Animal Studies in the Field of Transcranial Electric Stimulation

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Abstract

Dozens of animal studies of transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) have provided insight into the cellular mechanism of stimulation. Biomarkers of tDCS/tACS responses at the neurophysiological, behavioral, and molecular levels provide a basis to design clinical interventions that engage specific targets. This chapter provides a broad introduction to methods and insights from animal models. Both tDCS and tACS are sub-threshold techniques, producing membrane polarization rather than firing. If the nervous system is engaged during tDCS/tACS, for example by cognitive behavioral therapy, then tDCS/tACS modulate this ongoing activity. Animal models have supported the basis for polarity-specific effects of tDCS ("anodal" excitation, "cathodal" inhibition) while also indicating limitations of simplistic dose strategies. tACS studies have focused on boosting of oscillations. Both techniques can modulate ongoing plasticity leading to lasting changes in brain function. As an adjunct therapy, tDCS/tACS may thus increase brain capacity for plasticity enhancing the effects of neuropsychiatric therapies, and compensating for disease-related decline.

Keywords

Translation • Preclinical • Rodent • Safety • Neuromodulation

Experimental Design of tDCS and tACS Animal Studies

There is a general perception that the rate of clinical trials on tDCS and tACS for a range of indications, including neuropsychiatric disorders, has outpaced research on the basic mechanisms of tDCS. Over the last few decades, the mechanisms by which tDCS and tACS work have been

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extensively tested in animal models and backed their application for treatment of neuropsychiatric disorders. Efforts to increase the effectiveness of tDCS/tACS interventions (e.g., changing stimulation dose) should be guided by ongoing and modern animal research.

The overall motivation for animal research of tDCS/tACS is similar to other translational medical research efforts: to allow rapid and safer application of stimulation protocols in research and clinical settings. Improving clinical efficacy and safety would require a thorough understanding of the underlying mechanisms being altered. To have meaningful relevance to clinical tDCS/tACS, animal studies must be designed with consideration for (1) correctly emulating the delivery of direct current (DC) or alternating current (AC) 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/tACS and then highlight research on their application to psychiatric pathologies.

Like any model, direct current stimulation (DCS) and alternating current stimulation (ACS) of animals are intended to reproduce relevant features for human applications with the goal of (1) retrospectively providing a mechanistic explanation for findings in humans, and (2) prospectively progressing rational optimization of tDCS/tACS protocols. The tDCS/tACS parameter space is large, spanning dose selection (electrode montage, current intensity, duration, and, for ACS, frequency), the potential use of biomarkers to titrate and customize dose, subject selection, and pairing of tDCS/tACS with cognitive training. Comprehensively testing this parameter space in humans is impractical, thereby necessitating the use of animal models to guide tDCS/tACS development.

Classification of Animal Studies and Relevance to Clinical Protocols

The scope of this review includes animal studies testing the neurophysiological, behavioral, and molecular response of the brain to DCS and ACS, with a focus on macro-electrodes delivering sustained (seconds to minutes) rather than pulsed (milliseconds) waveforms. For the purpose of this review, studies referring to any type of direct electrical current to stimulate parts of the brain will be referred to as DCS or ACS. The term tDCS/tACS will be strictly reserved for referring to noninvasive DCS/ACS applications in human settings, and studies involving behaving animals. DCS/ACS animal studies can be broadly classified by method of stimulation (namely where the electrodes are placed) as (1) stimulation in animals using surface electrodes; (2) in vivo intracranial stimulation, with one electrode on the cortex; and (3) in vitro stimulation of tissues, such as brain slices. While these classifications underpin the decisions by animal experimentalists, understanding the rationale and limitations of animal models of DCS/ACS is important for any effort to leverage them in the understanding and design of tDCS/tACS treatment protocols.

1. Modern animal studies on DCS/ACS typically use transcranial stimulation with a skull screw which functions as the electrode, or skull-mounted electrolyte-filled cup and electrode [1–4]. DCS/ACS using surface electrodes are least invasive of the three outlined methodologies and can be subdivided into applications with electrodes that leave the scalp intact and those that do not. Electrodes that leave the scalp intact typically use adhesives as fixatives and require conductive solutions to interface the electrode with the skin. Subcutaneous electrodes are typically fixed with skull screws, but if the electrode penetrates completely through the skull, the stimulation method is no longer considered transcranial. The advantages of transcranial stimulation include preventing electrode electrochemical side products from reaching the brain which would confound any results. Rodents are typically used but cats are also sometimes used as well [5]. In rodent models, an “active” electrode is placed on the head and a “passive” return electrode is mounted on the body—this setup is typically used for unipolar stimulation which is used to provide a more uniform electric field throughout the brain. In a study on

anesthetized rabbits, four silver ball electrodes formed a single virtual electrode to stimulate the target brain region [6]. Alternatively, two cranial electrodes produce bipolar stimulation [7] that results in an electric field spectrum between the electrodes. Since the cranium is not penetrated, the effects of DCS are quantified through behavioral tests, noninvasive recordings (electroencephalogram, EEG), noninvasive electrical interrogation (e.g., transcranial magnetic stimulation, TMS; transcranial electrical stimulation, TES), or histology after sacrifice.

