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Title: Animal Studies on the Mechanisms of Low-Intensity Transcranial Electric Stimulation

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Abstract: Experimentations with animal models spanning decades have characterized a number of effects of weak electric stimulation (ES) on neurons and brain activity. By “weak” we mean electric fields that are too weak to activate otherwise quiescent neurons. Low-intensity (few mA) transcranial electrical stimulation (tES) produces weak electric fields in the brain. The effects that have been observed for such weak fields include acute polarization of neurons, lasting modulation of neuronal excitability, network effects which can amplify small single-neuron effects, synaptic plasticity, effects on glia, and other non-neuronal effects such as inflammation, although the latter tend to occur mostly for stronger fields at longer stimulation durations. These effects will be reviewed here as candidates for the mechanisms that underlie the effects observed of tES in humans. We further review animal studies that are relevant to specific clinical pathologies.

Keywords: Neuronal Polarization, DCS, ACS, Synaptic Plasticity, LTP, LTD, Network Effects, Mechanisms, BDNF, Inflammation, Addiction, Alzheimer’s disease, Chronic stress,

1. Neuronal Polarization and Need for Amplification

In this section, we will discuss the acute effects of weak electric fields at the level of a single neuron. While electric fields produced in the brain during low-intensity transcranial stimulation in humans (e.g. tDCS, tACS) are generally below 1 V/m, we consider here fields of up to 20 V/m as “weak” as they are not expected to activate individual neurons in isolation.

First, we will describe the dominant view of the “somatic doctrine” that considers how electric fields affect neurons by incrementally polarizing the soma. Second, we review the somatic doctrine’s origin in classical animal studies. Third, we describe the recent advancement in our understanding of the important role played by polarization of dendrites and axons by electric fields. Next, we summarize recent efforts to quantify neuronal polarization. We then outline possible amplification mechanisms of the electric field stimulation, which are generally single-neuron level.

1.1 The Somatic Doctrine

Electric stimulation causes current to flow across the brain, which is reflected in voltage differences across the brain [1]. As it flows across the brain, any current that passes through a neuron will cause neuronal membrane polarization. Importantly, electric stimulation does not result in a pure depolarization or hyperpolarization across a neuron. Rather, inward current, that flows from outside to the inside of the neuron, hyperpolarizes the membrane and outward current, that flows from inside to the outside of the neuron, depolarizes the membrane [2,3]. Since current that enters a neuron must also exist, the polarization of every neuron should be considered in terms of neuronal compartments, in which each compartment (e.g. the soma, a given dendrite branch) experiences its own direction and magnitude of polarization [3].

It is often the case that the compartments at one end of a neuron are hyperpolarized while the compartments at the other end are depolarized, so that the profile of membrane polarization appears as a gradient from one end of the neuron to the other [3]. The relative direction between the neuron morphology and the electric field determines the sign of polarization across compartments. As it has been demonstrated in single-neuron recording, DCS in the “anodal” direction results in depolarization of soma and basal dendrites but hyperpolarization of the apical dendrites in an L5 pyramidal neuron, with polarization maximal when the electric field direction is parallel to the somatodendritic axis [4]. Reversing the direction of current flow to the “cathodal” direction, inverts this polarization profile (Fig 6.1). For ACS, opposite ends of a neuron remain polarized in opposite directions, with the polarities alternating with each ACS phase [5–7] - as the direction of current flow switches between the anodal and cathodal directions.

Polarization of soma can shape the excitability of a neuron as it plays an important role in action potential generation. The “somatic doctrine” tries to explain the effects of the electric field based on somatic polarization alone. As summarized below, early studies in animal models supported soma-centered explanation for changes in firing rate, and ongoing studies expanded on such

effects. At the same time, polarization by electric fields of other neuronal compartments such as dendrites, axon hillock, and axon, has been shown to affect the excitability of neurons. Human studies are predominantly designed and interpreted in light of somatic polarization. Indeed the canonical human neurophysiology tDCS that heralded our contemporary area of low-intensity tES studies, reported polarity-specific effects consistent with the somatic doctrine [8]; though ongoing human studies show nuanced dose and polarity response [9]. Thus, for all its limitations, the somatic doctrine remains an important basis to start understanding weak stimulation mechanisms. .

We emphasize that the current has a complex spatial flow in the brain during electric stimulation. In an lissencephalic brain, brain regions under the anode electrode and cathode electrode are exposed to radially-inward (anodal) and radially-outward (cathodal) direct current flow, however intermediate brain regions are stimulated with tangentially-direct current flow [10]. For folded cortex, current crossing across gyri can create a highly mixed pattern of directionality even directly under electrodes, though overall there is more inward/outward radial current near the anode/cathode [11]. The application of the somatic doctrine, as used in explaining tDCS clinical studies, assumes a consistently directed radial current flow in a region of interest, and assigns no somatic polarization in regions with tangential current flow. Tangential currents cannot simply be overlooked as animal studies have demonstrated that tangential current flow affected synaptic efficacy acutely during DCS in hippocampal and cortical slices [3,11,12]. Cortical folding is thus a complication concerning the directionality of the current flow and resulting polarity-specific soma polarization. Electrode montage influences the non-uniformity of the electric field across cortical patches. Consequently, the somatic doctrine is dependent on electrode montage and this can complicate the interpretation of clinical results [31].

1.2 Early Evidence on Modulation of Excitability, Polarity Specific Effects

While a historical review of electric stimulation is beyond the scope of this chapter, it is helpful to review a few early studies, which motivated the somatic doctrine. In 1870, a report on the effects of electric stimulation on the brain by Fritsch and Hitzig demonstrated that a negative current can suppress cortical excitability while a positive current can enhance it [13]. (Positive and negative here means inward and outward flow of positive charges, respectively. In clinical studies, they are referred to as anodal and cathodal stimulation). This early finding suggested that the brain is electrically excitable. Later, it was revealed that electric stimulation is capable of modulating ongoing firing patterns whereby a positive current stimulation increased neuronal firing rate and a negative current stimulation had an inhibitory effect on neural discharges [14,15]. To explain their observation, Creutzfeldt claimed that changes in neural excitability are epiphenomenal results of electric stimulation [15]. Terzuolo and Bullock assigned a physiological role to electric field stimulation [14]. The recent work on the effects of electric field stimulation is mostly in agreement with the latter hypothesis suggesting a direct effect of electric field on excitability [16–19]. The polarity of these effects is consistent with the polarization of the soma, namely, positive currents will depolarize the soma and therefore facilitate firing [12]. However, there has been recently an ongoing debate on whether peripheral nerve stimulation can have a causal role in the reported effect with regard to tACS [20,21].

The polarity specific effects on neural firing were confirmed by further animal studies in the early 1960s [22,23]. Additionally, the change in excitability due to the electric field seems to accumulate over time and can outlast the period of stimulation. These results alongside other findings such as modulation of epileptic discharges [24] and lasting effects through protein synthesis [25] supported the importance of the somatic doctrine in explaining the effectiveness of the electric field.

1.3 Polarization of Non-somatic Components

One might wonder whether the somatic doctrine can explain the range of effects that have been reported due to electric field stimulation. To answer this question, we need to emphasize that other compartments, such as dendrites, axon, and terminals, also undergo membrane polarization during electric field stimulation and they are overlooked in the somatic doctrine (Fig. 6.1). There is a risk that this simplification is misleading when interpreting results or designing a new experimental setup.

Dendritic trees are also electrically excitable membranes and electric stimulation can influence them to evoke subthreshold or suprathreshold activities [26]. In a pyramidal neuron, membrane polarization of basal dendrites has the same sign as the soma, while the apical dendrites are polarized in the opposite direction [2,3]. Subthreshold stimulation can influence the synaptic input since electric fields can change the strength of dendritic input in the postsynaptic neurons [27]. This can be a key factor in the modulation of synaptic plasticity and it will be discussed in more detail in **Sec 6.2.** Dendrites are also capable of exhibiting activities such as spiking during a suprathreshold stimulation [2,28–30]. It is worth noting that star-shaped neurons such as basal ganglia neurons and thalamocortical cells can be polarized in the same way with different electric field directions [31].

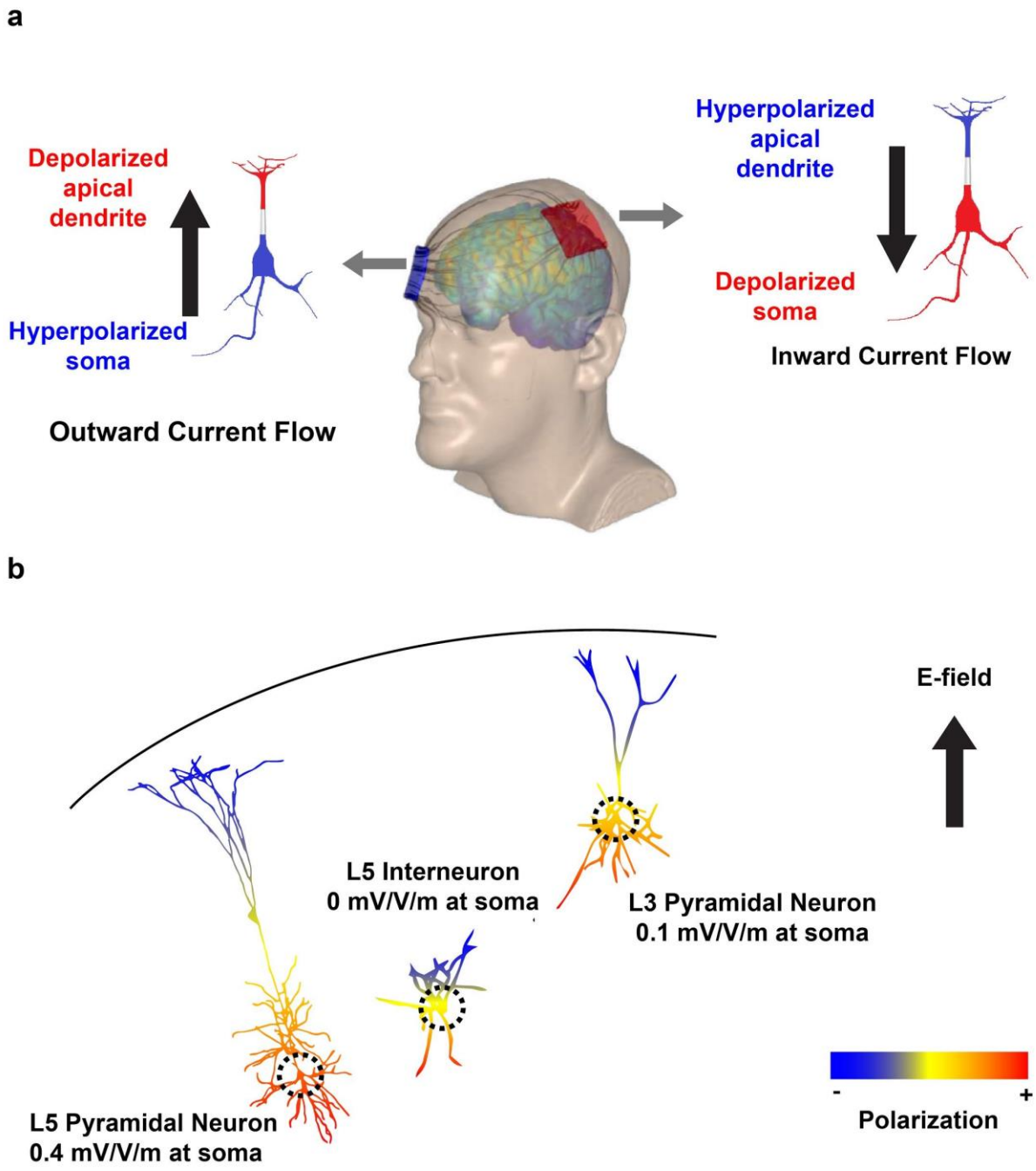

Fig. 6.1 The principle of somatic doctrine and polarization (Adapted from [37]). **(a)** Schematic of how a neuron will be polarized under different electric field polarities. **(b)** Quantification of somatic polarization in cortical neurons of rats during DCS.

Axons are also sensitive to electric fields. The magnitude and sign of axonal polarization depend on their morphology [32–34]. While the initial segment of the axon is most likely polarized with the same sign as soma [28], this assumption does not necessarily hold up for the rest of the axon. Acute brain slice studies indicate that electric field stimulation can modulate the excitability

of axons by measuring changes in presynaptic (antidromic) volley [3,12,35]. While the previous studies conducted extracellular recordings, an interesting finding in a recent study in mouse cortical slices demonstrated that the axonal terminals are 4 times more sensitive to electric field stimulation compared to the soma [36]. They also showed that modulating membrane potential of axonal terminals can shape action potential dynamics and synaptic input.

1.4 Membrane Polarization and Coupling Constants

Quantifying polarization of various compartments is a key step towards developing a predictive understanding of the effects of electric stimulation. Using electrophysiological recording from the turtle cerebellum, Chan et al. measured the amount of polarization during stimulation with a very low-frequency sinusoidal current [28,38]. They reported morphology details of a neuron are key factors determining the sensitivity of a neuron to electric stimulation. Using the rat brain slice, this work has been extended to hippocampal and cortical neurons with the approach of intracellular recording polarization during weak DCS [3,11,39,40]. The basic observation is that membrane polarization increases linearly with field magnitude [3], provided the fields are small enough, i.e. <30 V/m [4], to not engage non-linear channel properties. In other words, stimulation intensities are not strong enough to significantly activate voltage-gated channels and thus the passive properties of membrane determine the amount of polarization.

