Target Engagement with Transcranial Current Stimulation

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Abstract

Transcranial electric stimulation (tES) applies a weak electric current to the scalp, which causes an electric field that changes brain activity and behavior. Despite the rapidly growing number of studies that report successful modulation of behavior in both healthy participants and patients, little is known about how tES modulates brain activity. In this chapter, we discuss what we know and what we do not know about the targeting of brain networks with tES. We provide an in-depth review of studies that use computational models, *in vitro* and *in vivo* animal models, and human participants to elucidate the mechanism of action of tES. The main emerging

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themes are (1) that the stimulation interacts with endogenous network dynamics, (2) functional connectivity represents an attractive and underexplored target for tES, and (3) that low-frequency cortical oscillations during sleep and anesthesia have become the flagship network target to elucidate the mechanisms of tES.

Keywords

Transcranial current stimulation • tACS • tDCS • Noninvasive brain stimulation • Cortical oscillations • Sleep oscillations • Functional connectivity • Electric fields • Entrainment • Plasticity

It has been known for a long time that electricity interacts with both the central and peripheral nervous systems. Today, electric brain stimulation is used both as a research tool for the study of brain function and as a clinical tool for the treatment of neurological and psychiatric disorders. In this chapter, we will focus on one form of noninvasive brain stimulation, transcranial electric stimulation (tES, also referred to as transcranial current stimulation, tCS), which has recently attracted broad attention due to a large number of promising results.

TES applies a weak electric current to the scalp. We will focus on two main types of tES: transcranial direct current stimulation (tDCS) which applies a constant current and transcranial alternating current stimulation (tACS) which uses a sine-wave stimulation waveform. The aim of tES is to modulate brain function; the *target* of tES is the electrical activity in brain circuits. Most tES studies, however, only use behavioral outcomes and do not measure the changes in brain activity caused by stimulation. Therefore, the questions of how and by what mechanism tES engages network-level targets in the brain have remained mostly unanswered.

Here, we will review the research that is aimed at uncovering the mechanisms by which tES modulates neuronal network dynamics and behavior. As we will see, the mechanisms of action by which the application of weak electric fields modulates neuronal activity have been studied with a range of different methods. In vitro studies using live slices of hippocampus and neocortex have contributed to a mechanistic understanding of the effect of weak electric fields on neuronal activity at the cellular and microcircuit levels. *In vivo* studies in animals have enabled the characterization of the effects of tES on intact brains with invasive recording methods. Noninvasive electrophysiology and imaging studies have contributed insights into how stimulation interacts with endogenous network activity in humans. In addition to these experimental approaches, computational modeling studies have provided important insights into targeting of specific networks and their endogenous dynamics. The combination of these methods has proven to be very useful to understand how a weak electric field can change brain function.

In this chapter, we will provide an overview of the potential mechanisms of tES that have been uncovered using these diverse methodological approaches. First, we will review animal studies. This is followed by a discussion of computational modeling studies, which provide mechanistic insights on the effects of tES at a cellular and network level. Next, we will focus on human studies that measured changes in brain activity by tES. Then, we turn our attention to the future and delineate what we believe are the rising new areas of tES research that deserve particular attention by the field. First, we propose that functional connectivity, which measures how different brain areas interact, is one of the most promising new targets for tES. Second, we look at one promising network target where the different methodological approaches discussed here have come together in a synergistic way: low frequency oscillations during sleep and anesthesia. Together, this chapter aims to equip the reader with a comprehensive understanding of how tES engages network targets and of what the future of tES may look like.

Mechanistic Insights from Animal Studies

Although tES is a noninvasive stimulation modality with an outstanding safety track record for the use in humans, studies in animal models are of high importance. Animal studies play a crucial role in understanding the mechanisms by which tES modulates brain activity. First, animal experiments allow for the use of invasive electrophysiology such as the insertion of recording microelectrodes into the brain. Such recordings overcome the technical difficulties of simultaneously stimulating and recording electric activity since action potential signals occur in a different frequency band (typically 300–5000 Hz) than the stimulation artifacts, which exhibit a spectral peak at the stimulation frequency (typically below 100 Hz). Therefore, the stimulation artifact can be removed by high-pass filtering for the study of neuronal firing. Second, reduced *in vitro* preparations such as the slice preparation offer the opportunity to study the effects of weak electric fields under controlled experimental conditions.

Effect of Electric Fields on Individual Neurons

One of the first observations of the effect of electric fields on neurons goes back many decades when Terzuolo and Bullock [1] applied a 1 mV/mm field to spontaneously active cardiac ganglion neurons of a lobster. The spontaneous firing rate of the cells was increased by the electric field. Similar modulation of neuronal firing rates by constant electric fields was also reported for other species [2, 3]. In 1988, Chan and colleagues [4] demonstrated that an applied electric field depolarizes the membrane voltage even when action potentials were blocked with the sodium-channel blocker tetrodotoxin. This demonstrated that the membrane depolarization caused by electric fields was a passive event, i.e. no opening or closing of ion channels was required. Rather, the ions within neurons change position in the presence of an external electric field. As the charge carriers redistribute within the cell to compensate for the applied field, the intracellular potential changes. The two distal poles of the structure aligned with electric field exhibit a depolarization and a hyperpolarization, respectively. This process is called *polarization* and depends on the overall length of the neuron as measured along the direction of the applied electric field (Fig. 11.1). Therefore, the orientation and size of the cell play a role in the response to the application of electric fields.

In addition, the change in the membrane voltage also depends on both the amplitude and frequency of the applied field. To demonstrate that the change in membrane voltage is dependent on the strength of the electric field, fields ranging from −40 to +60 mV/mm were applied along the somato-dendritic axis of CA1 cells and the change in membrane voltage at somata recorded in acute hippocampal slices [5]. The resulting polarization linearly depended on the strength of the applied electric field. This work was then extended to sine-wave (AC) electric fields in CA3 pyramidal cells [6]. The change in membrane potentials resulting from AC electric fields were less than those of DC fields of the same strength. The relationship between the field strength and the membrane depolarization was still linear but the slope, which quantifies the change in membrane voltage for every V/m of electric field, was decreased with increased frequency. Frequencies ranging from 5 to 100 Hz were applied and the change in the slope exponentially decays with the frequency of the applied electric field. This frequency dependence is caused by the low-pass filtering property of the passive cell membrane.

Interactions of Network Oscillations and Electric Fields

The change in membrane voltage of a single neuron by tES electric fields is too small to evoke action potentials in a cell at its resting potential in absence of synaptic input. Therefore, the effects of tDCS and tACS depend on the interaction of the applied stimulation and the endogenous network dynamics [7].

