Modulating Hippocampal Plasticity with In Vivo Brain Stimulation

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Investigations into the use of transcranial direct current stimulation (tDCS) in relieving symptoms of neurological disorders and enhancing cognitive or motor performance have exhibited promising results. However, the mechanisms by which tDCS effects brain function remain under scrutiny. We have demonstrated that in vivo tDCS in rats produced a lasting effect on hippocampal synaptic plasticity, as measured using extracellular recordings. Ex vivo preparations of hippocampal slices from rats that have been subjected to tDCS of 0.10 or 0.25 mA for 30 min followed by 30 min of recovery time displayed a robust twofold enhancement in long-term potentiation (LTP) induction accompanied by a 30% increase in paired-pulse facilitation (PPF). The magnitude of the LTP effect was greater with 0.25 mA compared with 0.10 mA stimulations, suggesting a dose-dependent relationship between tDCS intensity and its effect on synaptic plasticity. To test the persistence of these observed effects, animals were stimulated in vivo for 30 min at 0.25 mA and then allowed to return to their home cage for 24 h. Observation of the enhanced LTP induction, but not the enhanced PPF, continued 24 h after completion of 0.25 mA of tDCS. Addition of the NMDA blocker AP-5 abolished LTP in both control and stimulated rats but maintained the PPF enhancement in stimulated rats. The observation of enhanced LTP and PPF after tDCS demonstrates that non-invasive electrical stimulation is capable of modifying synaptic plasticity.

Key words: brain stimulation; extracellular recording; hippocampus; long term potentiation; rat; tDCS

Significance Statement
Researchers have used brain stimulation such as transcranial direct current stimulation on human subjects to alleviate symptoms of neurological disorders and enhance their performance. Here, using rats, we have investigated the potential mechanisms of how in vivo brain stimulation can produce such effect. We recorded directly on viable brain slices from rats after brain stimulation to detect lasting changes in pattern of neuronal activity. Our results showed that 30 min of brain stimulation in rats induced a robust enhancement in synaptic plasticity, a neuronal process critical for learning and memory. Understanding such molecular effects will lead to a better understanding of the mechanisms by which brain stimulation produces its effects on cognition and performance.
tDCS on cellular LTP. Here, we show that polarizing current applied to the exposed cortex of an anesthetized rat for at least 5 min produced enhancement in evoked response and spontaneous activity that persisted for at least 3 h after cessation of polarizing current stimulation (Bindman et al., 1962). Follow-up studies in humans later indicated that tDCS lasting at least 5 min applied to the motor cortex induced a significant increase in motor-evoked potential that lasted 90 min after the end of stimulation (Nitsche and Paulus, 2001). However, excitability enhancement in the motor cortex lasting >24 h could be induced by periodical anodal tDCS (Monte-Silva et al., 2013).

Recent work using rats subjected to in vivo anodal tDCS corroborates human studies, revealing increased cortical excitability and improvements in working memory, skill learning, and motor coordination as assessed using a variety of behavioral tests (Dockery et al., 2011; Binder et al., 2014; Romero Luaro et al., 2014). Also consistent with human studies, anodal tDCS has been demonstrated to possess therapeutic potential in rat models of Alzheimer’s disease (Yu et al., 2014) and stroke (Jiang et al., 2012). However, the cellular mechanism by which anodal tDCS exerts its effects remains elusive. Based on past studies on the enhancement of learning and memory in both human and animals, there is a general consensus that anodal tDCS could enhance synaptic plasticity, especially long-term potentiation (LTP). In vivo application of tDCS in human subjects produced LTP-like effects in the human cerebral cortex that are dependent on the glutamatergic system and calcium (Nitsche et al., 2008; Stagg and Nitsche, 2011). Similarly, in vivo stimulation in rabbits suggested that tDCS can modify synapses at presynaptic sites that are essential for associative learning (Márquez-Ruiz et al., 2012). In vitro exposures of brain slices to anodal current stimulation enhanced synaptic plasticity in mouse motor cortex (Fritsch et al., 2010) and in CA1 neurons of rat hippocampus (Ranieri et al., 2012). Furthermore, in vitro current stimulation applied directly to rat hippocampal slices has been shown to alter amplitude and frequency of gamma oscillations, mathematically predicted to be induced by changes in synaptic function (Reato et al., 2015).

