Title: Epigenetic mechanisms linking diabetes and synaptic impairments

Running title: Diabetes and synaptic dysfunction

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ABSTRACT

Diabetes is one of the major risk factors for dementia. However, the molecular mechanism underlying the risk of diabetes for dementia is largely unknown. Recent studies revealed that epigenetic modifications may play a role in the pathogenesis of diabetes. We hypothesized that diabetes may cause epigenetic changes in the brain that may adversely affect synaptic function. We found significant elevation in the expression of histone deacetylases (HDACs) class IIa in the brains of diabetic subjects compared to control subjects, and these changes coincide with altered expression of synaptic proteins. In a mouse model of diet-induced type II diabetes mellitus (T2DM), we found that, similar to humans, T2DM mice also showed increased expression of HDAC IIa in the brain and these alterations were associated with increased susceptibility to oligomeric Aβ-induced synaptic impairments in the hippocampal formation and eventually led to synaptic dysfunction. Pharmacological inhibition of HDAC IIa was able to restore synaptic plasticity. Our study demonstrated that diabetes may induce epigenetic modifications affecting neuropathological mechanisms in the brain leading to increased susceptibility to insults associated with neurodegenerative or vascular impairments. Our study provides, for the first time, an epigenetic explanation for the increased risk of diabetic patients to develop dementia.
INTRODUCTION

The prevalence of dementia increases with age. It is estimated that over 36 million people worldwide are currently living with this devastating disease and the prevalence is expected to increase to 66 million by 2030 and 115 million by 2050 (1). Dementia can be caused by a number of progressive diseases such as Alzheimer’s disease (AD) and vascular dementia (VaD). Mounting evidence suggests that cognitive impairments associated with dementia may be traced back to neuropathological conditions initiated several decades before clinical onset. Therefore, clinical development of novel interventions for dementia is now based on mechanisms associated with primary and/or secondary prevention.

Diabetes increases the risk of both AD dementia and VaD (2-9). Based on the National Diabetes Health Fact Sheet, approximately 8.3% of Americans have diabetes and, like dementia, the prevalence of diabetes increases with age; currently 26.9% of people 65 years or older have diabetes (10). Despite national and international campaign efforts to counter T2DM, its prevalence is still rising. T2DM and hyperglycemia are associated with cognitive dysfunction and an increased risk for dementia, including AD-type dementia. Three longitudinal studies conducted by Yaffe (11), Komulainen (12) and Dik (13) independently showed that T2DM is related to a higher risk of cognitive decline. A meta-analysis performed by Profenno and colleagues (14) reviewed thirteen longitudinal epidemiological studies that examined the association between diabetes and dementia, and found that diabetes significantly and independently increases one’s risk for dementia.

Emerging evidence has demonstrated that diabetes-associated chromatin modifications pertinent to epigenetic mechanisms may play an important role in the pathogenesis of diabetes (15;16). For example, epigenetic alterations of histone deacetylases (HDACs) cause decreases in H3 and H4 acetylation at the proximal promoter of pancreatic and duodenal homeobox factor 1 (Pdx-1) in pancreatic β-cells, leading to defects in glucose homeostasis and insulin resistance and eventually leading to T2DM (17). Similarly, histone modifications of glut4 mediated by DNA methyltransferase
(Dnmt) and HDACs result in reduced transcription of glucose transporter 4 in skeleton muscle in intrauterine growth-restricted offsprings (18).

Epigenetic mechanisms have also been widely studied in the brain. Histone methylation and histone acetylation in the hippocampal formation play an important role in modulating synaptic strength following neuronal activation (19-26). Mice that lack both Dnmt1 and Dnmt3a exhibited abnormalities in long-term potentiation (LTP) and long-term depression (LTD) following stimulations in CA1 synapses, and these dysfunctions were associated with impaired learning and memory (19). HDAC2 has emerged as a major histone modification enzyme in modulating synaptic plasticity and long-term memory. Overexpression of HDAC2 is associated with structural dysfunction including reduced number of dendritic spine and synapses as well as functional deficits such as impaired synaptic plasticity and memory function (27). Moreover, pharmacological inhibition of HDACs by HDACi such as trichostatin A (TSA) resulted in increased LTP responses, further confirming the role of histone acetylation in brain function (23).