The amount of polarization that is induced by an applied electric field in this linear regime can be quantified by the coupling constant, also referred to as the ‘coupling strength’ or ‘polarization length’ [4]. Under a uniform electric field, the membrane polarization, V_{tm} (in Volts) can be expressed as: $V_{tm}=G*E$ where G is the coupling constant (in V per V/m, or simply: m) and E is the electric field (in V/m) along the somatodendritic axis. Based on experimental results, the somatic coupling constant, G, was reported to be in the range of 0.1-0.3 mm for hippocampal and cortical pyramidal neurons in rats [3–5]. Additionally, the measured coupling constant of ferret cortical neurons is approximately 0.25 mm [16].

The orientation of a neuron with regard to the electric field affects both sign and magnitude of coupling strength. In other words, the maximum magnitude of polarization across the somatodendritic axis occurs when the electric field is parallel to this axis [3,28]. This corresponds to an electric field radial to the cortical surface. Additionally, the magnitude of somatic polarization depends on the length of the neuron and the dendritic asymmetry around the soma in accordance with both experimental [4,41] and computational studies [42]. Polarization is strongest at the distal ends of a neuron, whereas there is no polarization of the middle compartments of the neuronal structure. Therefore, interneurons with a soma in the center of the cell will not experience somatic polarization, whereas pyramidal neurons that have a soma located at the basal end of the cell will experience relatively stronger somatic polarization [4].

So far, we have discussed findings on the amount of polarization during DCS. To address the same issue during ACS, Deans et al demonstrated an approximate inverse relationship between the amount of polarization and frequency of stimulation [5]. According to their results, the effects

of ACS at 10 Hz were similar to those of DCS while the effectiveness of the electric field decreased with frequency due to the capacitive properties of the neuronal membrane. This inverse relationship suggests that high-frequency stimulation should be less effective.

The coupling constant has thus far only been measured in animal models and we have no direct measures of human cortical neurons. Biophysically realistic models of cortical L5 neurons suggest that somatic membrane polarization does not vary considerably between rats and humans [42]. Nonetheless, generally longer human neurons may polarize more strongly and it would be important to make direct empirical measures of this important variable.

Measurement of the electric field in the human brain revealed that the peak of the electric field in the brain is about 0.3 V/m for 1 mA current intensities [43]. Considering this electric field intensity, the maximum somatic polarization for the most sensitive neurons is about 0.1 mV. With 2 mA tES which is fairly prevalent in clinical trials, somatic polarization will be less than 0.2 mV. Compared with the endogenous background activity in the brain, this amount of polarization is relatively small. Alongside the results from animal studies and the minimum electric field needed to observe a meaningful effect of electric stimulation, one might ask how this small amount of electric stimulation can alter behavioral outcomes in humans. In what follows, we will try to explore the possible answers to this question on the level of a single neuron or a network of neurons.

1.5 Amplification through both Timing and Rate

At the level of a single neuron, a weak electric field can modulate the occurrence of action potentials. An action potential is an all-or-none response that occurs when the somatic membrane potential is sufficiently depolarized. Once this threshold is reached the neuron is said to “fire” or “spike”. The threshold of depolarization needed to generate a spike in action potential varies with the type of neurons but in general, is about 20 mV above the resting potential. Since weak tES is unable to polarize a neuron to this extent, the effect of polarization can only modulate ongoing activity by facilitating or suppressing neuronal firing. One frequent argument is that neurons are close to the threshold of firing due to ongoing activity. Consequently, a small amount of polarization can make a significant change in spiking behavior. Animal experiments have demonstrated modulation of both the firing rate and the specific timing of action potentials [5,6,14,16,17,19,44].

In 1965, Terzuolo and Bullock used a preparation of the non-adapting stretch receptor of the crayfish abdomen and also of the cardiac ganglion of the lobster to study the effect of electric fields on neural firing [14]. They were able to show that neural firing in an active state was remarkably influenced by DCS as low as 1 V/m in a single neuron. In addition, current intensities of more than 20 times of this amount were needed to elicit a spike when a neuron is at rest state. Reato et al demonstrated an effect of weak electric stimulation on gamma activity in vitro with field magnitudes as low as 0.2 V/m [17]. Computational modeling suggested that this modulation was significantly affected by network interactions. Recently, Vöröslakos et al reported that fields of 1 V/m can achieve a significant effect on spiking activity in deep layers of the rat visual cortex [44]. However, this intensity was not strong enough to modulate network oscillations.

As we mentioned earlier, weak electric fields can also modulate spike timing when a neuron is in an active state. Using intracellular depolarizing current injection into CA1 pyramidal neurons (Fig. 6.2a), Radman et al found that DCS can modulate the latency of spiking depending on its polarity [6]. Positive DCS can shorten this latency while negative DCS can increase it compared to the control condition. Moreover, they also reported that changes in spike latency can be quantified by multiplying electric field-induced membrane polarization by the inverse of the current ramp slope (Fig. 6.2b and 6.2c). Therefore, the slope of the current ramp is the gain for the amplification, and DCS can be more effective in spike latency modulation when this slope is smaller. Another interesting finding in their work is how ACS can modulate oscillatory responses generated by current injection in a neuron. They found that an alternating electric field as low as 1 V/m is able to induce coherent spiking in neurons oscillating at 30 Hz, i.e. gamma oscillation. Later, a more detailed quantification was introduced to describe the relationship between the spike timing phase and the coupling constant for biophysically realistic CA1 pyramidal neurons [45]. It is essential to note that the reported effect can only be due to amplification at a single neuron level. Research on entrainment in neural networks during ACS has indicated electric fields as low as 0.2 V/m can be significantly effective [17]. By comparing these values, one might suggest that amplification can be enhanced more at the network level.

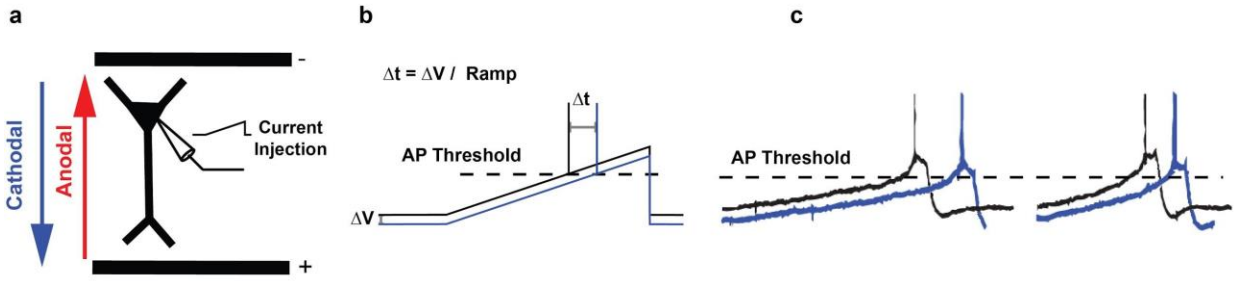

Fig. 6.2. (a) Schematic of the experimental setup in which a depolarizing current ramp is injected into a pyramidal neuron during DCS or control condition. **(b)** Schematic of timing amplification. **(c)** Intracellular response to depolarizing current ramp with 0.4 nA/s (left) and 0.7 nA/s (right) in control (black) and cathodal DCS (blue) conditions (Adapted from [6]).

It is not clear how these in vitro results in rodent slices translate to clinical studies. Krause et al performed single- and multi-unit activity recording from the prefrontal cortex of macaque [46]. Their gyrencephalic cortex and skull thickness, that is close to that of humans, make macaque an ideal animal model. Their study revealed that the spike timing could only be modulated by tDCS with intensities similar to those used in clinical trials while the firing rate is not affected. In addition, intracellular recording from neurons in deep brain structures demonstrated that tACS with intensities within the range of human experiments affected spike timing but not spiking rate in alert nonhuman primate [47]. Overall, these results suggest that low-intensity electric fields are capable of shaping neural activity in the human brain.

What can explain these aforementioned sensitivities to weak electric stimulation in an active state? One frequent argument is that neurons are close to the threshold of firing due to ongoing activity. Consequently, a small amount of polarization can make a significant change in spiking behavior. It is important to emphasize that the level of amplification can depend on neuron types since the coupling constant is not the same for all types of neurons.

While low-intensity tES has subthreshold effects on neurons, simultaneous electric field stimulation with ongoing neural activity can result in suprathreshold responses and long-lasting changes in neurons. However, it is still an ongoing debate on whether to apply the electric field before or during a behavioral or cognitive task [48].

1.6 Seizure Threshold and Modulation

Since somatic polarization is a key factor in triggering seizures during stimulation, considering the coupling constant can be insightful with regard to field intensities capable of modulating seizure. tDCS can generate <1 V/m electric field in the brain resulting in subthreshold membrane polarization. In contrast, TMS or Deep Brain Stimulation is able to produce fields of 100 V/m which generates suprathreshold activation of neurons. In vitro animal studies showed DCS over 20 V/m, which corresponds to >60 mA tDCS, can generate spikes in neurons with the most sensitivity to electric fields [6]. Additionally, it is reported that electric fields about 100 V/m, which corresponds to >500 mA tDCS, are capable of triggering epileptiform activity in acute hippocampal slices [3]. It is noteworthy that these values were recorded in quiescent brain slices and they may vary in an active network. In line with somatic doctrine, experimental results suggest electric fields as low as 1 mV can affect ongoing epileptiform activity [49–53]. In particular, negative DCS can suppress ongoing epileptiform activity due to hyperpolarizing soma and positive DCS can enhance this activity because of further somatic depolarization.

2. Synaptic Processing and Plasticity

Many of the effects discussed above are acute, i.e. they are observed during the period of stimulation and disappear when stimulation stops. However, clinically we are interested in changes that outlast the period of stimulation. Synaptic plasticity is known to be one of the underlying mechanisms of learning and memory formation [54]. It is often argued that the lasting effects of stimulation of tDCS may be mediated by synaptic plasticity [40,55–58]. This section addresses the contribution of animal studies to understanding plasticity generated by weak DC electric fields only. We do not have enough data on AC electric fields.

Animal studies in the 1960s established that weak DCS could produce lasting physical changes in neural activity. These sustained changes could not be explained as persistent “reverberating circuit” of activation [59,60]. Especially notable are the animal studies by Bindman and colleagues that showed that prolonged DCS can produce polarity-specific and lasting changes in cortical excitability [22]. This motivated their early work treating depressive patients with tDCS [61,62]. Persistent changes in excitability were observed in a study using stimulation protocols

lasting up to 13 min in humans [63,64]. These multi-minute protocols are frequently adopted in tDCS research. Lasting changes with ACS have recently been demonstrated in animals when endogenous neural oscillations were present [65]. Long-lasting changes beyond the transient effects of DCS- and ACS-induced polarization would require synaptic changes or changes in neuronal excitability [40,66–68]. In a recent study, the impairment of LTP of cerebellar purkinje cells resulted in the elimination of the effect of andoal DCS on vestibulo-ocular reflex habituation [69]. This study depicts the dependency of DCS-induced positive effect on underlying plasticity during a cerebellar task. Moreover, both in humans and animal studies, changes in synaptically mediated evoked responses are considered reliable hallmarks of long-term plastic changes that could support lasting clinical effects [40,46,67,70–72].

2.1. Paradigms for Modulation of Synaptic Plasticity by Electric Stimulation

Animal studies of tES allow us to formulate and test distinct theories on how stimulation can lead to lasting changes in function. Electric fields generated by tES are subthreshold. They are too weak to trigger an action potential in quiescent neurons, resulting in only transient polarizations. These acute effects can lead to lasting changes in synaptic efficacy mediated through different paradigms such as the following:

1. Modulation of membrane polarization due to the electric field may induce changes in synaptic efficacy regardless of any past, ongoing or future synaptic activity or state of the neuron. However, weak polarization was not sufficient to induce plastic changes in synaptic efficacy in cortical brain slice models, when there is no background activity [40].

2. Changes in action potential rate or timing, secondary to neuronal polarization, may affect synaptic efficacy since they are important factors in determining synaptic plasticity. Classic animal studies indicated that weak DCS is sufficient to induce short- and long-term plastic changes [23,25]. However, these studies do not directly provide a causal link between altered neuronal activity during stimulation and prolonged after-effects.

3. Incremental polarization of the membrane in combination with ongoing synaptic activity may induce synaptic plasticity. The theory is that the induction of plasticity requires synaptic coactivation during ES. It has been shown that in vitro synaptic potentiation under anodal stimulation only occurs with concurrent synaptic stimulation at specific frequencies [40]. In a rabbit study, DCS was combined with repeated somatosensory stimulation leading to polarity-specific lasting changes with cathodal stimulation [67]. If one assumes that tES exerts a postsynaptic priming effect, i.e., polarization of soma, then coactivation of afferent synaptic input could be conceived as Hebbian reinforcement. This learning mechanism has been shown in cortical slice models as well as in vivo [73,74]. Clinically this plasticity paradigm is broadly analogous to combining tES with a cognitive task or specific behavior that coactivates a targeted network or combining tES with TMS [75–78].

4. Incremental polarization of the membrane may boost ongoing endogenous synaptic plasticity similar to a model of associative learning [67] and has been shown to follow the rules of Hebbian plasticity — specificity and associativity in hippocampal slices [55].

Clinically this paradigm is analogous to combining tES with training [79]. Synaptic plasticity experiments typically distinguish between a long-term potentiation (LTP) of synaptic efficacy and long term depression (LTD). It has been shown in rat visual cortex slices that the same tetanic stimulation induced LTD or LTP depending on the level of polarization of the postsynaptic neuron [80]. Hence incremental polarization of the membrane may modulate LTP/LTD.

