# 3 Cellular and Network Effects of Transcranial Direct Current Stimulation

## *Insights from Animal Models and Brain Slice*

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**CONTENTS**

This chapter addresses the contribution of animal research on direct current (DC) stimulation to current understanding of transcranial direct current stimulation (tDCS) mechanisms and prospects and pitfalls for ongoing translational research. Though we attempt to put in perspective key experiments in animals from the 1960s to the present, our goal is not an exhaustive cataloging of relevant animal studies, but rather to put them in the context of ongoing effort to improve tDCS. Similarly, though we point out essential features of meaningful animal studies, we refer readers to original work for methodological details. Though tDCS produces specific clinical neurophysiological changes and is therapeutically promising, fundamental questions remain about the mechanisms of tDCS and on the optimization of dose. As a result, a majority of clinical studies using tDCS employ a simplistic dose strategy where "excitability" is increased or decreased under the anode and cathode respectively. We discuss how this strategy, itself based on classic animal studies, may not account for the complexity of normal and pathological brain function, and how recent studies have already indicated more sophisticated approaches.

## 3.1 MEANINGFUL ANIMAL STUDIES OF tDCS AND THE QUASI-UNIFORM ASSUMPTION

The motivation for animal research of tDCS is evident and similar to other translational medical research efforts: to allow rapid and risk-free screening of stimulation protocols and to address the mechanisms of tDCS with the ultimate goal of informing clinical tDCS efficacy and safety. To have a meaningful relevance to clinical tDCS, animal studies must be designed with consideration of: (1) conducting animal studies by correctly emulating the delivery of DC stimulation to the brain; and (2) measuring responses that can be used to draw clinically relevant inferences. Before reviewing the main insights drawn from animal studies, we outline the basis and pitfalls of translational animal research on tDCS.

### 3.1.1 Classification of Animal Studies

The scope of this review includes any animal study exploring the behavioral, neurophysiological, or molecular response of the brain to DC currents; with a focus on macro-electrodes, relatively low intensity stimulation, and sustained (seconds to minutes) rather than pulsed (millisecond or less) waveforms. Animal studies can be broadly classified by the preparation and related method of stimulation, namely

where the electrodes are placed, as: (1) transcranial stimulation in animals; (2) intracranial stimulation in vivo including with one electrode on the cortex (3) stimulation of tissue in vitro, including brain slices.

1. Modern animal studies on tDCS use transcranial stimulation with a skull screw or skull mounted cup (Liebetanz et al. 2006; Cambiaghi et al. 2010; Yoon et al. 2012)—advantages of transcranial stimulation include preventing electrochemical products from the electrodes from reaching the brain (which would confound any results). If the screw penetrates completely through the skull, stimulation is no longer in the transcranial category (see next). Rodents are typically used. A return electrode on the body, mounted in a "jacket" is typically used for "unipolar stimulation" (which is broadly analogous to a human tDCS extracephalic electrode). In a rabbit study four silver ball electrodes formed a single virtual electrode over the target (Marquez-Ruiz et al. 2012). Alternatively, two cranial electrodes produce bipolar stimulation (Ozen et al. 2010). Since the cranium is not penetrated, the effects of DC stimulation are probed through behavior, non-invasive recording (electroencephalogram, EEG), non-invasive electrical interrogation (e.g., transcranial magnetic stimulation, TMS; transcranial electrical stimulation, TES), or histology after sacrifice. In some older studies, transcranial DC stimulation was also applied in larger animals (monkeys) (Toleikis et al. 1974) but should be interpreted with caution when the skull was penetrated for recording electrodes (which distorts current flow) (Datta et al. 2010) or when recording between electrodes without control for cortical folding (leading to variation in current flow direction through cortical gyrations and inconsistent effects). Replacement of removed skull with insulating filler (e.g., dental cement) may correct shunting through the hole (Marquez-Ruiz et al. 2012). AQ1
2. Classic animal studies typically used an electrode on the brain (Creutzfeldt et al. 1962; Bindman et al. 1964), where the intracranial electrode was covered in something like a cotton wick (Redfearn et al. 1964) to buffer electrochemical changes. Cats and monkeys were typically used. Note that the protection of the skin from electrochemical product at the electrode is why saline-soaked sponges (or gel) are used in tDCS (Minhas et al. 2010)—and though improper set-up can result in skin irritation, these products can not reach the brain and so are not part of tDCS mechanisms. When an electrode is placed inside the cranium (on the animal brain) then potential interference from electrochemical changes at the electrodes diffusing into the brain cannot be automatically ignored. These electrochemical products can even be polarity specific (Merrill et al. 2005) and produce reversible changes, but still have no relevance to tDCS. Steps to reduce interference include using suitable electrode (e.g., Ag/AgCl) and wrapping the electrode in cotton to buffer chemical changes; protocols that where rationally used in many studies. Passage of prolonged DC current through a poorly selected electrode material (e.g., screw) is expected to produce significant electrochemical changes near the metal. It is generally assumed with cortical electrodes

that current flow through nearby cortex will be unidirectional (inward for anode, outward for cathode; see conventions below); however, the presence of CSF in convoluted gyri (especially in larger animals) will distort current flow patterns and can produce local direction inversions (Creutzfeldt et al. 1962). Despite these concerns, the rationale for invasive stimulation in classical animal studies may simply be that the cranium must be exposed regardless to facilitate insertion of recording electrodes, and a majority of these studies were interested in the general effects of DC current on brain function and not necessarily clinical *transcranial* DCS.

3. The use of brain slices to study the effects of weak DC stimulation dates to work by John G.R. Jefferys in 1981 (Jefferys 1981; Nitsche and Paulus 2000; Ardolino et al. 2005), with experimental techniques used to the present day established by Bruce Gluckman and Steven Schiff (Gluckman et al. 1996) and adapted by Dominique Durand and our group (Durand and Bikson 2001). The rationale for using a brain slice (usually rodents and ferrets) is the ability to probe brain function in detail using a range of electrophysiological, pharmacological, molecular, and imaging techniques. In isolated tissue, the direction of current flow is also known and precisely controlled. Lopez-Quintero et al. (2010) described techniques for stimulating cultured monolayers. In a seminal series of papers Chan and Nicholson used isolated turtle cerebellum (Chan and Nicholson 1986; Chan et al. 1988). For in vitro DC stimulation studies, electrodes are placed in the bath at some distance from the tissue to buffer electrochemical changes. As emphasized below, tDCS delivers electrical current not chemicals to the brain.

### 3.1.2 tDCS Dose in Human and Animals, and the Quasi-Uniform Assumption

The clinical "dose" of tDCS has been defined as those aspects of stimulation that are externally controlled by the operator (Bikson et al. 2008; Peterchev et al. 2011), namely electrode montage (shape, location, etc.) and the specifics of the DC waveform (duration, intensity in mA applied, ramp, etc.). As explained next, it would be fundamentally misguided to simply replicate these dose parameters in animal studies. tDCS produces a complex pattern of current flow across the brain, which results in dose-specific electric field (current density) that varies significantly across brain regions. This brain electric-field distribution represents and determines the electrical actions of tDCS. The brain electric field is not a simple function of any dose parameter, for example the current density at the electrodes (total current/area) does not map simply to peak brain electric field (Miranda et al. 2007). There are fortunately well-established methods to predict the electric field generated in the brain using computational models (Miranda et al. 2006; Datta et al. 2009); though methodological approaches across groups vary those modeling studies using realistic anatomy have converged that the peak electrical field generated during tDCS is 0.2–0.5 V/m (0.05–0.14 A/m$^2$ current density) for a 1 mA intensity (Miranda et al. 2006; Datta et al. 2009; Sadleir et al. 2010). The electric field would scale linearly with current intensity such that 2 mA would produce up to 0.4–1 V/m (0.1–0.28 A/m$^2$ current density).

These peaks represent specific hot-spots. Using conventional tDCS montages weaker electric fields are generated across much of the brain. In addition, due to subject-specific idiosyncratic cortical folding, the electric field is "clustered" (Datta et al. 2009), with many local maxima (Figure 3.1). There is thus no single electric field generated in the brain during tDCS but rather a range of distributed electric fields across the brain. The question therefore is: given this complexity of electric field distribution across brain structures, what should (and can) be mimicked in animal models? It is important to emphasize that simply mimicking tDCS clinical dose in animal models, or adjusting dose guidelines by an arbitrary rule of thumb (e.g., by head volume), may not be prudent.

One solution (which we term an aspect of the "quasi-uniform" assumption) is to consider only the peak electric field generated in the brain, or only the electric field in one brain region of interest, and then to replicate the electric field across an area of the animal brain or the entire animal brain/tissue. (It is impractical to replicate the electric field induced in each brain region during tDCS in all corresponding brain region in an animal model.) As it turns out, because of practical consideration, the quasi-uniform assumption is already adopted implicitly in most animal research of tDCS. This approach is partly supported by electric fields generated during tDCS being largely uniform across any specific cortical column (neuronal dendritic tree) of interest (Figure 3.1, inset)—hence one can speak of a single electric field in reference to a region of interest.

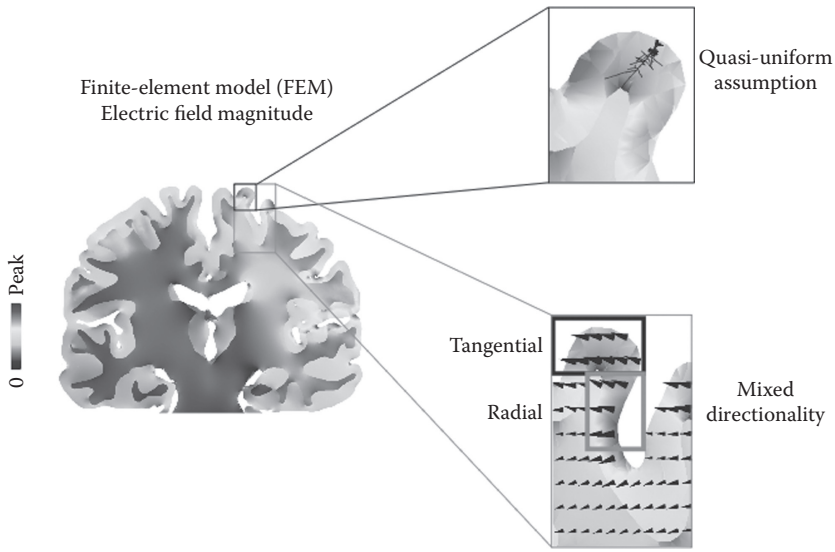


**FIGURE 3.1** The quasi-uniform assumption is implicit in the majority of modeling and animal studies of tDCS. The first aspect of the quasi-uniform assumption is based on the electric field generated in the brain to not significantly change (be uniform) on the scale of a single cortical column or neuronal dendritic tree. Only in this way it is meaningful to represent, for a first approximation, neuromodulation by regional electric field. This assumption underpins the rational basis for replicating an electric field of interest in an animal model as described in the text. Shown is a high-resolution finite element model (FEM) computational model of current flow through the head with overlaid neuronal morphology.