In the present study, we tested whether diabetic condition might alter the epigenetic mechanisms in the brain, which may lead to structural or functional changes that ultimately influence the ability of the brain to cope with conditions associated with neurological disorders, such as AD.
RESEARCH DESIGN AND METHODS

Brain specimen Human postmortem brain specimens from T2DM and age-matched non-diabetic (control) cases were obtained from the Icahn School of Medicine at Mount Sinai, Department of Psychiatry Brain Bank. The brains were donated by deceased residents of the Jewish Home and Hospital (JHH, Manhattan, NY and Bronx, NY). Diabetic or non-diabetic cases were identified using criteria previously described (28). Specifically, patients were diagnosed with diabetes either by a geriatrician or an internist based on the American Diabetes Association criteria; symptoms of diabetes plus casual plasma glucose concentration > 200 mg/dl; fasting plasma glucose > 126 mg/dl; 2-hour plasma glucose > 200 mg/dl during an oral glucose tolerance test. We only included the subjects with type 2 diabetes. Detailed demographic information is presented in Table 1. Clinical Dementia Rating (CDR) scale was used to assess cognitive and functional impairments associated with dementia. The cohort used in this study was devoid of any significantly cerebrovascular disease (CVD) neuropathology.

Assessment of gene expression Total RNA from brain tissues (hippocampus) was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA) and reverse transcribed using SuperScript III first-strand synthesis supermix for qRT-PCR (invitrogen, Carlsbad, CA) according to manufacturer’s instruction. Epigenetic chromatin modification enzymes PCR array for human or mouse (Qiagen, Valencia, CA) were used to evaluate the levels of gene expression. Expression of synaptic plasticity gene PSD95 and synaptophysin in the brains of T2DM mice were measured in 4 replicates by quantitative RT-PCR using Maxima SYBR Green master mix (Fermentas) in ABI Prism 7900HT with primers amplifying the target genes: PSD95 (forward: CGGGAGAAAATGGAGAAGGAC, reverse: GCATTGGCTGAGACATCAAG); Synaptophysin (forward: AGTGCCCTCAACATCGAAG, reverse: GCCACGGTGACAAAGAATTC). Mouse hypoxanthine phosphoribosyltransferase (HPRT, forward: CCCCAAAATGGTTAAGGTTGC, reverse: AACAAAGTCTGGCCTGTACC) expression level was used as an internal control. Data were
normalized using the $2^{-\Delta \Delta Ct}$ method (29). Levels of target gene mRNAs were expressed relative to those in control groups and plotted in GraphPad Prism.

**Assessment of protein expression by Western blotting analysis** Brain samples (the same hippocampal tissue as used in the gene expression studies) were extracted with radio immunoprecipitation assay (RIPA) buffer and boiled in Laemmli sample buffer for 5 min. Proteins were separated by gel electrophoresis using 10% sodium dodecyl sulfate–polyacrylamide gels and electrotransferred onto nitrocellulose membranes, blocking with 5% nonfat dried milk (Sigma-Aldrich, St. Louis, MO) in Tris-buffered saline. Membranes were probed with primary antibodies anti-PSD95 (Millipore, Billerica, MA, 1:2500) or anti-synaptophysin (Sigma-Aldrich, 1:5000) at 4 °C overnight, followed by HRP-conjugated secondary antibodies. Specific protein signals were detected using the enhanced chemiluminescence system (ECL plus; PerkinElmer LAS, Inc., Boston, MA, USA) and signals was quantified by densitometric scanning (GS-800; Bio-Rad, Hercules, CA) and analyzed using Quantity One software (Bio-Rad). Beta-actin (Sigma-Aldrich, 1:3000) was served as the loading control.

