Chapter 2
Principles of Transcranial Direct Current Stimulation (tDCS): Introduction to the Biophysics of tDCS

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Human research on transcranial electrical stimulation provides direct evidence that weak electric currents can affect brain function in health and disease. However, limitations on both the control of stimulation delivery (including, e.g., dose/repetition and anatomical variations), factors known to influence modulation (e.g., brain state) and variability of outcome measures make it difficult to delineate a general framework to explain the effects of the stimulation based solely on human research. In this regard, computational models of tDCS and animal studies, either in vivo or in vitro, can help to develop a specific biophysical framework while being informed by results from humans.

The biophysics of tDCS, and more broadly neuromodulation, is based on specific and quantitative (equation-based) models of brain stimulation with explicit parameters (preferably based on measurable physical quantities such as field strength, membrane potential) and well-defined brain signals whose neuronal substrates are known. This is required to guarantee testable and refutable hypothesis.

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Each biophysical model of tDCS must support an incremental establishment of a comprehensive theory for tDCS. This is in contrast to more heuristic or qualitative descriptions of tDCS (e.g. "anodal stimulation makes the brain more excitable which increases function.") – such theories are typically a priori used to justify trials (e.g. "anode over dorsolateral prefrontal cortex [dLPFC] to boost mood") rather than test refutable mechanistic hypothesis.

In this chapter we describe the biophysics of tDCS. In the first section we review the basic physical principles that describe how computational models relate the electric current applied at the electrodes to electric field generated inside the brain. In the second part, we illustrate how such electric fields affect neuronal activity, focusing on results from animal studies because they allow a direct link between stimulation parameters and neuronal substrate.

Physical Principles

Transcranial direct current stimulation (tDCS) is a non-invasive technique which has been shown to modulate cortical excitability (Nitsche and Paulus 2000, 2001) and is currently envisioned as a promising tool in several neurological and psychiatric disorders, as well as stroke recovery and chronic pain (Fregni et al. 2006; Nitsche et al. 2009; Nitsche and Paulus 2009; Schlaug et al. 2008). The neuromodulatory effects elicited by tDCS depend on the electric field (E-field, measured in *Volts per meter*, *V/m*) induced in the nervous system. This field is induced by two or more electrodes placed in contact with the scalp and connected to a stimulation device. The electrodes consist of conductive materials, such as metal or conductive rubber, connected to stimulator leads. This material is in contact with a conductive solution, the electrolyte, which is usually a conductive fluid or a gel. Examples of the latter include large (25 or 35 cm^2) "sponge-sock" electrodes soaked in physiological saline solution, with a conductive rubber pad, which is connected to the stimulator wires, located inside (Minhas et al. 2010; Nitsche et al. 2008; Ruffini et al. 2013; Saturnino et al. 2015). Smaller electrodes usually use gel as an electrolyte (Ruffini et al. 2014; Sehm et al. 2013). In modern current-controlled stimulators, the current (I measured in *Amperes*, A) that enters the volume (via the electrodes) is controlled during the stimulation (Peterchev et al. 2012). In these stimulators, the voltage difference between the electrodes is controlled by the device so that the current reaches the intensity specified by the user regardless of the time-varying impedance at the electrode-skin interface. The current flows from the anode to the cathode and the voltage difference between these two electrodes is always positive in tDCS (although not constant [Minhas et al. 2010]).

A weak, 1–2 mA, and long lasting, 1–30 min, current is usually chosen in tDCS (Nitsche et al. 2008). The current is kept constant throughout the protocol, except at the beginning/end, where it increases/decreases linearly in time: ramp-up/down period. The duration of these ramp periods is usually 10 s (Minhas et al. 2010;

Nitsche et al. 2008). For purely resistive tissues, a valid approximation for DC signals (as discussed below), the E-field induced in the head during tDCS is proportional to the applied current (Peterchev et al. 2012). The spatial distribution of the E-field and its direction depend on several other parameters, like the shape and positions of the electrodes (Saturnino et al. 2015), the current injected by each electrode, the geometry of the head tissues (Opitz et al. 2015) and their electrical conductivity properties (Datta et al. 2009; Miranda et al. 2006; Miranda et al. 2013). The way neurons are affected by the E-field depends on its magnitude and direction, as will be discussed in more detail later in this chapter, as well as on the duration of stimulation. The calculation of the E-field in the head volume for a given electrode montage is deemed the "forward problem" in tDCS. The mathematical formulation of the forward problem in tDCS is well known from electrostatics: the E-field induced in the head can be obtained from the gradient of a scalar function (the electric potential, V measured in *Volts*) which is a solution of the Laplace equation (Rush and Driscoll 1968, 1969). However, analytical solutions of the resulting equations can usually only be obtained in simple approximations for the head geometry (such as a spherical geometry (Dmochowski et al. 2012) and hence numerical methods are commonly employed to obtain the E-field (Datta et al. 2009; Miranda et al. 2013).

Electric Properties of Tissues

In general, for electrical stimulation using arbitrary waveforms, the current induced in the head can be divided into an ohmic (resistive) component and a displacement (capacitive) current. The first component arises from the movement of the free ions that exist in the intra and extracellular fluids of the head tissues. The property of materials that describes how well they can conduct electricity by means of free charges is called electrical conductivity (σ in *Siemens per meter*, *S/m*). The second component of the current results from the polarization of localized charge distributions in the cellular membrane (Pethig and Kell 1987). The permittivity (ε in *Farads per meter*, *F/m*) of a medium is a measure of how easy this polarization is induced by an applied E-field. The values of these dielectric properties (σ and ε) depend on the frequency of the currents: permittivity values decrease with frequency, whereas conductivity values increase with it (Pethig and Kell 1987).

For purely ohmic materials, the waveform of the E-field follows that of the current. When capacitive currents exist, this is no longer the case and strong distortions of the current's waveform can occur (Wagner et al. 2014a). The latter exist only when the current varies with time. Since, the current in tDCS is mostly constant during stimulation, the displacement current can be considered zero. Even during the ramp-up/down period, when the current changes in time, the relatively low rate of change with time will not give rise to a strong displacement current (Opitz et al. 2016).

Knowledge about the conductivity of biological tissues is therefore crucial in tDCS. Several studies have appeared reporting measurements of these properties in biological tissues in a wide range of frequencies (Baumann et al. 1997; Gabriel et al. 1996a, b; Geddes and Baker 1967; Koessler et al. 2016; Logothetis et al. 2007; Oostendorp et al. 2000). The disparity between recording methods, tissue preparation and types (*in vivo* vs *ex vivo*) however, has led to the appearance of inconsistent data among studies (Gabriel et al. 1996a; Wagner et al. 2014a). This is especially true in the DC to low frequency range because measuring the dielectric properties in that region is technically more challenging (Schwan 1966; Wagner et al. 2014a). These uncertainties are a major cause for concern regarding computational predictions of E-field distributions during tDCS since changes in tissue conductivity values have been shown to significantly affect the E-field peak values and distribution (Laakso et al. 2015; Salvador et al. 2012).

Another important aspect concerning the conductivity and permittivity values is the fact that they are anisotropic in some tissues, i.e. the dielectric properties of the tissues are different depending on direction. This is typically due to the presence of structures that limit the flow of ions along specific directions. In the white matter (WM) the limiting structures are the axons of the neurons that constitute this tissue. These typically constrain the movement of ions in a direction parallel to the fiber (Geddes and Baker 1967). In the skull, anisotropy results from the presence of three layers of different tissues: a layer of cancellous bone between two layers of more insulating compact bone in the top part of the skull (Akhtari et al. 2002). This arrangement results in a higher effective conductivity in a direction tangential to the skull surface compared to the effective conductivity perpendicular to it (e.g. Opitz et al. 2015; Rampersad et al. 2011; Wagner et al. 2014b).

For anisotropic media, the conductivity is described as a symmetric tensor. In the WM, the conductivity tensor can be estimated via diffusion tensor imaging (DTI) (Basser et al. 1994). DTI allows for the estimation of the water molecules' diffusion tensor by acquiring diffusion weighted images (DWI) along several directions (Huisman 2010). Since the flow of ions and water molecules is thought to be constrained by the same structures, the conductivity tensor can then be obtained from the diffusion tensor (Tuch et al. 2001). This method, however, is limited by the fact that the scaling of the diffusion tensor components can be done in a variety of image processing ways and each produce very different conductivity values which highly affects the E-field calculations (Opitz et al. 2011; Tuch et al. 2001).

The Spatial Distribution of the Electric Field: Insights from Modelling Studies

The E-field induced in the head during tDCS is a vector whose magnitude and direction changes from tissue to tissue but also within each individual tissue. Since most computational studies model the tissues as connected volumes bounded by

smooth surfaces (Datta et al. 2009; Miranda et al. 2006, 2013), discontinuities arise in the E-field's magnitude and direction at these surfaces, provided the two tissues that are separated by them have different conductivities (Miranda et al. 2003). The discontinuities are such that the magnitude of the E-field's component in the direction perpendicular to the surface (the normal component) is always higher in the side of the surface belonging to the tissue with the lowest conductivity. This discontinuity is proportional to the ratio between the difference and the sum of the conductivities of the two tissues (Miranda et al. 2003). No such effect occurs for the component of the E-field parallel to the surface (the tangential component), which is continuous across these interfaces (Tofts 1990). This also means that the E-field's principal direction tends to be perpendicular to the interfaces in the tissues with very low bulk conductivities (like the skull) and parallel to them in tissues with comparatively high conductivities (like the cerebrospinal fluid). In many modelling studies, the current density (J in *Ampere per squared meter*, A/m^2) is reported instead of the E-field (Sadleir et al. 2010). The latter is also a vector which, in isotropic media, is proportional to the E-field: J is the product of the electric conductivity and the E-field. For anisotropic media, since the conductivity can no longer be described by a scalar but by a matrix instead (conductivity tensor), the current's density direction is no longer the same as that of the E-field (Miranda et al. 2003).

Most of what is presently known about the E-field distribution comes from computational modelling studies. The results obtained in these models can sometimes be counterintuitive. An example of one of such result is the fact that the E-field magnitude on the scalp under each electrode is not homogeneous. This can be seen in Fig. 2.1a, where the maxima of the E-field's magnitude are seen to be located at the electrode's edges. This also shows that the metric reported in many different studies, the ratio of the current to the electrode's area, cannot be used to estimate the current density under the electrode since the latter, like the E-field, is not uniformly distributed under the electrodes (see also Miranda et al. 2009). The maxima on the scalp are also much higher than those attained in the brain.

Another counterintuitive aspect of the E-field's distribution in tDCS arises when one analyses it in the brain. The E-field shown in Fig. 2.1b, e displays properties in line with results from spherical head models (Datta et al. 2008; Miranda et al. 2006): a stronger field at the top of the gyri beneath the electrodes and with a direction perpendicular to the local cortical sheet and tangential to it in the region in between the electrodes. These results, however, were obtained for a fully homogeneous model (all tissues represented with the same conductivity value). A more realistic model for the conductivities of the tissues results in the E-field distributions shown in Fig. 2.1d, g. These results, which have been shown in a number of modelling studies (Datta et al. 2009; Miranda et al. 2013), arise from the effects of the low conductivity of the skull, which reduce the magnitude of the E-field in the brain (compare Fig. 2.1b with c). Another contribution comes from the combination of the high conductivity of the CSF, and the convoluted geometry of the corti-

