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Direct current stimulation modulates LTP and LTD: activity-dependence and dendritic effects

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Direct current stimulation modulates LTP and LTD: activity-dependence and dendritic effects

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Contents

1. Abstract

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 be mediated by altered synaptic plasticity. However, the effects of DCS during the induction of endogenous synaptic plasticity remain largely unexplored. To model endogenous plasticity I induced long-term potentiation (LTP) and depression (LTD) at Schaffer collateral synapses in CA1 of rat hippocampal slices. When induction was paired with concurrent DCS, the resulting plasticity was biased towards potentiation, such that LTP was enhanced and LTD was reduced. Remarkably, both anodal and cathodal stimulation can produce these effects, depending on the dendritic location of plasticity induction. DCS did not affect synapses that were weakly active or when NMDA receptors were blocked, suggesting a sensitivity for active synapses that are already undergoing endogenous plasticity. These results highlight the role of DCS as a modulator, rather than inducer of synaptic plasticity, as well as the complex dependence of DCS effects on the spatial and temporal properties of endogenous synaptic activity.

2. 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, neuro-physiological 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 analyzed effects when DCS precedes plasticity induction [9]–[11], or is paired with endogenous activity otherwise not known to induce plasticity [12]–[14]. Here I am interested in the effects of DCS applied during training, i.e. concurrent with synaptic plasticity induction. As a model of endogenous synaptic plasticity, I 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 I 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 I show that DCS can shift the FRF, facilitating LTP and diminishing LTD, similar to BCM-like metaplasticity [15].

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 I 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, I show that DCS did not induce plasticity, but rather acted only as a modulator of endogenous synaptic plasticity. These results motivate a more nuanced approach, which accounts for the properties of endogenous synaptic activity in predicting DCS effects.

3. Transcranial direct current stimulation

3.1 Overview

The common thread among tDCS applications in humans is the passage of weak (< 2 mA) direct electric current between electrodes placed on the scalp [25]. The rationale is that some current will reach the brain and influence cognitive function. Within this general paradigm, a large number of parameters have been shown to influence stimulation outcomes, including the subject's cognitive load [4], [26], the electrode placement [27], and the stimulation timing [28], duration, and intensity [27]. While the precise mechanisms for cognitive effects of tDCS remain poorly understood, it is widely thought that stimulation acts by polarizing neuronal membranes, thereby modulating neuronal excitability [21], [24], [25].

Seminal work in the early 2000s showed that tDCS could modulate motor evoked potentials (MEPs), a metric considered to reflect the excitability of motor neuron pools in the primary motor cortex (M1) [27]. A positive electrode (anode) placed over the primary motor cortex (M1) enhanced motor evoked potentials, while a negative electrode (cathode) over M1 diminished motor evoked potentials. These results were interpreted as evidence that tDCS could modulate the excitability of stimulated cortical regions. Subsequent work demonstrated that modulation of MEPs could last for over an hour after stimulation, depending on the duration and intensity of stimulation [29]. These early results sparked interest in tDCS as a tool to produce long-term neuromodulatory effects, leading to explorations of tDCS as a treatment for a range of neurological disorders and for the modulation of baseline cognitive functions [1], [3].

3.2 Effects on learning

tDCS has been shown to modulate various forms of learning [1], making it an attractive tool for cognitive enhancement. Early work demonstrated that anodal stimulation over M1 could improve implicit motor learning [30], which was later shown to improve motor skill acquisition when combined with training over multiple days [31]. Kincses et al. demonstrated one of the first non-motor effects of tDCS on learning, by showing anodal stimulation of the prefrontal cortex improved probabilistic classification learning [32]. tDCS effects have since been extended to language learning [33] and object recognition [34], [35]. A common paradigm is to apply stimulation over brain areas thought to be involved in the type of learning in the hopes that excitability changes modulate performance. However this idea is likely an oversimplification, as many studies report no effects of cathodal stimulation [36] or inhibition of learning with anodal stimulation [37], which run counter to the excitability hypothesis

Long-term learning and memory are thought be driven by synaptic plasticity, and there is now strong evidence for this from various animal models [38]–[42]. This has led to speculation that long-term effects of tDCS, may be similarly driven by altered synaptic plasticity [24]. The most well characterized cellular effect of tDCS is the membrane polarization that it induces, which is indeed cited as the source of exctiability changes observed in humans [21]. However, polarization of various neuronal compartments can influence synaptic plasticity through multiple complex mechanisms, perhaps explaining the variability of tDCS effects on learning. Although there is some evidence linking tDCS to synaptic plasticity, the precise cellular mechanisms that underlie tDCS effects on learning remain poorly understood. A better understanding of these mechanisms would help to improve and predict learning outcomes in humans.

4. Direct current stimulation and synaptic plasticity

4.1 NMDA receptor

Early theoretical work recognized that patterns of neural activity, and therefore memories, could be stored via changes in synaptic strength. Donald Hebb famously proposed that this synaptic plasticity might occur in response to correlated firing between pre and post-synaptic neurons [43]. The NMDA receptor (NMDAR) is uniquely suited to act as a detector of this correlated activity. At hyperpolarized membrane potentials the NMDAR channel pore is blocked by magnesium ions, allowing it to only open in response to simultaneous glutamate binding (presynaptic requirement) and postsynaptic depolarization (postsynaptic requirement)[44]. Indeed, long-term potentiation (LTP) and long-term depression (LTD), canonical models of synaptic plasticity, have been shown to be NMDAR-dependent at many synapses throughout the brain [45]. Moreover, blockade of NMDARs with pharmacological agents leads to deficits in multiple types of learning and memory [46], [47].

The NMDAR has been implicated in tDCS effects since the early 2000s when Nitsche et al. showed that long-term effects on MEPs were blocked by administration of an NMDAR antagonist [48]. This idea has been corroborated by a handful of animal studies showing that DCS modulates synaptic plasticity and that these effects were NMDAR-dependent [11], [12], [14]. However, an important limitation of these studies is that they show NMDAR-dependence by blocking NMDARs, which abolishes plasticity altogether. This makes it difficult to pinpoint precisely *how* DCS influences NMDAR-dependent plasticity.

DCS is known to modulate neuronal membrane potential [16], [17], [19], [49], [50]. Given the dependence of NMDAR current on membrane potential, this polarization is often cited as the source of tDCS plasticity effects. However, the connection between DCS-induced membrane polarization and NMDAR-dependent has yet to be demonstrated directly. Moreover, neurons during DCS do not experience a singular shift in membrane potential throughout their morphology. Rather, neuronal processes closest to the cathode will be depolarized, while those closest to the anode will be hyperpolarized, with a gradient of polarization in between [16], [49]–[51]. During DCS any neuron will simultaneously experience both hyperpolarization and

depolarization in different compartments, depending on the neuronal morphology and orientation relative to the electric field [51]. Thus, different neuronal compartments will experience different polarization and can make antagonistic contributions to NMDAR activity.

4.2 Compartmentalized membrane polarization

DCS has been shown to modulate the membrane potential at pyramidal neuron somas in both hippocampus and cortex [16], [17], [19], leading to changes in both firing rate and timing [18], [20], [52]. This is thought to result in the excitability changes observed during early work on the neurophysiology of tDCS in humans [27]. While NMDARs are primarily distributed throughout the dendritic arbor [53], rather than near the soma, NMDAR-dependent plasticity is sensitive to somatic firing, as action potentials can back-propagate to dendrites. If appropriately timed with presynaptic input, these back-propagating action potentials (bAP) can provide the depolarization that opens NMDARs and initiates intracellular synaptic plasticity cascades [54]. It follows that DCS-induced somatic polarization, and the resulting modulation of somatic firing should modulate NMDAR-dependent synaptic plasticity.

Concurrent with somatic polarization, pyramidal neuron dendrites are also polarized by DCS [16], [49]. Given that NMDARs are sensitive to dendritic membrane potential, this polarization may directly influence NMDAR current. Moreover, dendritic membrane potential dynamics are critical to the fidelity of bAP propagation through the dendritic tree [53]. Importantly, pyramidal neuron apical dendrites will experience an opposite polarization from the soma and basal dendrites [16], [49]. Therefore DCS may simultaneously produce dendritic and somatic effects that are antagonistic from the perspective of NMDARs. For example, canonical anodal stimulation of the cortex depolarizes pyramidal neuron somas, but hyperpolarizes their apical dendrites. Somatic depolarization would tend to increase NMDAR activity by raising the probability of bAPs coincident with presynaptic input [54]. However, hyperpolarization of apical dendrites opposes NMDAR activity, both directly [55] and through inhibition of bAP propagation through dendrites [56]. Somatic and

dendritic membrane polarization may therefore influence NMDAR activity and synaptic plasticity through highly nonlinear interactions that have yet to be studied.

