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Weak DCS causes a relatively strong cumulative boost of synaptic plasticity with spaced learning

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Abstract

Background: Electric fields generated during direct current stimulation (DCS) are known to modulate activity-dependent synaptic plasticity *in-vitro*. This provides a mechanistic explanation for the lasting behavioral effects observed with transcranial direct current stimulation (tDCS) in human learning experiments. However, previous *in-vitro* synaptic plasticity experiments show relatively small effects despite using strong fields compared to what is expected with conventional tDCS in humans (20 V/m vs. 1 V/m). There is therefore a need to improve the effectiveness of tDCS at realistic field intensities. Here we leverage the observation that effects of learning are known to accumulate over multiple bouts of learning, known as spaced learning.

Hypothesis: We propose that effects of DCS on synaptic long-term potentiation (LTP) accumulate over time in a spaced learning paradigm, thus revealing effects at more realistic field intensities.

Methods: We leverage a standard model for spaced learning by inducing LTP with repeated bouts of theta burst stimulation (TBS) in hippocampal slice preparations. We studied the cumulative effects of DCS paired with TBS at various intensities applied during the induction of LTP in the CA1 region of rat hippocampal slices.

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Author contributions

M.S, M.B. and L.C.P. designed research; M.S. performed experiments; M.S., F.F. and L.C.P. analyzed data; M.S. and L.C.P. wrote the paper.

Author declaration

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

CRedit authorship contribution statement

Mahima Sharma: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Forouzan Farahani:** Software, Formal analysis. **Marom Bikson:** Conceptualization, Writing – review & editing.

Lucas C. Parra: Conceptualization, Supervision, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

M.B and L.C.P. have a financial interest in Soterix Medical LLC and are listed as inventors in intellectual property related to transcranial direct current stimulation. M.S and F.F declare no conflict of interest.

Results: As predicted, DCS applied during repeated bouts of theta burst stimulation (TBS) resulted in an increase of LTP. This spaced learning effect is saturated quickly with strong TBS protocols and stronger fields. In contrast, weaker TBS and the weakest electric fields of 2.5 V/m resulted in the strongest relative efficacies (12% boost in LTP per 1 V/m applied).

Conclusions: Weak DCS causes a relatively strong cumulative effect of spaced learning on synaptic plasticity. Staturarion may have masked stronger effects sizes in previous *in-vitro* studies. Relative effect sizes of DCS are now closer in line with human tDCS experiments.

Keywords

Transcranial direct current stimulation; Direct current stimulation; Synaptic plasticity; LTP; TBS-LTP; Spaced learning

1. Introduction

Transcranial direct current stimulation (tDCS) has generated significant interest as a promising, easy-to-use therapeutic approach for various conditions, including psychological disorders, cognitive enhancement, and neurorehabilitation [1-5]. Modulation of cortical excitability is the prevailing mechanistic explanation, whereby anodal tDCS increases excitability and cathodal tDCS decreases excitability [6-9]. It is often argued that lasting effects of tDCS during behavioral learning may be mediated by the modulation of activity-dependent synaptic plasticity [1,10,11], in particular, long-term potentiation (LTP), as demonstrated by *in-vitro* studies [12-17]. Anodal tDCS is often expected to produce LTP-like effects [1,18,19]. Many *in-vitro* studies confirm the LTP boosting effect of anodal DCS [12-17]. While that may be true, these *in-vitro* studies use electric fields in the range of 20 V/m or above [12,13,15,17]. Conventional tDCS in humans uses 2 mA current that generates electric fields in the brain of less than 1 V/m [20]. This evident gap, along with the relatively small effect sizes observed *in-vitro*, challenges the hypothesis that a boost of activity-dependent synaptic plasticity is the underlying mechanism of tDCS effects on learning.

This focused study aims to narrow this gap by testing weaker field magnitudes and increasing efficacy by leveraging cumulative effects over multiple bouts of learning. Cumulative effects of behavioral learning protocols find their counterpart in *in-vitro* experiments referred to as spaced learning, which consists of multiple bouts of LTP induction [21]. We hypothesized that spaced learning will result in cumulative LTP effects of anodal DCS *in-vitro*. We test this here at conventional high field magnitudes (20 V/m) as well as lower intensities, which are closer to fields that might be achieved with contemporary tDCS approaches [22,23].