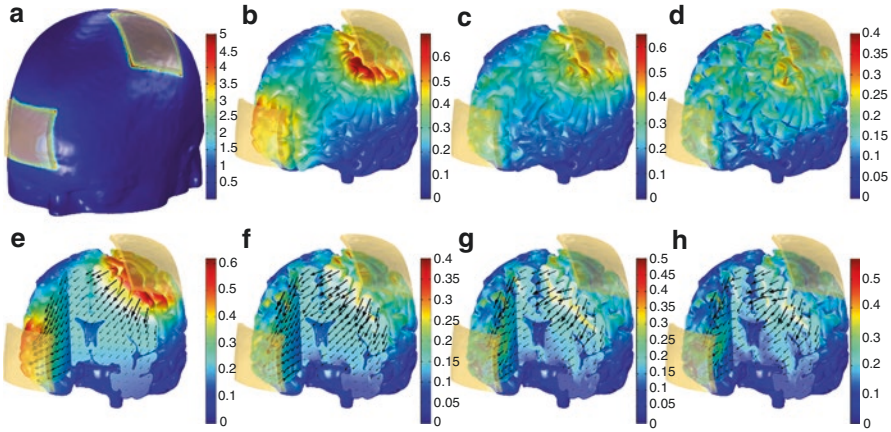

Fig. 2.1 Impact of the electrical conductivities of the tissues in E-field distribution in a realistic head model. The model contains two homogeneous electrodes ($\sigma_{electrodes} = 2$ *S*/*m*) located over the left hemisphere's hand-knob region (anode) and the right supra-orbital region (cathode). A current of 1 *mA* was injected at the anode. (**a**, **b**) E-fields distribution in the scalp (**a**) and the brain (**b**) in a homogeneous model where all tissues have an isotropic conductivity of 0.33 *S*/*m*. (**c**) Same as *B* but with the skull's conductivity set to 0.008 *S*/*m*. (**d**) Same as *C* but with the CSF's conductivity set to 1.79 *S*/*m*. (**e**) Same as *B* but now showing the direction of the E-field and its magnitude in a sagittal slice passing through the middle of the cathode and a coronal one passing through the middle of the anode. (**f**) Same as *E* but with the skull's and CSF's conductivities set to 0.008 *S*/*m* and 1.79 *S*/*m*, respectively. (**g**) Same as *F* but with the WM's conductivity set to 0.15 *S*/*m*. (**h**) Same as *G* but for an anisotropic conductivity for the GM and WM. False color: electric field (V/m)

cal surface. This reduces the E-field's magnitude in the brain, due to the shunting effect of the CSF, but creates localized maxima at the bottom of the sulci under the electrodes. The latter arise because the shunted current enters the GM perpendicularly at the bottom of the sulci (Miranda et al. 2013). The presence of the CSF therefore boosts the field at the bottom of the sulci in a direction perpendicular to the GM's outer surface, as shown in Fig. 2.1f. Finally, the inclusion of the WM as a tissue with different conductivities than the GM (Fig. 2.1g), introduces a discontinuity at the GM-WM interface which tends to increase the E-field in the WM (which has a lower isotropic conductivity) as compared to the one induced in a homogenous brain model (Fig. 2.1f). The inclusion of an anisotropic WM produces subtler changes in the results (compare Fig. 2.1g with h). In this case, the E-field tends to decrease along the main direction of the fibers since the latter corresponds to higher conductivity values compared to those of the isotropic case. The E-field in the direction perpendicular to the fibers tends to increase its value since the conductivity is much smaller than the ones in the isotropic model (see also Opitz et al. 2011).

Comparisons with Other Brain Stimulation Techniques

There are several other techniques which are used to induce an E-field in the brain non-invasively and thus affect the state of neurons. Two of them are closely related to tDCS because they use the same method to induce the E-field: transcranial alternating current stimulation (tACS) and random noise current stimulation (tRNS). tACS has been shown to interfere with ongoing brain waves or rhythms (Herrmann et al. 2013; Kanai et al. 2008; Zaehle et al. 2010), whereas high frequency tRNS has been shown to increase cortical excitability in the motor cortex (Moliadze et al. 2010; Terney et al. 2008). The difference is essentially related to the waveform of the current. The current remains constant in tDCS (apart from the ramp-up/down periods at the beginning and the end), whereas in tACS it varies sinusoidally in time with a low frequency (1–45 Hz) and in tRNS it follows a white-noise band-limited waveform ($0.1 - 640\ Hz$). For these low frequencies, the capacitive component of the current in the tissues is still much smaller than the resistive current, so the E-field waveform is in phase with that of the current as well (Plonsey and Heppner 1967). Since the current varies between a negative and a positive maximum value, the direction of the E-field will change in time, which does not occur in tDCS.

Another technique of interest is transcranial magnetic stimulation (TMS), which has been shown to be able to elicit motor responses when used over the primary motor cortex (Barker and Jalinous 1985; Hallett 2007). TMS produces a time-varying magnetic field which will induce a time-varying E-field, a processed described by Faraday's law of electromagnetic induction (Eaton 1992). The magnetic field is generated by the passage of a very high magnitude ($\sim 1 - 3\ kA$) and short lasting ($<1\ ms$) time-varying current through a coil located close to the target region in the head. The current is generated by a high-powered stimulator device connected to the coil (Peterchev et al. 2008). The E-field induced in the head depends not only on the coil's geometry and its position but also on the head geometry. Besides, it has very different properties than the one induced in tDCS, as shown in Fig. 2.2 for the field induced by a figure-8 coil in an orientation traditionally used to achieve stimulation of the motor cortex (Di Lazzaro et al. 1998). See also (Salvador et al. 2015) for a more detailed description. One of these differences is the induced E-field's magnitude, which is much higher in TMS ($\sim 100\ V/m$) than in tDCS ($\sim 0.4\ V/m$). The orientation of the field and the location of the maxima is also substantially different (compare Fig. 2.2c with d). The maxima in TMS are predominantly located at the top of the gyri under the coil and the E-field there is oriented tangentially to the cortical surface. In tDCS the orientation of the field is predominantly radial to this surface at the top of the gyri, and local maxima also appear at the bottom of the sulci where the E-field induced in TMS is already very low. The temporal variation of the induced E-field in TMS follows that of the rate of change of the current in the coil (Roth et al. 1991) which depends on stimulator

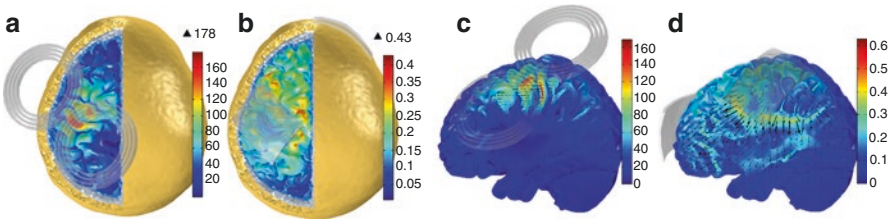

Fig. 2.2 E-field distribution in TMS (**a**, **c**) and tDCS (**b**, **d**). The first two figures show the geometry and position of the figure-8 coil (**a**) and 7×5 cm^2electrodes (**b**). (**c**, **d**) show the E-field distribution in a sagittal slice passing through the hand-knob cortical representation for TMS and tDCS respectively. The field induced in TMS was obtained for a value of dI/dt of 67 $A/\mu s$, whereas the current injected by the tDCS electrodes was set to 1 mA. The tissues in the head were given isotropic electrical conductivities based on values found in the literature, except the WM and GM which were modelled as anisotropic (for more details see Salvador et al. (2015). False color: electric field (V/m)

type (Peterchev et al. 2008). This has been disputed by recent studies which seem to indicate that at the frequency of variation of the E-field in TMS, the capacitive component of the induced current might be significant which could alter significantly the E-field waveform (Wagner et al. 2014a).

Effects of Weak Direct Current Stimulation on Neuronal Activity in Animal Models

The main advantage of using animal models is the possibility of directly measuring the effects of weak currents at multiple scales, from distinct compartments of single cells all the way to full populations responsible for measurable behaviors. At the same time, the stimulation parameters can be controlled usually with higher precision than human studies, pharmacological and genetic manipulations can be easily applied (in a manner dangerous or impossible in humans) and electrophysiology and imaging can be performed routinely (including small network, synapse, and single cell measurements).This section provides a review of the current experimental evidence on the effects of weak electrical direct current (DC) on neuronal activity and highlights the biophysical models that emerge from this data. Human literature on this matter, mainly coming from pharmacological interventions, is not explicitly considered here since this has been already discussed elsewhere in this book and in previous reviews (for example see Stagg and Nitsche 2011; Woods et al. 2016).

Animal research on the biophysics of DC stimulation started over a century ago. While studying the origin of voltage gradients in the brain, Fritsch and Hitzig in

1870 noticed that anodal stimulation increased the excitability of the brain while cathodal decreased it (Fritsch and Hitzig 1870). However, a first wave of quantitative research on the use of transcranial electrical stimulation to study brain function did not begin until the second half of the twentieth century. Studies using different animal preparations characterized in great details the effects of weak electric fields, such as those induced by transcranial stimulation, on neuronal activity. In the majority of these studies, however, the stimulation was used more as a tool to understand the origin of electric events/oscillations in the brain, not with the aim to validate a tool for neuromodulation (Bindman et al. 1964; Creutzfeldt et al. 1962; Terzuolo and Bullock 1956). A second wave of basic animal research on transcranial electrical stimulation started after seminal papers in humans showed that weak currents could modulate cortical excitability (Priori et al. 1998) and these changes could persist after the stimulation period (Nitsche and Paulus 2000). This second wave of animal research, that is still very active, does not aim at simply reproducing the results of human studies in animal models but, more importantly, at finding generic principles that explain how weak electric currents affect neurons and neuronal circuits.

The previous section of this chapter illustrated how computational current-flow models of transcranial electrical stimulation provide precise estimations of current densities (and electric fields) generated inside the brain. These estimates provide the numbers needed in animal studies to set the stimulation amplitudes and directions. However, knowing current flow by itself is not enough to predict the effects of such currents on neurons. Ultimately, the way a weak current affect brain function is determined by its interaction with neurons.

Brain function is evidently complex and determined by the concerted activity of large number of neurons and interconnected brain areas. These areas are composed of neuronal circuits made of different types of neurons and other non-neuronal cell types. To properly estimate the effects of weak electric currents on the brain it is therefore necessary to consider different scales: single neurons, how they are connected and interact, how they communicate with other neuronal and non-neuronal populations and how these populations ultimately support behavior usually in concert with other brain areas. As previously mentioned, animal research allows this type of multi-scale approach to study the temporal and spatial effects the stimulation.

This section describes the literature on the effects of weak direct currents on neuronal activity at these different scales in animal models. Building on prior reviews that addressed a selection of these aspects (Bikson et al. 2012; Krause et al. 2013; Márquez-Ruiz et al. 2014; Pelletier and Cicchetti 2015; Reato et al. 2013b; Woods et al. 2016), here the emphasis is on the different scales at which electric currents can affect neuronal activity. Moreover, apart for reviewing the known literature on this topic, new frontiers in this field of research and open questions are highlighted. The hope is that this may help guiding future research and that the list of open questions will look obsolete in a few years from now.

Effects of Weak Direct Current Stimulation on Membrane Potential, Firing Rate and Spike Timing

Whether neurons are passive or active affects how weak electrical stimulation affects their function. "Passive" here refers to those neurons whose membrane voltage is not close to the threshold for action potential generation (10–20 mV over resting membrane potential). In the literature, the effects of weak electric fields on this neuron would be called sub-threshold. "Active" neurons are those that receive massive synaptic inputs and are so depolarized that they occasionally (or often) generate action potentials.

Passive Neurons

The most widely accepted notion regarding the effects of DC currents on brain activity is that neurons under the anode are excited while neurons under the cathode are inhibited. This simple explanation of tDCS effects (anode: excitatory, cathode: inhibitory) is supported by seminal work of Jefferys (Jefferys 1981). By stimulating electrically granule cells in quiescent guinea-pig hippocampal slices, Jefferys showed that extracellular voltage fluctuations across a cell are able to modulate the membrane potential. This induced polarization is depolarizing (higher membrane potential) for the soma during anodal stimulation and hyperpolarizing (lower membrane potential) for cathodal, whenever neurons are aligned with their apical dendrite pointing towards the electrode. The membrane polarization at the soma affects the size of monosynaptic evoked potentials, with anodal stimulation increasing the response size while cathodal decreasing. Interestingly, Jefferys also found that the induced extracellular voltages are not uniform across neurons but changed depending on the cellular compartment. Similar results were found by Chan et al. for Purkinje cells in turtle cerebellar slices (Chan et al. 1988).

A later study by Bikson et al. (2004) further characterized Jefferys' hippocampal preparation by directly measuring the membrane polarization of hippocampal CA1 neurons and determining that the membrane potential at the soma changes linearly with the electric field magnitude in a polarity specific manner. The deflection of the membrane voltage at the soma is in the order of 0.1 mV of polarization per V/m electric field applied for pyramidal CA1 neurons. Moreover, using voltage sensitive dyes, the authors found that the polarization of neurons is compartment specific: soma depolarizing fields (anodal) hyperpolarize the dendrites and, vice-versa, soma hyperpolarizing fields (cathodal) depolarize the dendrites (Fig. 2.3a, b). These results were all consistent with the earlier findings of Jefferys (1981) and Chan et al. (1988). In addition, Bikson et al. showed that weak electric fields perpendicular to the main orientation of a neuron do not polarize the somatic membrane significantly (though may still influence function).

