Animal Models of tES: Methods, Techniques, and Safety

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4.1 Methods

Why Use Animal Models?

The efficacy and specificity of tES benefits from an enhanced understanding of the underlying mechanisms of action. A detailed investigation and isolated demonstration of independent mechanisms is not fully tractable using just human subjects. Animal models allow for isolation and characterization of specific tES cellular pathways. Evidently, there are differences between animals and humans. Like any model, animal experiments with direct current stimulation (DCS), alternating current stimulation (ACS), and other forms of electric stimulation are intended to reproduce relevant features of human applications, so as to have translational relevance. Therefore, the "why" and "how" of tDCS and tACS animal models depend on translational relevance—which is the focus of this chapter. Translational outcomes from animal experiments can then (1) retrospectively provide mechanistic explanations for findings in humans and (2) prospectively progress rational optimization of tES protocols. The benefits of using animal models include, but are not limited to, the following:

1. The tES parameter space is large, spanning dose selection (electrode montage, current intensity, duration, frequency for AC), the potential use of biomarkers to titrate and customize dose, subject selection, and pairing of tES with cognitive/motor/rehabilitation training. Comprehensively, testing this wide parameter space in humans is impractical, thereby necessitating the use of animal models to optimize tES development [1–5].
2. Animal models allow for the rapid screening of stimulation parameters and analysis of neurophysiological/molecular changes in ways not possible in humans. They also facilitate quantitative and qualitative assessment of the tES-related safety parameters, the underlying mechanisms, acute and aftereffects, and their application to psychiatric pathologies [6–10].
3. Animal models allow for modulation of synaptic efficacy to be characterized quantitatively with pathway specificity [11]. Given the interest to evaluate synaptic plasticity from electric stimulation (ES), the mechanisms of plasticity can be analyzed using specific pharmacology and detailed cellular and molecular analysis not possible in human experiments [12, 13]. Brain slices allow for a precise control of drug concentration, the background level and nature of the ongoing activity, and the electric field orientation relative to slice—the latter especially relevant for tDCS [14, 15].

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4. The role of specific neuronal cell types [16] and compartments (soma, dendrite, axon) within neurons [11, 17–19], as well as non-neuronal cells including glia [20–22] and endothelial cells [23, 24] in mediating tDCS/tACS responses, can be studied.
5. Animal models support dissociations of mechanisms that are readily explained by actions on single cells versus mechanisms that inherently depend on coupled neuronal networks [25–29]. In the latter case, the response of a connected and active system is unique from the response of single neurons in isolation.
6. A simplistic "sliding scale" explanation of anodal and cathodal tDCS, increasing and decreasing "excitability," respectively, seems unlikely to capture the nuance of brain function. Animal models can help advance a more thorough understanding of tDCS effects, including consideration for state-dependent changes as well as changes in information processing that are not simply explained by "less" or "more" activity [30]. Thus, while animal models helped underpin the notion of polarity specific excitability changes [31, 32], ongoing animal experiments have demonstrated complex dose-response [11, 15, 33–36].

To have meaningful relevance to human tES, animal studies must be designed with consideration for (1) correctly emulating the delivery of the current stimulation to the brain, and (2) measuring responses that can be used to draw translationally relevant inferences such that outcomes from animal models should relate to targeted brain processes in humans (Fig. 4.1a).

Classification of Animal Studies and Relevance to Clinical Protocols

In this chapter and the next one, we will cover the effects of tES on neurophysiology, behavior, and molecular response of the brain in animal studies. We will focus on macro-electrodes rather than microelectrodes and on sustained rather than pulsed waveforms lasting seconds to minutes rather than milliseconds. For the purpose of this chapter, studies referring to any type of electrical current applied directly to the brain (i.e., not through the skull) will be referred to as ES or DCS (for DC waveforms) or ACS (for sinusoidal waveforms). The term tES/tDCS/tACS will be reserved specifically for noninvasive stimulation in humans and animals. Animal studies can be broadly classified by the location of the stimulation electrodes. These classes are briefly described as follows:

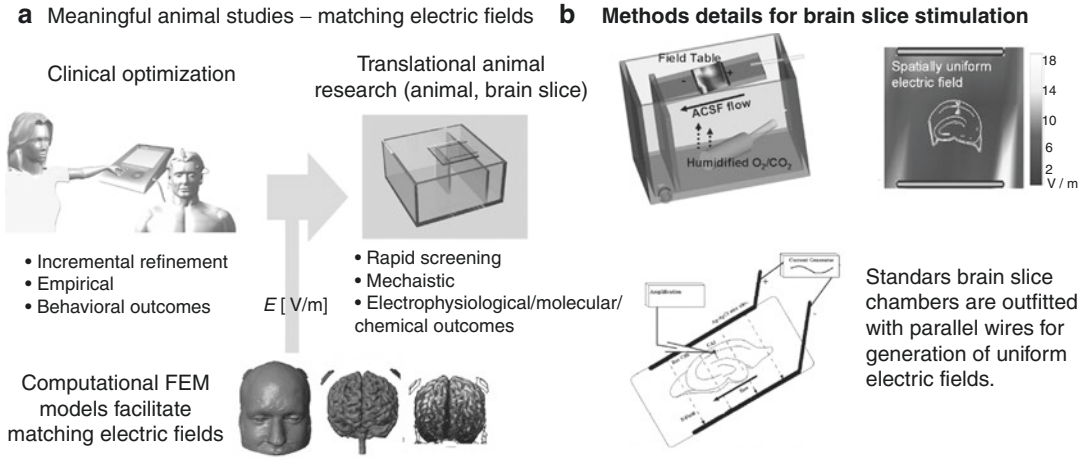


Fig. 4.1 Relevance of animal models to study tES mechanisms. (**a**) Meaningful translational research in animals requires replication of electric fields generated clinically in animal brain/tissue. (**b**) For in vitro brain slice studies, the generation of a uniform electric field with the use of two long parallel wires placed across a shallow bath allows for the replication of electrical fields. The uniform electric field in the chamber can be calibrated using a field-recording electrode. (Adapted from [9])