Stimulation across the skull in animals is the most relevant for informing tDCS/tACS clinical trials for neuropsychiatric disorders, as this class of studies offer the possibility to link neurophysiologic mechanisms with behavior [6]—though there are relatively few such studies at present and the relevance of animal behavior to clinical disorders remains debated (see below). Studies from this class are also the most relevant from the perspective of safety.

2. Classic DCS animal studies placed an electrode directly on the cortical surface [8, 9]. Cats, monkeys, and rats were typically used. When an electrode is placed inside the skull then potential interference from electrochemical changes at the electrode interface diffusing into the brain cannot be ruled out. While these electrochemical products can be polarity specific [10] and produce reversible changes, direct electrochemical diffusion from the electrode surface to the brain is not considered relevant for DCS. Steps to reduce interference from electrochemical by-products include using suitable electrodes (e.g., Ag/AgCl) and wrapping the electrodes in cotton to shield chemical changes [11]. Prolonged DCS through a poorly selected electrode material (e.g., steel) produces significant electrochemical accumulation on the metal, and would warrant careful scrutiny of results. For cortical electrodes, it is generally assumed that current flow through nearby cortex will be unidirectional. Passage of direct current through invasive electrodes is known to produce electrochemical lesions of the local tissue [5].This form of stimulation is relevant for informing the more fundamental aspects of DCS/ACS and excitability changes. For example the earliest notions about polarity-specific cortical excitability changes and the potential for lasting after effects when stimulation are sustained derives from this class of animal work. As mentioned above, studies from this class are less relevant from the perspective of safety than tDCS/tACS.
3. The use of brain slices to study the effects of weak DCS dates to work done in the 1980s [12–16], with comparable approaches used for ACS [17]. Brain slice models (usually rodent) allow probing of specific brain regions in detail using a range of quantitative electrophysiological, pharmacological, molecular, and imaging techniques. For in vitro DCS/ACS studies, the stimulation electrodes are typically placed in the bath at a distance from the tissue to shield electrochemical changes. In isolated tissue, the direction of current flow can also be precisely controlled. Techniques have been developed for stimulating in vitro monolayer cultures [18]. In a seminal series of papers, Chan and Nicholson used isolated turtle cerebellum to study DCS modulations of spiking patterns [19, 20]. Slice studies have provided the most quantitative and sophisticated insights into tDCS/tACS principles—leading to the development of hypothesis regarding mechanisms of actions regarding cell polarization, plasticity induction, and oscillation effects.

tDCS and tACS Dose

The dose of brain stimulation for tDCS and tACS has been defined by stimulation parameters that are controlled by the operator (Bikson et al. 2008; [21]), namely the electrode montage (shape, location, etc.) and the specifics of the waveform (duration, peak intensity in mA applied, and, for ACS, frequency). All the downstream effects of tDCS/tACS are a result of the current flow generated in the brain and are a direct function of dosage. Analogous to drug dosages, tDCS/tACS doses too small may lead to nonsignificant effects and doses too large have detrimental

consequences. Due to the convoluted structure of the head (that includes the skull, layers of meninges, and gyri surrounded with flowing cerebrospinal fluid), the electric field will vary considerably around different geometries and through different materials [22]. As a result, tDCS/tACS produce complex spatial current flow patterns across the brain, which results in a dose-specific electric field that varies significantly across brain regions. As a consequence, the current density at the electrodes does not homogeneously describe peak electric fields in the brain [23]. These electric field peaks represent centers of concentrated charge with weaker fields being generated in other parts of the brain. There are established methods to predict the electric field generated in the brain using computational models [22, 24]. Though methodological approaches vary, studies using realistic anatomy models have converged in their estimates of peak electrical fields generated during tDCS/tACS to 0.2–0.5 V/m (0.05–0.14 A/m2 current density) for a 1 mA peak tDCS/tACS dosage [22, 24, 25], though it has been proposed that tACS may produce significant larger fields [26]. The electric field scales linearly with current intensity such that 2 mA peak could produce intensities upwards of 0.4–1 V/m (0.1–0.28 A/m2 current density). There is no single electric field generated during tDCS/tACS but rather a range of electric field magnitudes are generated across the brain. This issue is further complicated by the fact that electric fields also vary as a function of head size, so applying the same dosage to a human and a mouse would not yield similar results. Therefore, the question is this: Given this complexity of current flow pattern (electric field distribution across brain structures), what are the best montages to be used in the treatment of neuropsychiatric disorders? This question is addressed further in the chapter of models.

The Quasi-Uniform Assumption

In creating an animal model, it is impractical to replicate the electric fields induced in each brain region during tDCS in all corresponding brain regions in a human. One solution is to only focus on the electric fields generated in the brain region of interest in the human, and then to locally apply the same fields on the corresponding brain region in an animal model. In doing so one implicitly adopts the assumption that fields are nearly uniform across small scales—this assumption has been termed the "quasi-uniform assumption" [27, 28] (Fig. 5.1). This approach is supported by the fact that electric fields generated are largely uniform across any specific cortical column (neuronal dendritic tree) of interest allowing a single electric field to describe a region of interest.

As previously explained, DCS experimental design falls into three categories (section "Classification of Animal Studies and Relevance to Clinical Protocols"). When using the quasi-uniform assumption to approximate the local electric fields in each of the experimental designs, oversimplifications in the assumption can result in substantial mismatches between calculated and actual electric field intensities. The limitations and methods to approach the issue are outlined below for each experimental design.