5. Meta-plasticity is defined as sustained polarization before or after the generation of endogenous plasticity that “primes” the brain to respond differently to potentiation [81]. Evidence from brain slices shows that priming with DCS modulates subsequent tetanus-induced synaptic plasticity in a polarity-specific manner [82].

6. Oscillatory network dynamics that induce LTP, which when modulated can result in lasting effects of electric fields [19,83]. Such modulation may reflect interference with the finely tuned excitatory-inhibitory synaptic balance during oscillations [17].

7. Synaptic tagging and capture hypothesis could offer another explanation for the observed effect of ES [84,85]. In this case, ES might be guiding the process of formation of molecular tags for some plasticity proteins whose synthesis is induced by successive strong synaptic stimulation, either tetanic or theta-burst. There might be different origins for the formation of these molecular tags ranging from ES exposure induced modification of existing proteins to changes in spontaneous neuronal spiking and/or miniature synaptic potentials, or even the expression of new proteins by early gene induction. DCSS is indeed shown to modulate the response to a successive protocol of synaptic potentiation in a polarity-specific manner [82]. The influence of DCS on cortical plasticity has also been demonstrated in humans [86,87].

Aside from these possible synaptic plasticity effects, there may be non-synaptic origins of lasting plastic changes following ES. Though the synapse is typically considered the locus of plastic changes, “non-synaptic” changes have been noted after DCS in peripheral axons [66]. In brain slice models, where background synaptic activity is absent, orthodromic synaptic and antidromic non-synaptic axonal inputs can be precisely isolated. This allows more precise isolation of synaptic and non-synaptic mechanisms. However, functional outcomes of non-synaptic changes in the CNS would still be expected to affect synaptic processing [88].

2.2. Effects of Direct Current Stimulation on LTP and LTD in vitro

A wide array of animal studies using tetanic stimulation to induce LTP/LTD have demonstrated multiple forms of plasticity involving distinct pre- and post-synaptic mechanisms on distinct time scales. DCS-induced lasting changes in excitability were reported [22] a decade before the well-lauded discovery of LTP by Bliss and Lomo [89]. However, the research on tetanic LTP outpaced the investigation of the DCS-induced plasticity changes.

LTP/LTD induced by either tetanic stimulation or DC may, unsurprisingly, share some common molecular substrates [25,82,90]. NMDA receptor-mediated LTP/LTD are the most common

forms [91], and have been implicated in lasting tDCS effects in both humans [92] and rodents in vivo [93], and in vitro DCS-induced plasticity [40,56].

DCS with the anode closer to CA1 apical dendrites is referred to as anodal stimulation as this corresponds to a positive inward current for cortical pyramidal neurons. Conversely, DCS with the cathode closer to CA1 apical dendrites is referred to as cathodal stimulation. So anodal DCS would depolarize soma and basal dendrites, and hyperpolarize apical dendrites. Conversely, cathodal DCS would hyperpolarize soma and basal dendrites, and depolarize apical dendrites. A study done by Grassi group in hippocampal CA3-CA1 synapses exhibited an increase and decrease in LTP with anodal and cathodal DCS, respectively [82]. Subsequent studies highlighted the fact that DCS modulation effects are not as binary and simple [55,56]. These studies identified DCS as a modulator of synaptic activity, not its inducer. They also brought attention to the dependency of the DCS-modulation effects on spatial and temporal patterns of endogenous synaptic activity. When DCS at 20V/m was coupled with tetanic plasticity induction, anodal stimulation enhanced LTP in basal dendrites while cathodal stimulation enhanced LTP in apical dendrites. Interestingly, both anodal and cathodal stimulation modulated LTD in the same direction [56]. This asymmetry of DCS effects might arise from ceiling effects of one/multiple cellular processes that design the endogenous state in a way that its modulation is allowed only in one direction.

Afferent axonal polarization is shown to drive the changes in synaptic activity during DCS [39]. The observed changes are probably due to the orientation of pre- and post-synaptic neurons relative to the electric field. Paired pulse analysis in both rabbit and rodent models also pointed to the presynaptic origin of these tDCS-induced effects [67,93], while the other studies did not find tDCS affecting the presynaptic component [56]. There is a unified emphasis on the DCS-induced change in the postsynaptic membrane potential during the endogenous synaptic activity that drives its effects on ongoing synaptic activity [39,55,56,82]. In any event of DCS, there is simultaneous depolarization and hyperpolarization of different compartments within the same neuron, i.e., the polarity of soma and the basal dendrites is opposed to that of apical dendrites and that leads to varying effects as discussed below.

Contrary to the DCS-induced effects on tetanic-LTP, the modulation of TBS-LTP isn't as complex, as depicted by the studies done in CA1 Schaffer Collateral synapses (Fig.6.3). Irrespective of the dendritic location of the electrodes, anodal stimulation augments the existing LTP whereas cathodal DCS seemed to not affect LTP in either of the compartments [55]. Why is this discrepancy in the observation of DCS effects in the two scenarios? This could be explained by the general principle proposed by Kronberg et al [68]. In an event of endogenous plasticity being primarily driven by the somatic sources of depolarization, e.g. spikes, as is the case with TBS-LTP, DCS-induced polarization at the soma determines the effects on plasticity. In this case, no matter the dendritic location, anodal stimulation will depolarize the soma that will result in an enhanced LTP. When dendritic sources of depolarization, e.g. subthreshold depolarization of dendritic spikes, primarily drive the endogenous plasticity, such as in most of the tetanic-LTP forms, DCS-induced polarization at the dendrite determines the effects on plasticity. Since different dendritic segments don't share the same sign of polarity, the observed modulation of synaptic plasticity varies depending on the type of stimulation and the location of

electrodes. It is, therefore, the interaction between the induced electric field, neuron morphology, and the endogenous brain dynamics that determines the DCS-mediated synaptic function output [55].

In the same study, ACS (5 Hz) when coupled with TBS bursts that were timed to either the peak or the trough of the sinusoidal AC, resulted in the modulation of TBS-LTP as described (Fig.6.3c). The applied electric field at the peak of the AC was identical to anodal constant current, whereas the one at the trough of the AC was identical to cathodal constant current. The effects of AC were similar to those of the analogous constant current paradigm, indicating that plasticity modulation is consistent with the instantaneous incremental membrane polarization on a millisecond timescale [55].

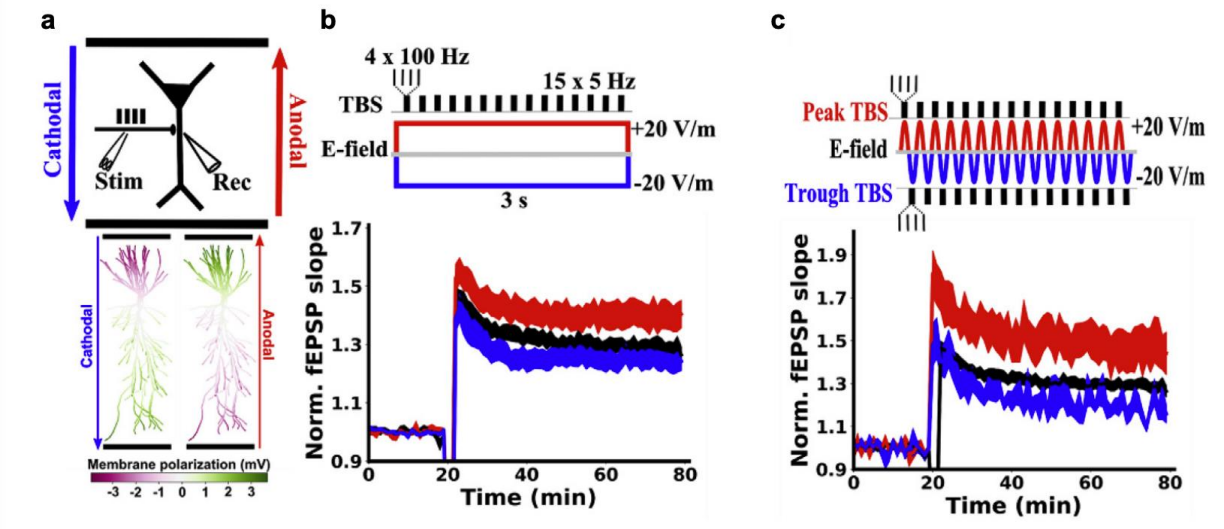

Fig. 6.3: Effect of electric fields on TBS-induced LTP in the hippocampal Schaffer Collateral pathway. a) Top: Schematic of the experimental setup, showing the orientation of electric fields generated by parallel wires (black). Location of stimulation (Stim) with TBS and recording (Rec) of fEPSP are indicated relative to a CA1 pyramidal neuron soma (black triangle). Bottom: Membrane polarization throughout a model pyramidal neuron in response to 20 V/m anodal (red) or cathodal (blue) DCS. Green compartments are depolarized due to DCS, while magenta compartments are hyperpolarized by DCS. b) Constant current stimulation applied during TBS modulates the resulting LTP. c) ACS (5 Hz) was applied and TBS bursts were timed to either the peak (red) or the trough (blue) of the sinusoidal AC. LTP was induced at the 20 min mark. (Adapted from [55]).

Another emerging aspect is the compliance of Hebbian rules by DCS modulation. The modulation effects of DCS are not only input specific but also exhibit associative properties [55]. These results align with the tDCS-induced facilitation of associative learning in the primate brain [46].

Since electric stimulation has a distinct modulatory effect on various compartments of the neuron (see Section 6.1), the interaction between the multiple compartments makes it difficult to predict the outcome on synaptic plasticity. Prolonged tDCS will trigger effects operating on both shorter

time scales, e.g. membrane polarization and plasticity induction, as well as longer time scale, e.g. cell motility and immune responses. Different mechanisms will then interact with each other to produce the results.

2.3. Molecular Mechanisms of tES induced effects on Synaptic plasticity

Since the tES-induced effects are primarily driven by the influence on the underlying synaptic plasticity, it is no surprise that the molecular underpinnings of these observed effects are similar to what forms the basis of induction and maintenance of synaptic plasticity. For example, the BDNF/TrKB pathway that is a potent modulator of these common forms of LTP/LTD [94], has also been implicated in lasting tDCS effects in both humans and in vitro animal studies [40,82]. BDNF/ TrkB was also shown to mediate the metaplastic effect of anodal DCS on the induction of hippocampal CA1 LTP [95]. In addition, BDNF val66met polymorphism, that partially affects activity-dependent BDNF secretion, impaired motor skill acquisition in both humans and mice [22]. The enhancement of anodal tDCS-induced motor learning was subjective to the secretion of activity-dependent BDNF.

Towards the end of the 20th century, stimulated brain slices were probed for different possible molecular targets. DCS was found to affect cyclic adenosine monophosphate ‘cAMP’, the protein kinase C family ‘PKC’, and calcium, each of which play a role in LTP/LTD [90,96]. Recent in vivo animal work has shown the dependency of lasting tDCS effects on the adenosine A1 receptor [67] and NMDA receptor activation [93]. In vitro current stimulation of brain slices led to an immediate increase in the c-*fos* and *zif268*, two of the immediate early genes known to regulate downstream target genes [82]. These genes play an important role in the maintenance of long-term neuronal changes and memory formation [97–101].

It is highly probable that multiple other signaling events, including but not limited to phosphorylation, recruitment, or shuffling of various synaptic proteins, mediate tDCS effects. The manner of interaction between the primary, polarizing effect of tDCS and the molecular mechanism still eludes us. We are yet to fully leverage the wealth of techniques and tools developed by tetanic stimulation LTP as well as TBS-LTP research to deconstruct the mechanistic pathway of tDCS-induced modulation of synaptic plasticity.

3. Morphological Changes

A plethora of in vivo and in vitro studies have highlighted the influence of high intensity electric fields of more than 50 V/m on nervous development and regeneration [102,103]. While not necessarily “weak” (as focused on in other sections of this chapter), and in some cases directed to peripheral nerves with micro-electrodes, these results suggest a novel mechanism that may impact tDCS/tACS outcomes. Electric fields are known to govern the directed migration of neuronal cells, also referred to as electrotaxis. This is further linked to development, membrane protein redistribution, cell proliferation, and recovery from injury [103–105]. A study in the medullary explants from chick embryos exposed to an electric field of ~60 V/m featured the preferential growth of neural processes towards the cathode, and their stunted development

towards the anode [106]. Electric fields also affected the growth rate of the neurites, as they could grow about three times faster towards the cathode at 70 V/m [107].

Electrotaxis has been extensively characterized in vivo. Application of 1 μA of current for 3 weeks to a sprouting rat nerve resulted in an increase in responsiveness with anodal stimulation, when cathode was placed in the direction of growth of the sprouting nerve, in the hind paw sensitivity assessment [108]. Physiological correlates have also been measured in association with the functional recovery of the neurons exposed to low intensity extracellular fields. Administration of 30-minute currents generating fields of approximately 10 V/m for 20 days, after the cut-suture intervention of the sciatic nerve, resulted in nerve regeneration and electromyographic (EMG) activity in 67% of the animals receiving stimulation. Here, growth was directed toward the cathode, as compared to only 17% growth towards the reversed polarity [109]. Subsequent studies further supported an increase in neurofilament growth towards the cathode in damaged sciatic nerves [110], morphological regeneration after nerve transection [111], and complete recovery of associated function [112].

