Chapter 3
Mechanisms of Acute and After Effects of Transcranial Direct Current Stimulation

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Introduction

Understanding the mechanisms of action of central nervous system modulation with direct current stimulation (DCS) is an important endeavor, given the increasing usage of tDCS as a research tool in basic and applied studies, including trials exploring clinical potential. The scale and breadth of tDCS research requires careful consideration of tDCS mechanisms, namely tDCS-induced alterations of physiology and morphology to understand trial results and develop a consensus of its application as a research or treatment tool. In the absence of such understanding, retrospective or meta-analysis can be misguided, for example grouping studies that use different tDCS protocols, which are known from mechanistic studies to produce different and sometimes even opposite functional changes. Leveraging insights on mechanism will support the design of stimulation protocols resulting in optimized functional outcome, especially for clinical application. The parameter space for tDCS protocols (spanning

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H. Knotkova et al. (eds.), *Practical Guide to Transcranial Direct Current Stimulation*, https://doi.org/10.1007/978-3-319-95948-1_3

not just variation in dose but also when/how tDCS is combined with training) makes discovery of best practices (by trial-and-error) in human trials impractical. Indeed, many ongoing trials are encouraging, as modern insights on mechanism are integrated into trial design and significant increases in efficacy can be expected. Similarly, real population level effects of tDCS are reduced in effect-size by inter-subject variability – understanding tDCS mechanisms helps to explain this variability and point the way forward to individualize tDCS (including use of biomarkers) (Strube et al. 2016).

There is a broad base of knowledge regarding the mechanisms of action of tDCS, which spans decades but has rapidly increased during the last years. The first critical description of physiological and functional effects of DCS dates back to the 50s and 60s of the twentieth century in animal models and humans (for an overview see Nitsche et al. (2003a)). This work helped to define basic mechanisms including established polarity specific effects on both acute and lasting activity. The early stimulation approaches were then nearly forgotten until the turn of the century. Interest in tDCS was then increasing again, mainly based on experiments in humans showing neuroplastic effects of tDCS. Based on established neurophysiological effects in man predominantly derived from motor evoked potential studies – including polarity specific lasting changes – additional trials demonstrated effects on behavior and cognitive processes, as well as clinical symptoms in patients suffering from neurological and psychiatric symptoms. The demonstration that tDCS could influence a wide range of behaviors and disorders, spurred further research regarding identification of the mechanistic foundations and thus modeling as well as animal studies and experiments in humans were conducted to this aim. This work reinforced the basic findings on polarity specific changes in acute function (e.g. synaptic efficacy) as well as the modulation of plasticity; but modern mechanistic studies have focused on developing a deeper and more subtle understanding of mechanism including identification of new cellular targets, molecular cascades, forms of plasticity (e.g. long-term potentiation [LTP] vs. long-term depression [LTD]) dose response (at time non-linear), and a more subtle understanding on how tDCS can be specific to various indications (e.g. “functional targeting”; (Bikson and Rahman 2013)).

Studying the mechanisms of tDCS can be approached from various scales and is relevant for understanding the effects of DCS; these can be discerned into studies exploring the effects of tDCS at the microscopic (molecular, cellular), mesoscopic (small neuronal networks, defined cortical areas), and macroscopic (whole brain effects including functional connectivity) level (Rahman et al. 2013). For comprehension of the effects of tDCS, combining all these levels ranging from single cells in animal brain slices to large-scale brain networks in human and ultimately cognition and behavior is relevant, and different experimental approaches are suited to explore tDCS effects at these different levels of complexity (Fig. 3.1). In addition to considerations of scale, in regards to time the mechanisms of tDCS can be discerned in acute or primary effects, which emerge directly during stimulation, and after or secondary effects, which develop during stimulation, but outlast the intervention. We will follow this structure, starting with acute effects of the different levels of complexity, and then going on with tDCS after-effects. We then consider tDCS effects at the network level. Furthermore, we will describe morphological effects of tDCS and effects of tDCS on non-neuronal tissue, which have been comparatively less studied.

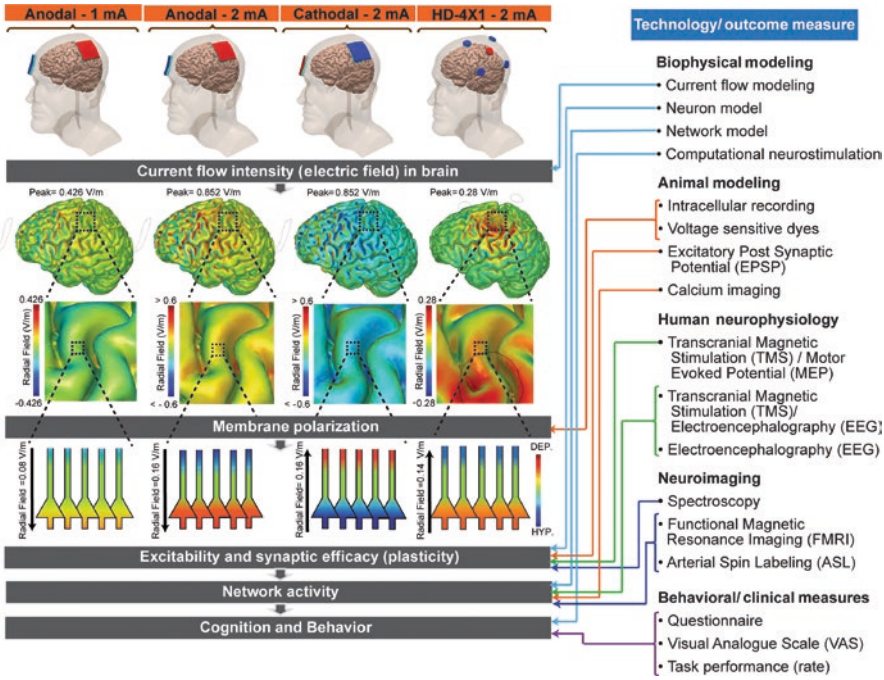

Fig. 3.1 Multi-scale effects and outcome measures of transcranial direct current stimulation. MRI-derived FEM models of current flow illustrate EF in cortex as a function of stimulation polarity, current intensity and electrode configuration. From macroscopic to microscopic level, a uniform EF along pyramidal neurons polarize membrane proportional to induced EF magnitude and direction. Neuronal excitability and plasticity is modulated by external electric field that in larger scale modulate network connectivity and ultimately cognition and behavior. tDCS effects can be probed using different techniques and experimental procedures regarding different scales

Regional Neuronal Effects of tDCS

Primary or Acute Effects

As with other forms of electrical stimulation (Mcintyre et al. 2004; Merrill et al. 2005; Rattay and Wenger 2010), the physiological effects of tDCS can be understood to derive from membrane polarization produced during stimulation. Weak DCS initiates the polarization of cell membranes; specifically the flow of electrical current produced by DCS results in sustained polarization of cell membranes exposed to this current flow (Bikson et al. 2004). Therefore, for the duration that DCS is applied the polarization is sustained. For example, if stimulation is applied for 20 min, then during that entire time the membranes of neurons would be slightly polarized. If tDCS is applied with a training task, then the polarization will be ongoing during the neuronal activity generated by the task. This in turn, would have the

effect of changing how neurons process information related to the task (Lafon et al. 2017; Rahman et al. 2017) and their propensity for plasticity (Fritsch et al. 2010; Jackson et al. 2016; Kronberg et al. 2017).

Characterizing which cells (principal cells, interneurons, glia, endothelial cells…) are polarized, and more specifically which compartments within these cells (soma, dendrite, axon) is thus central for understanding the effects of DCS. As discussed below, the consequences of membrane polarization are multi-faceted and complex, spanning changes in action potential threshold and timing following neuronal soma polarization (Radman et al. 2007b) to changes in network coherence (Polania et al. 2011a; Reato et al. 2010) to changes in synaptic efficacy (Bikson et al. 2004; Dudel 1971) and plasticity (Fritsch et al. 2010; Kronberg et al. 2017) to morphological and molecular changes (Pelletier and Cicchetti 2014). Early studies referred to tDCS/DCS as 'polarizing current' (Bindman et al. 1964), reinforcing the idea that transduction is by membrane polarization. Contrasting to other brain stimulation techniques, DCS has the inherent feature that the polarization is sustained (does not recover or reverse as consequence of change in stimulation waveform). The well-recognized time dependence of tDCS/DCS plastic changes (Nitsche and Paulus 2000, 2001) presumably results from the need for sustained polarization, and so in some aspects may be unique to DCS.

A range of alternate transduction mechanisms have been historically ventured as alternative to membrane polarization such as ionic concentration changes somehow generated directly by DCS (e.g. iontophoresis of charged molecules/ions; (Gardner-Medwin 1983)), but to our knowledge no quantitative analysis, much less experimental evidence, exists for tDCS. Rather, as detailed throughout the remainder of this chapter, our mechanistic considerations typically start with the well-established principle of membrane polarization induced by extracellular direct current flow, and all other changes are presumed secondary to this membrane polarization.

The Polarization Effect and Acute DCS Polarity-Specific Excitability Changes

DC stimulation with electrodes on the scalp leads to current flow across the brain (Datta et al. 2009; Huang et al. 2017; Miranda et al. 2006; Opitz et al. 2016), with current from the anode flowing into the brain and current exiting the brain to the cathode. The flow of current around neurons results in polarization of cell membranes when some of this current crosses the membrane. Flow into a specific membrane compartment (from outside the neuron into it) will result in local membrane hyperpolarization, and flow out of another membrane compartment (from inside to out) will result in local membrane depolarization (Andreasen and Nedergaard 1996; Bikson et al. 2004). An often overlooked concept is that the physics of electrical stimulation dictate that any neuron exposed to extracellular DC stimulation will have some compartments that are depolarized while others are hyperpolarized (Bikson et al. 2004; Chan et al. 1988). Which compartments are polarized in which

direction depends on the neuronal morphology relative to the DC electric field. Simplistically, for a typical cortical pyramidal cell, with a large apical dendrite pointed toward the cortical surface, a surface anode (positive electrode, generating a cortical inward current flow) will result in somatic (and basal dendrite) depolarization and apical dendrite hyperpolarization (Radman et al. 2009). For this same neuron, a surface cathode (negative electrode, generating cortical outward current flow) will result in opposite polarization effects (Fig. 3.2).

The importance of the somatic compartment in eliciting action potentials, and thereby determining cortical output, suggests somatic polarization plays a critical role in determining cortical excitability changes by DCS (Bikson et al. 2004; Bindman

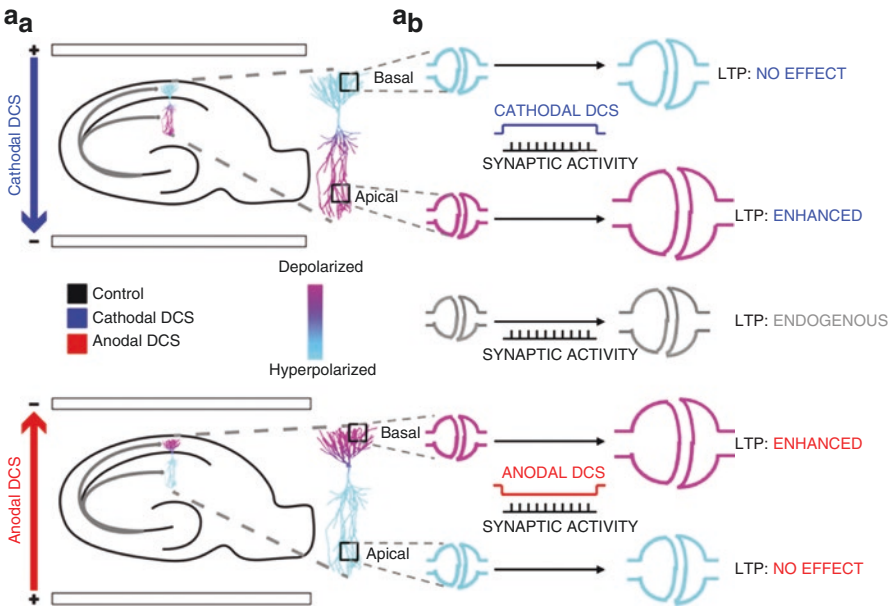

Fig. 3.2 DCS modulation of LTP and LTD depends on dendritic location and endogenous synaptic activity. DCS was applied during synaptic plasticity induction in hippocampal brain slices. The frequency of synaptic activity and dendritic location of plasticity induction were varied to study their role in determining DCS effects. (**a**$_a$) Schematic of hippocampal brain slice preparation, highlighting the membrane polarization of CA1 pyramidal neurons during each polarity of DCS. (**a**$_b$) Changes in synaptic strength in each dendritic compartment when DCS is applied during LTP induction. Both anodal and cathodal DCS can enhance LTP, but in different dendritic compartments, consistent with a pivotal role for DCS induced dendritic membrane depolarization. (**b**) In apical dendrites, cathodal DCS modulates both LTD and LTP induced by trains of synaptic activity at varying frequencies. Note that when synaptic activity is very weak (0.0167 Hz), DCS has no effect on synaptic strength. (**c**) DCS effects depend on the synaptic activity that stimulation is concurrent with (20 or 1 Hz) and the dendritic compartment (apical or basal). Plasticity modulation here is the ratio of the change in synaptic strength for each stimulation condition to the change in synaptic strength for corresponding control condition. Figure adapted from (Kronberg et al. 2017; with permission)