There is limited data available on the direct effects of in vivo tDCS on cellular LTP. Here, we show that in vivo application of anodal tDCS in rats (0.25 or 0.10 mA for 30 min) induced a significant enhancement in LTP and paired-pulse facilitation (PPF) in the Schaffer collateral–CA1 synapse of the hippocampus. The enhanced effect on LTP in hippocampal slices was dependent on tDCS intensity and persisted for at least 24 h after completion of tDCS. Additionally, we show that the observed tDCS-enhanced LTP at the Schaffer collateral–CA1 pathway is dependent on NMDA receptors, whereas tDCS-enhanced PPF is independent of NMDA receptors.

Materials and Methods
Animal handling. All rats were maintained according to National Institutes of Health and Wright Patterson Air Force Base (WPAFB) Institutional Animal Care and Use Committee guidelines. The study protocol was reviewed and approved in compliance with the Animal Welfare Act and with all applicable federal regulations governing the protection of animals in research.

All animals (7-week-old male Sprague Dawley rats) were purchased from Charles River and received a 10 d acclimation period on arrival to WPAFB facilities before surgical implantation of an electrode. A total of 34 rats were used for this study. Rats were monitored for 1 week to assess recovery before being randomly selected for sham or tDCS treatment.

Surgical implantation of cranial electrode. Animals were anesthetized with isoflurane (Med-Vet International) using 5% induction, followed by 2–3% isoflurane to maintain anesthetic depth. A head electrode of 0.25 cm² (1.25-inch-diameter circular electrode cut to 5 × 5 mm; Valu-Trode; Axelgaard Manufacturing) was applied to the skull with the center of the electrode resting on the midline 2.5 mm caudal to bregma (Fig. 1). A c-clamp was then placed on the skull with C&B Metabond Adhesive (Parkell). Acrylic dental cement (Sigma) was then applied. A minimum of 7 d recovery was permitted before tDCS treatment.

tDCS treatment. Five minutes before stimulation, animals were removed from the home cage, weighed, and brought to the experimental room. The head electrode was connected to experimental wires, and a reference electrode (8.04 cm²; ValuTrode; Axelgaard Manufacturing) was placed between the shoulders with Signagel electrode gel (Parker Laboratories) as the conducting medium. Once the electrodes were in place, the animal was placed into a novel environment made of Plexiglas, containing two novel objects for exploration. Animals were allowed to move freely throughout stimulation and were monitored via Ethovision software. The use of novel environment placement allowed for enhanced neuronal activity of the target areas during the application of tDCS. tDCS was then applied using a constant-current stimulator (Magstim DC-stimulator; Neuroconn) for 30 min at 250 or 100 μA. Previous work in our laboratory using histological analysis has shown that these intensities (100 and 250 μA) do not cause tissue damage. The animals in the sham group were connected the same way as the stimulation group but did not receive any current. After stimulation, the animals were returned to their home cage until time they were euthanized for brain slice preparation (30 min or 24 h after stimulation).

Brain slice preparation. Brain and brain slices were kept viable by keep- ing in ice-cold artificial CSF (ACSF) that was kept continuously oxygenated (95% O₂/5% CO₂). ACSF consisted of the following (in mM): 124 NaCl, 3 KCl, 1.25 KH₂PO₄, 10 D-glucose, 1 MgSO₄, 36 NaHCO₃, and 2 CaCl₂, pH 7.4. Cerebellum and ~1 cm of frontal cortex were removed, and the remaining brain was sectioned at 350 μm using a vibratome (VT1000S from Leica or OTS-4000 from FHC) in the transverse plane, at 20–30° laterally off the horizontal axis. Brain slices were maintained in warmed oxygenated ACSF and allowed to recover for at least 60 min before recording. A new batch of ACSF was prepared each morning of experimentation and continuously oxygenated with 95% O₂ and 5% CO₂.