In addition to postsynaptic dendrites and somas, DCS will also polarize presynaptic neuronal somas and axon terminals [51], [57]. Polarization of somas can modulate the rate of presynaptic transmitter release via changes in firing rate. This could in turn influence NMDAR current by affecting temporal summation of synaptic currents and the probability that presynaptic glutamate release precedes a bAP. Polarization of axon terminals can modulate the magnitude of each release event by affecting release probability [13], [57], which can similarly influence NMDAR currents through synaptically driven depolarization. Importantly, presynaptic axon terminals can be polarized independently of their postsynaptic counterparts (somas and dendrites) [51], potentially producing further antagonistic effects. Moreover, DCS can simultaneously polarize multiple presynaptic neurons converging on the same postsynaptic neuron, potentially amplifying effects.

The DCS effects on various pre and postsynaptic compartments likely interact in complex nonlinear ways to contribute to NMDAR activity and the induction of synaptic plasticity. Moreover, this interaction is likely not static, but rather a dynamic function of the endogenous neural activity at the time of stimulation. Given the relatively weak degree of DCS-induced polarization (< 1 mV in cortex)[51], DCS likely acts via modulation of endogenous activity, making active compartments more sensitive to DCS effects [58]. Moreover, some types of plasticity induction depend more on somatic membrane potential, while others depend more on dendritic membrane potential [59]. The effects of DCS-induced somatic and dendritic polarization is are therefore also likely shift between plasticity induction patterns.

While neuronal polarization is often speculated to be the source of tDCS effects on plasticity, no studies have directly examined this idea. Moreover, the distinction is rarely made between polarization of various neuronal compartments, removing a critical nuance from mechanistic discussions of tDCS. The polarization of different neuronal compartments, the interaction between compartment-specific effects, and their dependence

on endogenous neural activity in producing synaptic plasticity outcomes remain important areas for future research.

4.3 BDNF

BDNF is a critical neurotrophic factor during development and has more recently been investigated for its role in adult and juvenile synaptic plasticity. The mechanisms by which BDNF influences plasticity during normal brain function remain unclear, but BDNF appears to reduce LTD, facilitate LTP, and be involved in the maintenance of late, protein synthesis dependent forms of LTP (L-LTP) [60]. Multiple studies have implicated a role for BDNF in mediating effects of DCS on synaptic plasticity [9], [10], [12]. Fritsch et al. first showed that induction of DCS-induced LTP was BDNF-dependent and correlated with increased activation of the BDNF receptor TrkB [12]. Moreover, they showed that people with a polymorphism in a BDNF gene that reduces BDNF expression experienced diminished effects of tDCS on learning. More recently, Podda et al. showed DCS led to epigenetic modifications of BDNF promoter sequences, increased BDNF production, enhanced LTP, and improved hippocampal dependent memory [9].

Similar to NMDAR-dependent effects, it remains unclear precisely *how* DCS influences BDNF activity. BDNF can be released in response to standard LTP induction protocols, dependent on postsynaptic membrane depolarization and calcium influx [61], [62]. DCS may therefore affect BDNF indirectly, perhaps by modulating NMDAR. In this case, the relevant parameters for determining DCS effects on BDNF would be the same as for NMDARs. However, DCS could produce similar effects by acting on any process that is upstream of BDNF release in the cascade of events that produces plasticity. It will therefore be important for future research identify this point of action to optimize effects on synaptic plasticity.

4.4 Astrocytic calcium elevations

While astrocytes were originally thought to play a maintenance role at synapses, more recent evidence suggests that they are critical information processors and mediators of synaptic plasticity [63]. These

discoveries have led to the concept of a tripartite synapse, in which astrocytic, presynaptic, and postsynaptic compartments cooperate to process synaptic information [64]. Intracellular calcium levels in astrocytes appear to regulate the release of various gliotransmitters, which in term can regulate various pre and post-synaptic processes like neurotransmitter release and NMDAR activity [63].

A recent study in vivo study from Monai et al. demonstrated large tDCS-induced calcium elevations in cortical astrocytes [14]. They show further that tDCS induced LTP of evoked potentials and neuronal calcium signals in visual and barrel cortex. AStrocytic calcium signals require activation of alpha-1 adrenergic receptors (A1ARs) and intracellular inositol triphosphate type 2 (IP3R2) to release calcium from intracellular stores [65]–[67]. The authors disrupt this signaling via A1AR antagonists and IP3R2 knockout to show that tDCS effects are also removed. However, their conclusion that tDCS induces plasticity by modulating adrenergic input to astrocytes is faulty. Their manipulations abolish astrocytic calcium signals and NMDAR-dependent plasticity altogether [66]. tDCS could therefore act at any other point in the molecular pathways that govern these processes and one would still expect to see abolishment of all calcium elevations and NMDAR-dependent plasticity. While A1AR-dependent calcium elevations in glia are compelling effect of tDCS, it remains unclear how this effect is produced. A plausible mechanism that the authors mention is that DCS may modulate the release of noradrenaline by polarizing adrenergic axon terminals in the cortex or the somas of adrenergic neurons originating in the locus coeruleus. However no evidence is provided for these hypotheses, warranting further investigation.

4.5 Adenosine

Marquez-Ruiz et al. were able to modulate evoked responses and eye-blink conditioning with tDCS in awake rabbits [13]. Cathodal stimulation induced LTD of evoked responses, while anodal stimulation did not induce plasticity. Motivated by much earlier work that had shown effects of DCS on adenosine elicited cAMP accumulation [68], the authors blocked A1 adenosine receptors (A1AdRs) and found that the cathodal-induced

LTD was also blocked. Interestingly the authors also found that tDCS modulated paired-pulse ratio during stimulation, indicating that tDCS might alter presynaptic release probability [69]. Little is known however about the mechanisms of adenosine release or the role of A1 adenosine receptors in LTD [70], again making it difficult to infer how tDCS might exert these effects

5. Open questions

tDCS has shown promise as a powerful tool for cognitive therapy and enhancement [1], [3]. However, clinical results have been highly variable between studies and individuals, making it difficult to predict the efficacy of a given tDCS intervention and leading some to question its efficacy altogether [71]. Presumably, we can optimize stimulation outcomes and make results more predictable through a firmer understanding of the mechanisms by which tDCS produces long-term effects.

Effects on learning and memory are of particular interest in cognitive tDCS therapies, and synaptic plasticity is thought to be a fundamental mechanism that underlies such learning and memory [72]. Multiple studies have found DCS effects on synaptic plasticity in animals [9]–[14], and synaptic plasticity-like effects in humans [29], [48], making synaptic plasticity a promising target for tDCS therapies.

tDCS effects are presumably derived from the weak electric field that it induces in brain tissue. The parameters of this electric field (i.e. intensity and polarity, timing and duration, location and orientation) are the principal tool at the disposal of clinicians in controlling stimulation outcomes. To facilitate parameter choice in the clinical setting, it is therefore prudent to find direct links between DCS-induced electric fields and effects on synaptic plasticity. While a few studies have demonstrated phenomenological effects on synaptic plasticity, none have provided such a direct mechanistic link. This makes it difficult to develop a coherent mechanistic framework and extrapolate these results to different stimulation parameters, species (e.g. from rodents to humans), or endogenous states (e.g. during training or rest). For example, the demonstration of NMDAR, BDNF, A1AR, or A1AdR dependent effects that are discussed here provide little mechanistic insight into

whether the DCS electric field is acting directly on these signaling systems. This leaves little basis for predicting whether these effects would hold under different conditions, such as if the electric field were rotated in brain slice studies, or tDCS was applied to freely exploring animals during in vivo studies, or most importantly, in humans.

Membrane polarization of neuronal compartments is a principal and well-characterized effect of electric fields on brain tissue [16], [17], [19], [49]–[51]. Given the crucial role that membrane potential plays in many plasticity related processes [45], [54], [55], membrane polarization provides a potential point of interaction between the DCS electric field and synaptic plasticity processes. While often speculated about, this hypothesis is yet to be examined directly, providing an interesting direction for future research. DCS is known polarize multiple neuronal compartments simultaneously (e.g. dendrites, somas, axon terminals), having potentially synergistic or antagonistic influences on synaptic plasticity. It will therefore be crucial to determine how the polarization these compartments dynamically interact to produce an overall outcome on plasticity.

tDCS induces modest membrane polarization (< 1 mV), resulting from modest electric fields (< 1 V/m) [51]. As such, tDCS is considered to be a modulatory technique [21]. Supporting this notion, effects depend on the cognitive load [4], [26], [73] and endogenous neural activity that stimulation is paired with [12]. However, little attempt has been made to systematically investigate how endogenous neuronal activity factors into DCS effects on synaptic plasticity.

Many 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 analyzed effects when DCS precedes plasticity induction [9]–[11], or is paired with endogenous activity otherwise not known to induce plasticity [12]–[14]. A potential direction for future research would therefore be to characterize the interactions between polarized neuronal compartments during varying patterns of pre and post-synaptic activity, particularly those that induce plasticity.