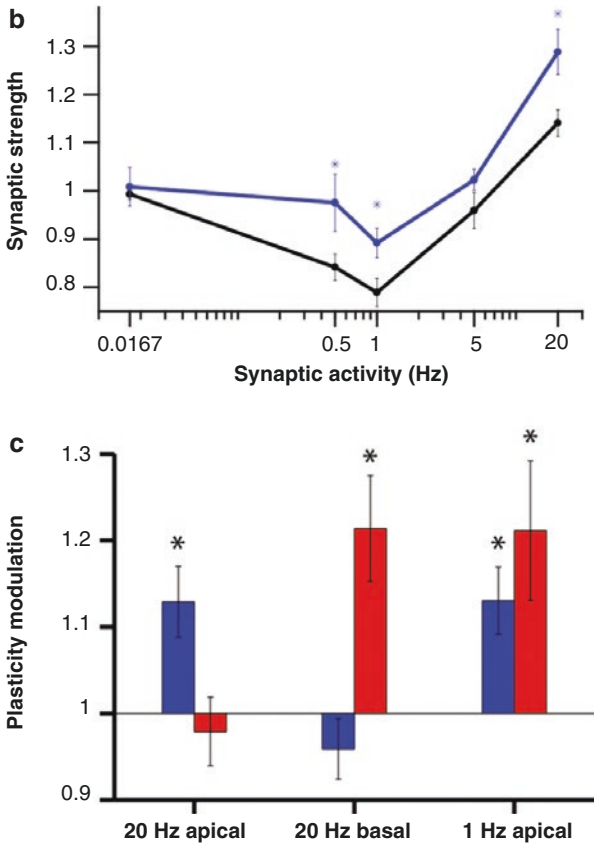

Fig. 3.2 (continued)

et al. 1964; Purpura and Mcmurtry 1965; Radman et al. 2007b), an idea we term the 'somatic doctrine.' Some of the earliest DCS findings in animals were changes in neuronal firing rate under electrodes consistent with surface-anode producing soma depolarization and surface-cathode producing soma hyper-polarization (Bindman et al. 1964; Purpura and Mcmurtry 1965). Ultimately, whether a neuron fires or not is not only determined by the soma, but by the integration of activity in all neuronal compartments including dendrites, axon, presynaptic terminal, axon hillock (see below). DC fields can modulate the functionality of these compartments, increasing the complexity of a purely 'somatic doctrine' (Kabakov et al. 2012; Kronberg et al. 2017; Rahman et al. 2013). None-the-less, the somatic doctrine has implicitly informed the rationale for most tDCS human trials – namely presumed excitation by the anode and inhibition by the cathode.

In accordance with a primary polarizing effect of DC stimulation, studies in humans have shown that tDCS for a few seconds, which does not induce after-effects, already induces stimulation polarity-dependent cortical excitability altera-

tions as probed by transcranial magnetic stimulation (TMS) (Nitsche et al. 2007a; Nitsche and Paulus 2000). These seem not to relevantly depend on synaptic effects, since block of glutamatergic NMDA receptors and enhancement of GABAergic activity – the main synaptic drivers of cortical excitability – do not affect acute DC-induced excitability alterations. Furthermore, intracortical inhibition and facilitation, which are driven by GABAergic and glutamatergic synapses, are not relevantly affected by stimulation protocols which do not induce after effects (Nitsche et al. 2005). In contrast, block of voltage-gated ion channels, which should affect the impact of depolarizing stimulation on cortical excitability, abolishes excitability-enhancing effects of tDCS (Nitsche et al. 2003a). Excitability-enhancing effects of anodal tDCS and –reducing effects with cathodal tDCS in the human motor cortex (Nitsche and Paulus 2000) are in accordance with respective de- and hyperpolarization of the soma of pyramidal cells. However, the respective experiments cannot rule out an effect of tDCS on other structures, since the primary measure of motor cortex excitability – single pulse transcranial magnetic stimulation-generated motor evoked potentials – is an unspecific measure of cortico-spinal excitability.

In analogy to findings that tDCS acutely changes response to TMS in man, studies in animal models have demonstrated that responses to afferent micro-stimulation are acutely changed in the target neurons by direct current (Bikson et al. 2004; Kabakov et al. 2012; Lafon et al. 2017; Rahman et al. 2013). The modulation is polarity specific and for short duration DCS, the effects on evoked responses do not outlast the DCS.

Quantification of Polarization Effects with Coupling Constant

Precisely understanding tDCS requires a quantitative model, beginning with quantification of somatic, as well axon and dendrite, polarization during tDCS. Here, the coupling constant (also termed polarization length) is an important concept. Assumed that for weak electric fields (well below the threshold for action potential generation) the membrane polarization at any given compartment, including the soma, produced by DCS is linear with electric field intensity, for uniform electric fields, the membrane potential polarization can thus be expressed as: $V_{tm} = G*E$ where V_{tm} is the polarization of the compartment of interest (in: Volts), G is the coupling constant (in: V per V/m, or simply: m) and E is the electric field (in: V/m). For rat hippocampus and cortical neurons, the somatic coupling constant is in the range of 0.1–0.3 mm (Bikson et al. 2004; Deans et al. 2007; Radman et al. 2009). In ferret cortical neurons the coupling constant is similarly approximately 0.25 mm (Fröhlich and Mccormick 2010). Note that for humans, assuming scaling of sensitivity with total neuronal length (Joucla and Yvert 2009) somatic depolarization per V/m may be higher. The finding that higher stimulation intensities result in stronger effects of stimulation, within specific limits, in case of motor cortex tDCS in humans is in principle accordance with this coupling constant, although respective intensity-dependent effects have been only explored for

after-effects of tDCS so far (Nitsche and Paulus 2000) and higher dose and altered brain state complicates dose response in humans (Giordano et al. 2017; Jamil et al. 2017).

An important consequence of the concept of the coupling constant (polarization length) is that a presumed linear polarization with DCS intensity means that there is no "threshold" for polarization; any field intensity will produce some level of polarization (Bikson et al. 2004). The central question is not if tDCS will polarize neurons at all but rather what is the consequence of that polarization, and specifically as tDCS is expected to produce only a small membrane potential change (e.g. less than a mV) what active brain processes "amplify" the effects of this polarization. Vice versa it has been argued that activation of neurons may via opening of ion channels shorten the time constant of the membrane and reduce the efficacy of tDCS (Paulus and Rothwell 2016). Characterizing the mechanisms of tDCS has thus focused on explaining how weak membrane polarization of specific cellular compartments leads to functional changes of ongoing activity.

Geometry of Stimulation Effects and Sensitivity of Soma, Dendrite, and Axon Compartments

Determining the coupling constant of the soma and other membrane compartments in humans to tDCS remains an important research question. The maximal depolarization of pyramidal neurons somas occurs when the electric field is parallel with the somato-dendritic axis which typically corresponds to an electric field radial (normal) to the cortical surface, while electric fields orthogonal to the somato-dendritic axis (along the cortical surface) do not produce significant somatic polarization (Bikson et al. 2004; Chan et al. 1988). The somatic coupling strength is generally related to the size of the cell and the dendritic asymmetry around the soma (Radman et al. 2009; Svirskis et al. 1997) making layer II/IV and layer 5 pyramidal neurons relatively sensitive to DCS polarization. For cortical pyramidal neurons, the typical polarity of somatic polarization is consistent with the 'somatic doctrine' (e.g., positive somatic depolarization for positive electric field). The polarity of the coupling constant is inverted (using our field direction convention) for CA1 pyramidal neurons due to their inverted morphology of the apical-dendrite branches relative to the field direction.

Experiments in humans support the direction-dependency of tDCS effects not only for antagonistic stimulation polarities, but also for the relation of cortical current flow angle in relation to neuronal orientation. It was shown that the position of the return electrode, and thus electrical field orientation, critically determines the efficacy of tDCS (Nitsche and Paulus 2000). Furthermore, with the same target electrode position, antagonistically placed return electrodes, which convert the direction of electrical field orientation, result in roughly converted effects of visual cortex stimulation on visual evoked potentials (Accornero et al. 2007; Antal et al. 2004a). Finally, studies showing that stimulation of distant, but connected areas

affect primary motor cortex excitability are compatible with the concept that tDCS might affect primarily pyramidal output neurons (Boros et al. 2008; Rivera-Urbina et al. 2015).

A presumption of the somatic doctrine is that at the cortex under the anode electrode currents are radial and inward producing somatic depolarization, while at the cortex under the cathode current is radial and outward, producing somatic hyperpolarization. However, high-resolution modeling suggests that in convoluted human cortex, current is neither unidirectional nor dominantly radial (Rahman et al. 2013). Though the 'somatic doctrine' is based only on radially directed electrical current flow (normal to the cortical surface), during tDCS significant tangential current flow is also generated (along the cortical surface) (Rahman et al. 2013). Indeed, recent work suggests tangential currents may be more prevalent between and even under electrodes. Tangential currents cannot be ignored in considering the effects of tDCS. Moreover, due to cortical folding the direction of radial current flow under tDCS electrodes is not consistent, meaning there are clusters of both inward (depolarizing) and outward (hyperpolarizing) cortical current flow under either the anode or the cathode (Rahman et al. 2013). Due to the cortical convolutions, current is not unidirectional under electrodes, thus, under the cathode there may be isolated regions of inward cortical flow, and in those regions neuronal excitability may increase (Creutzfeldt et al. 1962).

For dendritic effects of DCS, the basal dendrite of pyramidal neurons will be polarized similarly as the soma, however the apical dendrite will be polarized in the opposite direction (Fig. 3.2) (Andreasen and Nedergaard 1996; Bikson et al. 2004). The dendrites are also electrically excitable. Animal studies with high intensity applied DC fields (~100 V/m) have shown that with sufficiently strong stimulation, active processes (spikes) can be triggered in the dendrites (Andreasen and Nedergaard 1996; Chan et al. 1988; Delgado-Lezama et al. 1999; Wong and Stewart 1992). Even if the electric fields induced during tDCS are not sufficient in themselves to trigger dendritic spikes, they are likely to alter ongoing voltage-dependent mechanisms and synaptic integration in dendrites (Cavarretta et al. 2014). Indeed, recent work suggests that DCS modulates synaptic plasticity in a manner consistent with dendritic polarization (Fig. 3.3; (Kronberg et al. 2017)). The role of dendritic polarization during tDCS remains an open question especially when considering processing of synaptic input.

It is also well established that axons are sensitive to applied electric fields (see below); the magnitude and direction of polarization is a function of neuronal and axonal morphology (Bullock and Hagiwara 1957; Salvador et al. 2011; Takeuchi and Takeuchi 1962). While the axon initial segment would likely be polarized in the same direction as the soma (Chan et al. 1988), for distal regions of long axons, this is not necessarily the case. Hence, it is useful to separately consider the axon initial segments (within a membrane space constant of the soma) and more distal axonal processes, which can be further divided into 'axons-of-passage' and afferent axons with terminations. Notably, for long straight axons-of-passage (e.g., Peripheral Nervous System, PNS) cathodal stimulation will be more effective than anodal stimulation in inducing depolarization (opposite to the somatic doctrine; (Bishop

and Erlanger 1926)). It has been shown that weak DC fields can produce acute changes in CNS axon excitability (pre-synaptic/antidromic volley; (Bikson et al. 2004; Jefferys 1981; Kabakov et al. 2012)). The relevance of alteration of dendritic and axonal excitability by DC stimulation is underscored further by suggestions that not only radial, on which the somatic doctrine is based, but also tangential current flow might be relevant for DCS effects.

