Repeated transcranial direct current stimulation improves cognitive dysfunction and synaptic plasticity deficit in the prefrontal cortex of streptozotocin-induced diabetic rats

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Abstract

Background: Cognitive dysfunction is commonly observed in diabetic patients. We have previously reported that anodal transcranial direct current stimulation (tDCS) over the dorsolateral prefrontal cortex can facilitate visuospatial working memory in diabetic patients with concomitant diabetic peripheral neuropathy and mild cognitive impairment, but the underlying mechanisms remain unclear.  
Objective: We investigated the cellular mechanisms underlying the effect of tDCS on cognitive decline in streptozotocin (STZ)-induced diabetic rats.  
Methods: STZ-induced diabetic rats were subjected to either repeated anodal tDCS or sham stimulation over the medial prefrontal cortex (mPFC). Spatial working memory performance in delayed nonmatch-to-place T maze task (DNMT), the induction of long-term potentiation (LTP) in the mPFC, and dendritic morphology of Golgi-stained pyramidal neurons in the mPFC were assessed.  
Results: Repeated applications of prefrontal anodal tDCS improved spatial working memory performance in DNMT and restored the impaired mPFC LTP of diabetic rats. The mPFC of tDCS-treated diabetic rats exhibited higher levels of brain-derived neurotrophic factor (BDNF) protein and N-Methyl-D-aspartate receptor (NMDAR) subunit mRNA and protein compared to sham stimulation group. Furthermore, anodal tDCS significantly increased dendritic spine density on the apical dendrites of mPFC layer V pyramidal cells in diabetic rats, whereas the complexity of basal and apical dendritic trees was unaltered.  
Conclusions: Our findings suggest that repeated anodal tDCS may improve spatial working memory performance in streptozotocin-induced diabetic rats through augmentation of synaptic plasticity that requires BDNF secretion and transcription/translation of NMDARs in the mPFC, and support the therapeutic potential of tDCS for cognitive decline in diabetes mellitus patients. © 2017 Elsevier Inc. All rights reserved.

Introduction

Diabetes mellitus (DM) is a common metabolic disorder with neurological complications in the peripheral and central nervous systems [1]. Diabetic polyneuropathy (DPN) is the most common form of neuropathy that occurs in up to 50% of diabetic patients leading to sensory, motor, and/or autonomic dysfunction [2,3]. DM also contributes to central nervous system neurodegenerative processes, including brain atrophy and cognitive decline [4]. Recent studies have revealed that DM can accelerate age-related cognitive decline and is a risk factor for both vascular dementia and Alzheimer’s disease (AD) [5,6]. The common cognitive dysfunctions identified in DM patients include mental and motor slowing,
worsened executive functioning such as planning, problem-solving and working memory [7]. Functional neuroimaging studies have provided important insights into the neural mechanisms associated with cognitive decline in type 2 DM patients. It has been shown that type 2 DM patients exhibited gray matter atrophy in the hippocampus, amygdala, prefrontal and parietal cortex, and reduced integrity of cerebral white matter [8–11]. However, the therapeutic interventions for preventing and/or ameliorating cognitive dysfunction in DM patients remain undefined.

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulation technique providing a focal polarity-specific direct current electric field, either anodal or cathodal, through the skull to modulate brain function [12]. Over the past decade, tDCS has been increasingly used in clinical trials to improve motor dysfunction caused by stroke or Parkinson’s disease, and cognitive performances in patients with neuropsychiatric disorders such as schizophrenia, depression, epilepsy and AD [13–17]. Besides these potential clinical benefits, tDCS has been shown to improve declarative and working memory capacity in healthy subjects [18–20]. Animal studies have reported similar beneficial effects of tDCS on visuospatial working memory training and skill learning in normal rats [21], spatial memory in rats with traumatic brain injury [22], and object recognition before a genetically modulated attention-deficit hyperactivity disorder [23]. Furthermore, electrophysiological experiments have also shown that tDCS can induce a sustained response in the form of a long-term potentiation (LTP)– or long-term depression (LTD)-like plasticity in the human motor cortex [24]. Given that LTP- and LTD-like plasticity are considered to be the cellular correlates of learning and memory [25], tDCS might be an attractive method to overcome learning- and memory-related deficits associated with cognitive decline in DM patients. Indeed, our recent study has demonstrated that anodal tDCS over the dorsolateral prefrontal cortex can improve visuospatial working memory performance in patients with concomitant DPN and mild cognitive impairment [26]. However, it remains unclear how tDCS produces its effect. The present study was designed to study the cellular mechanisms underlying the effect of tDCS on cognitive decline in streptozotocin (STZ)-induced diabetic rats. Because repeated sessions of tDCS may have cumulative effects associated with greater magnitude and duration of effects [27], repeated tDCS protocol was used. We hypothesized that repeated anodal tDCS over the medial prefrontal cortex (mPFC) may alter expression profiles of molecules importantly involved in maintaining synaptic structure and function, to improve spatial working memory performance in STZ-induced diabetic rats. Because decreased brain-derived neurotrophic factor (BDNF) levels have been associated with the cognitive decline in STZ-induced diabetic rats [28], we examined the role of BDNF in mediating the effects of tDCS.

