (B) I–V plot of the IPSC recorded at different membrane potentials in the visual cortex of brain slices from 8-week-old HDAC2f/f and HDAC2f/f;Pv-cre mice. (Inset) Typical traces of IPSCs recorded at different membrane potentials from HDAC2f/f and HDAC2f/f;Pv-cre mice (scale bars, 200 pA and 20 milliseconds). Analysis of covariance; $P < .0001$; n = 24 cells from 5 mice (HDAC2f/f), 25 cells, 5 mice (HDAC2f/f;Pv-cre). (C) Cumulative probability distribution of mIPSC amplitude of layer II/III pyramidal cells in the visual cortex of 8-week-old HDAC2f/f and HDAC2f/f;Pv-cre mice. (Inset) Column bar graph represents the average mIPSC amplitude from all cells recorded. Two-tailed *t* test; $P < .05$; n = 15 cells, 2 mice (HDAC2f/f), 13 cells, 2 mice (HDAC2f/f;Pv-cre). (D) Cumulative probability distribution of mIPSC interevent interval as in C. (Inset) Column bar graph represents the average mIPSC frequency from all cells recorded. Two-tailed *t* test; $P > .05$; n = 15 cells, 2 mice (HDAC2f/f), 13 cells, 2 mice (HDAC2f/f;Pv-cre). (E) Traces represent mIPSCs recorded from HDAC2f/f (blue) and HDAC2f/f;Pv-cre mice from C and D.

Inhibitory synaptic plasticity is reduced in the visual cortex of HDAC2f/f;Pv-cre mice

To examine whether loss of HDAC2 in Pv-expressing interneurons affected the strength of inhibitory synapses, we measured the evoked postsynaptic responses of pyramidal neurons in the visual cortex from brain slices of wild-type HDAC2f/f and mutant HDAC2f/f;Pv-cre mice. We determined the maximal IPSCs and EPSCs in layer II/III pyramidal neurons evoked by layer IV stimulation. To isolate maximal IPSCs, the membrane potential was held at 0 mV (I_0), which is the reversal potential for α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and NMDAR. Maximal EPSCs were measured by holding the same pyramidal cells at −60 mV (I_{-60}), which is the approximate reversal membrane potential for IPSCs. The amplitude of maximal IPSC (Fig. 2A) was significantly reduced in HDAC2f/f;Pv-cre mice (635.4 pA ± 87 pA; n = 16) compared to wild-type HDAC2f/f mice (933.5 ± 100.4 pA; n = 16), whereas no significant difference was observed for maximal EPSC between wild-type HDAC2f/f mice (1175 ± 170.4 pA; n = 16) and HDAC2f/f;Pv-cre mice (1093 ± 179.4 pA; n = 16). These results are indicative of a reduction in the evoked GABAergic inhibition of pyramidal cells in the visual cortex in adult mice that lack HDAC2 in Pv-expressing interneurons. This finding mirrors that of rats reared in the dark for 5 weeks, which have profoundly reduced maximal IPSCs compared to normally reared animals (Morales et al., 2002).

The peak conductance underlying the maximal IPSC was estimated from the slope of the linear fit using the I-V plot in Fig. 2B. The peak conductance was significantly reduced in the visual cortex of HDAC2f/f;Pv-cre mice (6.145 ± 0.2014 nS; n = 25) compared to wild-type HDAC2f/f mice (9.817 ± 0.4442 nS; n = 24). This is in accordance with rodents that have been dark reared for 5 weeks and have a reduced peak conductance compared to 5-week

normally reared animals (Morales et al., 2002). The data obtained from 5-week dark reared animals closely reflect that of 3-week-old normally reared animals, an age within the critical period of the rodent visual cortex (Morales et al., 2002). The reduced peak conductance observed in adult HDAC2f/f;Pv-cre mice is in agreement with Fig. 2A and collectively shows that the inhibitory postsynaptic response of pyramidal neurons is reduced in the visual cortex of adult HDAC2f/f;Pv-cre mice.