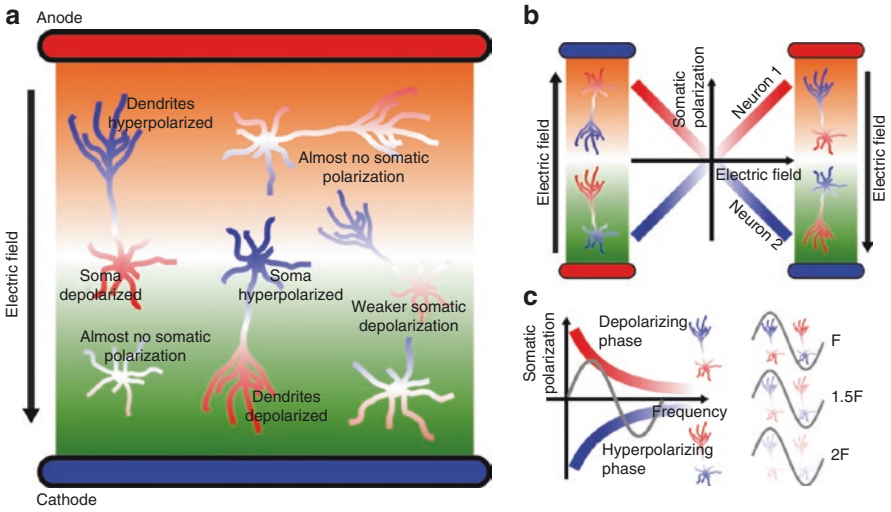

Fig. 2.3 Weak electric fields applied extracellularly polarize neuronal membrane. (**a**) Induced polarization is polarity- and compartment-specific and strongly depends on neuronal orientation relative to the electric field applied and morphology. (**b**) Somatic polarization depends linearly on the electric field amplitude. (**c**) Polarization decreases exponentialthe equation is called the ay with the frequency of the field applied. (**a** and **b** are based on data from Jefferys (1981), Chan et al. (1988) and Radman et al. (2009). **c** is based on data from Deans et al. (2007))

The sensitivity of neurons to extracellular electric fields as measured in Bikson et al. is called the coupling constant (how many millivolts the somatic voltage of a neuron changes per V/m electric field applied). The estimation provided by Bikson et al. (0.1–0.2 mV/V/m) was confirmed for pyramidal cortical neurons in ferret slices by Fröhlich and McCormick (Frohlich and Mccormick 2010) and for CA3 pyramidal neurons in hippocampus by Deans et al. (2007). In the latter study, the coupling constant was directly measured varying the frequency of the field applied. Because of the membrane capacitative and resistive properties, the response of a neuron to an electric field is low-pass filtered: high frequency stimulation induces a small polarization compared to low frequencies. Therefore, the study by Deans et al. confirmed that the coupling constant depends on the frequency of the stimulation applied (Fig. 2.3c). A couple of years later, Radman et al. added another key element to consider when evaluating the effects of electric fields on neurons (Radman et al. 2009). By performing a morphologic reconstruction of biocytin-filled neurons, the authors found that the coupling constant strongly depends on neuronal morphology. Neurons with a symmetric dendritic arbor, like fast spiking interneurons, were polarized by external electric fields much less than neurons with a more asymmetric morphology, such as pyramidal neurons. Similar results were also reported in another study for hippocampal neurons (Berzhanskaya et al. 2013).

To summarize, the biophysical model that emerges from these studies is that the voltage fluctuations (ΔV, units: V or mV) at the soma induced by spatially uniform DC electric fields (E, units: V/m or mV/mm) oriented along the primary dendritic axis can be described by.

$$\Delta V = c_E(M)E,$$

where c_E is the coupling constant (units: m or mm). The coupling constant is in general a complex function of neuronal morphology (M). A field that is oriented perpendicularly to the primary dendritic axis has no effect on the voltage at the soma, while its effect is maximum for parallel orientations.

The effect of an external applied electric field on the membrane potential can be determined by a formulation known as cable theory (for a recent review see (Rahman et al. 2015)). The generic equation that describes how the membrane potential of a neuron (V_m) is linked to the extracellular potential (V_e) as a function of time (t) and space (x) is the following:

$$\frac{\partial V_m}{\partial t} + \frac{\partial^2 V_m(x)}{\partial x^2} - V_m = \lambda^2 \frac{\partial^2 V_e(x)}{\partial x^2},$$

where the right side of the equation is called the activating function. Here, λ is the membrane length constant, which depends only on the electrophysiological properties of the membrane. This relatively complex equation can be simplified in particular conditions and solved analytically. In general, however, numerical methods can be used to solve it for multi-compartment neuronal models. There is a large amount of theoretical work in which cable theory was used to estimate polarization profiles of neurons subjected to an extracellular electric field (V_e) (Basser and Roth 2000; Chan and Nicholson 1986; Hause 1975; Joucla and Yvert 2009; Mcintyre and Grill 1999; Miranda et al. 2007; Plonsey and Barr 1998; Rahman et al. 2013; Ranck 1975; Svirskis et al. 1997; Tranchina and Nicholson 1986). However, one inevitable outcome of this classic theory is that the polarization profile produced by extracellular fields is not simple, even for tDCS. In the specific case in which a neuron compartment can be approximated as a very long (>5λ) straight cylindrical segment, as in the case of long dendrite or axon (terminal) processes of cortical or hippocampal neurons, the coupling constant c_E can be expressed directly as a function of the polarization length and the angle between the main neuronal axis and the electric field (θ):

$$c_E = \lambda \cos\theta$$

While the estimation of somatic membrane polarization is robust across brain regions and species, a sophisticated analysis of tDCS effects must account for the distributed profile of polarization (Fig. 2.3a). Though it is correct that an “anodal”

direct field will depolarize the soma of cortical pyramidal neurons in a hippocampal slice, it will inevitable hyper-polarize their dendrites (Bikson et al. 2004), which can change dendritic processing (Fig. 2.3b). In addition, in tDCS, cortical folding will cause local changes in the orientation of neurons relative to the electric field such that neurons in adjacent cortical regions may be polarized in opposite direction (Rahman et al. 2013; Reato et al. 2013a). This was also anticipated by Terzuolo and Bullock as early as 1956, who wrote "*Finally, current flowing along the surface of the grey matter (tangential directed flow as opposed to inward/outward radial flow) may influence brain function by polarizing structures oriented along the surface, namely afferent axons.*" (Terzuolo and Bullock 1956).

The effects of stimulation on neuronal physiology can be understood by using computational models of single neurons. These models are based on a set of equations that describes how the membrane potential of a compartment of a neuron, V, evolves in time. The best-known is the Hodgkin-Huxley model (Hodgkin and Huxley 1952). The general formulation of a Hodgkin-Huxley-like model is:

$$C\frac{dV}{dt} = -\sum_x I_x + I,$$

where C is the membrane capacitance, I an applied current and I_x describes in general all the possible currents, either ionic, synaptic, due to input currents from other compartments, etc.

In their original formulation, Hodgkin and Huxley considered current contributions from sodium, potassium and a leakage current, such that the equation is:

$$C\frac{dV}{dt} = -g_{Na}m^3h\left(V - E_{Na}\right) - g_K n^4\left(V - E_K\right) - g_L\left(V - E_L\right) + I,$$

where $g_{Na,K,L}$ are the maximum conductances for sodium and potassium and leakage currents, m and h describe the probability that sodium channels are open or inactivated, n the probability that potassium channels are open and $E_{Na,K,L}$ the reversal potential for sodium, potassium and leakage channels respectively, and I is an applied current. Importantly for tDCS, the driving force terms $(V - E_X)$ are directly affected by changes in membrane potential, such that, for example if an externally applied electric field increases the voltage by ΔV (i.e. polarizes the membrane), then the driving force for all the conductances will be altered to $(V + \Delta V - E_X)$. Additionally, all the probabilities for channels to be open or inactivated are also time and voltage dependent. Therefore, changes in membrane potential affect directly ionic currents in two ways.

Synaptic conductances are also voltage dependent because their magnitude can be expressed as:

$$I_{syn} = g_{syn}\left(V - E_{syn}\right),$$

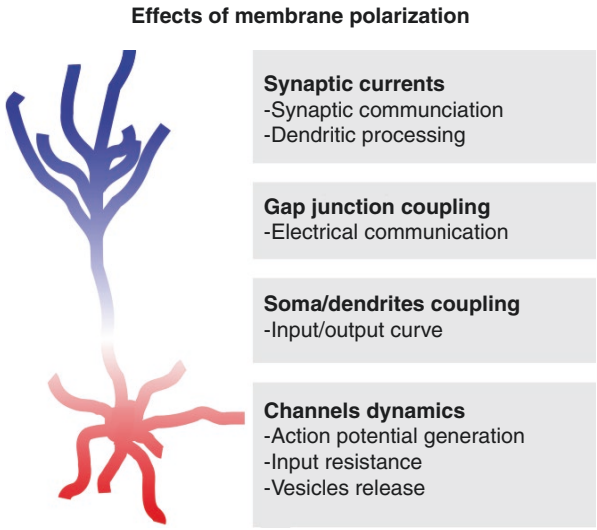

Fig. 2.4 Summary of the multiple potential effects of membrane polarization on the electrical and synaptic activity of neurons

where the values of the parameters depends on the type of synaptic current (AMPA, NMDA, GABAa, GABAb, etc.). Current through gap-junctions connecting neurons or electrotonic coupling of neuronal compartments also depend on voltage differences. Finally the release on synaptic vesicles depends also on voltage changes.

In summary, any effect on membrane voltage affects potentially every aspect of neuronal, electrical and synaptic activity (Fig. 2.4). Therefore, the notion that tDCS affects neuronal function by inducing a membrane polarization must be extended by considering how that voltage fluctuation modulates the neuronal activity of interest. For example, depolarization of the somatic compartment is usually associated with hyperpolarization of the dendrites. Depolarization of the soma increases the excitability of the neuron and hyperpolarization of the dendrites increases the driving force for excitatory synaptic inputs, while reducing the one for inhibitory inputs. How this dichotomy may be solved is an intense area of research (see next sections).

Active Neurons

Assuming no synaptic inputs (as in many in vitro models) the polarization induced by electric fields generated during tDCS is too small (0.1–0.5 mV) to increase the membrane voltage of a neuron from rest sufficiently to generate an action potential (10–20 mV over resting membrane voltage). How does tDCS therefore affect neuronal activity at all?

In contrast to typical in vitro conditions, neurons in the brain are often spontaneously active even when animals do not receive any specific sensory stimulus or are engaged in any specific task. The general level of activity depends strongly on the

behavioral state of the animal and specific patterns of neuronal firing are determined by intrinsic cellular and network properties (ion channel expression, number, type and strength of synaptic inputs, etc.). When animals are explicitly engaged in a task, neurons are usually highly depolarized, exhibit spiking activity and are in a high conductance state (Destexhe et al. 2003). Therefore, when a neuron is already active, it seems more appropriate to consider the effects of the stimulation on the firing activity. This intuitive idea was already proposed and demonstrated a long time ago. In fact, Terzuolo and Bullock in 1956 (Terzuolo and Bullock 1956) already pushed forward ideas that are nowadays at the core of our understanding of the biophysics of transcranial electrical stimulation. In their study, they used crayfish and lobsters to test the effects of weak currents applied extracellularly on neurons while keeping the synaptic inputs under tight control. They used electric fields of the order of 1 V/m, a value completely reasonable for transcranial electrical stimulation applied with common stimulation protocols. Interestingly, some of the sentences from that paper contain already the majority of key concepts for describing the effects of electric fields on neurons. Here we report a few of those. *"We have not seen in the literature, however, a quantitative evaluation of the sensitivity of nerve cells to electric fields in terms of voltage gradient across some appropriate dimension of the neuron. We have undertaken to estimate the threshold value as being the unique value of greatest interest and have found this to be far lower for modulation of the frequency of an already active neuron than for the excitation of a silent one."* Already then, it was recognized that: *"it will be realized that there will be no characteristic value for this membrane potential change, since in an equatorial region of the cell, with respect to the axis of polarization, the potential across the membrane will not be changed at all during polarization, and on one side of this line it will be increased and on the other side decreased."* Finally, *"These values of voltage gradient were all obtained in the best axis of polarization of the neuron. When the field was rotated, a significant increase of the applied current was necessary in order to reproduce the same effect as that obtained in the axono-dendritic axis"* It is quite astonishing that as early as 60 years ago the biophysics of DCS was already quite understood. The findings of Terzuolo and Bullock were then confirmed in vivo in anesthetized rats and in cat encéphale isolé. Bindman et al. (1964) applied electrical stimulation transcranially and found that firing rates are increased/decreased by anodal/cathodal stimulation (Fig. 2.5a). They also found that evoked potentials are similarly affected in a polarity-specific manner by the stimulation. Importantly, the authors found that stimulation applied for longer than 5 min induces long-lasting changes in firing rates. Purpura and Mcmurtry (1965) also reported changes in firing rates induced in a polarity specific-manner and linked the results to the orientation of neurons and induced polarization (even if the currents applied were high enough to directly generate action potentials). Similar results were also found a few years before by Creutzfeldt et al. (1962) by recording from motor and visual cortex of cat encéphale isolé while applying currents of the order of 1 mA transcortically. In particular, they also found that the relationship between firing rate changes and current applied is approximately linear. Consistently with previous studies, they also found that electrically evoked activity is modulated by weak electrical stimulation.