Methods

Animals

Eight-week-old male Sprague-Dawley rats (280–300 g; n = 130) were used. Hyperglycemia was induced by a single tail vein injection of STZ (50 mg/kg; Sigma-Aldrich, St. Louis, MO) dissolved in freshly prepared 0.05 M citrate buffer (pH 4.5) [29]. Control rats received an equivalent volume of citrate buffer. The rats were randomly assigned to the conditions of STZ or citrate buffer injection and were housed two per cage. The plasma glucose was sampled from the tail vein before and 1 week after STZ injection. STZ-treated rats with hyperglycemia above 300 mg/dl were considered as diabetic rats [30]. All experimental procedures were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University. Investigators were blind to the group allocations while performing the behavioral ratings and morphological analysis.

Electrode implantation and tDCS in vivo

Seven weeks after STZ injection, diabetic rats were anesthetized with intraperitoneal injection of Zoletil® (20–40 mg/kg) and Rompun® (5–10 mg/kg). The anesthetized rats were placed in a stereotaxic apparatus and a plastic cannula (1 cm-height and 1 mm-inner radius) was fixed at a defined circular surface on skull (center located at 2.5 mm anterior to Bregma on the sagittal fissure with 1 mm-radius) to cover the mPFC [31]. The plastic cannula was anchored onto the skull with four surrounding screws and dental acrylic resin to form an open end which was filled with 0.9% saline solution and served as the plugin site of the anodal electrode pin to conduct the current via the contact area on the skull. A conventional 5 mm-radius electroencephalography electrode (Genuine Grass) was placed at the anterior chest of the rat with conductive gel and fixed by an elastic bandage to serve as the cathodal electrode (Fig. 1B). Both anodal and cathodal electrodes were connected to a direct current stimulator (DC-Stimulator Plus, NeuroConn) for electric current stimulation. All diabetic rats acquired post-operation rest for 1 week before experiment, when rats were randomized into two groups receiving anodal tDCS (200 μA/3.14 mm², 30 min) or sham (200 μA/3.14 mm², 30 s) stimulation, respectively [32]. Both tDCS and sham procedures were conducted once daily under isoflurane gas anesthesia before delayed nonmatch-to-place T maze task (DNMT). All procedures of tDCS and sham including the duration of current fade-in (10 s) and fade-out (10 s), current strength, stimulation days and gas anesthesia period (30 min during stimulation) were identical except the stimulation duration.

DNMT

The apparatus was made of three black acrylic plastic arms arranged in a T shape with two horizontal choice arms and one vertical start arm. Each arm was composed of a 10 cm × 50 cm straight alley with 20 cm high walls and was positioned 90 cm above the ground. All rats underwent a preceding fasting period to achieve 85% baseline body weight [33,34], and maintained diet control to keep the body weight through the whole experiment period. After fasting period, rats were habituated for 2 days and trained for 10 days. Ten trials were performed each day for 10 days of training. A trial was composed of a forced choice and a following free choice. The force choice only allowed the rat to enter one choice arm where a sugar pellet was served as a reward at the end of this arm. After an interval of 10 s, as the free choice, the rat was placed back to the start arm and provided with two open choice arms. The correct free trial was defined as when rats entered the opposite choice arm other than the open one in the force choice. The number of the correct free choice among the 10 trials was recorded daily for the consecutive 10 days of DNMT. DNMT was performed immediately after the procedure of tDCS or sham stimulation and recovery from anesthesia. Theawaking state was defined by the intact righting reflex when the rat could immediately recover posture from a supine position within 5 s.