1. *Transcranial stimulation*: Recent animal studies with tES used transcranial stimulation with a skull screw as the electrode, or skull-mounted electrolyte-filled cup and electrode [12, 37–39]. Surface electrodes are in principle less invasive than other methods, although even for surface electrodes there are different levels of invasiveness. Electrodes that leave the scalp intact typically use adhesives 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.

   One advantage of transcranial stimulation is to prevent electrochemical products from reaching the brain. Recent experiments mostly use rodents [7, 12, 24, 31, 37, 38, 40], but cats [41] and other animal models have been tested as well. In rodent models, an "active" electrode is placed on the head and a "passive" return electrode is mounted on the body [10]. This setup is typically used for "unipolar" stimulation in the sense the polarity of the "active" electrode determines if stimulation is "anodal" or "cathodal." However, as with human tDCS, both electrodes are active and "anodal"/"cathodal" reflects the hypothesis that outcomes are determined by stimulation of the brain region under a given electrode. In a study using anesthetized rabbits, four silver ball electrodes formed a single virtual electrode to stimulate the targeted brain region [42]. Alternatively, two cranial electrodes produced bipolar stimulation [40].

   Since the cranium is not penetrated, the effects of ES are quantified through behavioral tests [4, 43–46], noninvasive recordings with electroencephalograms [4, 5, 47], transcranial imaging techniques that require methods to increase skull transparency [20, 21, 24], intracranial electrophysiology while accounting for skull defects from recording electrode penetration [3, 48–50], noninvasive electrical interrogation with external stimulations such as transcranial electrical stimulation [38], or histology after sacrifice [51–55]. In principle, animal experiments with transcranial stimulation have special relevance from a translational point of view, as they can link neurophysiologic mechanisms with behavior [42]. However, there are relatively few such studies at present [1, 12, 56–58] and the relevance of animal behavior to clinical disorders remains debated. Transcranial studies are quite important from the perspective of clinical safety as they come closest to the clinical use of tES [6–8, 51, 59].
2. *Intracranial stimulation*: In older DCS animal studies, typically done on cats, monkeys, and rats, an electrode was placed directly on the cortical surface [31, 32]. When an electrode is placed inside the skull, then one cannot rule out potential confounds from electrochemical changes at the electrode interface which can diffuse into the brain. This is less of a concern with ACS, which is typically charge-balanced and avoids buildup of electrochemical byproducts. For DCS, these byproducts are polarity specific and can produce changes that reverse with polarity [60]. Electrochemical byproducts can be reduced with suitable electrodes (e.g., Ag/AgCl) or wrapping the electrodes in cotton [61]. Prolonged DCS through a poorly selected electrode material (e.g., steel) produces significant accumulation of electrochemical products on the metal [60]. For cortical electrodes, it is generally assumed that current flow through the nearby cortex will be unidirectional. Passage of direct current through invasive electrodes is known to produce electrochemical lesions of the local tissue [9]. Thus, in terms of clinical safety of tES, these studies are less relevant. Nevertheless, this form of stimulation has revealed some fundamental aspects of ES. Two important findings from this early work are polarity-specific cortical excitability changes and lasting aftereffects when stimulation is sustained [31, 62].
3. *In vitro stimulation*: The use of brain slices to study the effects of weak DCS dates back to work done in the 1980s [63–67], with comparable approaches adapted for ACS [26, 68]. Brain slice models, usually rodents, allow for

detailed probing of specific brain regions using a range of quantitative electrophysiological, pharmacological, molecular, and imaging techniques [1, 14, 15, 34, 46, 69–71]. For in vitro studies, the stimulation electrodes are typically placed in the bath distanced from the tissue to shield from electrochemical products at the electrodes and to produce a controlled uniform field across the tissue (Fig. 4.1b). In isolated tissue, the direction of current flow can also be precisely controlled. Techniques have also been developed for stimulating in vitro monolayer cultures [72] including in transwell (membrane used for cell cultures) monolayer models [73]. In a seminal series of papers, Chan and Nicholson used isolated turtle cerebellum to study ACS modulations of spiking patterns [74, 75]. Slice studies have provided the most quantitative and sophisticated insights into tES principles—leading to the development of hypotheses regarding mechanisms of actions such as cell polarization [11, 16, 18, 35], plasticity induction [14, 15, 34], and oscillation effects [26–28, 76, 77].

4.2 Modes of Noninvasive Electrical Brain Stimulation

In this section, we will briefly introduce different modes of electric field stimulation which have been used in animal studies of noninvasive electrical brain stimulation.