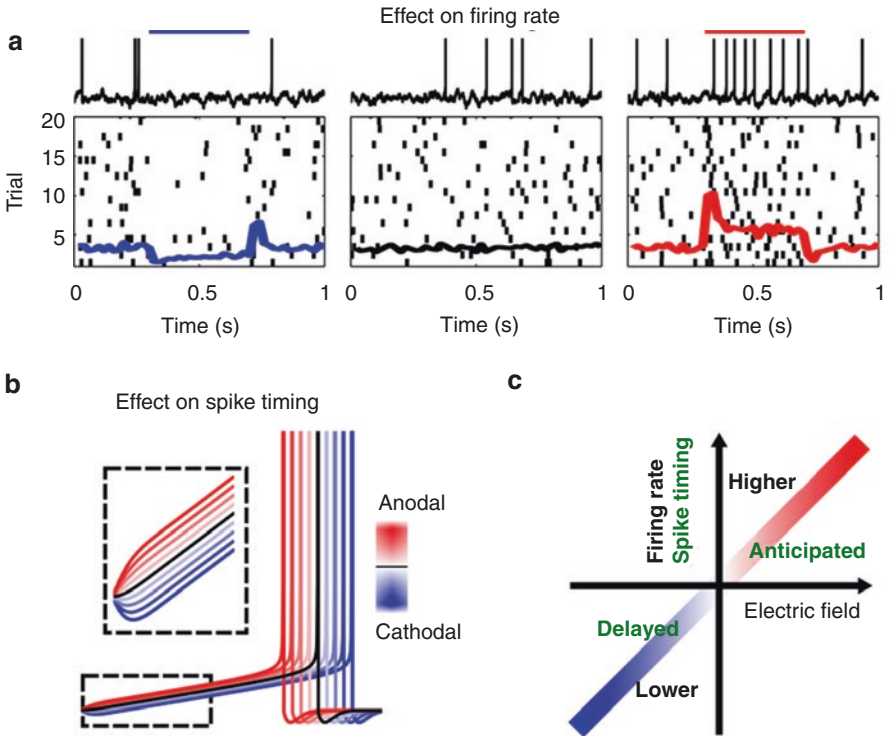

Fig. 2.5 Schematics of the effects of weak DC currents on active neurons. (**a**) Anodal/cathodal (blue/red) stimulation increases/decreases firing rate compared to control conditions (black). Top row: membrane voltage during the application of the stimulation. Bottom row: raster plot (each line represents an action potential) and average firing rate change across trials during stimulation or control conditions. (**b**) Anodal stimulation anticipates action potential generation while cathodal delays it. Control condition in black. (**c**) The effects on both firing rate and spike timing are linear with electric field amplitude. (**a** is based on data from Bindman et al. (1964) and Reato et al. (2010). **b** is based on data from Radman et al. (2007); **c** is based on data from all the previous)

The work of Gartside added more key elements to the after-stimulation effects of electric fields on neuronal firing (Gartside 1968). After inducing lasting effects as in Bindman's work, the author cooled the whole body of rats to completely abolish neuronal activity. After the temperature was left free to rise again to normal levels, the changes in firing rate induced by the electrical stimulation were still present. The author therefore suggested that these persisting changes are not driven by reverberation of the activity but *"The underlying mechanism must involve some type of synaptic modification."*

Years later, Chan and Nicholson (1986) found that the firing rate of Purkinje cells in the cerebellum is very sensitive to weak electric fields (even though the stimulation was alternating current). Firing rate increases by about 6 spikes per second per millivolt depolarization applied, a value that is consistent with results in cat visual

cortex (but no electrical stimulation was applied (Carandini and Ferster 2000) and rat hippocampal slices (Reato et al. 2010)).

Apart from changes in firing rate, weak electric fields can also affect spike timing. Changes in spike timing do not necessary imply changes in rate, such that even if the average rate is the same, the timing of these events can be altered by electrical stimulation. A clear evidence of this phenomenon was provided by Radman et al. (2007). They patched hippocampal neurons and then linearly drove the membrane towards the threshold for action potential generation. In some trials, they applied a spatially uniform electric field on the top of that depolarization. They found that somatic anodal stimulation sped up the threshold crossing, while cathodal slowed it down (Fig. 2.5b, c). Furthermore, Radman et al. also showed that AC stimulation can entrain the spiking activity of single neurons, a key result for explaining how weak electric currents can entrain full neuronal populations (Deans et al. 2007; Frohlich and Mccormick 2010; Ozen et al. 2010; Reato et al. 2010).

Changes in firing rate and spike timing produced by weak electric stimulation have been modeled throughout the years often using single-neuron descriptions that are simplified compared to the Hodgkin-Huxley formalism. These models assume that neurons can be described as a single compartment (the soma) and are particularly suited for implementation in large populations of synaptically connected neurons.

Parra and Bikson (Parra and Bikson 2004) used an integrate-and-fire (IF) neuron model to show that the spike coherence in a neuronal population increased when small polarizations where applied to the whole network. Their model was described by:

$$\tau \frac{dV}{dt} = -V + RI,$$

where R is the membrane resistance, τ the time constant, V the membrane voltage, and the term I includes both the synaptic currents from other neurons and the contribution from an external electric field. We can refer to this as "RI" formalism, with direct analogy to how compartment-based biophysical models of electrical stimulation incorporate the effects of electric fields as equivalent intracellular current injection (Lafon et al. 2016; Park et al. 2005). Expanding on this formalism to describe the effects of weak electric fields, Reato et al. (2010) implemented Izhikevich's single neuron model (Izhikevich 2003, 2007) to reproduce the effects of electric fields on a network of excitatory and inhibitory neurons (see following section). The differential equation that describes the voltage is

$$\frac{dV}{dt} = f\left(V\right) - u\left(V\right) + I_{syn} + I_E$$

where I_{syn} is the sum of the synaptic inputs from other neurons, $u(V)$ an adaptation variable and $f(V)$ is a combination of a linear and quadratic function of the voltage that also give rise to the action potential generation (a reset of the voltage

is then necessary). Electrical stimulation can be implemented as a current term I_E, such that

$$I_E = k_E E,$$

where k_E is the conversion factor that must be set to reproduce the correct polarization levels expected by the application of the electric field. In other words, if the value of the somatic membrane potential is V without stimulation and $V + \Delta V$ when the electric field is applied, the parameter k_E must be tuned such that the current I_E induces a change equal to ΔV. This type of simple modeling formalism has been broadly adopted including in simulate the effects of gamma oscillations in vitro (Reato et al. 2010, 2015) as well as slow-waves in humans (Reato et al. 2013a) and in ferrets (Ali et al. 2013).

A similar simplified approach has been recently used to simulate the effects of electric fields on neuronal populations underlying decision-making processes (Bonaiuto and Bestmann 2015; Hammerer et al. 2016). Bonaiuto and colleagues used the exponential leaky integrate-and-fire (LIF) (Brette and Gerstner 2005), where the voltage dynamics is described by:

$$C\frac{dV}{dt} = g\left(V\right) + I_{syn} + I_E$$

The function $g(V)$ is a combination of linear and exponential functions. Similarly to Reato et al.'s approach using Izhikevich's model, the effects of electric fields can be implemented by directly adding an external current input I_E.

The use of simplified single-neuron models allows for the simulation of large neuronal populations. However, when full populations of neurons are stimulated, the average synaptic inputs in the network must be considered to estimate or predict the effects of weak currents on single neurons. In fact, as suggested in a recent review (Paulus and Rothwell 2016), if a neuron receives multiple synaptic inputs, the membrane becomes leakier. This translates to lower input resistance and therefore a smaller direct polarization induced by electric fields. Thus, while population activity can amplify the small effects of weak currents on neurons (Reato et al. 2010), strong synaptic tone decreases the polarization induced on single neurons. None-the-less, since active neurons are often near firing threshold, active systems are expected to be significantly more sensitive to polarization. This is turn leads to the notion of "functional targeting" discussed in the next sections.

Summary of the Effects of Weak Direct Current Stimulation on Membrane Potential, Firing Rate and Spike Timing and Open Questions

The summarized literature delineates a precise view on the effects of weak electric fields, such those induced by tDCS, on single neurons. When neurons are not active, weak stimulation induces a small polarization of the membrane. When neurons are

active, the effects of fields are on firing rate and spike timing. Somatic anodal stimulation increases firing rate and shorten the time required to reach the threshold for action potential generation. Somatic cathodal stimulation has the opposite effect. However, many open questions and debates remain on the effects of tDCS on single neurons:

1. The dichotomy anodal/excitatory vs cathodal/inhibitory is not precise. Modulation of membrane potential does not directly translate to increased/decreased excitability, since these concepts are linked to the desired effect of the stimulation. For example, depolarization of the soma may lead to easier generation of action potential, an effect that may be considered excitatory. Depolarization of the dendrites however may not be beneficial for post-synaptic neurons. An increase in membrane potential decreases the synaptic response of post-synaptic neurons because it reduces the driving force. Considering that neurons constantly experience compartment-specific polarizations, it cannot be assumed that electric fields always have a net excitatory or inhibitory effect.
2. The polarization of dendrites and axons has been predicted by modelling studies but never measured experimentally. A common assumption, for example, is that stimulation does not affect morphologically symmetric neurons. However, this assumption is mainly based on somatic polarization (the so-called somatic doctrine [Bikson et al. 2012]). It cannot be excluded that polarization of axons and dendrites may be very effective in modulating cellular functions ([Rahman et al. 2013], see next paragraph).
3. The coupling constant has not been measured directly in vivo. This experiment is quite critical to assure that the results from the in vitro literature can really be used to guide and support human research. Moreover, whether brain state and therefore high or low conductance neuronal states affect coupling constant is not known.
4. Effects of weak electric fields on non-neuronal type of cells have not been exhaustively studied yet (Monai et al. 2016). For example, coupling constant for glial cells has never been measured before. These types of cells are critical for neuronal function and seem to mediate some of the lasting effects of electric fields (see next section).

Effects of Weak Direct Current Stimulation on Synapses and Neuronal Populations

Ultimately, to affect brain function weak electric fields must exert significant effects on whole neuronal populations. A priori, the effects of stimulation on single neurons could be altered, amplified or damped (or completely disappear) at the population level. It is therefore not surprising that many studies on the biophysics of tDCS have now focused on neuronal populations and the effects of weak electric fields on synapses. The majority of animal studies in vitro on this topic involved the use of

evoked responses or analyzed the effects of the stimulation on neuronal oscillations. In some of these studies, plastic effects were reported. In vivo studies on the other hand, provide great opportunities to study the effects of electrical stimulation on behavior.

Evoked Responses In Vitro

The most commonly studied animal model of transcranial stimulation is the modulation by applied electric fields of evoked population responses, which, to a first approximation, provide a measurement of synaptic currents on post-synaptic neurons. A stimulating electrode, usually bipolar, is placed close to fibers tract in vitro or in vivo. A very short (<1 ms) current pulse is then applied to generate action potentials in axons. An extracellular recording electrode is used to record the population response (local field potential, LFP) around the dendrites or somas of the post-synaptic neurons. A weak electric field is then applied to modulate the population response. Many of the studies mentioned in the previous paragraph reported modulation of evoked responses by weak electric currents. In particular, electric fields whose orientation is parallel to the somatodendritic axis of a neuron and pointing towards the soma (anodal stimulation for cortical cells) increase the evoked response, while fields with opposite orientation (cathodal for cortical cells) decrease the response (Fig. 2.6a). These results were found consistently for cortical (Bindman et al. 1964; Creutzfeldt et al. 1962; Purpura and Mcmurtry 1965) and hippocampal CA1 neurons (Bikson et al. 2004; Jefferys 1981). In recent years, new studies helped deepening our understanding on the effects of weak currents in this preparation.