The involvement of non-pyramidal neurons in the effects of DC stimulation remains an open question. Because of their relatively symmetric dendritic morphology, interneuron somas are expected to polarize less than pyramidal neurons (Radman et al. 2009). Based on the 'somatic doctrine' their importance might then be assumed diminished. However, one cannot exclude polarizing effects of fields on dendrites and axons of interneurons. Moreover, interneurons represent a wide range of morphologies and size, including more asymmetric morphologies (Freund and Buzsaki 1996). An impact of DC fields on interneuron excitability has been shown in animal experimentation (Purpura and Mcmurtry 1965). Interneurons exert a powerful regional effect, including a role in plasticity and oscillations. An effect of paired-pulse facilitation in hippocampal slices may also suggest modulation of the activity of interneurons (Kabakov et al. 2012). Similarly at least for after-effects of tDCS, alteration of GABAergic-driven processes seems to be relevant, as shown in experiments in humans (Nitsche et al. 2005; Stagg et al. 2009a), although these experiments do not allow to conclude if these are direct or secondary effects of DC stimulation. Thus, the specific role of interneurons in the direct effect of tDCS remains an open question.

In summary, while it is convenient to assume a consistent direction of current flow under electrodes, such that brain regions under anode/cathode have uniform inward/outward direct current across the cortex, the situation in humans is more complex. The convoluted cortical surface in fact produces mixed directed currents even directly under each electrode (Lafon et al. 2017; Rahman et al. 2013). This in turn means that neurons will experience a mixed polarity of polarization. The morphology of neuronal processes is itself heterogeneous, and the role of dendrite and axon polarization independent of soma, should be considered.

Amplification: Enhancing Neuronal Sensitivity to DCS

Work quantifying how much current reaches the brain during tDCS (Datta et al. 2009; Huang et al. 2017; Miranda et al. 2006; Opitz et al. 2016) and the sensitivity of neurons to weak DCS has raised questions about how such minimal polarization (<1 mV) could result in functional/clinical changes especially considering that endogenous 'background' synaptic noise can exceed these levels (Magee and Cook 2000). In recent years, motivated by increased evidence that transcranial stimulation with weak currents has functional effects (Floel 2014), as well as ongoing questions about the role of endogenous electric fields that can have comparable electric fields (Fröhlich and Mccormick 2010), the mechanisms of amplification have been explored in animal studies.

At the level of a single neuron, the most evident non-linear response that could serve as a substrate for acute amplification is the threshold-based all-or-none action potential. Importantly, as the electric fields generated in the brain during tDCS are too weak to trigger action potentials in neurons at rest (e.g. ~20 mV membrane depolarization from rest to action potential threshold) we should consider instead modulation of ongoing action potential activity. At the single cell level, amplification could affect either: (1) the rate of action potential generation (rate effects); and/or (2) amplification through change in the timing of action potentials (timing effects). As discussed above, classic animal studies on weak direct current stimulation showed a change in ongoing action potential discharge rate that is roughly linear with electric field intensity and so membrane polarization by DCS (Bindman et al. 1964; Creutzfeldt et al. 1962; Purpura and Mcmurtry 1965; Terzuolo and Bullock 1956). In this sense, the amplification (gain) would relate to the sensitivity of discharge rate to DCS-induced membrane polarization. Interestingly, Terzuolo and Bullock (Terzuolo and Bullock 1956) reported a detectable change in neuronal firing rate at electric fields as small as 0.8 V/m, and postulated that this detection threshold would likely decrease with longer and more sophisticated experiments. Assuming that a 2 mA tDCS protocol generated a peak electric field in the brain of 0.5 V/m (Huang et al. 2017) leading to ~0.15 mV somatic polarization (Radman et al. 2009), and that across animal studies changes in firing rates of 7 Hz per mV membrane polarization have been reported (Carandini and Ferster 2000), a change in firing rate of approximately 1 Hz during conventional tDCS is plausible. Remarkably, recent work has shown that sub-mV depolarization of pyramidal neuron somas was sufficient to convert silent cells into place cells in the hippocampus (Lee et al. 2012).

Changes in AP timing (rather than discharge rate) could also serve to amplify the effects of weak membrane polarization produced by weak direct current stimulation (Radman et al. 2007b). In acute brain slice recordings and in a simple neuron model it was demonstrated that the resulting change in timing could be quantified simply by the induced membrane polarization times the inverse of the ramp slope. Thus, the inverse of the ramp-slope is a "gain/amplification" term because the shallower a ramp, the larger the timing change for any given small polarization by direct current stimulation. For example, based on an approximate 0.2 mV somatic polarization during 2 mA tDCS, then in response to a 1 mV/ms ramp slope, timing would change by 0.3 ms. Therefore, the amplification in this case can be understood as a larger change in action potential timing for a small DCS membrane polarization. This coupling sensitivity and the resulting timing changes were further confirmed by Anastassiou and colleagues (Anastassiou et al. 2010) using a more complex model. Though the basic principle of timing amplification is expected to generalize to other neuron types responding to an increasing synaptic input (Bikson et al. 2004), the most simple amplification equation makes specific assumptions about membrane properties and dynamics (Radman et al. 2007a) that may not extend to all neurons types (Radman et al. 2009). For acute effects of DC stimulation, amplification has not been studied in the human brain, but amplification seems to play a role in net-

work after effects of tDCS (see below). For reasons not entirely clear, the maintenance of tDCS for minutes appears to play a key role in the generation of after-effects and thus increasing sensitivity, as discussed next.

DCS Modulation of Synaptic Efficacy and Polarization of Axon Terminals

A compelling topic of investigation about probable mechanisms of excitability changes induced by tDCS, is which types of neurons, and which neuronal compartments are involved. Regarding changes in synaptic efficacy a key question is: as invariably during tDCS half the dendrite will be polarized in the same direction as the soma and half of the dendrite will be polarized in the opposite direction (see above), how do polarity-specific changes arise? This question has been addressed in detail in animal models examining acute changes in evoked synaptic efficacy (excitability) during DCS.

Early work probing evoked responses in animal models indicated modulation in excitability, with the direction of evoked response change consistent with the somatic doctrine (Bindman et al. 1964; Creutzfeldt et al. 1962), though Bishop and O'Leary (1950) already noted deviations. Recent studies aimed at developing and validating animal models of transcranial electrical stimulation have shown modulation of TMS evoked potentials and visual evoked potentials consistent with the somatic doctrine (Cambiaghi et al. 2010, 2011). In a pioneering work using uniform electric fields in brain slices, Jefferys showed acute modulation of excitability (synaptically driven population spikes) in the dentate gyrus of hippocampal slices when electric fields were parallel to the primary target cell dendritic axis. The detected polarity-specific changes were consistent with somatic polarization, and no modulation occurred when the electric field was applied orthogonal to the primary dendritic axis (Jefferys 1981). The precise control of electric field angle is possible in brain slices and was leveraged in subsequent work.

For the hippocampal slice preparation, several deviations from the somatic doctrine were found (Bikson et al. 2004). Optical imaging with voltage sensitive dyes provided direct evidence that DC electric fields always produce bimodal polarization across target neurons such that somatic depolarization is associated with apical dendrite hyperpolarization, and vice-versa – yet weak interactions across compartments were observed. In addition, for synaptic inputs to the apical dendritic tuft, we reported modulation inconsistent with the somatic doctrine. Also in hippocampal slices, Kabakov et al. (2012) reported modulation of synaptic efficacy in a direction opposite to that expected from the somatic doctrine (noting inversion of dendrite morphology in CA1 pyramids relative to cortex). In this case, one may speculate that apical dendrite depolarization determines the direction of modulation despite somatic hyperpolarization (Bikson et al. 2004); though Kabakov et al. (2012) provides evidence suggesting dendritic polarization affects the magnitude but not direction of modulation. As noted, in cortical slices by Fritsch et al. (2010), modulation of

evoked responses is indeed consistent with the somatic doctrine – a finding we have confirmed for four distinct afferent cortical synaptic pathways (Rahman et al. 2013). Variations across animal studies could be simply ascribed to differences in region/preparation, timescale (acute, long-term), and different forms of plasticity (BDNF dependent/independent), but this is speculative and provides little insight into tDCS. Rather, in attempt to reconcile these findings in a single framework, we cite evidence for and define the 'terminal-doctrine' to compliment the 'somatic-doctrine'.

The effects of tangential fields on synaptic efficacy were also explored (Bikson et al. 2004). Tangential fields are oriented perpendicular to the primary somatodendritic axis, so they are expected to produce little somatic polarization, which was directly confirmed with intracellular recording. Surprisingly, electric fields applied tangentially were as effective at modulating synaptic efficacy as radially directed fields. The afferent axons run tangentially, so we speculated they might be the targets of stimulation. Exploring different pathways, we found that axon pathways with terminals pointed toward the anode were potentiated, while axon pathways with terminals pointed toward the cathode were inhibited. Kabakov et al. (2012) reported similar pathway specific dependence summarizing "the fEPSP is maximally suppressed when the AP travels toward the cathode, and either facilitated or remains unchanged when the excitatory signal [AP] propagates toward the anode". In addition, Kabakov et al. (2012) observed changes in paired-pulse facilitation that are consistent with pre-synaptic vesicular glutamate release. In a variety of tDCS studies different tDCS polarity resulted in behavioral effects in one direction only. E.g. in an implicit motor learning paradigm anodal tDCS facilitated reaction times (Nitsche et al. 2003a) whereas cathodal tDCS also induced a trendwise facilitation. One explanation could be that in case of anodal tDCS the somatic doctrine dominated whereas with cathodal tDCS more superficial horizontal afferents were facilitated.

We recently confirmed a similar directional sensitivity in cortical slices across 4 distinct pathways where electric fields applied tangentially to the surface (and so producing minimal somatic polarization) (Radman et al. 2009), modulated synaptic efficacy (Rahman et al. 2013). An impact of premotor and posterior parietal tDCS on primary motor cortex plasticity was reported for the human brain, which is in accordance with an involvement of afferent terminals in the plasticity effects of tDCS (Boros et al. 2008; Rivera-Urbina et al. 2015). A role for pre-synaptic modulation during DC stimulation is indeed not surprising and has been also historically observed. Purpura and McMurtry (1965) noted "although the [somatic] membrane changes produced by transcortical polarization current satisfactorily explains alterations in spontaneous discharges and evoked synaptic activities in [pyramidal tract] cell, it must be emphasized that the effects of polarizing current on other elements constituting the 'pre-synaptic,' interneuronal pathway to [pyramidal tract] cells also appear to be determinants of the overt changes observed in [pyramidal tract] cells activities." Bishop and O'Leary (1950) not only quantified pre-synaptic effects during DC stimulation in animals, they noted that pre-synaptic effects would

complicate the interpretation of post-synaptic changes as well as themselves induce long-lasting aftereffects.

Cellular process terminals including axon terminals are especially sensitive to electric fields as a result of their morphology, and terminal polarization can modulate synaptic efficacy (independent of target soma polarization) (Awatramani et al. 2005; Bullock and Hagiwara 1957; Del Castillo and Katz 1954; Hubbard and Willis 1962a, b; Takeuchi and Takeuchi 1962). Moreover, this modulation is cumulative in time and endures after stimulation (Hubbard and Willis 1962b), has a temporal profile noted in classic DC experiments (Bindman et al. 1964), and suggests the possibility for plasticity. The direction of modulation in brain slice studies consistently suggests that terminal hyperpolarization enhanced efficacy, while depolarization inhibited efficacy. Paired-pulse analysis in a rabbit model suggested tDCS influences pre-synaptic sites (Márquez-Ruiz et al. 2012). Since tDCS induces significant tangential fields, the role of terminal polarization (independent of the 'somatic doctrine') remains a compelling and open question especially when taken together with the need for amplification and the role of synapses in plasticity.

Secondary or After-Effects

Beyond the acute effects of DC stimulation on membrane polarity, sufficiently long stimulation (for some minutes) induces after-effects, which can last for over 1 h, and under specific conditions more than 24 h after stimulation (Monte-Silva et al. 2013; Nitsche et al. 2003a; Nitsche and Paulus 2001). Several animal and human studies have speculated that processes linked to the dendrites are involved in the long-term effects of tDCS (e.g., glutamatergic receptors like n-methyl-D-aspartic receptor, NMDAR) (Liebetanz et al. 2002; Nitsche et al. 2003a; Yoon et al. 2012). Animal studies, some decades old, have suggested lasting changes in brain excitability by DCS. Animal studies in the 1960's established that weak DC current can produce lasting physical changes in neural activity, which cannot be explained as persistent 'reverberating circuit' of activation (Gartside 1968a, b). Especially notable are animal studies by Bindman and colleagues (Bindman et al. 1962), who recognized the importance of prolonged DC stimulation to produce polarity-specific lasting cortical excitability changes (>5 h) which informed their early work in tDCS of psychiatric disorders (Costain et al. 1964; Redfearn et al. 1964). Multi-minute stimulation was later adopted in humans to demonstrate polarity-specific lasting changes in cortical excitability by TMS (Nitsche et al. 2003a; Nitsche and Paulus 2000, 2001). Though these multi-minute protocols are now universally adopted in tDCS research, the mechanisms by which specifically prolonged stimulation protocols trigger plasticity have not been completely clarified.