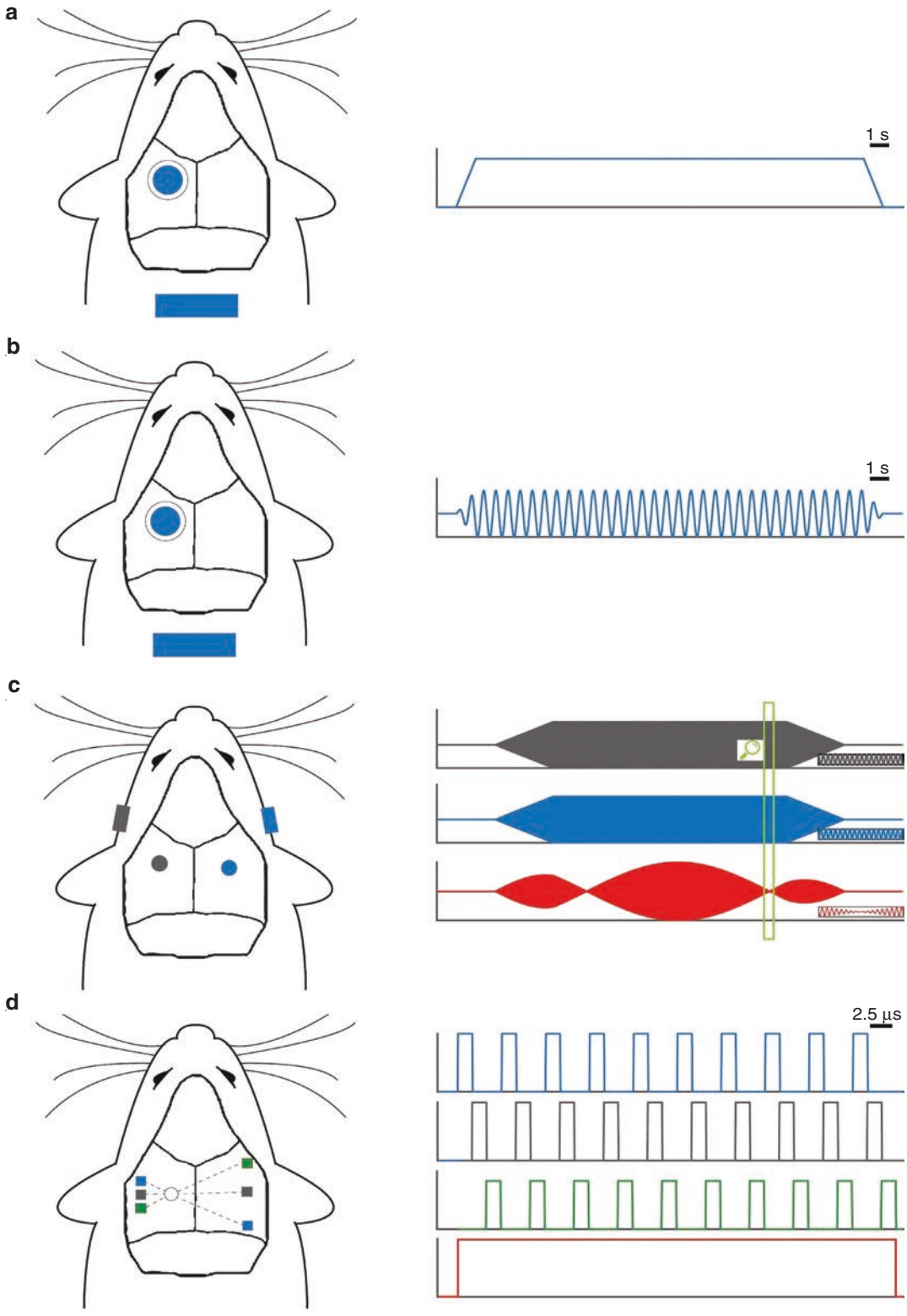
Direct Current Stimulation (DCS) and Alternating Current Stimulation (ACS)

Direct current stimulation (DCS) and alternating current stimulation (ACS) are two conventional waveforms used in animal studies. In DCS, a constant and unidirectional direct current is used to generate the static electric field between anode and cathode electrodes (Fig. 4.2a). In ACS, an alternating current flows between the pair of electrodes (Fig. 4.2b). Applied ACS generally refers to sinusoidal waveforms. When different pulses such as monophasic, charge-balanced biphasic, or charge-imbalanced biphasic are used, this is typically not called ACS (tACS) in the literature. While most research conducted on animals predominantly studied the effects of DCS, there is also a considerable number of studies on the effects of ACS.

High-Definition Stimulation (HD)

Datta et al. first proposed to use multiple small electrodes to achieve more focal stimulation as compared to conventional stimulation with large sponge electrodes [78]. These small electrodes are now often referred to as "high-definition" electrodes. Dmochowski et al. suggested an optimization method for where to best place these multiple small electrodes to obtain more focal stimulation in a specific brain area of interest [79]. The approach can also be used to maximize the intensity of stimulation on a target in the brain, with fixed constraints on the scalp currents. This method can also be used to increase the total intensity of stimulation by distributing currents across multiple electrodes [80]. Since any waveform can be applied using HD electrodes (HD-tDCS, HD-tACS, pulsed), this mode of stimulation should be thought of as an electrode configuration method [81].