In particular, slice experiments have provided important insights on the interactions between the ongoing network activity and the applied

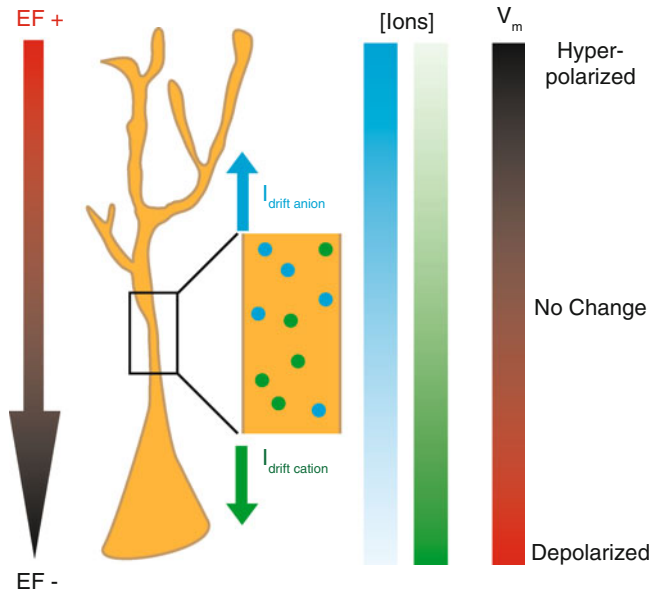

Fig. 11.1 Schematic illustration of how an electric field changes the membrane voltage of a neuron. The electric field (EF) is indicated with the arrow to the left of the neuron. When an electric field is applied (parallel to the somato-dendritic axis of a neuron), cations and anions move in opposite direction to cancel out the electric potential gradient imposed by the field within the neuron. The membrane voltage is defined as the difference between the electric potentials inside and outside the cell. Therefore, the gradient in the extracellular potential leads to a net depolarization of one end of the neuron (cell body) and a hyperpolarization of the other end of the neuron (distal apical dendrites)

electric fields. Few slice preparations exhibit spontaneous network oscillations, presumably because of (1) the relative lack of synaptic inputs due to the deafferentation inherent to this preparation and (2) impaired neuromodulatory tone in tissue slices in comparison to the intact brain. However, oscillations may occur spontaneously in the slice preparation in more in vivo-like ionic conditions [10] and in response to pharmacological activation [11]. More recently, optogenetic stimulation has uncovered in vivo-like activity patterns in the slice preparation [12]. Therefore, these experimental strategies can be combined with the application of external electric fields for the study of the mechanisms of tES. For example, pharmacological activation of hippocampal slices caused the emergence of gamma oscillations that were susceptible to weak DC electric fields [13]. Interestingly, the effect of the DC field was asymmetric with regards to the polarity. Hyperpolarizing fields were more effective at suppressing this network oscillation than depolarizing fields which were more effective at enhancing the same activity pattern. In case of AC fields, for sufficiently low stimulation frequency, the amplitude of the gamma oscillation was periodically modulated, reminiscent of the theta-nested gamma oscillation [14]. The most complex effect occurred if the stimulation frequency was similar to the frequency of the endogenous oscillation. In this case, three simultaneous frequencies were observed. The endogenous oscillation was reduced (but still present) while oscillations half a harmonic above and below the endogenous frequency appeared. Thus, the effects of AC stimulation can be highly nonlinear since in linear systems the observed output exhibits the same frequency as the input signal. In other words, neuronal networks may act as an energy transfer filter whereby energy in one frequency may be shifted into different frequency bands.

The interaction of electric field stimulation and endogenous oscillation appears to not only depend on the frequencies of both but their relative amplitudes. In a study of low frequency (1 Hz) oscillations evoked by optogenetic stimulation, it was observed that electric fields of a mismatched frequency would enhance the power of the endogenous

oscillation often without increasing power at the frequency of the electric field [15]. This occurred when the optogenetic drive and therefore the "endogenous" oscillations were strong and the electric field was relatively weak. However, the power of the oscillations at the stimulation frequency was enhanced when the magnitude of the endogenous oscillation was reduced (lower light intensity for optogenetic stimulation) or the strength of the electric field was increased. Taken together, the response of neural networks depends both on the frequency and the power (relative to the endogenous oscillation) of the electric field used for stimulation. Furthermore, these results demonstrate that the response of cortical networks to tES may be nonlinear in nature.

So far, we have focused on the response to stationary stimulation waveforms; however, endogenous neural activity is not stationary. To this end, endogenous activity may be better manipulated with feedback control algorithms than with static pre-programmed stimulation waveforms. One such example is the modulation of seizure-like, epileptiform electric events in slices. The application of DC fields can suppress epileptiform activity in hippocampal slices, which exhibit spontaneous seizure-like activity, however the network quickly adapted to the stimulation and epileptiform activity returned [16]. In a follow-up study, nonstationary electric stimulation was applied to suppress seizure-like activity [17]. The authors were able to suppress seizure activity for 16 min using a negative feedback stimulation paradigm on a hippocampal slice which exhibited seizure events every 40 s. Critically, spontaneous activity still occurred while epileptiform activity was suppressed. Thus, in the case of suppression of epileptiform activity with tES, these studies show that adaptive feedback stimulation may have greater effect on network dynamics than constant stimulation. Indeed, there is also evidence that feedback stimulation has uses outside of suppression of aberrant activity. In spontaneously oscillating slices of ferret visual cortex, positive feedback stimulation with electric field was shown to decrease the length of time between cortical up states and increase the strength of the endogenous oscillation [18]. Conversely, the application of negative feedback stimulation to the slices reduced strength of the endogenous oscillation. Interestingly, this effect was accomplished with stimulation amplitudes similar to the amplitude of endogenous electric fields recorded in vivo (1 mV/mm).

Outlasting Effects of Electric Fields

One of the most exciting aspects of tES is that the effects of stimulation can outlast the stimulation as demonstrated by sustained modulation of motor-evoked potentials after completion of stimulation [19]. This "outlasting effect" of tDCS has been studied in animal models and slice preparations. Most in vitro studies have reported no outlasting effects of weak electric fields, however the stimulation duration in these studies was typically short. With a longer stimulation duration, outlasting effects were observed more than 10 min after the end of 10 min DC stimulation with higher field amplitudes (i.e. 10 mV/mm and higher) than what can be expected to occur with tES in humans [20]. In vivo, tDCS over somatosensory cortex applied to rabbits modulated eye blink conditioning; however, an outlasting effect of tDCS only occurred for cathodal stimulation [21]. The underlying mechanism was probed by paired pulse experiments which revealed that spike-time-dependent long-term depression (LTD) was activated by tDCS. Moreover, the resulting LTD was suppressed by pharmacological blockade of adenosine receptors by a local injection. Similarly, evoked potentials were enhanced by application of electric fields in vivo in anesthetized rats, with effects that outlasted the stimulation for hours [22]. Both long-term potentiation (LTP) and paired-pulse facilitation (PPF) were increased after DC field application in hippocampal slices [23]. Intriguingly, LTP (but not PPF) was also enhanced in hippocampal slices of rats which had received anodal tDCS 24 h earlier. Application of an NMDA antagonist prevented LTP induction but not paired pulse facilitation. In slices of mouse motor cortex, the application of DC field enhanced synaptic strength when paired with a low-frequency electric stimulation of afferent pathways [24]. Importantly, this observed form of LTP depended on NMDA receptors and

brain-derived neurotrophic factor (BDNF). Today's limited evidence therefore suggests that tDCS activates multiple, diverse plasticity mechanisms, both pre- and postsynaptic, depending on the brain region, polarity (anodal vs cathodal) of stimulation, and other poorly understood factors. In addition, enhancement of oscillation following tACS has also been attributed to plasticity [8], however direct experimental evidence for such a mechanism is lacking.