Slice preparations and electrophysiology

Slice preparations and electrophysiological recording were performed as previously described [35]. We focused on the mPFC because of its prominent role in DNMT task [36]. Postsynaptic responses were evoked by stimulating the layer II-III of the mPFC
slices with the afferents at 0.033 Hz by a bipolar stimulating electrode. Field excitatory postsynaptic potential (fEPSP) was recorded at the layer V of the mPFC by a glass pipette filled with 1 M NaCl. LTP was induced by two 1-sec trains of stimuli separated by an intertrain interval of 20 s at 100 Hz.

Western blotting

Western blotting was performed as previously described [37]. Each sample from tissue homogenate was separated by 8% SDS-PAGE gel. Following the transfer on nitrocellulose membranes, blots were blocked in buffer solution of 5% BSA and 0.1% Tween-20 that recognize GluN1 (1:2000; Santa Cruz Biotechnology, Santa Cruz, CA), GluN2A (1:1000; Cell Signaling Technology, Danvers, MA), GluN2B (1:1000; Cell Signaling Technology or β-actin (1:5000, Millipore, Billerica, MA). It was then probed with HRP-conjugated secondary antibody for 1 h and developed using the ECL Plus™ immunoblotting detection system (Amersham Biosciences, Piscataway, NJ).

Quantitative real-time RT-PCR

Quantitative Real-Time RT-PCR was performed on the Roche LightCycler instrument (Roche Diagnostics, Indianapolis, IN) using the FastStart DNA Master SYBR Green I kit (Roche Applied Science). The following primers were used: GluN1, 5'-CTGCAACCT-CACCTTTTGGAG-3' (forward) and 5'-TGCAAAAGCCAGCTGCATCT-3' (reverse); GluN2A, 5'-GACGGTCTTG GGATCTTAAC-3' (forward) and 5'-CAACTTTCGATGGTAGTCGC-3' (reverse); GluN2B, 5'-TGCA-CAATTACTTCCGTCCAGC-3' (forward) and 5'-TCCGATTCTTCTTCTCT-GACC-3' (reverse); 18S rRNA, 5'-CAACTTTCGATGGTAGTCGC-3' (forward) and 5'-TCGGTCTTCCCTCTCTTCTGACC-3' (reverse); GluN2B, 5'-TGCA-CAATTACTTCCGTCCAGC-3' (forward) and 5'-TCCGATTCTTCTTCTCT-GACC-3' (reverse); 18S rRNA, 5'-CAACTTTCGATGGTAGTCGC-3' (forward) and 5'-TGCA-CAATTACTTCCGTCCAGC-3' (reverse); GluN2B, 5'-TGCA-CAATTACTTCCGTCCAGC-3' (forward) and 5'-TCCGATTCTTCTTCTCTGACC-3' (reverse); 18S rRNA, 5'-CAACTTTCGATGGTAGTCGC-3' (forward) and 5'-TGCA-CAATTACTTCCGTCCAGC-3' (forward) and 5'-TCCGATTCTTCTTCTCTGACC-3' (reverse); Samples were amplified for 35 cycles consisting of 95 °C (10 s), 55 °C (15 s) and 68 °C (20 s). Data were analyzed by LightCycler quantification software to determine the threshold cycle above background for each reaction. The relative transcript amount of GluN1, GluN2A and GluN2B was normalized to expression of 18S rRNA.

Brain-derived neurotrophic factor immunoassay

The BDNF protein levels were determined with a conventional BDNF Rapid ELISA kit (Biosensis, Thebarton, SA) according to the manufacturer’s protocol.

Golgi impregnation

Golgi-Cox staining and measurement of dendritic morphology were performed as previously described [37]. Pyramidal neurons were reconstructed by a computer-assisted neuron tracing system (Neurolucida, Microbrightfield Inc., Williston, VT) attached to an Olympus BX51 microscope with a 20 × objective and analyzed with NIH software ImageJ running the three-dimensional Sholl analysis. Spine was defined as dendritic protrusions ≤2 μm in length, with an obvious head structure.