1. In the first case of transcranial stimulation of animals, the same modeling approaches that predict electric fields during clinical tDCS can be used to model and guide stimulation design [29]. In applying tDCS to animals it is important to consider how the position of the reference electrode influences current flow under the active electrode [30, 31]. 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 [6]. In the absence of specific modeling of current flow in animals, 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 [32]. However, it is important to recognize that the direction of the electric fields generated across the brain, including in deep brain structures (particularly in higher animals with increasing convoluted cortex), may also vary. The electric field in a region of interest may also be measured with invasive electrodes [7], though

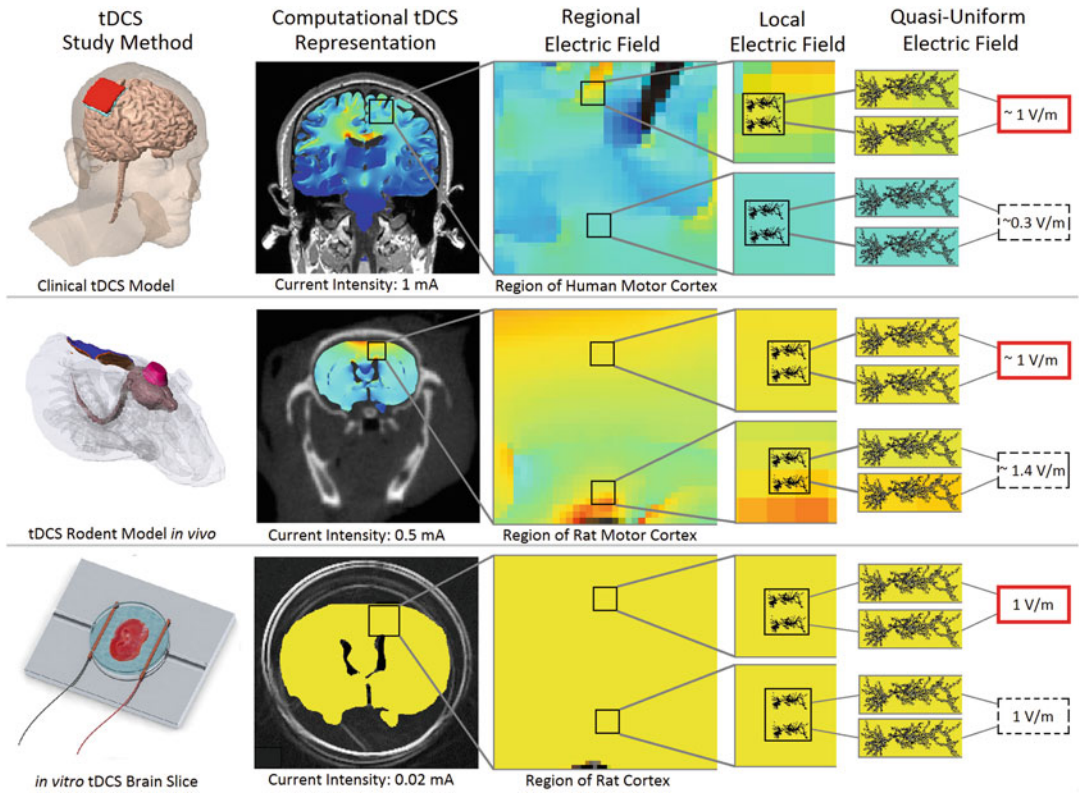

Fig. 5.1 The quasi-uniform assumption in animal models of tDCS. Current flow patterns predicted by FEM models are shown for human tDCS (*top row*), animal epicranial DCS (*middle row*), and brain slice DCS (*bottom row*). *Second column*: For human and epicranial stimulation, stimulation produces a globally nonuniform electric field, with higher electric field intensities generally in regions near the electrodes (indicated by hotter false *color map*); though local hotspots can be distributed for brain slice stimulation a uniform electric field is generated using large parallel wires. *Third column*: Consideration of regional electric field shows the electric field generation gradually over space. *Fourth column*: On the scale of single neuron, the electric field is largely ("quasi") uniform. *Fifth column*: The electric field is thus not uniform across the brain as a whole, but uniform across each neuron and indeed across local network of neurons. It is possible to match the electric field in a given region of human cortex, with electric field in a given region of animal brain, with an electric field generated in vitro. For illustration, at the current intensities used in each case (see values in *second column*) and the subregions selected in each animal model there is equivalence at 1 V/m. The equivalence, and more generally the local uniform nature of the fields, is the quasi-uniform assumption which implicitly informs every translational model of tDCS. Equivalence is not achieved by matching the applied current but by matching an electric field only in a specific region of interest

the insertion and presence of electrodes may itself distort current flow. It should also be noted that because the coupling constant also is much higher in humans than in other animals and will result in much larger polarizations in humans even when electric fields are matched—this discrepancy will be further discussed later.

