HHS Public Access

Author manuscript

Curr Behav Neurosci Rep. Author manuscript; available in PMC 2019 June 01.

Published in final edited form as:

Curr Behav Neurosci Rep. 2018 June ; 5(2): 125–135. doi:10.1007/s40473-018-0149-6.

Using animal models to improve the design and application of transcranial electrical stimulation in humans

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Abstract

Purpose of Review—Transcranial electrical stimulation (tES) is a non-invasive stimulation technique used for modulating brain function in humans. To help tES reach its full therapeutic potential, it is necessary to address a number of critical gaps in our knowledge. Here, we review studies that have taken advantage of animal models to provide invaluable insight about the basic science behind tES.

Recent Findings—Animal studies are playing a key role in elucidating the mechanisms implicated in tES, defining safety limits, validating computational models, inspiring new stimulation protocols, enhancing brain function and exploring new therapeutic applications.

Summary—Animal models provide a wealth of information that can facilitate the successful utilization of tES for clinical interventions in human subjects. To this end, tES experiments in animals should be carefully designed to maximize opportunities for applying discoveries to the treatment of human disease.

Keywords

transcranial electrical stimulation; tDCS; brain stimulation; neuromodulation; animal models; plasticity

Introduction

The technique of transcranial electrical stimulation (tES) relies on the application of weak electrical currents on the scalp. These currents can have different spatiotemporal patterns. In the last 18 years, transcranial direct-current (DC), alternating-current (AC) and random-

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Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

The authors declare that they have no competing interests.

noise current stimulation (commonly known as tDCS, tACS, and tRNS, respectively) have been used to neuromodulate different regions of the brain and their associated functions [1]. Despite the high number of published studies (especially for tDCS) [2,3], the neural mechanisms underlying tES and the rationale behind the choice of a specific tES modality, as well as the selection of the specific stimulation parameters (duration, polarity, intensity, frequency or location), remain unclear.

The main objective of this review is to summarize studies that have used animal models to improve stimulation protocols and to develop tES-based applications for enhancing normal brain function, and for treating a variety of neurological disorders. These studies in animal models have helped bridge a number of gaps in our knowledge of tES, such as basic mechanisms mediating its effects, the definition of safety limits, and the experimental validation of computational models. In the final section, we will discuss existing limitations for translating animal-based results to human studies and how we could possibly minimize them.

Understanding basic mechanisms of tES

A full understanding of the mechanisms underlying the effects of tES on brain activity requires electrophysiological measurements, local pharmacological manipulation, fine histological characterization and well-established behavioral tasks [4]. Animal models, including *in vitro* slice preparations as well as *in vivo* whole animal, anesthetized or awake, allow the application of powerful tools required to achieve a clear understanding of the impact of tES on brain function. The study of tES effects comprises (1) immediate effects observed in neural activity when the externally applied electric field enforces the displacement of intracellular ions altering the internal charge distribution and modifying the membrane potential of the neuron [4,5], (2) and the long-term effects mediated by protein modifications. Recent studies have begun to clarify how the immediate and long-term effects of tES are linked to related changes in local pre- and postsynaptic elements, including specific receptors, neurotransmitter systems, and glial cells, as well as the global impact of these changes on large-scale neural networks, and general blood perfusion levels in the brain [4,6].

After seminal investigations demonstrating the modulatory effect of epidural DC stimulation in anesthetized animals in the 1960s [7–9], and posterior descriptions about the underlying molecular mechanisms in the 1990s [10–12], various animal models appeared as a consequence of the new interest in transcranial application of DC for human brain modulation [13,14]. Early work indicated that anodal and cathodal DC stimulation leads to an increase and a decrease of neuronal excitability respectively [7,8,13]. Since then, *in vitro* studies have revealed a more complicated picture, by demonstrating the importance of different neuronal features, such as the orientation of somato-dendritic axes with respect to the electrical field [15], the neuronal morphology [16] or the axonal orientation [17]. In addition to investigating how single-neuron excitability is modified in response to externally applied electrical fields, other studies have focused on the modulation of synaptic events (at both presynaptic and postsynaptic sites). *In vitro* [16–22•] and *in vivo* animal models [23,24] have been used to demonstrate modulatory effects of DCS on excitatory postsynaptic evoked