One hippocampal slice was placed onto the precoated MED64 probe, using small weights to anchor the slices down. The probe containing the
brain slice was then assembled with the MED64 system, as specified in the MED64 instruction manual. A perfusion cap was used to circulate fresh oxygenated ACSF into the probe and prevent the slices from drying. The ACSF solution and oxygen entering the probe chamber were maintained at 32–34°C. Flow rates were maintained at ~0.5–1.0 ml/min while ensuring a liquid-air interphase. Humidified oxygen entered the probe at ~0.3–0.5 L/min.

Electrophysiology recording. All electrophysiology recordings were blinded experiments, in which the exposure condition of the rat (tDCS or sham) was not identified until the completion of recordings from all rats in the same cohort. A cohort is one group of rats of the same age that has undergone electrode placement surgery on the same day.

All electrophysiology data were obtained using AlphaMed MED64 (Automate), an extracellular recording system containing 64 planar microelectrodes arranged in an 8 × 8 array. Data acquisition and stimulation protocols were performed using Mobius software (Automate). A stimulating current of 10–100 μA was applied to the Schaffer collateral region of the hippocampus to obtain an input/output relationship curve (Fig. 2). Evoked field potentials in the form of field EPSPs (fEPSPs) and population spikes were obtained in the CA1 region. Amplitudes of population spikes were also calculated. Slope rise of the evoked response and calculating the slope in the CA1 region. Percentages LTP or percentage potentiation was calculated by computing the percentage difference in population spike amplitude or fEPSP amplitude at either 30 or 60 min after LTP induction by TBS from baseline. Averages of five data points were calculated to obtain baseline and LTP values. PPF was obtained by delivering two consecutive stimuli at 50 μA that are 40 ms apart. To ensure that facilitation was not present at 24 h, additional recordings were made with paired stimuli at 30 and 40 μA. Responses mediated by the AMPA and kainate receptors were blocked using 30 μM 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX; Sigma). NMDA receptors were blocked using 50 μM 2-amino-5-phosphonopentanoic acid (AP-5; Sigma).

Data analysis. fEPSPs were calculated by fitting a line to the initial rise of the evoked response and calculating the slope in the CA1 region. Amplitudes of population spikes were also calculated. Slope and amplitude calculations were performed using Mobius software (Automate). Quantitation of LTP was obtained by averaging five data points at the indicated times (just before LTP induction by TBS and at 30 or 60 min after TBS). Percentage LTP or percentage potentiation refers to the slope or amplitude of baseline fEPSP values. Normalized fEPSP data refers to the slope or amplitude of fEPSP divided by the average slope or amplitude of all fEPSP points before TBS. Quantita-
Effects of tDCS on synaptic plasticity. Rats were subjected to tDCS for 30 min at 250 μA, followed by 30 min additional recovery time. A–D, Effects of tDCS on LTP. A, Graph of average, normalized slopes of evoked responses from CA1 region of hippocampus from control (Sham, black trace, n = 6 rats, 8 slices) or stimulated (tDCS, red trace, n = 6 rats, 7 slices) rats. Data are presented as means ± SEMs. Arrow denotes induction of LTP by TBS. Sample trace of evoked response before (black) and 30 min after (red) LTP induction by TBS is shown to the right (inset). Calibration: 0.5 mV, 5 ms. B, Graph of average, normalized amplitudes of evoked responses from CA1 region of hippocampus from control (Sham, black trace, n = 6 rats, 8 slices) or stimulated (tDCS, red trace, n = 6 rats, 7 slices) rats. Arrow denotes induction of LTP by TBS. Data are presented as means ± SEMs. C, D, Bar graph representing the average percentage LTP calculated using slopes (solid fill) and amplitudes (pattern fill) of evoked responses at 60 min after LTP induction (C) or 30 min after LTP induction (D). Significant enhancements were observed in hippocampal slices from tDCS-treated rats (red) compared with sham-treated rats (black) (slope data, p = 0.002 and 0.01 for 60 and 30 min, respectively; amplitude data, p = 0.0005 and 0.0002 for 60 and 30 min, respectively; df = 13). E, Effects of tDCS on PPF. The PPF ratio was calculated as slope (solid fill) or amplitude (pattern fill) of response resulting from the second stimulus divided by the respective slope or amplitude of response resulting from the first stimulus. There was a significant increase in PPF ratio in the CA1 region of the hippocampus from rats treated with tDCS (n = 5 rats, 6 slices) compared with that from control (n = 5 rats, 7 slices; p = 0.003 and 0.005 for slope and amplitude data, respectively; df = 65). Data are presented as means ± SEMs. *p < 0.05.