2. In the second case, animal studies using a surface cortical electrode assume that the current density in the brain directly under the electrodes equals that *average* current density at the electrode. When scalp electrodes, such as sponges or cotton wrappers, are used, the total contact areas should be used in calculations. Depending on the electrode design, current density may be orders of magnitude higher at electrode edges than at the center of the electrode [24, 33, 34]. This is an issue aggravated for small electrodes where electric field near a monopole source can be very high leading to further potential complications [8]. As with unipolar stimulation, current spread throughout the brain (affecting both cortical and

subcortical structures) should be assumed when using return electrodes located away from the head [35].

3. In the third approach, including in vitro brain slice studies, the task is simplified by using long parallel wires placed in a bath. This setup generates a truly uniform electric field across the entire slice that can be readily calibrated to match tDCS levels [14, 36, 37]. The uniformity of the electric field across brain slices has been verified though exceptions have been reported. Inhomogeneities in the field may be due to the presence of stagnant conductive fluid around the brain slices that would alter current flow through the slice. Typically, the placement of the electrodes in the bath, away from the tissue of interest, protects the sample from electrochemical by-products. The simplicity and versatility of this technique make control of DC parameters in slice straightforward and allow for direct investigation of mechanisms not possible with other techniques.

Translation from Animal Studies to Clinical Applications: The Importance of Intensity

Many proposals for tDCS/tACS mechanisms fail to consider the much higher DCS/ACS intensities and/or durations used in some animal experiments. Recognizing that tDCS may produce weak outcomes, high intensities not reasonably used in humans are often intentionally applied to animal models in order to more reliably detect effects. Dosages are calculated by scaling down effect sizes based on the linear responses measured in animal models [17, 38]. In vitro studies also indicate a surprisingly linear response curve over low intensities [36, 39]. The in vitro studies that have explicitly explored the lower electric field limit of sensitivity to fields (see network effects; [37, 39, 40] report statistically significant responses at <0.2 V/m—fields within clinical tDCS/tACS ranges. Regardless, a cautious, rational approach reading dose–response should be taken.

Throughout this review, we emphasize caution when exploiting conclusions from studies using large DC/AC currents in animals that (far) exceed electric field magnitudes comparable to those generated during clinical tDCS/tACS—which is the overwhelming majority of them. While DCS and ACS can generate seizures, clinical tDCS/tACS intensities are orders of magnitude lower than those necessary to produce epiliform activity [41]. While these experiments are invaluable in suggesting tDCS/tACS mechanisms, just as with drugs, increasing dose beyond clinical levels 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 [42, 43], but both the duration and intensity of electric fields were often orders of magnitude greater than tDCS. Mechanisms such as electroporation and joule heating can be produced by some forms of electrical stimulation, but the waveforms required to produce these effects are not relevant for tDCS [22, 32, 44].

Safety Concerns

Any attempt to develop safety standards for tDCS/tACS requires assumptions about dose–response and variability in its effects. One approach uses the lowest documented current intensity to produce a measurable brain tissue response in an animal model for any stimulation duration. However, there may be a nonlinear minimum threshold for tissue damage or the dose–response curve may not be monotonic with very low intensities. However, animal studies are often for short term making long-term side effects of tDCS/tACS difficult to discern. They are also limited in time points for measurement—since the collection of tissue for analysis often requires terminal procedures we must assume that damage was irreversible and cannot exclude delayed damage responses.

Studies investigating the safety limits of prolonged DCS have shown that current densities above 15 A/m2 for durations longer than 10 min resulted in brain lesions in rats [44]. However, it

is unclear how this threshold for injury translates from animals to human brain tissue. In developing human safety guidelines it is prudent not to approach injury thresholds in clinical settings, especially given montage and individual differences. Consolidated animal DCS safety data scaled to humans using computational models indicate that current conventional clinical tDCS protocols are orders of magnitude below the threshold for tissue damage [32].

Relating Biomarkers from Animal Models to Humans

In considering the use of tDCS/tACS in clinical treatment, animal models of disease can be used, not simply to validate outcomes, but to characterize mechanisms and optimize stimulation protocols [4, 45]. To quantify tDCS/tACS efficacy, researchers have used noninvasive biomarkers of tDCS including spontaneous EEG [46–51] and TMS motor-evoked responses [15, 52], screening different dosage and time courses. These generic clinical measures of activity and excitability have rough animal analogs such as spontaneous firing rate, oscillations, and evoked responses—though these measures may not have the same range in animals and humans. More invasive measures of tDCS/tACS effects include protocols to measure gene expression and protein synthesis.

The primary human neurophysiology metric used to establish the acute and lasting effects of tDCS/tACS in humans is the transcranial magnetic stimulation (TMS) motor-evoked potential (MEP). Indeed, the establishment of modern tDCS can be traced to the discovery that tDCS can modulate TMS-MEP in a polarity- and montage-specific manner [15]. The development of other weak tES approaches, including tACS, followed. A common metric in animal trials is synaptic responses evoked by micro-electrical stimulation (e.g., field excitatory postsynaptic potentials, fEPSPs). These micro-recordings show how DCS/ACS effects evoked synaptic potentials in slice models and have served as the basis for the characterization of cellular mechanisms [6, 36, 38, 53]. Both TMS and microelectrode stimulation use suprathreshold stimulation of afferent pathways to assess how DCS/ACS modulates postsynaptic responses to the stimulation. These studies have given us insight into neural pathways and dose-specific modulation of excitability [6, 36, 38, 53] and emerging data suggests that there is a pathway dependence in humans as well [54]. For example, micro-electrical stimulation in brain slice models has shown that DCS outcomes vary depending on the specific fiber volley activated [5]. TMS is the preferred method for human use because it is noninvasive but the spatial resolution is much lower than with micro-electrode stimulation, which may account for some of the variability observed in clinical studies.