General Framework for Synaptic Plasticity Modulation by DCS

Synaptic plasticity is considered central in brain plasticity, so synapses are an evident focus to explain lasting tDCS effects. Both in humans and animal studies changes in synaptically mediated evoked responses are considered reliable hallmarks of long-term plastic changes. Thus, much of modern animal studies on tDCS plasticity considered lasting changes in synaptic efficacy.

Electric fields generated by tDCS are sub-threshold, in the sense that they are too weak to trigger action potential in quiescent neurons – in the brain where neurons are not quiescent the actions of tDCS are considered to modulate ongoing activity. Modulatory effects on firing rate, timing, and synaptic efficacy have been demonstrated. Lasting changes in synaptic efficacy could be mediated through different paradigms, which are not necessarily exclusive:

1. Membrane polarization may trigger plastic synaptic changes in a manner independent of any ongoing, future, or past synaptic input or action potential generation (i.e., simply holding the membrane at an offset polarization initiates changes). However, in a cortical brain slice model (with no background activity), weak polarization was not sufficient to induce plastic changes in synaptic efficacy (Fritsch et al. 2010) (c.f. Ranieri et al. 2012). The concept is mute in humans since the cortex is always active; it was shown that alteration of cortical activity levels modulates tDCS effects (Antal et al. 2007; Thirugnanasambandam et al. 2011).
2. Changes in action potential rate or timing, secondary to neuronal polarization, may affect synaptic plasticity. Bindman et al. (1964) already stated "There is some evidence that a determining factor in producing long-lasting after effects is the change in the firing rate of neurons rather than the current flow that produces the changes." Classic animal studies indicated weak DC stimulation is sufficient to induce plastic changes (Bindman et al. 1964; Gartside 1968a).
3. Incremental polarization of the membrane in combination with ongoing synaptic activity may induce synaptic plasticity. The specific hypothesis here is that the generation of plasticity requires synaptic co-activation during DC stimulation. Fritsch et al. (2010) shows synaptic potentiation in-vitro under anodal stimulation only during synaptic stimulation of specific frequencies. In a rabbit study, DCS was combined with repeated somatosensory stimulation in-vivo, leading to acute polarity-specific changes, and lasting changes for the cathodal case (Márquez-Ruiz et al. 2012). If dependent on combined polarization and synaptic input, then synapse specific changes are plausible. If one assumes DCS exerts a post-synaptic priming effect (polarization of soma/dendrite) then co-activation of afferent synaptic input could be conceived as Hebbian reinforcement. This plasticity paradigm is broadly analogous to combining tDCS with a cognitive task or specific behavior that co-activates a targeted network or combining tDCS with TMS. Indeed, work showing the importance of

co-activation in cortical slices (Hess and Donoghue 1999; Rioult-Pedotti et al. 1998), influenced Nitsche and Paulus (2000) in developing tDCS. Importantly, unlike in brain slice and anesthetized animal models, the human cortex is constantly active such that tDCS is always applied in conjunction with ongoing synaptic input even if it is not explicitly paired with another intervention. However, plasticity-increasing effects are seen when tDCS is combined with peripheral nerve stimulation in humans (Rizzo et al. 2014), which supports this concept.

4. Incremental polarization of the membrane may boost ongoing endogenous synaptic plasticity. Clinically this fourth paradigm is analogous to combing tDCS with learning/training (Bolognini et al. 2010). For example, in the aforementioned rabbit study, DCS modulated ongoing synaptic habituation, similar to a model of associative learning (Márquez-Ruiz et al. 2012). An important implication of this paradigm is that DCS effects will depend on the nature of the endogenous plasticity that is paired with. For example, recent work in brain slices showed that DCS can modulate endogenous synaptic plasticity, but the direction and magnitude of this modulation depends on the dendritic location and pattern of endogenous synaptic activity (Fig. 3.3; Kronberg et al. 2017). As a result, both anodal and cathodal stimulation can enhance and diminish LTP depending on these parameters. In human motor cortex, tDCS modulates simultaneous LTP induction via paired associative stimulation (PAS) (Nitsche et al. 2007b). Moreover, LTP-like plasticity-inducing tDCS has been shown to foster motor learning, which is thought to critically depend on LTP, if applied synchronously with task performance (Nitsche et al. 2003b; Reis et al. 2009).
5. Meta-plasticity is defined as sustained polarization before, or potentially after, the generation of endogenous LTP that "primes" the brain to respond differently to potentiation. Evidence from brain slices (Ranieri et al. 2012) and in vivo animal experiments (Podda et al. 2016; Rohan et al. 2015) shows priming with DCS modulates subsequent tetanus-induced LTP in a polarity specific manner. A similar effect has been shown for the human motor cortex in case of priming PAS-induced LTP-like plasticity via anodal and cathodal tDCS. However, whether priming stimulation reduces or enhances subsequently induced plasticity might also critically depend on the inter-intervention interval (Fricke et al. 2011; Monte-Silva et al. 2010).
6. Changes in network dynamics where the generation of LTD/LTP is explained through intervention with ongoing oscillations and may manifest as lasting changes in oscillation dynamics (Reato et al. 2013a, 2015). Such modulation may reflect interference with the finely tuned excitatory-inhibitory synaptic balance during oscillations (Reato et al. 2010). Indeed, tDCS in humans was shown to alter oscillatory brain activity during (Hanley et al. 2016), but also after stimulation (Ardolino et al. 2005; Zaehle et al. 2010), and might also affect phase-coupling of oscillatory activity (Carter et al. 2015).

Decades of Research Characterizing DCS Changes of Neuronal Plasticity

It is remarkable that a decade before the widely-credited "discovery" of Long-Term Potentiation by trains of suprathreshold pulses by Bliss and Lomo (1973), animal studies had shown lasting changes in excitability following DCS lasting up to hours (Bindman et al. 1962). Moreover, DCS researchers had begun to address the underlying molecular mechanisms (Gartside 1968a, b) and translating results to humans. LTP/LTD induced by tetanic stimulation and by DC current may share some common molecular substrates (Gartside 1968b; Islam et al. 1995b; Ranieri et al. 2012).

Common forms of LTP/LTD are mediated by the NMDA receptor (Malenka and Bear 2004), which has been implicated in both long-term tDCS effects in humans (Liebetanz et al. 2002; Nitsche et al. 2003a, 2004) and in-vitro DCS-induced plasticity (Fritsch et al. 2010). Moreover, GABAergic activity – which reduces glutamatergic plasticity in animal slice preparations (Castro-Alamancos et al. 1995) seems to be reduced by both, cathodal and anodal tDCS, as shown for the human motor cortex (Stagg et al. 2009a). This combined mechanism might enhance the propensity of tDCS to induce plasticity in the human brain in vivo. Given the relevant involvement of calcium in NMDA receptor-dependent glutamatergic plasticity, it is not surprising that intracellular calcium content is increased by LTP-like plasticity-inducing DC stimulation in animal models (Islam et al. 1995), and that calcium channel block abolishes tDCS-induced LTP-like plasticity in humans (Nitsche et al. 2003a). The dependency of LTP and LTD induction on the amount of calcium influx – low increase results in LTD, high increase in LTP (Lisman 2001), and even higher increase might again diminish plasticity due to compensatory mechanisms – explains furthermore the switch from LTP- to LTD-like plasticity if stimulation lasts too long (Monte-Silva et al. 2013), or is accompanied by pharmacological intervention increasing calcium influx (Lugon et al. 2015).

Beyond these potential drivers of DC stimulation-induced plasticity, experiments in humans have revealed an important impact of neuromodulators, such as dopamine, acetylcholine, and serotonin. Alteration of the activity of these systems prominently impact the plasticity-inducing effects of DC stimulation (Fresnoza et al. 2014a, b; Grundey et al. 2012; Nitsche et al. 2012). Similarly, the BDNF/TrKB pathway is known to be a potent modulator of these common forms of LTP/LTD (Lu 2003) and this pathway has also been implicated in long-term tDCS effects in both humans and animals (Fritsch et al. 2010; Podda et al. 2016; Ranieri et al. 2012). Earlier work looked at accumulation of potential molecular targets of stimulated brain tissue, and beyond the impact of calcium (Islam et al. 1995a) found effects of DC stimulation on adenosine-sensitive cAMP (Hattori et al. 1990), and protein kinase C (Islam et al. 1995b), each of which play a role in LTP/LTD. Building on this, more recent in vivo animal work has shown long-term tDCS effects to be dependent on the adenosine A1 receptor (Márquez-Ruiz et al. 2012). While evidence is accumulating that DCS-induced plasticity shares molecular mechanisms with classic LTP/LTD, the manner in which the primary, polarizing effect of tDCS interacts with this molecular machinery remains an important area of research. Here experiments in humans show that combination of anodal tDCS and voltage-gated

ion channel block not only abolish acute, polarization-dependent effects of tDCS, but also after-effects, which suggests an important role of polarization for the development of neuroplasticity (Nitsche et al. 2003a).

Furthermore, regarding contributing neurons, motor cortex studies in humans deliver relevant information adding to the results of pharmacological studies. Here, tDCS polarity-dependently alters intracortical inhibition and facilitation, which are driven by glutamatergic and GABAergic neurons. However, tDCS polarity-independently enhances I-wave facilitation, which is suppressed by GABAergic activity (Nitsche et al. 2005). Modulation of afferent activity to the primary motor cortex might be involved, since modulation of premotor activity by DC stimulation modifies intracortical inhibition and facilitation in a similar manner as primary motor cortex stimulation (Boros et al. 2008).

Given the complexity of plasticity, and how it underpins learning, there are open questions about how tDCS modulates synaptic function. Importantly, valuable research in this direction should not be confused with the absence of decades of literature (summarized above). Similarly, exhaustive work over the past decade showing nuance in how DCS modulates synaptic efficacy (such as state dependent effects) should not be conflated with a deficiency in existing knowledge. Rather, ongoing research on tDCS modulation of plasticity is more advanced than most other neuromodulation tools and indeed many drugs. These studies reflect the detail of ongoing work. Many of these investigations relate to variation in the direction of modulation, if anodal and cathodal stimulation always exhibit and inhibit synaptic efficacy – consistent with the 'somatic doctrine' – or if there are dose and brain-state specific reversals in direction.

Synaptic Plasticity and Galvanotropism

The kind of plasticity induced by tDCS so far refers to functional or synaptic plasticity. Another plasticity mechanism includes morphological alterations, like axonal growth and guidance, which might also be affected by DCS. It is well established that electric fields play a role as signals in the development and regeneration of the nervous system (Mccaig et al. 2005). Several studies have shown endogenous electric fields within growing and recovering tissue. Whether similar mechanisms may be relevant during DCS may come down to the sensitivity of growth to DCS relevant electric fields. As we review, axonal growth in vivo and in vitro has been demonstrated with applied fields at significantly higher intensities and for longer durations than tDCS (Mccaig and Rajnicek 1991).

The study of electric fields and cellular galvanotropism (induced growth by an electrical stimulus) has been linked to cell proliferation, development, membrane protein redistribution, and recovery from injury (Mccaig et al. 2005). We will focus here on the role of galvanotropism for tDCS relevant field intensities and durations. The first quantitative study in vitro by Marsh and Beams in 1946, exposed medullary explants from chicken embryos to ~60 V/m electric fields and demonstrated

that neural processes grow preferentially towards the cathode and their development is suppressed towards the anode (Marsh and Beams 1946). In 1979 Jaffe and Poo assessed that neurites grow about three times faster towards the cathode at 70 V/m (Jaffe and Poo 1979). The lowest reported field values to induce galvanotropis are: 3 V/m applied for 20 min for locally induced fields (Patel and Poo 1984) and for uniform fields from 7 V/m applied during 16–20 h (Hinkle et al. 1981) to 10–50 V/m applied for 24 h (Patel and Poo 1982). The mean growth induced by DC fields is 0.4 μm per V/m per minute for local fields and 0.12 nm per V/m per minute for uniform fields (Patel and Poo 1982).