6. 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).

6.1 Brain slice preparation

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.

6.2 Evoked responses

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 milliseconds 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.

6.3 Direct current stimulation

DCS was applied between two parallel Ag-AgCl wires (1 mm in diameter and 12 mm in 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 analog 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 (Figure 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).

6.4 NMDA receptor antagonist

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 [74].

6.5 Data acquisition and analysis

Data acquisition and stimulation waveforms were controlled with PowerLab hardware and LabChart software (AD Systems). Extracellular fEPSPs were amplified (100x), 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). Two-tailed student's t-tests were used to determine the significance of differences in synaptic plasticity between conditions, with $p < 0.05$ considered significant.

6.5 Stimulation polarity

Here I 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], [51], [75]. 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 (Figure 1A).

6.6 Analysis of acute effects

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. Fiber volley amplitude was taken as the difference between the trough of the fiber volley and the mean of the two surrounding peaks. Paired pulse ratio was taken as ratio of the second and first fEPSP slopes during 20 Hz HFS (50 ms inter-pulse interval) in each condition.

7. Results

7.1 DCS shifts the frequency-response function

Trains of synaptic activity have conventionally been used to induce synaptic plasticity in hippocampal slices [45]. 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 (Figure 1B) maps the degree of synaptic activity during induction to the degree of resulting synaptic plasticity and is consistent with existing literature [15].

DCS was then applied during plasticity induction at each frequency. Our group's previous experiments with the present preparation demonstrate that cathodal DCS depolarizes CA1 apical dendrites (Figure 1A; Bikson et al. 2004, figure 10), and was therefore expected to facilitate LTP in this dendritic region [45]. DCS significantly attenuated LTD induced by 0.5 Hz (Figure 1C; 97.5±5.9%, $p = 0.04$, n = 8) and 1 Hz LFS (Figure 1D; 89.2±3.1%, $p = 0.03$, n = 10) and enhanced LTP induced by 20 Hz HFS (Figure 1F; 128.8±4.7%, $p = 0.01$, n = 14). The DCS effect at 5 Hz trended towards significance (Figure 1E; 102.3±2.2%, $p = 0.14$, n = 11), consistent with smaller effects observed previously at the threshold between LTP and LTD [76]–[78]. The resulting DCS FRF was significantly shifted compared to control ($F=17.93$, $df=1$, $p<0.0001$). Similar shifts of the FRF have been associated with enhanced learning in cortex [15], [79].

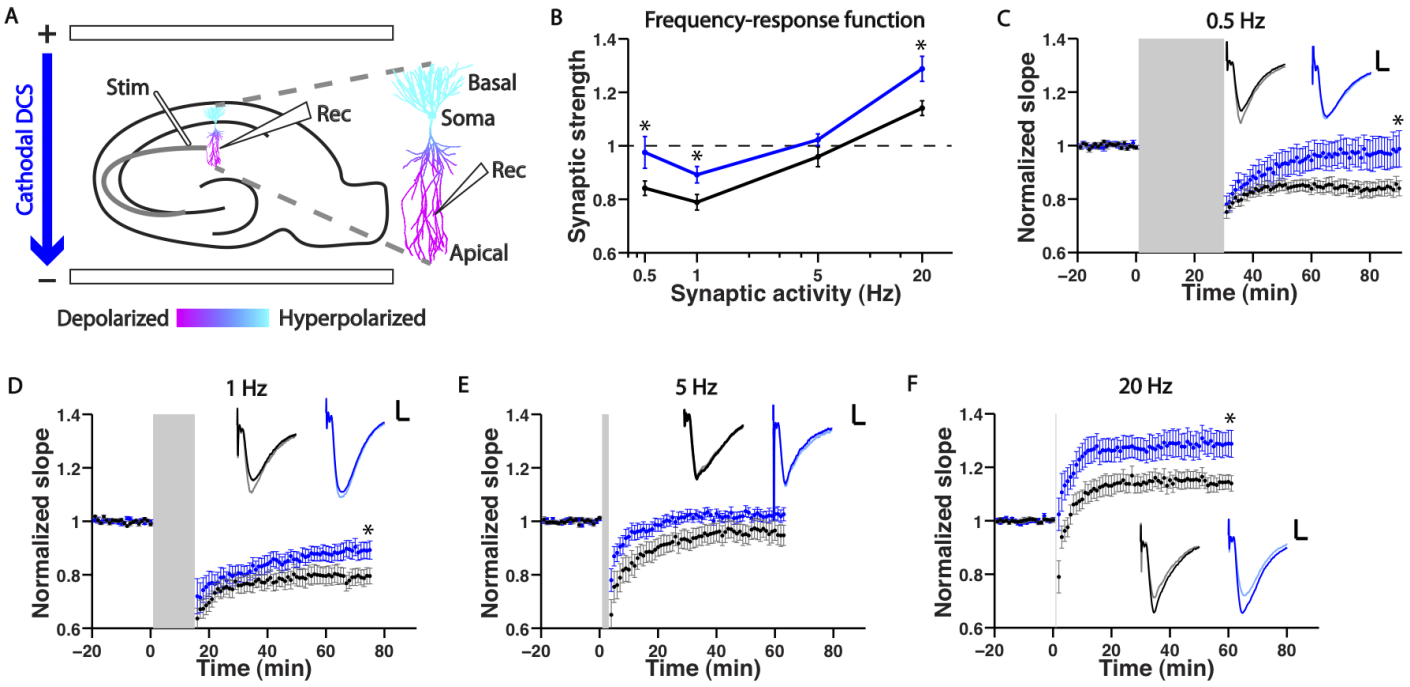

Figure 1. DCS shifts synaptic plasticity in apical dendrites towards potentiation. A: Schematic depicts cathodal DCS of a hippocampal slice, with expected membrane polarization of CA1 pyramidal neuron (enlarged at right; prediction based on computational model as in Rahman et al. 2013). Arrow indicates the direction of positive current flow between electric field wires placed in the recording chamber (horizontal bars above and below hippocampal slice). **B:** Cathodal DCS (blue) shifts the BCM-like frequency-response function towards potentiation. **C-F:** DCS applied during plasticity-inducing LFS attenuated LTD (**C,D**) and enhanced LTP (**F**), but the effect was not significant near the crossover point between LTD and LTP (**E**). Sample fEPSP traces are provided for each condition (grey/black: before/after control; light blue/blue: before/after cathodal; scale bars: 1 mV, 4 ms). Synaptic strength in (**B**) is the average of the last ten normalized fEPSP slopes in each condition (51-60 minutes post-induction). Grey bars indicate the duration of plasticity induction and concurrent DCS. Data are represented as mean ± SEM across slices. * = $p < 0.05$.

7.2 DCS effects on LTP depend on dendritic location

DCS is known to modulate the membrane potential of neuronal compartments [16], [19], [50], [51], [75] and dendritic membrane potential is known to be a critical determinant of NMDAR-dependent plasticity [45]. Other DCS effects in humans and animals have been shown to be NMDAR-dependent, and it is widely speculated that tDCS exerts long-term effects through membrane polarization and NMDARs [24]. An important subtlety that is often lost in this discussion is that DCS will simultaneously depolarize and hyperpolarize different compartments within the same neuron. Indeed, previous work from our own group with a similar experimental setup showed that cathodal DCS simultaneously depolarizes CA1 apical dendrites while hyperpolarizing their basal dendrites and soma. Conversely, anodal DCS hyperpolarizes CA1 apical dendrites while depolarizing their basal dendrites and soma [16]. I therefore expected that the effects of anodal and cathodal stimulation would vary with dendritic location. To test this I paired both anodal and cathodal DCS with 20 Hz HFS in both CA1 apical and basal dendrites. In apical dendrites, cathodal DCS enhanced LTP, while anodal DCS had no significant effect (Figure 2C,D; control: 114.1±2.7%,n=13; cathodal: 128.8±4.7%,p=0.01,n=14; anodal: 111.7±4.5%,p=0.63, n=8). In basal dendrites, anodal DCS now enhanced LTP while cathodal DCS had no significant effect (Figure 2E,F; control: 148.6±3.6%,n=10; cathodal:142.5±5.2%, p=0.34,n=10; anodal: 180.4±9.1%, p<0.01,n=5). As expected, the effects of anodal and cathodal DCS were dependent on dendritic location.

7.3 DCS effects are polarity dependent for LTP but not LTD

Anodal and cathodal DCS apply stimulation with opposite polarity and are canonically expected to produce opposite effects [25]. As reported above, I find that cathodal and anodal DCS have asymmetric effects on LTP for a given dendritic location. Moreover, when paired with 1Hz LFS I observe no polarity dependence of effects. LTD is reduced by both anodal and cathodal DCS, i.e. synaptic strength is increased compared to control (Figure 2G,H; control: 78.9±2.9%,n=9; cathodal: 89.2±3.1%,p=0.03,n=10; anodal: 95.6±5.9%,n=8,p=0.04). These results reveal that modulation of synaptic plasticity by DCS depends on both

the physical location of concurrently active synapses (basal or apical dendrites) and the rate of their activity (LFS or HFS) (Figure 2B).