Immunofluorescence

Immunofluorescence was performed as previously described [37]. Coronal brain sections (40 μm thick) were incubated with the primary antibodies against c-Fos (1:2000; Santa Cruz Biotechnology) and neuronal nuclei (NeuN; 1:2000; Chemicon, Temecula, CA) overnight at 4 °C in blocking solution. Sections were washed with 0.3% Tween-20 in PBS and then incubated with the secondary Alexa Fluor 488 and Alexa Fluor 594 antibodies (Life Technologies, Grand island, NY) for 2 h at room temperature. Fluorescence
microscopic images of neurons were obtained using an Olympus BX51 microscope coupled to an Olympus DP70 digital camera.

Statistical analysis

The results are presented as mean ± SEM. All statistical analyses were performed using the Prism 6 software package. The number of animals used is indicated by n. The significance of any difference between two groups was calculated using the unpaired Student’s t-test. The significance of the difference between multiple groups was calculated by two-way ANOVA. For LTP experiments, statistical analysis was performed using the non-parametric Mann-Whitney U test. LTP values across multiple slices from the same animal were averaged to yield a single value for each animal. Probability values of p < 0.05 were considered to represent significant differences.

Results

Repeated applications of prefrontal tDCS improve spatial working memory performance in STZ-induced diabetic rats

Assessment of cognitive function was carried out by investigating performance in the DNMT, a powerful behavioral task for evaluating spatial working memory in rodents [38]. STZ treatment induced a significant increase in the levels of plasma glucose compared with vehicle-treated group (p < 0.001; Fig. 1A). The experimental setup for the tDCS treatment is depicted in Fig. 1B. STZ-induced diabetic rats exhibited impaired performance in the DNMT task, as confirmed by a two-way repeated measure ANOVA, a significant effect of STZ versus vehicle treatment (p < 0.001; 17 STZ rats and 9 vehicle rats) and a significant effect of time (p < 0.001), but a non-significant effect for the interaction between treatment and time (p = 0.45; Fig. 1C). Repeated application of anodal tDCS over the mPFC significantly improved performance of the DNMT task in STZ-induced diabetic rats. A two-way repeated measure ANOVA revealed a significant effect of tDCS versus sham treatment (p < 0.001; 26 STZ-tDCS rats and 18 STZ-sham rats) and a significant effect of time (p < 0.001), but a non-significant effect for the interaction between treatment and time (p = 0.74; Fig. 1D). There were no significant differences between the groups (STZ vs vehicle or tDCS vs sham) in time to reach choice arm on the free choice trial of DNMT.

Because tDCS has somewhat limited spatial precision [39], we then assessed the impact of repeated prefrontal tDCS on neuronal excitation in various brain areas, including the mPFC (tDCS target region), MC (neighborhood region of the mPFC) and ventral hippocampus (a remote but connected region of the mPFC). We examined the expression of c-Fos immunoreactivity, a marker for recent neuronal excitation, in the mPFC, MC and hippocampus of normal rats received 8 consecutive days of sham or anodal tDCS over the mPFC. The remarkable increases in the expression of c-Fos immunoreactivity were observed in the mPFC and MC following application of repeated prefrontal tDCS compared to sham stimulation (Fig. 2A,B). Repeated applications of prefrontal tDCS elicited only a slight increase of c-Fos expression in the ventral hippocampus (Fig. 2C).

Fig. 2. Repeated applications of prefrontal tDCS elevate neuronal excitation in the mPFC, MC and hippocampus. (A) Top panel, the square area representing the mPFC region sampled for immunofluorescence photomicrographs from normal rats received repeated sham or anodal tDCS over 8 consecutive days. Bottom panels, augmented figures showing c-Fos (green) and NeuN (red) double-labeling results in the square area. (B) Top panel, the square area representing the coronal MC region sampled for immunofluorescence photomicrographs from normal rats received repeated sham or anodal tDCS over 8 consecutive days. Bottom panels, augmented figures showing c-Fos and NeuN double-labeling results in the square area. (C) Top panel, the square area representing the ventral hippocampus (HIP) sampled for immunofluorescence photomicrographs from normal rats received repeated sham or anodal tDCS over 8 consecutive days. Bottom panels, augmented figures showing c-Fos and NeuN double-labeling results in the square area. Similar results were obtained from three additional independent experiments in each group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Repeated anodal tDCS restores impaired long-term potentiation in the mPFC of diabetic rats