The effects of extracellular fields on nerve migration have been extensively characterized in vivo. In 1984, Pomeranz et al. applied 1 μA of current for 3 weeks to a sprouting rat nerve (Pomeranz et al. 1984). Hindpaw sensitivity was assessed before and after applying the field, finding an increase in responsiveness only when the cathode was placed in the direction of growth of the sprouting nerve (anodal stimulation). Physiological correlates were measured through histological studies showing an elevated number of neural fibers for anodal stimulation. In 1987, McDevitt et al. were the first to describe re-growth in mammals. They did a cut-suture intervention of the sciatic nerve and applied currents that generated fields of approximately 10 V/m for 20 days, each session lasting 30 min. Electromyographic activity was present in 67% of the animals that received stimulation with growth directed toward the cathode, and only in 17% with the reversed polarity (Mcdevitt et al. 1987). Supporting evidence is shown for an increase in neurofilament growth towards the cathode in damaged sciatic nerves (Politis et al. 1988) and for morphological regeneration after nerve transection (Roman et al. 1987). In addition, functional recovery was assessed by measuring various parameters of the rat's gait (Beveridge and Politis 1988).

Even endogenous injury potentials, which are presumed to have a functional role, are over an order of magnitude above tDCS fields (~10 V/m compared to <0.5 V/m). Given that studies on galvanotropism use much higher magnitude and longer duration DCS (typically ~100 V/m; (Palmer et al. 2000)), at first glance, effects of tDCS-relevant dose might be dismissed. However, assuming a linear dose-response (e.g. 0.12 nm per V/m minute) and considering the scale of individual synapse/dendrite spines, it is possible that even small morphological modifications have an important role in plastic changes underlying long term effects induced by tDCS. Indeed, the need for long-duration tDCS to produce after-effects may reflect cumulative galvanotropism. For example, 2 mA tDCS would, in theory, result in local electric fields of ~ 0.5 V/m, which over 20 min, could displace a neuronal process by 1.2 nm. Thus, during tDCS morphological reorientation of axon terminals and dendritic spines at synapses (rather than growth of axons over long distance) may be significant. To distinguish this local synaptic-cleft phenomena from conventional long range axon guidance, we call this "nano-galvanotropis". This conjecture reinforces our overall methodological theme that the relevance of animal studies to tDCS relies on both dose response (e.g. change per V/m) and outcome measures (e.g. plasticity vs. migration).

Network Effects of tDCS: Amplification and Recruitment

The consideration of how tDCS interacts with active networks (e.g. oscillations) is a major area of ongoing research: just as networks of coupled active neurons exhibit "emergent" network activity not apparent in isolated neurons, the application of DCS to active networks can produce responses not expected by single neurons. These responses are specific to the network architecture and activity (Reato et al. 2013b; Schmidt et al. 2014). Networks also provide an important substrate for amplification beyond the cell/synapse level. Ongoing studies on tDCS in humans has addressed modulation of EEG oscillations, while reports that DCS can alter "spontaneous rhythm" in animals span decades (Antal et al. 2004b; Dubner 1939; Marshall et al. 2011). Finally, modulation of oscillations are a substrate for changes in plasticity (Reato et al. 2013a, 2015).

Beyond the single neuronal level, amplification of networks might play a role in DCS effects. As discussed above, the initial action of DC stimulation remains to polarize all neurons subjected to the electric field (current flow inside the head). Our emphasis here is that tDCS generates electric fields across large areas of cortex and that polarization acts on every neuron in these brain regions. In considering the effects of tDCS on networks, a key concept is that the entire population of coupled neurons is polarized- this *coherent* polarization of the population provides a substrate for signal detection and for amplification (Parra and Bikson 2004; Reato et al. 2013b; Schmidt et al. 2014). In an oscillating network, DCS polarization of even a sub-set of neurons effects the whole population – in this way cells that in isolation might be less sensitive (e.g. interneurons) might be recruited to respond to tDCS (Reato et al. 2010).

Interestingly, at the single neuron scale the effective coupling constant for a neuron immersed in an active network may be enhanced compared to that of neurons in isolation (Reato et al. 2010) – meaning that by virtue of being in a network, a given compartment (soma) may be polarized directly by the field and indirectly by field actions on a collection of afferent neurons. In addition, in a network if tDCS is effective on a (more sensitive) upstream neuron, this will change synaptic activity at downstream neurons (Boros et al. 2008).

As described above, the concept that the threshold for electric field sensitivity would be "lower for modulation of the frequency of an already active neuron than for excitation of a silent one" (Terzuolo and Bullock 1956) is well established, but network activity adds another dimension to this. During many network activities, notably oscillations, neurons are near threshold and thus primed for firing. If a neuron is near threshold by virtue of network drive, then a small polarization may be influential in modulating the likelihood of firing. For example, a relatively small depolarization may be sufficient to trigger an action potential. Moreover, because the network is interconnected, activated neurons could synaptically trigger action potentials in other neurons. The whole process can be feed-forward such that a small DC electric field can induce a robust action potential discharge in a population. This has been demonstrated in brain slices and explained with quantitative

models (Reato et al. 2010). This concept is interesting because it blurs the distinction between "supra-threshold" stimulation, such as TMS, and "sub-threshold" stimulation, as tDCS is commonly considered.

Mechanisms of network amplification are difficult to explore in the human brain directly, but functional imaging data are in accordance with enhanced glutamatergic activity during stimulation (Alekseichuk et al. 2016; Hone-Blanchet et al. 2016). Moreover, electroencephalography shows that DC stimulation enhances individual alpha activity (Spitoni et al. 2013), which is in good accordance with network amplification mechanisms of tDCS (Polania et al. 2011a).

Network Effects of tDCS: Consequences for Spread of Neuromodulation

Apart from the regional network effects of tDCS under or near the stimulation electrodes, remote effects on topographically distant cortical and subcortical areas were described relatively early for the human brain (Lang et al. 2005). There is no *a priori* rationale to ignore these regions in interpreting the behavioral and cognitive consequences of tDCS. These brain-wide changes might also further support network-scale amplification.

However, it was unclear whether those effects are caused by physiological spreading of cortical activity (i.e. one region being activated by tDCS and subsequently driving another region) or by physical current spread (i.e. during tDCS current flow). Simulation studies, which have been recently validated, are in favor of at least a partial contribution of spread of current flow (Datta et al. 2009; Huang et al. 2017). In addition, clear physiological effects of tDCS on remote areas have been described. Premotor anodal tDCS enhances intracortical facilitation of M1, most probably due to the activation of premotor-primary motor cortex afferents (Boros et al. 2008). Similarly, combined dorsal premotor and supplementary motor area (SMA) stimulation alters motor and somatosensory evoked potentials (Kirimoto et al. 2011). For parietal cortex stimulation, anodal tDCS enhanced, but cathodal tDCS reduced MEP amplitudes elicited by motor cortex TMS. Moreover, anodal tDCS over the posterior parietal cortex increased ipsilateral M1 intracortical inhibition and facilitation, as well as parietal-motor cortical connectivity (Rivera-Urbina et al. 2015). Furthermore, anodal tDCS over posterior parietal cortex increased cortico-cortical potentials elicited by TMS in both local and surrounding or contralateral regions (Romero Lauro et al. 2014).

Recently, functional connectivity approaches have been applied to explore cortical network alterations induced by tDCS in humans. For motor cortex stimulation under resting conditions, a fMRI study revealed that nodal minimum path length increased after anodal tDCS over M1, which means that functional connectivity of this area with topographically distant regions of the whole brain significantly decreased. In contrast to this generally reduced whole brain connectivity of M1, functional connectivity was enhanced between the primary motor cortex on the one

hand, and premotor and superior parietal areas on the other (Polania et al. 2011b). In another study, cathodal tDCS of the primary motor cortex increased functional connectivity between the stimulated M1, and the contralateral M1 and premotor cortices (Stagg et al. 2009b). A similar effect of tDCS was described for anodal stimulation combined with motor practice in an EEG study, where functional connectivity was enhanced between primary motor, premotor, and sensorimotor areas in the high gamma band (Polania et al. 2011a). Moreover, anodal tDCS of the primary motor cortex alters cortico-subcortical connectivity of the motor cortex at rest. Specifically, it was shown to enhance connectivity with the ipsilateral caudate nucleus, and thalamus (Polania et al. 2012a). Alterations of intrinsic motor cortex connectivity by tDCS have also been demonstrated: cathodal stimulation increased local connectivity, most likely due to cortical noise reduction accomplished by the respective excitability and activity diminution, while anodal tDCS enhanced long-distance connectivity within this area (Polania et al. 2012b). Therefore it can be concluded by the results of these studies that motor cortex tDCS alters the connectivity of large parts of the motor network, and thus regional stimulation has network effects.

Such effects are not restricted to motor cortex tDCS. Stimulation of the dorsolateral prefrontal cortex has been demonstrated to induce widespread alterations of functional connectivity, including the default mode network, and attention-related networks in healthy subjects (Keeser et al. 2011; Pena-Gomez et al. 2012). Thus it can be concluded that the effects of DC stimulation in vivo are not restricted to the primary target area, but involve a larger set of connected areas. Since these effects are assumed to be activity-related, the impact of tDCS on remote areas might nevertheless differ from those stimulated directly by the intervention, because the polarizing effect by external application of an electrical field is missing. If and in which way this leads to different functional and physiological effects in these secondary areas remains to be shown.

Non-neuronal Effects of tDCS

So far, most research on DC stimulation was focused on neurons. However, additional cell types – including glia and endothelial cells – are affected by DC fields and might contribute to the neuromodulation outcomes. Here, it can be distinguished between (1) direct stimulation effects, reflecting direct polarization and modulation of these cell types by direct current fields; (2) indirect stimulation effects, reflecting changes in function secondary to direct excitatory neuronal activation that then influences these other cell types; and (3) modulatory effects, where the sensitivity of neurons to direct effects (e.g., their excitability) is influenced by these other cell types.

Glia cells represent the majority of cells in the CNS – the concept that they are just ‘passive’ support cells is outdated (Haydon and Carmignoto 2006) and their essential role in neuronal functions such as plasticity are being elucidated (Di Castro et al. 2011; Panatier et al. 2011). Astrocytes are particularly crucial in regulating

synaptic transmission and plasticity, leading to the recent idea of a 'tripartite synapse' (Perea et al. 2009). While astrocytes are sensitive to small changes in membrane potential (Amzica et al. 2002) and their elongated processes are susceptible to polarization by DCS (Ruohonen and Karhu 2012), the effects of weak DCS on these cells remain relatively neglected in the literature (Gellner et al. 2016). However, a recent in vivo study in mice showed that tDCS induced astrocytic calcium waves in visual cortex, which appeared to drive plasticity of visually evoked potentials (Monai et al. 2016). It was unclear whether this effect was due to direct or indirect action on astrocytes, but this motivates more work in understanding the role of astrocytes in tDCS induced plasticity. In addition to effects in individual glia cells, a glial syncytium (an electrically coupled population of glial cells) might act to amplify field polarization. Just as a single cell (glia) experiences a biphasic polarization in response to DCS, the glial syncytium may experience a net biphasic polarization across the network axis. Another possible mechanism for DC modulation through glia cells relates to the concept of potassium 'spatial buffering'. Glia cells are thought to regulate extracellular potassium concentration through a polarization imbalance across their membrane, and the biphasic polarization induced by DC fields would be expected to drive the collection and release of potassium across the glia or glial syncytium ends. Indeed, Gardner-Medwin induced extracellular potassium transport by passing DC current and noted concentration changes in saline near the electrodes, which is mechanistically distinct from tissue changes (Gardner-Medwin 1983). Studies in brain slices however show no changes in extracellular potassium concentration with DC fields (Lian et al. 2003), though the brain slice preparation has distorted extracellular concentration control mechanisms (An et al. 2008).

Endothelial cells form the blood-brain barrier (BBB) that tightly regulates transport between the brain extracellular space and blood. Any direct action of DC stimulation on endothelial cells would thus have important consequence for brain function. Endothelial cells do not have processes and their spherical shape indicates peak polarization will be related to cell diameter (Kotnik et al. 2010). However, a compelling hypothesis is that the blood vessel network formed by the BBB might channel current flow in a manner that concentrates electric field across the BBB. The direct effects of tDCS current on vascular response remain an open and compelling question. There is abundant evidence that DC current affects vascular function in skin (Berliner 1997; Ledger 1992; Malty and Petrofsky 2007; Prausnitz 1996) and skin redness is inevitable under tDCS electrodes (Minhas et al. 2010) – with a component that is pressure related but a component that is in response to current flow (Ezquerro et al. 2016).