Fig. 4.2 Schematic of different tES techniques applied to in vivo animal models [50, 82]. (**a**, **b**) The active electrode is placed over the area of interest and the returning electrode is usually attached on the neck or the chest to deliver (**a**) conventional tDCS waveform or (**b**) conventional tACS with an alternating waveform as examples. (**c**) TIS in which two pairs of electrodes are used to apply two high-frequency sinusoidal current waveforms (black and blue waveform). An amplitude-modulated signal will be generated in deep brain structures (red waveform). (**d**) IPS. Multiplexing between different pairs of electrodes. Each waveform depicts one of these short pulses. Note, in conventional tDCS and tACS, the resulting brain electric field waveform directly tracked the applied current (same trace) with a weight dependent on the brain region location, while in TIS and IPS the resulting brain electric field is a weighted sum (for each region) of the applied currents


a
1 s
b
1 s
c
d
2.5 µs

Temporal Interference Stimulation (TIS)
Temporal interference stimulation (TIS) consists of at least two pairs of electrodes delivering high-frequency sinusoidal AC stimulation on the scalp. The stimulation frequency of electrodes differs from each other slightly, such as 2 and 2.01 kHz, causing interference that can result in amplitude-modulated electric fields in deep structures of the brain (Fig 4.2c). The amplitude of fields is modulated at the difference frequency, 10 Hz in the example. Grossman et al. have argued the unmodulated kHz frequency component has little or no effect on neurons with a slow membrane response of ~30 ms [82]. On the other hand, amplitude-modulated (AM) electric fields can modulate neural firing rates. However, recent in vitro experiments suggest that field magnitudes required for this response to amplitude-modulated fields need to be significantly larger than the ones used in other tES approaches [77]. This study aims to understand the mechanisms governing both sensitivity and selectivity to TIS. Computational modeling of field distribution in the brain suggests that one may in fact achieve focal amplitude modulation in deep brain areas [83, 84]. However, the intensity of modulation is smaller than with conventional HD stimulation, and the unmodulated high-frequency fields are much stronger on the cortical surface [77, 84].

Intersectional Short Pulse (ISP)
Vöröslakos et al. suggested a new tES protocol to distribute current spatially similar to conventional HD-tES [50]. In this technique, which is called "intersectional short pulse" stimulation, current pulses are delivered in temporal succession across a sequence of scalp electrode pairs. While each pair is active for only ~60 μs, the polarization of the neuronal membrane sums up the effect of the electric fields of all pulses due to a slow membrane time constant (Fig. 4.2d). One suggested advantage of ISP is the ability to deliver higher current intensities while limiting the average current delivered through each electrode. The net effect is similar to the HD stimulation whereby scalp currents are distributed in space by virtue of controlling the maximum current through each electrode, while with ISP the current is distributed in time [80]. For both ISP and TIS, the argument is made that the high-frequency currents at the scalp surface minimize peripheral sensation. However, a recent study on skin sensations with various waveforms challenges this claim (under preparation).

4.3 Stimulation Artifact in Recording

Electric stimulation generates voltages in the tissue that are several orders of magnitude larger than electrophysiological signals: several volts of artifact caused by stimulation versus millivolts of neural activity for intracranial recordings, and microvolts for scalp recordings. Therefore, a frequent problem when attempting to record neural signals during stimulation is the distortion or saturation of the recording amplifier. To avoid this, (1) the amplifiers need to have a sufficiently large dynamic range and intensity resolution to resolve the smaller neural signals; (2) appropriate analog filters can be implemented; and/or (3) additional steps to minimize or correct for stimulation artifacts can be implemented. Overall, any approaches to manage stimulation artifacts should consider the features of interest in the neural signals. For example, if the DC component of the recording is not important for the objective of the study, a high-pass filter can remove the voltage artifact caused by DCS. Measuring the slope of fEPSP is an example of such a recording [35]. Moreover, aspects of the recording apparatus itself, such as drift in electrode conditions and field uniformity, may result in artifacts even under DCS.

A standard approach to reduce stimulation artifacts in neural recordings is to place a second recording electrode as a reference close to the electrode of interest. For example, when recording the transmembrane potential, one can subtract the adjacent extracellular electrode signal from the intracellular electrode since both electrodes have identical artifacts due to proximity. Another possible approach is to place the second electrode on the isopotential line with the

first one, where the iso-potential electrode location is selected as a region with comparable artifact as the recording electrode but not comparable electrophysiological signal of interest. The above approach has proven effective for extracellular potential recording and current-clamp recording under diverse conditions [11, 16]. Voltage-clamp recording under conditions of ongoing extracellular stimulation should only be conducted with caution over the possibility the amplifier will "correct" for the artifact producing a "signal" that reflects the artifact.

An additional source of distortions for relatively high-frequency stimulation is capacitive coupling at the electrode. This occurs for kilohertz-frequency stimulation as well as any kind of rectangular or pulsed waveform which contains broad-band components that are difficult to remove. Examples of such capacitive effects are capacitive-walled glass recording electrodes [85]. This distortion is magnified in patch-clamp and even sharp intracellular recording electrodes since they have higher resistance and capacitance [85]. In addition, amplifiers can be another source of distortion such as patch-clamp amplifiers [86].

For in vivo recordings, one should also note that nonstationarity of the current flow pattern due to movement, including cardioballistic, can cause large irregular voltage fluctuations even under DCS, that is the simplest of all waveforms [87, 88]. An example of that is the pulsing of the blood that causes large voltage fluctuations during DCS, which are particularly pronounced in scalp recordings [89]. A recent study using intracranial recordings and sinusoidal AC stimulation found it difficult to remove the AC artifacts due to nonstationarity, for example, subject movements [90]. AC stimulation with sinusoidal waveforms is narrowband and can in theory be removed. However, in practice, even small nonlinear distortions can lead to harmonics that contaminate the signal across the frequency spectrum. One of the few neural features that can be measured with little risk for stimulation artifacts is neuronal firing with microelectrodes. The distinct unitary spiking events are distinguishable enough from stimulation artifacts so that they can readily be identified [11, 27, 40, 50, 68]. Otherwise, local field potentials or EEG activity in concurrent stimulation should always be evaluated with great care. The only way to really rule out confounds from stimulation artifacts is to measure effects on the neural activity before and after stimulation.