potentials, suggesting the existence of tES mechanisms which affect the vesicle release probability at presynaptic terminals [17,23,25]. Moreover, the implication of glial cells [26], and different receptors, such as NMDA [27], mGluR5 [28], AMPA [29], and adenosine receptors [10–12,23], together with the involvement of neurotrophic BDNF [18,27] and the activation of early genes participating in protein synthesis [18,30], have been demonstrated in numerous animal-based experiments.

In addition to local effects, the impact of tES on neuronal populations distant to the electrically stimulated region has also been reported in animal models. Thus, neural processing in the pyramidal tract as well as the reticulo- and rubrospinal pathways is facilitated when tES is applied over different skull regions in anesthetized animals [24,31,32]. More recently, it has been reported that tDCS applied to the prefrontal cortex affects LFP coherence inducing a decrease in low frequencies between distant cortical sites and an increase in high frequencies between local sites [33•]. Finally, polarity-specific effects induced by tES on blood flow over wide areas of the brain [34,35] have also been characterized in animals. Based on these animal studies, it is clear that clinical application of tES must take into account that neuromodulation will not be restricted to the local brain area being stimulated.

Defining safety limits

Although tES is delivered with low-intensity currents in humans, concerns may arise about the technique when considering its safety limits, especially when protocols aim to increase intensity or duration of the applied electrical field, the number of stimulating sessions or when studies are performed in susceptible individuals (e.g., children) [36]. Nevertheless, only a few studies have addressed the issue of safety in human trials, mainly using computational models [37], or by assessing behavioral changes [38], the occurrence of skin erythema [39], and injury-related alterations of the blood-brain barrier or cerebral tissue detected by MRI [40]. However, the establishment of safety parameters for tES requires characterization of a dose-response curve, determination of a density threshold for histological damage and the impact of electrical fields on molecular markers that mediate neuroinflammatory processes [36].

Despite the obvious interest in the use of animal models for defining safety limits, it was not until 2009 that the first systematic study about safety aspects of tES was published. In this work, Liebetanz and colleagues evaluated the minimum current density (intensity/electrode surface area), as well as the minimum charge density (current density × time) necessary to cause first tissue damage by epicranial tDCS in rats [41]. Testing different current intensities and durations of cathodal tDCS, the authors estimated a charge density threshold of 52400 C/m^2 for histological damage, with no detectable tissue lesions below a current density of 28.6 A/m^2. On the other hand, brain tissue remained lesion-free with current densities between 142.9 and 287.0 A/m^2 when the charge density was set below 52400 C/m^2. This data suggests that the current densities usually applied in human studies (171 – 480 C/m^2) are situated approximately two orders of magnitude below the calculated lesion threshold. Similarly, different tDCS protocols employed in subsequent animal-based studies have revealed harmful effects when current densities surpassed a threshold still significantly

above the values applied in humans [42–45•]. A recent study performed in rats reported a histological lesion threshold of about 20 A/m^2 of current density for anodal tDCS being slightly below the previously established lesion threshold of 28.6 A/m^2 [45•]. However, the threshold of charge density in this study was 72000 C/m^2 (vs. 52400 C/m^2 determined for cathodal tDCS), which highlights the importance of taking into account different parameters (stimulation time and density current) to assess the safety of tES.

Monitoring exclusively macroscopic lesions in brain tissue is not sufficient to define safety standards for the use of tES. Changes in the level of different molecular mediators involved in brain injury processes as a consequence of current electrical field application should be considered. Neuroinflammatory responses with density currents below established lesion thresholds have been observed, consisting of an upregulation of the innate immune response after both anodal and cathodal stimulation, as well as an increased number of neural stem cells with a directly proportional relationship to cathodal tDCS sessions [42]. A good example of tES safety characterization in mice has been recently reported for temporally interfering electric fields. In this inspiring study the authors demonstrate the safety profile of this new stimulation protocol by comparing immunohistochemically cellular and synaptic molecular profiles (apoptotic, DNA damage, microglial and astrocyte markers) in the stimulated vs. the non-stimulated hemisphere and with respect to a sham condition [46••].