To examine whether the observed decrease of maximal IPSCs in visual cortex of HDAC2f/f;Pv-cre mice is due to an alteration in the number of GABAergic inputs and/or an alteration in the number of synaptic contacts, miniature IPSCs (mIPSCs) were measured. Recordings were performed using conditions that block Na^+ channels, AMPA receptor and NMDAR to inhibit the generation of action potentials and to eliminate the contribution of excitatory inputs; this allows quantification of spontaneous inhibitory responses. An effect was observed with mIPSC amplitude, which was significantly higher in the visual cortex of HDAC2f/f;Pv-cre mice (19.5 ± 1.062 pA; Fig. 2C and E) compared to wild-type HDAC2f/f mice (16.04 ± 1.064 pA; Fig. 2C and E). There was no significant change in the mIPSC frequency of recordings from the visual cortex of brain slices from HDAC2f/f;Pv-cre mice (7.273 ± 0.0.9934 Hz; Fig. 2D and E) compared to wild-type HDAC2f/f mice (6.378 ± 0.5579 Hz; Fig. 2D and E). These results indicate that loss of HDAC2 leads to an increase in the amplitude of unitary inhibitory responses. These data are in keeping with observations of 5-week-old rodents that have been reared in the dark, which exhibit higher mIPSC amplitudes compared to 5-week-old normally reared animals, and are similar to recordings of 3-week-old animals. Thus, similar to observations during sensory deprivation, loss of HDAC2 appears to reduce the total potency of evoked GABAergic