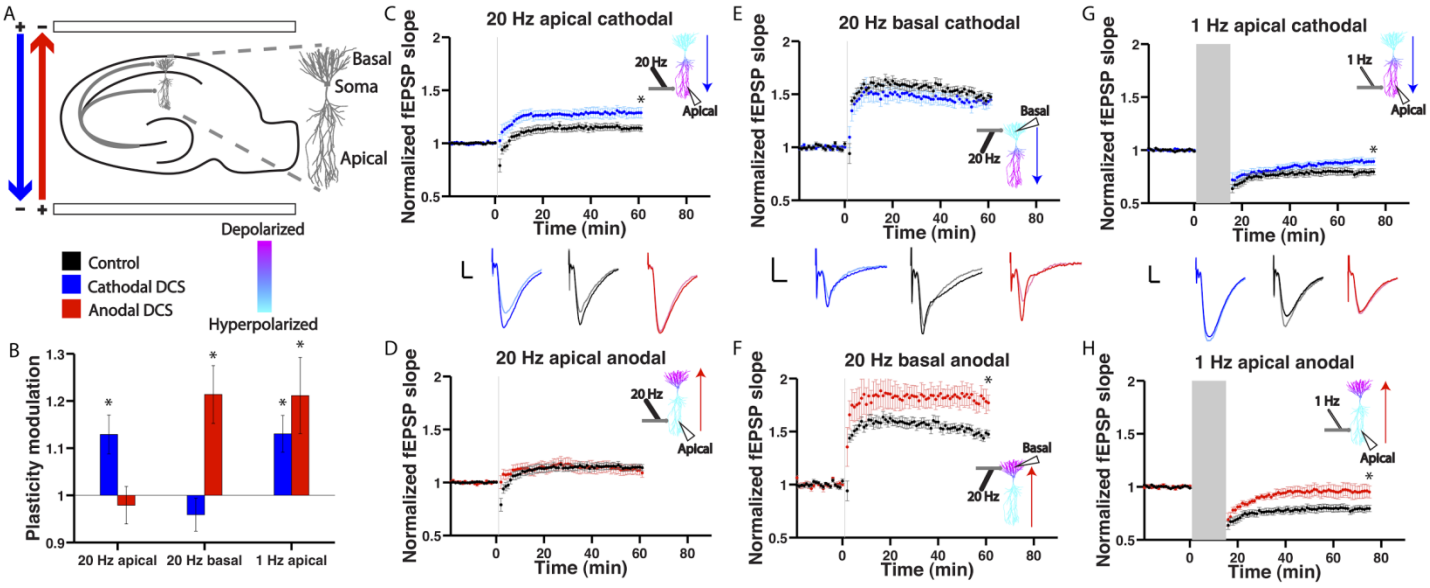

Figure 2. Mixed of effects of anodal and cathodal DCS. A: Schematic depicts anodal (red) and cathodal (blue) DCS of a hippocampal slice. Arrows indicate the direction of positive current flow between DCS electrodes. Reconstruction of a CA1 pyramidal neuron with dendritic compartments labeled at right. **B:** Modulation of synaptic plasticity depends on polarity, dendritic location and rate of plasticity induction. **C-D:** In apical dendrites cathodal DCS enhances LTP, but anodal has no significant effect. **E-F:** Changing dendritic location to basal dendrites, anodal DCS now enhances LTP, but cathodal DCS has no effect. **G-H:** Changing induction frequency to 1 Hz LFS (in apical dendrites), both anodal and cathodal reduce LTD. **C-H:** Example traces for each condition are given in the center of each column (grey/light blue/pink traces are before plasticity induction; black/blue/red traces are after plasticity induction; scale bars: 1 mV, 4 ms). Insets depict the reconstructed CA1 pyramidal neuron in (**A**) with expected membrane polarization (prediction based on computational model as in Rahman et al. 2013), induction and recording sites, and orientation of DCS electric

field. Grey bars indicate the duration of plasticity induction and concurrent DCS. Plasticity modulation in (**B**) is the resulting plasticity in each DCS condition normalized to the mean of the plasticity in the corresponding control condition. Data are represented as mean ± SEM across slices. * = $p < 0.05$.

7.4 DCS effects require a concurrent endogenous source of NMDAR plasticity

tDCS is often applied under the assumption that stimulation alone is sufficient to induce plasticity [5], [6], [23]. However, given the modest effects on membrane potential for typical stimulation intensities [19], [51], [75], I propose that DCS instead acts as a modulator of NMDAR plasticity. DCS would therefore require a concurrent endogenous source of plasticity to modulate. To test this requirement I again applied cathodal DCS, but removed endogenous NMDAR-dependent plasticity in two ways: first by weakening synaptic activity to well below the plasticity threshold, and second by directly blocking NMDAR current during strong synaptic activity. When applied during weak synaptic activity (30 pulses, 1/60 Hz), cathodal DCS had no effect (Figure 3C; control: 99.3±1.1%, n=9; cathodal DCS: 100.8±4.0%, n=7; p = 0.68). When paired with strong synaptic activity (20 Hz HFS) but NMDARs were blocked with antagonist MK-801, cathodal DCS also had no effect (Figure 3B, control: 92.0±1.6%, n=10; cathodal DCS: 94.3±2.3%, n=9; p = 0.42). These results suggest that DCS may act as a modulator of endogenous synaptic plasticity, rather than an inducer of de novo synaptic plasticity.

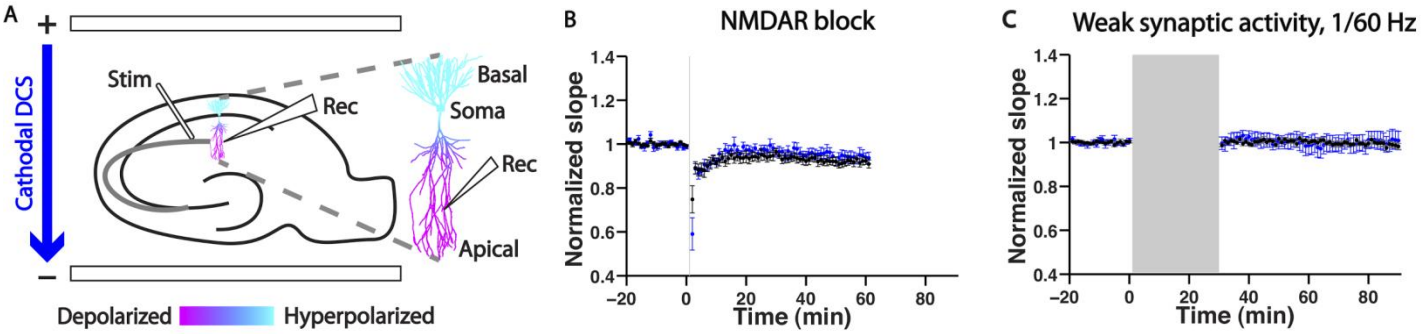

Figure 3. DCS requires an endogenous source of NMDAR plasticity. A: Schematic depicts cathodal DCS (blue) of a hippocampal slice, with expected membrane polarization of CA1 pyramidal neuron (enlarged at right; prediction based on computational model created as described in Rahman et al. 2013). **B**: Cathodal DCS with 20 Hz HFS has no effect on synaptic strength when NMDARs are blocked with antagonist MK-801 **C**: Cathodal DCS applied during synaptic activity that is too weak to induce plasticity (30 pulses at 1/60 Hz) has no effect on synaptic strength. Grey bars indicate duration of induction and concurrent DCS. Data are represented as mean ± SEM across slices.

7.5 Acute effects of DCS on synaptic transmission

To determine whether the effects of DCS were already apparent in acute synaptic effects , I examined several measures of baseline synaptic transmission. However, one-way ANOVAs yielded no significant effect of stimulation on fEPSP slope (Figure 4A; F=0.23,df=1,p=0.63), fiber volley amplitude (Figure 4B, F=0.33,df=1,p=0.57), or paired pulse ratio (Figure 4C; F=0.11,df=1,p=0.74).