4.4 Safety

4.4.1 Dose-Response and Safety

Any attempt to develop safety standards for any tES protocol requires assumptions to be made about dose-response. One approach to the dose-response curve is to use the lowest documented current intensity that produces a measurable destructive brain tissue response in an animal model at any stimulation duration. Animal studies have so far presented a wide range of thresholds that may be considered "safe." It is difficult to establish a single lowest threshold for tissue damage because of differences in methods across animal studies. Studies differ in stimulation setups, the number of animals used, the state of the animals undergoing tES, the time at which an animal is euthanized post stimulation, etc. [6–8, 51]. Animal studies are also limited in time points for measurement of tissue damage since the collection of tissue for analysis often requires terminal procedures. Therefore, there is a general lack of long-term follow-up. But perhaps the strongest limitation is the difficulty in equating invasive animal studies with noninvasive tES in humans. It is not clear if the relevant translational measure is current density, field magnitude, total current, total charge, or total charge per volume or per area of tissue [10].

In addition, the relative sensitivity of animal versus human tissue to tES injury is unclear. While developing safety guidelines could be challenging, rodent studies focusing on brain injury are summarized here. It is prudent not to approach injury thresholds derived from rodent studies when developing human safety guidelines. Given the electrode montage and interindividual differences, and scaling consolidated

animal tES safety data to humans, computational models have indicated that conventional tES protocols are orders of magnitude below the threshold for damage [91]. Since most in vivo animal studies investigated the safety limits of tDCS, we will focus most of the next section on the available findings of tDCS safety limits.

4.4.2 Safety Limits for Tissue Injury

Animal studies have been used to identify the intensity and duration of tDCS at which brain damage first manifests. Data establishing the safety limits solely focus on current intensity or charge density [6, 92]. Results from the three main studies investigating the safety thresholds for epicranial tDCS, measured in terms of brain lesions, are summarized in Table 4.1 [6–8]. All studies applied tDCS using an electrode on the surface of the rat skull. This epicranial electrode contact area was smaller relative to the return electrode positioned on the body. Given the variation in stimulation parameters summarized in Table 4.1, the lowest tDCS current intensity at which histological damage was recorded for each study was: (1) Liebetanz: 500 μA applied through 2.1 mm diameter circular electrode (3.5 mm^2 surface area) for 10 min; (2) Fritsch: 600 μA applied through 4 mm diameter circular electrode (12.5 mm^2 surface area) for 20 min; and (3) Jackson: 500 μA applied through 5 × 5 mm square electrode (25 mm^2 surface area) for 60 min. The discrepancies between the results of the three studies might arise from the variability of electrode montage, that is, size and location of the return electrode.

One might argue that the presence of lesions indicates that the brain has already undergone damage. Are there more sensitive safety measures than brain lesions? The inflammatory response is one of the sub-lesion predictors of brain injury, which has been evaluated in a few studies [7, 8, 51]. However, these three studies

Table 4.1 In vivo animal studies deriving the safety limit for tDCS-mediated tissue injury

Author	Liebetanz et al. [6]	Jackson et al. [7]	Fritsch et al. [8]
Species	Rat	Rat	Rat
Stimulation method	Epicranial	Epicranial	Epicranial
Stimulation polarity	Cathodal	Anodal	Anodal
Area of stimulation	Frontal cortex	−2.5 mm Bregma	Motor cortex
Return electrode	Rubber plate on chest (with jacket)	On the neck	Implanted platinum plate on the chest
Stimulation duration	10, 30, 90 or 270 min	60 min	20 min
Electrode surface area	3.5 mm^2	5.3, 10.6 and 25 mm^2	12.56 mm^2
Current intensity	1,10, 50, 100, 500, and 1000 μA	150, 300, 500, 100 and 2500 μA	600
Damage detection	H&E staining	H&E, Iba1	Fluoro-Jade C stain
Brain state	Anesthetized	Anesthetized	Anesthetized and alert
Threshold for neurodegeneration (electrode current density)	143 A/m^2 (10 min of stimulation)	20 A/m^2	47.8 A/m^2
Threshold for neurodegeneration (electrode charge density)	52,400 C/m^2	72,000 C/m^2	57,325 C/m^2
Threshold for neurodegeneration (electrode current intensity and surface area, duration)	500 μA 3.5 mm^2 10 min	500 μA 25 mm^2 60 min	600 μA 12.5 mm^2 20 min
Scaling factor	240	134	288
Estimated current intensity threshold for humans	120 mA	67 mA	173 mA

Scaling factor and resulting human thresholds are adapted from [9]

had a different timeline for euthanasia after tDCS for pre-lesion analysis which may affect the result. Nonetheless, an increase in immune and inflammatory biomarkers such as microglia is observed at the current intensities higher than the ones used in behavioral studies. It is worth noting that these intensities are also close to the lesion thresholds. Fritsch et al. reported the activation of microglia 24 h after tDCS at the electrode current density of 31.8 A/m^2. They found this value to be lesser than the electrode current density threshold needed for neurodegeneration, that is, 47.8 A/m^2 [8]. They also suggested that the current density threshold ranging between microglial activation and neurodegeneration can evoke a pre-lesional inflammatory response. An earlier rodent study reported an increase in the density of microglia after both anodal and cathodal tDCS within the stimulated brain region [51]. This increased density would suggest microglia shift toward their active state during tDCS. Another study on microglial activation also used both anodal and cathodal tDCS on mice at the current intensity of 0.1 mA and found that the microglial processes were shorter, indicating their activation, when observed immediately after tDCS but normal when observed 3 h post tDCS [20]. Both studies indicated that tDCS shifts microglia to their more active state in two different ways. One possible way is that morphological changes in microglial cells occur as the primary results of tDCS or as the result of tDCS-induced neurodegeneration.