Two plausible mechanisms underlie the axonal growth and guidance. First, the number of cytoplasmic projections that guide axonal growth, also referred to as filopodia, towards the cathode is double that of the ones growing towards the anode [113]. This might be serving as an augmentation mechanism, not a necessary one, since galvanotropic behavior is seen without filopodia. The second mechanism is electric field-induced receptor migration [113]. Acetylcholine (Ach) receptors clustered towards both the anode and the cathode during DC stimulation of Xenopus muscle cells at 400 V/m for 20-40 min, followed by continued accumulation towards the cathode [114,115]. These receptors can increase intracellular calcium concentration via second messenger pathways. This localized shift of intracellular calcium then promotes the growth of neural processes.

In addition to affecting the axonal growth and guidance, electric fields are known to affect the dendritic spines as well. In an ischemia rat model, daily 10 Hz, 0.1 mA tDCS over a period of two weeks increased spine density and improved motor function [116]. Anodal tDCS at 2.2 mA/m^2, when combined with electrical forepaw stimulation, increased spine density and enlarged head sizes of new spines in the sensorimotor (M1/S1) cortex [117]. This tDCS induced regrowth of dendrites and axons was further supported by the upregulation of MAP-2, a critical protein in dendritic outgrowth and remodeling, and GAP-43, a protein found in axonal growth cones [118]. DCS at 25 V/m and 50 V/m applied to differentiated neurons in vitro increased GAP-43 expression as well [105].

4. Network effects:

How electric field stimulation can modulate a neural network has been an active area of research. The activity of neurons in an active network are different than those of neurons in a quiescent state. Similarly, electric field stimulation can produce responses in an active network not expected from single neurons. These responses are specific to the network's architecture and level of activity. A key aspect of network activity is rhythmic firing which results in oscillatory brain signals. Both clinical trials and animal experiments reported that electric stimulation modulates

oscillations in the brain [83,119]. It is essential to emphasize that the underlying mechanisms are not the same for different endogenous oscillations in the brain, e.g. slow-wave, alpha, or gamma oscillations have entirely different physiological origins. Consequently, the effects of electric field stimulation on active networks are likely to depend on network dynamics leading to each type of oscillation. In this section, we will summarize animal studies on how electric stimulation can affect activities within neural networks with different techniques of stimulation and we will outline the suggested explanatory mechanisms to this date.

Slow-wave oscillations that are common during sleep consist of a succession of high firing activity (Up state) and almost no spiking state (Down state) [120]. Frölich et al showed that anodal DCS (soma-depolarizing) can significantly reduce the duration of the Down state, while the Up state was unchanged by weak electric fields. Based on their hypothesis, this resulted in a reduction of the oscillation period. In rat hippocampal slices, gamma oscillations can be modulated in amplitude with DCS and the modulation is strongest when applied fields are oscillating at theta frequencies [17]. Later, the same group showed that prolonged DCS caused lasting effects on gamma oscillations and multi-unit activity [19]. Recordings in non-human primates showed that tDCS with 2 mA had a significant effect on local field potentials in a broad range of frequencies [46], although there are some concerns that this may be the result of physiological artifacts also observed in humans EEG [121].

tACS has been used as an intervention to target specific oscillatory patterns in the brain [122,123] (Fig. 6.4a). Studies on mechanisms of action include both in vivo and in vitro experiments. One such mechanism is resonance whereby ACS modulates endogenous activity at the same frequency of the stimulation [16,17,124]. For instance, fields as weak as 0.2 V/m are able to enhance the firing activity during gamma oscillation in hippocampal CA3 if the frequency of oscillation matches that of the endogenous rhythm [17]. ACS within the frequency range of cortical slow oscillations can also entrain endogenous activity in anesthetized rats [125]. Stronger fields managed to entrain a larger number of neurons, consistent with findings from in vitro experiment [5]. A study in awake head-fixed ferrets [126] suggests that low-intensity electric fields (<0.5 V/m) can selectively entrain alpha oscillations (11-17 Hz).

What are the mechanisms underlying the aforementioned effects of ACS? The proposed explanations are often tied to the specific mechanism underlying a given endogenous oscillation. The temporal biasing of spikes is one possible mechanism for the ACS-induced effects. Small amounts of polarization generated by an exogenous electric field can shift spike occurrence or spike timing when a neuron is close to the threshold of action potential generation. Network entrainment is another way ACS can influence oscillatory behaviors, particularly in a network with coherent oscillation. When the frequency of weak ACS is matched with the endogenous oscillation, time shifts can accumulate over several cycles. This results in a temporal alignment of spiking activity in a network whereby there will be a constant phase difference between applied ACS and native rhythm. In the case of a network with less regular oscillation, ACS can exert its effects through enforcing a firing pattern. In this manner, the exogenous electric field counteracts with the endogenous oscillation. Imposing a firing pattern requires the external electric field to be strong enough to overpower native rhythm.

These endogenous oscillations can be the result of balanced interactions between excitatory and inhibitory neurons. In such networks, excitatory inputs drive inhibitory neurons to control the timing of the network. ACS, when applied, can enhance the temporal alignment of firing patterns of excitatory neurons. This ACS-induced elevated level of synchrony is followed by stronger activation of inhibitory neurons, resulting in increased suppression of excitatory neurons. This suppression can cause the network to “skip a beat” resulting in half as many cycles, i.e., half harmonic [5,17]. For example, the strong ACS-induced modulation of gamma oscillations is a result of an overshoot of the dynamic balance between excitatory and inhibitory interactions. A similar effect was observed with amplitude-modulated ACS [127]. The high-frequency carrier (2kHz) had only minimal effects, but once modulated in amplitude at lower frequencies (10Hz) there was a strong modulation of the endogenous gamma rhythm. In temporal interferential stimulation (TIS) a similar amplitude modulated high frequency oscillation is generated. Grossman et al have argued that the spatial selectivity observed in TIS is the result of this specificity of AM modulated fields [128]. It is not yet clear how the high-frequency stimulation of the carrier becomes more effective when it is modulated in amplitude at lower frequencies. One modeling study suggests an interaction of axonal activation with the high frequency and network adaptation effects as lower frequencies [128].

Networks with slow-wave oscillations (0.5-4 Hz) were also reported to be sensitive to applied ACS [16,124]. Slow-wave oscillations are identified by synchronized neural activities alternating between Up and Down states. The high level of neural activities during Up state are governed by excitatory interactions. This heightened level of activity is followed by depletion of the available cellular resources and the collapse of the excitatory activities. This leads the network to transition to Down state, where the neurons become quiescent and there is simultaneous recovery of the resources. Consequently, a small amount of depolarization can shift the network to the active states again.

Another proposed mechanism of tACS is the attenuation of neural adaptation. In a non-human primate study, Kar et al applied tACS with 2 mA peak-to-peak at 10 Hz to surface electrodes over the vertex and lateral to the middle temporal area (MT) on the scalp [129]. While they did not observe neural entrainment, they reported tACS-induced attenuation in spiking adaptation to the visual input. Sodium and calcium-gated potassium channels have been implicated in this adaptation mechanism, therefore, Kar et al. suggest that the 10Hz field oscillation may affect these channels.

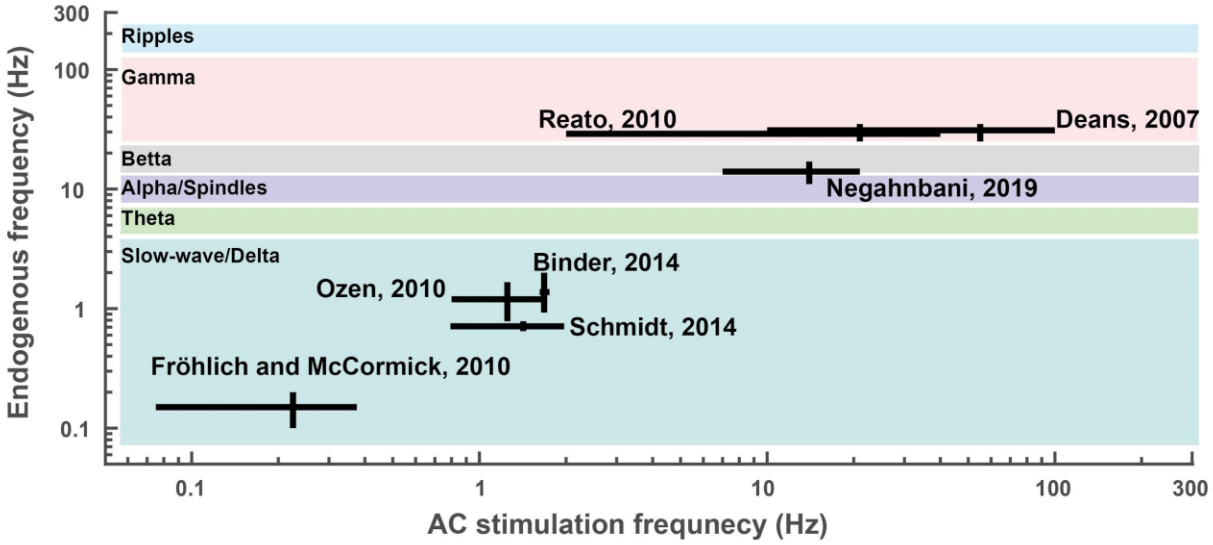

Fig. 6.4 Effects of ACS on a network of neurons (Adapted from [83]). Schematics of the in vitro and in vivo animal models applying AC (sinusoidal) stimulation on oscillatory rhythms. Colors indicate frequency bands.

5. Interneurons and Non-Neuronal Effects

5.1. Interneurons

Many interneurons have a relatively symmetric morphology compared with pyramidal neurons. This will result in weaker somatic polarization as reported in both experimental results in cortical slices [4] and biophysically realistic computational models [42]. However, we cannot ignore the effects of polarization of other compartments such as dendrites and axon during stimulation. Additionally, interneurons have a great variety of morphology which includes neurons with asymmetric dendritic trees [130]. The study of interneuron is particularly important because they play a pivotal role in plasticity and brain oscillations [131]. Recent studies have explored the effects of weak electric stimulation on interneurons. For example, DCS modulated paired-pulse facilitation in hippocampal slices suggest an effect of DCS interneurons [12]. Similarly, computational modelling suggests that ACS could modulate the activity of fast-spiking interneurons through indirect network effects [126]. Further studies are needed to fully characterize the effects of DCS and ACS on the functional and morphological attributes of interneurons.

5.2. Glia

Glial cells represent about half of cells in the human brain while the precise glia-neuron ratio varies in different brain regions [132]. Astrocyte, microglia, and oligodendrocytes are three different glial cell types in the CNS. It is increasingly recognized and understood that glial cells do not act only passively as a supportive role for neurons, but rather are actively involved in information processing [133]. There are few studies that have investigated the primary effects

of electric stimulation on glia. Any activation of neurons or synaptic function will trigger a secondary glial response - in this sense any tDCS effects on neurons includes glial-neuronal interactions. This section however focuses on evidence for primary glial response to electrical stimulation.

Several studies reported that DCS can cause protrusion elongation in both astrocytes and microglia in culture preparations. In addition, cell alignment is possible at higher intensities of stimulation [105]. In such a case, the orientation of microglia is parallel and the orientation of astrocyte is perpendicular to the electric field direction [134]. These studies offered evidence for the responsiveness of glial cells to electric field stimulation as direct effects [134]. Since most in vivo studies focusing on how electric field stimulation affects glial cells have investigated the inflammatory response, we will review them in the next section. Here, we will point out other possible mechanisms for modulation of glial cell activities due to electric stimulation.

A computational model suggested that applied electric fields can produce polarization in astrocytes which are within the range of their ongoing endogenous polarization [135]. This polarization is further influenced by the presence of voltage-sensitive channels across the membrane of astrocytes. Astrocytes play a role in the regulation and reuptake of excess extracellular potassium and sodium changes produced by neuronal activity, including through processes such as potassium spatial buffering that is driven by glial membrane polarization [136]. The application of direct current in vivo can activate these ionic clearance processes [137]. While it has been reported that extracellular potassium concentration doesn't change during DCS in vitro [138], it should be noted that the brain slice preparation interferes with extracellular concentration mechanisms [139].

Calcium signaling is a means of communication between astrocytes and neurons [140]. Electrical activation of one astrocyte can cause activation of others in a local astrocytic network and thereby affect the neuronal processing [141]. In addition, learning can be impaired in the absence of astrocytic calcium signaling, highlighting the importance of astrocytes in learning [142]. Interestingly, the application of anodal tDCS with a current density of 50 A/mm^2 for 10 minutes induced a high-level of astrocytic Ca^{+2} surge across cortical areas of awake mice. As the authors did not observe a significant change in local field potential, they concluded that this is a direct effect of tDCS on astrocytes leading to metaplasticity mediated by noradrenergic transmission [143].

Microglial cells function as immune cells and phagocytes in CNS; however, there is a growing body of evidence showing their active role in synaptic plasticity [144]. A recent study showed that in vivo anodal tDCS caused morphological changes of microglia such as enlargement of soma and decreased their motility in mice. These results were obtained after 3 hours after tDCS and they were absent if the animals were under anesthesia during tDCS. The authors speculated that tDCS could slow down the surveillance of microglia and this might help the initiation of synaptic changes.

Myelination and metabolic support are the main functions of oligodendrocyte in CNS. In vivo ACS study in adult rats showed that stimulation of corticospinal axons can promote the

proliferation and differentiation of oligodendrocyte-specific progenitors after multiple sessions of stimulation [145]. In addition, it has been reported that cathodal tDCS over the ischemic region recruited oligodendrocytes precursor toward the lesion in adult rats while tDCS promoted neurogenesis regardless of its polarity [146].

