Prefrontal Cortical Dysfunction After Overexpression of Histone Deacetylase 1

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Background: Postmortem brain studies have shown that *HDAC1*—a lysine deacetylase with broad activity against histones and nonhistone proteins—is frequently expressed at increased levels in prefrontal cortex (PFC) of subjects diagnosed with schizophrenia and related disease. However, it remains unclear whether upregulated expression of *Hdac1* in the PFC could affect cognition and behavior.

Methods: Using adeno-associated virus, an *Hdac1* transgene was expressed in young adult mouse PFC, followed by behavioral assays for working and long-term memory, repetitive activity, and response to novelty. Prefrontal cortex transcriptomes were profiled by microarray. Antipsychotic drug effects were explored in mice treated for 21 days with haloperidol or clozapine.

Results: *Hdac1* overexpression in PFC neurons and astrocytes resulted in robust impairments in working memory, increased repetitive behaviors, and abnormal locomotor response profiles in novel environments. Long-term memory remained intact. Over 300 transcripts showed subtle but significant changes in *Hdac1*-overexpressing PFC. Major histocompatibility complex class II (MHC II)-related transcripts, including *HLA-DQA1/H2-Aa*, *HLA-DQB1/H2-Ab1*, and *HLA-DRB1/H2-Eb1*, located in the chromosome 6p21.3-22.1 schizophrenia and bipolar disorder risk locus, were among the subset of genes with a more robust (>1.5-fold) downregulation in expression. *Hdac1* levels declined during the course of normal PFC development. Antipsychotic drug treatment, including the atypical clozapine, did not affect *Hdac1* levels in PFC but induced expression of multiple MHC II transcripts.

Conclusions: Excessive HDAC1 activity, due to developmental defects or other factors, is associated with behavioral alterations and dysregulated expression of MHC II and other gene transcripts in the PFC.

Key Words: Bipolar disorder, gene expression, major histocompatibility complex II, prefrontal cortex, protein deacetylase, schizophrenia

Postmortem brain studies implicate epigenetic alterations involving DNA methylation and histone modifications and other determinants of chromatin structure and function in the neurobiology of schizophrenia. In particular, dysregulated methylation of DNA cytosines and of histone lysine and arginine residues in the prefrontal cortex (PFC) and other corticolimbic circuitry has been reported for select gene promoters important for neurotransmission, myelination, and various other functions (1–8). Some of these epigenetic alterations were subsequently confirmed in nucleated blood cells of subjects with schizophrenia (9–11). These changes in chromatin architectures at specific loci may ultimately be driven by genetic factors (4,5,12) or may reflect aberrant epigenetic signatures in a parental germline (13,14) or adverse events during prenatal or early postnatal development (15,16). Furthermore, epigenetic dysregulation of gene expression may result from exposure to nicotine and alcohol (17,18), psychostimulants, and various other drugs of abuse (19,20).

The above findings, taken together, leave little doubt that maladaptive mechanisms in the brain's epigenetic machinery could be a critical factor in the etiology of at least some cases on the psychosis spectrum. However, with the exception of rare cases of schizophrenia caused by deleterious mutations in genes encoding chromatin regulators such as the histone H3-lysine 9 specific methyltransferase *KMT1D/EHMT1* (21) or the methyl-CpG-binding protein *MECP2* (22), the molecular pathways associated with epigenetic dysregulation leading to clinical symptoms and molecular changes in the psychotic brain, including the PFC, remain unknown.

The balance between histone acetylation and deacetylation is highly regulated in brain cells and of pivotal importance for behavioral plasticity in the brain's learning and reward circuitry (19,23–25) and could profoundly affect motivational and affective states (26,27). Sharma *et al.* (28) previously noticed that in a publicly accessible microarray collection from the Harvard Brain Tissue Resource Center, expression of the class I histone deacetylase, *HDAC1*, was significantly increased (on average 30% to 50%) in the PFC of a cohort of 19 subjects with schizophrenia compared with 25 control subjects (28) (Figure S1 in Supplement 1). Similar changes may affect a subset of patients diagnosed with bipolar disorder (28). Furthermore, microarray datasets from Narayan *et al.* (29), who profiled transcriptomes in PFC specimens from an Australian collection, also revealed a significant increase in *HDAC1* transcript levels in 30 schizophrenia subjects compared with 29 control subjects (Figure S1 in Supplement 1). Finally, upregulated *HDAC1* expression has also been reported for the neuronal layers of the hippocampus and medial temporal lobe in a third cohort of schizophrenia subjects (30). Therefore, abnormal *HDAC1* expression in corticolimbic circuitry is a type of molecular pathology representative of a significant portion of cases on the mood and psychosis spectrum. However, it remains unclear

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whether this type of molecular alteration is detrimental to brain function, or a neutral epiphenomenon, or a medication side effect. To distinguish between these possibilities was the goal of the present study.

Methods and Materials

Analysis of human and mouse microarray data and details on adeno-associated virus vector preparation and delivery, animal surgery and antipsychotic drug treatments (APD), and behavioral studies, cell culture work, immunoblotting and immunohistochemistry procedures, quantitative reverse transcriptase-polymerase chain reaction, and statistical analysis are provided in the Supplemental Methods (in Supplement 1).

Results

Adeno-associated Virus, Serotype 9 Capsid-Mediated Expression of *Hdac1* and *LacZ* in Adult PFC

We used an adeno-associated virus, serotype 9 capsid (AAV9)-based system for long-lasting transgene expression following bilateral injections into mouse anterior medial cortex, considered the broad functional homolog to human PFC (31). Similar to the spreading pattern described for juvenile animals (32), injection of AAV9-LacZ reporter in young adult (12-week-old) C57BL/6J animals resulted, over the course of 3 weeks, in a gradual increase in numbers of cells expressing the transgene, covering at least 2.5 mm along the rostrocaudal axis of the PFC and involving all cortical layers I to VI (Figure 1A). Additional staining was found in the underlying white matter and portions of the dorsal and lateral septum (Figure 1A). A few scattered cells, positioned in dorsal hippocampus and rostral thalamus, were also labeled (Figure S2 in Supplement 1).