Results

In vivo anodal tDCS in rats enhanced LTP in acutely prepared hippocampal slices

To determine the optimum stimulation intensity for LTP experiments, we applied multiple stimulating currents to the Schaffer collateral region of rat hippocampal slices ranging from 10 to 100 μA in intensity (Fig. 2A). We found that a current of 50 μA consistently induced half-maximal response, and thus a 50 μA current was used as stimulus for our electrophysiological experiments. There was no obvious effect on the size and shapes of the evoked response in the hippocampus of control or stimulated rats (Fig. 2B). Furthermore, tDCS did not induce significant changes in the frequency of spontaneous spiking activity in the CA1 region of the hippocampus resulting from tDCS (Fig. 2C). There may be subtle activation of fast spiking interneurons surrounding CA1 region (Fig. 2C,D), but this activation was only observed in two of four slices and was not statistically significant. Follow-up study is worth pursuing to determine tDCS effects on the fast spiking interneurons of the hippocampal CA3 region, in which high-frequency spiking has been shown to be more prominent and can be induced reliably.

LTP was induced using three trains of TBS. We found that there was a significant increase in the degree of LTP in rats that were subjected to tDCS compared with control rats (Fig. 3). By calculating the initial slope of the field potentials at 30 min after LTP induction using TBS, there was a 63.7 ± 6% potentiation in control rats (sham) but a 129.6 ± 16% potentiation in stimulated rats (tDCS; Fig. 3A,C). At 60 min, the difference is further enhanced, resulting in 52.9 ± 5% potentiation in control rats and 135.2 ± 14% in stimulated rats (Fig. 3A,D). Statistical analysis using unpaired, two-tailed t test yielded p values of 0.01 and 0.002 for the 30 and 60 min slope data, respectively. Amplitudes of field potentials were also calculated, yielding a 42.8 ± 3 and 93.5 ± 9% potentiation in control and tDCS-treated rats, respectively, at the 30 min time point, and 42.3 ± 4 and 92.3 ± 9% potentiation in control and tDCS-treated rats, respectively, at the 60 min time point (Fig. 3B,E,F). p values were <0.001 for both the 30 and 60 min amplitude data. The differences observed at 30 min were always observed at 60 min and were of a greater magnitude (Fig. 3D). Therefore, in subsequent experiments, the percentage LTP po-
tentiation of evoked responses were calculated only at the 30 min time point after tDCS induction.

Anodal tDCS in rats enhanced PPF in acutely prepared hippocampal slices
PPF measurements were obtained by delivering two 50 μA stimuli that are 40 ms apart to the Schaffer collateral region of the hippocampus, and evoked responses from CA1 region were recorded. We found that there was significantly greater PPF in stimulated rats compared with control rats (Fig. 3E). Rats subjected to 30 min of tDCS (250 μA) followed by 30 min recovery time displayed a PPF ratio of 1.5 ± 0.04 compared with 1.1 ± 0.09 of control rats when slope measurements were used (p = 0.003). Similarly, PPF ratio values based on amplitude calculations were 1.5 ± 0.1 and 2.2 ± 0.07 for control and tDCS-treated rats, respectively (p = 0.005).