Vascular and neuronal functions in the brain are closely interrelated, as evidenced by functional Magnetic Resonance Imaging (fMRI). The relation is also complex, and it can be difficult to disentangle direct neuronal and potential direct vascular effects (Takano et al. 2011), including during tDCS. Wachter and colleagues (2011) reported a polarity specific change in blood perfusion during tDCS in the rat, in a direction consistent with the somatic doctrine, and speculated the direction specificity was consistent with a primary neuronal action. Furthermore, it

was shown that high-intensity electrical stimulation could increase transport across the blood-brain barrier. This phenomenon was termed "electro-permeation" between cells, to distinguish it from electroporation of single cells (Lopez-Quintero et al. 2010). Taken together, there are reasons to assume that application of DC fields affect also non-neuronal cells of the CNS, but the paucity of experimental evidence requires further investigation on the ultimate impact on tDCS outcomes.

Concluding Remarks: Building on an Extensive Foundation of Mechanistic Studies

This chapter gave an overview about the current state of knowledge of the physiological effects of brain stimulation with weak DC fields. As can be derived from the available studies and concepts, knowledge is extensive but far from being complete. Whereas basic general mechanisms of action have been identified, especially at the microscopic cellular level and clinical neurophysiology, important identified questions await yet to be answered. The effects of tDCS may be complex in the sense that they are brain-state and dose (montage, current intensity, duration) dependent, such that different mechanisms are operant depending on the application. Nonetheless, certain basic principles, as highlighted in this review, are likely universal. Especially integration of knowledge across animal and human experiments at different levels of organization, is important to address this complexity.

What seems to be clear even at different physiological scales (from cellular to human neurophysiology), is that the general assumption that anodal DC stimulation enhances excitability and cathodal stimulation diminishes excitability is an oversimplification. Rather, the outcome of stimulation is to be qualified by protocol specifics. At the same time it's important to recognize that such over-simplifications are not germane to tDCS and exist across neuromodulation technologies (e.g. DBS) and pharmacology. tDCS research, more than other domains, has (1) over decades established a scientific foundation; (2) in this process addressed head-on limitations in existing understanding. It is a mistake to confuse ongoing discovery of nuance in DCS effects with a crisis in the fundamentals.

For example, ongoing experiments in animal models of direct current stimulation are beginning to provide insight into how neuromodulation by tDCS cannot be explained as a monolithic "sliding-scale" of excitability (where regions under the anode are "excited" while regions under the cathode are "de-excited"). Brain function and disease are complex and so their influence by DC stimulation is similarly complex. Moving beyond the "somatic doctrine", polarization of dendrites, axon terminals, and astrocytes can no longer be ignored. The effects of polarization in each of these compartments are likely to vary with their activity state (e.g. membrane potential, neurotransmitter tone, ion channel state), with effects being amplified by increased ongoing activity. Importantly, this may support modulation of plasticity specifically in the most active synapses. This also implies that tDCS may

have vastly different effects depending on the form of endogenous plasticity (e.g. driven by dendritic or somatic spikes).

Which neuronal processes are modulated and how, will depend on the tDCS montage used and the state of the underlying network. The rational advancement of tDCS thus requires progressing from the sliding-scale approach (applied indiscriminately across cognitive applications and indications) and addressing these mechanistic and targeting issues. With increased recognition of complexity, the need for translational animal studies, that are properly designed, becomes increasingly clear. Following the organization in this chapter, this includes considering the effects of DCS at three scales: membrane compartment polarization, synaptic efficacy, and network effects. While brain function is evidently understood to span across these levels, this among other structures introduced here, provide a path forward toward framing of new hypotheses. Combining animal experimentation with human experimental work, and new approaches like computational neurostimulation (Bestmann 2015) will help to comprehend the mechanisms of action of DC stimulation further, which will be the essential pre-condition to develop stimulation protocols which allow clearly defined and targeted interventions in basic and applied neuroscience.

Literature

Accornero, N., Voti, P. L., La Riccia, M., & Gregori, B. (2007). Visual evoked potentials modulation during direct current cortical polarization. *Experimental Brain Research, 178*(2), 261–266.

Alekseichuk, I., Diers, K., Paulus, W., & Antal, A. (2016). Transcranial electrical stimulation of the occipital cortex during visual perception modifies the magnitude of BOLD activity: A combined tES-fMRI approach. *NeuroImage, 140*, 110–117.

Amzica, F., Massimini, M., & Manfridi, A. (2002). Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. *The Journal of Neuroscience, 22*(3), 1042–1053.

An, J. H., Su, Y., Radman, T., & Bikson, M. (2008). Effects of glucose and glutamine concentration in the formulation of the artificial cerebrospinal fluid (ACSF). *Brain Research, 1218*, 77–86.

Anastassiou, C. A., Montgomery, S. M., Barahona, M., Buzsáki, G., & Koch, C. (2010). The effect of spatially inhomogeneous extracellular electric fields on neurons. *Journal of Neuroscience, 30*(5), 1925–1936.

Andreasen, M., & Nedergaard, S. (1996). Dendritic electrogenesis in rat hippocampal CA1 pyramidal neurons: Functional aspects of Na+ and Ca2+ currents in apical dendrites. *Hippocampus, 6*(1), 79–95.

Antal, A., Nitsche, M. A., Kruse, W., Kincses, T. Z., Hoffmann, K.-P., & Paulus, W. (2004a). Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. *Journal of Cognitive Neuroscience, 16*(4), 521–527.

Antal, A., Terney, D., Poreisz, C., & Paulus, W. (2007). Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. *European Journal of Neuroscience, 26*(9), 2687–2691.

Antal, A., Varga, E. T., Kincses, T. Z., Nitsche, M. A., & Paulus, W. (2004b). Oscillatory brain activity and transcranial direct current stimulation in humans. *Neuroreport, 15*(8), 1307–1310.

Ardolino, G., Bossi, B., Barbieri, S., & Priori, A. (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *The Journal of Physiology, 568*(Pt 2), 653–663.

Awatramani, G. B., Price, G. D., & Trussell, L. O. (2005). Modulation of transmitter release by presynaptic resting potential and background calcium levels. *Neuron, 48*(1), 109–121.

Berliner, M. N. (1997). Skin microcirculation during tapwater iontophoresis in humans: Cathode stimulates more than anode. *Microvascular Research, 54*(1), 74–80.

Bestmann, S. (2015). Computational neurostimulation: Models for bridging physiology and behavior. Brain stimulation: Basic, translational, and clinical research in. *Neuromodulation, 8*(2), 435.

Beveridge, J. A., & Politis, M. J. (1988). Use of exogenous electric current in the treatment of delayed lesions in peripheral nerves. *Plastic and Reconstructive Surgery, 82*(4), 573–579.

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H., & Jefferys, J. G. (2004). Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *The Journal of Physiology, 557*(1), 175–190.

Bikson, M., & Rahman, A. (2013). Origins of specificity during tDCS: Anatomical, activity-selective, and input-bias mechanisms. *Frontiers in Human Neuroscience, 7*, 688.

Bindman, L. J., Lippold, O., & Redfearn, J. (1962). Long-lasting changes in the level of the electrical activity of the cerebral cortex produced by polarizing currents. *Nature, 196*(4854), 584–585.

Bindman, L. J., Lippold, O., & Redfearn, J. (1964). 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. *The Journal of Physiology, 172*(3), 369–382.

Bishop, G., & Erlanger, J. (1926). The effects of polarization upon the activity of vertebrate nerve. *American Journal of Physiology--Legacy Content, 78*(3), 630–657.

Bishop, G., & O'Leary, J. (1950). The effects of polarizing currents on cell potentials and their significance in the interpretation of central nervous system activity. *Electroencephalography and Clinical Neurophysiology, 2*(1), 401–416.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology, 232*(2), 331–356.

Bolognini, N., Fregni, F., Casati, C., Olgiati, E., & Vallar, G. (2010). Brain polarization of parietal cortex augments training-induced improvement of visual exploratory and attentional skills. *Brain Research, 1349*, 76–89.

Boros, K., Poreisz, C., Munchau, A., Paulus, W., & Nitsche, M. A. (2008). Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. *The European Journal of Neuroscience, 27*(5), 1292–1300.

Bullock, T. H., & Hagiwara, S. (1957). Intracellular recording from the giant synapse of the squid. *The Journal of General Physiology, 40*(4), 565–577.

Cambiaghi, M., Teneud, L., Velikova, S., Gonzalez-Rosa, J., Cursi, M., Comi, G., & Leocani, L. (2011). Flash visual evoked potentials in mice can be modulated by transcranial direct current stimulation. *Neuroscience, 185*, 161–165.

Cambiaghi, M., Velikova, S., Gonzalez-Rosa, J. J., Cursi, M., Comi, G., & Leocani, L. (2010). Brain transcranial direct current stimulation modulates motor excitability in mice. *European Journal of Neuroscience, 31*(4), 704–709.

Carandini, M., & Ferster, D. (2000). Membrane potential and firing rate in cat primary visual cortex. *Journal of Neuroscience, 20*(1), 470–484.

Carter, M. J., Maslovat, D., & Carlsen, A. N. (2015). Anodal transcranial direct current stimulation applied over the supplementary motor area delays spontaneous antiphase-to-in-phase transitions. *Journal of Neurophysiology, 113*(3), 780–785.

Castro-Alamancos, M. A., Donoghue, J. P., & Connors, B. W. (1995). Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. *The Journal of Neuroscience, 15*(7 Pt 2), 5324–5333.

Cavarretta, F., Carnevale, N. T., Tegolo, D., & Migliore, M. (2014). Effects of low frequency electric fields on synaptic integration in hippocampal CA1 pyramidal neurons: Implications for power line emissions. *Frontiers in Cellular Neuroscience, 8*, 310.

Chan, C., Hounsgaard, J., & Nicholson, C. (1988). Effects of electric fields on transmembrane potential and excitability of turtle cerebellar Purkinje cells in vitro. *The Journal of Physiology, 402*, 751–771.

Costain, R., Redfearn, J., & Lippold, O. (1964). A controlled trial of the therapeutic effects of polarization of the brain in depressive illness. *The British Journal of Psychiatry, 110* (469),786–799.

Creutzfeldt, O. D., Fromm, G. H., & Kapp, H. (1962). Influence of transcortical dc currents on cortical neuronal activity. *Experimental Neurology, 5*(6), 436–452.

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., & Bikson, M. (2009). Gyri-precise head model of transcranial direct current stimulation: Improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimulation, 2*(4), 201–207. e201.

Deans, J. K., Powell, A. D., & Jefferys, J. G. (2007). Sensitivity of coherent oscillations in rat hippocampus to AC electric fields. *The Journal of Physiology, 583*(2), 555–565.

Del Castillo, J., & Katz, B. (1954). Changes in end-plate activity produced by pre-synaptic polarization. *The Journal of Physiology, 124*(3), 586–604.

Delgado-Lezama, R., Perrier, J. F., & Hounsgaard, J. (1999). Local facilitation of plateau potentials in dendrites of turtle motoneurones by synaptic activation of metabotropic receptors. *The Journal of Physiology, 515*(Pt 1), 203–207.

Di Castro, M. A., Chuquet, J., Liaudet, N., Bhaukaurally, K., Santello, M., Bouvier, D., … Volterra, A. (2011). Local Ca2+ detection and modulation of synaptic release by astrocytes. *Nature Neuroscience, 14*(10), 1276–1284.

Dubner, H. H. (1939). Factors controlling brain potentials in the cat. Ph D, University of Chicago, Springfield, IL.

Dudel, J. (1971). The effect of polarizing current on action potential and transmitter release in crayfish motor nerve terminals. *Pflügers Archiv, 324*(3), 227–248.

Ezquerro, F., Moffa, A. H., Bikson, M., Khadka, N., Aparicio, L. V., Bd, S.-J., … Pereira, A. C. (2016). *The influence of skin redness on blinding in transcranial direct current stimulation studies: A crossover trial*. Neuromodulation: Technology at the Neural Interface.

Floel, A. (2014). tDCS-enhanced motor and cognitive function in neurological diseases. *NeuroImage, 85*(Pt 3), 934–947.

Fresnoza, S., Paulus, W., Nitsche, M. A., & Kuo, M. F. (2014a). Nonlinear dose-dependent impact of D1 receptor activation on motor cortex plasticity in humans. *The Journal of Neuroscience, 34*(7), 2744–2753.