To investigate the potential neurophysiological basis of tDCS to improve cognitive function in STZ-induced diabetic rats, we compared the magnitude of LTP in mPFC slices taken from one day after the last tDCS. In slices from vehicle-treated rats, HFS induced a robust LTP (138.3 ± 4.6%; n = 5 rats), whereas a significant impairment of LTP was observed in slices from STZ-induced diabetic rats (108.2 ± 3.2%, n = 7 rats, p < 0.001; Fig. 3A,C). Repeated applications of anodal tDCS over the mPFC significantly restored LTP in STZ-induced diabetic rats (STZ-sham rats: 108.0 ± 1.7%, n = 6 rats; STZ-tDCS rats: 127.9 ± 3.1%, n = 8 rats; p < 0.001; Fig. 3B,C).

Repeated anodal tDCS increases N-Methyl-D-aspartate receptor expression in the mPFC and hippocampus of diabetic rats

Since LTP induction in the mPFC is N-Methyl-D-aspartate receptor (NMDAR)-dependent [40,41], we reasoned that LTP impairment observed in STZ-induced diabetic rats could be associated with reduced expression of NMDARs. To examine this possibility, we measured the protein expression patterns of NMDAR subunits in the PFC, MC and hippocampus of vehicle- and STZ-induced diabetic rats by Western blot analysis. We did not observe significant differences between vehicle- and STZ-treated rats in the expression levels of the three major NMDAR subunits, GluN1, GluN2A and GluN2B, in the PFC and MC (p > 0.05; n = 13).
Anodal repeated tDCS increases NMDAR protein expression in the mPFC and hippocampus of diabetic rats. (A) Representative immunoblots and corresponding densitometric analysis depicting protein levels of GluN1, GluN2A, GluN2B and β-actin in the MC (unpaired Student’s t-test, GluN1, t20 = 0.2, p = 0.84; GluN2A, t20 = 1.5, p = 0.15; GluN2B, t20 = 0, p > 0.99), PFC (unpaired Student’s t-test, GluN1, t14 = 0.8, p = 0.45; GluN2A, t14 = 1.6, p = 0.12; GluN2B, t14 = 0.2, p = 0.87) and HIP (unpaired Student’s t-test, GluN1, t20 = 4.0, p < 0.001; GluN2A, t20 = 4.3, p < 0.001; GluN2B, t20 = 7.0, p < 0.001) tissue homogenates from rats treated with Veh or STZ. (B) Representative immunoblots and corresponding densitometric analysis depicting protein levels of GluN1, GluN2A, GluN2B and β-actin in the MC (unpaired Student’s t-test, GluN1, t20 = 2.0, p = 0.07; GluN2A, t20 = 1.2, p = 0.25; GluN2B, t20 = 1.2, p = 0.27), PFC (unpaired Student’s t-test, GluN1, t12 = 2.3, p < 0.05; GluN2A, t12 = 1.2, p = 0.10; GluN2B, t12 = 2.7, p < 0.05) and HIP (unpaired Student’s t-test, GluN1, t14 = 2.7, p = 0.05; GluN2A, t14 = 2.8, p < 0.05; GluN2B, t14 = 4.8, p < 0.001) tissue homogenates from STZ-induced diabetic rats received repeated sham or anodal tDCS over 8 consecutive days. The numbers in parentheses indicate the number of animals examined. Data are presented as mean ± SEM. *p < 0.05 and **p < 0.001 compared with Veh or STZ-Sham group by the unpaired Student’s t-test.

Repetitive anodal tDCS increases BDNF expression in various brain areas of diabetic rats

Given that a single anodal tDCS application over the hippocampus elevates hippocampal BDNF levels [42], and that BDNF has been shown to increase mRNA transcription of NMDA receptor subunits [44, 45], we then measured the levels of BDNF proteins in the PFC, MC and hippocampus of STZ-induced diabetic rats following repeated applications of anodal prefrontal tDCS or sham stimulation. ELISA analysis revealed significant increases in BDNF protein levels in the mPFC, MC and hippocampus of tDCS-treated diabetic rats compared to those of sham stimulation diabetic rats (p < 0.05; n = 7–8 diabetic rats in each group; Fig. 5).