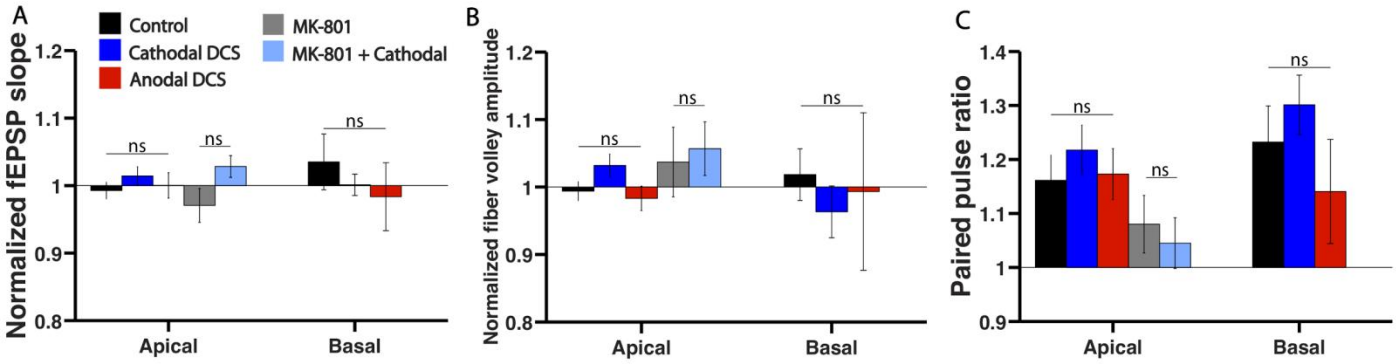

Figure 4. No significant effects on baseline synaptic transmission. DCS had no significant effect on fEPSP slope (**A**), fiber volley amplitude (**B**), or paired pulse ratio (**C**), in apical or basal dendrites, or when NMDARs were blocked with MK-801. Data are represented as mean ± SEM across slices. ns = $p > 0.05$.

8. Discussion

8.1 LTP, LTD, and learning

There is now strong evidence for a role of both LTP and LTD-like processes in various types of learning and memory [38]–[40], [80]–[82]. At the behavioral level, learning is likely to involve both of these processes, with the precise degree of each depending on the specific behavior. For example, some learned behaviors directly require habituation to a familiar stimulus and are specifically dependent on LTD [83], [84]. Other learned behaviors involve formation of new associations and responses to the environment, which require LTP and are eliminated by LTD [38], [39]. I observed that LTP is facilitated in dendrites that are depolarized by DCS. This cellular DCS effect may contribute to enhanced learning when tDCS is paired with training that induces plasticity, such as motor rehabilitation [40], [85]. Indeed, similar shifts in the FRF have been linked to facilitation of learning on both theoretical and experimental grounds [15]. I also observed a reduction of LTD for both stimulation polarities (Figure 2B). One may therefore expect that these effects would disrupt learning that requires LTD.

8.2 Plasticity dependence may underlie task-specific effects

When tDCS is paired with training, the observed effects are often specific to the trained task [4], [86]. While electrodes are typically placed over an intended target region, it is unlikely that task specificity is solely the result of spatial selectivity of current flow. Even in the most focal tDCS applications (e.g. HD-tDCS), current flow through the brain is diffuse, reaching large swaths of cortex and subcortical structures [87], [88]. Moreover, within any particular brain region, there are likely to be neurons involved in many disparate memory engrams or behaviors. The common assumption that tDCS induces plastic effects indiscriminately [25], or even at weakly active synapses [12], therefore implies broad effects on any cognitive output in the stimulated brain regions. This is at odds with the observed specificity of effects. Instead, to explain task-specificity tDCS may act as a selective modulator of endogenous synaptic plasticity. These results support this hypothesis, as DCS

had no effect when synaptic input was too weak (Figure 3C) or when NMDARs were blocked during strong synaptic input (Figure 3B), indicating that synaptic efficacy is modulated by DCS only when NMDAR-dependent plasticity is present. This provides a basis for effects to be task-specific, as synapses associated with the paired task are more likely to be undergoing plasticity and therefore subject to modulation during tDCS. Moreover, this predicts that tDCS effects should be enhanced when paired with tasks that induce synaptic plasticity. Indeed, there is some evidence for this [4], [89]. The precise role of endogenous synaptic activity in DCS effects remains an important area for future research.

8.3 A potential role for dendritic membrane polarization

Under the conventional excitability hypothesis, 'anodal tDCS' is assumed to produce inward cortical current flow, which depolarizes pyramidal neuron somas and hence increases cortical excitability. 'Cathodal tDCS' is soma-hyperpolarizing and thus should reduce cortical excitability [25]. However, it is becoming increasingly clear that this reasoning is an oversimplification, particularly when it comes to long-term effects and learning [36], [90], [91]. While effects on somatic membrane potential must still be considered, these results here point to a potential role for dendritic membrane polarization in determining DCS effects on synaptic plasticity.

Membrane polarization due to DCS can in principle affect the function of all voltage-dependent channels distributed throughout a neuron, particularly the relief of NMDARs from magnesium blockade. This influence may be most pronounced in dendrites, where DCS has been shown to modulate excitability involving multiple voltage-dependent channels [75]. While I do not directly measure membrane polarization in the present experiments, our group has done this previously with the same preparation, showing membrane polarization to be maximal in dendrites (Bikson et al. 2004, figure 10), with opposite polarization in apical and basal dendrites. Indeed I observe modulation of synaptic plasticity that is consistent with this variable dendritic, rather than a singular somatic polarization effect (Figure 2).

Given that DCS effects can vary with dendritic location, tasks that activate synaptic pathways with different dendritic locations may respond differently to the same stimulation. A lack of control over the location of active pathways could therefore lead to highly variable results in clinical studies. Attention to dendritic polarization may therefore help to explain mixed effects observed in tDCS outcomes [36], [71], [73], [90]. This motivates a shift away from the conventional focus on somatic polarization, which implies fixed excitability changes for an entire brain region.

While these results are consistent with a role for DCS-induced dendritic polarization, I cannot rule out the involvement of other cellular DCS effects, such as on inhibitory interneurons, glia, neuromodulators systems, or immune response [6]. Further investigation into the involvement of these systems is an important area for future work. The lack of effects observed on fiber volleys and paired pulse ratio suggest that DCS does not affect recruitment or vesicle release probability at presynaptic terminals. This is expected, as Schaffer collateral fibers are oriented perpendicular to the applied DCS electric field vector. While previous studies have reported effects on fEPSP slope, the lack of an effect here may result from a smaller sample size and weaker fields [16], [51], [57].

8.4 Low frequency stimulation effects

The horizontal axis of the FRF is often equated with the degree of postsynaptic calcium influx during induction. HFS leads to strong calcium influx and triggers LTP, while LFS leads to moderate calcium influx and LTD. Based on this calcium control hypothesis, I expected DCS-induced dendritic polarization to modulate calcium influx through NMDARs and produce horizontal shifts in the FRF [15]. The effects I observe with 1 Hz LFS may therefore be expected, as a horizontal shift of the FRF in either direction would result in less LTD if 1 Hz is near the point of maximum LTD (minimum synaptic strength). This interpretation is less adequate in accounting for the effect observed at 0.5 Hz LFS (Figure 1C), as a left horizontal shift would produce more LTD at 0.5 Hz. However, recent evidence suggests a deviation from the calcium control hypothesis, as LTD

can be induced by metabotropic NMDAR function rather than calcium influx [74], [92]–[95]. The calcium and voltage dependence of LTD remains controversial though [94], [96], making it more difficult to interpret results with LFS. I also note that the duration of DCS was particularly long with 0.5 Hz LFS (30 minutes), potentially producing effects that occur on longer time scales, such as on protein synthesis. For example, priming of BDNF synthesis at the start of DCS [9] may lead to increased BDNF release later on during DCS, which reduces LTD [77]. Future experiments directly measuring calcium influx during these induction protocols may provide some resolution to these issues.

8.5 Effect asymmetry

These results demonstrate an asymmetric DCS effect on synaptic plasticity, such that DCS was only able to increase synaptic strength (enhance LTP, reduce LTD). Asymmetries have been found in other animal studies [10], [13] and human studies [1], [36]. In parallel work in our lab, we find an asymmetry in acute DCS effects on cellular excitability. This nonlinearity could be the result of the nonlinear voltage dependence of NMDARs [97]. Similarly, these asymmetries may reflect floor or ceiling effects of any number of cellular processes, where the endogenous state is such that it can only be modulated in one direction.

8.6 Conclusions and context

DCS is likely to affect many cellular processes simultaneously [6]. Previous studies in animals [9]–[14], [98] and humans [48], [99], [100] have implicated various effects related to synaptic plasticity (NMDAR, BDNF, adenosine, norepinephrine). However, it remains unknown exactly how the DCS electric field interacts with cellular activity to produce these effects. The brain slice preparation used here allows for precise control over the electric field with respect to neuronal morphology and synaptic activity, facilitating a bottom-up approach. It will be important for future work to synthesize in vitro mechanisms with in vivo tDCS effects in both rodent models and humans.

DCS-induced membrane polarization is often cited as a source of effects [24], but the polarization profile of a neuron during DCS will be as complex as its morphology [51] and the effects of polarization are likely to vary with the endogenous activity of the neuron. I demonstrate here that the effects of DCS vary with the spatial and temporal properties of the endogenous synaptic activity that it is paired with (Figure 2). These results highlight the complexity of DCS interactions with neuronal morphology and activity, motivating a departure from the canonical excitability hypothesis, which implies singular effects for an entire brain region.