However, it is worthwhile to point out that considering the *peak* of the electric field (either across the whole brain or in a sub-region) as basis for the field amplitude may be misleading. The field amplitude can change by orders of magnitudes in different brain areas and dramatically even across local gyri (Datta et al. 2009). The average (or median) value of the electric field can be up to 10 times smaller than the field peak (depending on local geometry and conductivity properties). Also, since usually the electric fields used in animal experiments are based on estimations from humans (using finite element models), it is also necessary to consider how the coupling constant between neuronal polarization and electric field applied can vary across species (see next). For that reason, while the average electric field can be smaller than the peak value, the polarization of neurons in humans could be higher, assuming a higher coupling constant in humans (see next).

Generally, once a clinical (quasi-uniform) tDCS electric field that is to be replicated in an animal model is decided, three approaches have been taken in rational experimental design. These three approaches typically relate to: (1) Transcranial stimulation in animal; (2) Invasive stimulation of animals with intracranial electrodes; (3) Stimulation of tissue/brain slices with bath electrodes. In each case, the quasi-uniform assumption is re-applied in the generation and control of a (quasi)uniform electric field in a targeted region of the animal brain or across isolated tissue.

1. In the first case of transcranial stimulation of animal, the same modeling approaches that predict electric fields during clinical tDCS can be used to model and guide stimulation design (Gasca et al. 2010). As the case in clinical tDCS, in DC transcranial stimulation in animals it is important to consider how the position of the "return/reference" electrode influences current flow even under the "active" electrode (Bikson et al. 2010; Brunoni et al. 2011). As anatomically precise animal models are under development, concentric sphere models (simply scaled to size) can be used to determine electric field intensity generated in the animal brain (Marquez-Ruiz et al. 2012); free tool available through neuralengr.com/BONSAI). In the absence of an specific modeling of current flow in animal, and in cases where the electrode is placed directly on the skull, one can, to a first approximation, assume a maximum potential brain current density equal to the average electrode current density (total current/electrode area; (Bikson et al. 2009). However, it is important to recognize that the direction (inward or outward) of the electric fields generated across the brain, including in deep brain structures (particularly in higher animals with increasing convoluted cortex) may also vary (as it does in human tDCS). The electric field in a region of interest may also be measured with invasive electrodes (Ozen et al. 2010), recognizing it is not uniform throughout the animal brain, and the insertion and presence of electrodes may itself distort current flow.
2. In the second case, for animal studies with an electrode placed on the brain surface, one might again assume that the (quasi-uniform) current density in the brain directly under the electrodes equals that *average* current density at the electrode (total current/electrode area). As with scalp electrodes in tDCS, when a sponge of cotton wrapper is used, its contact areas should be

used in calculations. But depending on the electrode design, current density may in fact be (orders of magnitude) higher at electrode edges (Miranda et al. 2006; Minhas et al. 2011)—an issue aggravated for small electrodes where electric field near a monopolar source can be very high leading to further potential complications (see discussion in Bindman et al. 1964). As with transcranial stimulation, current spread throughout the brain should be assumed with any return outside the head (Islam et al. 1995).

3. In the third approach, including in vitro brain slice studies, the task is simplified because using long parallel wires (or plates) placed in a bath across the entire tissue—with proper care, this generates a uniform electric field across the entire tissue (a truly uniform electric field) that can be readily calibrated to match tDCS levels (Gluckman et al. 1996; Francis et al. 2003; Bikson et al. 2004). Typically, the placement of the electrodes in the bath, away from the tissue of interest, protects from electrochemical products. The simplicity and versatility of this techniques, makes control of DC parameters in slice straightforward and allows analysis of function in detail not possible with other techniques (see next). It is interesting that the generation of uniform fields across an entire brain region can make the most invasive in vitro approaches analogous to regional electric field induced by tDCS.

In each of the aforementioned cases the quasi-uniform assumption applies by (1) assuming a uniform electric field in a region of interest during tDCS that is a function of scalp electrode position and applied current (Figure 3.1) though recognizing that the electric field varies across the brain; and (2) positioning electrodes and selecting current in translational research that replicate this electric field in a specific region of the animal brain (recognizing that electric field will vary across the entire animal brain) or across a brain slice in vitro (recognizing that the entire brain slice will be exposed to a single electric field; Figure 3.2).

### 3.1.3 Stimulator and Electrode Techniques, and Nomenclature

On a technical note, our opinion is that for reproducibility and precision current-controlled stimulation should be used in animal studies. Indeed, for the same reason tDCS with current-controlled stimulation is used in almost all translational studies of DC stimulation. The electrode-solution interface represents an unknown and changing impedance in series with the stimulator (Merrill et al. 2005). It is well established that current-control guarantees consistent stimulation of tissue through this interface; the electric field in the brain tracks the applied current and can be simply scaled to match clinical electric field values. Using voltage control, especially during DC stimulation, may result in an unknown variable, and indeed changing (not DC) electric field. If voltage control is used, the electric field generated in the tissue during the entire course of stimulation should be monitored. Simply using current control does not cancel the importance of considering (1) electrode size and position, which determines brain current flow pattern; and (2) electrode material and use of any buffer which determine electrochemical changes. Moreover, the two can

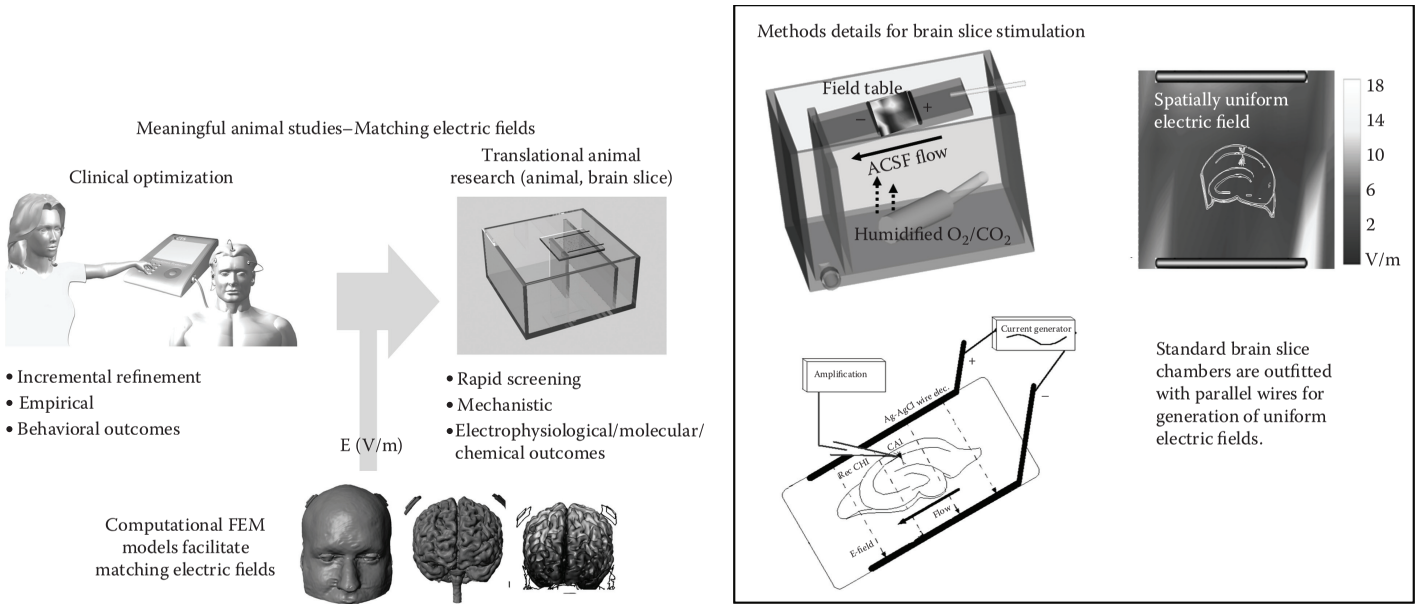


**FIGURE 3.2** Animal studies on tDCS mechanism allow rapid screening of stimulation parameters and analysis of neurophysiological and molecular changes in ways not possible clinically. Meaningful translation research in animals required replication of electric fields generated clinically in animal brain/tissue. The electric field generated in the brain during tDCS is dependent on the stimulation dose (current intensity, electrode montage) and head anatomy. It is not trivial to relate externally controlled dose with internally generated electric fields (i.e., the current density in the brain is not the same as at the electrodes), but FEM computational models provide a method to do so. In the experimental design of animal studies, the electric field generated should correspond in intensity to that generated clinically; otherwise results should be applied to the clinical case with caution. In the case of in vitro brain slice studies, the replication of clinical electrical fields is experimentally straightforward with the use of two long parallel wires placed across the bath, generating uniform electric field. The single uniform electric field in the chamber can be simply calibrated, using a field-recording electrode, to the current applied to the wires. The position of the brain slice in the uniform field is not important to control; moreover, multiple slices can be screened at once.

be interrelated as electrode degradation will make a portion of the electrode inactive causing current re-distribution, while increasing electrode size (in particular electrode contact area) reduces electrochemical burden. We caution: as more diverse research groups apply increasingly sophisticated techniques to analyze the effects of DC stimulation to understand the mechanisms of tDCS, it is simultaneously necessary to apply rigor in the methods used to delivery stimulation for each animal model. At a minimum, the "dose" of stimulation needs to be reported in a manner that allows reproduction consistent with clinical rules of dose reporting and control (Peterchev et al. 2011).