We test this hypothesis in rodent hippocampal slices, since LTP recording in the hippocampus is a widely recognized cellular model for the study of memory related mechanisms [24-26]. Indeed, DCS effects are well studied in the hippocampus as compared to cortex *in-vitro* [13,17]. Using repeated bouts of theta burst stimulation (TBS), we find cumulative effects of DCS over time. Interestingly, we find a saturation effect such that weaker induction together with weaker electric fields results in the strongest relative

effect sizes. With this brief report, we have narrowed the gap between *in-vitro* plasticity experiments and *in-vivo* human learning protocols, both in terms of effect size as well as in physiological realism.

2. Materials and methods

All animal experiments were performed 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 846.3 and 2016–24). For electrophysiological recordings, a total of 110 hippocampal brain slices were prepared from 85 male Wistar rats aged 25–32 days old. Two animals were housed per cage under 12 h light/dark conditions with food and water available *ad libitum*. Brain slice preparation, field excitatory postsynaptic potential (fEPSP) recordings and DCS application were done as described [13] except that we used 2.5% isoflurane for anesthesia.

2.1. Brain slice preparation

Following cervical dislocation of the anaesthetized rat, the brain was quickly removed and immersed in chilled (2–6 °C) dissecting artificial cerebrospinal fluid (aCSF) solution containing (in mM): Choline chloride, 110; KCl, 3.2; NaH₂PO₄, 1.25; MgCl₂, 7; CaCl₂, 0.5; NaHCO₃, 26; D-glucose, 10; sodium ascorbate, 2; sodium pyruvate, 3. Transverse slices (400 μm thick) were cut using a manual tissue chopper and transferred to a chamber containing a recovery aCSF at 34 °C (in mM): NaCl, 124; KCl, 3.2; NaH₂PO₄, 1.25; MgCl₂, 1.3; CaCl₂, 2.5; NaHCO₃, 26; D-glucose, 25; sodium ascorbate, 2; sodium pyruvate, 3. After 30 min in the recovery solution, slices were transferred to a holding chamber containing recording aCSF, the same composition as recovery aCSF without sodium pyruvate and sodium ascorbate, at 30 °C for at least 30 min. Finally, slices were transferred to a fluid-gas interface chamber (Harvard Apparatus) perfused with warmed recording aCSF (30.0 ± 0.1 °C) at 1.5 ml/min. The humidified atmosphere over the slices was saturated with a mixture of 95% O₂/5% CO₂. All aCSF solutions were bubbled with a mixture of 95% O₂/5% CO₂. Slices were allowed to acclimate to the recording chamber for at least 30 min before recording started.

2.2. fEPSP recordings

fEPSPs were evoked as described [13], using a platinum iridium bipolar stimulating electrode placed in stratum radiatum of CA1 within 200 μm of the somatic layer. Recording electrode was placed in stratum radiatum approximately 400 μm from the stimulating electrode in CA1 to record fEPSP (Fig. 1A and B). 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). Test stimulation intensity was adjusted to elicit fEPSP slope of 30% of the maximal slope response.

2.3. LTP induction

To induce LTP, TBS (4 pulses at 100 Hz repeated for 15 bursts at 5 Hz, 3 s total; referred to as “stronger” TBS in Fig. 1C) was used. For another series of experiments, “weaker”

TBS (as before but with 10 instead of 15 bursts, 2 s total; Fig. 1C) was used. Four trains of TBS were applied, separated by intervals of 60, 45 and 45 min, respectively (Fig. 1D). The customary interval between TBS trains for spaced learning experiments is 60 min. However, we were limited in total duration by our DCS stimulation equipment. We had promising preliminary data with 45 min and the literature suggested that spaced learning with 45–60 min is appropriate to show cumulative effects [21]. The final protocol with initial 60 min and subsequent 45 min interval was a compromise between these constraints.

LTP was measured in terms of the slope of fEPSP, averaged over the last 10 min of recording, and normalized by a 20 min baseline. All data are reported as the mean \pm standard error of the mean (SEM).