Interaction of Cellular and Network Mechanisms

The main target of tES is cortical networks due to their positions closest to the stimulation electrodes. The circuits in neocortex are composed of different cell types that exhibit distinct morphology and electrophysiological properties. Importantly, not all cell types respond equally to weak electric fields. This was demonstrated by the combination of patch recordings of the somatic membrane voltage with careful reconstruction of cell morphology [25]. Layer 5 (L5) pyramidal cells were shown to have the largest change in membrane voltage in response to externally applied electric fields due to their morphology and orientation within cortex. These cells exhibit an elongated somato-dendritic axis that spans from L5 to L1. In addition, the somato-dendritic axis is approximately perpendicular to the surface of the brain meaning that the cells are properly aligned to receive energy from an external electric field orthogonal to the skull. Note that the folding of cortex introduces additional complexity, which for the purpose of this section we do not further discuss. Because L5 pyramidal neurons are the likely primary targets of tES, we can expect that their response to stimulation plays a critical role in the modulation of cortical network dynamics. Therefore, considering the intrinsic dynamics of this cell type will provide clues with regards to the network-level effects of stimulation. The response of L5 pyramidal cells to subthreshold changes in membrane voltages, particularly in the prefrontal cortex, has been well studied by current-clamp whole-cell patch clamp experiments; these cells respond best to subthreshold perturbations in the theta frequency (4–8 Hz) band [26, 27]. This suggests that electric fields of a given strength will cause the largest subthreshold oscillations in the theta band and that AC field stimulation preferentially modulates low-frequency oscillation in the cortex. However, direct experimental evidence confirming this link between single cell excitability, cell morphology, and network level effects has not yet been reported.

Computational Models

Despite the extensive investigation of cognitive and clinical applications of tES, the exact mechanisms of tES in modulating neuronal activity in humans have remained only partially understood. In the above section, we have discussed key findings on mechanisms of tES from animal experiments. Here, we provide an up-to-date review of computational models of tES, focusing on recent advances in modeling techniques and their applications.

Forward Models

Computational forward models determine the current flow in biological tissue and can predict the resulting electric field during tES. The current density distribution in the head depends on a number of dose parameters, including electrode number, position, size, shape, and electric current amplitude and waveform. Different electrode *montages*, positioning of the stimulation electrodes, result in distinct current flow through the brain. Although such flexibility allows for customization and optimization of tES paradigms, it also renders the optimal choice for engaging a specific brain circuit more difficult to identify. Perhaps most importantly, forward models allow us to relate the amount of current applied to the scalp to the magnitude and the direction of the resulting electric field in the targeted brain areas [28]. By calculating current density distributions, forward models provide accurate and detailed description of current flow patterns, thus greatly facilitating the rational design and optimization of tES parameters.

Computational forward models of tES have evolved from the simple concentric sphere models

assuming simplified geometries to low-resolution anatomy-based models to high-resolution, anatomically accurate models based on individual structural magnetic resonance imaging (MRI) scan. Lacking regional anatomical differences, the concentric sphere models were successfully used to determine the main effects of different electrode montages [28]. Such simplified models are particularly beneficial for initial evaluation of the effects of different electrode configurations. For example, a finite-element concentric sphere human head model for simulating a range of different electrode configurations showed that concentric ring electrode causes electric field distributions with higher spatial focality than more commonly used electrode types and montages [29]. In contrast, low-resolution anatomy-based models incorporate both anatomical structure and individual patient-specific features, but the anatomical accuracy is limited because cortical folding, ventricles, and tissue anisotropy are usually not taken into account. Consequently, such models are not able to capture local nonuniformities in electrical field distribution [30]. Despite these limitations, low-resolution models have offered valuable insights in informing tES montage design and how pathological changes of brain and skull anatomy affects current density distribution. A number of low-resolution models developed by Wagner et al. (2004, 2006 and [31]) serve this purpose. In one tDCS study [31], the comparison of several electrode montages commonly used in clinical application showed that smaller electrodes led to greater current shunting through the scalp. In the same study, the analysis of current density distribution between healthy and stroke head models under tDCS demonstrated that lesions substantially altered spatial targeting, which may interfere with the treatment outcome. Lastly, high-resolution anatomically accurate models based on MRI scans have become a promising tool in assisting the design of customized and individualized tES protocols as they allow for accurate representation of current density distribution in the brain (for a comprehensive review, see [32]). These high-resolution models advance our understanding of tES effects and may eventually lead to improved stimulation for optimized and customized therapy. Below we review a few examples to illustrate the merit and utility of high-resolution models in the design and analysis of tES. It is important to note that most of these modeling results are awaiting physiological proof.

The actual pattern of current flow produced by tES is greatly shaped by anatomy and tissue properties [28]. To achieve similar treatment outcome despite patient-to-patient variability in head and brain anatomy, it is important to know the sensitivity of electrical field distributions to normal anatomy variation for a given electrode montage. High-resolution models provide an ideal tool to analyze the underlying basis for individual variation during tES. For example, a detailed analysis of the influence of cerebrospinal fluid (CSF) showed that electric fields may be clustered at distinct gyri/sulci sites due to details of CSF flow [33]. Together with other high-resolution models [34–36], this study suggested that individual variability in dosing of tES could arise primarily due to gyri-specific dispersion of current flow more than differential skull dispersion as previously thought.

High-resolution models have contributed significantly to the design and validation of new tDCS montages. The conventional tDCS applies weak direct currents to the scalp via sponge-based rectangular pads. High-definition tDCS (HD-tDCS) uses arrays of small scalp electrodes for stimulation [27]. A high-resolution MRI-based finite element model of the human head demonstrated that the 4×1 ring electrode configuration [four "return" (cathode) disk electrodes arranged in a circular fashion around an "active" (anode) center electrode] resulted in significant improvement of spatial focality [33]. To what extent such increased spatial focality improves treatment outcomes remains an open question.