Dependence of synaptic plasticity on tDCS intensity
The enhancing effect on LTP and PPF was still observed, albeit to a smaller extent, when tDCS intensity was decreased from 250 to 100 μA (Fig. 4A, D). Hippocampal slices obtained from rats treated with 30 min of 100 μA tDCS followed by 30 min recovery time resulted in a percentage LTP of 118.5 ± 16% compared with 83 ± 7% from control rats (sham) as measured by calculating the slopes of field potentials (p = 0.01; Fig. 4B). Amplitude measurements were 54 ± 7% LTP for control rats and 88 ± 17% for stimulated rats (p = 0.04). Furthermore, slopes of evoked responses in hippocampal slices from tDCS-treated rats still displayed a greater PPF ratio (1.7 ± 0.1) compared with sham-treated rats (1.3 ± 0.05; Fig. 4D; p = 0.002). Amplitude values for the PPF ratio were 2.4 ± 0.06 and 1.6 ± 0.2 for tDCS- and sham-treated rats, respectively (Fig. 4D; p = 0.006).

Lasting effects of tDCS on synaptic plasticity
To determine whether the effects to tDCS were persistent, animals received in vivo tDCS stimulation for 30 min and then were returned to their home cage for 24 h. We observed that the effect of tDCS on LTP was still maintained 24 h after tDCS (Fig. 5). Using field potential slope values, the sham group experienced an average LTP of 76 ± 9%, whereas the tDCS group experienced an average LTP of 154 ± 35% (p = 0.03, n = 7 rats, 8 slices). Similarly, using amplitude values, the sham group experienced an average LTP of 47 ± 8%, whereas the tDCS group experienced an average LTP of 155 ± 48% (p = 0.02). Unlike the data obtained from rats 30 min after tDCS, data obtained from rats 24 h after tDCS did not reveal significant changes in PPF. We stimulated hippocampal slices with two consecutive stimuli that are 40 ms apart at three different intensities (30, 40, and 50 μA) and did not detect any significant effects on the PPF ratio (p > 0.1).

Dependence of tDCS-mediated plasticity effects on NMDA receptors
The observed field potentials from CA1 neurons were mediated predominantly by ionotropic glutamate receptors (iGluRs) because the evoked response was abolished quickly by the perfusion of 30 μM DNQX and 50 μM AP-5 in hippocampal slices from both control and tDCS rats (Fig. 6). Application of 50 μM AP-5 only minimally reduced the evoked response but prevented LTP induction in both sham and tDCS-treated rats (Fig. 6A-C). However, the enhanced effect on PPF was still observed in the presence of AP-5 (Fig. 6D). This suggests that the tDCS-induced increase in PPF is not NMDA dependent, because blockade of NMDA receptor still induced PPF ratios of 1.4 ± 0.1 in tDCS-treated rats compared with a PPF ratio of 1.1 ± 0.08 in control rats when slope values were used (p = 0.02) and PPF ratios of 2.7 ± 0.5 and 1.6 ± 0.2 in stimulated and control rats, respectively, when amplitude values were used (p = 0.03). Our data are consistent with the previously established principles claiming that calcium accumulation in the presynaptic terminal is critical in establishing
PPF (Zucker, 1989; Zucker and Regehr, 2002; Madroñal et al., 2009). We believe this is the first account of in vivo tDCS enhancing plasticity of neurons at both the presynaptic and postsynaptic sites of rat hippocampus.