**Mouse model and treatment** Female C57BL6/J mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed in the centralized animal care facility of the Center for Comparative Medicine and Surgery (CCMS) at Icahn School of Medicine at Mount Sinai. The C57BL/6J mouse develops hyperinsulinemia and hyperglycemia when fed a high fat diet (30). Mice were randomly grouped into two groups and received the following treatment starting at approximately 3 months of age: Control mice were treated with regular diet (CTRL group, 10 kcal% fat, Research Diets D12450B), T2DM mice were treated with a high fat diet (T2DM group, 60 kcal% fat, Research Diets, D12492). All animals were maintained on a 12:12-h light/dark cycle with lights on at 07:00 h in a temperature-controlled (20 ± 2 °C) vivarium and all procedures were approved by the MSSM IACUC.

**Intra-peritoneal glucose tolerance test (IGTT) and fasting insulin measurements** IGTT was
performed as previously reported (31;32). Specifically, mice were given a single dose of intraperitoneal glucose (2 g/kg BW) postprandially, and blood was collected from the tail vein periodically over a 3 h period. Blood glucose content was assessed using the Contour blood glucose System (Bayer, IN). Insulin was measured using the mouse serum adipokine multiplex MAP kit from Millipore (Billerica, MA).

**Electrophysiological Recordings** Mice were sacrificed by decapitation and the brains were quickly removed. Hippocampal slices (350 µm) were placed into oxygenated artificial cerebrospinal fluid (ACSF) at 29 °C for a minimum of 90 min to acclimatize. Slices were then transferred to a recording chamber and perfused continuously with oxygenated-ACSF in the presence or absence of 60nM of oligomeric Aβ (oAβ) at 32 °C. For extracellular recordings: CA1 field excitatory postsynaptic potentials (fEPSPs) were recorded by placing stimulating and recording electrodes in CA1 stratum radiatum. Basal synaptic transmission was assayed by plotting the stimulus voltages against slopes of fEPSP. For long term potentiation (LTP) experiments, a 15 min baseline was recorded every min at an intensity that evokes a response ~35% of the maximum evoked response. LTP was induced using θ-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including three 10-burst trains separated by 15 s) and fEPSPs were monitored for 60 min to assess the magnitude of potentiation.

**Functional Magnetic Resonance Imaging (fMRI) Imaging** The Bruker Biospec 70/30 system, a 7T micro MRI scanner, was used. Prior to scanning, mice were anesthetized with 2% Isoflurane in NO₂ (75%) and O₂ (23%). They were then positioned onto the scanner bed. Anesthesia was maintained at 1.5% isoflurane throughout the scan time. Data acquisition was done using a 4 channel mouse brain-dedicated phased array coil as follows: after a localizer we manually shimmed the brain region for optimal signal and signal distortion. The resting state fMRI was acquired using an EPI BOLD sequence.
with the following protocol: Segmented EPI (4x), TR=1500ms, TE=18.9ms, Matrix=96x96, FOV=12.8mm, slice thickness=1.1mm, 11 slices. Total acquisition time was around 5 mins per scan; 3 scans were acquired for each mouse. **Analysis** Preprocessing of the functional images began by excluding scans where there was movement. Manual brain extraction was then performed on the mean functional image. A study specific template was created based on all the mean functional images using FSL. Independent component analysis (ICA) was used to identify unique networks of resting state activity using MELODIC (33) as implemented in FSL. We visually identified the DMN from the networks produced by FSL. The default mode network (DMN) was defined as containing orbital cortex, cingulate cortex and hippocampal regions according to the literature (34). Group ICA was performed and the DMN and sensory motor were visually identified for dual regression analysis. General linear modeling and permutation-based inference testing (RANDOMISE) were used to test for group differences and symptom severity correlates of the DMN (35;36).

**Primary neuron culture and MC1568 treatment** Embryonic-day 16 cortico-hippocampal neuronal cultures were prepared from C57BL6/J mice as previously described (32). Neurons were seeded on poly-D-lysine–coated 6-well plates and cultured in Neurobasal medium supplemented with 2% B27, 0.5 mM L-glutamine and 1% penicillin-streptomycin (Gibco-BRL) in the tissue culture incubator at 37 °C with 5% CO\textsubscript{2}. Following 5 days in vitro culturing, the neurons were treated either with 200nM MC1568 (Selleckchem) or vehicle for 16-18 hours. Cells were washed once with cold PBS before RNA isolation.