Furthermore, high-resolution models allow for safety and efficiency analysis of tES application in populations at increased risk of negative side-effects. For example, there is a growing interest in applying tES in children for the treatment of disorders such as autism and epilepsy. However, due to anatomical differences, the same stimulation dose that is safe for adults may be hazardous to children. In order to establish the comparable safety and tolerability dose in children,

cortical electric field maps at different stimulation intensities and electrode configurations were determined using a high-resolution MRI-derived finite element model of a typically developing, anatomically normal 12-year-old child [37]. Simulation results indicated that, for a given stimulus intensity, the maximal electric fields in the adolescent brain were twice as high as in the adult brain for conventional tDCS and nearly four times as high for a 4×1 high-definition tDCS electrode configuration. Thus, special caution needs to be taken when applying tES to the pediatric population. Another vulnerable population is patients with traumatic brain injury or decompressive craniectomy, who often have skull defects or surgically implanted plates. To safely apply tES in these patients, safety guidelines need to be established. In order to evaluate the impact of skull defect on current density distribution under tDCS, a MRI-derived finite element head model with several conceptualized skull injuries including two types of skull defects and two types of skull plates was developed [38]. Interestingly, simulation results indicated that skull defect provided a preferential pathway for current flow to concentrate in the brain. Under such conditions, the underlying cortex would be exposed to a higher intensity of focused current flow, raising important clinical and safety considerations. Together, these studies show that computational forward models are an essential tool for safe (and optimal) targeting of the brain structure of interests.

Computational Neural Models

Different from computational forward models, computational neural models of tES focus on the effects of electrical stimulation on neuronal excitability and network dynamics. Neural models of tES are desirable since they provide a solid computational framework to readily explore the neural mechanisms underlying tES-induced behavioral/treatment outcome and the effects of stimulation parameters such as frequency and amplitude in the case of tACS. Although there exist a number of cellular and network models of electrical stimulation [39–47], few are dedicated to the study of tES. Below, we focus on three neuronal network models that specifically investigate the effects of tES on cortical activity [45–47].

During neural activity, the superimposition of electrical currents from a large population of neurons that have similar spatial orientation gives rise to a potential in the extracellular medium. This electric field is the source of the electroencephalogram (EEG) recorded from the scalp [48, 49]. Scalp EEG activity shows oscillations in a variety of frequency bands which reflect the synchronous activity of thousands or millions of cortical neurons [50] and are associated with different behavioral states (e.g. waking and sleep [51]). Abnormal or disrupted cortical oscillations are a hallmark of a number of neurological and psychiatric disorders including schizophrenia and depression [52]. The mechanisms by which externally applied fields modulate the activity of cortical neurons remain unclear. The three computational studies [45–47] aim to elucidate how cortical dynamics are modulated by tES.

The computational study by Molaee-Ardekani and colleagues [47] analyzed in detail how cortical neuronal assemblies are affected by the electrical field induced by tDCS and how local field potentials (LFPs) respond to the applied electrical field. The authors constructed a macroscopic computational model (neural mass model) of the cerebral cortex including subpopulations of pyramidal cells and inhibitory interneurons connected with realistic models of synapses. Model parameters were adjusted to reproduce evoked potentials (EPs) recorded from the somatosensory cortex of the rabbit in response to air-puffs applied to the whiskers. The application of tDCS was modeled as a perturbation on the mean membrane potentials of pyramidal cells and/or interneurons. Simulation results demonstrated (1) that a feedforward inhibition mechanism must be included in the model to accurately replicate the actual EP and (2) that electric fields had to modulate interneurons to replicate the experimental findings.

EEG signals usually contain oscillations in multiple frequency bands that can be analyzed by power spectrum. To capture the origin of tDCS-induced alterations in the EEG power spectrum, Dutta and Nitsche [46] developed a thalamocortical neural mass model that contained four

subpopulations of cortical cells (excitatory pyramidal cells, excitatory interneurons, slow inhibitory interneurons, and fast inhibitory interneurons) and two subpopulations of thalamic neurons (excitatory thalamo-cortical cells and inhibitory reticular thalamic neurons). This thalamo-cortical network model was used to simulate the subject-specific EEG power spectrum changes during and following tDCS by varying synaptic parameters. Model simulation showed that anodal tDCS enhanced activity and excitability of the excitatory pyramidal neurons at a population level in a nonspecific manner and led to mu-rhythm (9–11 Hz) desynchronization. The model further showed that the tDCS effects on mu-rhythm desynchronization depended on the stimulation polarity, consistent with experimental observations [53].

Recent human studies have demonstrated that sine-wave stimulation waveforms (tACS) induce frequency-specific effects on brain dynamics measured by EEG [54–56], suggesting that tACS may present a more targeted stimulation paradigm for the enhancement of cortical oscillations than tDCS. However, it remains unknown how periodic, weak global electric fields alter the spatiotemporal dynamics of large-scale cortical networks. To address this question, Ali and colleagues [45] developed a large-scale two-dimensional cortical network consisting of 160,000 (400×400) pyramidal cells and 40,000 (200×200) interneurons modeled by Izhikevich neural dynamics [57, 58]. Simulations revealed distinct roles of the depolarizing and hyperpolarizing phases of tACS in oscillation entrainment, which entailed moving the network activity toward and away from a strong nonlinearity provided by the local excitatory coupling of pyramidal cells. Interestingly, the model demonstrated that recovery of synaptic depression played an important role in the entrainment of network activity by tACS and that sparse global stimulation was more effective than spatially localized stimulation. The simulations further revealed that entrainment by tACS was mediated by "Arnold tongue" dynamics so that stimulation frequency matched with the endogenous frequency was most effective in entraining the oscillating network. These findings provide a detailed mechanistic understanding of tACS at the level of large-scale network dynamics and give support for tACS as a more targeted stimulation paradigm for the treatment of neuropsychiatric illnesses with impaired cortical oscillations.

Future Directions

Together, computational models of tES play a critical role in visualizing the electrical field distribution, understanding the mechanistic action of tES on neuronal network dynamics, and optimizing stimulation parameters to guide the design of the next generation of tES. While anatomically accurate high-resolution MRI-based forward models guide the rational design and optimization of tES electrode montages, neuronal models constrained by neurophysiological measurements provide a mechanistic understanding of the effects of tES on cellular and network dynamics and thereby provide guidance for the rational design of the stimulation waveform. As most existing neural models of tES are either neural mass models or simplified spiking models that lack accurate ion channel dynamics, it is desirable to construct biophysically realistic neuronal models of tES. We anticipate that such models will further illustrate at both the cellular and network levels how the stimulation dynamics interact with the intrinsic neuronal dynamics to give rise to the state-dependent effects of tES. Furthermore, there is an increasing demand for the incorporation of neural models into computational forward models of electric current flow to thoroughly explore how tES-induced electric fields modulate cellular excitability and network dynamics as a function of time and space.

Effects of Weak Electric Fields on the Human Brain

Even before observations of interactions between electricity and brain activity, electrical currents have been used for treating various disorders such as headache and epilepsy. Initial treatments involved using live electric rays and electric catfishes [59]. Efforts by a number of pioneers

including Walsh, Galvani, Volta, and Aldini lead to the establishment of the field of bioelectricity and subsequently the development of *electrotherapy* [60]. Interest in electrically polarizing brain regions using transcranial weak current stimulation for treating symptoms of psychiatric disorders increased in the 1960s and 1970s with a number of studies showing positive outcomes [61–64]. However, development of drugs which appeared to be more effective in treating psychiatric disorders led to waning interest in transcranial stimulation.