Fig. 4. Anodal repeated tDCS increases NMDAR protein expression in the mPFC and hippocampus of diabetic rats. (A) Representative immunoblots and corresponding densitometric analysis depicting protein levels of GluN1, GluN2A, GluN2B and β-actin in the MC (unpaired Student’s t-test, GluN1, t20 = 0.2, p = 0.84; GluN2A, t20 = 1.5, p = 0.15; GluN2B, t20 = 0, p > 0.99), PFC (unpaired Student’s t-test, GluN1, t14 = 0.8, p = 0.45; GluN2A, t14 = 1.6, p = 0.12; GluN2B, t14 = 0.2, p = 0.87) and HIP (unpaired Student’s t-test, GluN1, t20 = 4.0, p < 0.001; GluN2A, t20 = 4.3, p < 0.001; GluN2B, t20 = 7.0, p < 0.001) tissue homogenates from rats treated with Veh or STZ. (B) Representative immunoblots and corresponding densitometric analysis depicting protein levels of GluN1, GluN2A, GluN2B and β-actin in the MC (unpaired Student’s t-test, GluN1, t20 = 2.0, p = 0.07; GluN2A, t20 = 1.2, p = 0.25; GluN2B, t20 = 1.2, p = 0.27), PFC (unpaired Student’s t-test, GluN1, t12 = 2.3, p < 0.05; GluN2A, t12 = 1.2, p = 0.10; GluN2B, t12 = 2.7, p < 0.05) and HIP (unpaired Student’s t-test, GluN1, t14 = 2.7, p = 0.05; GluN2A, t14 = 2.8, p < 0.05; GluN2B, t14 = 4.8, p < 0.001) tissue homogenates from STZ-induced diabetic rats received repeated sham or anodal tDCS over 8 consecutive days. The numbers in parentheses indicate the number of animals examined. Data are presented as mean ± SEM. *p < 0.05 and **p < 0.001 compared with Veh or STZ-Sham group by the unpaired Student’s t-test.

Fig. 5. Anodal repeated tDCS increases NMDAR subunit mRNA expression in the mPFC and hippocampus of diabetic rats. Summary graph depicting the relative mRNA levels of GluN1, GluN2A and GluN2B in the MC (unpaired Student’s t-test, GluN1, t14 = 2.9, p < 0.05; GluN2A, t14 = 1.0, p = 0.33; GluN2B, t14 = 1.2, p = 0.25), PFC (unpaired Student’s t-test, GluN1, t12 = 4.3, p < 0.01; GluN2A, t12 = 2.4, p < 0.05; GluN2B, t12 = 2.7, p < 0.05) and HIP (unpaired Student’s t-test, GluN1, t12 = 5.5, p < 0.001; GluN2A, t14 = 2.5, p < 0.05; GluN2B, t14 = 3.5, p < 0.01) tissue homogenates from STZ-induced diabetic rats received repeated anodal tDCS over 8 consecutive days compared to sham group. The numbers in parentheses indicate the number of animals examined. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001 compared with STZ-Sham group by the unpaired Student’s t-test.

Anodal repeated tDCS increases dendritic spine number of mPFC layer V pyramidal neurons in diabetic rats

We finally evaluated whether the beneficial effects of tDCS on spatial working memory and LTP were attributed to changes in dendritic morphology of mPFC pyramidal neurons. We used Golgi-Cox staining to visualize individual mPFC layer V pyramidal neurons (Fig. 7A), and analyzed the complexity of their dendritic trees. Sholl analysis of reconstructed mPFC layer V pyramidal neurons revealed that repeated applications of prefrontal tDCS had no significant effect on the complexity of apical dendrites (p = 0.98; n = 47 and 40 neurons from 5 mice in each group; Fig. 7B) or basal dendrite branching (p = 0.13; n = 40 and 47 neurons from 5 mice in each group; Fig. 7C). However, a significantly higher number of protrusions per unit length was detected on secondary and tertiary branches of the apical dendrites of mPFC layer V pyramidal neurons.
in tDCS-treated diabetic rats compared to sham stimulation diabetic rats \( (p < 0.01; n = 114 \text{ dendrites from 38 neurons of 4 STZ-sham rats and 78 dendrites from 26 neurons of 5 STZ-tDCS rats}; \text{Fig. 7D,E}). \)