5.3. Inflammation, Angiogenesis and Apoptosis

In addition to the above-stated effects, tES is known to modulate other vital physiological processes of inflammation, angiogenesis and apoptosis. In vitro studies demonstrated the high intensity DCS-induced accelerated and polarized migration of different peripheral immune cells, including neutrophils [147], lymphocytes [148], macrophages [149], and polymorphonuclear cells [150] [151]. Stimulation of cultured primary astrocytes as well as astrocytic cell lines resulted in increased energy metabolism [152] and perpendicular alignment to the electric field [153,154]. Depending on the intensity and direction, tDCS effects could be pro-inflammatory or anti-inflammatory in nature. Both cathodal and anodal stimulation at 500 μA, 15 min for 10 sessions resulted in increased proliferation of activated microglia in the ipsilateral side of motor cortex [155]. In another study, anodal tDCS at current strength of 200 μA, 30 min for ten days in the rat model of vascular dementia reduced the number of activated microglia and astroglia. There was a reduced expression of pro-inflammatory factors such as IL-1β, IL-6, and TNF-α, indicating the attenuated inflammatory response in the hippocampus [156]. Cathodal tDCS at 500 μA for 15 min for five consecutive days attenuated the activation of astrocyte and microglia, reduced the expression of pro-inflammatory IL-1β, IL-6, and TNF-α, and upregulated the anti-inflammatory IL-10 in rat model of middle cerebral artery occlusion [157]. Bicephalic tDCS at the current density of 33.4 A/m^2 also reduced the levels of IL-1β and TNF-α in the cerebral cortices of obese rats [158]. It is to be noted that most of these studies used higher intensity electric fields than expected in human tDCS.

Large electric fields (50-400 V/m) applied for long periods of time are known to direct the migration, reorientation, cell-division and elongation of endothelial cells in culture [159–165]. In vitro DCS also stimulated the secretion of vascular endothelial growth factor (VEGF) [159,162,164], nitric oxide, and interleukin-8 [163,166]. All three are critical players in angiogenesis. Furthermore, DCS-induced increase in capillary density in a rabbit model of myocardial infarction [167] and a rat model of hindlimb ischemia [159] suggests a positive modulatory effect on angiogenesis. Electric fields may induce significant angiogenesis through the increased expression of VEGF [159,168], activation of VEGF receptor 2 (VEGFR2) and downstream activation of phosphoinositide 3-kinase (PI3K)/ Akt, extracellular regulated kinase 1,2 (Erk1/2), as well as the c-Jun NH2-terminal kinase (JNK) signalling pathways [161,162,164,166]. DCS induced the upregulation and increased activation of chemokine receptors CXCR4 and CXCR2 in an in vitro study [165]. Both the chemokine receptors are necessary for endothelial cell chemotaxis [169,170]. Endothelial cells form the blood–brain barrier (BBB) that tightly regulates transport between the brain extracellular space and blood. As such, any action of DCS on endothelial cells would significantly affect the brain function. tDCS with a current density of 8.0 mA/cm^2 increased the permeability of BBB and this modulation is dependent on nitric oxide [171].

Electric stimulation has been shown to affect apoptotic processes. In ischemic mice, cathodal tDCS significantly decreased the number of cortical and striatal caspase-3 positive cells but anodal stimulation had an opposite effect [172]. ACS (100 μA, 2Hz) decreased the number of apoptotic cells in the cortex, but not in the striatum of ischemic rats, and these anti-apoptotic effects were exerted through Akt phosphorylation [168]. In vitro study with fibroblasts exposed to a 100V/m stimulation demonstrating the upregulation of anti-apoptotic proteins, namely apoptosis inhibitor 5, caspase 8, and Fas-associated death domain-like apoptosis regulator and the protein kinase C epsilon [173], further highlights the ES induced attenuation of apoptosis. DCS at 100 V/m, when applied to injured rat dorsal root ganglion (DRG) cells for an hour, decreased the apoptotic rate of DRG cells [174]. In an in-vitro study with biofilms, low-frequency low voltage AC accelerated the apoptotic process in bioelectrical reactor biofilms [175].

6. Applications to Clinical Pathologies

The noninvasiveness and low cost of tES methods have made it versatile and widely studied as a potential treatment for various diseases [176,177]. tES is especially favorable as a treatment tool for psychiatric disorder because of low-cost, portability and ease of use. tES effects can be directly assessed with behavioral and cognitive tests, which are more direct and informative in humans than animals [46,156,178–183]. It is easy to interpret human behavior and assess their feelings as compared to animals. For these reasons, a majority of published findings are of tDCS effects in humans and relatively few are in animal models. Amongst the animal studies, most involved highly invasive methodologies (e.g., tissue damage, brain slice, and protein-synthesis experiments). Nonetheless, some studies treating animal models of psychiatric disorders with tDCS are briefly outlined below.

6.1. Stroke:

Since the application of tES and more specifically tDCS has shown promising results as a therapeutic intervention in stroke patients, several groups have attempted to use animal models of stroke to study the effectiveness of the electric field stimulation and the underlying functional and cellular mechanisms explaining this efficacy. One important factor to consider is when tES should be applied after a stroke. While there are clinical studies that have delineated beneficial effects of tACS in patients during recovery after stroke [184], animal studies have not attempted to investigate the efficacy of this technique of stimulation in stroke models yet. We can categorize studies based on the time of intervention into acute,i.e., less than<24 hours after stroke and subacute, 1-7 days after stroke groups [185].

Different reports outlined the potential benefit of ipsilateral cathodal tDCS within a few hours following the stroke induction, namely reduction in various stroke-related outcome measures such as infarct growth, edema, inflammation, and the number of apoptotic cells [172,186]. Additionally, DCS can be used for the purpose of rehabilitation to regain cognitive and motor performances when it is applied in the subacute phase. While there is a debate on the effectiveness of DCS with regard to the polarity of the electric field, there is accumulating evidence suggesting an improvement in the recovery and neural growth such as elevated levels

of microtubule and growth-associated protein due to DCS application [118]. Overall, these results suggest a preventive and rehabilitative benefit of tDCS for stroke patients.

6.2. Addiction

A handful of studies using tDCS as a treatment for addiction in animals have been conducted. Anodal tDCS at 0.2 mA, when applied to the frontal cortex for 20 min twice a day for 5 consecutive days was sufficient to reduce anxiety-like and depression-like behavior in nicotine-addicted mice [187]. Repeated anodal tDCS impaired cocaine-induced place preference conditioning and locomotor activation [188]. In this study, repeated anodal tDCS also reduced cocaine-induced expression of Zif268 in specific corticostriatal circuits for three weeks in female mice. tDCS mediated modulation of cortical excitability is shown to treat food addiction as well, and the underlying biochemical response involves lipid, protein and metal/non-metal ion driven mechanisms [189].

6.3. Alzheimer's Disease

The main methods of noninvasive brain stimulation for Alzheimer's disease are TMS and anodal tDCS and preliminary findings suggest that both techniques reduced cognitive deficits in Alzheimer's patients [124–126]. To replicate the cognitive symptoms of Alzheimer's, intraperitoneal injections of scopolamine were given to rats that subsequently received 0.1 mA of anodal tDCS for 20 minutes twice a day, five times a week [190]. After 2 weeks of treatment, rats treated with tDCS had slightly increased cognitive function in comparison to the rats just treated with tacrine. After the 4 weeks of treatment, rats that receive tDCS therapy had motor behavior improvements and increased acetylcholine activity. Improved cognitive function and memory performance effects of repetitive anodal tDCS lasted for two months in a rat model of Alzheimer's [191]. In another study, tDCS when delivered for 20 min/day, 5 days/week over three weeks at 50 μA to triple transgenic (3X Tg) Alzheimer's mice failed to improve memory performance and alter the expression of neuropathological hallmarks of Alzheimer's [192]. Anodal tDCS when delivered for 30 minutes/day over five days at 200 μA alleviated the cognitive impairment, assessed by Morris Water Maze task, in a rat model of vascular dementia [193].

6.4. Chronic Stress and Depression

Though numerous tDCS studies have shown a therapeutic effect in humans and in animal models, the limits to tDCS effects were only recently tested [194]. In this study, tDCS efficacy was measured in chronic stress mice models. After subjecting rats to chronic restraint-induced stress (CRS) for 11 weeks, rats were given 20 min anodal tDCS treatment sessions for 8 days. Behavioral tests were performed after the 11 weeks of CRS, immediately after and 24 h after tDCS treatment. Control rats were not subject to CRS but were randomly given either sham or tDCS treatment. tDCS treatment reversed the stress induced allodynia and increased the pain threshold in unstressed animals. tDCS was only able to decrease BDNF release in the spinal cord and brainstem of unstressed rats. Interestingly, CRS rats treated with tDCS had a weak reduction in pain sensitivity even though no change of BDNF levels was detected indicating that a different mechanism may be involved in the attenuation of pain sensitivity. The results from this study highlight that tDCS treatments alone may not be sufficient to produce long-term effects

when chronic stress is present. Chronic stress-induced pain threshold in rats was evaluated using a hot plate and tail flick latency (TFL) tests. In this study, active bicephalic tDCS increased the pain threshold and thereby reduced stress-induced hyperalgesia [195].

Anodal tDCS, when delivered at 200 μA for ten sessions attenuated depression-like behavior induced by chronic corticosterone exposure in mice and these effects were long-lasting [196]. tDCS at 0.1 mA for 10 minutes was also shown to alleviate depression-like behavior induced by chronic restrained stress in mice [197].

7. Prospects for Animal Research in tDCS/tACS Informing Ongoing Human Trials

A central challenge for tDCS/tACS studies is translating data collected from animal models of tDCS/tACS to inform the interpretation and design of human protocols. Historically, tDCS/ tACS animal studies have informed human testing. Notably, the demonstration that prolonged DCS/ACS protocols, lasting for minutes, in animals can lead to short- and long-term plasticity encouraged the use of such protocols in humans [198]. The polarity dependence of DCS was first demonstrated in animal models [14,23,28]. Animal models demonstrated that low-intensity DCS/ACS can modulate ongoing neuronal activity, which provides a possible physiological substrate for the effects observed in human clinical trials [3]—countering the argument that weak fields, such as those applied in tDCS/tACS, are physiologically inert. In some cases, animal studies of DCS/ACS were conducted contemporaneously with human testing providing confirmatory evidence, for example, that AC can entrain oscillations [180,199] or that tDCS plasticity is NMDA dependent [200]. On the other hand, there are scarce examples of modern animal tES studies influencing how human trials are conducted and analyzed. This reflects how tES protocols have remained largely unchanged with the majority of protocols applying 1–2 mA over 10–30 min using two large pad electrodes without any customization based on an individual's biomarkers.

Developments in tES protocols were driven by clinical neurophysiology [201] rather than extrapolated from animal models. Often animal studies confirm findings in humans rather than suggesting novel improvements to the current protocols; a notable example being the identification of the role of BDNF polymorphism [40]. We believe development in animal tES studies combined with an increased emphasis on designing these experiments for clinical relevance would accelerate the development and application of tES in humans. This includes an increased emphasis of the plastic, rather than acute effects of stimulation [40,202]. Simultaneously, results from human trials also point to a need to critically address issues such as nonlinear dose–response, state dependency, and inter-subject variability.

Animal experiments provide a degree of cellular resolution, state control, and rapid screening not available in human subjects to help detangle complex interactions [3]. We propose that meaningful translation to human applications would be accelerated by the exploration of data that appears, at first glance, to be conflicting between animals and humans. For example, the

acute effects of DCS in animal are monotonic across a very wide intensity and brain-state range, e.g., anodal/cathodal almost always results in excitatory/inhibitory effects after accounting for orientation of neurons relative to the field [14,82]. This is in direct contrast with clinical neurophysiology studies showing that many pharmacological, dose-dependent, and brain-state perpetrators can qualitatively change the direction of neuromodulation [17,201]. As another example, ACS in animals can influence ongoing oscillations in a myriad of ways and is dependent on the nature of endogenous activity and stimulation frequency [19,119,199], while human testing with tACS and EEG are typically limited to testing one or a few frequencies [203]. Rather than speculating which protocols are effective, it would be useful to consider cellular effects from animals in comparison to network effects observed in human studies. The most impactful translational animal studies will be those that explain results from humans in previously unexpected ways and that can suggest non trivial methods to optimize tES outcome in human trials.