**Overall statistics** In these studies, all values are expressed as mean and standard error of the mean (SEM). Differences between means were analyzed using either two-way repeated measures ANOVAs or two-tailed student t-test. In all analyses, the null hypothesis was rejected at the 0.05 level. All statistical analyses were performed using the Prism Stat program (GraphPad Software, Inc.).
RESULTS

Differential regulation of histone deacetylase IIa is associated with impaired synaptic integrity in the brains of diabetic subjects To test whether there are epigenetic changes in the brains of diabetic patients, we measured the expressions of epigenetic chromatin modification enzymes in the brains of diabetic patients and normal controls using a PCR based array. We found that there are significant changes in the expression of select chromatin modification enzymes, particularly HDAC class IIa (Fig. 1A), while there are no changes with regard to the expression of HDAC class I (Fig. 1B). Specifically, over 50% upregulation of HDAC4, 7 and 9 were observed in the brains of diabetic subjects, as well as an almost two fold increase in HDAC5 expression compared to the non-diabetic controls.

We next tested whether diabetes-induced epigenetic modifications in the brain might be associated with the modulation of neuronal activity at the level of synapse. We examined the levels of proteins involved in synaptic plasticity in the pre- and postsynaptic compartments as indices for synaptic integrity in the control and T2DM subjects. Western blot was used to examine the levels of synaptophysin, an important presynaptic protein responsible for synaptic vesicle biogenesis and recycling, and postsynaptic density protein 95 (PSD-95), the most abundant scaffolding protein involved in recruiting and anchoring glutamate receptor subunits in PSD. We found that the levels of synaptophysin and PSD-95 were both significantly lower in postmortem brain specimens from the T2DM subjects compared to the control subjects (Fig. 1C and inset). Moreover, correlation studies showed that the expression of HDAC5 was significantly reverse correlated with PSD95 (p<0.05) and synaptophysin (p<0.01, Fig. 1D). The expression of HDAC7 was significantly reverse correlated with synaptophysin (p<0.05) but had no correlation with PSD95. We also found that the expression of HDAC9 showed non-significant reverse correlations with the expression of PSD95 (p=0.085) and synaptophysin (p=0.084). We did not find any correlation between HDAC4 and the expression of synaptic proteins.
Elevated HDAC IIa in the brain of a mouse model of diabetes

Based on the specific HDAC changes observed in the post mortem brains from diabetic subjects, we tested whether diabetic conditions can causally induce HDAC IIa alteration in the brain. We used the C57BL/6J mouse as a model of human T2DM, because it develops hyperinsulinemia and hyperglycemia when fed a diabetogenic diet but remains physically normal when fed low-fat chow (30). We treated mice with a regular low-fat diet or the diabetogenic diet starting at 2 months of age. Following 6 months of chronic diet treatment, the T2DM mice developed severe diabetes including increased baseline fasting glucose, and impaired insulin sensitivity and glucose tolerance as compared to the CTRL mice (Fig. 2A, B and C). Examination of chromatin modification enzyme expressions in the brains following 6 months diabetogenic diet treatment revealed that, similar to the human data, two of the class IIa HDACs are upregulated in the brains of T2DM mice. Most notably, HDAC5 was significantly upregulated, 4 fold, and HDAC9 by 2 fold (Fig. 2D). We did not find any changes in the expression of Class I HDACs including HDAC 1, 2, 3 and 8 in the brain. HDAC 10, which belongs to HDAC IIb, was significantly reduced in the T2DM mouse brains, while there was no change in the expression of HDAC 6 and HDAC 11.