High-resolution computational modeling has been helpful to scale the results from animal studies to approximate the safety thresholds in tDCS applications on humans. However, these estimated safety thresholds have to be considered with caution due to some limitations including what we outline here. It is possible that the susceptibility of humans and tissue to damage from tDCS is different. In addition, there are experimental limits for detecting various modes of damage, including dose-response assumptions. Moreover, anatomical differences can complicate scaling rodent results from rat to human predictions. Finally, variations in the method of stimulation, that is, transdermal versus epicranial, can lead to different safety limits [93]. In spite of the limitations of basing human safety standards on rat histology, including lack of long-term data and associated behavioral changes, this data represent an outer safety limit that cannot be approached during clinical tDCS.

The computational rat model by Jackson et al. predicts the current produced in the brain for the three studies summarized in Table 4.1 [9]. They derived a scaling factor by comparing the resulting peak electric field in the brain per mA at the electrode in rats to the peak electric field produced in the brain per mA at the electrode in humans. This scaling factor allows for the prediction of current magnitude that needs to be applied in the human using a common montage (M1-SO) to approximate the electric field produced in the brain of a rat for a given current. Applying this scaling factor to the damage threshold observed in each of these rodent studies allows us to predict a current intensity damage threshold in humans. The estimated scaling factors are within the range of 134–288 for the three studies in Table 4.1 [7]. Utilizing the reported current intensity thresholds for damage in animal models and the aforementioned scaling factors, Jackson et al. reported the range of 67–120 mA as the predicted human damage threshold. While there is considerable variability in these thresholds, they are still approximately two orders of magnitude above maximum currents intensities used during tDCS on humans.

Prior studies determined the tDCS safety thresholds by changing current intensity, electrode surface area, and stimulation duration (Table 4.1). It is worth noting that a similar current intensity threshold, with similar parameters and tDCS method, leads to considerable neuronal damage in awake animals as compared to the anesthetized ones [8]. This will have bearing on scaling the rodent data to direct human tDCS safety measures as human experiments are conducted on subjects in an awake state.

What could be the exact mechanism for the tDCS induced lesions? Even though excitotoxicity and heat generated by stimulation are among the suggested mechanisms [6, 94], there is insufficient experimental evidence to support the claim.

There is a scarcity of animal models explicitly considering the safety limits of tACS. It is not clear that injury mechanisms for DCS and ACS are comparable and so how much studies of tDCS safety informs tACS. There are hundreds of studies that did not explicitly address safety but did not report any damaging, lasting aftereffects following application of clinically relevant intensities [26–29, 40]. Among these are many studies that applied intensities much higher than used in humans [26, 28]. For both tDCS, tACS, and other forms of noninvasive electrical brain stimulation, one can rationally consider these studies as providing indirect evidence for safety. However, it should be noted that many human studies did report lasting aftereffects following application of clinically relevant intensities [95–98].

Our knowledge of the only safety data on transcranial TIS (tTIS) comes from a study in awake mice [9]. In this study, tTIS was applied with a current intensity of 250 μA for 20 min distributed over two electrode pairs. This did not cause measurable tissue damage as assessed with neuronal density, number of apoptotic cells, or DNA damage. In their functional evaluation, however, currents were three times stronger, which would have generated fields in the order of 400 V/m [11].

Another safety concern is with regard to the effect of tES on preexisting neurological conditions. A few studies have investigated the effects of tES on animal stroke models. Kim et al. assessed whether DCS increased preexisting infarct volume in a rat stroke model [99]. Their results showed no increase at the doses tested at 100 μA for 20 min and 0.785 cm^2 surface area of the epicranial electrode. But they found a potential neuroprotective effect in the form of reduced neuronal axon deterioration. Another group also reported protective effects of intracranial cathodal stimulation, that is, DC, 2 and 10 Hz at 100 μA, in ischemic stroke rats while they did not observe any significant effect at 50 Hz stimulation [100]. However, results from a study in a mouse model presented different effects of DCS on postischemic lesion volume [101]. According to Peruzzotti-Jametti et al., anodal DCS at 250 μA for 40 min with 4.52 mm^2 surface area of the epicranial electrode worsened the lesion volume and exacerbated the dysregulation of postischemic blood-brain barrier, whereas the cathodal DCS had a neuroprotective effect. This discrepancy between the results obtained from rat versus mouse study could be associated with the smaller size of a mouse's brain compared to that of a rat [91].

4.5 The Quasi-Uniform Assumption

Replication of tES human experiments in animal studies cannot merely be done by using the same stimulation parameters or by scaling down the stimulation parameters by some (arbitrary) factor (e.g., mice are X smaller than humans, so tDCS is applied to mice with X less current and X less electrode size). These clinical parameters include stimulation waveforms (tDCS, tACS), electrode montage, that is, shape and location, and the specifics of the waveform, such as duration, intensity in mA applied, and ramp. It is noteworthy that the electric field varies across different brain regions as the current flow has a complex spatial pattern across the brain. This results in a dose-specific electric field (current density) that varies significantly across the brain regions. The electric field distribution across the brain represents and determines the electrical actions of tDCS.