During this period, the predominant understanding of how stimulation produces such effects was based on evoked potentials observed in animal studies. When a positive polarization is applied across the cortex, there is an increase in evoked response amplitude and conversely, there is decrease in evoked potential amplitude when a negative polarization is applied [65, 66]. In essence, stimulation was thought to affect the excitability of neurons. In humans, one of the first studies to look at excitability change after transcranial direct current stimulation (tDCS) was performed by Priori et al. [67]. Weak DC current (< 0.5 mA) was applied over motor cortex and excitability was tested using single pulse transcranial magnetic stimulation (TMS) to trigger an evoked response. The resulting motor-evoked potential (MEP) amplitudes served as a physiological measure of change in excitability. Anodal and cathodal stimulation indeed modulated the MEP amplitude, however factors such as the temporal order of the stimulation paradigm appeared to matter. A clearer result emerged from a more comprehensive study by Nitsche and Paulus [19] where they showed that anodal stimulation led to an increase in MEP amplitude and conversely cathodal stimulation led to a decrease in MEP amplitude. Interestingly, the change in amplitude lasted for a few minutes after completion of tDCS and returned to baseline after 5 min. Also, the size and duration of the after-effect depended on the stimulation duration and current intensity.

Neurophysiology of tDCS in Humans

Increasing interest in tDCS has led to an exploration of possible modalities that can provide more insight into neurophysiological effects. Consequently, tDCS has been used in conjunction with other neurophysiological approaches. Electroencephalography (EEG), the earliest approach for measuring brain activity in humans, was also one of the earliest modalities used in studying the effect of current stimulation [68].

Analogous to the approach of using MEPs for evaluating excitability change in motor cortex, Antal et al. [69] used visual-evoked potentials (VEPs) to study excitability change caused by tDCS. They found that the amplitude of N70 component of the VEP in EEG was increased by anodal stimulation and conversely, decreased by cathodal stimulation over visual cortex. In another study [70], tDCS was found to affect the P100 component (anodal tDCS caused decrease in amplitude while cathodal tDCS caused increase in amplitude) of the VEP and the duration of the after-effect of tDCS depended on the duration of stimulation. Of note, as so often in this literature, the choice of return electrode was different. This may explain the different findings across studies. In both studies, stimulation did not affect the latency of the VEP. Similarly, the effects of tDCS on somatosensory-evoked potentials (SEPs) have been studied. A 9-min application of cathodal tDCS to somatosensory cortex decreased the N20 component of the SEP for up to an hour after stimulation while there was no significant change with anodal tDCS [71]. In another study, tDCS applied over motor association areas produced changes in SEP amplitudes as well as MEP amplitudes. Interestingly, the effects were inversely related. Anodal stimulation decreased amplitudes of MEPs while amplitudes of SEP components increased compared to cathodal stimulation [72]. Other studies have evaluated pain perception using laser-evoked potentials (LEPs) after tDCS and found that only cathodal stimulation produced a change in the amplitudes of N2 and P2 components of LEPs [73, 74]. The effects of tDCS on auditory-evoked potentials (AEPs) have also been evaluated and significant effects of stimulation polarity and stimulation locations (temporal vs temporo-parietal) have been found [75].

Apart from evoked potentials, EEG oscillations have also been investigated for elucidating the effect of tDCS. In a study accompanying the

previously mentioned study by Antal et al., cathodal tDCS was found to decrease power in the beta band (15.625–31.25 Hz) as well as the gamma band (31.25–62.5 Hz) related to VEPs [76]. A study by Ardolino et al. [77] evaluated the changes in spontaneous EEG activity following application of cathodal tDCS over motor cortex and found increases in power in the delta and theta bands. In another study, the effect of tDCS on mu event-related desynchronization (ERD) caused by imagined hand movements was studied [53]. The change in power of mu rhythms was used as a measure of ERD. Anodal tDCS increased mu ERD while cathodal tDCS decreased mu ERD. The changes were attributed to the change in excitability caused by tDCS. There have also been studies which evaluated tDCS-induced changes in EEG activity patterns observed during sleep. These are covered in detail in the last section of this chapter.

The use of tDCS and EEG can be divided into two approaches—the *offline* approach, where EEG is collected after tDCS treatment, and the *online* approach, where EEG is collected concurrently with tDCS application. The former approach allows evaluation of the after-effects of stimulation while the latter approach allows study of the effect of stimulation on ongoing dynamics. Most of the studies described above fall under the offline category. A few of the studies have attempted to concurrently record EEG signals when stimulating with tDCS and have found noise to be the limiting factor. In a study assessing the efficacy of tDCS as a treatment for epilepsy, tDCS produced high-frequency artifacts that contaminated the EEG [78]. These artifacts were removed using an independent component analysis (ICA) algorithm. In another study [79], tDCS electrodes were placed between EEG electrodes and a band-pass filter between 0.5 and 70 Hz was found sufficient to remove the artifacts produced by tDCS.

Magnetoencephalography (MEG), which records brain activity by measuring magnetic fields produced by neuronal activity, is a similar modality that has been used with tDCS. MEG (at least partially) overcomes the main limitation of using tDCS concurrently with EEG, namely the limited source localization capability due to volume conduction. Soekadar et al. [80] applied tDCS over motor cortical areas of healthy volunteers performing a button-press task and assessed task-related changes in alpha and beta frequency bands from concurrently recorded MEG. Using a mathematical approach that provided spatially selective noise reduction and source localization, they were able to successfully isolate the stimulation current as a source. By separating this identified source from other sources that corresponded to brain oscillations, they were able to remove the stimulation artifacts.

Functional magnetic resonance imaging (fMRI) which relies on blood oxygenation level dependent (BOLD) signal to detect changes in activity in different brain regions is another commonly used approach to measure neurophysiological changes associated with tDCS. Compared to EEG and MEG, fMRI provides higher spatial resolution in terms of identifying the anatomical regions affected by stimulation. However, the temporal resolution is poorer than EEG/MEG as the changes in BOLD signals are observed a few seconds after neuronal activation. In one of the earliest studies, cathodal tDCS over motor cortex was shown to produce decreased activation [81]. As in the case with early tDCS-EEG studies, this study used an offline approach, i.e., there was no stimulation during fMRI data acquisition. This was due to the potential safety hazard caused by magnetic fields from the MRI scanner inducing currents in the stimulation electrodes. Once this concern was resolved by the addition of current limiting resistors, it became possible to perform concurrent fMRI-tDCS studies [82]. Overall, such studies have helped to elucidate the spatial distribution of the effects of tDCS in terms of motor and visual functions as well as functional connectivity between different regions. The latter topic is covered in detail in the Functional Connectivity section.