To determine which of the major cell populations express the transgene (which was driven by a generic cytomegalovirus promoter) when packaged into the AAV9 capsid, we employed co-localization studies on brain sections of mice injected with the AAV9-LacZ reporter by double staining for β-galactosidase in combination with the neuronal marker NeuN (Millipore, Schwalbach, Germany), the astrocytic marker S-100B (Abcam, Cambridge UK), and the oligodendrocyte marker CNPase (Sigma Aldrich, Munich, Germany). We consistently found co-localization for NeuN and S-100B with β-galactosidase in approximately 50% of neurons and a slightly lower percentage of astrocytes. However, for CNPase and β-galactosidase, the overlapping signal was observed in less than 5% of CNPase positive cells (Figure 1B; Figure S3 in Supplement 1). We concluded that AAV9 primarily transduces neurons and astrocytes but only a very small portion of the oligodendrocyte population.

Using the above system, a full-length *Hdac1* complementary DNA transfected into adult mouse PFC and N1E-115 mouse neuroblastoma cells resulted in robust upregulation of HDAC1 protein levels (Figure 2A,B; Figure S4 in Supplement 1). This increase in HDAC1 protein was associated with a 20% to 25% reduction of bulk histone H3 and H4 acetylation levels in the cell lysate, suggesting that the transgene indeed conveys a histone deacetylase activity (Figure 2C). Expression and protein levels of neuronal housekeeping genes, including NeuN and *Synl*, remained unaffected in adeno-associated virus (AAV)-Hdac1 cortex even several weeks after the injection (Figure S5 in Supplement 1), which is consistent with previous reports (33).

Working Memory Performance, Repetitive Behaviors, and Response to Novelty Are Altered after AAV-Mediated *Hdac1* Expression in the PFC

To test whether *Hdac1* overexpression would model the working memory deficits and other alterations attributed to a

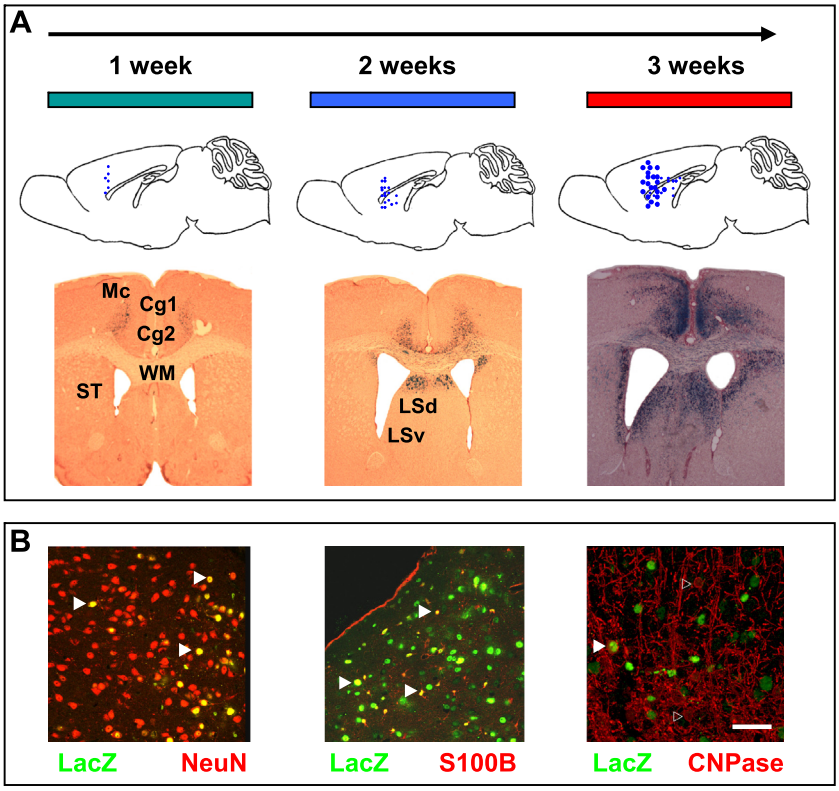

Figure 1. Localized spreading and cellular expression patterns of adeno-associated virus-LacZ reporter in adult mouse prefrontal cortex (PFC). **(A)** (Top): Graphical representation (sagittal plane) of dorsoventral spread of virus between weeks 1 to 3 postinjection. (Bottom): Coronal sections, stained with β-galactosidase enzyme histochemistry, from adeno-associated virus-LacZ injected brains. Notice regional spreading and increased numbers of transduced cells during the time periods tested. **(B)** Cellular specificity of adeno-associated virus, serotype 9 capsid transductions in PFC (LacZ transgene with nuclear localization signal). Photomicrographs from PFC sections double-stained for anti-β-galactosidase (green) with (red, from left to right) anti-NeuN neuronal marker, anti-S100B for astrocytes, and anti-CNPase for oligodendrocytes. Notice double-labeled (yellow, marked by filled arrows) neurons and astrocytes but no double-labeled (unfilled arrows) oligodendroglia. Scale bar, 75 μm. Cg1, cingulate cortex (area 1); Cg2, cingulate cortex (area 2); LSd, lateral septum (dorsal part); LSv, lateral septum (ventral part); Mc, motor cortex; ST, striatum; WM, white matter.

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