References:

1. Datta A, Bansal V, Diaz J, Patel J, Reato D, Bikson M. Gyri-precise head model of transcranial direct current stimulation: Improved spatial focality using a ring electrode versus conventional rectangular pad. Brain Stimulat. 2009;2:201-207.e1.
2. Andreasen M, Nedergaard S. Dendritic electrogenesis in rat hippocampal CA1 pyramidal neurons: functional aspects of Na+ and Ca2+ currents in apical dendrites. Hippocampus. 1996;6:79–95.
3. Bikson M, Inoue M, Akiyama H, Deans JK, Fox JE, Miyakawa H, et al. Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices *in vitro*: Modulation of neuronal function by electric fields. J Physiol. 2004;557:175–90.
4. Radman T, Ramos RL, Brumberg JC, Bikson M. Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. Brain Stimulat. 2009;2:215-228.e3.
5. Deans JK, Powell AD, Jefferys JGR. Sensitivity of coherent oscillations in rat hippocampus to AC electric fields: AC electric fields. J Physiol. 2007;583:555–65.
6. Radman T, Su Y, An JH, Parra LC, Bikson M. Spike Timing Amplifies the Effect of Electric Fields on Neurons: Implications for Endogenous Field Effects. J Neurosci. 2007;27:3030–6.
7. Maeda K, Maruyama R, Nagae T, Inoue M, Aonishi T, Miyakawa H. Weak sinusoidal electric fields entrain spontaneous Ca transients in the dendritic tufts of CA1 pyramidal cells in rat hippocampal slice preparations. PloS One. 2015;10:e0122263.
8. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 2000;527 Pt 3:633–9.
9. Esmaeilpour Z, Marangolo P, Hampstead BM, Bestmann S, Galletta E, Knotkova H, et al. Incomplete evidence that increasing current intensity of tDCS boosts outcomes. Brain Stimulat. 2018;11:310–21.
10. Datta A, Elwassif M, Battaglia F, Bikson M. Transcranial current stimulation focality using disc and ring electrode configurations: FEM analysis. J Neural Eng. 2008;5:163–74.
11. Rahman A, Reato D, Arlotti M, Gasca F, Datta A, Parra LC, et al. Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects: Somatic and terminal origin of DCS effects. J Physiol. 2013;591:2563–78.
12. Kabakov AY, Muller PA, Pascual-Leone A, Jensen FE, Rotenberg A. Contribution of axonal

orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus. J Neurophysiol. 2012;107:1881–9.
13. Carlson C, Devinsky O. The excitable cerebral cortex. Epilepsy Behav. 2009;15:131–2.
14. Terzuolo CA, Bullock TH. MEASUREMENT OF IMPOSED VOLTAGE GRADIENT ADEQUATE TO MODULATE NEURONAL FIRING. Proc Natl Acad Sci. 1956;42:687–94.
15. Creutzfeldt OD, Fromm GH, Kapp H. Influence of transcortical d-c currents on cortical neuronal activity. Exp Neurol. 1962;5:436–52.
16. Fröhlich F, McCormick DA. Endogenous Electric Fields May Guide Neocortical Network Activity. Neuron. 2010;67:129–43.
17. Reato D, Rahman A, Bikson M, Parra LC. Low-Intensity Electrical Stimulation Affects Network Dynamics by Modulating Population Rate and Spike Timing. J Neurosci. 2010;30:15067–79.
18. Ali MM, Sellers KK, Frohlich F. Transcranial Alternating Current Stimulation Modulates Large-Scale Cortical Network Activity by Network Resonance. J Neurosci. 2013;33:11262–75.
19. Reato D, Bikson M, Parra LC. Lasting modulation of in vitro oscillatory activity with weak direct current stimulation. J Neurophysiol. 2015;113:1334–41.
20. Asamoah B, Khatoun A, Mc Laughlin M. tACS motor system effects can be caused by transcutaneous stimulation of peripheral nerves. Nat Commun. 2019;10:266.
21. Vieira PG, Krause MR, Pack CC. tACS entrains neural activity while somatosensory input is blocked [Internet]. Neuroscience; 2019 Jul. Available from: http://biorxiv.org/lookup/doi/10.1101/691022
22. Bindman LJ, Lippold OCJ, Redfearn JWT. Long-lasting Changes in the Level of the Electrical Activity of the Cerebral Cortex produced by Polarizing Currents. Nature. 1962;196:584–5.
23. Bindman LJ, Lippold OCJ, Redfearn JWT. 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. 1964;172:369–82.
24. Purpura DP, Mcmurtry JG. INTRACELLULAR ACTIVITIES AND EVOKED POTENTIAL CHANGES DURING POLARIZATION OF MOTOR CORTEX. J Neurophysiol. 1965;28:166–85.
25. Gartside IB. Mechanisms of Sustained Increases of Firing Rate of Neurones in the Rat Cerebral Cortex after Polarization: Role of Protein Synthesis. Nature. 1968;220:383–4.
26. Kandel ER, editor. Principles of neural science. 5th ed. New York: McGraw-Hill; 2013.
27. Lafon B, Rahman A, Bikson M, Parra LC. Direct Current Stimulation Alters Neuronal Input/Output Function. Brain Stimul Basic Transl Clin Res Neuromodulation. Elsevier; 2017;10:36–45.
28. Chan CY, Hounsgaard J, Nicholson C. Effects of electric fields on transmembrane potential and excitability of turtle cerebellar Purkinje cells in vitro. J Physiol. 1988;402:751–71.
29. Wong RK, Stewart M. Different firing patterns generated in dendrites and somata of CA1 pyramidal neurones in guinea-pig hippocampus. J Physiol. 1992;457:675–87.
30. Delgado-Lezama R, Perrier J-F, Hounsgaard J. Local facilitation of plateau potentials in dendrites of turtle motoneurones by synaptic activation of metabotropic receptors. J Physiol. 1999;515:203–7.
31. Liu A, Vöröslakos M, Kronberg G, Henin S, Krause MR, Huang Y, et al. Immediate neurophysiological effects of transcranial electrical stimulation. Nat Commun. 2018;9:5092.
32. Bullock TH, Hagiwara S. INTRACELLULAR RECORDING FROM THE GIANT SYNAPSE OF THE SQUID. J Gen Physiol. 1957;40:565–77.
33. Takeuchi A, Takeuchi N. Electrical changes in pre- and postsynaptic axons of the giant synapse of Loligo. J Gen Physiol. 1962;45:1181–93.
34. Salvador R, Mekonnen A, Ruffini G, Miranda PC. Modeling the electric field induced in a high resolution realistic head model during transcranial current stimulation. 2010 Annu Int Conf IEEE Eng Med Biol [Internet]. Buenos Aires: IEEE; 2010 [cited 2020 Jun 5]. p. 2073–6. Available from: http://ieeexplore.ieee.org/document/5626315/

35. Jefferys JG. Influence of electric fields on the excitability of granule cells in guinea-pig hippocampal slices. J Physiol. 1981;319:143–52.
36. Chakraborty D, Truong DQ, Bikson M, Kaphzan H. Neuromodulation of Axon Terminals. Cereb Cortex. 2018;28:2786–94.
37. Jackson MP, Rahman A, Lafon B, Kronberg G, Ling D, Parra LC, et al. Animal models of transcranial direct current stimulation: Methods and mechanisms. Clin Neurophysiol. 2016;127:3425–54.
38. Chan CY, Nicholson C. Modulation by applied electric fields of Purkinje and stellate cell activity in the isolated turtle cerebellum. J Physiol. 1986;371:89–114.
39. Rahman A, Lafon B, Parra LC, Bikson M. Direct current stimulation boosts synaptic gain and cooperativity in vitro. J Physiol. 2017;595:3535–47.
40. Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, et al. Direct Current Stimulation Promotes BDNF-Dependent Synaptic Plasticity: Potential Implications for Motor Learning. Neuron. 2010;66:198–204.
41. Svirskis G, Gutman A, Hounsgaard J. Detection of a Membrane Shunt by DC Field Polarization During Intracellular and Whole Cell Recording. J Neurophysiol. 1997;77:579–86.
42. Aberra AS, Peterchev AV, Grill WM. Biophysically realistic neuron models for simulation of cortical stimulation. J Neural Eng. 2018;15:066023.
43. Huang Y, Liu AA, Lafon B, Friedman D, Dayan M, Wang X, et al. Measurements and models of electric fields in the in vivo human brain during transcranial electric stimulation. eLife. 2017;6:e18834.
44. Vöröslakos M, Takeuchi Y, Brinyiczki K, Zombori T, Oliva A, Fernández-Ruiz A, et al. Direct effects of transcranial electric stimulation on brain circuits in rats and humans. Nat Commun. 2018;9:483.
45. Anastassiou CA, Montgomery SM, Barahona M, Buzsaki G, Koch C. The Effect of Spatially Inhomogeneous Extracellular Electric Fields on Neurons. J Neurosci. 2010;30:1925–36.
46. Krause MR, Zanos TP, Csorba BA, Pilly PK, Choe J, Phillips ME, et al. Transcranial Direct Current Stimulation Facilitates Associative Learning and Alters Functional Connectivity in the Primate Brain. Curr Biol. 2017;27:3086-3096.e3.
47. Krause MR, Vieira PG, Csorba BA, Pilly PK, Pack CC. Transcranial alternating current stimulation entrains single-neuron activity in the primate brain. Proc Natl Acad Sci. 2019;116:5747–55.
48. Cabral ME, Baltar A, Borba R, Galvão S, Santos L, Fregni F, et al. Transcranial direct current stimulation: before, during, or after motor training? Neuroreport. 2015;26:618–22.
49. Gluckman BJ, Neel EJ, Netoff TI, Ditto WL, Spano ML, Schiff SJ. Electric field suppression of epileptiform activity in hippocampal slices. J Neurophysiol. 1996;76:4202–5.
50. Ghai RS, Bikson M, Durand DM. Effects of Applied Electric Fields on Low-Calcium Epileptiform Activity in the CA1 Region of Rat Hippocampal Slices. J Neurophysiol. 2000;84:274–80.
51. Durand DM, Bikson M. Suppression and control of epileptiform activity by electrical stimulation: a review. Proc IEEE. 2001;89:1065–82.
52. Su Y, Radman T, Vaynshteyn J, Parra LC, Bikson M. Effects of high-frequency stimulation on epileptiform activity in vitro: ON/OFF control paradigm. Epilepsia. 2008;49:1586–93.
53. Sunderam S, Gluckman B, Reato D, Bikson M. Toward rational design of electrical stimulation strategies for epilepsy control. Epilepsy Behav. 2010;17:6–22.
54. Lisman JE, McIntyre CC. Synaptic plasticity: a molecular memory switch. Curr Biol CB. 2001;11:R788-791.
55. Kronberg G, Rahman A, Sharma M, Bikson M, Parra LC. Direct current stimulation boosts hebbian plasticity in vitro. Brain Stimulat. 2020;13:287–301.
56. Kronberg G, Bridi M, Abel T, Bikson M, Parra LC. Direct Current Stimulation Modulates LTP and LTD: Activity Dependence and Dendritic Effects. Brain Stimulat. 2017;10:51–8.

57. Nitsche MA, Müller-Dahlhaus F, Paulus W, Ziemann U. The pharmacology of neuroplasticity induced by non-invasive brain stimulation: building models for the clinical use of CNS active drugs. J Physiol. 2012;590:4641–62.
58. Mosayebi Samani M, Agboada D, Jamil A, Kuo M-F, Nitsche MA. Titrating the neuroplastic effects of cathodal transcranial direct current stimulation (tDCS) over the primary motor cortex. Cortex. 2019;119:350–61.
59. Gartside IB. Mechanisms of sustained increases of firing rate of neurones in the rat cerebral cortex after polarization: role of protein synthesis. Nature. 1968;220:383–4.
60. Gartside IB. Mechanisms of sustained increases of firing rate of neurons in the rat cerebral cortex after polarization: reverberating circuits or modification of synaptic conductance? Nature. 1968;220:382–3.
61. Redfearn JW, Lippold OC, Costain R. A PRELIMINARY ACCOUNT OF THE CLINICAL EFFECTS OF POLARIZING THE BRAIN IN CERTAIN PSYCHIATRIC DISORDERS. Br J Psychiatry J Ment Sci. 1964;110:773–85.
62. Costain R, Redfearn JW, Lippold OC. A CONTROLLED TRIAL OF THE THERAPEUTIC EFFECT OF POLARIZATION OF THE BRAIN IN DEPRESSIVE ILLNESS. Br J Psychiatry J Ment Sci. 1964;110:786–99.
63. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 2000;527 Pt 3:633–9.
64. Nitsche MA, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W. Safety criteria for transcranial direct current stimulation (tDCS) in humans. Clin Neurophysiol Off J Int Fed Clin Neurophysiol. 2003;114:2220–2; author reply 2222-2223.
65. Reato D, Bikson M, Parra LC. Lasting modulation of in vitro oscillatory activity with weak direct current stimulation. J Neurophysiol. 2015;113:1334–41.
66. Ardolino G, Bossi B, Barbieri S, Priori A. Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. J Physiol. 2005;568:653–63.
67. Marquez-Ruiz J, Leal-Campanario R, Sanchez-Campusano R, Molaee-Ardekani B, Wendling F, Miranda PC, et al. Transcranial direct-current stimulation modulates synaptic mechanisms involved in associative learning in behaving rabbits. Proc Natl Acad Sci. 2012;109:6710–5.
68. Kronberg G, Rahman A, Sharma M, Bikson M, Parra LC. Direct current stimulation boosts hebbian plasticity in vitro. Brain Stimulat. 2020;13:287–301.
69. Das S, Spoor M, Sibindi TM, Holland P, Schonewille M, De Zeeuw CI, et al. Impairment of Long-Term Plasticity of Cerebellar Purkinje Cells Eliminates the Effect of Anodal Direct Current Stimulation on Vestibulo-Ocular Reflex Habituation. Front Neurosci. 2017;11:444.
70. Boroda E, Sponheim SR, Fiecas M, Lim KO. Transcranial direct current stimulation (tDCS) elicits stimulus-specific enhancement of cortical plasticity. NeuroImage. 2020;211:116598.
71. Sun Y, Lipton JO, Boyle LM, Madsen JR, Goldenberg MC, Pascual-Leone A, et al. Direct current stimulation induces mGluR5-dependent neocortical plasticity. Ann Neurol. 2016;80:233–46.
72. Paciello F, Podda MV, Rolesi R, Cocco S, Petrosini L, Troiani D, et al. Anodal transcranial direct current stimulation affects auditory cortex plasticity in normal-hearing and noise-exposed rats. Brain Stimulat. 2018;11:1008–23.
73. Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP. Strengthening of horizontal cortical connections following skill learning. Nat Neurosci. 1998;1:230–4.
74. Hess G, Donoghue JP. Facilitation of long-term potentiation in layer II/III horizontal connections of rat motor cortex following layer I stimulation: route of effect and cholinergic contributions. Exp Brain Res. 1999;127:279–90.
75. Bae EB, Lee JH, Song J-J. Single-Session of Combined tDCS-TMS May Increase Therapeutic Effects in Subjects With Tinnitus. Front Neurol. 2020;11:160.