Mechanisms of tDCS in Humans

A common observation in most neurophysiological studies discussed above is that tDCS produces a change in excitability of the region being stimulated. Alterations in membrane potential changes

are thought to be the main mechanism underlying the change in excitability in both anodal and cathodal stimulations. Blocking sodium and calcium channels using pharmacological agents led to decrease or complete abolition of the effects of anodal tDCS in humans. While there was no change in the effects of cathodal tDCS, this still supported the hypothesized hyperpolarization effect of cathodal tDCS [83]. The outlasting effects of stimulation have been attributed to synaptic plasticity such as LTP that depends on NMDA receptors. Indeed, an NMDA antagonist suppressed the outlasting effects of tDCS [84]. The effect of cathodal tDCS is likely also the result of synaptic plasticity since it is also abolished by blockade of NMDA receptor blockade [83]. Synaptic long-term depression [85] is thus a strong candidate mechanism. Further supporting the idea that synaptic plasticity underlies the outlasting effects is the observation that individuals with brain-derived neurotrophic factor (BDNF) Val66Met polymorphism showed lower effect of tDCS-induced change in MEP compared to individuals without the polymorphism [24].

Moreover, studies involving magnetic resonance spectroscopy have shown that tDCS polarity affects local accumulation of neurotransmitters. Stagg et al. [86] showed that anodal tDCS reduced concentrations of GABA while cathodal tDCS reduces concentration of glutamate (with a correlated decrease in GABA concentrations as well). Given the fact that increased firing rates have been shown to decrease GAD-67 activity and decreased firing rate is correlated with decreased glutamate/glutamine cycling, the idea that anodal tDCS increases and cathodal tDCS decreases excitability (and consequently firing rate) is therefore further supported by these spectroscopy results. In another study by Clark et al. [87], application of anodal tDCS over parietal cortex led to an increase in glutamate and glutamine levels. The effect was local as only the region in the ipsilateral hemisphere showed an increase compared to the same region in the contralateral hemisphere. The relation between reduction in extracellular GABA concentration and motor learning suggests that modulation of GABA levels is another possible mechanism which explains the observed effects of tDCS. This idea has received further support in a recent study [88] which showed that the effect of anodal tDCS over primary motor cortex produced a local decrease in the GABA concentrations and the tDCS-induced concentration change predicted motor learning performance.

Neurophysiology of tACS in Humans

The renewed interest of the scientific community in tDCS has led to the recent development of novel tES paradigms. One particular approach, transcranial alternating current stimulation (tACS) has garnered considerable interest and is now the topic of a large and rapidly growing number of scientific studies [89–91]. Transcranial alternating current stimulation is a type of noninvasive electrical brain stimulation where oscillating, (typically) sinusoidal currents are applied to the scalp and underlying brain tissue of an individual. Many different frequencies have been used throughout the literature, but it is most common to apply currents in the frequency range of observed periodic phenomena in the brain such as local field potentials and EEG oscillations. This follows from the assumption that mimicking the structure of endogenous electrical brain activity is the best way to interact with and influence the sources of such activity. Various studies have combined neurophysiological measurements with tACS in attempts to show that oscillatory noninvasive brain stimulation indeed influences the activity of the human brain. Most of these studies have found outlasting effects of tACS when examining EEG before and after stimulation, providing the first evidence that approximately matching the stimulation frequency to the frequency of prominent endogenous oscillatory brain activity yields effects on EEG activity at that frequency. A smaller number of studies have also measured the effects of tACS during its administration.

One of the first studies to record EEG and apply tACS found no effect of tACS on EEG activity or motor-evoked potentials [92], but several subsequent studies found outlasting effects of theta-frequency tACS on EEG theta power [93], alpha-frequency tACS on EEG alpha power

[8, 56, 94], and gamma-frequency tACS on EEG gamma coherence [95, 96] and alpha power [95]. The first evidence for outlasting effects of tACS on EEG was found by Zaehle and colleagues [56]. In this study, participants performed a vigilance monitoring task for the stimulation portion of a single 16 min session (3 min of EEG recording, 10 min of stimulation, 3 min of EEG recording). During the task, participants were required to fixate on a crosshair on a computer monitor and press a button whenever the crosshair rotated 45°. At the beginning of the session, the authors determined the peak individual alpha frequency (IAF) from the single-channel EEG data by calculating the spectral peak in the alpha band during a 1 min closed-eyes recording. Either sham tACS or approximately 1 mA (peak-to-peak) tACS at the IAF was applied under the assumption that matching the stimulation frequency would best enhance endogenous alpha power. The tACS amplitude was titrated just below the thresholds of visual phosphene induction or skin sensation. They compared the average amplitude spectrum of 1 s windows between the baseline and the post-stimulation epochs for both stimulation conditions and found a significant increase in alpha power relative to baseline in the IAF-tACS condition and not for the sham stimulation condition. Specifically, this increase was found to be in the neighborhood of the IAF across participants (IAF ± 2 Hz). Neuling et al. then investigated if the effects of tACS were also dependent on the brain state of participant [94]. They utilized the well-known alpha power difference between the eyes-open and eyes-closed to test the hypothesis that the state of endogenous alpha oscillations would in part determine the EEG response to alpha-frequency tACS. The authors recorded 5 min of whole-head EEG activity, then applied the sham or verum IAF-tACS during an auditory oddball task, and finally recorded EEG for 30 min after the task. The protocol for the other experimental group was exactly the same except participants had their eyes closed for the entirety of the experiment. In this study, tACS enhanced the alpha power for the entire 30 min post-tACS recording window. This effect was specific to the eyes-open (low endogenous alpha power) experiment, and no such power enhancement occurred during the eyes-closed (high endogenous alpha power) experiment. They also found that IAF-tACS enhanced coherence between P3 and P4 alpha activity for the eyes-closed condition, but not the eyes-open condition. These electrophysiological changes did not result in a change in oddball task performance as measured by reaction time and sensitivity. While the authors argue that the effects seen in these studies result from the entrainment of endogenous alpha oscillators to the tACS frequency, Vossen et al. found similar alpha power enhancements in the absence of evidence for entrainment [8]. The authors conducted a 4-session within-participant study with three active tACS conditions and one sham tACS condition. During each session, participants performed a basic visual detection task for 22–30 min with a 2 min EEG recording before and after. During the task, the authors administered tACS at the individual alpha frequency (determined in the first session and used for all subsequent sessions) with individually adjusted intensity (1.35–2 mA peak-to-peak). Each tACS protocol consisted of intermittent bursts of tACS, two of which were 80 cycles on followed by 80 cycles off and the other 30 cycles on followed by 30 cycles off. The difference between the two 80 cycle on/off conditions was whether or not the tACS phase was continuous throughout the experiment relative to the phase of a virtual sine wave at the tACS frequency for the full duration of the task. This was termed the "long continuous condition". The "long discontinuous condition" shifted the start of each tACS burst such that the phase difference between the virtual sine wave and the administered tACS changed by a randomly selected 0, 90, 180, or 270°. For the 30 cycle burst condition the onset phase was not disrupted (short continuous). The comparison of the pre-stimulation and post-stimulation EEGs showed significant alpha power enhancement for both the long conditions and long discontinuous conditions relative to sham stimulation, but no significant difference between the two conditions. For the uncontaminated EEG epochs during the stimulation protocols, they assessed the degree of phase locking present after each burst of stimulation in terms of inter-trial phase coherence (ITPC) in the alpha band. They hypothesized that entrainment

"echoes", or brief periods of phase consistency in the alpha oscillation across trials, would likely be present if each tACS burst entrained the endogenous alpha oscillation to its phase. However, they found no difference in ITPC between the stimulation conditions or the sham condition (essentially measuring spontaneous phase consistency in the alpha oscillation). These results have been interpreted in favor of a spike-timing dependent plasticity framework to explain outlasting elevation of alpha power after tACS.