In addition to event-related potentials (ERPs) by electrical probes (TMS-MEP, TME-phosphenes, micro-stimulation), ERPs produced by environmental cues (e.g., light, SEP, VEP) can also be produced in human and animal models. Another direct neurophysiological marker found in animal DCS/ACS studies with human correlates is network oscillations which can be measured with EEG and field recordings. Despite differences in the etiology of oscillations between human and animal models (even when the frequency appears matched), mechanistic findings from animal studies on how DCS/ACS effects oscillations in a highly activity-dependent manner [39, 55] may help elucidate complex effects of tDCS/tACS in humans.

Neuronal Polarization

Any forms of electrical stimulation, including AC or DC, generate electric fields that lead to current flow across the brain [22, 56]. This current flow though the brain results in polarization of the neuron membranes which the current passes through. Finite-element models (FEM) have been used to incrementally approximate how neuron membrane potentials will react when exposed to such electric fields. Current flow into a specific membrane compartment will result in local membrane hyperpolarization, and flow out of another membrane compartment will result in

local membrane depolarization [36, 57]. It is fundamental to emphasize that the physics of electrical stimulation dictate that any neuron exposed to extracellular DCS/ACS will have some compartments that are depolarized and others that are hyperpolarized [19, 36]. The neuronal morphology relative to the DC/AC electric field determines the polarity of the neuronal compartments. Simplistically, during tDCS, for a typical cortical pyramidal cell, with a large apical dendrite pointed toward the cortical surface, proximity to a surface anode will result in somatic depolarization, and apical dendrite hyperpolarization [58]. For this same neuron, a surface cathode will result in somatic hyperpolarization and apical dendrite depolarization. For tACS the direction of current flow alternates and so the resulting membrane polarization also alternates—but at each instant, opposite poles of the cell are polarizing in opposite directions.

Though dendrites are polarized opposite to the soma, neuron excitability is conventionally assumed to most closely follow soma polarization. Since tDCS/tACS doses in humans are subthreshold—such that the level of polarization is insufficient to directly cause neuronal firing—polarizations in the somatic membrane potential are thought to influence excitability through modifications in the sensitivity to synaptic input [59].

The assumption that DC/AC electric fields induced somatic polarization are the leading driver of tDCS/tACS mechanisms (as opposed to dendrite polarization) is termed the "somatic doctrine" [38]. Though neuron activity is determined by the integration of activity in all neuronal compartments to varying degrees (dendrites, axon, presynaptic terminal, axon hillock), the somatic doctrine assumes that most functional outcomes can be directly correlated to the soma.

Polarity-Specific Effects for DCS and Implications for ACS

The concept that DCS produces polarity-specific effects is the most fundamental result from classic and ongoing animal studies, and underpins how tDCS protocols for neuropsychiatric disorders are rationalized. As early as 1870 Fritsch and Hitzig showed that application of a positive current (anode) to the cortex had stimulating effects, while a negative current (cathode) inhibits (a finding that itself contributed to early understanding that the cortex is electrically excitable; [60]—a finding that fits well with the somatic doctrine). Other studies [9, 61] helped establish that neural firing rate can be altered by DCS. In the early 1960s, animal studies [8, 62] confirmed polarity-specific changes in discharge rate and further showed excitability changes that are both cumulative with time and out-last stimulation. Early work testing tDCS for psychiatric disorders in fact derived from Bindman and colleagues. In 2000, when Nitsche and Paulus validated the polarity-specific effects of tDCS in humans using TMS, they were very much aware of these animal studies and their work established the convention of anode/cathode providing cortical excitation/inhibition. The earliest clinical trials with tDCS adopted strategies using the anode/cathode to enhance/inhibit function of underlying cortex [63], and this rationale continues to underpin most applications of tDCS to neuropsychiatric disorders (e.g., place anode electrode over left DLPFC to increase its function to treat depression; [64]). Though results from ongoing clinical trials designed based on the rationale anode/cathode excite/inhibit have been encouraging [36, 65], it is important to emphasize that more ongoing clinical neurophysiology and modeling studies suggest that changes in brain function with stimulation polarity are more complicated (e.g., drug-dependent increased cathode intensity from 1 to 2 mA can result in excitation; [66, 67]).

Quantifying Neuronal Polarization with Coupling Constants

In regard to quantifying how much polarization is produced by tDCS/tACS, the concept of the "coupling constant" is fundamental. In the 1980s, Chan and colleagues [19, 20] used turtle cerebellum recordings to model membrane polarization

under near-static electric fields. These monumental series of studies identified the basic morphological determinants for neuronal membrane polarization to applied DCS. However, considering the variety of neuronal morphologies within a brain and across species, one cannot assume that all neurons will polarize in the same manner. To address this, our group has quantified cell-specific polarization by weak DCS in hippocampus and cortex in rat brain slices [36, 58]. We assumed that for weak electric fields the membrane polarization produced by DCS/ACS is linear with electric field intensity along the primary neural axis. For uniform electric fields, the membrane potential polarization can thus be expressed as $Vtm = G \times E$ where Vtm is the polarization of the compartment of interest (volts), G is the "coupling constant" (meter), and E is the electric field (volts/meter) along the primary somatodendritic axis. The coupling constant is also referred to as the "coupling strength" or "polarization length."