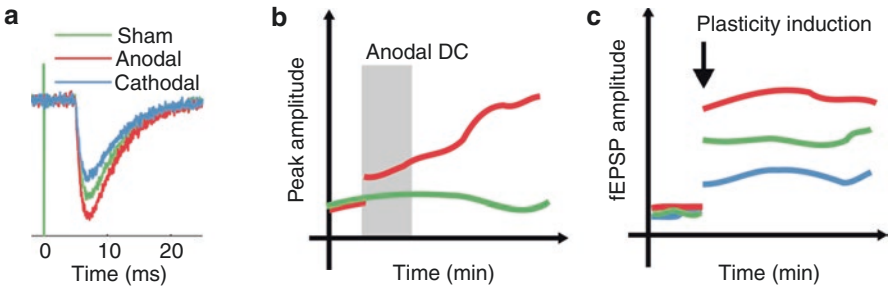

Fig. 2.6 Schematics of the effects of weak DC currents on evoked responses. (**a**) Anodal/cathodal (blue/red) stimulation increases/decreases the amplitude of evoked responses (green). (**b**) When evoked responses are combined with prolonged DC stimulation (~10 min), the amplitude of the response increases and this change outlasts the stimulation period. (**c**) When DC stimulation is applied during plasticity induction (LTP), the amount of potentiation is modulated bi-directionally by weak DC stimulation (fEPSP: field excitatory post-synaptic potential). The green line represents the control condition, red anodal stimulation and blue cathodal. (**a** is based on data from Creutzfeldt et al. 1962; Bindman et al. 1964; Purpura and Mcmurtry 1965; Jefferys 1981; Bikson et al. 2004; Rahman et al. 2013. **b** is based on data from Fritsch et al. 2010; **c** is based on data from Ranieri et al. 2012)

Rahman and colleagues used evoked responses in rat motor cortex to test the effects of weak DC stimulation (Rahman et al. 2013). The authors stimulated different cortical pathways and applied electric fields of different polarities, amplitudes and orientations relative to the stimulated neurons. They confirmed that when electric fields are oriented parallel to the dendrosomatic axis, the response is modulated in the same way as for hippocampal slices. They then tried to stimulate pathways perpendicular to the applied field. From what was previously known, such fields should induce no net polarization at the soma and therefore no effects on evoked responses. However, the authors reported a modulation of the responses comparable in magnitude to that found for the pathways parallel to the electric field. To understand this surprising result, Rahman et al. used a computational model of a single neuron in a spatially uniform electric field and found that terminal polarization could explain the experimental results. While these findings were not tested experimentally, they suggest that, at least in some cases, somatic polarization does not fully explain the effects of weak electrical stimulation. Importantly, these findings are consistent with a previous study in hippocampus (Kabakov et al. 2012).

Additional animal studies aimed at understanding how weak electrical stimulation can induce lasting effects on neuronal excitability as found in human studies. Fritsch and colleagues (Fritsch et al. 2010) combined electrophysiology, pharmacology and genetic tools to elucidate the cellular mechanisms underlying the lasting effects induced by tDCS. They evoked population responses in mouse motor cortex slices and applied weak DC stimulation extracellularly. They found that application of prolonged stimulation (15 min) induces a potentiation of the response (Fig. 2.6b). The change starts minutes after the stimulation onset and the magnitude of the responses continues to increase even after the cessation of the stimulation. Importantly, the effects are NMDA-dependent, and the lasting changes critically depend on whether or not synaptic co-activation is applied (and its frequency), suggesting that the state of the cortical network may dictate the susceptibility to the stimulation. Finally, by using genetic tools, the authors found that DC stimulation enhances the release of BDNF and that BDNF receptors are required for plasticity induction. In fact, when the authors repeated the same experiments using mice where these receptors were knocked down, they found that the effects of DC stimulation vanished.

Ranieri and colleagues, using evoked responses in hippocampal slices, critically improved our understanding on tDCS lasting effects (Ranieri et al. 2012). They applied a standard stimulation protocol to induce plasticity at the CA3 to CA1 synapse (Schaffer collateral) and found that anodal stimulation increased long-term potentiation (LTP) while cathodal decreased it. They further showed evidence that these effects may be due to an increased expression of zif268 protein (an early gene). The findings of this work suggested that weak electrical stimulation, while not inducing plasticity per se, may strongly modulate ongoing plasticity in a bidirectional manner (Fig. 2.6c).

Confirming and expanding this hypothesis, a study by Kronberg et al. (2016) showed that weak DC stimulation effectively modulates LTP and depression (LTD). Kronberg et al. used a typical experimental model of hippocampal plasticity in brain

slices (as in Ranieri et al. study, (2012) in which stimulation of axonal afferents (Schaffer collaterals) can lead to post-synaptic potentiation or depression depending on the frequency of pre-synaptic activation (Cooper and Bear 2012). The authors found that DC stimulation biases plasticity towards potentiation, such that LTP is enhanced and LTD is reduced. Importantly, the authors found that similar effects could be induced using either anodal or cathodal stimulation, but with the effects localized in different dendritic compartments (apical/basal dendrites). Finally, Kronberg et al. clearly showed that DC stimulation alone or applied when plasticity was blocked did not lead to any synaptic changes.

Taken together, these studies on the lasting effects induced by weak electric currents suggest that stimulation alone does not produce significant persisting synaptic effects if not paired with activity or ongoing plasticity. This underlies the concept of "functional targeting", in which stimulation paradigms can be targeted to specific neuronal populations depending on their activity and plasticity-permissive states (Jackson et al. 2016).

Finally, in the previous section we pointed out the possible issue that arises from compartment-specific polarization of the neuronal membrane. The polarity of somatic polarization (depolarization/hyperpolarization) is opposite than dendritic. A study by Lafon et al. (2016) shed light on this issue. By combining electrophysiology in hippocampal rat brain slices and computational models based on previous research (Park et al. 2005; Prescott et al. 2008; Yi et al. 2014), Lafon et al. found that weak constant electric fields affect neuronal input/output function, i.e. the relationship between strength of synaptic inputs and the firing they induce on the post-synaptic neuron. Moreover, the authors found that somatic and dendritic polarization may have a synergistic effect for anodal stimulation: soma-depolarizing electric fields increase the likelihood of neuronal firing while also increasing the driving force for synaptic input at the dendrites. However, the effects of electric fields of opposite polarity (cathodal) tend to cancel out. This result suggests how an asymmetry between the effects of anodal and cathodal stimulation may arise directly at the single neuron level.

Oscillations In Vitro

Another in vitro experimental model commonly used to study the effects of weak electric fields is pharmacologically-induced oscillations or seizure-like population activity. However, in almost all the studies in the field, alternating currents (Ali et al. 2013; Berenyi et al. 2012; Deans et al. 2007; Frohlich and Mccormick 2010; Ozen et al. 2010; Reato et al. 2010) or Gaussian waveforms (Francis et al. 2003) were applied. Reviews on tACS are already present in the literature (Herrmann et al. 2013; Reato et al. 2013b) and so here the focus is just on DC stimulation studies.

Using high extracellular concentration of potassium in hippocampal slices to generate seizure-like activity, Gluckman and colleagues (Gluckman et al. 1996) tested whether relatively weak electric fields could reduce epileptic activity. They

found that when fields were hyperpolarizing for the soma (cathodal stimulation), the seizure was temporarily stopped.

Pharmacologically induced beta/gamma oscillations in the hippocampus have been used to study the effects of AC stimulation (Deans et al. 2007). However, Reato and colleagues characterized in more detail the effects of the stimulation (Reato et al. 2010). By using many frequencies (AC) and amplitudes in brain slices and a computational model they found that the effects of stimulation on the oscillations presumably depend on the interplay between excitation and inhibition (Fig. 2.7a), whose balance is a critical feature of this type of rhythm (Atallah and Scanziani 2009). In particular, when using DC stimulation, Reato et al. found that soma depolarizing (anodal) stimulation increases the power of the oscillations while

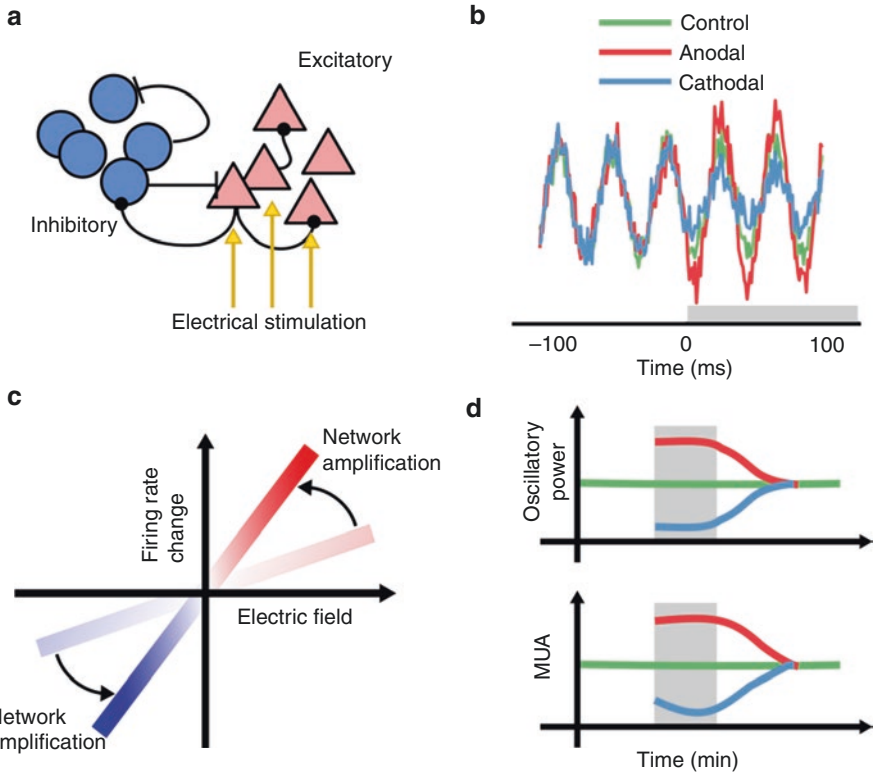

Fig. 2.7 Schematics of the effects of weak DC stimulation on gamma oscillations induced in vitro. (**a**) Gamma oscillations in vitro are generated by the interplay between excitatory and inhibitory neurons. (**b**) Anodal/cathodal (blue/red) stimulation increases/decreases the power of the oscillations compared to control experiments (green). (**c**) The modulation of the firing activity of single neurons by weak DC stimulation is amplified by the population. (**d**) When stimulation is applied for a prolonged time (10 min) the power of the oscillations and multi-unit activity modulation outlast the stimulation period for about 10 min. (**a**–**c** are based on data from Reato et al. 2010. **d** is based on Reato et al. 2015)

cathodal decreases it (Fig. 2.7b). They explained the results as evidence of altered excitation and inhibition. When the firing rate of excitatory neurons is increased, inhibition compensates. Because the inhibition is fed by excitation and sets the tone of the oscillations, the result at the population level is an increase in oscillatory power. The opposite is true for cathodal stimulation. Lower firing rates of excitatory neurons lead to lower balanced inhibition and decreased gamma power. Using a computational model (see previous section), the authors showed that the weak effects of DC stimulation on single neurons (for example firing rate modulation) are amplified by the population dynamics (Fig. 2.7c). Importantly, the same authors found a similar amplification at the population level when implementing a computational model of slow-wave activity (Reato et al. 2013a).

The same group used the same in vitro preparation in a later study in which DC stimulation was applied not just for few seconds but for 10 min (Reato et al. 2015). Monitoring power and frequency of the oscillations as well as multi-unit activity (a proxy for population firing rate), they found that the stimulation induces lasting effects on the neuronal population in a polarity-dependent manner (Fig. 2.7d). Anodal stimulation increases the power of the oscillations and multi-unit activity, while cathodal decreases both. Based on the hypothesis of balanced excitation and inhibition during gamma oscillations and the same computational model they used in their previous study, the authors suggested that the results could be explained by balanced synaptic changes of both excitatory and inhibitory synapses. While intriguing, however, this hypothesis has not been directly tested experimentally.