While studies that observe the after-effects of tACS have elucidated a robust set of neurophysiological changes attributable to oscillatory noninvasive brain stimulation, they can merely speculate about the changes that occur during stimulation to achieve the observed results. This is why studies that performed tACS while acquiring neurophysiological data such as EEG [97] and MEG [98] are of particular interest. Helfrich et al. [97] devised an artifact removal method that allowed them to measure EEG during a visual oddball task accompanied by the administration of 10 Hz tACS. In this study, participants performed a standard color-mismatch visual oddball paradigm where the presentation of each stimulus was aligned to one of four phase bins of the tACS waveform. The authors recorded 59-channel whole-head EEG while administering the 1 mA peak-to-peak current. To remove the artifact potential from the EEG, which is approximately, but not exactly, a sine wave at 10 Hz due to fluctuations in scalp impedance and various other sources of nonstationarity, the authors first constructed artifact templates from moving neighborhoods of recording epochs by a moving average approach. These artifact templates were then subtracted from their respective artifact-contaminated EEG segments to yield semi-cleaned EEG data. The remaining tACS artifacts were captured by decomposing each EEG time-series into its principal component subspace via principal component analysis (PCA). Components that were clearly artifactual in nature were removed and the time-series reconstructed from the remaining components in this final step. The authors assessed the validity of this approach by contaminating artifact-free data with similar artifacts found when they applied tACS (somewhat nonstationary 10 Hz sine waves 2–4 orders of magnitude greater than typical EEG potentials). The study of the preprocessed EEG showed an enhancement of mainly occipital alpha power during tACS application, and the enhancement was strongest at the stimulation frequency. The phase-locking value (PLV) between the tACS waveform and alpha-band frequencies of the EEG was significantly greater during tACS application than that during sham stimulation, and this PLV enhancement was constrained to occipital brain regions. Interestingly, the authors found a phasic modulation of oddball target detection accuracy as a function of the tACS phase during target presentation. Given that the phase of the alpha oscillation is known to influence the perception of visual stimuli [99–101], combined with the observed enhancement in endogenous alpha power, this study provides compelling evidence that 10 Hz tACS over occipital brain regions may entrain disparate endogenous alpha oscillations to a similar phase, resulting in an increase in occipital alpha synchronization. While this approach is a promising direction for the study of the neurophysiology of tACS, it has yet to be replicated in the literature.

More recently, a study by Neuling et al. [98] detailed a different approach to study the "online" effects during stimulation based on MEG. The authors applied IAF-tACS at weak (50 μA peak-to-peak) and strong (between 100 μApp and 1.5 mApp) current levels while acquiring 306-channel MEG. Participants performed several tasks well-established to induce alpha modulations and each participant completed three blocks consisting of sham stimulation, weak tACS, or strong tACS. The authors found substantial contamination of the sensor-level signals by tACS-induced magnetic artifacts, but were able to recover meaningful event responses by using linearly constrained minimum variance (LCMV) beamforming to project the measured magnetic fields into a grid of dipolar sources within the Montreal Neurological Institute (MNI) coordinate system. The source signals determined with this method showed alpha activations/suppressions and auditory/visual average event responses that were surprisingly similar to those obtained during sham stimulation. Importantly, these effects are all within-condition

and localized to the same regions as seen during sham tACS, whether or not that happened to be near or away from the stimulation electrodes. Furthermore, the presence of similar enhancements *and* reductions of alpha power during all three tACS conditions strongly supports the idea that measured source activity is physiological in nature during all three conditions.

Mechanism of tACS in Humans

The interest in tACS as a tool for manipulating cortical dynamics as well as a therapeutic option for treating CNS disorders with aberrant cortical and thalamo-cortical oscillations is relatively recent compared to tDCS. Correspondingly, the mechanisms by which tACS produces change are also less certain.

The primary targets for tACS in humans are oscillations observed in EEG and different studies have shown that tACS indeed alters the strength of oscillations [8, 56, 94, 97]. Given the periodic nature of stimulation as well as the stimulation target, concepts from dynamical systems are generally borrowed to explain the mechanism of action of tACS. The different cortical oscillations are considered to be generated by self-sustained oscillators with phase as a free parameter [102]. Depending on the level of abstraction, neurons or networks of neurons or individual brain regions are treated as these oscillators. One leading hypothesis is that the brain region targeted by tACS is composed of many oscillators and tACS produces a realignment of the phase of the oscillators to the phase of stimulation waveform. This is defined as entrainment [9]. Once the oscillators are aligned, it is assumed that oscillations continue even after the removal of stimulation until entropy of the system pulls them back to the initial state. An alternate hypothesis is that tACS preferentially strengthens synapses between neurons by spike-timing dependent plasticity (STDP) and this facilitates the effects of stimulation to be present after the removal of stimulation.

Studies involving tACS and EEG in humans have attempted to elucidate which of the above-mentioned mechanisms might be prevalent. The study by Helfrich et al., where healthy volunteers were stimulated with 10 Hz tACS during a visual oddball task, found an increase in phase-locking value between stimulation waveform and EEG waveform (after stimulation artifact removal) during stimulation [97]. This was postulated as evidence for entrainment as the results satisfied the key requirements for entrainment as proposed by Thut et al. [9]. In another study, tACS applied at the individual alpha frequency produced an enhancement in alpha power when the participants had their eyes open compared to the condition where they had their eyes closed [94]. This result provides additional support to the entrainment hypothesis. In the eyes-closed condition, the phases of the oscillators within the region targeted by tACS can be considered to be aligned to each other resulting in a strong endogenous alpha oscillation. In the eyes-open condition, however, the phases of the oscillators are not aligned with each other and tACS is able to cause synchronization of the phases of the oscillators resulting in stronger alpha oscillations. However, in the study where tACS was applied in an intermittent manner, scrambling the phase of stimulation current between consecutive trials did not produce effects different from the stimulation where the phase of the stimulation current was maintained to be continuous across all trials [8]. The authors argue that the results imply entrainment is not the underlying mechanism as the enhancement produced by stimulation with scrambled inter-trial phases should have been lesser than that produced by stimulation with continuous phase. Also, enhancement was stronger when stimulation frequency was close to the individual alpha frequency. If the entrainment hypothesis were true, the enhancement should have been higher at the stimulation frequency and not the individual alpha frequency. Additionally, as mentioned before, the absence of difference in inter-trial phase coherence between sham and stimulation conditions suggested that the outlasting effects of stimulation was not caused by entrainment. The authors propose a simplified STDP model to account for the effects of stimulation. Although plasticity is a plausible mechanism underlying the outlasting effects of tACS, there have been no studies in humans that explicitly show that this is indeed the case.