In conclusion, animal models constitute a valuable approach for defining safety limits allowing for a systematic and in-depth analysis of tES-related changes in the brain. Despite the low number of currently published papers focused on assessing safety thresholds for different types of non-invasive brain stimulation, the growing interest in human tES presumably will boost new animal studies in the near future.

Validating computational models

Two critical aspects of tES, namely the impact of the externally applied electrical field at the level of single neurons and neural networks [47], and the distribution of the electrical field in the brain [48,49], have been addressed by computational models that are based on the underlying biophysical properties of the brain. Because these biophysical models rely on physiological observations (i.e., neuronal response to exogenous electric fields or brain conductivity and geometry) obtained in both human subjects and animals [50], they provide valuable information for the optimization of tES protocols through the definition of proper electrode positions and size, as well as duration and intensity of current stimulation. In this context, computational studies can benefit from work in animal models, which supply fundamental physiological knowledge and facilitate the posterior validation of model predictions.

The synergy between experimental approaches based on computational and animal models is readily apparent in recent studies. For example, Bikson and colleagues have used data from animal experiments to make a number of major contributions to the design and development of biophysically-constrained computational models of tES [22•,45•,51]. One study used a combination of a multi-scale computational model and slices from rat cortex to determine the current diffusion and the effects of the electrical field orientation on the

polarization of different cellular compartments [51]. The authors based their simulations on physiological data previously obtained in animal models, which demonstrated a key role of the axo-dendritic axis orientation [15], as well as the morphology and orientation of the different subcellular compartments [15,51,52] in the electrical field. In another example, computational neural-mass models have been used to reveal a key role of polarization of interneuron populations in reproducing the effects of tES on real sensory potentials recorded in the cortex of alert rabbits [53].

Animal studies have also contributed to the success of computational models that explore electric field diffusion in the brain, by providing physiological data to represent stimulated tissues (scalp, skull, cerebrospinal fluid and brain) [23], as well as MRI and micro computed tomography scans that can be used to capture the underlying neural geometry [45•]. These models can be used to obtain an estimate of the strength of the electric field that is more realistic than the simple current density value applied to the stimulating electrode. Some of the key predictions of these computational models are supported by a recent study performed in human and nonhuman primates, which characterized the spatiotemporal distribution of intracranial electric fields induced by tES and found that electric fields act in a linear ohmic manner with greatest strengths in superficial brain regions [54••].

In summary, animal models provide an excellent opportunity to directly measure different parameters related to the strength of externally applied electrical fields during tES, helping to guide the design of future stimulation protocols by constraining the assumptions and validating the predictions of computational models.

Exploring new tES protocols

The animal-based validation of present tES protocols, together with other experimental approaches proposing a more unconventional application of tES, are of highest priority for the future application of the technique in human subjects.

The ubiquitous nature of oscillations in the brain [55] and the increasing interest in using tES protocols with higher physiological significance instigated the application of tACS in human subjects to enhance, or reduce, neuronal activity at specific electrocortical frequencies [56•,57]. Different experimental designs in animal models have been used to validate and improve tACS protocols. The effects of sinusoidal AC electric fields on brain tissue have been examined *in vitro* by using animal brain slices [58–60]. For instance, AC electric fields applied in rat hippocampal slices at frequencies ranging from 10 to 100 Hz resulted in sinusoidal membrane potential fluctuations with peak-to-peak amplitudes that decay exponentially as a function of frequency [58]. The same study also highlights the importance of considering naturally occurring endogenous fields, which have been shown to modulate the ongoing activity of local cortical networks in acute slices [61], and increase the sensitivity to specific stimulation frequencies during tACS [58]. Similarly, combining the application of AC electric fields (0.8–2.0 Hz) with optogenetic stimulation in mouse neocortical slices demonstrated that endogenously generated oscillations constrain the neuromodulatory effects of the externally applied sinusoidal electrical field [59].