2.4. Direct current stimulation

Uniform extracellular electric fields of varied intensities (2.5, 10, 20 V/m) were generated by passing constant current (D/A driven analog follower; A-M Systems, WA, USA controlled by PowerLab hardware and LabChart software (AD Instruments)) between two large Ag–AgCl wires of 1 mm diameter & 12 mm length, positioned in the bath across the slice separated by 10 mm. 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 and B). Before each recording, DCS current intensity was calibrated to produce the required electric field. To generate 2.5 V/m, 10 V/m and 20 V/m electric field across each slice, the current was adjusted so that two recording electrodes separated by 0.8 mm in the slice measured a voltage difference of 2 mV ($2 \text{ mV}/0.8 \text{ mm} = 2.5 \text{ V/m}$), 8 mV ($8 \text{ mV}/0.8 \text{ mm} = 10 \text{ V/m}$) and 16 mV ($16 \text{ mV}/0.8 \text{ mm} = 20 \text{ V/m}$), respectively. The required currents were in the range of 30–110 μA .

Anodal DCS of varying intensities (2.5 V/m, 20 V/m) was applied to the slices starting 3 s before the onset of “stronger” TBS and ending 3 s after the end of TBS (9s total) (Fig. 1A, C). For “weaker” TBS, anodal DCS of varying intensities (2.5 V/m, 10 V/m) was applied to the slices starting 3.5 s before the onset of TBS and ending 3.5 s after the end of TBS (9s total) to ensure that duration of DCS is constant across conditions.

2.5. Data analysis

Experimental groups were analyzed using two-way ANOVA. Statistical significance was considered when $p < 0.05$.

2.6. Relative efficacy

We obtain the effect size from two-way ANOVA - Partial eta-squared, denoted by η^2_p [27]. Partial eta-square is calculated as:

$$\eta^2_p = \frac{SS_{\text{effect}}}{(SS_{\text{effect}} + SS_{\text{error}})}$$

Where SS_{effect} denotes effect sum of squares, and SS_{error} denotes the error sum of squares.

To determine the relative efficacy of DCS, we compute efficacy relative to field magnitude:

$$\text{Relative Efficacy} = \frac{\text{Effect size}}{\text{Field Magnitude}} \times 100$$

Relative efficacy is reported as % effect per V/m.

3. Results

We first tested the effect of DCS on LTP induced by TBS (4 pulses at 100 Hz repeated for 15 bursts at 5 Hz, 3 s total; referred to as “stronger” TBS in Fig. 1C). LTP was measured in terms of the slope of the field-excitatory postsynaptic potentials (fEPSP), averaged over the last 10 min of recording, and normalized by a 20 min baseline (Fig. 1E). See Fig. 1F for representative fEPSP traces. As expected for spaced learning (21), repeated trains of TBS increase LTP and DCS seems to boost LTP above control. A two-way ANOVA shows a main effect of time (TBS1-TBS4: $F(1,54) = 188.23$, $p = 3.07E-19$, $\eta^2_p = 0.0424$) confirming the cumulative effect of spaced learning. However, the main effect of stimulation condition does not reach statistical significance (DCS at 0, 2.5, 20 V/m: $F(1,54) = 2.57$, $p = 0.11$, $\eta^2_p = 0.046$), as might have been expected from previous work, which paired DCS with individual trains of TBS [13].

The failure of fields to boost LTP may be the result of the saturation observed already after the first two TBS trains (Figs. 1E and 2A). We, therefore, repeated the experiment using a weaker TBS pulse train (as before but with 10 instead of 15 bursts, 2 s total, Fig. 1C). This was paired with concurrent DCS at 2.5 V/m and 10 V/m (Fig. 1G). A two-way ANOVA confirms the cumulative effect of repeated learning ($F(1,39) = 75.53$, $p = 1.16E-10$, $\eta^2_p = 0.659$). Now there is a clear effect of the stimulation condition ($F(1,39) = 5.70$, $p = 0.022$, $\eta^2_p = 0.1274$). Importantly, there was an interaction between the factors of time and stimulation condition ($F(1,39) = 5.37$, $p = 0.026$, $\eta^2_p = 0.1211$). This indicates that DCS not only boosted LTP but also enhanced the cumulative effect of spaced learning itself.

