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Direct Current Stimulation Modulates LTP and LTD: Activity Dependence and Dendritic Effects

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ABSTRACT

Background: Transcranial direct current stimulation (tDCS) has been reported to improve various forms of learning in humans. Stimulation is often applied during training, producing lasting enhancements that are specific to the learned task. These learning effects are thought to be mediated by altered synaptic plasticity. However, the effects of DCS during the induction of endogenous synaptic plasticity remain largely unexplored.
Objective/Hypothesis: Here we are interested in the effects of DCS applied during synaptic plasticity induction.
Methods: To model endogenous plasticity we induced long-term potentiation (LTP) and depression (LTD) at Schaffer collateral synapses in CA1 of rat hippocampal slices. Anodal and cathodal DCS at 20 V/m were applied throughout plasticity induction in both apical and basal dendritic compartments.
Results: When DCS was paired with concurrent plasticity induction, the resulting plasticity was biased towards potentiation, such that LTP was enhanced and LTD was reduced. Remarkably, both anodal and cathodal stimulation can produce this bias, depending on the dendritic location and type of plasticity induction. Cathodal DCS enhanced LTP in apical dendrites while anodal DCS enhanced LTP in basal dendrites. Both anodal and cathodal DCS reduced LTD in apical dendrites. DCS did not affect synapses that were weakly active or when NMDA receptors were blocked.
Conclusions: These results highlight the role of DCS as a modulator, rather than inducer of synaptic plasticity, as well as the dependence of DCS effects on the spatial and temporal properties of endogenous synaptic activity. The relevance of the present results to human tDCS should be validated in future studies.

Introduction

Transcranial direct current stimulation (tDCS) applies a weak constant current of 2 mA or less across the scalp. This apparently simple technique is currently under investigation for a wide variety of conditions, including psychiatric disorders, neurorehabilitation and cognitive enhancement [1–3]. Stimulation is often paired with a training task, leading to task-specific enhancements in learning performance [1,4]. Despite the observation of pharmacological, neurophysiological and imaging effects in humans [5] and animals [6], a coherent picture of the relevant cellular mechanisms is yet to emerge.

Learning and memory are thought to be mediated by synaptic plasticity [7] and training paradigms in humans presumably influence learning by inducing plasticity [8]. Despite the common practice of applying tDCS during training, cellular effects of DCS applied *during* endogenous plasticity induction remain largely unexplored. Instead, the majority of research has analysed effects when DCS precedes plasticity induction [9–11], or is paired with endogenous activity otherwise not known to induce plasticity [12–14]. Here we are interested in the effects of DCS applied during training, i.e. concurrent with synaptic plasticity induction. As a model of endogenous synaptic plasticity, we induced long-term potentiation (LTP) and depression (LTD) using canonical protocols (pulse trains delivered to Schaffer collateral synapses in CA1 of rat hippocampal slices). By sweeping across induction frequencies we capture a frequency–response function (FRF), which has been widely used to study the predictions of the Bienenstock, Cooper and Munro (BCM) theory of synaptic plasticity. Here we show that DCS can shift the FRF, facilitating LTP and diminishing LTD, similar to BCM-like metaplasticity [15].

Abbreviations: tDCS, transcranial direct current stimulation; LTP, long-term potentiation; LTD, long-term depression; FRF, frequency–response function; BCM theory, theoretical model proposed by Bienenstock, Cooper, and Munro; ACSF, artificial cerebrospinal fluid; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; LFS, low-frequency stimulation.

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A prevailing mechanistic explanation is that tDCS produces shifts in cortical excitability, with anodal stimulation increasing excitability and cathodal stimulation decreasing excitability [5]. This excitability hypothesis is rooted in physiological evidence that DCS modulates membrane potential at neuronal somas, leading to changes in firing rate and timing [16–20]. Based on these observations, anodal and cathodal tDCS are often assumed to produce LTP and LTD-like effects, respectively, for an entire brain region [21–24]. However, this reasoning ignores the gradient of membrane polarization induced in any neuron during DCS and the role of endogenous synaptic activity in determining effects.

Here we show that DCS effects vary greatly within a small population of neurons, depending on dendritic location and endogenous synaptic activity. Both anodal and cathodal DCS facilitated LTP, but in different dendritic compartments. Moreover, when paired with LTD, DCS effects were independent of polarity. Both anodal and cathodal DCS reduced LTD in the same dendritic compartment. Finally, we show that DCS did not induce plasticity, but rather acted only as a modulator of endogenous synaptic plasticity. Our results motivate a more nuanced approach, which accounts for the properties of endogenous synaptic activity in predicting DCS effects.

Materials and methods

All animal experiments were carried out in accordance with guidelines and protocols approved by the Institutional Animal Care and Use Committee (IACUC) at The City College of New York, CUNY (Protocol No: 846.3).

Hippocampal brain slices were prepared from male Wistar rats aged 3–5 weeks old, which were deeply anaesthetized with ketamine (7.4 mg kg^{-1}) and xylazine (0.7 mg kg^{-1}) applied I.P., and killed by cervical dislocation. The brain was quickly removed and immersed in chilled (2–6 °C) artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 125; KCl, 4.4; NaH_2PO_4, 1; $MgSO_4$, 1.5; CaCl, 2.5; $NaHCO_3$, 26; D-glucose, 10; bubbled with a mixture of 95% O_2–5% CO_2. Transverse slices (400 μm thick) were cut using a vibrating microtome (Campden Instruments) and transferred to a holding chamber for at least 1 h at ambient temperature. Slices were then transferred to a fluid–gas interface chamber (Harvard Apparatus) perfused with warmed ACSF (30.0 ± 0.1 °C) at 1.0 ml min^{-1}. The humidified atmosphere over the slices was saturated with a mixture of 95% O_2–5% CO_2. Recordings started 2–3 h after dissection.