Plasticity and Behavioral Effects In Vivo

A decade ago, Liebetanz et al. found that tDCS applied in rats is able to modulate pathological states. In a first study, cortical spreading depression (CSD) was induced in anesthetized rats using a high potassium chloride solution (Liebetanz et al. 2006a). Neither sham nor cathodal stimulation have any effect of the CSD while anodal stimulation significantly increases the propagation speed of the CSD. In another study, the authors showed that tDCS is effective in modulating the threshold for epileptic seizure generation (Liebetanz et al. 2006b). The threshold was determined by applying a biphasic pulse train to the cortex to induce seizures. When anodal stimulation was applied, there were no changes on the threshold. However, cathodal stimulation applied for 60 min or 30 min at double intensity decreased the threshold for more than 2 h (Fig. 2.8a)**.**

Another group also found that prolonged DC stimulation over the motor cortex of anesthetized mice induces an increase (anodal) or decrease (cathodal) of motor evoked responses (MEPs) that outlasts the stimulation period (Cambiaghi et al. 2010). The same group also later found, using anesthetized mice, that the amplitude of visually evoked potentials can be modulated with DC stimulation in a polarity-dependent manner (Cambiaghi et al. 2011). Anodal stimulation increases the amplitude of the evoked potential and cathodal decreases. The effects of the

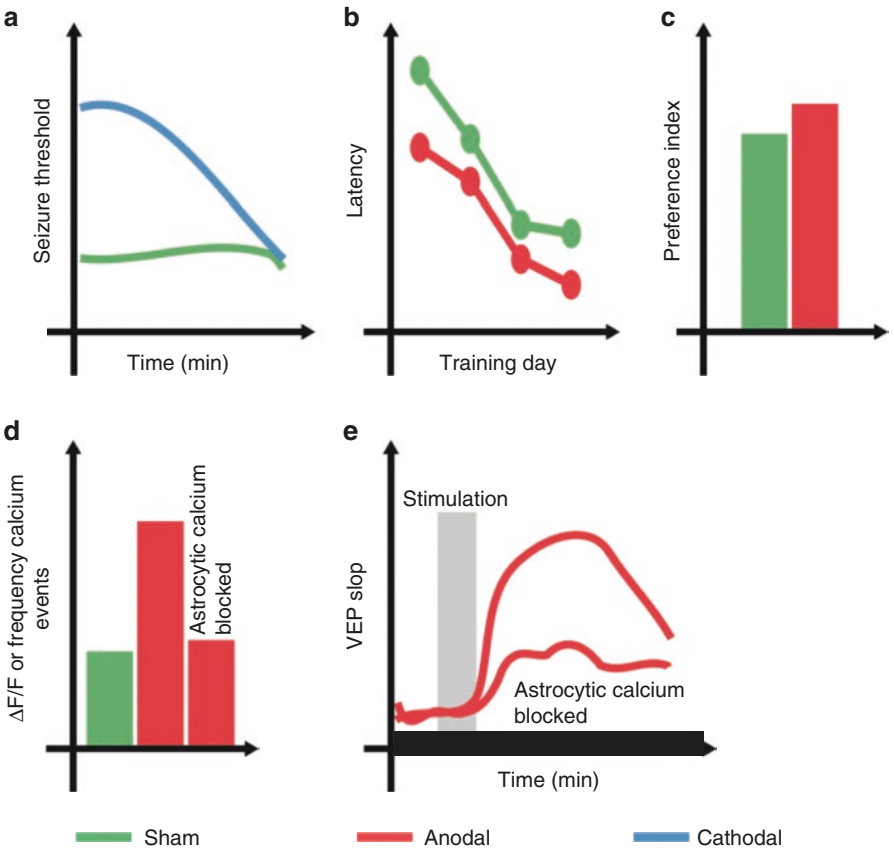

Fig. 2.8 Schematics of the effects of weak DC stimulation on in vivo animal models. (**a**) Cathodal stimulation increases the threshold for seizure generation (anticonvulsant effect). (**b**) Anodal tDCS improves performances in a Morris water maze test (lower latency) compared to non-stimulated animals. (**c**) Anodal tDCS improves performances in a novel object recognition task. (**d**) Anodal tDCS induces high amplitude and frequency calcium events in cortical populations that are driven by astrocytic calcium. (**e**) The lasting increase of visually evoked responses after tDCS is strongly limited when calcium transients in astrocytes are blocked. (**a** is based on data from Liebetanz et al. 2006a. **b** and **c** are based on data from Podda et al. 2016). **d** and **e** are based on data from Monai et al. 2016)

stimulation also persist for the 10 min following the termination of the current application.

An important study on tDCS effects on neuronal population in vivo came from Marquez-Ruiz and colleagues (Marquez-Ruiz et al. 2012). Instead of using electrically evoked responses (as usually done in in vitro models), the authors induced sensory responses in awake rabbits by delivering air puff to the whisker pad. When DC stimulation was applied over the somatosensory cortex, the LFP

induced by the sensory stimulus was increased in magnitude during anodal and decreased during cathodal stimulation. The effect of cathodal stimulation persists after the cessation of the stimulation and the effects were abolished after blocking adenosine A1 receptors. Importantly, stimulation was able to directionally modulate eye blink conditioning. Using paired-pulse stimuli, the authors further found that the effects of tDCS are mediated by the modulation of thalamo-cortical synapses at presynaptic sites.

Two recent studies reported effects of tDCS on hippocampal plasticity. Application of anodal tDCS in freely moving mice boosts the amount of LTP that can be induced in slices ex-vivo by stimulating the Shaffer collateral pathway (Rohan et al. 2015). The authors also reported an increase in pair-pulse facilitation (PPF). The effects are NMDA receptor-dependent for LTP but not for PPF. The modulation depends on the stimulation amplitude and significant LTP enhancement can be found also 1 week after the stimulation. Interestingly, there authors did not find any effects on evoked responses before LTP induction. Using a similar approach, a different group stimulated mice with tDCS for 20 min (Podda et al. 2016). They confirmed that anodal stimulation leads to stronger LTP induction while cathodal decreases the amount of LTP without affecting basal synaptic transmission. To test whether the stimulation had an effect on hippocampal learning, mice were then tested on two different behavioral tasks (Morris water maze test and novel object recognition test) a day after receiving anodal stimulation. In both cases, mice performed better than mice subjected to sham stimulation (Fig. 2.8b, c). To confirm the lasting effects of the stimulation, the same tests were then performed a week after the stimulation. The authors reported very similar effects on electrophysiological tests and behavior. The authors then tried to elucidate the molecular pathway by which tDCS affected hippocampal plasticity. They found that tDCS induces differential regulation of exon-specific BDNF mRNAs and BDNF expression is higher after tDCS due to increased histone acetylation promoting BDNF transcription.

While the results of all the previous studies are broadly consistent with each other, a new study just expanded the view on how tDCS may affect brain function. Using a transgenic mouse line expressing G-CaMP7 in astrocytes and a subpopulation of excitatory neurons, Monai and colleagues (Monai et al. 2016) found that DC stimulation over the visual cortex induces large calcium transients across the whole cortex (Fig. 2.8d). The transients are larger and longer than the spontaneous ones. Notably, the effects vanish when mice that lack the receptor responsible for calcium elevations in astrocytes (IP_3R2) are used (Fig. 2.8e). When the authors used a cranial to image the target area at single-cell resolution, they found that the modulation of transients is due to effects mainly on astrocytes. With further experiments, the authors found that the effects on calcium surges are mediated by noradrenergic activation of adenosine A1 receptors. The authors further reported that lasting enhancements of sensory responses (either visual or somatosensory) by transcranial stimulation vanish when calcium transients are blocked in astrocytes.

Summary of the Effects of Weak Direct Current Stimulation on Synapses and Neuronal Populations and Open Questions

While the effects of weak electric fields on single neurons are well characterized, both experimentally and theoretically, a general framework for neuronal populations is still lacking. Evoked responses can be modulated bi-directionally by weak electric fields both in vitro and in vivo. The effects appear to depend not just on somatic but possibly also on terminal polarization. DC stimulation increases or decreases the power of gamma oscillations presumably by affecting firing rate of single neurons. When applying stimulation on large neuronal populations, the effects on single neurons seem to be amplified by the network endogenous dynamics.

Until a few years ago, it was not clear how weak currents could induce lasting effects on neuronal activity. The literature presented here clarified the mechanisms. BDNF has been consistently linked to the lasting effects of DC stimulation, and NMDA receptors also play a major role in plasticity induction. Glial cells may also mediate the lasting effects of DC stimulation. Electrical stimulation can induce behavioral effects, including boosting learning and modulating sensory responses and pathological states. However, many questions remain open:

1. Experimental and modeling results suggest that the weak effects of electric fields on single neurons are amplified by the network. Under which conditions this is true is not clear. Does it apply for all or only specific types of oscillations? What is the role of brain state in determining the effects of the DC stimulation? (for AC stimulation, see Alagapan et al. 2016).
2. The modulation of gamma oscillations seems to depend on the interplay between excitatory and inhibitory neurons. A common assumption is that the effects on inhibition are indirect, through modulations of the excitatory population. What are the direct effects on inhibitory neurons at the population level? If inhibitory neurons are affected by DC stimulation, do the effects outlast the stimulation period?
3. Current flow during tDCS is such that neurons are depolarized or hyperpolarized depending on their orientation relative to the electric field. The mixed effects across a population pose a critical problem to understand tDCS effects: do the effects just cancel out such that there is no net effect on brain activity? Human studies show that this is not the case. Supported by a computational model, it was previously hypothesized that population activity may rectify the effects of mixed polarizations in the case of slow-wave oscillations and monophasic quasi-DC stimulation (Reato et al. 2013a). However, the results may be specific to the specific oscillatory rhythm and may not apply to others. Understanding how to interfere with neuronal population dynamic with specific stimulation parameters is crucial to improve the specificity of the stimulation.
4. The brain is always spontaneously active but often without showing any clear oscillatory activity. There is a full line of research on the dynamics of neuronal

networks and the computations they can perform even without synchronous activity (Renart et al. 2010; Vogels et al. 2005). How DC stimulation may affect non-oscillatory brain activity remains unknown.

5. Until now, all the animal research on tDCS has focused almost exclusively on the temporal aspects of the stimulation (but see Xu et al. 2014) without considering in detail the possible spatial aspects. For example, oscillatory activity can be generated in localized positions in a brain area and so the general effects of DC stimulation may depend on the distribution of fields across the brain relative to the pools of neurons that generate the activity within the network.
6. Cortical and hippocampal networks have been the main subject of studies on the effects of weak electric fields on neuronal populations. However, many networks in the brain do not show the same architecture. For example, the cerebellum, an area that can be easily stimulated transcranially (Galea et al. 2009; Grimaldi et al. 2016; Jayaram et al. 2012), has a very different neuronal organization. The lack of recurrent connections across Purkinje cells, presumably the most polarizable cells in the cerebellar cortex, implies that weak electric fields may easily regulate the output of the cerebellar cortex without unexpected non-linearities.
7. Glial cells and neurons form what has been called a tri-partite synapse (Araque et al. 1999). Their role in synaptic transmission and plasticity is now well-established (Di Castro et al. 2011; Panatier et al. 2011). Glial cells are electrotonically connected through gap-junctions and can feedback into neuronal activity through calcium-dependent glutamate release (Haydon and Carmignoto 2006). This feedback can strongly affect the dynamics of neuronal activity, including in pathological conditions like epileptic seizures (Gomez-Gonzalo et al. 2010; Reato et al. 2012). The effects of weak electric fields on the glial cells' potassium buffering activity has been studied in only one work (Gardner-Medwin and Nicholson 1983). It is certain that any direct effect of stimulation on glial cells may affect the dynamics of neuronal populations.
8. What is the relationship between BDNF and glial cells-mediated lasting effects of transcranial electrical stimulation? Are there many plasticity mechanisms that are induced/affected by DC stimulation?

Emerging Framework: Functional Targeting by tDCS

In the last few years, our understanding of the mechanisms of interaction between electric fields and cells in the brain has been boosted by detailed animal studies. The body of literature reviewed in this chapter converges to suggest that tDCS may functionally target neuronal function (Jackson et al. 2016) (Rahman et al. 2017) – the cellular concept of "functional targeting" provide an important substrate for how tDCS may boost specific tasks/learning. For acute effects, DCS modulations depend on the specific targeted neuronal population and its own ongoing dynamics, usually determined by the interplay between different cell types. At the same time, lasting effects of DCS are also mediated by modulation of ongoing neuronal activity and

plasticity. In this view, it seems that the effects of tDCS alone are not sufficient to generate lasting changes if not coupled with ongoing activity and/or plasticity. Thus, the cellular framework of "functional targeting" may provide the foundation for explaining at the single neuron/population/synapse levels why tDCS seems to be effective in humans mainly when applied during tasks/training paradigms.