In addition to the *in vitro* approach, the modulatory effects of AC electrical fields at low frequencies (0.8–1.7 Hz) have also been reported in anesthetized rats showing reliably entrained neurons in widespread cortical areas [60]. The same study revealed that the effects of tACS on the discharge of single neurons depend on the animal's behavioral state, showing no response in the awake animal in contrast to a 25–50% of entrained neurons during sleep (tACS at 1.25 Hz). Importantly, tACS has been successfully used in behaving animal models to enhance sleep-dependent memory consolidation in rats [62] and more recently, to generate tactile perception when applied over the whisker somatosensory cortex of awake rabbits at specific frequencies (100 Hz) similar to the frequencies recorded during somatosensation [56•]. Also in this last study, tACS application at different frequencies (0.05–200 Hz) over the motor cortex of the awake animal suggested that the sub- or supra-threshold nature of tACS-associated effects depends on the frequency of the applied current.

Finally, information gained from animal models about the impact of oscillatory AC electrical fields on neuronal networks has instigated a movement toward more sophisticated stimulation approaches using arbitrary stimulating waveforms or even the combination of multiple electric fields. Thus, work in epileptic rats has demonstrated how seizure-triggered feedback tES (consisting of a 50-ms Gaussian waveform) can be used to successfully reduce spike-and-wave episodes [63]. In another application, the combination of slightly different frequency AC electrical fields (2.01 and 2 kHz) applied over two distant electrodes on the mouse scalp has been shown to stimulate neurons in deeper structures like the hippocampus without recruiting superficially located cortical neurons [46••].

Results from animal studies suggest an extensive margin of improvement in the application of non-invasive tES. Considering the current state of the art, it is not an exaggeration to affirm that the success of this technique in human application for basic research and clinical treatments will rely to a large extent on the findings we will make for optimizing and refining stimulation protocols in animal models.

Improving brain function

The possibility of enhancing brain function in healthy subjects through the application of tES is enticing. Indeed, it has been demonstrated that tES can improve visual contrast perception [64], spatial tactile acuity [65], motor performance [66,67], and learning and memory processes [68] in human subjects. Animal models offer a unique opportunity to explore new stimulation protocols aimed to boost brain function, particularly those that are unconventional and may be associated with higher risk. In this regard, animal models have shown tES-induced enhancement of sensory-motor, learning and memory processes, constituting a "proving ground" for future applications in human subjects.

Modulatory effects of tES on sensory processing have been demonstrated in the somatosensory and visual cortices of anesthetized [69,70] and awake [23,53,56•,71,72] animals. For example, a recent study performed in macaque monkeys showed that tACS at 10 Hz applied 4 cm anterior to the vertex attenuated sensory adaptation to visual stimuli [72]. In addition, the application of anodal and cathodal tDCS over S1 or primary visual cortex, respectively, increased and decreased simultaneous sensory evoked potentials

induced by tactile [23,56•,70] or visual [69] stimuli. The functional significance of the observed LFP enhancement during anodal tDCS has been examined by using whisker stimulation as conditioned stimulus in a delay paradigm in the classical eyeblink conditioning learning protocol [23]. The authors described long-term effects of tDCS over S1 after 20 min of cathodal currents (but not after anodal) consisting in a decreased amplitude of sensory LFPs in response to both whisker pad or ventroposterior medial thalamic nucleus electrical stimulation [23]. These asymmetric long-term effects of tDCS on sensory cortex constrain the underlying mechanisms to the thalamocortical pathway.