Diabetes induced HDAC IIa upregulation is associated with increased synaptic susceptibility to oligomeric Aβ insults

We next tested whether the epigenetic changes in the brain of T2DM mice coincide with synaptic impairments. Electrophysiological studies were performed on the hippocampal slices isolated from the T2DM mice. We found that hippocampal slices derived from the T2DM mice following 6 months of diabetogenic diet treatment had normal electrophysiological responses following tetanus stimulation (data not shown). However, these hippocampal slices had a much lower threshold to oligomeric Aβ1-42 (oAβ)-induced LTP deficits. Normally, healthy hippocampal slices show compromised LTP when exposed to 200 nM oAβ (data not shown). However, hippocampal slices derived from T2DM mice showed limited potentiation following tetanic stimulation in the presence of 60 nM of oAβ, while slices from strain-, age- and gender-matched mice on the control diet showed robust LTP under the same conditions (Fig. 3A). Consistent with previous observations, oAβ had no effect on
basal synaptic transmission (37) (Fig. 3B). Synaptic deficits were exacerbated with prolonged diabetic conditions. While hippocampal slices isolated from T2DM mice, following 6 months of diabetogenic diet treatment, showed normal LTP response in the absence of oAβ, slices isolated from mice following 10 months of treatment showed compromised LTP responses (Fig 3C). In the diabetic mice, the LTP responses were 153.48±5.05% 60 minutes after the theta burst stimulus while in the CTRL mice, the responses were 233.97±14.82% (p<0.01), further stressing that diabetic conditions might adversely influence brain synaptic function and may eventually lead to cognitive impairments.

**LTP deficits coincide with abnormal brain connectivity in T2DM mice** To test whether diabetic condition-induced epigenetic alterations/synaptic impairments in the brain might be associated with brain biology changes, we used MRI imaging to objectively measure the biological effects of diabetic condition on the brain. We used fMRI with blood oxygenation level-dependent (BOLD) contrast to examine the brain connectivity in T2DM and control mice, following 10 months treatment. We found significant difference between the CTRL and T2DM mice in the Default Mode Network (DMN). Compared to CTRL mice, T2DM mice had significantly stronger signal intensities in the DMN (Fig. 4A) as well as in the sensory/motor cortex regions (Fig. 4B). The green color represents the group resting state network and the dark blue color (red arrows) represents significant difference between CTRL and T2DM.

**Inhibition of HDAC class IIa restores LTP response in brain slices isolated from T2DM mice and increases the expression of synaptic structure proteins in primary neurons** To confirm whether HDAC IIa might be responsible for the synaptic impairment we observed in the electrophysiology study on the T2DM mice treated with diabetogenic diet for 10 months (Fig. 3C), we treated hippocampal slices isolated from these mice with an HDAC IIa specific inhibitor, MC1568 (38;39), and recorded the LTP responses. We found that ex. vivo treatment with MC1568 significantly improved LTP responses compared to the vehicle treated slices. In the vehicle treated diabetic brain slices, 60 minutes after the
theta burst stimulus, the LTP responses were 151.85±8.64% while in the MC1568 treated diabetic slices, the responses were 202.51±12.08% (p<0.01, Fig.5A). There was no difference in basal synaptic transmission (Fig. 5B). Similarly, we also found that ex. vivo MC1568 treatment promoted the LTP in brain slices from T2DM mice following 6 months diabetogenic diet treatment when challenged with 60nM of oAβ (from 156.58±4.48% to 198.02±10.28%, p<0.01). In control studies, we found that 200nM MC1568 treatment did not affect LTP responses in hippocampal slices from mice treated with regular diet.

We also tested whether pharmacological inhibition of HDAC IIa could influence some of the indices of neuronal activity. In primary neurons derived from C57BL6/J mice, treatment with MC1568 significantly increased the transcription level of the synaptic structure proteins PSD-95, synaptophysin and brain-derived neurotrophic factor (BDNF) (Fig. 5C), which are important molecules for learning and memory, thus confirming the role of HDAC IIa in the regulation of synaptic activity.
DISCUSSION

Diabetes mellitus has become an epidemic and the prevalence in the U.S. is projected to reach 39 million in 2050. As one of the major causes of functional disability, this disease poses tremendous burden on the health system including medical expenses, costs for caregivers and reduced productivity. Diabetes is one of the major causes of morbidity and mortality and is associated with long-term complications that affect the peripheral as well as the central nervous system.