The electric field across the brain is not a simple function of any dose parameter. For example, the electrode current density does not map simply to the peak electric field in the brain [102]. Datta et al. estimated the electric fields generated in the brain using computational modeling [78]. They introduced computational models using realistic anatomy, and their estimation of peak electric field generated during tDCS has converged to between 0.2 and 0.5 V/m (0.05–0.14 A/m^2 current density) for a 1 mA intensity. Electric field scales linearly with a current intensity such that 2 mA would produce a range of 0.4–1 V/m (0.1–0.28 A/m^2 current density). These peaks represent local electric field maximum, and weaker electric fields are generated across much of the brain using conventional tDCS montages.

In addition, due to subject-specific idiosyncratic cortical folding, the electric field is clustered [78], with many local maxima (Fig. 4.3a). There is thus no single uniform electric field generated in the brain during tDCS but rather a range of electric field magnitudes varying across the brain. Therefore, the question is: Given this complexity of electric field distribution across brain structures, what can and should be mimicked in animal models?

One solution is to calculate the electric field in the brain region of interest, and then to replicate the selected electric field in the animal model (Fig. 4.3b, c). This approach replicates the electric field which is approximately uniform at the length scale of individual neurons [103] (Fig. 4.3a). This approach is supported by evidence suggesting electric fields generated during tDCS are largely uniform across any specific cortical column (neuronal dendritic tree) of interest (Fig. 4.3b); hence, one can speak of a single electric field in reference to a region of interest.

However, it is important to realize the limitations of the quasi-uniform assumption. Considering the peak of the electric field either across the whole brain or in a subregion can result in a discrepancy between expected and actual electric field. One reason for this mismatch is that field amplitude can change by orders of magnitudes in different brain regions and even across local gyri [30, 40]. The average and/or median value of the electric field can be up to ten times smaller than the peak amplitudes depending on local geometry and conductivity properties. Another consideration is that the coupling constant might vary across species. For example, given the same electric field stimulation to both a human and a rat cortical neuron, the amount of

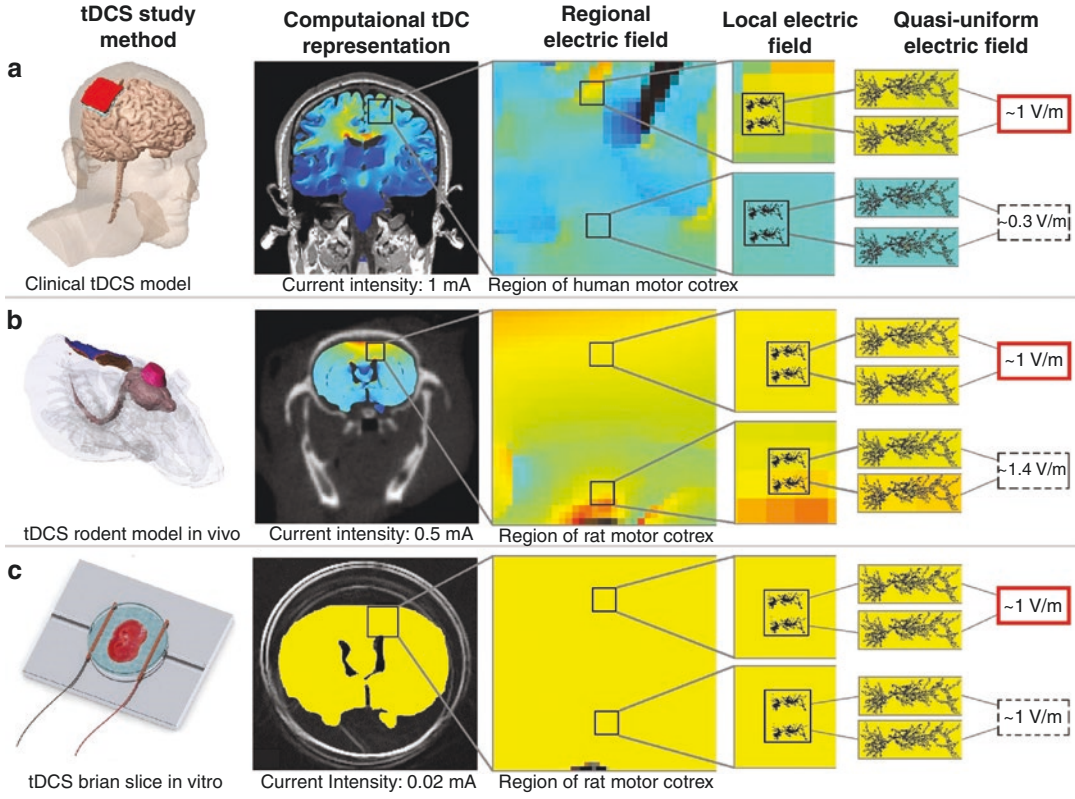


Fig. 4.3 The quasi-uniform assumption in modeling and animal studies. A high-resolution finite-element method (FEM) computational model of predicted current distribution during tDCS in a slice of the whole brain, a cortical column, and a neuron in (**a**) human, (**b**) rat in vivo, and (**c**) rat brain slice in vitro

neuronal polarization can be different. This species-dependent discrepancy is due to different size and geometry of neurons as will be explained more in detail in Chap. 6.