Increasing DCS field magnitude increased LTP, but the effect saturates at higher DCS intensities (Fig. 2B). To determine the relative efficacy of DCS, we compute efficacy relative to field magnitude (% effect per V/m, see Methods). A schematic summary of relative efficacy across all experiments is shown in Fig. 2C-E. In this view, the strongest effects of 12% per V/m are seen at 2.5 V/m, while increasing field intensity only diminishes the relative efficacy (Fig. 2C). Similarly, stronger relative effects are obtained for weaker TBS (Fig. 2D). Finally, LTP increases with repeated bouts of TBS (Fig. 2E). In total, the efficacy of 12% per V/m benefits from spaced learning, but only when using relatively weak induction protocols and weaker DCS fields.

4. Discussion

The relative efficacy reported here is significantly stronger than previous reports of *in-vitro* experiments, which generally are in the range of 1% per V/m [13]. Importantly, weaker fields as well as weaker TBS are physiologically more realistic than previous work [12,13]. Additionally, the field strength of 2.5 V/m used here is close to the values that can be

achieved with modern tDCS protocols in humans [22,23]. Our *in-vitro* results are in better agreement with the efficacy of tDCS observed for behavioral learning in humans, which is in the range of 10–40% per V/m, e.g. Refs. [3,5,28,29]. They also align with the increased effectiveness reported for multi-session tDCS in humans [30-33] and animals [11,34,35]. One caveat of our study is that we have studied these effects in hippocampal slices, whereas tDCS in humans is thought to primarily reach cortical structures [18,19,30,36-38].

Based on our finding that weak fields are strong enough to cause measurable plasticity, we expect that *in-vivo* animal experiments at 2.5 V/m should give measurable effects. Most *in-vivo* animal experiments did not measure field magnitudes [16,34,39,40] and modeling efforts to predict fields in animals are yet to be experimentally validated [41,42]. Future work needs to establish field magnitudes for *in-vivo* experiments as well as demonstrate modulation of plasticity at low magnitudes.

Earlier work on spaced learning showed an increase in LTP even after the third TBS and provided evidence that the cumulative effect results from the recruitment of previously missed synapses [21]. In our case, at least for the control condition, further potentiation after the second TBS seems limited, possibly due to methodological differences in the two studies. In the case of DCS, it is highly likely that a combination of field and TBS recruits most of the synapses with the second induction. It is, therefore, quite remarkable that further induction appears to elevate the ceiling of LTP, when paired with DCS. The ceiling effect of LTP induced by TBS has been attributed to long-lasting afterhyperpolarization (AHP). Indeed, this ceiling can be increased by pharmacologically reducing AHP [43-45].

AHP is known to be modulated by a number of factors, including BDNF, calcium concentration, receptor signaling and various voltage gated ion channels [43,46]. Interestingly enough, all these factors are implicated in DCS mediated long-lasting after effects [1,2,10,11]. It is possible that weak DCS diminishes AHP and therefore increases the ceiling of LTP. In particular, anodal electric fields incrementally depolarize basal and somatic compartments [13,47,48], and are therefore likely to directly reduce the magnitude of the AHP. Thus, anodal DCS not only increases the likelihood of firing for the initial spikes during TBS, but is also likely to facilitate later spikes within later TBS bursts [13] that are typically suppressed due to the AHP [44,45,49]. Increased firing is likely to have a number of downstream consequences that boost LTP, in particular, increased BDNF synthesis and release [34,50-52]. The activity dependent release of BDNF from the glutamatergic synapses [53,54] is known to modulate the synaptic modification threshold [55]. One of the mechanisms by which BDNF can increase the ceiling of LTP is by suppressing AHP [43]. Future experiments could determine the role of this indirect mechanism by pharmacologically inhibiting AHP during DCS and TBS (e.g. by blocking the SK channels using apamine [44,45,56]) or using intracellular recordings during DCS (as in Ref. [15]). Whatever the mechanism, they are likely to operate at higher field intensities as well. However, when strong fields are used, network activity may be induced which limits further potentiation via homeostatic mechanisms consistent with previous reports [12,15]. In summary, stimulation at physiological levels may conspire to optimally benefit from the cumulative effects of repeated TBS while extending the ceiling of LTP with weak DCS. Therefore, we have presented a physiological model that combines the effects of repeated

learning and weak electric fields, resulting in effect sizes that narrow the gap between animal studies and human clinical data.