### Discussion

Our previous findings demonstrated that anodal tDCS over the dorsolateral prefrontal cortex can improve visuospatial working memory performance in DM patients who suffer from both DPN and mild cognitive impairment \[26\]. Extending these clinical observations, our current results provide additional insight into the mechanistic basis for understanding how tDCS elicits improvement in DM-related cognitive dysfunction. We confirmed that STZ-induced diabetic rats exhibited significant deficits in spatial working memory and LTP induction in the mPFC. We have demonstrated, for the first time that repeated anodal tDCS over the mPFC significantly improves the cognitive performance in diabetic rats. In particular, we found that tDCS-induced cognitive improvement was consistent with the restoration of LTP deficit and the elevation of BDNF and NMDAR expression levels in the mPFC of diabetic rats.

Despite accumulating evidences support feasibility and clinical potential of tDCS for treating patients with a number of psychiatric and neurological disorders \[13–17\], the exact underlying mechanisms remain obscure. Due to the disadvantages inherent to experimental designs in clinical trials, research on the cellular and molecular mechanisms of action of tDCS has resorted to the use of animal models. The STZ-induced diabetic rodent model has been shown to recapitulate many of the clinical and pathological features caused by human DM \[46\]. Previous studies have shown that STZ-induced diabetic rats displayed deficits in spatial memory tasks, such as the Morris water maze \[47\] and novel object recognition \[48\]. Consistent with these findings, we observed that STZ-induced diabetic rats showed an impaired performance in the DNMT task, which is a valid indicator of spatial working memory \[38\]. Our data further suggest that repeated applications of prefrontal tDCS improved DNMT performance in STZ-induced diabetic rats, which is correlated with our previous clinical findings that anodal tDCS

**Fig. 6.** Anodal repeated tDCS increases BDNF expression in various brain regions of diabetic rats. Summary graph depicting the BDNF protein levels in the MC (unpaired Student’s t-test, \( t_{12} = 3.0, p < 0.05 \)), PFC (unpaired Student’s t-test, \( t_{12} = 6.5, p < 0.001 \)) and HIP (unpaired Student’s t-test, \( t_{12} = 2.8, p < 0.05 \)) tissue homogenates from STZ-induced diabetic rats received repeated sham or anodal tDCS over 8 consecutive days. The numbers in parentheses indicate the number of animals examined. Data are presented as mean ± SEM. *\( p < 0.05 \), and **\( p < 0.001 \) compared with STZ-Sham group by the unpaired Student’s t-test.

**Fig. 7.** Anodal repeated tDCS increases dendritic spine number of mPFC layer V pyramidal neurons in diabetic rats. (A) Representative camera lucida tracings of mPFC layer V pyramidal neurons from STZ-induced diabetic rats received repeated sham or anodal tDCS over 8 consecutive days. (B) and (C) Sholl analysis of apical (Two-way repeated measure ANOVA, treatment, \( F_{1,4250} = 5.0, p < 0.05 \); distance from soma, \( F_{29,4250} = 3.8, p < 0.05 \); distance from soma, \( F_{29,4250} = 28.7, p < 0.001 \); treatment and distance from soma, \( F_{29,4250} = 1.3, p = 0.11 \)) dendrites of mPFC layer V pyramidal neurons from STZ-induced diabetic rats received repeated sham or anodal tDCS over 8 consecutive days. (D) Representative images of the secondary branch of mPFC layer V pyramidal neurons from STZ-induced diabetic rats received repeated sham or anodal tDCS over 8 consecutive days. (E) Summary bar graphs depicting the density of protrusions in apical dendrites of mPFC layer V pyramidal neurons from STZ-induced diabetic rats received repeated sham (114 dendrites from 38 neurons of 4 rats) or anodal tDCS (78 dendrites from 26 neurons of 5 rats) over 8 consecutive days (unpaired Student’s t-test, \( t_{62} = 4.2, p < 0.01 \)). Data represent the mean ± SEM. The total number of neuron examined is indicated by \( n \) in parenthesis. **\( p < 0.01 \) compared with STZ-Sham group by the unpaired Student’s t-test.
over the dorsolateral prefrontal cortex improved visuospatial working memory performance in patients with concomitant DPN and mild cognitive impairment [26]. An immediate question that follows these observations is where the brain area is targeted by tDCS to improve the DNMT task. Given that both the mPFC and hippocampus are crucial for performing the DNMT task [49], it is possible that our repeated anodal tDCS may modulate neural activity of these two brain areas to improve DNMT performance deficit in STZ-induced diabetic rats. This idea is supported by the findings that repeated anodal tDCS led to a significant increase in c-Fos expression in the PFC and ventral hippocampus (Fig. 2). Interestingly, we observed that the elevation of c-Fos expression by tDCS was less prominent in the ventral hippocampus than that in the mPFC. This could be due to the locational difference in responsiveness to tDCS. Indeed, the mPFC is the brain region directly under the electrode and the ventral hippocampus could be indirectly activated through the functional connectivity. In rodents, the PFC projects directly to the ventral hippocampus and indirectly to the dorsal hippocampus via the thalamus [50,51].