Some comments on conventions used to indicate the polarity of stimulation may be useful. Firstly, in brain electrical stimulation *anode* and *cathode* terminology should always be used consistently for indicating the electrode where positive current is entering the body (anode) and the electrode where positive current is exiting the body (cathode)—there is no basis to confuse these terms in electrical stimulation literature (Merrill et al. 2005). For conventional tDCS with two electrodes, there is simply one anode and one cathode, with the anode at a positive voltage relative to the cathode. In clinical and animal studies, *anodal stimulation* or *cathodal stimulation* would indicate that a cortical region of interest (target) was nearer the anode or the cathode, respectively. In earlier animal literature the terms *surface positive* and *surface negative*, correspond to an anode or cathode, respectively, electrode placed the surface of the cortex, with the other electrode often placed on the neck or body. Considering the cortical surface, *inward current* and *outward current* are typically expected under the anode and cathode respectively (though cortical anatomy may produce deviations). When discussing *electric field*, the direction needs to be specified. In our FEM studies we use the convention that an inward current will produce a positive electric field measured from outside pointed in, while an outward current will produce a negative electric field measured from outside pointed in (Datta et al. 2008) (unless otherwise stated, it is implied that the current and electric fields are normal/orthogonal to the cortical surface, rather than tangential/parallel). *Current density* will always be in the same polarity/direction as the electric field, for example current density flow is positive inward (into the cortex) under the anode. In tissue/brain slices, though the terms anode and cathode remain unambiguous in regards to the electrodes, the electric field reference direction is arbitrary and needs to be defined. In our studies where uniform DC stimulation is applied to cortical slices, we always define the electric field as positive when the anode is on the pia surface side of the slice and the cathode on the midbrain side—while a negative electric field indicates the cathode on the pia side (Radman et al. 2009). In this way a positive electric field indicates stimulation polarity (direction) associated with clinical anodal tDCS, while negative electric field indicates a polarity associated with cathodal tDCS. Typically, in hippocampal slice studies using parallel wires, a positive electric field indicates the anode on the alveus side of CA1 (Gluckman et al. 1996; Ghai et al. 2000; Bikson et al. 2004; Ranieri et al. 2012). Finally, the term *polarizing*, or polarizing current, is used in classic animal literature and modern tDCS, and appears to refer to the use of prolonged (not pulsed) DC stimulation applied with macro-electrodes, with the polarization related to the electrodes, brain, and/or neurons.

### 3.1.4 Dose Control and Meaningful Animal Studies

We emphasize caution when drawing conclusions from studies using any DC currents in animals that do not produces electric field magnitudes comparable to those generated during tDCS. These studies are valuable in suggesting mechanisms for tDCS but, just as with drugs, increasing dose beyond clinical levels (by orders of magnitude) can induce physiological changes not relevant clinically. For example, some animal studies have shown that application of DC can control neuronal process orientation and growth direction (Alexander et al. 2006; Li et al. 2008); however, both the intensity and duration of electric fields were orders of magnitude greater than tDCS. Similarly, electroporation and joule heating can be caused by electricity in general, but do not seem relevant for clinical tDCS electric fields (Bikson et al. 2009; Datta et al. 2009; Liebetanz et al. 2009). Thus, these mechanisms and related animal studies are not considered further here. Additional theories have been ventured regarding the role of concentration changes induced by DC current (e.g., iontophoresis of charged molecules/ions) (Gardner-Medwin 1983), and though intriguing, to our knowledge no quantitative analysis of plausibility, and much less experimental evidence, exists for tDCS relevant electric fields. Speculative direct electrochemical changes in the brain should not be confused with: (1) established electrochemical reactions that occur at the electrode interface, which would not reach the brain using scalp electrodes (Merrill et al. 2005; Minhas et al. 2010); (2) indirect chemical and molecular changes secondary to neuronal activation (Stagg and Nitsche 2011). We also caution against any theories that suggest violation of electroneutrality during DC stimulation (e.g., "accumulation" of positive charge near the cathode). Rather, as explained in the next section, our mechanistic considerations start with the well-established principle of membrane polarization induced by extracellular DC current flow, with all other changes secondary to this polarization. Interestingly, in this context, the coupling sensitivity for human neurons may be higher than animals.

Though important to our understanding of tDCS mechanism, most animal work on DC stimulation in the 1960s used current densities with invasive electrodes higher than used in tDCS at the scalp (most of these studies did not intend to mimic tDCS). Recent animal studies often used transcranial DC stimulation with current density at the skull higher than used in tDCS at electrodes (Fregni et al. 2007; Brunoni et al. 2011). Perhaps also motivated by magnifying effect size (and not necessarily motivated only by tDCS) many recent in vitro studies, including those by our own group, used electric fields higher than those generated clinically (Andreasen and Nedergaard 1996; Bikson et al. 2004). Because of the complexity (nonlinearity) of the nervous system function one cannot automatically assume a monotonic (more field = more response) relationship between intensity and outcome; however, in vitro studies that explore field strength-response curves indicate a surprisingly linear response curve over low intensities (Bikson et al. 2004; Reato et al. 2010), and membrane coupling constant certainly appears linear with field strength (see next). Those in vitro studies that have explicitly explored the lower electric field limit of sensitivity to fields (see Section 3.4; Francis et al. 2003; Jefferys et al. 2003; Reato et al. 2010) report statistically significant responses at <0.2 V/m, within tDCS ranges.

One example of how the use of high DC current intensities can produce effects opposite than expected at DC relevant intensities is noted. As discussed later, in a polarity-specific manner, DC fields can increase excitability and evoked responses (synaptic efficacy). But if the intensity of DC current is increased significantly, it may increase excitability to the point that the neuron generates (high frequency) discharges—the responsiveness of the very active neuron to an evoked response may then *decrease* because it is often in a refractory state. This was shown in brain slice (Bikson et al. 2004) and may explain results in animal (Purpura and McMurtry 1965) using high DC current intensities.

### 3.1.5 Outcome Measures

The second issue to consider in the design of translational studies is the appropriateness of the outcome measure in animal models, which is not specific to tDCS and will not be discussed in detail here. In considering the use of tDCS in clinical treatment, animal models of disease can be used, not simply to validate outcomes, but to characterize mechanisms and optimize stimulation protocols (Sunderam et al. 2010; Yoon et al. 2012). One factor facilitating quantitative translational research is the noteworthy emphasis by tDCS clinical researcher to determine neurophysiological markers of tDCS including spontaneous EEG (Marshall et al. 2004; Marshall et al. 2006) and TMS motor evoked responses (Nitsche and Paulus 2000), including while screening different dosage and time-course. These generic clinical measures of "excitability" have rough animal analog in spontaneous firing rate, oscillations, and evoked responses—though "evoked responses" or oscillations of a given frequency may not have the same origin in animals and humans. Animal research in tDCS has only started to access the breadth of behavior and disease models that are available. As summarized by Brunoni et al. (2011)

> Although pre-clinical studies, including experiments with animals, are critical in developing novel human therapies, translational research also has several challenging aspects, as animal and human studies can differ in characteristics of disease (i.e., 'human disease' vs. 'experimental animal model'), definition of outcomes (especially for neurological research that often rely heavily on behavioral outcomes….

Having outlined potential pitfalls in translational tDCS studies, the need and value of well-designed animal research remains evident. Contributions of animal studies to our current understanding of tDCS and their importance as tDCS becomes more sophisticated are discussed in the next sections.

## 3.2 SOMATIC DOCTRINE AND NEED FOR AMPLIFICATION

Since 2000 (Nitsche and Paulus 2000; Ardolino et al. 2005; Fregni et al. 2005, 2007), there has been rapid acceleration in the use of tDCS in both clinical and cognitive-neuroscience research, encouraged by the simplicity of the technique (two electrodes and a battery powered stimulator) and the perception that tDCS protocols can be simply designed by placing the anode over the cortex to "excite," and the cathode over cortex to "inhibit." Starting with the consideration of single neurons and acute

effects, in this section we (1) define this simplistic and ubiquitous "somatic doctrine"; (2) consider its origin in classic animal studies; (3) describe modern efforts to quantify somatic polarization using brain slices; this in turn leads to an appreciation of the need for amplification mechanisms. Discussions focused specifically on mechanism of lasting changes following tDCS (e.g., plasticity) and consideration of network activity are left for the next sections.

### 3.2.1 Neuronal Polarization

DC stimulation with electrodes on the scalp leads to current flow across the brain, which in turn, results in polarization of cell membranes when some of this current crosses the membrane (Ohms' law). Flow into a specific compartment of membrane will result in local membrane hyperpolarization, and flow out of another compartment of membrane will result in local membrane depolarization (Andreasen and Nedergaard 1996; Bikson et al. 2004). It is fundamental to emphasize (especially as this concept is overlooked in clinical literature) that there is no such thing a purely depolarizing or purely hyperpolarizing weak DC stimulation—the physics of electrical stimulation dictate that any neuron exposed to extracellular DC stimulation will have some compartments that are depolarized and some that are hyperpolarized (Chan et al. 1988; Bikson et al. 2004). Which compartments are polarized in which direction depends on the neuronal morphology relative to the DC electric field. Simplistically, for a pyramidal type neuron, with a large apical dendrite pointed toward the cortical surface, a surface anode (positive electrode, generating a cortical inward current flow) will result in somatic (and basal dendrite) depolarization and apical dendrite hyperpolarization (Radman et al. 2009). For this same neuron, a surface cathode (negative electrode, generating cortical outward current flow) will result in somatic (and basal dendrite) hyperpolarization and apical dendrite depolarization. tDCS protocols based on the "somatic doctrine" simply assume that somatic polarization determines all relevant functional/clinical outcomes. This consensus of a generic excitation/inhibition by anodic/cathodic stimulation underpins a majority of clinical tDCS study design (Figure 3.2a)—combined with the concept that brain (dis)function is a sliding scale of excitability that can be controlled in this fashion.

### 3.2.2 Modulation of Excitability, Polarity-Specific Effects

The application of DC stimulation (often as short pulses) to the neuro-muscular system dates to the origin of batteries (indeed, as electrical energy sources must predate any electrical devices, human and animals made natural targets). The review of the history of DC stimulation is well beyond the scope of this chapter, but some highlights help position the origin of the "somatic doctrine." In 1870 Fritsch and Hitzig may have been the first to show that application of a positive current to the cortex had stimulating effects, while a negative current inhibits (a finding that itself contributed to early understanding that the cortex is electrically excitable) (Fritsch 1870; Carlson and Devinsky 2009). Terzuolo and Bullock (1956) and Creutzfeldt et al. (1962) helped establish that ongoing discharge frequency is enhanced by

surface-positive current and decreased by surface-negative currents (it is curious how the debate over the role of endogenous electric fields is reflected in these early works in which Creutzfeldt et al. suggested they are epiphenomena while Terzuolo and Bullock suggested a physiological role—indeed modern work with weak transcranial stimulation has provided the strongest clinical evidence for a plausible role). The concept that threshold for electric field sensitivity would be "lower for modulation of the frequency of an already active neuron than for excitation of a silent one" was thus well established, with early observation of changes in discharge rate with fields as low as 0.8 V/m (Terzuolo and Bullock 1956).