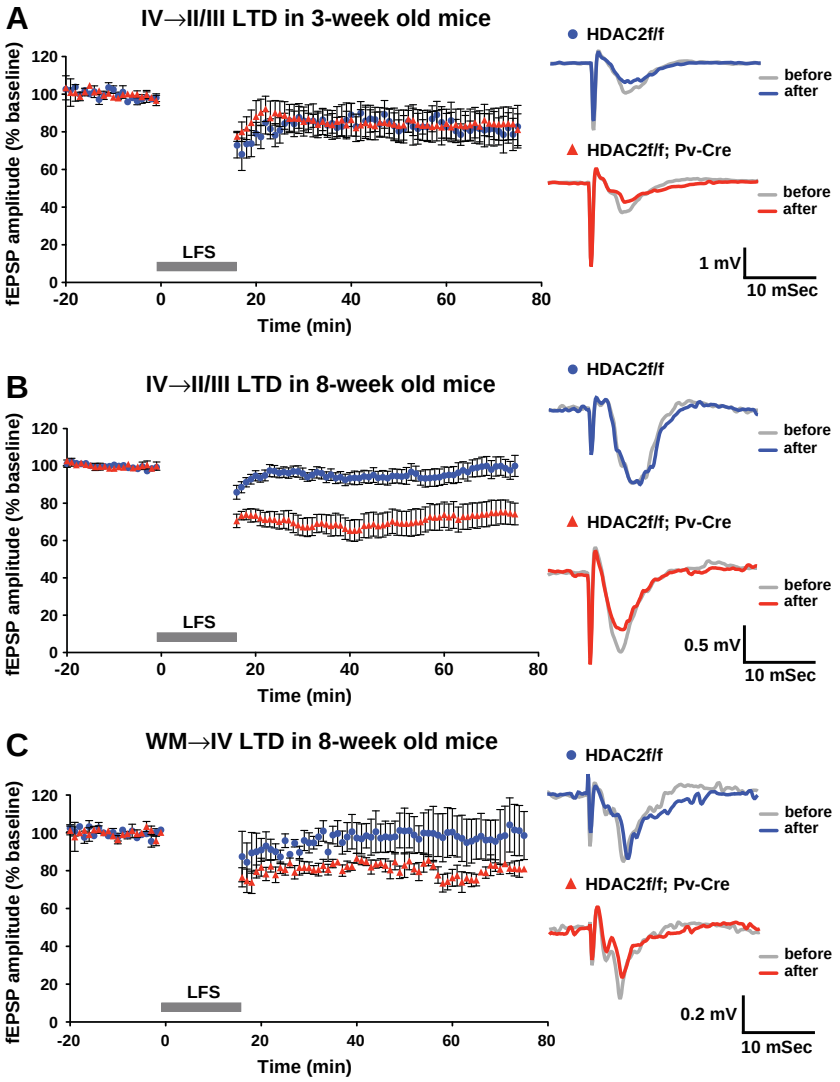

Fig. 3. Effects of HDAC2 deletion from Pv interneurons on LTD recorded in the visual cortex. (A) LTD in layer II/III induced by 1-Hz stimulation (900 pulses) in layer IV in the visual cortex of brain slices from 3-week-old HDAC2f/f and HDAC2f/f;Pv-cre mice (HDAC2f/f: 81.01% ± 8.56% of baseline during final 10 minutes after SP-LFS, n = 5 slices, 3 mice; HDAC2f/f;Pv-cre: 84.12% ± 8.23% of baseline during final 10 minutes after SP-LFS, n = 5 slices, 3 mice; 2-tailed *t* test; $P > .05$). (Right) Sample traces of field excitatory postsynaptic potentials (fEPSP) before and 1 hour after SP-LFS are superimposed. (B) LTD in layer II/III induced by 1-Hz stimulation (900 pulses) in layer IV in the visual cortex of brain slices from 8-week-old HDAC2f/f and HDAC2f/f;Pv-cre mice (HDAC2f/f: 99.14% ± 4.92% of baseline during final 10 minutes after SP-LFS, n = 10 slices, 4 mice; HDAC2f/f;Pv-cre: 75.24% ± 6.05% of baseline during final 10 minutes after SP-LFS, n = 9 slices, 5 mice; 2-tailed *t* test; $P < .01$). (Right) Sample traces of fEPSP before and 1 hour after SP-LFS are superimposed. (C) LTD in layer IV induced by 1-Hz stimulation (900 pulses) of the WM–layer VI border in the visual cortex of brain slices from 8-week-old HDAC2f/f and HDAC2f/f;Pv-cre mice (HDAC2f/f: 98.84% ± 12.71% of baseline during final 10 minutes after SP-LFS, n = 4 slices, 3 mice; HDAC2f/f;Pv-cre: 81.30% ± 2.717% of baseline during final 10 minutes after SP-LFS, n = 4 slices, 2 mice; 2-tailed *t* test; $P > .05$). (Right) Sample traces of fEPSP before and 1 hour after SP-LFS are superimposed.

inputs as measured by maximal IPSC and peak conductance, whereas the amplitude of spontaneous GABAergic responses appears to be up-regulated. In the case of dark rearing, this is suggested to occur through a compensatory mechanism similar to that described for excitatory synapses (Turrigiano, 1998; Murthy 2001).

Delayed developmental decline of visual cortical LTD of HDAC2f/f;Pv-cre mice

A period of enhanced long-term plasticity in the visual cortex requires visual stimulation (Jiang et al., 2010; Kirkwood et al., 1995) and coincides with a 3-fold increase in the number of GABAergic inputs that converge onto pyramidal cells (Morales et al., 2002). To examine whether HDAC2 expression in Pv-expressing interneurons affects previously observed postnatal reductions in NMDAR-mediated synaptic plasticity in the visual cortex, we utilized an LTD paradigm. An SP-LFS procedure of delivering 900 stimuli at 1 Hz has been shown to induce homosynaptic depression in the visual cortex of young mice that is subsequently absent in the visual cortex of adult mice (Jiang et al., 2007; Kirkwood et al., 1997).

First, we determined whether LTD in the visual cortex of layer II/III induced with SP-LFS stimulation in layer IV (IV → II/III LTD) of young mice is altered by deletion of HDAC2 in Pv-expressing cells. Brain slices from 3-week-old wild-type HDAC2f/f and age-matched littermate HDAC2f/f;Pv-cre mice were subjected to the layer IV → II/III LTD protocol. SP-LFS of layer IV induced an LTD in brain slices of wild-type mice (Fig. 3A; 81.01 % ± 8.56 % of baseline during final 10 minutes after SP-LFS), as has been previously reported for mice at 3 weeks of age (Jiang et al., 2007; Kirkwood et al., 1997). A similar magnitude of LTD was induced in the brain slices of HDAC2f/f;Pv-cre mice (Fig. 3A; 84.12 % ± 8.23 % of baseline during final 10 minutes after SP-LFS) as observed in the wild-type mice. This indicates that loss of HDAC2 from Pv-expressing cells has no effect on layer IV → II/III LTD during the critical period in the mouse visual cortex.

To determine whether HDAC2 expression in Pv-positive interneurons is important for NMDAR-mediated synaptic plasticity in adult mice, layer IV → II/III LTD was measured in 8-week-old mice. Previous studies have shown that after 5 weeks of age, there is a closure of the critical period in the visual cortex, and this correlates with a large reduction in the magnitude of LTD recorded compared to 3-week-old mice (Jiang et al., 2007; Kirkwood et al., 1997). In agreement with this observation, in 8-week-old wild-type HDAC2f/f mice, the induction of layer IV → II/III LTD was of very small magnitude (Fig. 3B; 99.14 % ± 4.92 % of baseline during final 10 minutes after SP-LFS). However, application of SP-LFS in layer IV in littermate HDAC2f/f;Pv-cre mice elicited induction of layer IV → II/III LTD with a magnitude comparable to that observed in mice that are 3 weeks of age (Fig. 3B; 75.24 % ± 6.05 % of baseline during final 10 minutes after SP-LFS). This is reminiscent of enhanced long-term plasticity measured in the visual cortex of dark-reared adult mice but is almost absent in normally reared adult mice (Jiang et al., 2010; Kirkwood et al., 1995). This would suggest that HDAC2 expression in Pv-expressing interneurons is important for the developmental reduction in plasticity of the visual cortex.