Discussion

We have demonstrated that in vivo tDCS in rats can enhance LTP and PPF, two distinct types of synaptic plasticity in the rat hippocampus. Reduction of tDCS intensity from 200 to 100 μA decreased the LTP enhancement to ~1.4-fold, supporting the possibility of dose-dependent effects. This is consistent with previous findings in which modulation of cortical excitability was dependent on current stimulation intensity (Nitsche and Paulus, 2000; Bastani and Jaberzadeh, 2013; Murray et al., 2015). Of interest, the effect on PPF appears to be all-or-none, enhancing PPF by ~30–50% at both the high and low tDCS intensities. Although the increase in PPF can no longer be detected at 24 h after completion of tDCS, the LTP enhancement still persists, suggesting the possibility that tDCS-mediated enhancements of LTP and PPF occur through distinct mechanisms. Our data further suggest that the tDCS-induced increase in LTP is NMDA dependent, which is consistent with previously established principles that postsynaptic NMDA receptors play a critical role in LTP at the CA1 region of the hippocampus, as well as with other studies demonstrating that tDCS-induced effects can be blocked by NMDA blockers (Liebetanz et al., 2002; Nitsche et al., 2003). Although we did not observe significant changes in spike frequency attributable to tDCS, there may be some activation of high-frequency responses most likely attributable to the fast-spiking hippocampal interneurons. Investigation of tDCS effects on interneuron activity will be pursued. Components of evoked responses in the CA1 region were mediated primarily by iGluRs, because perfusion of the kainate and AMPA blocker DNQX, combined with the NMDA blocker AP-5, blocked all excitation-evoked responses from both control and stimulated rats. Our data rule out the mechanistic possibility of tDCS producing effects that recruit or enhance other nonglutamatergic synapses in the measured CA1 region of the hippocampus.

A rigorously studied form of synaptic plasticity is LTP, discovered first in the perforant path of an anesthetized rabbit (Bliss and Lomo, 1973). Additional work in brain slices revealed that the CA1 pyramidal cells of the rat hippocampus consistently undergo LTP during high-frequency stimulation (Dunwiddie and Lynch, 1978). Since then, LTP has been studied extensively not only in the hippocampus but also in other brain regions, and it has been widely accepted as the molecular basis for learning and memory (Izquierdo, 1994; Gruart et al., 2006; Whitlock et al., 2006; Neves et al., 2008). Although the complete molecular mechanisms of LTP remain under investigation, many of the key players have been identified (Baudry and Lynch, 2001). Data suggest that high-frequency stimulation induced recruitments of postsynaptic iGluRs onto the postsynaptic cell, as well as gene expression changes (Baudry and Lynch, 2001). Although there are different types of LTP, a robust and well studied form is the NMDA-dependent LTP that persists in the Schaffer collateral–CA1 region of the hippocampus. NMDA-dependent LTP has been shown to be essential for learning and memory administration of NMDA receptor blocker AP-5 prevented LTP induction and impaired learning (Izquierdo, 1994; Gruart and Delgado-Garcia, 2007; Caroni et al., 2012).

The tDCS-induced enhancement of LTP observed in this study is consistent with previous findings of improved cognitive functions and plasticity in diseased and healthy subjects resulting from in vitro non-invasive stimulations (Nitsche et al., 2008; Stagg and Nitsche, 2011; Bastani and Jaberzadeh, 2013; Heise et al., 2014). Furthermore, our data are also in agreement with previous work that in vitro current stimulation directly on brain slices resulted in immediate augmentation of NMDA-dependent LTP in the Schaffer collateral–CA1 pathway of rat hippocampus (Ranieri et al., 2012) and the NMDA and BDNF-dependent LTP in the mouse motor cortex (Fritsch et al., 2010). We measured LTP only on the Schaffer collateral–CA1 pathway of the hippocampus that is mainly NMDA dependent. Therefore, we questioned whether the observed effect on LTP was attributable to increased recruitment of other glutamate receptors to the postsynaptic site to generate other types of LTP that is not dependent on NMDA receptors. However, this idea was ruled out by the fact that per-
fusion of AP-5 blocks LTP in both control and tDCS-treated rats. Although this does not eliminate the possibility that tDCS can also affect other non-NMDA forms of LTP, it strengthens the hypothesis that the NMDA receptor is an essential target whereby tDCS exerts its effect, at least in the Schaffer collateral–CA1 pathway.

PPF is another form of synaptic plasticity, but, in contrast to LTP, it is short-lived and mediated presynaptically, resulting from accumulation of calcium ions attributable to two stimulating pulses delivered within a short interstimulus duration (Zucker 1989; Zucker and Regehr, 2002). PPF is observed when two consecutive stimuli are delivered, within tens of milliseconds of each other, resulting in a potentiated postsynaptic response elicited by the second stimulus. The prevailing mechanistic explanation of PPF is the transient buildup of calcium ions during two consecutive stimuli (Katz and Miledi, 1968; Thomson, 2000). The second stimulus produces an unusually larger calcium pool, which will subsequently trigger greater release of neurotransmitter molecules. This is unlike LTP in which the likely mechanism involves mainly postsynaptic events, such as the recruitment of more iGluRs.