Note, the electric fields induced by tDCS are considered far too weak to trigger action potentials in quiescent neurons (compare >100 V/m induced by TMS to <1 V/m by tDCS). It is thus not surprising that early animal studies on lower-intensity DC stimulation addressed modulation of ongoing normal or pathological neuronal firing rate, as well as evoked response. In the early 1960s, animal studies by Bindman and colleagues (Bindman et al. 1962, 1964) confirmed polarity-specific changes in discharge rate and further showed excitability changes that are both cumulative with time and out-last stimulation (discussed in next section)—this group went on to explore long-duration stimulation in early psychiatric treatment. It was also recognized that the direction of changes in discharge rate were consistent with presumed somatic polarization (and dependent on the orientation of the apical dendrites). Furthermore, animal studies in the 1950s and 1960s examining control of epileptic discharges (Purpura et al. 1966), evoked responses (Creutzfeldt et al. 1962; Bindman et al. 1964; Purpura and McMurtry 1965), lasting effects and related molecular changes (see also next; Gartside 1968), also reinforced the concept that the direction of somatic polarization determined the net effect on excitability/functional outcomes (Figure 3.3).

### 3.2.3 Quantifying Polarization with Coupling Constants

A specific and predictive understanding of tDCS requires quantitative model, beginning with quantification of somatic (and dendritic) polarization during tDCS. In the 1980s, Chan and colleagues (Chan and Nicholson 1986; Chan et al. 1988) used electrophysiological recordings from turtle cerebellum and analytical modeling to quantify polarization under quasi-static (low-frequency sinusoid electric fields)—these seminal studies identified morphological determinants of neuron sensitivity to applied DC fields. We extended this work to rat hippocampal CA1 neurons and then to cortical neurons with the approach of quantifying cell-specific polarization by weak DC fields using a single number—the "coupling constant" (also called the "coupling strength" or "polarization length.") We assumed that for weak electric fields (stimulation intensities too weak to significantly activate voltage gated membrane channels, and well below action potential threshold) that the resulting membrane polarization at any given compartment, including the soma, is linear with stimulation intensity. For uniform electric fields, the membrane potential polarization can be expressed as: $V_{tm} = G * E$ where $V_{tm}$ is the polarization of the compartment of interest (in: V), G is the coupling constant (in: V per V/m, or simply: m) and E is the electric field (in: V/m) along the primary dendritic axis. For rat hippocampus and cortical neurons the

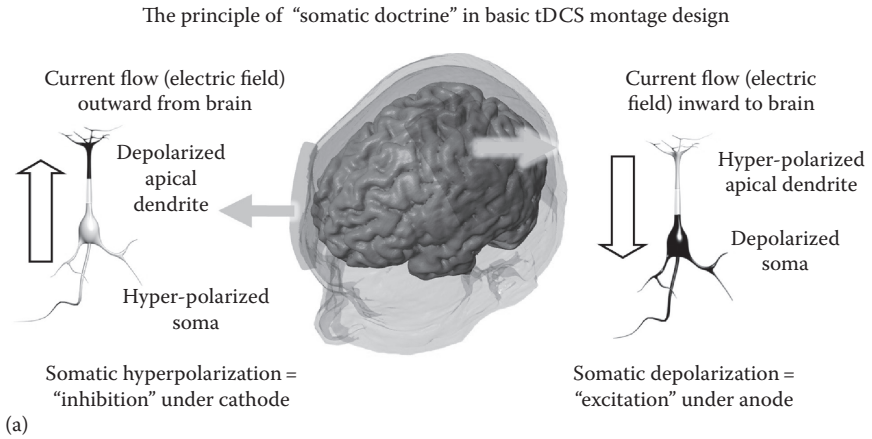


**FIGURE 3.3** The principle and quantification of the somatic doctrine. (a) The somatic doctrine simplifies tDCS design by assuming inward current flow under the anode, leading to somatic depolarization, and a generic increase in excitability and function. Under the cathode, an outward current leads to somatic hyperpolarization and a generic decrease in excitability and function. (b) Modern efforts to quantify somatic polarization in animal models have confirmed some aspects of the somatic doctrine, at least under specific controlled and tested conditions, but indicated that the polarization produced by tDCS would be small.

somatic coupling constant is in the range of 0.1–0.3 mV polarization per V/m electric field (Figure 3.2c; Bikson et al. 2004; Deans et al. 2007; Radman et al. 2009). For ferret cortical neurons the coupling is similarly ~0.25 mV per V/m (Frohlich and McCormick 2010). For humans, assuming scaling of sensitivity with total neuronal length (Joucla and Yvert 2009) somatic depolarization per V/m might be higher than in animals.

The maximal depolarization occurs when the electric field is parallel with the somatic-dendritic axis which corresponds to radial to the cortical surface, while electric field orthogonal to the somatic-dendritic axis do not produce significant somatic polarization (Chan et al. 1988; Bikson et al. 2004; but see axon terminal polarization next). The somatic coupling strength is roughly related to the size of the cell and the dendritic asymmetry around the soma (Svirskis et al. 1997; Radman et al. 2009) making pyramidal neurons relatively sensitive. For cortical pyramidal neurons, the typical polarity of somatic polarization is consistent with the "somatic doctrine" (e.g., positive somatic depolarization for positive electric field). The polarity of the coupling constant is inverted (using our field direction convention) for CA1 pyramidal neurons due to their inverted morphology. Using experimental and modeling techniques the coupling constant of dendritic compartments can also be investigated; generally the maximal polarization is expected at dendritic tufts (Bikson et al. 2004), but should not exceed, in animals, ~1 mV polarization per V/m electric field (Chan et al. 1988; Radman et al. 2007, 2009). AQ6

If tDCS produces a peak electric field of 0.3 V/m at 1 mA (with the majority of cortex at reduced values) then the maximal somatic polarization for the most sensitive cells is ~0.1 mV. Similarly, for 2 mA tDCS stimulation, the most sensitive cells in the brain region with the highest electric field would have somatic polarization of ~0.3 mV. Far from "closing the book" on tDCS mechanism, work by our group and others quantifying the sensitivity of neuron to weak DC fields, has raised questions about how such minimal polarization could result in functional/clinical changes especially considering that endogenous "background" synaptic noise can exceed these levels. In recent years, motivated by increased evidence that transcranial stimulation with weak currents has functional effects, as well as ongoing questions about the role of endogenous electric fields which can have comparable electric fields, the mechanisms of amplification have been explored in animal studies; we organize these efforts by non-linear single cell properties (discussed next) as well as synaptic processing and network processing (addressed in the next sections).

### 3.2.4 Amplification through Rate and Timing

At the single cell level the most obvious non-linear response that could provide a substrate for acute amplification is the action potential. As the electric fields induced by tDCS are far too weak to trigger action potentials (AP) in neurons at rest (i.e., ~15 mV depolarization from rest to AP thereshold, one can consider instead modulation of ongoing AP activity. At the single cell level we (1) consider acute implication through the rate of action potential generation (rate effects); and (2) develop the concept of amplification through change in the timing of action potential (timing effects). As already discussed classic animal studies on weak DC stimulation addressed the rate of change of spontaneous action potential discharge rate in many systems changes roughly linearly with membrane polarization. The amplification (gain) would relate to sensitivity of discharge rate to membrane polarization. Terzuolo and Bullock (1956) reported a detectable change in discharge rate down to 0.8 V/m, and this detection threshold would likely decrease with longer experiments. Assuming a 0.6 V/m peak electric field during 2 mA tDCS leading to ~0.2 mV somatic polarization, and that AQ7

across animal studies changes in firing rates of 7 Hz per mV membrane polarization are reported (Carandini and Ferster 2000), a change in firing rate of ~1.5 Hz is plausible. The aforementioned consideration is for isolated neurons, for neurons in an active network (see Section 3.4).

In 2007, we proposed that changes in action potential timing (rather than discharge rate) can act to amplify the effects of weak polarization (Radman et al. 2007). Specifically we showed in brain slice recording and in a simple neuron model that the resulting change in timing was simply the induced membrane polarization times the inverse of the ramp slope (Figure 3.4a and b); the inverse of the ramp slope is thus a "gain/amplification" term because the more shallow a ramp, the larger the timing change for given small polarization (Figure 3.4c and d). For example, assuming as aforementioned a ~0.2 mV somatic polarization during 2 mA tDCS, then in response to a 1 mV/ms electric field, timing would change by 0.3 ms. We also extended these findings to AC fields (Radman et al. 2007). Both the coupling sensitivity and the

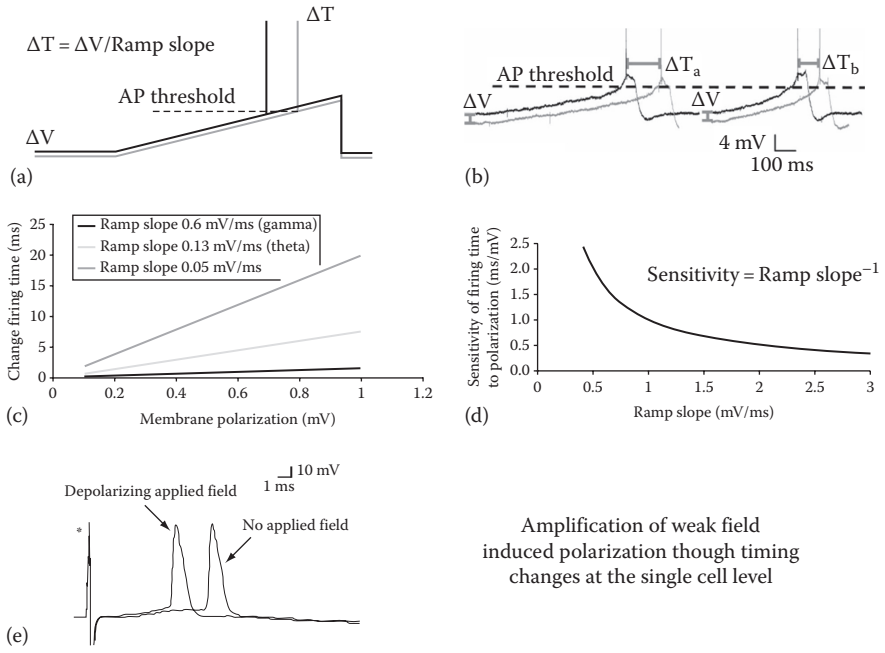


**FIGURE 3.4** Incremental membrane polarization produced by tDCS may significantly affect the timing of action potentials in response to a ramp (synaptic, oscillation) input. Moreover, the amplification of effect (change in timing per change in membrane polarization) increased for more gradual input. (a) Schematic illustrates the principle of timing amplification. (From Radman, T. et al., *J. Neurosci.,* 27, 3030, 2007b.) (b) The timing amplification was validated in hippocampal CA1 neurons using intracellular injected current ramp of various slopes. (c and d) The timing change increased with membrane polarization with a sensitivity (amplification) that is the inverse of the input ramp slope. The amplification would function during processing of incoming synaptic input including oscillations. (e) Demonstration of timing change in response to an incoming EPSP. nA incremental depolarization produced by direct current led to significant change in action potential timing in response to a synaptic input.

timing changes were confirmed by Anastassiou et al. (2010) using a more complex model. Though the principle of timing amplification generalizes to other cell types and to synaptic input (Figure 3.4e; Bikson et al. 2004), the simple amplification equation (Figure 3.4a) makes specific assumptions about membrane dynamics (Radman et al. 2007) that may not extend to all cell types (Radman et al. 2009).