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Abbreviations:

tDCS	transcranial direct current stimulation
DCS	Direct current stimulation
TBS	Theta burst stimulation
LTP	Long-term potentiation
fEPSP	Field excitatory postsynaptic potential
aCSF	Artificial cerebrospinal fluid
BDNF	Brain derived neurotrophic factor
AHP	Afterhyperpolarization

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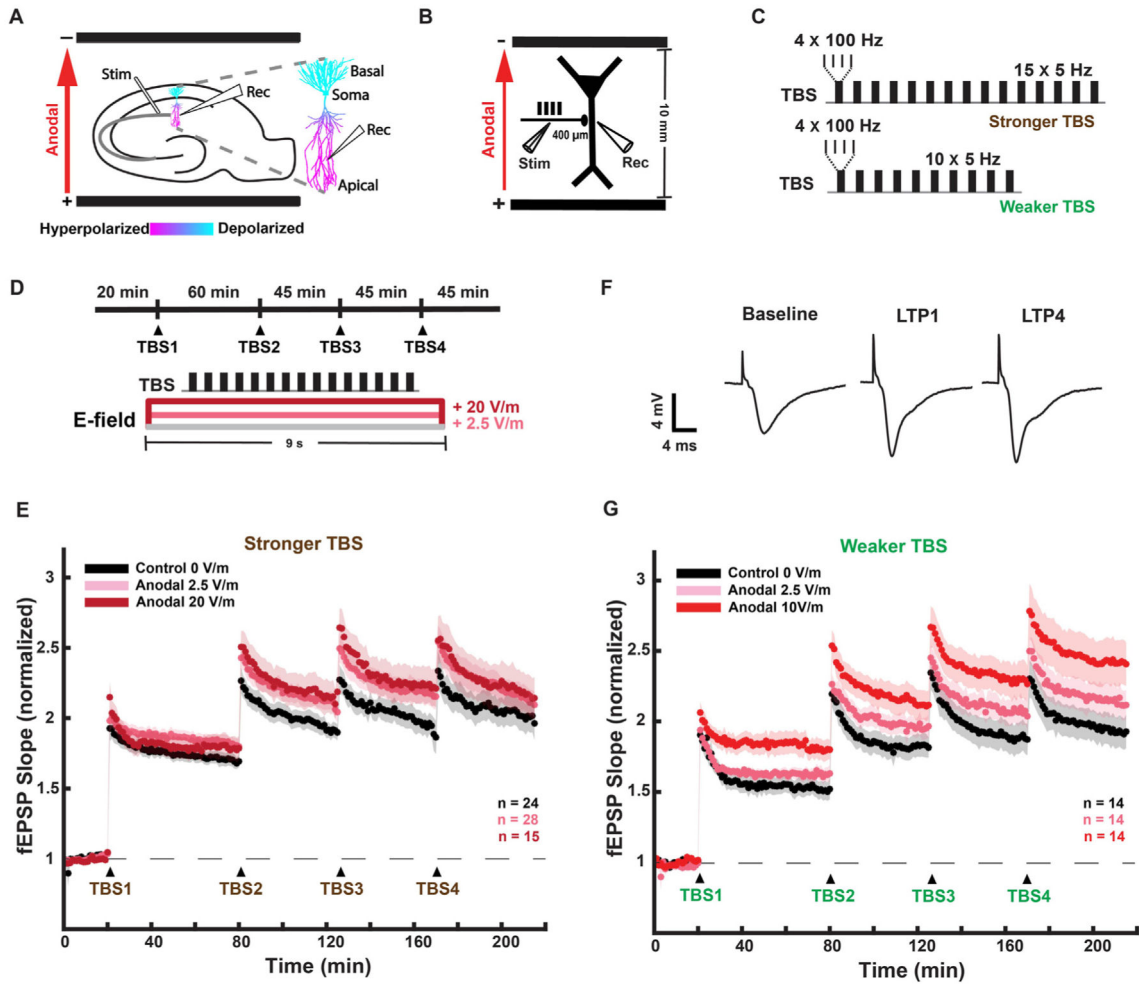