9. References

[1] B. A. Coffman, V. P. Clark, and R. Parasuraman, "Battery powered thought: Enhancement of attention, learning, and memory in healthy adults using transcranial direct current stimulation," *NeuroImage*, vol. 85, pp. 895–908, Jan. 2014.

[2] A. Gomez Palacio Schjetnan, J. Faraji, G. A. Metz, M. Tatsuno, and A. Luczak, "Transcranial Direct Current Stimulation in Stroke Rehabilitation: A Review of Recent Advancements," *Stroke Res. Treat.*, vol. 2013, pp. 1–14, 2013.

[3] M.-F. Kuo, W. Paulus, and M. A. Nitsche, "Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases," *NeuroImage*, vol. 85 Pt 3, pp. 948–960, Jan. 2014.

[4] J. Gill, P. P. Shah-Basak, and R. Hamilton, "It's the Thought That Counts: Examining the Task-dependent Effects of Transcranial Direct Current Stimulation on Executive Function," *Brain Stimulat.*, vol. 8, no. 2, pp. 253–259, Mar. 2015.

[5] A. R. Brunoni, M. A. Nitsche, N. Bolognini, M. Bikson, T. Wagner, L. Merabet, D. J. Edwards, A. Valero-Cabre, A. Rotenberg, A. Pascual-Leone, R. Ferrucci, A. Priori, P. S. Boggio, and F. Fregni, "Clinical research with transcranial direct current stimulation (tDCS): Challenges and future directions," *Brain Stimulat.*, vol. 5, no. 3, pp. 175–195, Jul. 2012.

[6] S. J. Pelletier and F. Cicchetti, “Cellular and Molecular Mechanisms of Action of Transcranial Direct Current Stimulation: Evidence from In Vitro and In Vivo Models,” *Int. J. Neuropsychopharmacol.*, vol. 18, no. 2, pp. pyu047–pyu047, Jan. 2015.

[7] T. Takeuchi, A. J. Duszkiewicz, and R. G. M. Morris, “The synaptic plasticity and memory hypothesis: encoding, storage and persistence,” *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, vol. 369, no. 1633, p. 20130288, Jan. 2014.

[8] B. Draganski and A. May, “Training-induced structural changes in the adult human brain,” *Behav. Brain Res.*, vol. 192, no. 1, pp. 137–142, Sep. 2008.

[9] M. V. Podda, S. Cocco, A. Mastrodonato, S. Fusco, L. Leone, S. A. Barbati, C. Colussi, C. Ripoli, and C. Grassi, “Anodal transcranial direct current stimulation boosts synaptic plasticity and memory in mice via epigenetic regulation of Bdnf expression,” *Sci. Rep.*, vol. 6, p. 22180, Feb. 2016.

[10] F. Ranieri, M. V. Podda, E. Riccardi, G. Frisullo, M. Dileone, P. Profice, F. Pilato, V. Di Lazzaro, and C. Grassi, “Modulation of LTP at rat hippocampal CA3-CA1 synapses by direct current stimulation,” *J. Neurophysiol.*, vol. 107, no. 7, pp. 1868–1880, Apr. 2012.

[11] J. G. Rohan, K. A. Carhuatanta, S. M. McInturf, M. K. Miklasevich, and R. Jankord, “Modulating Hippocampal Plasticity with In Vivo Brain Stimulation,” *J. Neurosci.*, vol. 35, no. 37, pp. 12824–12832, Sep. 2015.

[12] B. Fritsch, J. Reis, K. Martinowich, H. M. Schambra, Y. Ji, L. G. Cohen, and B. Lu, “Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning,” *Neuron*, vol. 66, no. 2, pp. 198–204, Apr. 2010.

[13] J. Marquez-Ruiz, R. Leal-Campanario, R. Sanchez-Campusano, B. Molaee-Ardekani, F. Wendling, P. C. Miranda, G. Ruffini, A. Gruart, and J. M. Delgado-Garcia, “Transcranial direct-current stimulation modulates synaptic mechanisms involved in associative learning in behaving rabbits,” *Proc. Natl. Acad. Sci.*, vol. 109, no. 17, pp. 6710–6715, Apr. 2012.

[14] H. Monai, M. Ohkura, M. Tanaka, Y. Oe, A. Konno, H. Hirai, K. Mikoshiba, S. Itohara, J. Nakai, Y. Iwai, and H. Hirase, “Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain,” *Nat. Commun.*, vol. 7, p. 11100, Mar. 2016.

[15] L. N. Cooper and M. F. Bear, “The BCM theory of synapse modification at 30: interaction of theory with experiment,” *Nat. Rev. Neurosci.*, vol. 13, no. 11, pp. 798–810, Oct. 2012.

[16] M. Bikson, M. Inoue, H. Akiyama, J. K. Deans, J. E. Fox, H. Miyakawa, and J. G. R. Jefferys, “Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro,” *J. Physiol.*, vol. 557, no. Pt 1, pp. 175–190, May 2004.

[17] D. P. Purpura and J. G. Mcmurtry, “Intracellular activities and evoked potential changes during polarization of motor cortex,” *J. Neurophysiol.*, vol. 28, pp. 166–185, Jan. 1965.

[18] T. Radman, Y. Su, J. H. An, L. C. Parra, and M. Bikson, “Spike timing amplifies the effect of electric fields on neurons: implications for endogenous field effects,” *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 27, no. 11, pp. 3030–3036, Mar. 2007.

[19] T. Radman, R. L. Ramos, J. C. Brumberg, and M. Bikson, “Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro,” *Brain Stimulat.*, vol. 2, no. 4, pp. 215–228, 228.e1–3, Oct. 2009.

[20] D. Reato, A. Rahman, M. Bikson, and L. C. Parra, “Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing,” *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 30, no. 45, pp. 15067–15079, Nov. 2010.

[21] E. Dayan, N. Censor, E. R. Buch, M. Sandrini, and L. G. Cohen, “Noninvasive brain stimulation: from physiology to network dynamics and back,” *Nat. Neurosci.*, vol. 16, no. 7, pp. 838–844, Jun. 2013.

[22] K. Monte-Silva, M.-F. Kuo, S. Hessenthaler, S. Fresnoza, D. Liebetanz, W. Paulus, and M. A. Nitsche, “Induction of Late LTP-Like Plasticity in the Human Motor Cortex by Repeated Non-Invasive Brain Stimulation,” *Brain Stimulat.*, vol. 6, no. 3, pp. 424–432, May 2013.

[23] M. C. Ridding and U. Ziemann, “Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects: Induction of cortical plasticity by non-invasive brain stimulation,” *J. Physiol.*, vol. 588, no. 13, pp. 2291–2304, Jul. 2010.

[24] C. J. Stagg and M. A. Nitsche, “Physiological basis of transcranial direct current stimulation,” *Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry*, vol. 17, no. 1, pp. 37–53, Feb. 2011.

[25] M. A. Nitsche, L. G. Cohen, E. M. Wassermann, A. Priori, N. Lang, A. Antal, W. Paulus, F. Hummel, P. S. Boggio, F. Fregni, and A. Pascual-Leone, "Transcranial direct current stimulation: State of the art 2008," *Brain Stimulat.*, vol. 1, no. 3, pp. 206–223, Jul. 2008.

[26] S. C. Andrews, K. E. Hoy, P. G. Enticott, Z. J. Daskalakis, and P. B. Fitzgerald, "Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex," *Brain Stimulat.*, vol. 4, no. 2, pp. 84–89, Apr. 2011.

[27] M. A. Nitsche and W. Paulus, "Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation," *J. Physiol.*, vol. 527 Pt 3, pp. 633–639, Sep. 2000.

[28] M.-F. Kuo, M. Unger, D. Liebetanz, N. Lang, F. Tergau, W. Paulus, and M. A. Nitsche, "Limited impact of homeostatic plasticity on motor learning in humans," *Neuropsychologia*, vol. 46, no. 8, pp. 2122–2128, Jul. 2008.

[29] M. A. Nitsche and W. Paulus, "Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans," *Neurology*, vol. 57, no. 10, pp. 1899–1901, Nov. 2001.

[30] M. A. Nitsche, A. Schauenburg, N. Lang, D. Liebetanz, C. Exner, W. Paulus, and F. Tergau, "Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human," *J. Cogn. Neurosci.*, vol. 15, no. 4, pp. 619–626, May 2003.

[31] J. Reis, H. M. Schambra, L. G. Cohen, E. R. Buch, B. Fritsch, E. Zarahn, P. A. Celnik, and J. W. Krakauer, "Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation," *Proc. Natl. Acad. Sci.*, vol. 106, no. 5, pp. 1590–1595, Feb. 2009.