Fresnoza, S., Stiksrud, E., Klinker, F., Liebetanz, D., Paulus, W., Kuo, M. F., & Nitsche, M. A. (2014b). Dosage-dependent effect of dopamine D2 receptor activation on motor cortex plasticity in humans. *The Journal of Neuroscience, 34*(32), 10701–10709.

Freund, T. F., & Buzsaki, G. (1996). Interneurons of the hippocampus. *Hippocampus, 6*(4), 347–470.

Fricke, K., Seeber, A. A., Thirugnanasambandam, N., Paulus, W., Nitsche, M. A., & Rothwell, J. C. (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. *Journal of Neurophysiology, 105*(3), 1141–1149.

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., & Lu, B. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: Potential implications for motor learning. *Neuron, 66*(2), 198–204.

Fröhlich, F., & Mccormick, D. A. (2010). Endogenous electric fields may guide neocortical network activity. *Neuron, 67*(1), 129–143.

Gardner-Medwin, A. (1983). Analysis of potassium dynamics in mammalian brain tissue. *The Journal of Physiology, 335*, 393–426.

Gartside, I. B. (1968a). Mechanisms of sustained increases of firing rate of neurones in the rat cerebral cortex after polarization: Reverberating circuits or modification of synaptic conductance? *Nature, 220*(5165), 382–383.

Gartside, I. B. (1968b). Mechanisms of sustained increases of firing rate of neurones in the rat cerebral cortex after polarization: Role of protein synthesis. *Nature, 220*(5165), 383–384.

Gellner, A. K., Reis, J., & Fritsch, B. (2016). Glia: A neglected player in non-invasive direct current brain stimulation. *Frontiers in Cellular Neuroscience, 10*, 188.

Giordano, J., Bikson, M., Kappenman, E. S., Clark, V. P., Coslett, H. B., Hamblin, M. R., … Mckinley, R. A. (2017). Mechanisms and effects of transcranial direct current stimulation. *Dose-Response, 15*(1), 1559325816685467.

Grundey, J., Thirugnanasambandam, N., Kaminsky, K., Drees, A., Skwirba, A. C., Lang, N., … Nitsche, M. A. (2012). Rapid effect of nicotine intake on neuroplasticity in non-smoking humans. *Frontiers in Pharmacology, 3*, 186.

Hanley, C. J., Singh, K. D., & Mcgonigle, D. J. (2016). Transcranial modulation of brain oscillatory responses: A concurrent tDCS-MEG investigation. *NeuroImage, 140*, 20–32.

Hattori, Y., Moriwaki, A., & Hori, Y. (1990). Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic AMP in rat cerebral cortex. *Neuroscience Letters, 116*(3), 320–324.

Haydon, P. G., & Carmignoto, G. (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiological Reviews, 86*(3), 1009–1031.

Hess, G., & Donoghue, J. P. (1999). 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. *Experimental Brain Research, 127*(3), 279–290.

Hinkle, L., Mccaig, C. D., & Robinson, K. R. (1981). The direction of growth of differentiating neurones and myoblasts from frog embryos in an applied electric field. *The Journal of Physiology, 314*, 121–135.

Hone-Blanchet, A., Edden, R. A., & Fecteau, S. (2016). Online effects of transcranial direct current stimulation in real time on human prefrontal and striatal metabolites. *Biological Psychiatry, 80*(6), 432–438.

Huang, Y., Liu, A. A., Lafon, B., Friedman, D., Dayan, M., Wang, X., … Parra, L. C. (2017). Measurements and models of electric fields in the in vivo human brain during transcranial electric stimulation. *eLife, 6*, e18834.

Hubbard, J., & Willis, W. (1962a). Hyperpolarization of mammalian motor nerve terminals. *The Journal of Physiology, 163*(1), 115–137.

Hubbard, J. I., & Willis, W. D. (1962b). Mobilization of transmitter by hyperpolarization. *Nature, 193*(4811), 174–175.

Islam, N., Aftabuddin, M., Moriwaki, A., Hattori, Y., & Hori, Y. (1995a). Increase in the calcium level following anodal polarization in the rat brain. *Brain Research, 684*(2), 206–208.

Islam, N., Moriwaki, A., & Hori, Y. (1995b). Co-localization of c-fos protein and protein kinase C gamma in the rat brain following anodal polarization. *Indian Journal of Physiology and Pharmacology, 39*(3), 209–215.

Jackson, M. P., Rahman, A., Lafon, B., Kronberg, G., Ling, D., Parra, L. C., & Bikson, M. (2016). Animal models of transcranial direct current stimulation: Methods and mechanisms. *Clinical Neurophysiology, 127*(11), 3425–3454.

Jaffe, L. F., & Poo, M. M. (1979). Neurites grow faster towards the cathode than the anode in a steady field. *The Journal of Experimental Zoology, 209*(1), 115–128.

Jamil, A., Batsikadze, G., Kuo, H. I., Labruna, L., Hasan, A., Paulus, W., & Nitsche, M. A. (2017). Systematic evaluation of the impact of stimulation intensity on neuroplastic after-effects induced by transcranial direct current stimulation. *The Journal of Physiology, 595*(4), 1273–1288.

Jefferys, J. (1981). Influence of electric fields on the excitability of granule cells in Guinea-pig hippocampal slices. *The Journal of Physiology, 319*, 143–152.

Joucla, S., & Yvert, B. (2009). The "mirror" estimate: An intuitive predictor of membrane polarization during extracellular stimulation. *Biophysical Journal, 96*(9), 3495–3508.

Kabakov, A. Y., Muller, P. A., Pascual-Leone, A., Jensen, F. E., & Rotenberg, A. (2012). Contribution of axonal orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus. *Journal of Neurophysiology, 107*(7), 1881–1889.

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., … Padberg, F. (2011). Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *The Journal of Neuroscience, 31*(43), 15284–15293.

Kirimoto, H., Ogata, K., Onishi, H., Oyama, M., Goto, Y., & Tobimatsu, S. (2011). Transcranial direct current stimulation over the motor association cortex induces plastic changes in ipsilateral primary motor and somatosensory cortices. *Clinical Neurophysiology, 122*(4), 777–783.

Kotnik, T., Pucihar, G., & Miklavcic, D. (2010). Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. *The Journal of Membrane Biology, 236*(1), 3–13.

Kronberg, G., Bridi, M., Abel, T., Bikson, M., & Parra, L. C. (2017). Direct current stimulation modulates LTP and LTD: Activity dependence and dendritic effects. *Brain Stimulation, 10*(1), 51–58.

Lafon, B., Rahman, A., Bikson, M., & Parra, L. C. (2017). Direct current stimulation alters neuronal input/output function. *Brain Stimulation, 10*(1), 36–45.

Lang, N., Siebner, H. R., Ward, N. S., Lee, L., Nitsche, M. A., Paulus, W., … Frackowiak, R. S. (2005). How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *The European Journal of Neuroscience, 22*(2), 495–504.

Ledger, P. W. (1992). Skin biological issues in electrically enhanced transdermal delivery. *Advanced Drug Delivery Reviews, 9*(2–3), 289–307.

Lee, D., Lin, B. J., & Lee, A. K. (2012). Hippocampal place fields emerge upon single-cell manipulation of excitability during behavior. *Science, 337*(6096), 849–853.

Lian, J., Bikson, M., Sciortino, C., Stacey, W. C., & Durand, D. M. (2003). Local suppression of epileptiform activity by electrical stimulation in rat hippocampus in vitro. *The Journal of Physiology, 547*(Pt 2), 427–434.

Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain, 125*(10), 2238–2247.

Lisman, J. E. (2001). Three Ca2+ levels affect plasticity differently: The LTP zone, the LTD zone and no man's land. *The Journal of Physiology, 532*(Pt 2), 285.

Lopez-Quintero, S. V., Datta, A., Amaya, R., Elwassif, M., Bikson, M., & Tarbell, J. M. (2010). DBS-relevant electric fields increase hydraulic conductivity of in vitro endothelial monolayers. *Journal of Neural Engineering, 7*(1), 16005.

Lu, B. (2003). BDNF and activity-dependent synaptic modulation. *Learning & Memory, 10*(2), 86–98.

Lugon, M. D. M. V., Batsikadze, G., Fresnoza, S., Grundey, J., Kuo, M.-F., Paulus, W., … Nitsche, M. A. (2015). Mechanisms of nicotinic modulation of glutamatergic neuroplasticity in humans. *Cerebral Cortex*, bhv252.

Magee, J. C., & Cook, E. P. (2000). Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nature Neuroscience, 3*(9), 895–903.

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: An embarrassment of riches. *Neuron, 44*(1), 5–21.

Malty, A. M., & Petrofsky, J. (2007). The effect of electrical stimulation on a normal skin blood flow in active young and older adults. *Medical Science Monitor, 13*(4), CR147–CR155.

Márquez-Ruiz, J., Leal-Campanario, R., Sánchez-Campusano, R., Molaee-Ardekani, B., Wendling, F., Miranda, P. C., … Delgado-García, J. M. (2012). Transcranial direct-current stimulation modulates synaptic mechanisms involved in associative learning in behaving rabbits. *Proceedings of the National Academy of Sciences, 109*(17), 6710–6715.

Marsh, G., & Beams, H. W. (1946). In vitro control of growing chick nerve fibers by applied electric currents. *Journal of Cellular and Comparative Physiology, 27*, 139–157.

Marshall, L., Kirov, R., Brade, J., Molle, M., & Born, J. (2011). Transcranial electrical currents to probe EEG brain rhythms and memory consolidation during sleep in humans. *PLoS One, 6*(2), e16905.

Mccaig, C. D., & Rajnicek, A. M. (1991). Electrical fields, nerve growth and nerve regeneration. *Experimental Physiology, 76*(4), 473–494.

Mccaig, C. D., Rajnicek, A. M., Song, B., & Zhao, M. (2005). Controlling cell behavior electrically: Current views and future potential. *Physiological Reviews, 85*(3), 943–978.

Mcdevitt, L., Fortner, P., & Pomeranz, B. (1987). Application of weak electric field to the hindpaw enhances sciatic motor nerve regeneration in the adult rat. *Brain Research, 416*(2), 308–314.

Mcintyre, C. C., Grill, W. M., Sherman, D. L., & Thakor, N. V. (2004). Cellular effects of deep brain stimulation: Model-based analysis of activation and inhibition. *Journal of Neurophysiology, 91*(4), 1457–1469.

Merrill, D. R., Bikson, M., & Jefferys, J. G. (2005). Electrical stimulation of excitable tissue: Design of efficacious and safe protocols. *Journal of Neuroscience Methods, 141*(2), 171–198.

Minhas, P., Bansal, V., Patel, J., Ho, J. S., Diaz, J., Datta, A., & Bikson, M. (2010). Electrodes for high-definition transcutaneous DC stimulation for applications in drug delivery and electrotherapy, including tDCS. *Journal of Neuroscience Methods, 190*(2), 188–197.

Miranda, P. C., Lomarev, M., & Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clinical Neurophysiology, 117*(7), 1623–1629.

Monai, H., Ohkura, M., Tanaka, M., Oe, Y., Konno, A., Hirai, H., … Hirase, H. (2016). Calcium imaging reveals glial involvement in transcranial direct current stimulation-induced plasticity in mouse brain. *Nature Communications, 7*, 11100.

Monte-Silva, K., Kuo, M.-F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., & Nitsche, M. A. (2013). Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimulation, 6*(3), 424–432.

Monte-Silva, K., Kuo, M. F., Liebetanz, D., Paulus, W., & Nitsche, M. A. (2010). Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). *Journal of Neurophysiology, 103*(4), 1735–1740.

Nitsche, M., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., … Paulus, W. (2003a). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of Physiology, 553*(1), 293–301.

Nitsche, M. A., Doemkes, S., Karakoese, T., Antal, A., Liebetanz, D., Lang, N., … Paulus, W. (2007a). Shaping the effects of transcranial direct current stimulation of the human motor cortex. *Journal of Neurophysiology, 97*(4), 3109–3117.

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2004). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology, 29*(8), 1573–1578.

Nitsche, M. A., Muller-Dahlhaus, F., Paulus, W., & Ziemann, U. (2012). The pharmacology of neuroplasticity induced by non-invasive brain stimulation: Building models for the clinical use of CNS active drugs. *The Journal of Physiology, 590*(19), 4641–4662.

Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology, 527*(3), 633–639.

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*(10), 1899–1901.