Initially described by Nitsche and Paulus in humans [13], modulatory effects of tDCS on motor function have been successfully reproduced in anesthetized mice showing increased or decreased amplitude of motor-evoked potentials in response to anodal or cathodal tDCS in M1 [73]. A recent study in anesthetized rats, using tACS over the hindlimb area of the motor cortex, highlighted the importance of the cortical excitation/inhibition balance for determining whether tACS ultimately leads to increases or decreases in cortical excitability [74]. Besides the modulation of cortical neurons, tDCS applied over the sensory-motor cortex can also have an impact on the excitability of subcortical motor systems, which has been demonstrated by recording neck-muscle EMG and descending volleys from the surface of the spinal cord in response to electrical stimulation of the red nucleus, medial longitudinal fascicle and the pyramidal tract in anesthetized cats and rats [24,31,75]. Interestingly, the long-lasting effects of anodal and cathodal tDCS on subcortical neurons resulted, respectively, in facilitation and depression of evoked motor responses in the cat [24], whereas the opposite modulation has been observed in the rat [31].

One potential of tES, actively studied in human subjects but less in animals, is the capability of improving learning processes and memory formation [68,76]. Based on the proposed capacity to modulate the functional connectivity of the brain [33•], and to enhance synaptic plasticity processes [20•,25,27] such as long-term potentiation (LTP), investigations using animal models have attempted to apply tDCS to boost performance in a variety of learning protocols. The frontal cortex has received much attention, likely due to the fact that frontal cortex neurons project to numerous brain areas [77–79], i.e., their modulation could affect performance of a variety of tasks. The encoding and retrieval of spatial memory rely on the correct functioning of the frontal cortex [80], and applying three sessions of pre-training cathodal tDCS in this brain area in rats resulted in improved long-term task retention [81], whereas in a different study twice-daily anodal tDCS for five consecutive days had a minimal impact on subsequent long-term spatial learning and memory [82]. Interestingly, this last study found a significantly enhanced exploration rate of a new item in a novel object recognition task, used to evaluate working memory [83], four weeks after the repeated exposure to anodal tDCS in the frontal cortex [82]. A different modality of tES combining tDCS with slowly oscillating electric currents at a frequency characteristic for slow wave sleep has been applied over the frontal cortex during post-learning non-rapid eye movement sleep in rats. The findings showed modulated endogenous neural oscillations together with improved hippocampus-dependent memory consolidation after receiving tDCS [62,84], confirming learning-related interactions between hippocampus and frontal cortex [85,86]. Notably, a single-session of anodal tDCS on the cortical area above the hippocampal formation improves both spatial learning and working memory in the short-term (2–24h) and

the long-term (7 days), verifying previously described tDCS effects on deeper brain structures [25,31]. Regarding immediate online effects of tDCS on learning, recent findings showed significantly facilitated associative learning performance with anodal stimulation over the prefrontal cortex during behavioral training in non-human primates. This observation was accompanied by an increase of low-frequency oscillations within the stimulated area, which possibly modified the functional connectivity of different brain regions [33•]. In a different study, repeated (20 consecutive days) bilateral anodal tDCS in S1 resulted in an improved accuracy of movements in rats performing a skilled reaching task [87].

Clearly, tES can have an impact on a variety of brain functions, even if the exact way tES is able to modulate behavior and enhance performance is not yet fully understood. Animal models provide an optimal approach to explore the use of tES for boosting specific brain functions and to start elucidating the underlying cortical and subcortical neuronal mechanisms. Although recent findings encourage the use of tES to improve different neuronal processes and treat different neurological disorders linked to impaired brain function in human subjects [88], animal studies are necessary in order to discard possible adverse effects.

Exploring potential therapeutic applications

Animal models are being used to study possible benefits of tDCS for treating a number of neurological and neuropsychiatric disorders that result from brain injury or pathology.

Rodent models of acute cerebral ischemia have shown that repeated applications of anodal tDCS over the affected area not only improved motor function [89–91•] and cognitive recovery [89], but also resulted in a neuroprotective effect, established by an augmented level of axonal plasticity [89], intensified dendritic outgrowth [89] and spine density [90], diminished abnormal membrane permeability and ionic dysregulation [90], and induced neurogenesis [91•]. However, the effects of tDCS appear to be very sensitive to the parameters of the stimulation protocol because in a different study a single session of anodal tDCS resulted in increased post-ischemic lesion volume and blood brain barrier imbalance [92].