Additional mechanisms of amplification at the single cell level remain an area of active investigation, especially when considering how exposure to long-duration fields (e.g., minutes as used in tDCS) may produce cumulative effects not observed during short term application (e.g., molecular changes; Gartside 1968; Fritsch et al. 2010; Ranieri et al. 2012). It remains an open and key question how prolonged (minutes) polarization of both the soma and dendrites can then trigger specific chemical and molecular cascades, thereby leading to the induction of plasticity (see next section).

### 3.2.5 Seizure Threshold and Modulation

The coupling constant also provides insight into the safety of tDCS in regards to triggering of seizures during stimulation. Whereas TMS produces 100 V/m pulsed electric fields that are suprathreshold, tDCS results in a static electric field <1 V/m at 2 mA producing <1 mV of polarization. Animal studies indicate that only application of DC fields >20 V/m (corresponding to >60 mA tDCS) would trigger action potential in the most sensitive quiescent cortical cells (Radman et al. 2009) while electric fields of ~100 V/m (corresponding to >500 mA tDCS) in the somatic depolarizing direction can trigger epileptiform activity in hippocampal slices (Bikson et al. 2004). This threshold would decrease for already active neurons. In brain slices, weak DC stimulation (on the order of 1 V/m) can modulate ongoing epileptiform activity (Gluckman et al. 1996; Ghai et al. 2000; Durand and Bikson 2001; Su et al. 2008; Sunderam et al. 2010), such that the cathodal tDCS may control ongoing seizures while anodal tDCS may aggravate seizure activity. In a polarity-specific fashion (consistent with somatic polarization) DC stimulation can also modulate the propagation of epileptiform activity in slices (Gluckman et al. 1996), spreading depression in vivo (Liebetanz et al. 2006), and perhaps clinical epileptiform activity (Varga et al. 2011). Though the acute (during stimulation) effects of weak DC currents on epileptiform activity are well established in animal models, it remains an open question if and how prolonged DC stimulation modulates seizure propensity. Animal studies suggest that prolonged cathodal DC stimulation can be anti-convulsant, while reports of the effect of anodal tDCS are mixed (Hayashi et al. 1988; Liebetanz et al. 2006), and pilot human studies suggest an anti-epileptic effect.

### 3.2.6 Limitations of the Somatic Doctrine

The most evident limitation of the somatic doctrine is precisely what cell compartments it ignores: the dendrites and axons. While the basal dendrite will be polarized similarly as the soma, the apical dendrite will be polarized in the opposite direction (Figure 3.3; Andreasen and Nedergaard 1996; Bikson et al. 2004). The dendrites are electrically excitable. Animal studies with high-intensity applied DC

fields (~100 V/m) have shown that with sufficiently strong stimulation, active processes (spikes) can be triggered in the dendrites (Chan et al. 1988; Wong and Stewart 1992; Andreasen and Nedergaard 1996; Delgado-Lezama et al. 1999). Even if the electric fields induced during tDCS are not sufficient in themselves to trigger dendritic spikes, the role of dendritic polarization during tDCS remains an open question especially when considering processing of synaptic input (next section).

It is well established that axons are sensitive to applied electric fields; the magnitude and direction of polarization is a function of neuronal and axonal morphology (Bullock and Hagiwara 1957; Takeuchi and Takeuchi 1962; Salvador et al. 2010). While the axon initial segment would likely be polarized in the same direction as the soma (Chan et al. 1988), for long axons this is not necessarily the case. Thus it is useful to separately consider the axon initial segments (within a membrane space constant of the soma) and more distal axonal processes, which can be further divided into "axons-of-passage" and afferent axons with terminations (discussed in the next section). Notably, for long straight axons-of-passage (e.g., Peripheral Nervous System, PNS) cathodal stimulation will be more effective than anodal stimulation in inducing depolarization (opposite to the somatic doctrine; Bishop and Erlanger 1926). It has been shown that lasting changes can be induced in PNS axons in humans (so by implication in CNS axons independent of somatic actions) and also, in brain slices, that weak DC fields can produce acute changes in CNS axon excitability (presynaptic/antidromic volley) (Jefferys 1981; Bikson et al. 2004; Kabakov et al. 2012). An important role for axon terminal polarization is introduced in the next section.

A presumption of the somatic doctrine is that under the anode currents are radial and inward through the cortex, while under the cathode current is radial and outward (Figure 3.3). However, high-resolution modeling suggests that in convoluted human cortex, current is neither unidirectional nor dominantly radial. Though the "somatic doctrine" is based only on radially directed electrical current flow (normal to the cortical surface), during tDCS significant tangential current flow is also generated (along the cortical surface). Indeed, recent work by our group suggests tangential currents may be more prevalent between and even under electrodes (Figure 3.1). As discussed next, tangential currents cannot be ignored in considering the effects of tDCS. Moreover, due to cortical folding the direction or radial current flow under tDCS electrodes is not consistent, meaning there are cluster of both inward (depolarizing) and outward (hyperpolarizing) cortical current flow under either the anode or the cathode! Due to the cortical convolutions, current is not unidirectional under electrodes thus under the cathode there may be isolated regions of inward cortical flow, and in those regions neuronal excitability may increase (Creutzfeldt et al. 1962). The relative uniformity of direction across a given patch of cortex depends on the electrode montage, with electrode across the head producing the most consistent polarization under each electrode (Turkeltaub et al. 2011) and closer electrodes, such as the classic M1-SO (anode on motor strip, cathode on contralateral supraorbital area) montage, producing bidirectional current flow with a slight directionality preference *on average* in some regions under the electrodes (Figure 3.1). This seems puzzling in light of the dependence on the somatic doctrine in tDCS montage design and study interpretation. The role of tangential and bidirectional current flow is addressed in the next two sections.

## 3.3 PLASTICITY, SYNAPTIC PROCESSING, AND A "TERMINAL DOCTRINE"

The clinical need for lasting changes by tDCS relates to the impracticality of constant stimulation with non-invasive technology (i.e., wearing a stimulation cap all the time). The desire for lasting change means tDCS should influence plasticity during or after stimulation in cognitive/therapeutic relevant way (Yoon et al. 2012). This section addresses the contribution of animal studies to understanding plasticity generated by weak DC electric fields. The contribution of early translational studies to tDCS protocols is notable as Bindman and colleagues (Bindman et al. 1962) recognized the importance or prolonged DC stimulation to produce lasting effects (>5 min), which informed their early work in tDCS of psychiatric disorders (Costain et al. 1964; Redfearn et al. 1964) and the multi-minute stimulation required in the Nitsche and Paulus (2000) report, that in turn established the use of prolonged stimulation across modern tDCS studies. The need for prolonged (minutes) stimulation to induce plasticity (mechanism) and protocols for optimizing long-lasting changes (clinical utility) remains a central question in tDCS research (Monte-Silva et al. 2010). Marquez-Ruiz recently summarized (Marquez-Ruiz et al. 2012)

> When tDCS is of sufficient length, synaptically driven after-effects are induced. The mechanisms underlying these after-effects are largely unknown, and there is a compelling need for animal models to test the immediate effects and after-effects induced by tDCS in different cortical areas and evaluate the implications in complex cerebral processes.

Animal studies in the 1960s also helped established that weak DC current produces plastic changes (a lasting physical change in the brain rather than a "reverberating circuit" of activation) (Gartside 1968). As noted earlier, early animal studies also contributed to establishing the "somatic doctrine" in tDCS but modern clinical studies on tDCS have suggested that changes in excitability are not necessarily polarity specific (Marquez-Ruiz et al. 2012), or monotonic with intensity such that cathodal or anodal stimulation can produce variable effects depending on intensity, duration, or underlying activity. In the past decade, animal and computational studies are beginning to address these issues. Both in humans and animal studies changes in evoked (synaptically mediated) neurophysiological responses are considered reliable hallmarks of plastic changes that could support behavioral or clinical lasting changes (and are thus a focus of this section), though one recent report in rabbits indicated this was not simply the case (Marquez-Ruiz et al. 2012).

### 3.3.1 Paradigms for DC Modulation of Synaptic Efficacy

In the previous section it was discussed that as tDCS electric fields are sub-threshold (too weak to trigger action potential in quiescent neurons); their acute role is thus truly neuromodulation through either rate or timing effects. Weak DC stimulation

may generate plasticity through different paradigms, which are not necessarily exclusive:

1. Membrane polarization may trigger plastic changes in a manner independent of any ongoing synaptic input or action potential generation (i.e., simply holding the membrane at an offset polarization initiates changes). Though in a cortical brain slice model (with no background activity), weak polarization was not sufficient to induce plastic changes (Fritsch et al. 2010).
2. Changes in action potential discharge rate or timing, secondary to neuronal polarization, where the firing change (which is dependent on many factors including the direct polarization) actually determines plasticity. "There is some evidence that a determining factor in producing long-lasting after-effects is the change in the firing rate of neurones rather than the … current flow that produces the changes" (Bindman et al. 1964). Though classic animal studies indicated weak DC stimulation is sufficient to induce plastic changes (Gartside 1968) is it important to note that polarization would affect a network of neurons such that increased firing in afferents would in fact increase synaptic input (see next point).
3. Polarization of the membrane in combination with ongoing synaptic input. Though it is established that weak DC stimulation can lead to acute and lasting changes in synaptic efficacy (see next), the specific hypothesis here is that the generation of plasticity requires synaptic co-activation during DC stimulation. Evidence from brain slices (Fritsch et al. 2010) shows potentiation under anodal stimulation only during specificity matched patterns (frequencies) of synaptic input. In a rabbit study, DC was combined with repeated somatosensory stimulation, leading to acute polarity-specific changes, and lasting changes for the cathodal case (Marquez-Ruiz et al. 2012). If dependent on combined polarization and synaptic input, then synapse specific changes are plausible. If one assumes DC exerts a post-synaptic priming effect (polarization of soma/dendrite) than co-activation of afferent synaptic input could be conceived as Hebbian reinforcement (except post-synaptic action potentials may or may not be required). Clinically this plasticity paradigm is broadly analogous to combining tDCS with a cognitive task or specific behavior that co-activates a targeted network or combining tDCS with TMS. Indeed, work showing the importance of co-activation in cortical slice (Rioult-Pedotti et al. 1998; Hess and Donoghue 1999), influenced Nitsche and Paulus (2000) in developing tDCS. However, though TMS is used to probe the effects of tDCS, it turned out not to be necessary to apply it during tDCS. We would note that, unlike in brain slice and anesthetized animal models, the human cortex is constantly active such that tDCS is always applied on conjunction with ongoing synaptic input.
4. Polarization of the membrane in combination with ongoing activity that itself is independently leading to potentiation (i.e., modulation of ongoing plasticity). For example, in the aforementioned rabbit study, DC stimulation modulated ongoing synaptic habituation, a model of associative learning (Marquez-Ruiz et al. 2012). Clinically this fourth paradigm is analogous to combing tDCS with learning/training (Bolognini et al. 2010). Evidence from brain slices (Ranieri

et al. 2012) shows DC modulation of LTP induced by tetanic stimulation, in a polarity-specific manner apparently opposite to the somatic doctrine (when one considers that CA1 neuron morphology results in "anodal" stimulation producing somatic hyperpolarization). Using intracellular current injection, Artola et al. (1990) showed that depending on the level of polarization of the post-synaptic neuron, the same tetanic stimulation can induce LTD or LTP.

A separate classification includes lasting changes that are: (1) "synaptic": occur at synaptic processes (e.g., increased vesicle release, receptor density) and are blocked by synaptic antagonists; versus (2) "non-synaptic": occur independent of synaptic processes (e.g., membrane polarization). Though the synapse is typically considered the locus of plastic changes, so called "non-synaptic" changes have been noted after DC stimulation in peripheral axons away from any synapse; importantly, in this case, cathodal stimulation inducing potentiation (Ardolino et al. 2005) which is consistent with cathodal stimulation induced preferential depolarization in long axons. Clinically, the question of "synaptic" versus "non-synaptic" origin of tDCS modulation in the CNS has been explored and debated by distinguishing modulation of TMS versus TES evoked potentials—both of which, in our opinion, have variable origin (depending on methods). Moreover, using either TES or TMS, the presence of and role of background synaptic activity in priming excitability during tDCS, regardless of "synaptic" or "non-synaptic" locus, muddles any effort to disambiguate the mechanism along these lines. However, in brain slice models, where background synaptic activity is absent, synaptic (orthodromic) and non-synaptic (axon, antidromic) can be precisely isolated—see discussion on axon effects given earlier. It is important to note that as far as outcome, in the CNS changes of "non-synaptic" origin would be expected to affect synaptic processing (Mozzachiodi and Byrne 2010).

When considering the complexity of (multiple forms of) tDCS plasticity, the need for animal models is evident. Animal models allow for synaptic efficacy to be quantitatively probed with pathway specify—the importance of which we discuss next. The mechanisms of plasticity can be analyzed using specific pharmacology not practical in people (for toxicity), not to mention the ability to resect tissue for detailed cellular and molecular analysis (Islam et al. 1995; Yoon et al. 2012). Though limited to timescales of hours, the use of brain slice further facilitates imaging, precise drug concentration control, control of the background level and nature of ongoing activity (from quiescent, to transient activation at specific frequencies, to oscillations, to epileptiform) and, especially relevant for tDCS, the control of electric field orientation relative to slice (Figure 3.5). It may not be prudent to revert to a one-dimensional "sliding scale of excitability" explanation where anodal/cathodal tDCS increases/decreases "function" leading to lasting increases/deceases in generic synaptic plasticity, which are then related to cognitive/behavior changes—This approach seems simplistic and unlikely to ultimately advance tDCS sophistication and efficacy.

### 3.3.2 Relation with Tetanic Stimulation Induced LTP/LTD

Animal studies using tetanic stimulation to induce long-term potentiation/depression (LTP/LTD) have suggested multiple forms of plasticity, involving distinct pre- and

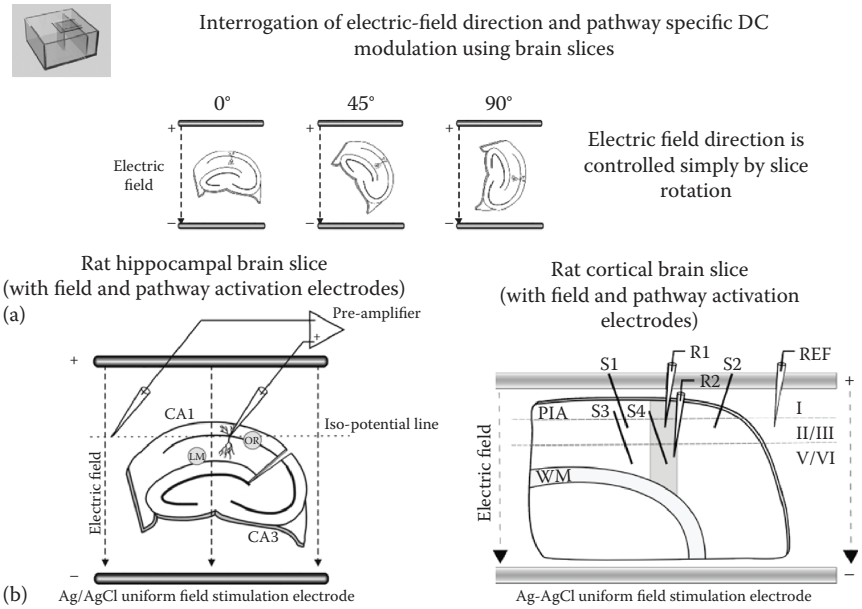


**FIGURE 3.5** Further advantages of the brain slice preparation in studying mechanisms of weak DC stimulation. (a) A discussion in the text, the direction of the applied electric field relative to the somato-dendritic axis can be precisely controlled. (Adapted from Bikson, M. et al., *J. Physiol.,* 557(Pt 1), 175, 2004.) The effects of DC current on brain function may vary with orientation. (b) Synaptic function/efficacy is not "one thing," rather there are multiple distinct synaptic afferent to any brain region which can be evaluated in isolation in brain slices. The effects of DC current on synaptic function may be highly pathway specific.

post-synaptic mechanisms, on distinct time scales. When comparing the thousands of studies on tetanic LTP compared with the <50 animal studies on DC induced plasticity, one may speculate that, despite recent progress, there is much to investigate about (multiple potential forms) of weak DC stimulation induced plasticity (Bindman and colleague's conclusion in 1964 that "at the moment we are not in a position to discuss the way in which polarizing currents acts on neurons to alter long-term excitability" comes to mind [Bindman et al. 1964]). LTP/LTD induced by tetanic stimulation and by DC current may, not surprisingly, share some common molecular substrates (Gartside 1968; Islam et al. 1995; Ranieri et al. 2012). It is remarkable that a decade before the lauded discovery of Long Term Potentiation by trains of suprathreshold pulses by Bliss and Lomo (1973), animal studies had shown lasting changes in excitability following DC stimulation lasting up to hours (Bindman et al. 1962) and moreover had begun established plastic changes and started to address the underlying molecular mechanisms (Gartside 1968) and translating results to humans! It was recently suggested that tDCS shares some of the classical molecular mechanisms associated with tetanic stimulation LTP/LTD (Marquez-Ruiz et al. 2012) including adenosine-elicited accumulation of cAMP (Hattori et al. 1990) inducing increased protein kinase C and calcium levels (Islam et al. 1994, 1995). The wealth of techniques and tools developed by the "cottage industry" of tetanic stimulation LTP have

yet to be fully leveraged to dissect the mechanisms of tDCS—but this seems a matter of time. In the context of translational importance, it is also interesting that protocols using tetanic stimulation in animals have influenced the design of TMS protocols (LTP/LTD, theta-burst, etc.).

### 3.3.3 Which Compartments are Influenced by DC Stimulation: Soma and Dendrites

As discussed earlier, we assume that the actions of DC stimulation initiate with membrane polarization, with all other (complex) changes secondary to this polarization. We noted that DC polarization will influence all neurons in areas of the brain with current flow, with equal portions membrane depolarization and hyperpolarization. Our research is thus focused on which membrane compartments (soma, dendrites, axons process, axon terminals) when polarized by weak DC stimulation are relevant from both the perspective of locus of change and mechanism. Can the somatic doctrine be used to predict plasticity changes? Or is plasticity related to polarization of specific dendritic or axonal compartments?

If one considers the lasting effects of tDCS to be generally analogous to long-term potentiation then tDCS effects how information is processed by neurons though altered synaptic efficacy. Though the soma is important for integration of synaptic input, the dendrites evidently play a central role in synaptic processing and in the induction of plasticity. Several animal and clinical studies have implicated processes linked to the dendrites in tDCS (e.g., glutamatergic receptors like n-methyl-D-aspartic receptor, NMDAR) (Liebetanz et al. 2002; Nitsche et al. 2003; Ranieri et al. 2012; Yoon et al. 2012). A key question is thus: as half the dendrite will be polarized in the same direction at the soma and half of the dendrite will be polarized in the opposite direction (Figure 3.3), how do polarity-specific changes arise? Are changes in synaptic processing/plasticity always consistent with the somatic doctrine? As summarized next, the answer seems to be: "it depends."

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

In Bikson et al. (2004) we used the hippocampal slice preparation, which was initially conceived as a series of straightforward experiments to confirm the validity of the somatic doctrine in predicting acute changes in excitability—to our surprise we found several deviations. Optical imaging with voltage sensitive dyes provided direct

evidence that DC electric fields always produces bimodal polarization across target neurons such that somatic depolarization is associated with apical dendrite hyperpolarization, and vice versa—yet over longer timescales interactions across compartments were observed. In addition, for synaptic inputs to the soma and basal dendrite, we reported modulation consistent with the somatic doctrine (considering the inversion relative). But for strong synaptic input on to the apical dendritic tuft both DC field polarities enhanced synaptic efficacy—such that dendrite depolarization with somatic hyperpolarization also enhanced synaptic efficacy. Also in hippocampal slices, both Kabakov et al. (2012) and Ranieri et al. (2012) reported modulation of synaptic efficacy in a direction opposite to that expected from the somatic doctrine (again noting inversion of dendrite morphology in CA1 pyramids relative to cortex). In these cases, one may speculate the apical dendrite depolarization (despite somatic hyperpolarization) determines the direction of modulation (Bikson 2004, p. 74); though Kabakov et al. (2012) provides evidence suggesting dendritic polarization effects the magnitude but not direction of modulation. As noted, in cortical slices by Fritsch et al. (2010), modulation of evoked responses is indeed consistent with the somatic doctrine—a finding we have confirmed for four distinct afferent cortical synaptic pathways. These variations across animal studies could be simply ascribed to different in region/preparation, timescale (acute, long-term), and different forms of plasticity (BDNF dependent/independent), but this is speculative and provides little insight into tDCS. Rather, in attempt to reconcile these findings in a single framework, we site evidence for and define the "terminal doctrine" to compliment the "somatic doctrine."