76. Milot M-H, Palimeris S, Corriveau H, Tremblay F, Boudrias M-H. Effects of a tailored strength training program of the upper limb combined with transcranial direct current stimulation (tDCS) in chronic stroke patients: study protocol for a randomised, double-blind, controlled trial. BMC Sports Sci Med Rehabil. 2019;11:8.
77. Han T, Xu Z, Liu C, Li S, Song P, Huang Q, et al. Simultaneously applying cathodal tDCS with low frequency rTMS at the motor cortex boosts inhibitory aftereffects. J Neurosci Methods. 2019;324:108308.
78. Nemanich ST, Rich TL, Chen C-Y, Menk J, Rudser K, Chen M, et al. Influence of Combined Transcranial Direct Current Stimulation and Motor Training on Corticospinal Excitability in Children With Unilateral Cerebral Palsy. Front Hum Neurosci. 2019;13:137.
79. Bolognini N, Fregni F, Casati C, Olgiati E, Vallar G. Brain polarization of parietal cortex augments training-induced improvement of visual exploratory and attentional skills. Brain Res. 2010;1349:76–89.
80. Artola A, Bröcher S, Singer W. Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. Nature. 1990;347:69–72.
81. Abraham WC. Metaplasticity: tuning synapses and networks for plasticity. Nat Rev Neurosci. 2008;9:387.
82. Ranieri F, Podda MV, Riccardi E, Frisullo G, Dileone M, Profice P, et al. Modulation of LTP at rat hippocampal CA3-CA1 synapses by direct current stimulation. J Neurophysiol. 2012;107:1868–80.
83. Reato D, Rahman A, Bikson M, Parra LC. Effects of weak transcranial alternating current stimulation on brain activity—a review of known mechanisms from animal studies. Front Hum Neurosci [Internet]. 2013 [cited 2020 Jun 5];7. Available from: http://journal.frontiersin.org/article/10.3389/fnhum.2013.00687/abstract
84. Frey U, Morris RG. Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. Trends Neurosci. 1998;21:181–8.
85. Barco A, Lopez de Armentia M, Alarcon JM. Synapse-specific stabilization of plasticity processes: the synaptic tagging and capture hypothesis revisited 10 years later. Neurosci Biobehav Rev. 2008;32:831–51.
86. Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, et al. Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. J Neurosci Off J Soc Neurosci. 2004;24:3379–85.
87. Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. Exp Brain Res. 2004;156:439–43.
88. Mozzachiodi R, Byrne JH. More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. Trends Neurosci. 2010;33:17–26.
89. Bliss TV, Gardner-Medwin AR. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol. 1973;232:357–74.
90. Islam N, Aftabuddin M, Moriwaki A, Hattori Y, Hori Y. Increase in the calcium level following anodal polarization in the rat brain. Brain Res. 1995;684:206–8.
91. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004;44:5–21.
92. Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol. 2003;553:293–301.
93. Rohan JG, Carhuatanta KA, McInturf SM, Miklasevich MK, Jankord R. Modulating Hippocampal Plasticity with In Vivo Brain Stimulation. J Neurosci Off J Soc Neurosci. 2015;35:12824–32.
94. Lu B. BDNF and activity-dependent synaptic modulation. Learn Mem Cold Spring Harb N.

2003;10:86–98.
95. Yu T-H, Wu Y-J, Chien M-E, Hsu K-S. Transcranial direct current stimulation induces hippocampal metaplasticity mediated by brain-derived neurotrophic factor. Neuropharmacology. 2019;144:358–67.
96. Islam N, Aftabuddin M, Moriwaki A, Hori Y. Induction of N-methyl-D-asparate receptor mediated c-fos protein in the rat brain by incomplete ischaemia. Indian J Med Res. 1994;100:281–8.
97. Pérez-Cadahía B, Drobic B, Davie JR. Activation and function of immediate-early genes in the nervous system. Biochem Cell Biol Biochim Biol Cell. 2011;89:61–73.
98. Abraham WC, Dragunow M, Tate WP. The role of immediate early genes in the stabilization of long-term potentiation. Mol Neurobiol. 1991;5:297–314.
99. Abraham WC, Christie BR, Logan B, Lawlor P, Dragunow M. Immediate early gene expression associated with the persistence of heterosynaptic long-term depression in the hippocampus. Proc Natl Acad Sci U S A. 1994;91:10049–53.
100. Cole AJ, Saffen DW, Baraban JM, Worley PF. Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. Nature. 1989;340:474–6.
101. Jones MW, Errington ML, French PJ, Fine A, Bliss TV, Garel S, et al. A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. Nat Neurosci. 2001;4:289–96.
102. McCaig CD, Rajnicek AM. Electrical fields, nerve growth and nerve regeneration. Exp Physiol. 1991;76:473–94.
103. McCaig CD, Rajnicek AM, Song B, Zhao M. Controlling cell behavior electrically: current views and future potential. Physiol Rev. 2005;85:943–78.
104. Zhao M. Electrical fields in wound healing-An overriding signal that directs cell migration. Semin Cell Dev Biol. 2009;20:674–82.
105. Pelletier SJ, Lagacé M, St-Amour I, Arsenault D, Cisbani G, Chabrat A, et al. The morphological and molecular changes of brain cells exposed to direct current electric field stimulation. Int J Neuropsychopharmacol. 2014;18.
106. Marsh G, Beams HW. In vitro control of growing chick nerve fibers by applied electric currents. J Cell Comp Physiol. 1946;27:139–57.
107. Jaffe LF, Poo MM. Neurites grow faster towards the cathode than the anode in a steady field. J Exp Zool. 1979;209:115–28.
108. Pomeranz B, Mullen M, Markus H. Effect of applied electrical fields on sprouting of intact saphenous nerve in adult rat. Brain Res. 1984;303:331–6.
109. McDevitt L, Fortner P, Pomeranz B. Application of weak electric field to the hindpaw enhances sciatic motor nerve regeneration in the adult rat. Brain Res. 1987;416:308–14.
110. Politis MJ, Zanakis MF, Albala BJ. Facilitated regeneration in the rat peripheral nervous system using applied electric fields. J Trauma. 1988;28:1375–81.
111. Román GC, Strahlendorf HK, Coates PW, Rowley BA. Stimulation of sciatic nerve regeneration in the adult rat by low-intensity electric current. Exp Neurol. 1987;98:222–32.
112. Beveridge JA, Politis MJ. Use of exogenous electric current in the treatment of delayed lesions in peripheral nerves. Plast Reconstr Surg. 1988;82:573–9.
113. McCaig CD. Dynamic aspects of amphibian neurite growth and the effects of an applied electric field. J Physiol. 1986;375:55–69.
114. Stollberg J, Fraser SE. Acetylcholine receptors and concanavalin A-binding sites on cultured Xenopus muscle cells: electrophoresis, diffusion, and aggregation. J Cell Biol. 1988;107:1397–408.
115. Stollberg J, Fraser SE. Local accumulation of acetylcholine receptors is neither necessary nor sufficient to induce cluster formation. J Neurosci Off J Soc Neurosci. 1990;10:247–55.
116. Jiang T, Xu RX, Zhang AW, Di W, Xiao ZJ, Miao JY, et al. Effects of transcranial direct

current stimulation on hemichannel pannexin-1 and neural plasticity in rat model of cerebral infarction. Neuroscience. 2012;226:421–6.
117. Gellner A-K, Reis J, Holtick C, Schubert C, Fritsch B. Direct current stimulation-induced synaptic plasticity in the sensorimotor cortex: structure follows function. Brain Stimulat. 2020;13:80–8.
118. Yoon KJ, Oh B-M, Kim D-Y. Functional improvement and neuroplastic effects of anodal transcranial direct current stimulation (tDCS) delivered 1day vs. 1week after cerebral ischemia in rats. Brain Res. 2012;1452:61–72.
119. Fröhlich F. Experiments and models of cortical oscillations as a target for noninvasive brain stimulation. Prog Brain Res [Internet]. Elsevier; 2015 [cited 2020 Jun 7]. p. 41–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0079612315001314
120. Sejnowski TJ, Destexhe A. Why do we sleep?11Published on the World Wide Web on 7 November 2000. Brain Res. 2000;886:208–23.
121. Gebodh N, Esmaeilpour Z, Adair D, Chelette K, Dmochowski J, Woods AJ, et al. Inherent physiological artifacts in EEG during tDCS. NeuroImage. 2019;185:408–24.
122. Antal A, Varga ET, Kincses TZ, Nitsche MA, Paulus W. Oscillatory brain activity and transcranial direct current stimulation in humans. Neuroreport. 2004;15:1307–10.
123. Herrmann CS, Rach S, Neuling T, Strüber D. Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. Front Hum Neurosci. 2013;7:279.
124. Schmidt SL, Iyengar AK, Foulser AA, Boyle MR, Fröhlich F. Endogenous Cortical Oscillations Constrain Neuromodulation by Weak Electric Fields. Brain Stimulat. 2014;7:878–89.
125. Ozen S, Sirota A, Belluscio MA, Anastassiou CA, Stark E, Koch C, et al. Transcranial Electric Stimulation Entrains Cortical Neuronal Populations in Rats. J Neurosci. 2010;30:11476–85.
126. Negahbani E, Stitt IM, Davey M, Doan TT, Dannhauer M, Hoover AC, et al. Transcranial Alternating Current Stimulation (tACS) Entrains Alpha Oscillations by Preferential Phase Synchronization of Fast-Spiking Cortical Neurons to Stimulation Waveform [Internet]. Neuroscience; 2019 Feb. Available from: http://biorxiv.org/lookup/doi/10.1101/563163
127. Esmaeilpour Z, Kronberg G, Reato D, Parra LC, Bikson M. Temporal interference stimulation targets deep brain regions by modulating neural oscillations [Internet]. Neuroscience; 2019 Dec. Available from: http://biorxiv.org/lookup/doi/10.1101/2019.12.25.888412
128. Grossman N, Bono D, Dedic N, Kodandaramaiah SB, Rudenko A, Suk H-J, et al. Noninvasive Deep Brain Stimulation via Temporally Interfering Electric Fields. Cell. 2017;169:1029-1041.e16.
129. Kar K, Duijnhouwer J, Krekelberg B. Transcranial Alternating Current Stimulation Attenuates Neuronal Adaptation. J Neurosci. 2017;37:2325–35.
130. Freund TF, Buzsáki G. Interneurons of the hippocampus. Hippocampus. 1996;6:347–470.
131. Kepecs A, Fishell G. Interneuron cell types are fit to function. Nature. 2014;505:318–26.
132. von Bartheld CS, Bahney J, Herculano-Houzel S. The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting: Quantifying neurons and glia in human brain. J Comp Neurol. 2016;524:3865–95.
133. Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D, et al. Local Ca2+ detection and modulation of synaptic release by astrocytes. Nat Neurosci. 2011;14:1276–84.
134. Gellner A-K, Reis J, Fritsch B. Glia: A Neglected Player in Non-invasive Direct Current Brain Stimulation. Front Cell Neurosci [Internet]. 2016 [cited 2020 Jun 5];10. Available from: http://journal.frontiersin.org/Article/10.3389/fncel.2016.00188/abstract
135. Ruohonen J, Karhu J. tDCS possibly stimulates glial cells. Clin Neurophysiol Off J Int Fed Clin Neurophysiol. 2012;123:2006–9.
136. Dallérac G, Chever O, Rouach N. How do astrocytes shape synaptic transmission? Insights

from electrophysiology. Front Cell Neurosci [Internet]. 2013 [cited 2020 Jun 4];7. Available from: http://journal.frontiersin.org/article/10.3389/fncel.2013.00159/abstract
137. Gardner-Medwin AR, Nicholson C. Changes of extracellular potassium activity induced by electric current through brain tissue in the rat. J Physiol. 1983;335:375–92.
138. Lian J, Bikson M, Sciortino C, Stacey WC, Durand DM. Local suppression of epileptiform activity by electrical stimulation in rat hippocampus in vitro. J Physiol. 2003;547:427–34.
139. An JH, Su Y, Radman T, Bikson M. Effects of glucose and glutamine concentration in the formulation of the artificial cerebrospinal fluid (ACSF). Brain Res. 2008;1218:77–86.
140. Pasti L, Volterra A, Pozzan T, Carmignoto G. Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes in situ. J Neurosci Off J Soc Neurosci. 1997;17:7817–30.
141. Poskanzer KE, Yuste R. Astrocytic regulation of cortical UP states. Proc Natl Acad Sci. 2011;108:18453–8.
142. Padmashri R, Suresh A, Boska MD, Dunaevsky A. Motor-Skill Learning Is Dependent on Astrocytic Activity. Neural Plast. 2015;2015:938023.
143. Monai H, Ohkura M, Tanaka M, Oe Y, Konno A, Hirai H, et al. Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. Nat Commun. 2016;7:11100.
144. Schafer DP, Lehrman EK, Stevens B. The “quad-partite” synapse: microglia-synapse interactions in the developing and mature CNS. Glia. 2013;61:24–36.
145. Li Q, Brus-Ramer M, Martin JH, McDonald JW. Electrical stimulation of the medullary pyramid promotes proliferation and differentiation of oligodendrocyte progenitor cells in the corticospinal tract of the adult rat. Neurosci Lett. 2010;479:128–33.
146. Braun R, Klein R, Walter HL, Ohren M, Freudenmacher L, Getachew K, et al. Transcranial direct current stimulation accelerates recovery of function, induces neurogenesis and recruits oligodendrocyte precursors in a rat model of stroke. Exp Neurol. 2016;279:127–36.
147. Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, et al. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature. 2006;442:457–60.
148. Li J, Nandagopal S, Wu D, Romanuik SF, Paul K, Thomson DJ, et al. Activated T lymphocytes migrate toward the cathode of DC electric fields in microfluidic devices. Lab Chip. 2011;11:1298–304.
149. Orida N, Feldman JD. Directional protrusive pseudopodial activity and motility in macrophages induced by extracellular electric fields. Cell Motil. 1982;2:243–55.
150. Franke K, Gruler H. Galvanotaxis of human granulocytes: electric field jump studies. Eur Biophys J EBJ. 1990;18:335–46.
151. Pelletier SJ, Cicchetti F. Cellular and molecular mechanisms of action of transcranial direct current stimulation: evidence from in vitro and in vivo models. Int J Neuropsychopharmacol. 2014;18.
152. Huang R, Peng L, Hertz L. Effects of a low-voltage static electric field on energy metabolism in astrocytes. Bioelectromagnetics. 1997;18:77–80.
153. Borgens RB, Shi R, Mohr TJ, Jaeger CB. Mammalian cortical astrocytes align themselves in a physiological voltage gradient. Exp Neurol. 1994;128:41–9.
154. Alexander JK, Fuss B, Colello RJ. Electric field-induced astrocyte alignment directs neurite outgrowth. Neuron Glia Biol. 2006;2:93–103.
155. Rueger MA, Keuters MH, Walberer M, Braun R, Klein R, Sparing R, et al. Multi-session transcranial direct current stimulation (tDCS) elicits inflammatory and regenerative processes in the rat brain. PloS One. 2012;7:e43776.
156. Guo T, Fang J, Tong ZY, He S, Luo Y. Transcranial Direct Current Stimulation Ameliorates Cognitive Impairment via Modulating Oxidative Stress, Inflammation, and Autophagy in a Rat Model of Vascular Dementia. Front Neurosci. 2020;14:28.
157. Zhang K, Guo L, Zhang J, Rui G, An G, Zhou Y, et al. tDCS Accelerates the Rehabilitation