Further analysis of coupling constants reveals that the maximal depolarization occurs when the electric fields are parallel with the somatodendritic axis, while electric fields orthogonal to the somatodendritic axis do not produce significant somatic polarization [19, 36]. The coupling strength is roughly related to the size of the cell and the dendritic asymmetry around the soma [58, 68] making pyramidal neurons relatively sensitive to polarization. For cortical pyramidal neurons, the typical polarity of somatic polarization is consistent with those predicted by the somatic doctrine (e.g., positive somatic depolarization for positive electric field). For rat hippocampus and cortical neurons the coupling constant for DCS was in the range of 0.1–0.3 mm [17, 36, 58]. In ferret cortical neurons the DCS coupling constant was approximately 0.25 mm [69]. Generally the maximal polarization is expected at dendritic tufts [36], but in animals should not exceed ~1 mV polarization per V/m electric field [19, 58, 59]. For ACS the coupling constant decreases with increasing stimulation frequency [17] as would be predicted by the RC behavior of the membrane (as evidence by step response experiments; [36]). In humans, assuming scaling of sensitivity with total neuronal length [70], somatic depolarization per V/m may be higher. Experimentally measuring the coupling constant of the soma and other membrane compartments in humans to tDCS remains a fundamental research question.

Synaptic Plasticity

There is a clinical need for lasting changes by tDCS/tACS, as it is impractical to stimulate continuously with electrodes on the head. The desire for lasting change means tDCS should influence plasticity during or after stimulation in the relevant pathway [4]. This section addresses the contribution of animal studies to understanding plasticity generated by weak DC and AC electric fields.

Animal studies in the 1960s established that weak DC current can produce lasting physical change in neural activity, which cannot be explained as persistent "reverberating circuit" of activation [71, 72]. Especially notable are animal studies by Bindman and colleagues [62] that showed that prolonged DCS can produce polarity-specific lasting cortical excitability changes. This study motivated their early work treating depressive patients with tDCS [11, 73]. Persistent polarity-specific excitability alterations were observed in a study using long stimulation protocols lasting up to 13 min [74, 75]. These multiminute protocols are frequently adopted in tDCS research. Lasting changes with AC stimulation have recently been demonstrated in animals when endogenous neural oscillations are present [55].

Long-lasting changes beyond the transient effects of DCS- and ACS-induced polarization would require synaptic changes. Moreover, both in humans and animal studies, changes in synaptically mediated evoked responses (see above) are considered reliable hallmarks of long-term plastic changes that could support lasting clinical effects.

Animal studies of tDCS/tACS allow us to formulate distinct theories on how stimulation can lead to lasting changes in function. Electric fields

generated by tDCS/tACS are subthreshold, in the sense that they are too weak to trigger action potential in quiescent neurons, resulting in only transient polarizations. These acute effects can lead to lasting changes in synaptic efficacy mediated through different paradigms such as the following:

1. Membrane polarization may trigger plastic synaptic changes in a manner independent of action potential generation—simply holding the membrane at an offset polarization initiates synaptic changes. However, in cortical brain slice models (with no background activity), weak polarization was not sufficient to induce plastic changes in synaptic efficacy [76].
2. Changes in action potential rate or timing, secondary to neuronal polarization, may affect synaptic plasticity. Classic animal studies indicated that weak DC stimulation is sufficient to induce short- and long-term plastic changes [8, 71]. However, these studies do not directly provide a causal link between altered neuronal activity during stimulation and prolonged after effects.
3. Incremental polarization of the membrane in combination with ongoing synaptic activity may induce synaptic plasticity. The theory is that the generation of plasticity requires synaptic coactivation during DC stimulation. It has been shown that in vitro synaptic potentiation under anodal stimulation only occurs with concurrent synaptic stimulation at specific frequencies [76]. In a rabbit study, DCS was combined with repeated somatosensory stimulation leading to polarity-specific lasting changes with cathodal stimulation [6]. If one assumes that DCS/ACS exerts a postsynaptic priming effect (polarization of soma) then coactivation of afferent synaptic input could be conceived as Hebbian reinforcement. This learning mechanism has been shown in brain slice models as well in vivo [77, 78]. Clinically this plasticity paradigm is broadly analogous to combining tDCS/tACS with a cognitive task or specific behavior that coactivates a targeted network or combining tDCS/tACS with TMS.
4. Incremental polarization of the membrane may boost ongoing endogenous synaptic plasticity similar to a model of associative learning [6]. Clinically this fourth paradigm is analogous to combining tDCS/tACS with training [79]. It has been shown in rat visual cortex slices that the same tetanic stimulation can induce LTD or LTP depending on the level of polarization of the postsynaptic neuron [80].
5. Meta-plasticity is defined as sustained polarization before, or potentially after, the generation of endogenous LTP that "primes" the brain to respond differently to potentiation. Evidence from brain slices [81] shows that priming with DCS modulates subsequent tetanus-induced LTP in a polarity-specific manner—though opposite to convention with soma hyperpolarization ("cathodal tDCS") enhancing plasticity.
6. Changes in network dynamics where the generation of LTD/LTP is explained through intervention with ongoing oscillations and may manifest as lasting changes in oscillation dynamics [55, 82]: Such modulation may reflect interference with the finely tuned excitatory-inhibitory synaptic balance during oscillations [39].