Nitsche, M. A., Roth, A., Kuo, M. F., Fischer, A. K., Liebetanz, D., Lang, N., … Paulus, W. (2007b). Timing-dependent modulation of associative plasticity by general network excitability in the human motor cortex. *The Journal of Neuroscience, 27*(14), 3807–3812.

Nitsche, M. A., Schauenburg, A., Lang, N., Liebetanz, D., Exner, C., Paulus, W., & Tergau, F. (2003b). Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *Journal of Cognitive Neuroscience, 15*(4), 619–626.

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., … Antal, A. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *The Journal of Physiology, 568*(1), 291–303.

Opitz, A., Falchier, A., Yan, C.-G., Yeagle, E. M., Linn, G. S., Megevand, P., & Thielscher, A. (2016). Spatiotemporal structure of intracranial electric fields induced by transcranial electric stimulation in humans and nonhuman primates. *Scientific Reports, 6,* 31236.

Palmer, A. M., Messerli, M. A., & Robinson, K. R. (2000). Neuronal galvanotropism is independent of external ca(2+) entry or internal ca(2+) gradients. *Journal of Neurobiology, 45*(1), 30–38.

Panatier, A., Vallee, J., Haber, M., Murai, K. K., Lacaille, J. C., & Robitaille, R. (2011). Astrocytes are endogenous regulators of basal transmission at central synapses. *Cell, 146*(5), 785–798.

Parra, L. C., & Bikson, M. (2004). Model of the effect of extracellular fields on spike time coherence. In *Engineering in Medicine and Biology Society. IEMBS'04. 26th Annual International Conference of the IEEE, 2004. IEEE* (pp. 4584–4587).

Patel, N., & Poo, M. M. (1982). Orientation of neurite growth by extracellular electric fields. *The Journal of Neuroscience, 2*(4), 483–496.

Patel, N. B., & Poo, M. M. (1984). Perturbation of the direction of neurite growth by pulsed and focal electric fields. *The Journal of Neuroscience, 4*(12), 2939–2947.

Paulus, W., & Rothwell, J. C. (2016). Membrane resistance and shunting inhibition: where biophysics meets state–dependent human neurophysiology. *The Journal of Physiology, 594*(10), 2719–2728.

Pelletier, S. J., & Cicchetti, F. (2014). Cellular and molecular mechanisms of action of transcranial direct current stimulation: Evidence from in vitro and in vivo models. *The International Journal of Neuropsychopharmacology, 18*(2), pyu047.

Pena-Gomez, C., Sala-Lonch, R., Junque, C., Clemente, I. C., Vidal, D., Bargallo, N., … Bartres-Faz, D. (2012). Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI. *Brain Stimulation, 5*(3), 252–263.

Perea, G., Navarrete, M., & Araque, A. (2009). Tripartite synapses: Astrocytes process and control synaptic information. *Trends in Neurosciences, 32*(8), 421–431.

Podda, M. V., Cocco, S., Mastrodonato, A., Fusco, S., Leone, L., Barbati, S. A., … Grassi, C. (2016). Anodal transcranial direct current stimulation boosts synaptic plasticity and memory in mice via epigenetic regulation of Bdnf expression. *Scientific Reports, 6*, 22180.

Polania, R., Nitsche, M. A., & Paulus, W. (2011a). Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. *Human Brain Mapping, 32*(8), 1236–1249.

Polania, R., Paulus, W., Antal, A., & Nitsche, M. A. (2011b). Introducing graph theory to track for neuroplastic alterations in the resting human brain: A transcranial direct current stimulation study. *NeuroImage, 54*(3), 2287–2296.

Polania, R., Paulus, W., & Nitsche, M. A. (2012a). Modulating cortico-striatal and thalamo-cortical functional connectivity with transcranial direct current stimulation. *Human Brain Mapping, 33*(10), 2499–2508.

Polania, R., Paulus, W., & Nitsche, M. A. (2012b). Reorganizing the intrinsic functional architecture of the human primary motor cortex during rest with non-invasive cortical stimulation. *PLoS One, 7*(1), e30971.

Politis, M. J., Zanakis, M. F., & Albala, B. J. (1988). Facilitated regeneration in the rat peripheral nervous system using applied electric fields. *The Journal of Trauma, 28*(9), 1375–1381.

Pomeranz, B., Mullen, M., & Markus, H. (1984). Effect of applied electrical fields on sprouting of intact saphenous nerve in adult rat. *Brain Research, 303*(2), 331–336.

Prausnitz, M. R. (1996). Do high-voltage pulses cause changes in skin structure? *Journal of Controlled Release, 40*(3), 321–326.

Purpura, D. P., & Mcmurtry, J. G. (1965). Intracellular activities and evoked potential changes during polarization of motor cortex. *Journal of Neurophysiology, 28*(1), 166–185.

Radman, T., Datta, A., & Peterchev, A. V. (2007a). In vitro modulation of endogenous rhythms by AC electric fields: Syncing with clinical brain stimulation. *The Journal of Physiology, 584*(2), 369–370.

Radman, T., Ramos, R. L., Brumberg, J. C., & Bikson, M. (2009). Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. *Brain Stimulation, 2*(4), 215–228.e3.

Radman, T., Su, Y., An, J. H., Parra, L. C., & Bikson, M. (2007b). Spike timing amplifies the effect of electric fields on neurons: Implications for endogenous field effects. *Journal of Neuroscience, 27*(11), 3030–3036.

Rahman, A., Lafon, B., Parra, L. C., & Bikson, M. (2017). Direct current stimulation boosts synaptic gain and cooperativity in vitro. *The Journal of Physiology, 595*, 3535–3547.

Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., & Bikson, M. (2013). Cellular effects of acute direct current stimulation: Somatic and synaptic terminal effects. *The Journal of Physiology, 591*(10), 2563–2578.

Ranieri, F., Podda, M. V., Riccardi, E., Frisullo, G., Dileone, M., Profice, P., … Grassi, C. (2012). Modulation of LTP at rat hippocampal CA3-CA1 synapses by direct current stimulation. *Journal of Neurophysiology, 107*(7), 1868–1880.

Rattay, F., & Wenger, C. (2010). Which elements of the mammalian central nervous system are excited by low current stimulation with microelectrodes? *Neuroscience, 170*(2), 399–407.

Reato, D., Bikson, M., & Parra, L. C. (2015). Lasting modulation of in vitro oscillatory activity with weak direct current stimulation. *Journal of Neurophysiology, 113*(5), 1334–1341.

Reato, D., Gasca, F., Datta, A., Bikson, M., Marshall, L., & Parra, L. C. (2013a). Transcranial electrical stimulation accelerates human sleep homeostasis. *PLoS Computational Biology, 9*(2), e1002898.

Reato, D., Rahman, A., Bikson, M., & Parra, L. C. (2010). Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. *Journal of Neuroscience, 30*(45), 15067–15079.

Reato, D., Rahman, A., Bikson, M., & Parra, L. C. (2013b). Effects of weak transcranial alternating current stimulation on brain activity—A review of known mechanisms from animal studies. *Frontiers in Human Neuroscience, 7*, 687.

Redfearn, J., Lippold, O., & Costain, R. (1964). Preliminary account of the clinical effects of polarizing the brain in certain psychiatric disorders. *The British Journal of Psychiatry, 110*(469), 773–785.

Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., … Krakauer, J. W. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proceedings of the National Academy of Sciences of the United States of America, 106*(5), 1590–1595.

Rioult-Pedotti, M.-S., Friedman, D., Hess, G., & Donoghue, J. P. (1998). Strengthening of horizontal cortical connections following skill learning. *Nature Neuroscience, 1*(3), 230–234.

Rivera-Urbina, G. N., Batsikadze, G., Molero-Chamizo, A., Paulus, W., Kuo, M. F., & Nitsche, M. A. (2015). Parietal transcranial direct current stimulation modulates primary motor cortex excitability. *The European Journal of Neuroscience, 41*(6), 845–855.

Rizzo, V., Terranova, C., Crupi, D., Sant'angelo, A., Girlanda, P., & Quartarone, A. (2014). Increased transcranial direct current stimulation after effects during concurrent peripheral electrical nerve stimulation. *Brain stimulation, 7*(1), 113–121.

Rohan, J. G., Carhuatanta, K. A., Mcinturf, S. M., Miklasevich, M. K., & Jankord, R. (2015). Modulating hippocampal plasticity with in vivo brain stimulation. *The Journal of Neuroscience, 35*(37), 12824–12832.

Roman, G. C., Strahlendorf, H. K., Coates, P. W., & Rowley, B. A. (1987). Stimulation of sciatic nerve regeneration in the adult rat by low-intensity electric current. *Experimental Neurology, 98*(2), 222–232.

Romero Lauro, L. J., Rosanova, M., Mattavelli, G., Convento, S., Pisoni, A., Opitz, A., … Vallar, G. (2014). TDCS increases cortical excitability: Direct evidence from TMS-EEG. *Cortex, 58*, 99–111.

Ruohonen, J., & Karhu, J. (2012). tDCS possibly stimulates glial cells. *Clinical Neurophysiology, 123*(10), 2006–2009.

Salvador, R., Silva, S., Basser, P. J., & Miranda, P. C. (2011). Determining which mechanisms lead to activation in the motor cortex: A modeling study of transcranial magnetic stimulation using realistic stimulus waveforms and sulcal geometry. *Clinical Neurophysiology, 122*(4), 748–758.

Schmidt, S. L., Iyengar, A. K., Foulser, A. A., Boyle, M. R., & Fröhlich, F. (2014). Endogenous cortical oscillations constrain neuromodulation by weak electric fields. *Brain Stimulation, 7*(6), 878–889.

Spitoni, G. F., Cimmino, R. L., Bozzacchi, C., Pizzamiglio, L., & Di Russo, F. (2013). Modulation of spontaneous alpha brain rhythms using low-intensity transcranial direct-current stimulation. *Frontiers in Human Neuroscience, 7*, 529.

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., … Johansen-Berg, H. (2009a). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *Journal of Neuroscience, 29*(16), 5202–5206.

Stagg, C. J., O'Shea, J., Kincses, Z. T., Woolrich, M., Matthews, P. M., & Johansen-Berg, H. (2009b). Modulation of movement-associated cortical activation by transcranial direct current stimulation. *The European Journal of Neuroscience, 30*(7), 1412–1423.

Strube, W., Bunse, T., Nitsche, M. A., Nikolaeva, A., Palm, U., Padberg, F., … Hasan, A. (2016). Bidirectional variability in motor cortex excitability modulation following 1 mA transcranial direct current stimulation in healthy participants. *Physiology Reports, 4*(15), e12884.

Svirskis, G., Gutman, A., & Hounsgaard, J. (1997). Detection of a membrane shunt by DC field polarization during intracellular and whole cell recording. *Journal of Neurophysiology, 77*(2), 579–586.

Takano, Y., Yokawa, T., Masuda, A., Niimi, J., Tanaka, S., & Hironaka, N. (2011). A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. *Neuroscience Letters, 491*(1), 40–43.

Takeuchi, A., & Takeuchi, N. (1962). Electrical changes in pre-and postsynaptic axons of the giant synapse of Loligo. *The Journal of General Physiology, 45*(6), 1181–1193.

Terzuolo, C., & Bullock, T. (1956). Measurement of imposed voltage gradient adequate to modulate neuronal firing. *Proceedings of the National Academy of Sciences, 42*(9), 687–694.

Thirugnanasambandam, N., Grundey, J., Adam, K., Drees, A., Skwirba, A. C., Lang, N., … Nitsche, M. A. (2011). Nicotinergic impact on focal and non-focal neuroplasticity induced by non-invasive brain stimulation in non-smoking humans. *Neuropsychopharmacology, 36*(4), 879–886.

Wachter, D., Wrede, A., Schulz-Schaeffer, W., Taghizadeh-Waghefi, A., Nitsche, M. A., Kutschenko, A., … Liebetanz, D. (2011). Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. *Experimental Neurology, 227*(2), 322–327.

Wong, R. K., & Stewart, M. (1992). Different firing patterns generated in dendrites and somata of CA1 pyramidal neurones in Guinea-pig hippocampus. *The Journal of Physiology, 457*, 675–687.

Yoon, K. J., Oh, B.-M., & Kim, D.-Y. (2012). Functional improvement and neuroplastic effects of anodal transcranial direct current stimulation (tDCS) delivered 1day vs. 1week after cerebral ischemia in rats. *Brain Research, 1452*, 61–72.

Zaehle, T., Rach, S., & Herrmann, C. S. (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS One, 5*(11), e13766.