In the following, we address the limitations and approaches to estimating field magnitudes for each category of animal research:

1. *Transcranial stimulation*: Similar to the procedures in human tES, the computational approaches can be used to model the electric field across the brain and guide the stimulation design [104–106]. For example, the position of the return/reference electrode affects the current flow even under the active electrode [107, 108]. The recent development of anatomically precise animal models can be helpful for the design of future studies [83, 109–111]. An alternative method is to incorporate concentric sphere models scaled to size to determine the electric field intensity generated in the animal brain [42]. In cases where the electrode is placed directly on the skull, one can, to a first approximation, assume a maximum potential current density in the brain is equal to the average electrode current density [92]. However, it is important to address the direction of current flow as the direction of the electric field may vary across the brain. This can be more complicated in deep structures of the brain or animals with a more gyrated cortex. To measure the electric field directly, intracerebral electrodes must be placed in a region of interest [40, 50]. It is important to realize that the electric field is not uniform throughout the animal brain, and the insertion and presence of electrodes may itself distort current flow.
2. *Intracranial stimulation*: Here similar considerations apply as above. One could assume that current density under the electrode in the brain is equal to the average current density at the electrode. However, depending on the electrode design, the current density may be orders of magnitude higher at electrode edges [112–114]. This is an issue that is aggravated for small electrodes where the electric field near a monopolar source can be very high leading to further complications [31]. As with scalp electrodes in tES, when a sponge of cotton wrapper is used, its contact areas should be used in calculations [9].
3. *In vitro studies*: Experimental design is more straightforward in this category. In these experiments, long parallel wires or plates are placed in a bath across the entire tissue (Fig. 4.3c). If it is done carefully, this method generates a uniform electric field across the entire tissue and can be readily calibrated to match tES levels [11, 65, 115]. The uniformity of the electric field across brain slices has been verified [11], though exceptions have been reported [36]. The presence of conductive fluid around the brain slices may dull any laminar inhomogeneity effects to resistivity. Due to electrochemical reactions at the interface of electrodes and the fluid, the electrodes should be placed away from the tissue of interest in the bath.

4.6 Dose Translation and Meaningful Animal Studies

One of the most fundamental sources of ambiguity in interpreting and designing meaningful animal tES experiments relates to dose. Many proposed mechanisms of action are based on animal studies in which the electric field intensities or durations are higher than those of clinical trials. It is not clear that these high-intensity experiments scale proportionally to lower dose human experiments. Animal experiments often intentionally select high intensities for stimulation so as to more reliably detect small effects, for example, [11, 15, 19, 82, 116]. Though early animal studies remain informative about tES mechanisms, their techniques were invasive and intensities of electric field stimulation were higher than during tES on the human scalp [117]. Recent in vivo animal studies have often used higher current densities compared to human experiments while adopting a noninvasive method of tES [8, 118].

The assumption of a monotonic relationship between intensity and outcome can be problem-

atic due to the nonlinear nature of nervous systems. One possible issue is the asymmetry in the strength of the electric stimulation effects with changing polarity [15, 19]. According to these results, effects achieved under one electric polarity cannot be simply reversed by changing the polarity. Some have argued that high-stimulation intensities can produce opposite effects [119]. As discussed later, DC electric fields can increase excitability and elevate evoked responses (e.g., synaptic efficacy) in a polarity specific manner. But if the DC intensity is increased significantly, neuronal excitability may increase to a point where the neuron generates high-frequency discharges, and the responsiveness of a very active neuron to a stimulus may then decrease. This phenomenon has been shown in brain slices [11] and may explain in vivo results using high DC current intensities [120]. One example of this type of nonlinearity has been reported in the application of tDCS to the motor cortex to modulate motor evoked response (MEP) in human experiments [121]. Based on their results, cathodal tDCS at two different current intensities had the opposite effect on MEP, that is, switching from excitability diminution to enhancement. Overall, the nonlinearity and state dependence of dose-response may be pertinent to the understanding of mechanisms and rational optimization of tES techniques.

However, in vitro studies that explored field strength-response curves did indicate a surprisingly linear response curve over low intensities in their results [11, 15, 28]. In particular, membrane polarization appears to be linear with electric field strength, which is quantified by the neuronal coupling constant [11, 16, 28]. In vitro studies that have explicitly explored the lower electric field limit of sensitivity to fields reported statistically significant responses at <0.2 V/m, which is within human tDCS range [28, 115, 122].

Regardless, we urge caution when transferring conclusions from animal studies with high field magnitudes (>5 V/m) to clinical tES with lower intensities (<1 V/m). While these experiments are valuable for suggesting tES mechanisms, just as with drugs, increasing the dose beyond clinical levels by orders of magnitude can induce physiological changes that are not clinically relevant. For example, some animal studies have shown DC application can control the orientation of neuronal processes and their growth direction [123, 124], but both the duration and intensity of electric fields were often orders of magnitude greater than tDCS used in clinical settings. Additionally, mechanisms such as electroporation and joule heating can be produced by some forms of electric stimulation, but the waveforms required to produce these effects are not relevant to tES [6, 92, 125]. Thus, some mechanisms which require waveforms incompatible with tES, and their associated animal studies, are not considered further here.

The issues surrounding dose-response are important yet are often overlooked when translating from animal to human tES. Dose translation is inherently linked with mechanism, affecting experimental design. Deciding which stimulation parameters are considered relevant for scaling, and the insights from animal models can shape clinical practice, including dose optimization.

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