Field excitatory postsynaptic potentials (fEPSPs) were evoked using a platinum–iridium bipolar stimulating electrode placed in either stratum radiatum or stratum oriens of CA1. Recording electrodes made from glass micropipettes pulled by a Sutter Instruments P-97 and filled with ACSF (resistance 1–8 MΩ) were placed in either stratum radiatum or stratum oriens approximately 250 μm from the stimulating electrode in CA1 to record fEPSPs. fEPSPs were quantified by the average initial slope, taken during the first 0.5 ms after the onset of the fEPSP. Stimulus intensity was set to evoke fEPSPs with 40% of the maximum slope, which was determined at the onset of recording. Stable baseline fEPSPs were recorded every minute for at least 20 minutes before any plasticity induction was applied. fEPSPs were then recorded again every minute for 60 minutes after plasticity induction. Plasticity was induced by evoking a train of 900 fEPSPs at varying frequency, based on previous studies of bidirectional synaptic plasticity [25]. Induction frequencies were chosen to be 0.5, 1, 5, and 20 Hz. DCS was applied throughout plasticity induction, lasting 30, 15, 3, and 0.75 minutes for 0.5, 1, 5, and 20 Hz induction, respectively.

DCS was applied between two parallel Ag–AgCl wires (1 mm diameter, 12 mm length) placed in the bath on opposite sides of the brain slice separated by 10 mm with the recording site approximately equidistant from each wire. DCS wires were connected to a current-controlled analogue stimulus isolator (A-M Systems) that was controlled by PowerLab hardware and LabChart software (AD Instruments). Slices were oriented such that the somato-dendritic axis of CA1 pyramidal neurons was parallel to the electric field between the DCS wires (Fig. 1A). Before each recording, DCS current intensity was calibrated to produce a 20 V/m electric field across each slice (typically 100–200 μA) by adjusting the current so that two recording electrodes separated by 0.8 mm in the slice measured a voltage difference of 16 mV (16 mV/0.8 mm = 20 V/m).

For NMDAR antagonist experiments, 100 μM MK-801 (Sigma Aldrich) was included in the ACSF perfused in the recording chamber throughout the experiment. Because MK-801 is an open channel blocker, baseline fEPSPs were recorded for at least 40 minutes to ensure complete blockade of NMDAR channels [26].

Data acquisition and stimulation waveforms were controlled with PowerLab hardware and LabChart software (AD Systems). Extracellular fEPSPs were amplified (100×), low pass filtered (3 kHz), and digitized (10 kHz). Synaptic plasticity was quantified for each slice by taking the average of the last ten fEPSP slopes (51–60 minutes after plasticity induction) and normalizing to the average of baseline fEPSP slopes (20–1 minutes before plasticity induction). All data are reported as the mean ± standard error of the mean (SEM). The statistical difference between groups (critical value = 0.05) was estimated using two-tailed Student's t-tests, after checking for normality in each group (Lilliefors test for normality, $p > 0.05$ in all cases). Conditions were not repeated in slices taken from the same animal. Reported n values therefore represent the number of slices and animals used in each condition.

Here we name the polarity of stimulation based on the orientation of DCS relative to pyramidal neurons. Following convention in human tDCS, DCS with the anode closer to CA1 apical dendrites is referred to as anodal stimulation. Conversely, DCS with the cathode closer to CA1 apical dendrites is referred to cathodal stimulation. Importantly, apical dendrites are polarized oppositely from basal dendrites and somas, regardless of DCS polarity [16,27,28]. So anodal DCS will depolarize somas and basal dendrites, while hyperpolarizing apical dendrites. Conversely, cathodal DCS will hyperpolarize somas and basal dendrites, while depolarizing apical dendrites (Fig. 1A).

Acute effects were determined based on the first response (two responses for paired pulse data) during DCS and were normalized to the average of baseline responses. Fibre volley amplitude was taken as the difference between the trough of the fibre volley and the mean of the two surrounding peaks. Paired pulse ratio was taken as the ratio of the second and first fEPSP slopes during 20 Hz HFS (50 ms inter-pulse interval) in each condition.

Results

DCS shifts the frequency–response function

Trains of synaptic activity have conventionally been used to induce synaptic plasticity in hippocampal slices [25,29]. As a model of endogenous synaptic plasticity, trains of 900 pulses at varying frequencies (0.5, 1, 5, 20 Hz) were applied to the Schaffer collateral pathway synapsing on CA1 apical dendrites. Low frequency stimulation (LFS) generated LTD (0.5 Hz: 84.1 ± 2.7%, $p < 0.001$, $n = 10$; 1 Hz: 78.9 ± 2.9%, $p < 0.0001$, $n = 9$), while high frequency stimulation (HFS) generated LTP (20 Hz: 114.1 ± 2.7%, $p < 0.001$, $n = 13$), and an intermediate frequency marked the transition between LTD and LTP (5 Hz: 95.9 ± 3.7%, $p = 0.30$, $n = 9$). The resulting FRF (Fig. 1B) maps the degree of synaptic activity during induction to the degree of resulting synaptic plasticity and is consistent with existing literature [15].