Fig. 1. Effects of Anodal DCS accumulate over time

(A) Schematic depicting anodal DCS of a hippocampal slice, which is oriented such that the somato-dendritic axis of CA1 pyramidal neurons is parallel to the electric field between the DCS wires. Arrow indicates the direction of positive current flow between electric field wires (horizontal black bars above and below hippocampal slice) placed in the recording chamber. (B) Schematic depiction of the location of stimulation (Stim) of Schaffer-collateral fibers with TBS in the presence of an electric field (red arrow) caused by anodal DCS and recording from dendrite (Rec) of fEPSP relative to a CA1 pyramidal neuron soma (black triangle). (C) Depiction of stronger and weaker TBS pulse patterns. (D) Top: Experimental timeline; Bottom: Depiction of constant current stimulation of varying intensities applied during TBS. (E) Strength of fEPSP slope over the course of the spaced learning experiment for different DCS intensities with stronger TBS (0 V/m, n = 24; 2.5 V/m, n = 28; 20 V/m, n = 15). Shaded areas indicate SEM. Timing of four spaced TBS trains indicated with black triangle. (F) Representative fEPSP traces for LTP recording. (G) Same as E but with weaker TBS (0 V/m, n = 14; 2.5 V/m, n = 14; 10 V/m, n = 14).

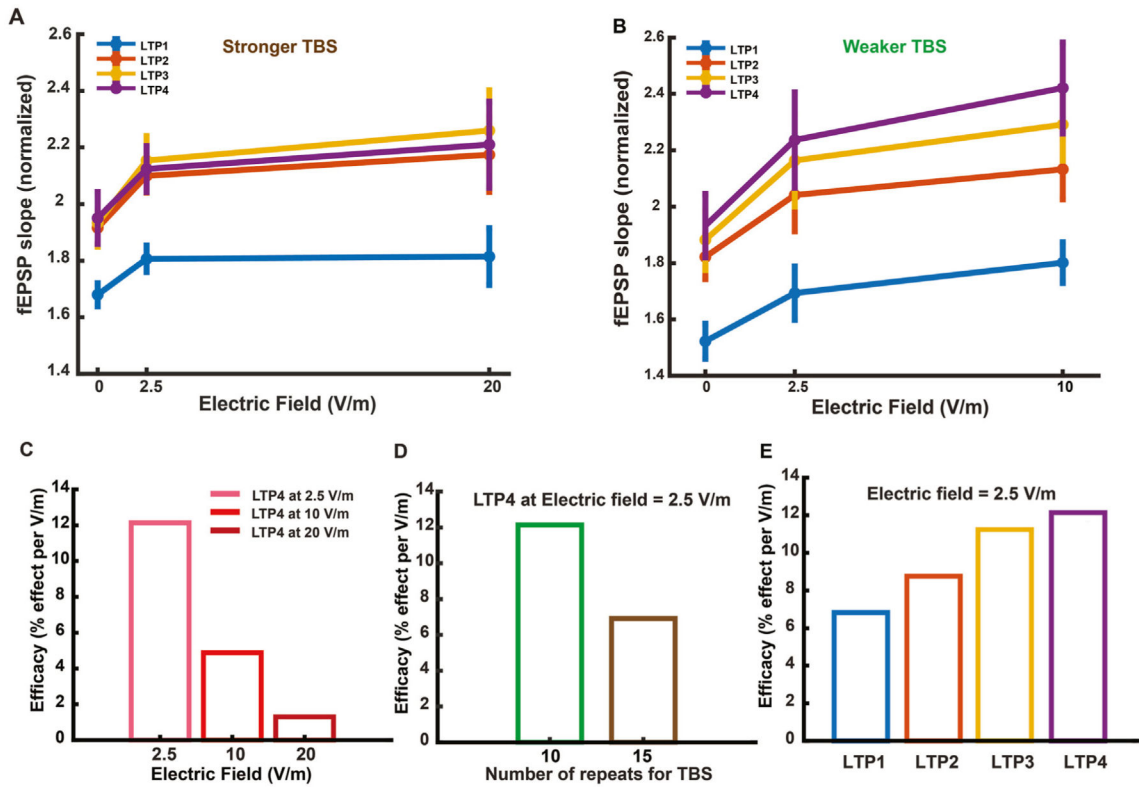


Fig. 2. Relative efficacy of DCS depends on field magnitude, TBS strength and spaced stimulation.

(A) LTP vs. DCS dose-response curves for stronger TBS and for (B) weaker TBS. Illustration of relative efficacy for (C) different DCS field intensities at the LTP4 timepoint, (D) different TBS protocols for LTP4 at 2.5 V/m, and (E) as a function of repeats during spaced learning at 2.5 V/m.