To test whether increased plasticity in the HDAC2f/f;Pv-cre mice at 8 weeks of age was specific to layer IV → II/III LTD, LTD of layer IV synaptic responses was induced by application of SP-LFS to the WM–layer VI border of the visual cortex (WM → IV LTD). As expected, 8-week-old wild-type HDAC2f/f mice did not exhibited substantial WM → IV LTD (Fig. 3C; 98.84 % ± 12.71 % of baseline during final 10 minutes after SP-LFS). However, littermate age-matched HDAC2f/f;Pv-cre mice elicited WM → IV LTD (Fig. 3C; 81.30 % ± 2.717 % of baseline during final 10 minutes after SP-LFS), which is comparable to that observed in 3-week-old mice for IV → II/III LTD (Fig. 3A). Together, these data indicate that HDAC2-mediated epigenetic control of Pv-expressing cells is important for regulating inhibitory inputs that contribute to defining the temporal closure of the critical period in the mouse visual cortex.

Discussion

An experience-dependent maturation in GABAergic inhibition during postnatal development contributes to the closure of a critical period of enhanced synaptic plasticity in the mouse visual cortex. Little is known regarding the transcriptional regulation of this process; however, studies have indicated that increases in histone acetylation play an important role in mediating increased cortical plasticity in adult mice and humans (Gervain et al., 2013; Putignano et al., 2007; Silingardi et al., 2010; Yang et al., 2012). Here, we have shown that loss of HDAC2 from Pv-expressing inhibitory cells leads to a reduction in the potency of inhibitory inputs in the visual cortex in adult mice, whereas the potency of excitatory inputs appears to be unaffected. These data are reminiscent of previous studies showing that visual deprivation by dark-rearing prevents the normal developmental increase in GABAergic inputs in the visual cortex and is thought to delay the maturation of intracortical inhibitory circuits (Morales et al., 2002). Likewise, an accelerated maturation of Pv inhibitory cells leads to a premature increase in inhibitory synaptic strength, with little effect on excitatory inputs (Huang et al., 1999; Sugiyama et al., 2008).

A growing consensus is that the closure of the critical period in the mouse visual cortex, as measured by reduced NMDAR-mediated synaptic plasticity, is modulated by the maturation of GABAergic cells. The critical period timing can be manipulated by genetic manipulations that enhance or reduce GABAergic inhibition, which respectively leads to a premature or delayed closure of the critical period (Di Cristo et al., 2007; Hensch et al., 1998; Sugiyama et al., 2008). This effect of inhibitory maturation on the critical period has been demonstrated specifically for accelerated maturation of Pv-expressing cells (Huang et al., 1999; Sugiyama et al., 2008). However, little is known regarding the transcriptional control mediating this developmental increase in cortical inhibition. Along with a delayed developmental increase in inhibitory inputs in the HDAC2f/f;Pv-cre adult mice, we observe a robust LTD in the visual cortex of adult mice lacking HDAC2 in Pv-expressing cells. As previously demonstrated by others, we find that LTD appears to be absent in HDAC2f/f littermate control adult mice (Jiang et al., 2007; Kirkwood et al., 1997). Collectively, these findings suggest that HDAC2-mediated transcriptional control of Pv-expressing neurons is important for the developmental increase in cortical inhibition and the temporal closure of the critical period in the visual cortex.

HDAC2 has been shown to be an important factor that mediates an epigenetic blockade of synaptic plasticity mechanisms required for learning and memory (Gräff et al., 2012; Guan et al., 2009). The chromatin function of HDAC2 can be negatively regulated following a posttranslational modification by nitric oxide, called *S*-nitrosylation (Nott et al., 2008). HDAC2 nitrosylation has been shown to have a role in regulating both cortical development and memory formation (Gräff et al., 2014; Nott et al., 2008, 2013). Interestingly, neuronal nitric oxide synthase (nNOS), an enzyme that provides a major source of nitric oxide in neurons, is highly expressed specifically in interneuron cell populations in the adult mouse cortex (Karagiannis et al., 2009; Kubota et al., 2011). Furthermore, nitric oxide has been demonstrated to have positive effects on neuronal plasticity (Garthwaite and Boulton, 1995; Massaad and Klann, 2011) and learning and memory (Gräff et al., 2014; Harooni et al., 2009; Kendrick et al., 1997; Li et al., 2012; Telegdy and Kokavszky, 1997; Walton et al., 2013). An appealing hypothesis

that waits to be tested is that negative regulation of HDAC2 by nitric oxide and its effect on transcription may modulate the activity of inhibitory circuits in a manner that is permissive for enhanced synaptic plasticity.

Conflict of interest

No conflict of interest.

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