Animal studies provide valuable information about the critical periods after brain damage that are most amenable to tES interventions. For example, anodal tDCS applied one to two weeks after an ischemic lesion ameliorated both motor deficits [89,90], and memory impairments [93]. However, other animal-based studies have intervened with tES immediately after a brain injury (from a few hours up to three days). In this context, a single session of anodal tDCS promoted early recovery of consciousness and motor function when applied immediately after a mild traumatic brain injury in rats [94]. Similarly, cathodal tDCS applied directly after cerebral infarction revealed a neuroprotective effect [92,95,96], inducing functional improvement and neurogenesis with concurrent migration of oligodendrocyte precursors towards the ischemic region [91•]. This tDCS-induced enhancement (particularly with cathodal stimulation) of proliferation and mobility of neural stem cells has been repeatedly shown in animal models [42,43,97], along with activated

innate inflammatory responses through repeated tDCS [42,97], promoting the use of restorative tDCS-based therapies. However, increased neuroinflammation, characterized as a "two-edged sword", can also be neurotoxic for the affected brain tissue [98], leaving open the question of whether interventions aimed at reducing neuroinflammation might be the better choice to promote recovery after brain injury, as observed by Peruzzotti-Jametti and colleagues after applying cathodal tDCS in ischemic mice [92].

The chronic presentation of inflammatory mediators results in an increase of nociceptors' sensitivity, inducing pain [99]. The therapeutic significance of tDCS for chronic-inflammatory pain treatment is derived from the anti-hyperalgesic effect observed after repeated anodal stimulations applied to the rat motor cortex [100]. The underlying mechanism of this antinociceptive tDCS effects seems to be a top-down modulation of descending inhibitory pathways resulting in pain suppression [101]. Several studies using different tDCS protocols, such as combined DC:AC electric fields applied to the cortex of healthy rats [102], repeated anodal tDCS in rat models of chronic stress [103,104], and cathodal tDCS in both ovariectomized [105] and healthy rats [106] demonstrated pain-reducing effects. Likewise, tDCS is able to reduce neuropathic pain induced by partial sciatic nerve ligation in mice [107], or by chronic constriction injury in rats [108–110], eliciting enhanced exploratory activity and reduced anxiety-like behavior [109].

Therapeutic applications of tDCS in mental disorders such as anxiety, depression, addiction, epilepsy or attention deficit hyperactivity disorder (ADHD) have been widely investigated in humans [111], and are now being examined in animals [112]. A sparse number of studies using animal models but with significant findings showed that repeated application of anodal tDCS in frontal cortex attenuated molecular and behavioral responses associated with addiction or abstinence [82,113,114]. One of the observed tDCS effects was a reduced long-lasting antidepressant behavior in Nicotine-treated mice [82], which has been reproduced in both healthy mice and a mouse model of depression [115], and is possibly mediated by the involvement of astrocytic Ca^{2+} signaling [26,116•]. Beneficial tDCS-effects on short-term memory of an ADHD rat model, possibly via dopaminergic modulation have also been reported [117]. In addition, cathodal tDCS has been shown to suppress *in vitro* epileptic excitatory postsynaptic currents [118], reduce spontaneously occurring spike and slow-wave discharges in a genetic rat model of absence epilepsy [119], and act as an anticonvulsant in rodents [120–123], while seizure-triggered sinusoid tES (consisting of a 50-ms Gaussian waveform) significantly reduced spike-and-wave episodes in a generalized epilepsy model [63]. Finally, tDCS has been used to improve behavioral performance, as well as learning and memory in animals with brain injury [89,124] and diabetic rats [125], and to ameliorate the pathological symptoms in animal models of Alzheimer's disease [126,127] and Parkinson's disease (PD) [128–130]. AD rats received 20 min of single-session [126] or twice-daily [127] anodal tDCS applied five times a week for up to four weeks to the frontal cortex. The stimulation protocols resulted in improved cognition and spatial memory, and induced a protective effect on neurons, reducing the probability of neuronal damage through the β-amyloid neurotoxicity. Regarding PD models, a single-session of anodal tDCS was shown to alleviate the unilateral bias in PD rats [128], whereas a 12-day treatment of anodal tDCS over the motor cortex of PD monkeys enhanced motor function via an augmented activation of primary motor cortex and substantia nigra neurons [129], and a three week