However, to better define this framework and take full advantage of its predictive power, it is necessary to define a clear link between functional targeting and basic biophysical principles. Ideally, the notion of functional targeting should emerge spontaneously by basic biophysical principles. If this was the case, findings about specific tDCS studies could become more easily generalizable to other stimulation applications. In the future, a single multi-scale biophysical model could be used to guide human research by providing the best stimulation parameters (electrode montage, applied current, waveform, etc.) to stimulate specific neuronal activity in brain areas of interest. This model should take into consideration not only single cells but also how they work together as populations to support behavior. The computational neurostimulation approach (Bestmann et al. 2015) aims exactly at providing a multi-scale approach that bridges effects on single neurons directly to behavioral consequences of stimulation. This may represent a first step to fully unravel how tDCS affects brain function and take full advantage of this powerful technique.

References

Akhtari, M., Bryant, H. C., Mamelak, A. N., Flynn, E. R., Heller, L., Shih, J. J., … Sutherling, W. W. (2002). Conductivities of three-layer live human skull. *Brain Topography, 14*(3), 151–167.

Alagapan, S., Schmidt, S. L., Lefebvre, J., Hadar, E., Shin, H. W., & Frhlich, F. (2016). Modulation of cortical oscillations by low-frequency direct cortical stimulation is state-dependent. *PLoS Biology, 14*(3), e1002424.

Ali, M. M., Sellers, K. K., & Frohlich, F. (2013). Transcranial alternating current stimulation modulates large-scale cortical network activity by network resonance. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 33*(27), 11262–11275.

Araque, A., Parpura, V., Sanzgiri, R. P., & Haydon, P. G. (1999). Tripartite synapses: Glia, the unacknowledged partner. *Trends in Neurosciences, 22*(5), 208–215.

Atallah, B. V., & Scanziani, M. (2009). Instantaneous modulation of gamma oscillation frequency by balancing excitation with inhibition. *Neuron, 62*(4), 566–577.

Barker, A. T., & Jalinous, R. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet, 1*(8437), 1106–1107.

Basser, P. J., Mattiello, J., & Lebihan, D. (1994). MR diffusion tensor spectroscopy and imaging. *Biophysical Journal, 66*(1), 259–267.

Basser, P. J., & Roth, B. J. (2000). New currents in electrical stimulation of excitable tissues. *Annual Review of Biomedical Engineering, 2*, 377–397.

Baumann, S. B., Wozny, D. R., Kelly, S. K., & Meno, F. M. (1997). The electrical conductivity of human cerebrospinal fluid at body temperature. *IEEE Transactions on Bio-medical Engineering, 44*(3), 220–223.

Berenyi, A., Belluscio, M., Mao, D., & Buzsaki, G. (2012). Closed-loop control of epilepsy by transcranial electrical stimulation. *Science, 337*(6095), 735–737.

Berzhanskaya, J., Chernyy, N., Gluckman, B. J., Schiff, S. J., & Ascoli, G. A. (2013). Modulation of hippocampal rhythms by subthreshold electric fields and network topology. *Journal of Computational Neuroscience, 34*(3), 369–389.

Bestmann, S., de Berker, A. O., & Bonaiuto, J. (2015). Understanding the behavioural consequences of noninvasive brain stimulation. *Trends in Cognitive Sciences, 19*(1), 13–20.

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*. (Pt 1, 175–190.

Bikson, M., Reato, D., & Rahman, A. (2012). Cellular and network effects of transcranial direct current stimulation. In P. M. Rossini (Ed.), *Transcranial brain stimulation*. CRC Press (pp. 55–91).

Bindman, L. J., Lippold, O. C., & Redfearn, J. W. (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*, 369–382.

Bonaiuto, J. J., & Bestmann, S. (2015). Understanding the nonlinear physiological and behavioral effects of tDCS through computational neurostimulation. *Progress in Brain Research, 222*, 75–103.

Brette, R., & Gerstner, W. (2005). Adaptive exponential integrate-and-fire model as an effective description of neuronal activity. *Journal of Neurophysiology, 94*(5), 3637–3642.

Cambiaghi, M., Teneud, L., Velikova, S., Gonzalez-Rosa, J. 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. *The European Journal of Neuroscience, 31*(4), 704–709.

Carandini, M., & Ferster, D. (2000). Membrane potential and firing rate in cat primary visual cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 20*(1), 470–484.

Chan, C. Y., 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.

Chan, C. Y., & Nicholson, C. (1986). Modulation by applied electric fields of Purkinje and stellate cell activity in the isolated turtle cerebellum. *The Journal of Physiology, 371*, 89–114.

Cooper, L. N., & Bear, M. F. (2012). The BCM theory of synapse modification at 30: Interaction of theory with experiment. *Nature Reviews Neuroscience, 13*(11), 798–810.

Creutzfeldt, O. D., Fromm, G. H., & Kapp, H. (1962). Influence of transcortical d-c currents on cortical neuronal activity. *Experimental Neurology, 5*, 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.

Datta, A., Elwassif, M., Battaglia, F., & Bikson, M. (2008). Transcranial current stimulation focality using disc and ring electrode configurations: FEM analysis. *Journal of Neural Engineering, 5*(2), 163–174.

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*(Pt 2), 555–565.

Destexhe, A., Rudolph, M., & Pare, D. (2003). The high-conductance state of neocortical neurons in vivo. *Nature Reviews Neuroscience, 4*(9), 739–751.

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.

Di Lazzaro, V., Oliviero, A., Profice, P., Saturno, E., Pilato, F., Insola, A., … Rothwell, J. C. (1998). Comparison of descending volleys evoked by transcranial magnetic and electric stimulation

in conscious humans. *Electromyography and Motor Control-Electroencephalography and Clinical Neurophysiology, 109*(5), 397–401.

Dmochowski, J. P., Fau, B. M., Parra, L. C., & Parra, L. C. (2012). The point spread function of the human head and its implications for transcranial current stimulation. (1361-6560 (electronic)). D – NLM: NIHMS409753. *Physics in Medicine and Biology, 57*(20), 6459.

Eaton, H. (1992). Electric field induced in a spherical volume conductor from arbitrary coils: Application to magnetic stimulation and MEG. *Medical & Biological Engineering & Computing, 30*(4), 433–440.

Francis, J. T., Gluckman, B. J., & Schiff, S. J. (2003). Sensitivity of neurons to weak electric fields. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 23*(19), 7255–7261.

Fregni, F., Gimenes, R., Valle, A. C., Ferreira, M. J. L., Rocha, R. R., Natalle, L., … Boggio, P. S. (2006). A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. *Arthritis and Rheumatism, 54*(12), 3988–3998.

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.

Fritsch, G., & Hitzig, E. (1870). Über die elektrische Erregbarkeit des Grosshirns. *Archives Anatomy Physiololy Wissen, 37*, 300–332.

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

Gabriel, C., Gabriel, S., & Corthout, E. (1996a). The dielectric properties of biological tissues: I. Literature survey. *Physics in Medicine and Biology, 41*(11), 2231–2249.

Gabriel, S., Lau, R. W., & Gabriel, C. (1996b). The dielectric properties of biological tissues. 2. Measurements in the frequency range 10 Hz to 20 GHz. *Physics in Medicine and Biology, 41*(11), 2251–2269.

Galea, J. M., Jayaram, G., Ajagbe, L., & Celnik, P. (2009). Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. *The Journal of Neuroscience, 29*(28), 9115–9122.

Gardner-Medwin, A. R., & Nicholson, C. (1983). Changes of extracellular potassium activity induced by electric current through brain tissue in the rat. *The Journal of Physiology, 335*, 375–392.

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

Geddes, L. A., & Baker, L. E. (1967). The specific resistance of biological material--a compendium of data for the biomedical engineer and physiologist. *Medical & Biological Engineering, 5*(3), 271–293.

Gluckman, B. J., Neel, E. J., Netoff, T. I., Ditto, W. L., Spano, M. L., & Schiff, S. J. (1996). Electric field suppression of epileptiform activity in hippocampal slices. *Journal of Neurophysiology, 76*(6), 4202–4205.

Gomez-Gonzalo, M., Losi, G., Chiavegato, A., Zonta, M., Cammarota, M., Brondi, M., … Carmignoto, G. (2010). An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. *PLoS Biology, 8*(4), e1000352.

Grimaldi, G., Argyropoulos, G. P., Bastian, A., Cortes, M., Davis, N. J., Edwards, D. J., … Celnik, P. (2016). Cerebellar Transcranial direct current stimulation (ctDCS): A novel approach to understanding cerebellar function in health and disease. *The Neuroscientist, 22*(1), 83–97.

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron, 55*(2), 187–199.

Hammerer, D., Bonaiuto, J., Klein-Flugge, M., Bikson, M., & Bestmann, S. (2016). Selective alteration of human value decisions with medial frontal tDCS is predicted by changes in attractor dynamics. *Scientific Reports, 6*, 25160.

Hause, L. (1975). A mathematical model for transmembrane potentials secondary to extracellular fields. In *Electroanaesthesia: Biomedical and biophysical studies* (pp. 176–200). New York: Academic.

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

Herrmann, C. S., Rach, S., Neuling, T., & Struber, D. (2013). Transcranial alternating current stimulation: A review of the underlying mechanisms and modulation of cognitive processes. *Frontiers in Human Neuroscience, 7*, 279.

Hodgkin, A. L., & Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of Physiology, 117*(4), 500–544.

Huisman, T. A. (2010). Diffusion-weighted and diffusion tensor imaging of the brain, made easy. *Cancer Imag, 10 Spec no A*, S163–S171.

Izhikevich, E. M. (2003). Simple model of spiking neurons. *IEEE Transactions on Neural Networks/a Publication of the IEEE Neural Networks Council, 14*(6), 1569–1572.

Izhikevich, E. M. (2007). *Dynamical systems in neuroscience*. MIT Press.

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.

Jayaram, G., Tang, B., Pallegadda, R., Vasudevan, E. V. L., Celnik, P., & Bastian, A. (2012). Modulating locomotor adaptation with cerebellar stimulation. *Journal of Neurophysiology, 107*(11), 2950–2957.

Jefferys, J. G. (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.

Kanai, R., Chaieb, L., Antal, A., Walsh, V., & Paulus, W. (2008). Frequency-dependent electrical stimulation of the visual cortex. *Current Biology, 18*(23), 1839–1843.

Koessler, L., Colnat-Coulbois, S., Cecchin, T., Hofmanis, J., Dmochowski, J. P., Norcia, A. M., & Maillard, L. G. (2016). In-vivo measurements of human brain tissue conductivity using focal electrical current injection through intracerebral multicontact electrodes. LID. (1097-0193 (Electronic)). *Human Brain Mapping, 38*, 974–986.

Krause, B., Marquez-Ruiz, J., & Cohen Kadosh, R. (2013). The effect of transcranial direct current stimulation: A role for cortical excitation/inhibition balance? *Frontiers in Human Neuroscience, 7*, 602.

Kronberg, G., Bridi, M., Abel, T., Bikson, M., & Parra, L. C. (2016). Direct current stimulation modulates LTP and LTD: Activity dependence and dendritic effects. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*.

Laakso, I., Tanaka, S., Koyama, S., De Santis, V., & Hirata, A. (2015). Inter-subject variability in electric fields of motor cortical tDCS. *Brain Stimulation, 8*(5), 8.

Lafon, B., Rahman, A., Bikson, M., & Parra, L. C. (2016). Direct current stimulation alters neuronal input/output function. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation, 10*, 36–45.

Liebetanz, D., Fregni, F., Monte-Silva, K. K., Oliveira, M. B., Amancio-Dos-Santos, A., Nitsche, M. A., & Guedes, R. C. (2006a). After-effects of transcranial direct current stimulation (tDCS) on cortical spreading depression. *Neuroscience Letters, 398*(1–2), 85–90.

Liebetanz, D., Klinker, F., Hering, D., Koch, R., Nitsche, M. A., Potschka, H., … Tergau, F. (2006b). Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. *Epilepsia, 47*(7), 1216–1224.