[32] T. Z. Kincses, A. Antal, M. A. Nitsche, O. Bártfai, and W. Paulus, "Facilitation of probabilistic classification learning by transcranial direct current stimulation of the prefrontal cortex in the human," *Neuropsychologia*, vol. 42, no. 1, pp. 113–117, 2004.

[33] A. Flöel, N. Rösser, O. Michka, S. Knecht, and C. Breitenstein, "Noninvasive Brain Stimulation Improves Language Learning," *J. Cogn. Neurosci.*, vol. 20, no. 8, pp. 1415–1422, Aug. 2008.

[34] P. S. Boggio, L. P. Khoury, D. C. S. Martins, O. E. M. S. Martins, E. C. de Macedo, and F. Fregni, "Temporal cortex direct current stimulation enhances performance on a visual recognition memory task in Alzheimer disease," *J. Neurol. Neurosurg. Psychiatry*, vol. 80, no. 4, pp. 444–447, Dec. 2008.

[35] V. P. Clark, B. A. Coffman, A. R. Mayer, M. P. Weisend, T. D. R. Lane, V. D. Calhoun, E. M. Raybourn, C. M. Garcia, and E. M. Wassermann, "TDCS guided using fMRI significantly accelerates learning to identify concealed objects," *NeuroImage*, vol. 59, no. 1, pp. 117–128, Jan. 2012.

[36] L. Jacobson, M. Koslowsky, and M. Lavidor, "tDCS polarity effects in motor and cognitive domains: a meta-analytical review," *Exp. Brain Res.*, vol. 216, no. 1, pp. 1–10, Jan. 2012.

[37] S. Nikolin, C. K. Loo, S. Bai, S. Dokos, and D. M. Martin, "Focalised stimulation using high definition transcranial direct current stimulation (HD-tDCS) to investigate declarative verbal learning and memory functioning," *NeuroImage*, vol. 117, pp. 11–19, Aug. 2015.

[38] J. I. Broussard, K. Yang, A. T. Levine, T. Tsetsenis, D. Jenson, F. Cao, I. Garcia, B. R. Arenkiel, F.-M. Zhou, M. De Biasi, and J. A. Dani, "Dopamine Regulates Aversive Contextual Learning and Associated In Vivo Synaptic Plasticity in the Hippocampus," *Cell Rep.*, vol. 14, no. 8, pp. 1930–1939, Mar. 2016.

[39] S. Nabavi, R. Fox, C. D. Proulx, J. Y. Lin, R. Y. Tsien, and R. Malinow, "Engineering a memory with LTD and LTP," *Nature*, vol. 511, no. 7509, pp. 348–352, Jun. 2014.

[40] M.-S. Rioult-Pedotti, "Learning-Induced LTP in Neocortex," *Science*, vol. 290, no. 5491, pp. 533–536, Oct. 2000.

[41] D. S. Roy, A. Arons, T. I. Mitchell, M. Pignatelli, T. J. Ryan, and S. Tonegawa, "Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease," *Nature*, vol. 531, no. 7595, pp. 508–512, Mar. 2016.

[42] J. T. Trachtenberg, B. E. Chen, G. W. Knott, G. Feng, J. R. Sanes, E. Welker, and K. Svoboda, "Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex," *Nature*, vol. 420, no. 6917, pp. 788–794, Dec. 2002.

[43] D. O. Hebb, *The organization of behavior: A neuropsychological approach.* John Wiley & Sons, 1949.

[44] L. Nowak, P. Bregestovski, P. Ascher, A. Herbet, and A. Prochiantz, "Magnesium gates glutamate-activated channels in mouse central neurones," *Nature*, vol. 307, no. 5950, pp. 462–465, Feb. 1984.

[45] R. C. Malenka and M. F. Bear, "LTP and LTD," *Neuron*, vol. 44, no. 1, pp. 5–21, Sep. 2004.

[46] H. Lee and J. J. Kim, "Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats," *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 18, no. 20, pp. 8444–8454, Oct. 1998.

[47] R. G. M. Morris, E. Anderson, G. S. Lynch, and M. Baudry, “Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5,” *Nature*, vol. 319, no. 6056, pp. 774–776, Feb. 1986.

[48] M. A. Nitsche, K. Fricke, U. Henschke, A. Schlitterlau, D. Liebetanz, N. Lang, S. Henning, F. Tergau, and W. Paulus, “Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans,” *J. Physiol.*, vol. 553, no. Pt 1, pp. 293–301, Nov. 2003.

[49] M. Andreasen and S. Nedergaard, “Dendritic electrogenesis in rat hippocampal CA1 pyramidal neurons: functional aspects of Na+ and Ca2+ currents in apical dendrites,” *Hippocampus*, vol. 6, no. 1, pp. 79–95, 1996.

[50] C. Y. Chan, J. Hounsgaard, and C. Nicholson, “Effects of electric fields on transmembrane potential and excitability of turtle cerebellar Purkinje cells in vitro,” *J. Physiol.*, vol. 402, pp. 751–771, Aug. 1988.

[51] A. Rahman, D. Reato, M. Arlotti, F. Gasca, A. Datta, L. C. Parra, and M. Bikson, “Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects,” *J. Physiol.*, vol. 591, no. Pt 10, pp. 2563–2578, May 2013.

[52] L. J. Bindman, O. C. Lippold, and J. W. Redfearn, “the action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects.,” *J. Physiol.*, vol. 172, pp. 369–382, Aug. 1964.

[53] P. J. Sjostrom, E. A. Rancz, A. Roth, and M. Hausser, “Dendritic Excitability and Synaptic Plasticity,” *Physiol. Rev.*, vol. 88, no. 2, pp. 769–840, Apr. 2008.

[54] D. E. Feldman, “The Spike-Timing Dependence of Plasticity,” *Neuron*, vol. 75, no. 4, pp. 556–571, Aug. 2012.

[55] P. J. Sjöström, G. G. Turrigiano, and S. B. Nelson, “Rate, Timing, and Cooperativity Jointly Determine Cortical Synaptic Plasticity,” *Neuron*, vol. 32, no. 6, pp. 1149–1164, Dec. 2001.

[56] P. J. Sjöström and M. Häusser, “A Cooperative Switch Determines the Sign of Synaptic Plasticity in Distal Dendrites of Neocortical Pyramidal Neurons,” *Neuron*, vol. 51, no. 2, pp. 227–238, Jul. 2006.

[57] A. Y. Kabakov, P. A. Muller, A. Pascual-Leone, F. E. Jensen, and A. Rotenberg, “Contribution of axonal orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus,” *J. Neurophysiol.*, vol. 107, no. 7, pp. 1881–1889, Apr. 2012.

[58] C. A. Terzuolo and T. H. Bullock, “MEASUREMENT OF IMPOSED VOLTAGE GRADIENT ADEQUATE TO MODULATE NEURONAL FIRING,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 42, no. 9, pp. 687–694, Sep. 1956.

[59] J. Lisman and N. Spruston, “Postsynaptic depolarization requirements for LTP and LTD: a critique of spike timing-dependent plasticity,” *Nat. Neurosci.*, vol. 8, no. 7, pp. 839–841, Jul. 2005.

[60] G. Leal, P. M. Afonso, I. L. Salazar, and C. B. Duarte, “Regulation of hippocampal synaptic plasticity by BDNF,” *Brain Res.*, vol. 1621, pp. 82–101, Sep. 2015.

[61] A. Balkowiec and D. M. Katz, “Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons,” *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 22, no. 23, pp. 10399–10407, Dec. 2002.

[62] V. Lessmann and T. Brigadski, “Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update,” *Neurosci. Res.*, vol. 65, no. 1, pp. 11–22, Sep. 2009.

[63] M. De Pittà, N. Brunel, and A. Volterra, “Astrocytes: Orchestrating synaptic plasticity?,” *Neuroscience*, vol. 323, pp. 43–61, May 2016.

[64] G. Perea, M. Navarrete, and A. Araque, “Tripartite synapses: astrocytes process and control synaptic information,” *Trends Neurosci.*, vol. 32, no. 8, pp. 421–431, Aug. 2009.

[65] N. Chen, H. Sugihara, J. Sharma, G. Perea, J. Petravicz, C. Le, and M. Sur, “Nucleus basalis-enabled stimulus-specific plasticity in the visual cortex is mediated by astrocytes,” *Proc. Natl. Acad. Sci.*, vol. 109, no. 41, pp. E2832–E2841, Oct. 2012.

[66] Y. Pankratov and U. Lalo, “Role for astroglial α1-adrenoreceptors in gliotransmission and control of synaptic plasticity in the neocortex,” *Front. Cell. Neurosci.*, vol. 9, Jun. 2015.