treatment of daily 10-min anodal tDCS over the frontal cortex of PD mice improved motor coordination as well as reduced oxidative stress [130]. Moreover, 10 min of cathodal but not anodal tDCS was sufficient to increase extracellular dopamine levels in the normal rat striatum [131]. In this context, an elegant study recently revealed that a 14-day treatment of 20 min anodal but not cathodal tDCS enhanced survival and striatal reinnervation of dopaminergic cell transplants in a PD rat model [132••], providing highly valuable information for future cell transplantation therapies in PD patients.

In conclusion, therapeutically oriented studies addressing the applicability of tES in animal models of brain dysfunction contribute to our understanding of disease progression and recovery. In addition, these animal studies provide an opportunity to elucidate the mechanisms underlying the beneficial effects of tES, and to design systematic experiments for assessing the efficacy of different stimulation protocols for treating brain pathology.

Conclusions

The use of animal models for exploring tES effects and associated underlying basic mechanisms has undergone a resurgence in recent years that parallels the increased interest in the application of this methodology to treat and enhance human brain function. As emphasized in this review, animal models provide an invaluable scientific instrument to understand the mechanisms implicated in tES, to define safety limits through the detection of lesion thresholds and the impact on molecular mediators involved in brain injury, to validate computational models, to inspire the development of more effective stimulation protocols, to enhance normal brain function, and to explore new therapeutic applications. Much of the progress in addressing these fundamental questions has come from studies that have taken advantage of animal models to apply tES in combination with invasive neuroscientific tools that are difficult to implement in human subjects, like electrophysiological recording of neural activity, fluorescent and two-photon imaging, or optogenetic manipulation.

Nevertheless, tES applications in animal models present important differences with respect to tES interventions in humans, and these must be taken into account before basic research findings can be translated to the clinic. Some of these limitations are inherent to the brain anatomy and geometry of the selected animal species, whose brains often lack cortical circumvolutions (in rodents) or are smaller in size and contain fewer neurons. Other limitations are related to the specific details of the stimulation protocols, e.g., the application of higher density currents [4] or the exploration of induced tES effects on behavioral tests with no clear translation to human behavior. However, as commented in this review, some studies have already examined the impact of tES on behavior, as well as on the activity of neural networks [33•] and electrical field distribution, in the brains of non-human primates [54••], helping bridge the difference between animal and human work. In addition, investigators have already started reducing the applied current densities in alert animals to levels used in clinical trials [24,31], and some have explored the impact of tES on behavioral tasks that are commonly used to assess brain function in humans [23,56•,72].

In conclusion, the future of tES and its successful utilization in basic research and clinical interventions in human subjects partially rely on the correct use of animal models and on the extent that these animal studies can provide answers to fundamental questions about the mechanisms underlying tES effects in the human brain. To this end tES experiments in animals should be carefully designed to maximize opportunities for applying discoveries to the treatment of human disease.

Acknowledgments

Funding: This work was supported by grants from the Spanish MINECO-FEDER (BFU2014-53820-P) to JMR and from the US National Institutes of Health (RF1 MH114269) to JFM and JMR. Dr. Sánchez León reports grants from FPU13/04858, during the conduct of the study.

Abbreviations

AC	alternating current
ADHD	attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	brain-derived neurotrophic factor
DC	direct current
EEG	electroencephalography
EMG	electromyography
fMRI	functional magnetic resonance imaging
LFP	local field potential
M1	primary motor cortex
mGluR5	metabotropic glutamate receptor 5
MRI	magnetic resonance imaging
NMDA	N-methyl-D-aspartate
S1	primary somatosensory cortex
tACS	transcranial alternating-current stimulation
tDCS	transcranial direct-current stimulation
tES	transcranial electrical stimulation
tRNS	transcranial random-noise stimulation

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