Diabetes has been linked to cognitive impairment and an increased risk factor for dementia, including AD-type dementia. Hyperglycemia-induced metabolic and vascular disturbances are well acknowledged, however, the exact mechanisms underlying cognitive dysfunction in diabetes are unclear. We found that select chromatin modification enzymes, specifically HDAC class IIa, were upregulated in the brains of diabetic patients and this increase was associated with reduced expression of synaptic proteins that are important for synaptic structural integrity and function. Using an animal model of T2DM, we found similar HDAC IIa changes in the brains and these alterations coincided with increased susceptibility of synaptic function to amyloid toxicity. In addition, prolonged diabetic condition exacerbated the observed synaptic dysfunction. Since MRI has been widely used to assess hemodynamic responses related to neural activity in the brain; we used resting state functional MRI to investigate the regionally connected functional networks, e.g., motor/sensory cortex, hippocampus, amygdala, striatum, visual, etc. in the T2DM mouse brains. We found that T2DM mice exhibited increased signal intensity both in the hippocampal formation and the sensory motor in the cortex, suggesting that T2DM brain might not be functioning efficiently and requires much more energy to accomplish a task compared to the normal efficient brain (40;41). It is also possible that a T2DM brain may not be signaling coherently and the increase in signaling is a result of haphazard signaling. Our findings are consistent with the human imaging and epidemiology studies demonstrating that progressive cerebral atrophy is associated with prolonged diabetic condition (42-46) and diabetic
individuals have an increased risk of developing cognitive dysfunction, including mild cognitive impairment and dementia (6-8;47).

HDACs are a family of enzymes that remove the acetyl moiety of lysine residues from the N-terminus of histone proteins, leading to chromatin compaction and thus transcription repression. Both class I and class II HDACs are abundantly expressed in the brains and have been shown to regulate neuronal activity that are essential for synaptic function, as well as for learning and memory. Our study for the first time demonstrates that diabetic condition can causally induce changes in HDAC levels in the brain, which in turn, trigger a detrimental molecular cascade that leads to compromised neuronal structure integrity and synaptic plasticity. Our study also demonstrates that diabetic brains are more susceptible to oAβ induced synaptic toxicity. oAβ is increasingly accepted as one of the major culprits for AD dementia. Cognitive deterioration in AD is directly linked to the brain accumulation of extracellular soluble oligomeric Aβ species, rather than amyloid plaque deposition (49;50). Previous epidemiological studies showed that the age of onset, duration and severity of diabetes can influence the incidence of mild cognitive impairment (MCI) (48). This is consistent with our findings that six-month diabetogenic diet treatment resulted in increased susceptibility to oAβ, while prolonging the diabetic condition for an additional 4 months resulted in reduced synaptic response in the absence of exogenous insults. Thus our results may provide mechanistic explanations of the epidemiological observation that diabetic subjects have an increased risk of developing AD dementia. Notably, HDACs are capable of regulating many cellular and molecular pathways involved in synaptic function and learning and memory. It is possible that modulation of other molecular mechanisms, such as BDNF, cAMP response element-binding (CREB) signaling and protein kinase-mediated hyperphosphorylation of microtubule-binding protein tau, can all contribute to the phenomenon observed in our study. These mechanisms need to be further investigated.

Diabetes is a multifaceted metabolic disorder that affects both the peripheral as well as the central nervous system. Based on our findings, it is plausible that the epigenetic alterations in diabetic brains may not only make the brain more susceptible to oAβ, but also to other insults such as
aberrantly aggregated neurofibrillary tangles made of hyperphosphorylated tau, the other pathological hallmark of AD. It is also possible that these changes make the brains more vulnerable to infarction and small vessel disease, which may lead to vascular dementia and other insults such as inflammation and advanced glycation end-products that are prevalent in diabetes. Future research on the relationship of diabetes-induced alteration of epigenetic mechanisms and the susceptibility to these potential diabetes-associated pathologic changes will further elucidate the molecular mechanisms underlying the increased risk of diabetic patients to develop dementia.
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Author contributions: J.W, L.H and G.M.P designed the studies; J.W, B.G, W.Z, M.V, T.N, W.B, A.B, S.B, P.V and L.K performed experiments. J.W and G.M.P wrote the manuscript.