### 3.3.4 Which Compartments Are Influenced by DC Stimulation: Synaptic Terminals

In the 2004 study (Bikson et al. 2004) we also investigated the effects of tangential fields on synaptic efficacy—tangential fields are oriented perpendicular to the primary somato-dendritic axis, so are expected to produce little polarization (which we directly confirmed with intracellular recording). Electric fields applied tangentially were as effective at modulating synaptic efficacy as radially directed fields. The afferent axons run tangentially, so we speculated that they might be the targets of stimulation. Exploring different pathways we found that axon pathways with terminal pointed toward the anode were potentiated, while axon pathways with terminals pointed toward the cathode were inhibited. Kabakov et al. (2012) reported similar pathway specific dependence summarizing "the fEPSP is maximally suppressed when the AP travels toward the cathode, and either facilitated or remains unchanged when the excitatory signal [AP] propagates toward the anode." In addition, Kabakov et al. (2012) observed changes in paired-pulse facilitation potentially consistent with pre-synaptic vesicular glutamate release. We recently confirmed a similar directional sensitivity in cortical slices across four distinct pathways (Figure 3.5) where electric field applied tangentially to the surface (and so producing minimal somatic polarization) (Radman et al. 2009), modulated synaptic efficacy. Interestingly, an in vivo study suggested axonal

regrowth (as well as dendritic growth) in tDCS mediated neuroplasticity after cerebral ischemia (Yoon et al. 2012).

A role for pre-synaptic modulation during DC stimulation is indeed not surprising and historically noted. Purpura and McMurtry (1965) observed

> although the [somatic] membrane changes produced by transcortical polarization current satisfactorily explains alterations in spontaneous discharges and evoked synaptic activities in [pyramidal tract] cell, it must be emphasized that the effects of polarizing current on other elements constituting the 'pre-synaptic', interneuronal pathway to [pyramidal tract] cells also appear to be determinants of the overt changes observed in [pyramidal tract] cells activities.

Bishop and O'Leary (1950) not only quantified pre-synaptic effects during DC stimulation in animals, they noted that pre-synaptic effects would complicate the interpretation of post-synaptic changes as well as themselves induce long-lasting after-effects.

It is well established that cellular process terminals including axon terminals are especially sensitive to electric fields as a result of their morphology (DelCastillo and Katz 1954; Bullock and Hagiwara 1957; Hubbard and Willis 1962; Takeuchi and Takeuchi 1962; Awatramani et al. 2005) and that terminal polarization can modulate synaptic efficacy (independent of target soma polarization) (DelCastillo and Katz 1954; Bullock and Hagiwara 1957; Hubbard and Willis 1962; Takeuchi and Takeuchi 1962; Awatramani et al. 2005). Moreover, this modulation is cumulative in time and endures after stimulation if stopped (Hubbard and Willis 1962); a temporal profile noted in classic DC experiments (Bindman et al. 1964) and suggesting the possibility for plasticity. The direction of modulation in brain slice studies consistently suggests that terminal hyperpolarization enhanced efficacy, while depolarization inhibited efficacy. Paired-pulse analysis in a rabbit model suggested tDCS influences pre-synaptic sites (Marquez-Ruiz et al. 2012). Our proposed "terminal doctrine" postulates: afferent synaptic processes oriented toward the cathode (or more specifically parallel with the direction of electric field) will be potentiated (due to synaptic terminal hyperpolarization), while processes oriented toward the anode (or specifically antiparallel with the electric field) will be inhibited. As tDCS induced significant tangential fields (Figure 3.1), the role of terminal polarization (independent of the "somatic doctrine") remains a compelling and open question especially when taken together with the need for amplification and the role of synapses in plasticity.

This proposal of a "somatic doctrine" versus "terminal doctrine" can be conceptualized as generically analogous to the pre-/post-synaptic debate in tetanic stimulation induced LTD/LTP (Artola et al. 1990); and as with tetanic stimulation induced LTD/LTP, both mechanisms are likely to play a role. It is important to note that current crossing the grey matter is rarely purely radial or tangential, such that simultaneous somatic and terminal polarization is broadly expected. Even in the brain slice afferent axons and the target neurons are not perfectly orthogonal, which may explain some of the divergent findings in hippocampal brain slices noted earlier. During tDCS because of the complexity of current flow across the gray matter (Figure 3.1) the situation is still more complex, especially considering that whether

the terminal doctrine predicts excitation or inhibition depends on the direction of incoming axons. Perhaps careful considering on brain current flow patterns combing with extending thinking beyond the simple somatic doctrine to include both the role of (oppositely polarized) dendrite, axons, and axons terminals can reconcile divergent clinical findings showing inversion of classical direction effects (Nitsche and Paulus 2000; Ardolino et al. 2005) or direction-neutral effects (Nitsche et al. 2003). We emphasize that given the complexity of plasticity paradigms and stimulation targets, leading to potentially multiple forms of tDCS plasticity, translational animal studies are critical, alongside clinical neurophysiology, to understand tDCS and ultimately inform rational electrotherapy. Moreover, meaningful clinical outcome rely on specific and increasingly long-lasting changes; the basis of which can be studied in animals.

## 3.4 NETWORK EFFECTS

The consideration of how weak DC electric fields interact with active networks (e.g., oscillations) is a very compelling area of ongoing research because, just as network of coupled active neurons exhibit "emergent" network activity not apparent in isolated neurons, so does application of electrical stimulation to active networks often produces responses not expected by single neurons. These responses are specific to the architecture and activity of the network. Networks also provide a substrate for amplification beyond the cell/synapse level. Reports that DC current can alter "spontaneous rhythm" in animals span decades (Dubner and Gerard 1939; Antal et al. 2004; Marshall et al. 2011), while recent clinical work on tDCS has addressed modulation of EEG oscillations. New animal studies on DC stimulation, which addressed mechanism of this coupling, are reviewed in the section—with a focus on acute effects as the role of ongoing activity in plasticity is discussed earlier.

### 3.4.1 Further Amplification at through Active Networks

In principle, the initial action of DC stimulation remains to polarize all neurons sensing the electric field. As discussed earlier, pyramidal somas are more sensitive by virtue of their morphology, but axonal and dendritic polarization should not be ignored. Note that tDCS generates electric field across large areas of cortex. In networks, a key concept is that the entire population of coupled neurons is polarized—this *coherent* polarization of the population provides a substrate for signal detection and for amplification. Interestingly, the effective coupling constant for a neuron immersed in an active network may be enhanced compared to that neuron in isolated (Reato et al. 2010)—meaning that by virtue of being in a network a given compartment (soma) may be polarized directly by the field and indirectly by field actions on a collective of afferent neurons.

As noted earlier, the concept that threshold for electric field sensitivity would be "lower for modulation of the frequency of an already active neuron than for excitation of a silent one" (Terzuolo and Bullock 1956) is well established, but network activity add another dimension to this. During many network activities,

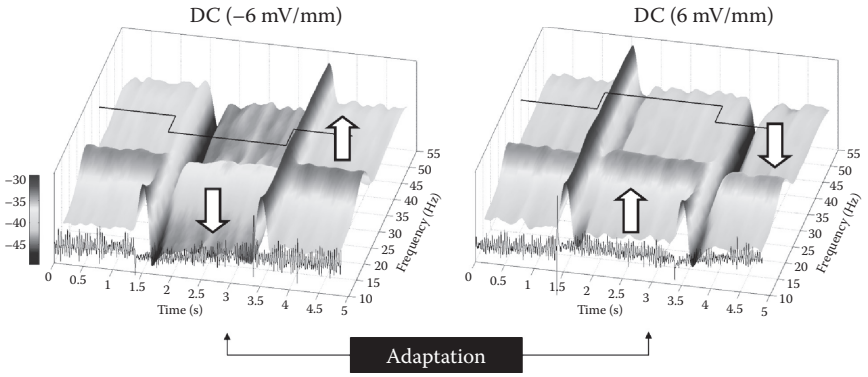


**FIGURE 3.6** Modulation of gamma oscillations in brain slice by weak DC fields. Gamma oscillations were induced in the CA3 region by perfusion with carbachol. Negative fields which produce hyperpolarization of CA1 pyramidal neuron soma, attenuated oscillation, but interestingly the attenuation was most pronounced when the fields where turned on, after which oscillation activity partly rebounded even though the field was still on. This suggests homeostatic "adaptation" (arrows) to the DC field by neuronal network system. After the field is turn off, there is an excitatory rebound response consistent with this adaption. An opposite effects is observed for positive fields that would be depolarizing the soma of CA3 pyramidal neurons. This adaption at the network level is not expected from single neurons, so reflects an emergent response of an active network to DC fields.

notably oscillations, neurons are constantly near threshold (e.g., primed for firing). If a neuron is near threshold by virtue of network drive, then a small polarization may be influential in modulating the likelihood of firing. For example, a relatively small depolarization may be sufficient to trigger an action potential. Moreover, because the network is interconnected, activated neurons could synaptically trigger action potentials in other neurons. The whole process can be feedforward such that a small DC electric field can induce a robust action potential discharge in a population. This has been shown in the brain slice (Reato et al. 2010). This concept is interesting because it clouds the entire distinction between "suprathreshold" stimulation, such as TMS, and "sub-threshold" stimulation, as tDCS is commonly considered. It remains the cases that the electric fields produced by tDCS are insufficient to trigger action potential in quiescent neurons (Figure 3.6).