Aside from these possible synaptic plasticity effects there may be non-synaptic origins of lasting plastic changes following DCS/ACS. Though the synapse is typically considered the locus of plastic changes, "non-synaptic" changes have been noted after DC stimulation in peripheral axons [12]. In brain slice models, where background synaptic activity is absent, synaptic (orthodromic) and non-synaptic (axon, antidromic) can be precisely isolated allowing more precise isolation of synaptic and non-synaptic mechanisms. However, functional outcomes of non-synaptic changes in the CNS would still be expected to affect synaptic processing [83].

Network Effects

The consideration of how weak electric fields modulate active networks (e.g., oscillations) is a compelling area of ongoing research. Electrical recordings, of both intact brains and dissociated in vitro cultures, show that neuronal firing activity tends to synchronize and desynchronize

in phases. These rhythmic firing patterns, termed "oscillations," have been recorded in many species but are primarily studied in humans and rats [46]. Oscillations span a wide range of frequencies in multiple brain regions and are thought to play roles in sleep and memory encoding [84]. In healthy subjects, there is a high level of synchrony between the oscillations that occur in different brain regions. However, in patients with neurological disorders, whether due to cell death or network dysfunction, there is a loss or modification of this synchronous order. Currently, transcranial electrical stimulation is being investigated as a means to resuscitate endogenous oscillations with the ultimate goal of functional improvement.

Up until now, this review has discussed tDCS-induced cellular and synaptic modifications. Considering the oscillatory nature of transcranial alternating current stimulation (tACS), we will also briefly discuss the effects of tACS on oscillations in neural networks.

tDCS and Oscillations

Reports that DCS can alter spontaneous oscillations in animals span decades [85–87]. A significant number of studies on weak DC electric fields and network oscillations addressed epileptiform activity using pathological oscillation models in brain slice models. For example, DC electric fields influence the propagation rate of epileptiform activity [37, 88]. It has also been shown that DC fields up-regulate gamma oscillations in rat brain slices [39]. Interestingly, this increased activity led to a delayed compensatory ("homeostatic") regulation of the network such that the activity levels were normalized to baseline levels. 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. Network-level mechanisms may thus provide a substrate for activity-dependent homeostatic-like observations during tDCS [89].

tACS and Oscillations

During ACS, a specific frequency is applied typically using similar electrode montages as used in DCS. Most of the applied stimulation frequencies are within the human EEG frequency range [46, 90]. Repetitive weak ACS can entrain native activity by aligning the phase of these oscillations with that of the AC stimulation [48, 82, 91]. By definition, during prolonged DCS there is no basis for entrainment (there is no phase to the DC), giving ACS a unique theoretical advantage in this regard. In line with effects on the phase of endogenous activity, tACS can selectively modulate spike-timing-dependent plasticity in oscillating networks with specific resonant frequencies [92]. This presents a mechanism for tACS modulation of network activity to produce long-term effects in synaptic plasticity.

In a mouse brain slice model, weak ACS enhanced intrinsic oscillations but failed to induce a frequency shift of the ongoing oscillations for stimulation frequencies that were not matched to native oscillations [51]. These results suggest that the primary tACS mechanism may be to amplify, not override, endogenous network dynamics. In a ferret hippocampal slice model, tACS will form positive- and negative-feedback loops with endogenous oscillating mechanisms in modulating pharmacologically evoked slow-wave oscillations [69]. The distinct roles of the depolarizing and hyperpolarizing phases of tACS in oscillation entrainment have been studied in large-scale computation models [93]. These findings were then verified in anesthetized ferrets, supporting the future of dynamically tailoring stimulation frequency to the endogenous activity.

Applications to Clinical Pathologies

The noninvasive and inexpensive methods of tDCS/tACS have made it versatile and widely studied as a potential treatment for various diseases [94, 95]. tDCS/tACS is especially favorable as a psychiatric disorder treatment because

the effects can be directly assessed with behavioral tests. For these reasons, a majority of published findings are of tDCS effects in humans and relatively few are in animal models. Of the handful of animal studies, most involved highly invasive methodologies or sacrifices (e.g., tissue damage, brain slice, and protein-synthesis experiments). Nonetheless, some studies treating animal models of psychiatric disorders with tDCS are briefly outlined below.

Addiction

A handful of studies using tDCS as a treatment for addiction in humans have been conducted [96]. The studies primarily show that anodal tDCS of the inferior frontal gyrus can reduce cravings better than stimulation of the left dorsolateral prefrontal cortex [97]. Other studies show that tDCS can improve impulse control [98] and reduce risky behavior [99]. In a meta-analysis of addiction in humans, rTMS and tDCS were found to be equally effective at treating addiction [100].