G.M.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Reference List


Table 1

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Table 1. Demographic information of control and diabetes cohorts for this study. A total of 48 cases, comprising 24 non-diabetic control subjects and 24 diabetic subjects were obtained from the brain bank. Key: CDR, Clinical Dementia Rating; CERAD, consortium to Establish a Registry for Alzheimer's Disease; CVD. Cerebrovasular Disease; F, female. Data are expressed as group mean (SD).

*Cerebrovascular disease (CVD) as primary or secondary CERAD diagnosis.
Figure Legend

Fig. 1. Differential expression of class IIa HDACs in the brains of CTRL vs. T2DM subjects and measurements of protein levels of synaptic structure proteins in the same brains. (A-B) Messenger RNA expression of (A) Class IIa HDACs (HDAC4, HDAC5, HDAC7 and HDAC9) and (B) Class I HDACs (HDAC1, HDAC2, HDAC3 and HDAC8) in the brains of CTRL and T2DM subjects. (C) Protein levels of PSD-95 (left panel) and synaptophysin (right panel) in the membrane fraction of brain tissues from the CTRL and T2DM subjects assessed by western blot analysis. Inset: representative Western blot. (D) Correlation of HDAC5 with PSD 95 (left, p=0.032) and synaptophysin (right, p=0.0074). For (A) and (C), *p<0.05, **p<0.01, n=21-24 per group.

Fig. 2. Increased expression of class IIa HDACs 5 and 9 in the brains of diabetogenic-induced T2DM C57BL/6J mice (A) Postprandial plasma glucose level (B) Fasting insulin level (C) Intra-peritoneal glucose tolerance test (D) Expression of HDAC IIa in the brain of mice following 6 months diabetogenic diet treatment; **p<0.01, ***p<0.001, n=7 for (A-C) and n=5 for (D).

Fig. 3. Increased susceptibility to oAβ-induced synaptic impairments in hippocampal slices isolated from T2DM mice. (A-B) The fEPSPs of (A) LTP and (B) Basal synaptic transmission were recorded from the CA1 region of hippocampal slices from CTRL (■) or T2DM (□) mice following 6 months dietary treatment and exposed to 60 nM of oAβ for 1 hour. (C) LTP and (D) basal synaptic transmission recorded from hippocampal slices from CTRL (■) or T2DM (□) mice following 10 months treatment. The arrow indicates the beginning of tetanus to induce LTP.

Fig.4. Voxel by voxel statistics of the default mode network (DMN) superimposed on anatomical resting state fMRI. Two networks were analyzed. (A): -DNM (arrows indicate cingulate gyrus and part of the hippocampus) and (B): sensory motor network (arrows indicate the bilateral sensory motor
cortices. Green color represents group resting state network. Blue color represents significantly stronger signals in T2DM mice compared to the control mice; n=8 per group.

**Fig. 5. Inhibition of HDAC class IIa restores LTP in the hippocampal slices from T2DM mice and increases expression of synaptic proteins in primary neurons** (A) Hippocampal slices from T2DM mice were treated with 200nM of MC1568 for 2 hours and LTP (left panel) and basal synaptic transmission (right panel) were recorded. (B) Primary neurons derived C57BL6/J mice were cultured for 5 days before treatment with vehicle or 5 µM MC1568 for 16 hours. Transcription levels of PSD-95 (left panel), synaptophysin (middle panel) and brain-derived neurotrophic factor (right panel) were normalized with hypoxanthine phosphoribosyltransferase (HPRT) *p<0.05; **p<0.01; n=4 per group.
Figure 1

Diabetes
Figure 2
Figure 3
Figure 4

88x61mm (300 x 300 DPI)
Figure 5