### 3.4.2 Oscillations

A majority of work on weak DC electric fields and network activity in slice addressed epileptiform activity (in investigation of methods for seizure control). These reports generally observed a change in the rate of epileptiform discharge generation (the likelihood an event would initiate) rather than a change in event waveform once initiated. This finding is consistent with the concept that weak field polarize neurons (Bikson et al. 1999, 2004) and that weak stimulation is more likely to influence stochastic initial recruitment of neurons in the robust regenerative epileptiform event. DC electric

fields may also influence the propagation rate (Francis et al. 2003; Varga et al. 2011). Reato et al. (2010) considered the effects of DC fields on gamma oscillations in brain slice and noted both transient effects when the field was turned on, and secondary sustained effects which are more relevant to tDCS (Figure 3.6). Sustained effects were characterized by a dramatic compensatory ("homeostatic") regulation by the network, such that the system tried to normalize activity to baseline levels despite the presence of the DC-field. This network adaptation was apparent when the DC field was turned off as the network was delayed in re-adjusting to the absence of the field—in this way, excitatory (somatic depolarizing) fields produced post-stimulation inhibition of oscillations, and vice versa. Network level mechanisms (as opposed to single neuron behavior) may thus provide a substrate for activity dependent homeostatic-like observations during tDCS (Cosentino et al. 2012).

Weak stimulation of "physiological" activity work with AC or pulsed stimulation is more common (Deans et al. 2007; Frohlich and McCormick 2010). Though Reato et al. (2010) proposed the effects of AC stimulation at different frequencies and DC could be explained in a single continuous framework, it is important to distinguish between studies exploring the limits of network sensitivity to weak AC or pulse fields, and prolonged DC current (tDCS). When a network is generating spontaneous oscillations of epileptiform activity (regenerative events), then it is well established that an electrical pulse can trigger a regenerative network event; moreover, repetitive weak pulses or AC stimulation can entrain activity by aligning the phase of these events with that of the repetitive stimulation. By definition, (except for at the start) during prolonged DC stimulation there basis for entrainment (there is no phase to the DC) such that tDCS can affect average discharge rate or waveform, but not phase. Thus, though entrainment is central in AC/pulsed stimulation studies in animals (as well as clinically) (Marshall et al. 2006), its relevance to tDCS is limited.

## 3.5 INTERNEURONS AND NON-NEURONAL EFFECTS

The role of interneurons and non-neuronal cells, such as glia and endothelial cells, in tDCS remains both a wide open and critical question. We distinguish between: (1) direct stimulation effects, reflecting direction polarization and modulation of these cell types by DC fields; (2) indirect stimulation effects, reflect change in function secondary to direct excitatory neuronal activation that then influences these other cell types; and (3) modulatory effects, where the sensitivity of neurons to direct effects (e.g., their excitability) is influenced by other cell types. In fact, the function of interneurons and non-neuronal cell types are so intricately wound together with excitatory neurons that the second and third aspects are presumed (though complex), and we here focus mostly on the first possibility of direct effects.

### 3.5.1 Interneurons

Because of their relatively symmetric dendritic morphology, interneuron somas are expected to polarize less than pyramidal neurons (Radman et al. 2009). Based on the "somatic doctrine" their importance might then be considered diminished.

However, we cannot exclude polarizing effects of fields on dendrites and axons. Moreover, interneurons represent a wide range of morphologies and size, including asymmetric morphologies (Freund and Buzsaki 1996). Interneurons exert a powerful regional effect, including playing role in plasticity and oscillations. An effect of paired-pulse facilitation in hippocampal slice may also suggest modulation of the activity of interneurons (Kabakov et al. 2012). The role of interneurons in the direct effect of tDCS remains then an open question.

### 3.5.2 Glia

Glia cells represent the majority of cell in the CNS—the concept that they are just "passive" support cells is outdated (Haydon and Carmignoto 2006) and their complex role in neuronal functions such as plasticity are being elucidated (DiCastro et al. 2011; Panatier et al. 2011). Some glia have distributed processes which would influence their sensitivity to applied electric fields (Ruohonen and Karhu 2012), but even more interesting is the notion that the glial syncytium (an electrically coupled population of glial cells), might act to amplify field polarization. One possible mechanism for DC modulation through glia cells relates to the concept of potassium "spatial buffering." Glia cells are thought to regulate extracellular potassium concentration through a polarization imbalance across their membrane, which is precisely the type of polarization induced by DC fields. How to explore possible effects of electric fields on this mechanism remains unclear. Gardner-Medwin induced extracellular potassium transport by passing DC current and noted concentration changes in saline near the electrodes, which is mechanistically distinct than tissue changes(Gardner-Medwin 1983). Studies in brain slice show no changes in extracellular potassium concentration with DC fields (Lian et al. 2003), though the brain slice preparation has distorted extracellular concentration control mechanisms (An et al. 2008). Neurons and glia can be cultured separately, but morphology and biophysics are altered in culture. In general, there are no "magic bullet" drugs for the glia function, and regardless any changes in glia function would influence neurons and so direct responses to DC fields may be difficult to determine. Still, given the growing interest in the role of glia cells in CNS function and the increased sophistication of experimental techniques, their role in tDCS is a worthwhile area of investigation (Ruohonen and Karhu 2012).

### 3.5.3 Endothelial Cells

Endothelial cells help form the blood-brain barrier that tightly regulates transport between the brain extracellular space and blood. Any direct action of DC stimulation on endothelial cells could have profound effects on brain function. Endothelial cells do not have processes and their spherical shape indicates peak polarization will be related to cell diameter (Kotnik and Miklavcic 2000)—during tDCS membrane polarization is expected to be well below the threshold for electroporation. The direct effects of tDCS current on vascular response are an open and compelling question. There are abundant evidences that DC current affects vascular function in skin (Ledger 1992; Prausnitz 1996; Berliner 1997; Malty and Petrofsky 2007) and indeed skin redness is typical under tDCS electrodes (Minhas et al. 2010). Vascular and neuronal functions

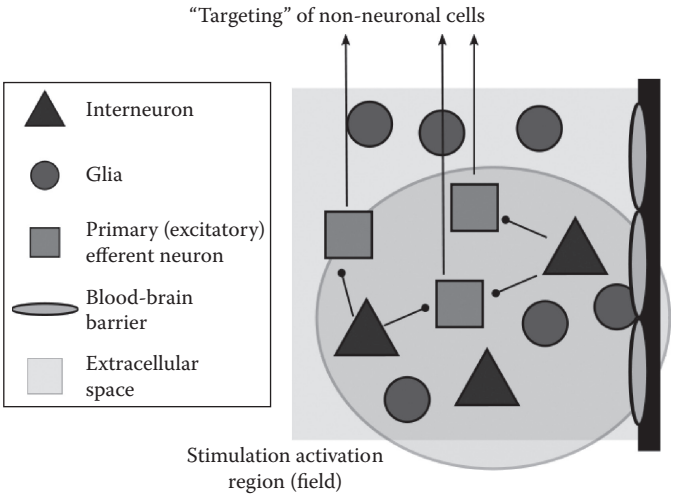


**FIGURE 3.7** Could tDCS directly modulate the functional of interneurons or non-neuronal cells? The direct response of interneurons, glia, and endothelial cells (that form the blood-brain barrier) remains an open question—that is difficult to address even in animal models. It is expected that these cell types, by influencing excitatory neuronal cells can indirectly modulate the effects of tDCS on excitatory neurons, and also that through direct actions on excitatory neurons, these cells types will be indirectly affected. A direct effect on interneurons and non-neuronal cells is possible but at this time speculative. (Adapted from Bikson, M. et al., *Conf. Proc. IEEE Eng. Med. Biol. Soc*., 1, 1616, 2006.)

in the brain are closely interrelated, as evidenced by functional Magnetic Resonance Imaging (fMRI). The relation is also complex, and it can be difficult to disentangle direct neuronal and potential direct vascular effects (a chicken-and-egg problem), including during tDCS. Wachter et al. (2011) found a polarity-specific change in blood perfusion during tDCS in rat, in a direction consistent with the somatic doctrine, and speculated the direction specificity was consistent with a primary neuronal action. The brain slice is compelling since the blood supply is not present such that findings in slice including acute (Bikson et al. 2004) and lasting synaptic efficacy (Fritsch et al. 2010) changes can exclude an endothelial contribution. Conversely, endothelial culture including models of the blood-brain-barrier can be electrically stimulated. We showed that high-intensity electrical stimulation could increase transport across such a model through a phenomena we called "electro-permeation" between cells, to distinguish it from electroporation of single cells (Lopez-Quintero et al. 2010). Investigation of DC stimulation in this model is ongoing (Figure 3.7).

## 3.6 SUMMARY AND A 3-TIER APPROACH

Clinical tDCS protocols continue to be largely designed and interpreted following the "somatic doctrine," namely that anode/cathode stimulation results in a generalized increase/decrease in neuronal excitability due to radial current flow and somatic polarization. Animal studies showed that current flow radial (normal) to the

cortical surface can modulate spontaneous neuronal activity in a polarity-specific manner, with inward current (corresponding to somatic depolarization) increasing firing rate, and outward current (corresponding to somatic hyperpolarization) reducing firing rate (Creutzfeldt et al. 1962; Bindman et al. 1964; Purpura and McMurtry 1965; Gartside 1968); because of the dependence on the neuronal target, we refer to this as the "somatic doctrine." Indeed, modern tDCS was motivated by neurophysiologic studies showing that anodal/cathodal tDCS increase/decrease, respectively, responses to TMS evoked cortical and muscle potentials, which is consistent with the aforementioned "somatic doctrine" (Nitsche and Paulus 2000). Extensive clinical and cognitive-neuroscience studies have been largely rationalized based on the somatic doctrine. However, despite positive outcomes from many of these studies, emerging evidence suggests that neuromodulation by tDCS may be more complex; stemming largely from the recognition that brain function is evidently not a monolithic "sliding scale of excitability."

Modern animal research is beginning to explicate how modulation by tDCS cannot be explained as a monolithic "sliding-scale" of excitability (anode = up, cathode = down). Brain function/disease and so its influence by DC stimulation is complex. Neither polarization of dendrites of synaptic terminals can be ignored, which may result in differential modulation of specific synaptic inputs. This in turn, may lead to distinct forms of tDCS-induced plasticity. Moreover, which neuronal processes are affected and how, will depend on the tDCS montage used and the state of the underlying network. The rational advancement of tDCS requires departing from the sliding-scale approach (applied indiscriminately across cognitive applications and indications) and addressing these mechanistic and targeting issues. With increased recognition of complexity, the need for translational animal studies, that are properly designed, becomes increasingly clear. At the same times, these issues make the investigation daunting. Our approach, reflected generally in the organization of this review has been to consider changes on "3-tiers": neuronal compartment polarization, synaptic processing, and network effects. While the brain function is evidently understood to span these integrated tiers, this chapter introduces how a 3-tier approach can allow organization of concepts and framing of hypothesis.

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