of MCAO-Induced Motor Function Deficits via Neurogenesis Modulated by the Notch1 Signaling Pathway. Neurorehabil Neural Repair. 2020;1545968320925474.
158. de Oliveira C, de Freitas JS, Macedo IC, Scarabelot VL, Ströher R, Santos DS, et al. Transcranial direct current stimulation (tDCS) modulates biometric and inflammatory parameters and anxiety-like behavior in obese rats. Neuropeptides. 2019;73:1–10.
159. Kanno S, Oda N, Abe M, Saito S, Hori K, Handa Y, et al. Establishment of a simple and practical procedure applicable to therapeutic angiogenesis. Circulation. 1999;99:2682–7.
160. Bai H, McCaig CD, Forrester JV, Zhao M. DC electric fields induce distinct preangiogenic responses in microvascular and macrovascular cells. Arterioscler Thromb Vasc Biol. 2004;24:1234–9.
161. Zhao M, Bai H, Wang E, Forrester JV, McCaig CD. Electrical stimulation directly induces pre-angiogenic responses in vascular endothelial cells by signaling through VEGF receptors. J Cell Sci. 2004;117:397–405.
162. Zhao Z, Qin L, Reid B, Pu J, Hara T, Zhao M. Directing migration of endothelial progenitor cells with applied DC electric fields. Stem Cell Res. 2012;8:38–48.
163. Long H, Yang G, Wang Z. Galvanotactic migration of EA.Hy926 endothelial cells in a novel designed electric field bioreactor. Cell Biochem Biophys. 2011;61:481–91.
164. Chen Y, Ye L, Guan L, Fan P, Liu R, Liu H, et al. Physiological electric field works via the VEGF receptor to stimulate neovessel formation of vascular endothelial cells in a 3D environment. Biol Open. 2018;7.
165. Cunha F, Rajnicek AM, McCaig CD. Electrical Stimulation Directs Migration, Enhances and Orients Cell Division and Upregulates the Chemokine Receptors CXCR4 and CXCR2 in Endothelial Cells. J Vasc Res. 2019;56:39–53.
166. Bai H, Forrester JV, Zhao M. DC electric stimulation upregulates angiogenic factors in endothelial cells through activation of VEGF receptors. Cytokine. 2011;55:110–5.
167. Zhang P, Liu Z-T, He G-X, Liu J-P, Feng J. Low-voltage direct-current stimulation is safe and promotes angiogenesis in rabbits with myocardial infarction. Cell Biochem Biophys. 2011;59:19–27.
168. Baba T, Kameda M, Yasuhara T, Morimoto T, Kondo A, Shingo T, et al. Electrical stimulation of the cerebral cortex exerts antiapoptotic, angiogenic, and anti-inflammatory effects in ischemic stroke rats through phosphoinositide 3-kinase/Akt signaling pathway. Stroke. 2009;40:e598-605.
169. Ganju RK, Brubaker SA, Meyer J, Dutt P, Yang Y, Qin S, et al. The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. J Biol Chem. 1998;273:23169–75.
170. Sai J, Fan G-H, Wang D, Richmond A. The C-terminal domain LLKIL motif of CXCR2 is required for ligand-mediated polarization of early signals during chemotaxis. J Cell Sci. 2004;117:5489–96.
171. Shin DW, Fan J, Luu E, Khalid W, Xia Y, Khadka N, et al. In Vivo Modulation of the Blood-Brain Barrier Permeability by Transcranial Direct Current Stimulation (tDCS). Ann Biomed Eng. 2020;48:1256–70.
172. Peruzzotti-Jametti L, Cambiaghi M, Bacigaluppi M, Gallizioli M, Gaude E, Mari S, et al. Safety and efficacy of transcranial direct current stimulation in acute experimental ischemic stroke. Stroke. 2013;44:3166–74.
173. Jennings J, Chen D, Feldman D. Transcriptional response of dermal fibroblasts in direct current electric fields. Bioelectromagnetics. 2008;29:394–405.
174. Hu M, Hong L, He S, Huang G, Cheng Y, Chen Q. Effects of electrical stimulation on cell activity, cell cycle, cell apoptosis and β- catenin pathway in the injured dorsal root ganglion cell. Mol Med Rep. 2020;21:2385–94.
175. Hoseinzadeh E, Wei C, Farzadkia M, Rezaee A. Effects of Low Frequency-Low Voltage Alternating Electric Current on Apoptosis Progression in Bioelectrical Reactor Biofilm. Front Bioeng Biotechnol. 2020;8:2.

176. Mondino M, Bennabi D, Poulet E, Galvao F, Brunelin J, Haffen E. Can transcranial direct current stimulation (tDCS) alleviate symptoms and improve cognition in psychiatric disorders? World J Biol Psychiatry Off J World Fed Soc Biol Psychiatry. 2014;15:261–75.
177. Tortella G, Casati R, Aparicio LVM, Mantovani A, Senço N, D'Urso G, et al. Transcranial direct current stimulation in psychiatric disorders. World J Psychiatry. 2015;5:88–102.
178. Dedoncker J, Brunoni AR, Baeken C, Vanderhasselt M-A. A Systematic Review and Meta-Analysis of the Effects of Transcranial Direct Current Stimulation (tDCS) Over the Dorsolateral Prefrontal Cortex in Healthy and Neuropsychiatric Samples: Influence of Stimulation Parameters. Brain Stimulat. 2016;9:501–17.
179. Harris DM, Rantalainen T, Muthalib M, Johnson L, Duckham RL, Smith ST, et al. Concurrent exergaming and transcranial direct current stimulation to improve balance in people with Parkinson's disease: study protocol for a randomised controlled trial. Trials. 2018;19:387.
180. Zaehle T, Rach S, Herrmann CS. Transcranial Alternating Current Stimulation Enhances Individual Alpha Activity in Human EEG. Aleman A, editor. PLoS ONE. 2010;5:e13766.
181. Esse Wilson J, Trumbo MC, Wilson JK, Tesche CD. Transcranial direct current stimulation (tDCS) over right temporoparietal junction (rTPJ) for social cognition and social skills in adults with autism spectrum disorder (ASD). J Neural Transm Vienna Austria 1996. 2018;125:1857–66.
182. Leffa DT, Bellaver B, Salvi AA, de Oliveira C, Caumo W, Grevet EH, et al. Transcranial direct current stimulation improves long-term memory deficits in an animal model of attention-deficit/hyperactivity disorder and modulates oxidative and inflammatory parameters. Brain Stimulat. 2018;11:743–51.
183. Valero-Cabré A, Sanches C, Godard J, Fracchia O, Dubois B, Levy R, et al. Language boosting by transcranial stimulation in progressive supranuclear palsy. Neurology. 2019;93:e537–47.
184. Fedorov A, Chibisova Y, Szymaszek A, Alexandrov M, Gall C, Sabel BA. Non-invasive alternating current stimulation induces recovery from stroke. Restor Neurol Neurosci. 2010;28:825–33.
185. Boonzaier J, van Tilborg GAF, Neggers SFW, Dijkhuizen RM. Noninvasive Brain Stimulation to Enhance Functional Recovery After Stroke: Studies in Animal Models. Neurorehabil Neural Repair. 2018;32:927–40.
186. Notturno F, Marzetti L, Pizzella V, Uncini A, Zappasodi F. Local and remote effects of transcranial direct current stimulation on the electrical activity of the motor cortical network: tDCS Effects on Motor Network EEG Activity. Hum Brain Mapp. 2014;35:2220–32.
187. Pedron S, Monnin J, Haffen E, Sechter D, Van Waes V. Repeated transcranial direct current stimulation prevents abnormal behaviors associated with abstinence from chronic nicotine consumption. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol. 2014;39:981–8.
188. Pedron S, Beverley J, Haffen E, Andrieu P, Steiner H, Van Waes V. Transcranial direct current stimulation produces long-lasting attenuation of cocaine-induced behavioral responses and gene regulation in corticostriatal circuits. Addict Biol. 2017;22:1267–78.
189. Surowka AD, Ziomber A, Czyzycki M, Migliori A, Kasper K, Szczerbowska-Boruchowska M. Molecular and elemental effects underlying the biochemical action of transcranial direct current stimulation (tDCS) in appetite control. Spectrochim Acta A Mol Biomol Spectrosc. 2018;195:199–209.
190. Yu SH, Park SD, Sim KC. The Effect of tDCS on Cognition and Neurologic Recovery of Rats with Alzheimer's Disease. J Phys Ther Sci. 2014;26:247–9.
191. Yang W-J, Wen H-Z, Zhou L-X, Luo Y-P, Hou W-S, Wang X, et al. After-effects of repetitive anodal transcranial direct current stimulation on learning and memory in a rat model of Alzheimer's disease. Neurobiol Learn Mem. 2019;161:37–45.
192. Gondard E, Soto-Montenegro ML, Cassol A, Lozano AM, Hamani C. Transcranial direct

current stimulation does not improve memory deficits or alter pathological hallmarks in a rodent model of Alzheimer's disease. J Psychiatr Res. 2019;114:93–8.
193. Guo T, Fang J, Tong ZY, He S, Luo Y. Transcranial Direct Current Stimulation Ameliorates Cognitive Impairment via Modulating Oxidative Stress, Inflammation, and Autophagy in a Rat Model of Vascular Dementia. Front Neurosci. 2020;14:28.
194. Spezia Adachi LN, Quevedo AS, de Souza A, Scarabelot VL, Rozisky JR, de Oliveira C, et al. Exogenously induced brain activation regulates neuronal activity by top-down modulation: conceptualized model for electrical brain stimulation. Exp Brain Res. 2015;233:1377–89.
195. Fregni F, Macedo IC, Spezia-Adachi LN, Scarabelot VL, Laste G, Souza A, et al. Transcranial direct current stimulation (tDCS) prevents chronic stress-induced hyperalgesia in rats. Brain Stimulat. 2018;11:299–301.
196. Peanlikhit T, Van Waes V, Pedron S, Risold P-Y, Haffen E, Etiévant A, et al. The antidepressant-like effect of tDCS in mice: A behavioral and neurobiological characterization. Brain Stimulat. 2017;10:748–56.
197. Monai H, Ohkura M, Tanaka M, Oe Y, Konno A, Hirai H, et al. Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. Nat Commun. 2016;7:11100.
198. Dhamne SC, Ekstein D, Zhuo Z, Gersner R, Zurakowski D, Loddenkemper T, et al. Acute seizure suppression by transcranial direct current stimulation in rats. Ann Clin Transl Neurol. 2015;2:843–56.
199. Abd Hamid AI, Gall C, Speck O, Antal A, Sabel BA. Effects of alternating current stimulation on the healthy and diseased brain. Front Neurosci. 2015;9:391.
200. Rroji O, van Kuyck K, Nuttin B, Wenderoth N. Anodal tDCS over the Primary Motor Cortex Facilitates Long-Term Memory Formation Reflecting Use-Dependent Plasticity. PloS One. 2015;10:e0127270.
201. Brunoni AR, Nitsche MA, Bolognini N, Bikson M, Wagner T, Merabet L, et al. Clinical research with transcranial direct current stimulation (tDCS): Challenges and future directions. Brain Stimulat. 2012;5:175–95.
202. Jefferys JGR, Deans J, Bikson M, Fox J. Effects of weak electric fields on the activity of neurons and neuronal networks. Radiat Prot Dosimetry. 2003;106:321–3.
203. Müller NG, Vellage A-K, Heinze H-J, Zaehle T. Entrainment of Human Alpha Oscillations Selectively Enhances Visual Conjunction Search. PloS One. 2015;10:e0143533.