We saw an enhancement in PPF of CA1 neurons from rats subjected to 30 min of tDCS that persists in the presence of the NMDA receptor blocker AP-5. This is consistent with the idea that facilitation is attributable to the accumulation of calcium in the presynaptic cell (Katz and Miledi, 1968; Thomson, 2000) and thereby NMDA receptor independent. However, our data of enhanced PPF contradicts previous work in rabbits, in which anodal stimulation induced a decrease in the PPF ratio (Márquez-Ruiz et al., 2012). The discrepancy may be accounted by the difference in the probability of neurotransmitter release resulting from the first pulse. We recorded evoked potentials from CA1 neurons in rat ex vivo hippocampal slices, which are known to have a low probability of initial release (Abbott and Regehr, 2004), whereas Márquez-Ruiz et al. (2012) recorded from the rabbit somatosensory cortex in vivo, which has a high probably of initial release (Silver et al., 2003).

In contrast to effects on LTP, the tDCS-induced enhancement on PPF can no longer be detected 24 h after cessation of stimulation. Although there is convincing correlation between anodal tDCS with increased cortical excitability, there is very limited data on whether stimulation can modify presynaptic machinery. Our data on PPF provide a glimpse on tDCS effect on the presynaptic cell, supporting the hypothesis that neurotransmitter levels at synapses could also be modulated by tDCS.

The unique aspect of our experimental approach is the combination of in vivo treatments with tDCS, followed by extracellular recordings of neurons in freshly prepared hippocampal slices. Such an ex vivo approach may explain why we did not observe lasting changes in our evoked potentials, inconsistent with a previous study (Bindman et al., 1964). In addition, the orientation specificity of tDCS effects (Kabakov et al., 2012) may mask effects on evoked potentials from a population of neurons arranged in various orientations in a hippocampal slice. Changes in synaptic plasticity were observed in these hippocampal slices hours after the application of tDCS.
brain extraction at 30 min and 24 h after completion of tDCS. For our experiments, brains from both control and stimulated rats were harvested, and hippocampal slices were prepared and placed in oxygenated ACSF for 1–6 h before any recording. Therefore, transient electrical field effects of local environment would have dissipated and could not account for the observed enhancements in synaptic plasticity. We observed no significant differences in the effects on LTP and PPF on slices recorded in the beginning of the day versus toward the end of the experiment (spanning 4–6 h). Averaged normalized responses from six CA1 regions within a hippocampal slice from a stimulated rat measured toward the beginning of an experiment showed similar levels of potentiation as those from another slice from the same rat measured toward the end of the day (data not shown).

We propose the possibility that the immediate effects of tDCS on local electrical environment induced additional downstream signaling events that persist for hours after brain extraction. Cellular changes attributable to tDCS have been documented previously. A previous study by Raneri et al. (2012) indicated that in vitro current stimulation of brain slices results in immediate increases in the c-fos and zif268, immediate early genes implicated in the maintenance of long-term neuronal changes and memory formation (Pérez-Cadahia et al., 2011). However, we cannot rule out the possibility of other faster signaling events, such as phosphorylation, recruitment, or shuffling of various synaptic proteins, in mediating tDCS effects. Experiments in which inhibitors or activators are used to block particular signaling cascades will be useful in determining the mechanistic pathway of tDCS-induced enhancements in synaptic plasticity.

We have demonstrated that in vivo tDCS induces a long-lasting enhancement of NMDA-dependent synaptic plasticity in the hippocampus of rats. These plastic changes may be the mechanism by which tDCS application facilitates performance in healthy human subjects or alleviates symptoms in patients suffering from neurological disorders. We believe that our approach of in vivo tDCS and direct recordings of neuronal signaling in acutely prepared hippocampal slices will continue to yield useful information pertaining to mechanisms of tDCS effects.

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