[67] N. Takata, T. Mishima, C. Hisatsune, T. Nagai, E. Ebisui, K. Mikoshiba, and H. Hirase, “Astrocyte Calcium Signaling Transforms Cholinergic Modulation to Cortical Plasticity In Vivo,” *J. Neurosci.*, vol. 31, no. 49, pp. 18155–18165, Dec. 2011.

[68] Y. Hattori, A. Moriwaki, and Y. Hori, “Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic AMP in rat cerebral cortex,” *Neurosci. Lett.*, vol. 116, no. 3, pp. 320–324, Aug. 1990.

[69] H. Kamiya and R. S. Zucker, “Residual Ca2 + and short-term synaptic plasticity,” *Nature*, vol. 371, no. 6498, pp. 603–606, Oct. 1994.

[70] A. M. Sebastião and J. A. Ribeiro, “Neuromodulation and metamodulation by adenosine: Impact and subtleties upon synaptic plasticity regulation,” *Brain Res.*, vol. 1621, pp. 102–113, Sep. 2015.

[71] J. C. Horvath, O. Carter, and J. D. Forte, “Transcranial direct current stimulation: five important issues we aren’t discussing (but probably should be),” *Front. Syst. Neurosci.*, vol. 8, 2014.

[72] J. P. Brasil-Neto, “Learning, Memory, and Transcranial Direct Current Stimulation,” *Front. Psychiatry*, vol. 3, 2012.

[73] C. M. Saucedo Marquez, X. Zhang, S. P. Swinnen, R. Meesen, and N. Wenderoth, “Task-Specific Effect of Transcranial Direct Current Stimulation on Motor Learning,” *Front. Hum. Neurosci.*, vol. 7, 2013.

[74] S. Nabavi, H. W. Kessels, S. Alfonso, J. Aow, R. Fox, and R. Malinow, “Metabotropic NMDA receptor function is required for NMDA receptor-dependent long-term depression,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, no. 10, pp. 4027–4032, Mar. 2013.

[75] M. Andreasen and S. Nedergaard, “Dendritic electrogenesis in rat hippocampal CA1 pyramidal neurons: functional aspects of Na+ and Ca2+ currents in apical dendrites,” *Hippocampus*, vol. 6, no. 1, pp. 79–95, 1996.

[76] S. R. Hulme, O. D. Jones, D. R. Ireland, and W. C. Abraham, “Calcium-Dependent But Action Potential-Independent BCM-Like Metaplasticity in the Hippocampus,” *J. Neurosci.*, vol. 32, no. 20, pp. 6785–6794, May 2012.

[77] Y. Ikegaya, Y. Ishizaka, and N. Matsuki, “BDNF attenuates hippocampal LTD via activation of phospholipase C: implications for a vertical shift in the frequency-response curve of synaptic plasticity,” *Eur. J. Neurosci.*, vol. 16, no. 1, pp. 145–148, Jul. 2002.

[78] J. E. Lisman, “Three Ca^{2+} levels affect plasticity differently: the LTP zone, the LTD zone and no man’s land,” *J. Physiol.*, vol. 532, no. 2, pp. 285–285, Apr. 2001.

[79] S. R. Hulme, O. D. Jones, and W. C. Abraham, “Emerging roles of metaplasticity in behaviour and disease,” *Trends Neurosci.*, vol. 36, no. 6, pp. 353–362, Jun. 2013.

[80] S. A. Connor and Y. T. Wang, “A Place at the Table: LTD as a Mediator of Memory Genesis,” *The Neuroscientist*, May 2015.

[81] Z. Dong, Y. Bai, X. Wu, H. Li, B. Gong, J. G. Howland, Y. Huang, W. He, T. Li, and Y. T. Wang, "Hippocampal long-term depression mediates spatial reversal learning in the Morris water maze," *Neuropharmacology*, vol. 64, pp. 65–73, Jan. 2013.

[82] S. J. Kuhlman, D. H. O'Connor, K. Fox, and K. Svoboda, "Structural Plasticity within the Barrel Cortex during Initial Phases of Whisker-Dependent Learning," *J. Neurosci.*, vol. 34, no. 17, pp. 6078–6083, Apr. 2014.

[83] S. Griffiths, H. Scott, C. Glover, A. Bienemann, M. T. Ghorbel, J. Uney, M. W. Brown, E. C. Warburton, and Z. I. Bashir, "Expression of Long-Term Depression Underlies Visual Recognition Memory," *Neuron*, vol. 58, no. 2, pp. 186–194, Apr. 2008.

[84] P. V. Massey and Z. I. Bashir, "Long-term depression: multiple forms and implications for brain function," *Trends Neurosci.*, vol. 30, no. 4, pp. 176–184, Apr. 2007.

[85] N. Kang, J. J. Summers, and J. H. Cauraugh, "Transcranial direct current stimulation facilitates motor learning post-stroke: a systematic review and meta-analysis," *J. Neurol. Neurosurg. Psychiatry*, vol. 87, no. 4, pp. 345–355, Apr. 2016.

[86] M. Bikson, A. name, and A. Rahman, "Origins of specificity during tDCS: anatomical, activity-selective, and input-bias mechanisms," *Front. Hum. Neurosci.*, vol. 7, 2013.

[87] J. J. Borckardt, M. Bikson, H. Frohman, S. T. Reeves, A. Datta, V. Bansal, A. Madan, K. Barth, and M. S. George, "A Pilot Study of the Tolerability and Effects of High-Definition Transcranial Direct Current Stimulation (HD-tDCS) on Pain Perception," *J. Pain*, vol. 13, no. 2, pp. 112–120, Feb. 2012.

[88] A. Datta, V. Bansal, J. Diaz, J. Patel, D. Reato, and M. Bikson, "Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad," *Brain Stimulat.*, vol. 2, no. 4, pp. 201–207, 207.e1, Oct. 2009.

[89] D. M. Martin, R. Liu, A. Alonzo, M. Green, and C. K. Loo, "Use of transcranial direct current stimulation (tDCS) to enhance cognitive training: effect of timing of stimulation," *Exp. Brain Res.*, vol. 232, no. 10, pp. 3345–3351, Oct. 2014.

[90] A. Antal, D. Terney, C. Poreisz, and W. Paulus, “Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex: Effect of tDCS is modified by mental activity and exercise,” *Eur. J. Neurosci.*, vol. 26, no. 9, pp. 2687–2691, Oct. 2007.

[91] B. W. Vines, D. Nair, and G. Schlaug, “Modulating activity in the motor cortex affects performance for the two hands differently depending upon which hemisphere is stimulated,” *Eur. J. Neurosci.*, vol. 28, no. 8, pp. 1667–1673, Oct. 2008.

[92] J. Aow, K. Dore, and R. Malinow, “Conformational signaling required for synaptic plasticity by the NMDA receptor complex,” *Proc. Natl. Acad. Sci.*, p. 201520029, Nov. 2015.

[93] K. Dore, J. Aow, and R. Malinow, “Agonist binding to the NMDA receptor drives movement of its cytoplasmic domain without ion flow,” *Proc. Natl. Acad. Sci.*, p. 201520023, Nov. 2015.

[94] S. Nabavi, R. Fox, S. Alfonso, J. Aow, and R. Malinow, “GluA1 trafficking and metabotropic NMDA: addressing results from other laboratories inconsistent with ours,” *Philos. Trans. R. Soc. B Biol. Sci.*, vol. 369, no. 1633, pp. 20130145–20130145, Dec. 2013.

[95] I. S. Stein, J. A. Gray, and K. Zito, “Non-Ionotropic NMDA Receptor Signaling Drives Activity-Induced Dendritic Spine Shrinkage,” *J. Neurosci.*, vol. 35, no. 35, pp. 12303–12308, Sep. 2015.

[96] W. E. Babiec, R. Guglietta, S. A. Jami, W. Morishita, R. C. Malenka, and T. J. O’Dell, “Ionotropic NMDA Receptor Signaling Is Required for the Induction of Long-Term Depression in the Mouse Hippocampal CA1 Region,” *J. Neurosci.*, vol. 34, no. 15, pp. 5285–5290, Apr. 2014.

[97] C. E. Jahr and C. F. Stevens, “Voltage dependence of NMDA-activated macroscopic conductances predicted by single-channel kinetics,” *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 10, no. 9, pp. 3178–3182, Sep. 1990.

[98] D. Reato, M. Bikson, and L. C. Parra, “Lasting modulation of in vitro oscillatory activity with weak direct current stimulation,” *J. Neurophysiol.*, vol. 113, no. 5, pp. 1334–1341, Mar. 2015.

[99] D. Liebetanz, “Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability,” *Brain*, vol. 125, no. 10, pp. 2238–2247, Oct. 2002.

[100] O. Rroji, K. van Kuyck, B. Nuttin, and N. Wenderoth, “Anodal tDCS over the Primary Motor Cortex Facilitates Long-Term Memory Formation Reflecting Use-Dependent Plasticity,” *PLOS ONE*, vol. 10, no. 5, p. e0127270, May 2015.