Animal models of addiction primarily involved rats treated with transcranial magnetic stimulation (TMS) in the frontal cortex [101]. In a pilot study, applying 0.2 mA anodal tDCS to the frontal cortex for 20 min twice a day for 5 consecutive days was sufficient to reduce anxiety-like and depression-like behavior in nicotine-addicted mice [102].

Alzheimer's Disease

The main methods of noninvasive brain stimulation for Alzheimer's disease are TMS and anodal tDCS and preliminary findings suggest that both techniques reduced cognitive deficits in Alzheimer's patients [103–105]. Visual recognition memory was also improved after five daily sessions of anodal tDCS and effects persisted for at least 4 weeks after therapy (Boggio). In another Alzheimer's disease memory study, memory was found to improve in Alzheimer's patients receiving memory training regardless if they received tDCS or sham-tDCS [106]. Transcranial electromagnetic treatment was also found to reverse cognitive impairments in Alzheimer's disease transgenic mice. It was also shown that deep brain stimulation (DBS) of the hypothalamus and nucleus basalis of Meynert may improve cognitive function in Alzheimer's patients.

To replicate the cognitive symptoms of Alzheimer's disease, intraperitoneal injections of scopolamine were given to rats that subsequently received 0.1 mA of anodal tDCS twice a day, five times a week [107]. After 2 weeks of treatment, rats treated with tDCS had slightly increased cognitive function in comparison to the rats just treated with tacrine. After the 4 weeks of treatment, rats that receive tDCS therapy had motor behavior improvements and increased acetylcholine activity.

Chronic Stress

Though numerous studies have been shown in tDCS to have a therapeutic effect in animal models and in humans, the limits to gainful tDCS effects were only recently tested [108]. In this study, tDCS efficacy was measured in chronic stress mice models. After subjecting rats to chronic restraint-induced stress (CRS) for 11 weeks, rats were given 20-min anodal tDCS treatment sessions for 8 days. Behavioral tests were performed after the 11 weeks of CRS, immediately after and 24 h after tDCS treatment. Control rats were not subject to CRS but were randomly given either sham or tDCS treatment. tDCS was only able to decrease BDNF release in the spinal cord and brainstem of unstressed rats. Interestingly, CRS rats treated with tDCS had a weak reduction in pain sensitivity even though no change of BDNF levels was detected indicating that a different mechanism may be involved in the attenuation of pain sensitivity. The results from this study highlight that tDCS treatments alone may not be sufficient to produce long-term effects when chronic stress is present.

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

A central challenge for tDCS/tACS studies is translating data collected from animal models of tDCS/tACS to inform the interpretation and design of human protocols. Historically, tDCS/tACS animal studies have informed human testing. The demonstration that prolonged (minutes) DCS/ACS protocols in animals can lead to short- and long-term plasticity encouraged the use of such protocols in humans [109]. The polarity dependence of DCS was first demonstrated in animal models. Animal models demonstrated that low-intensity DCS/ACS can modulate ongoing neuronal activity giving human technique credence of a cellular substrate [36]—countering the argument that weak fields, such as those applied in tDCS/tACS, are physiologically inert. In some cases, animal studies of DCS/ACS were conducted contemporaneously with human testing providing confirmatory evidence, for example, that AC can entrain oscillations [46, 92] of that tDCS plasticity is NMDA dependent [110].

On the other hand, there are scarce examples of modern animal tDCS/tACS studies influencing how human trials are conducted and analyzed. This reflects how tDCS/tACS protocols have remained largely unchanged with the majority of protocols applying 1–2 mA over 10–30 min using two large pad electrodes without any customization based on an individual's biomarkers. Developments in tDCS/tACS protocols were driven by clinical neurophysiology [65] rather than extrapolated from animal models. Often animal studies confirm findings in humans rather than suggesting novel improvements to the current protocols; a notable example being the identification of the role of BDNF polymorphism [76].

We believe development in animal tDCS/tACS studies combined with an increased emphasis on designing these experiments for clinical relevance would accelerate the development and application of tDCS/tACS in humans. This includes an increased emphasis of the plastic, rather than acute, effects of stimulation [40, 76]. Simultaneously, results from human trials also point to a need to critically address issues such as nonlinear dose–response, state dependency, and inter-subject variability. Animal experiments provide a degree of cellular resolution, state control, and rapid screening not available in human subjects to help detangle complex interactions [36].

We propose that meaningful translation to human applications would be the most accelerated by the exploration of data that *appears*, at first glance, to be conflicted between animals and humans. For example, the acute effects of DCS in animal are monotonic across a very wide intensity and brain-state range (e.g., anodal/cathodal almost always result in excitatory/inhibitory effects after accounting for orientation of neurons relative to field; [61, 81]). This is in direct contrast with clinical neurophysiology studies showing that many pharmacological, dose-dependent, and brain-state perpetrations can qualitatively change the direction of neuromodulation [39, 65]. As another example, ACS in animals can influence ongoing oscillations in a myriad of ways and is dependent on the nature of endogenous activity and stimulation frequency [46, 55, 90], while human testing with tACS and EEG usually explores only a basic single stimulation frequency [50]. Rather than speculating which protocols are ineffective, it would be useful to consider cellular effects from animals in comparison to network effects observed in human studies. The most impactful translational animal studies will be those that explain results from humans in previously unexpected ways and that can suggest nontrivial methods to optimize tDCS/tACS outcome in human trials.

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