How can tDCS improve cognitive decline in STZ-induced diabetic rats? Our results suggest that the elevation of mPFC BDNF and NMDAR protein levels could account, at least in part, for the beneficial effects of tDCS on cognitive performance in diabetic rats. Indeed, some studies have firmly established that both BDNF/TrkB signaling and NMDARs play a critical role in spatial working memory [52-54] and decreased BDNF levels have been associated with the cognitive decline in STZ-induced diabetic rats [28]. Considering that we did not observe significant changes in the levels of NMDARs in the mPFC of STZ-induced diabetic rats, it is conceivable that cognitive decline observed in our STZ-induced diabetic rats was not associated with the loss of NMDARs but can be improved by tDCS-induced elevations of NMDAR levels. Repeated anodal tDCS also increased the levels of GluN1, GluN2A and GluN2B mRNA in the mPFC, suggesting that tDCS regulates NMDAR expression via a transcriptional regulation mechanism. Furthermore, our data agree with previous work that has demonstrated a pivotal role of BDNF in mediating the effects of tDCS on motor skill learning [55] and hippocampal synaptic plasticity [33]. However, additional studies are required to clarify the interaction between BDNF and NMDARs in mediating the actions of tDCS. Because BDNF/TrkB signaling has also been shown to elicit an upregulation of GluN1 gene transcription in cultured cortical neurons [56], it will be interesting to know whether repeated anodal tDCS stimulates the release of BDNF, thereby triggering the transcription and translation of NMDARs.

Our data show that repeated anodal tDCS also restored impaired LTP in the mPFC of diabetic rats. Although the cellular and molecular mechanisms underlying the impairment of mPFC LTP in STZ-induced diabetic rats remain unclear, we propose that elevated expression of both BDNF and NMDARs by repeated anodal tDCS treatment may lead to increased TrkB-NMDAR interaction and enhanced mPFC LTP. Previous studies have established that BDNF/TrkB signaling and NMDARs play critical roles for mPFC LTP induction [40,41,57]. Another interesting finding is that repeated anodal tDCS increased dendritic spine density along the apical dendrites of mPFC layer V pyramidal neurons in STZ-induced diabetic rats. Considering that the number and structure of dendritic spines can affect synaptic plasticity and the efficacy of synaptic transmission [58], an elevation in spine density may also contribute to tDCS’s ability to enhance cognitive function. Our findings are consistent with the results from a previous study showing that daily tDCS over a period of 2 weeks following ischemia in rats significantly increased dendritic spine density of the remaining cortical neurons at the infarct site [59].

Conclusions

Our findings demonstrate that repeated anodal tDCS over the mPFC is effective to improve spatial working memory performance in STZ-induced diabetic rats, presumably resulting in augmented synaptic and structural plasticity that requires BDNF secretion and transcription/translation of NMDARs in mPFC layer V pyramidal neurons, which are essential for cognitive performance, especially memory formation [60]. These plastic changes may be the mechanism by which repeated tDCS application improves working memory in the diabetic subjects, and serve as an advanced understanding of the neurophysiological basis to optimize the clinical application of tDCS for the patients with cognitive dysfunction.

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Competing interests

The authors declare no competing interests.

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