Logothetis, N. K., Kayser, C., & Oeltermann, A. (2007). In vivo measurement of cortical impedance spectrum in monkeys: Implications for signal propagation. *Neuron, 55*(5), 809–823.

Marquez-Ruiz, J., Leal-Campanario, R., Sanchez-Campusano, R., Molaee-Ardekani, B., Wendling, F., Miranda, P. C., … Delgado-Garcia, J. M. (2012). Transcranial direct-current stimulation mod-

ulates synaptic mechanisms involved in associative learning in behaving rabbits. *Proceedings of the National Academy of Sciences of the United States of America, 109*(17), 6710–6715.

Márquez-Ruiz, J., Leal-Campanario, R., Wendling, F., Ruffini, G., Gruart, A., & Delgado-García, J. M. (2014). Transcranial electrical stimulation in animals. In R. Cohen Kadosh (Ed.), *The stimulated brain* (pp. 117–144). Academic.

Mcintyre, C. C., & Grill, W. M. (1999). Excitation of central nervous system neurons by nonuniform electric fields. *Biophysical Journal, 76*(2), 878–888.

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., Correia, L., Salvador, R., & Basser, P. J. (2007). The role of tissue heterogeneity in neural stimulation by applied electric fields. *Conference Proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Annual Conference, 2007*, 1715–1718.

Miranda, P. C., Faria, P., & Hallett, M. (2009). What does the ratio of injected current to electrode area tell us about current density in the brain during tDCS? *Clinical Neurophysiology, 120*(6), 1183–1187.

Miranda, P. C., Hallett, M., & Basser, P. J. (2003). The electric field induced in the brain by magnetic stimulation: A 3-D finite-element analysis of the effect of tissue heterogeneity and anisotropy. *IEEE Transactions on Bio-medical Engineering, 50*(9), 1074–1085.

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

Miranda, P. C., Mekonnen, A., Salvador, R., & Ruffini, G. (2013). The electric field in the cortex during transcranial current stimulation. *NeuroImage, 70*, 48–58.

Moliadze, V., Antal, A., & Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clinical Neurophysiology, 121*(12), 2165–2171.

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.

Nitsche, M. A., Boggio, P. S., Fregni, F., & Pascual-Leone, A. (2009). Treatment of depression with transcranial direct current stimulation (tDCS): A review. *Experimental Neurology, 219*(1), 14–19.

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., … Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation, 1*(3), 206–223.

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*(Pt 3), 633–639.

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. (0028–3878 (Print)). *Neurology, 57*, 1899.

Nitsche, M. A., & Paulus, W. (2009). Noninvasive brain stimulation protocols in the treatment of epilepsy: Current state and perspectives. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics, 6*(2), 244–250.

Oostendorp, T. F., Delbeke, J., & Stegeman, D. F. (2000). The conductivity of the human skull: Results of in vivo and in vitro measurements. *IEEE Transactions on Bio-medical Engineering, 47*(11), 1487–1492.

Opitz, A., Falchier, A., Yan, C. G., Yeagle, E. M., Linn, G. S., Megevand, P., … Schroeder, C. E. (2016). Spatiotemporal structure of intracranial electric fields induced by transcranial electric stimulation in humans and nonhuman primates. (2045-2322 (electronic)). *Scientific Reports, 6*, 31236. PST – epublish.

Opitz, A., Paulus, W., Will, S., Antunes, A., & Thielscher, A. (2015). Determinants of the electric field during transcranial direct current stimulation. (1095–9572 (Electronic)). *Neuroimage, 109*, 140.

Opitz, A., Windhoff, M., Heidemann, R. M., Turner, R., & Thielscher, A. (2011). How the brain tissue shapes the electric field induced by transcranial magnetic stimulation. *NeuroImage, 58*(3), 849–859.

Ozen, S., Sirota, A., Belluscio, M. A., Anastassiou, C. A., Stark, E., Koch, C., & Buzsaki, G. (2010). Transcranial electric stimulation entrains cortical neuronal populations in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 30*(34), 11476–11485.

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.

Park, E.-H., Barreto, E., Gluckman, B. J., Schiff, S. J., & So, P. (2005). A model of the effects of applied electric fields on neuronal synchronization. *Journal of Computational Neuroscience, 19*(1), 53–70.

Parra, L. C., & Bikson, M. (2004). Model of the effect of extracellular fields on spike time coherence. *Conference Proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Annual Conference, 6*, 4584–4587.

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

Pelletier, S. J., & Cicchetti, F. (2015). Cellular and molecular mechanisms of action of transcranial direct current stimulation: Evidence from in vitro and in vivo models. *The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum, 18*(2).

Peterchev, A. V., Jalinous, R., & Lisanby, S. H. (2008). A transcranial magnetic stimulator inducing near-rectangular pulses with controllable pulse width (cTMS). *IEEE Transactions on Biomedical Engineering, 55*(1), 257–266.

Peterchev, A. V., Wagner, T. A., Miranda, P. C., Nitsche, M. A., Paulus, W., Lisanby, S. H., … Bikson, M. (2012). Fundamentals of transcranial electric and magnetic stimulation dose: Definition, selection, and reporting practices. *Brain Stimulation, 5*(4), 435–453.

Pethig, R., & Kell, D. B. (1987). The passive electrical properties of biological systems: Their significance in physiology, biophysics and biotechnology. *Physics in Medicine and Biology, 32*(8), 933–970.

Plonsey, R., & Barr, R. C. (1998). Electric field stimulation of excitable tissue. *IEEE Engineering in Medicine and Biology Magazine: The Quarterly Magazine of the Engineering in Medicine & Biology Society, 17*(5), 130–137.

Plonsey, R., & Heppner, D. B. (1967). Considerations of quasi-Stationarity in electrophysiological systems. *B Math Biophys, 29*(4), 657–664.

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.

Prescott, S. A., De Koninck, Y., & Sejnowski, T. J. (2008). Biophysical basis for three distinct dynamical mechanisms of action potential initiation. *PLoS Computational Biology, 4*(10), e1000198.

Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *Neuroreport, 9*(10), 2257–2260.

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

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.

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

Rahman, A., Lafon, B., & Bikson, M. (2015). Multilevel computational models for predicting the cellular effects of noninvasive brain stimulation. *Progress in Brain Research, 222*, 25–40.

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.

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.

Rampersad, S., Fau, S. D., Oostendorp, T., & Oostendorp, T. (2011). On handling the layered structure of the skull in transcranial direct current stimulation models. (1557-170X (Print)). *Conference Proceedings IEEE Engineering in Medicine and Biology Society, 2011*, 1989.

Ranck, J. B., Jr. (1975). Which elements are excited in electrical stimulation of mammalian central nervous system: A review. *Brain Research, 98*(3), 417–440.

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.

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., Cammarota, M., Parra, L. C., & Carmignoto, G. (2012). Computational model of neuron-astrocyte interactions during focal seizure generation. *Frontiers in Computational Neuroscience, 6*, 81.

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. *The Journal of neuroscience : the official journal of the Society for 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.

Renart, A., de la Rocha, J., Bartho, P., Hollender, L., Parga, N., Reyes, A., & Harris, K. D. (2010). The asynchronous state in cortical circuits. *Science, 327*(5965), 587–590.

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: The Official Journal of the Society for Neuroscience, 35*(37), 12824–12832.

Roth, B. J., Cohen, L. G., & Hallett, M. (1991). The electric field induced during magnetic stimulation. *Electroencephalography and Clinical Neurophysiology, 43*, 268–278.

Ruffini, G., Fox, M. D., Ripolles, O., Miranda, P. C., & Pascual-Leone, A. (2014). Optimization of multifocal transcranial current stimulation for weighted cortical pattern targeting from realistic modeling of electric fields. *NeuroImage, 89*, 216–225.

Ruffini, G., Wendling, F., Merlet, I., Molaee-Ardekani, B., Mekkonen, A., Salvador, R., … Miranda, P. (2013). Transcranial current brain stimulation (tCS):Models and technologies. *IEEE Transactions on Neural Systems and Rehabilitation Engineering, 21*(3), 333–345.

Rush, S., & Driscoll, D. A. (1968). Current distribution in the brain from surface electrodes. *Anesthesia and Analgesia, 47*(6), 717-723.

Rush, S., & Driscoll, D. A. (1969). Eeg electrode sensitivity – an application of reciprocity. *IEEE Transactions on Bio-medical Engineering, 16*(1), 15.

Sadleir, R. J., Vannorsdall, T. D., Schretlen, D. J., & Gordon, B. (2010). Transcranial direct current stimulation (tDCS) in a realistic head model. *NeuroImage, 51*(4), 1310–1318.

Salvador, R., Ramirez, F., V'Yacheslavovna, M., & Miranda, P. C. (2012). Effects of tissue dielectric properties on the electric field induced in tDCS: A sensitivity analysis. *34th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), San Diego*, 787–790.

Salvador, R., Wenger, C., & Miranda, P. C. (2015). Investigating the cortical regions involved in MEP modulation in tDCS. *Frontiers in Cellular Neuroscience, 9*.

Saturnino, G. B., Antunes, A., & Thielscher, A. (2015). On the importance of electrode parameters for shaping electric field patterns generated by tDCS. (1095–9572 (Electronic)). *Neuroimage, 120*, 25.

Schlaug, G., Renga, V., & Nair, D. (2008). Transcranial direct current stimulation in stroke recovery. *Archives of Neurology, 65*(12), 1571–1576.

Schwan, H. P. (1966). Alternating current electrode polarization. *Biophysik, 3*(2), 181–201.

Sehm, B., Hoff, M., Gundlach, C., Taubert, M., Conde, V., Villringer, A., & Ragert, P. (2013). A novel ring electrode setup for the recording of somatosensory evoked potentials during transcranial direct current stimulation (tDCS). *Journal of Neuroscience Methods, 212*(2), 234–236.

Stagg, C. J., & Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry, 17*(1), 37–53.

Svirskis, G., Baginskas, A., Hounsgaard, J., & Gutman, A. (1997). Electrotonic measurements by electric field-induced polarization in neurons: Theory and experimental estimation. *Biophysical Journal, 73*(6), 3004–3015.

Terney, D., Chaieb, L., Moliadze, V., Antal, A., & Paulus, W. (2008). Increasing human brain excitability by transcranial high-frequency random noise stimulation. *The Journal of Neuroscience, 28*(52), 14147–14155.

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

Tofts, P. S. (1990). The distribution of induced currents in magnetic stimulation of the nervous-system. *Physics in Medicine and Biology, 35*(8), 1119–1128.

Tranchina, D., & Nicholson, C. (1986). A model for the polarization of neurons by extrinsically applied electric fields. *Biophysical Journal, 50*(6), 1139–1156.

Tuch, D. S., Wedeen, V. J., Dale, A. M., George, J. S., & Belliveau, J. W. (2001). Conductivity tensor mapping of the human brain using diffusion tensor MRI. *Proceedings of the National Academy of Sciences of the United States of America, 98*(20), 11697–11701.

Vogels, T. P., Rajan, K., & Abbott, L. F. (2005). Neural network dynamics. *Annual Review of Neuroscience, 28*, 357–376.

Wagner, S., Fau, R. S., Aydin, U., Fau, A. U., Vorwerk, J., Fau, V. J., … Wolters, C. H. (2014a). Investigation of tDCS volume conduction effects in a highly realistic head model. (1741–2552 (Electronic)). *Journal of Neural Engineering, 11*, 016002.

Wagner, T., Eden, U., Rushmore, J., Russo, C. J., Dipietro, L., Fregni, F., … Valero-Cabre, A. (2014b). Impact of brain tissue filtering on neurostimulation fields: A modeling study. *NeuroImage, 85*(Pt 3), 1048–1057.

Woods, A. J., Antal, A., Bikson, M., Boggio, P. S., Brunoni, A. R., Celnik, P., … Nitsche, M. A. (2016). A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology, 127*(2), 1031–1048.

Xu, W., Wolff, B. S., & Wu, J. Y. (2014). Low-intensity electric fields induce two distinct response components in neocortical neuronal populations. *Journal of Neurophysiology, 112*(10), 2446–2456.

Yi, G.-S., Wang, J., Wei, X.-L., Tsang, K.-M., Chan, W.-L., Deng, B., & Han, C.-X. (2014). Exploring how extracellular electric field modulates neuron activity through dynamical analysis of a two-compartment neuron model. *Journal of Computational Neuroscience, 36*(3), 383–399.

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