Thus, there is no clear consensus as to the mechanism underlying tACS. While the ideas of entrainment and plasticity seem mutually exclusive, this is not necessarily true. A realignment of phase may lead to strengthening of synaptic connections between the neurons because of STDP. Conversely, strengthening of synapses may lead to increased phase locking and consequently entrainment. Future studies trying to answer this question will be well served to include this consideration when designing the study as well as when trying to interpret the results.

Probing Functional Connectivity with tES

In this section, we will discuss a promising new target for tES, namely the dynamic interaction of neuronal networks within the brain. We will first introduce functional connectivity that quantifies such interactions and then discuss how tES could be used to modulate functional connectivity. The brain can be viewed of as a complex, high-dimensional network that dynamically changes over time. This network consists of billions of neurons with links, or connections, existing between individual neural cells, populations of neurons as well as different regions within the brain [103]. The network connectivity is not random, thus suggesting that specific connections are crucial for the processing and integration of new information [104, 105]. This idea is reinforced by the ability of the brain to form new connections during development as well as in response to input from the environment or induced trauma, a process known as neuroplasticity [106]. In this process, connections which are infrequently utilized are eliminated while those frequently used for information transfer are strengthened, essentially "pruning" synaptic connections in an activity-dependent process [107, 108].

We can think of the functional connectivity in the brain on three distinct levels as described by Polania et al. [106]: connectivity between individual cells (micro-scale level), connectivity between neuronal populations (meso-scale level), and connectivity between brain regions (large-scale level). Analysis on these different scales has allowed researchers to address a wide range of questions about the fundamental dynamics of the brain in physiological and pathological states.

The identification of network connectivity on the micro-scale level has received considerable attention from the computational community. Numerous methods have developed for the analysis of network connectivity on this level, an interest that in particular has been driven by the development of the multielectrode array (MEA) platform [109, 110]. Whether used in vitro or implanted in vivo the MEA allows for the recording of putative single-cell neuronal activity, often in the form of neuron spike trains, thus permitting individual cell-to-cell connectivity analysis. The techniques used to analyze these data can be characteristically divided into three classes. On one end of the spectrum, nonparametric methods assume no underlying model of the cell dynamics or of the interactions between cells. Cross-correlation and transfer entropy are two popular nonparametric approaches (see [111, 112] for a review and comparison of these methods). On the other end of the spectrum, parametric methods exist, which assume an underlying model for the cell dynamics as well as a model for the interaction between individual cells. For example, in [113] the authors considered the network connectivity problem in the state space framework whereby network connectivity was estimated using nonlinear Kalman filtering and a generic spiking neuron model. In between these two opposite ends of the spectrum, semiparametric methods exist as a mixture of both nonparametric and parametric approaches. For example, a semiparametric method may make no assumption about the cell dynamics, but it may assume an underlying model for the cell-to-cell interactions. In particular, the Cox connectivity method as explored in [114, 115] assumes that the interactions between neurons are modeled by a proportional hazard function.

The application of micro-scale connectivity analysis is limited in its scale and by the invasive nature of the recording technique it relies on. More applicable in the context of studying the human brain is analysis of connectivity on the meso- and large-scale levels. On this level, EEG and fMRI

have been used to determine functional connectivity at a larger spatial scale. Both methods allow for the noninvasive collection of signals related to neuronal activity; importantly, both offer specific spatial and temporal limitations in regards to their implementation [106]. EEG, which records electrophysiological neural activity through electrodes placed on the scalp, offers a high temporal resolution although its spatial resolution is poor. On the other hand fMRI, a neuroimaging technique capable of capturing hemodynamic activity which has been correlated with neural activity [116, 117], offers a much poorer temporal resolution but an improved spatial resolution. Regardless of their limitations, these techniques have been used to great effect in whole-brain functional connectivity analysis. Here the use of methods such as seed-based connectivity, independent component analysis, and graph theory has played a prominent role. In particular, the use of graph theory as a way of quantifying functional connectivity has gained increasing popularity [118, 119]. Mathematically, a graph consists of nodes which are linked by edges or connections. In the case of EEG the nodes are represented by the electrodes on the scalp and in the case of fMRI the nodes are represented by the blood-oxygen-level dependence, or BOLD. The connections within the network are then detected by linear or nonlinear correlations between the individual nodes. Specifically, these techniques have been used together to identify the resting-state functional connectivity of networks (see [120–122] for several reviews on the method).

Noninvasive brain stimulation techniques such as TMS and tDCS have been shown to significantly affect network functional connectivity. A large body of literature has examined the role of TMS in altering network connectivity (see [123] and the references within). Application of tDCS to the prefrontal cortex resulted in a significant change in the resting state functional connectivity [124]. Anodal tDCS improved [125] cognitive performance, paralleled by an increase in connectivity of the left inferior frontal gyrus, an area believed to be responsible for language functions. Application of tDCS to the left primary motor cortex was shown to alter the functional connectivity of cortico-striatal and thalamo-cortical circuits [126]. A promising direction in the field of noninvasive brain stimulation for therapeutic purposes is the use of tACS, as discussed in detail in the above section. There are only few studies that targeted functional connectivity with tACS. In-phase tACS of two fronto-parietal sites versus anti-phase tACS improved working memory [127], in agreement with previous EEG work. In a recent work by Helfrich et al. [95], the authors showed that tACS could be used to modulate interhemispheric brain connectivity.

While brain stimulation has been used with great success for the treatment of psychiatric and neurological disorders [128], the exact underlying mechanisms behind the success or failure of the stimulation for treatment remain mostly unclear [129]. Recent research has suggested that the pathology driving a range of neuropsychiatric diseases is network-based [116]. Abnormality in network connectivity has been implicated in particular for patients suffering from stroke [130–132], depression, and schizophrenia [133–135]. Given the potential effects of noninvasive brain stimulation on connectivity, and the prevailing belief that several neuropsychiatric diseases are driven by network abnormalities, it seems natural to address the question of therapeutic intervention not only from a brain stimulation framework but also from a network connectivity framework. In a paper by Fox et al. [128], the authors were able to map relationships between successful and unsuccessful stimulation sites across the treatment of 14 different neurological and psychiatric diseases. Their analysis revealed that sites where invasive deep brain stimulation (DBS) was effective for treatment were functionally connected to sites where TMS or tDCS were implemented effectively. These findings strongly suggest the importance of brain functional connectivity in stimulation procedure. While the integration of connectivity analysis and brain stimulation for therapeutic purposes is still in its very early stages, the initial results are encouraging [116].

The future role of functional connectivity in combination with brain stimulation is promising and thought-provoking at the same time. We must ask ourselves to what extent these functional connectivity mappings of the brain can use to guide our treatment of the various neuropsychiatric

diseases. In considering this, several future areas of inquiry come to mind. As mentioned earlier, tACS has been identified as a promising therapeutic treatment for disorders characterized by rhythmic cortical disturbance due to its frequency-specific modulation [95], and has been recently utilized for tremor suppression in Parkinson's patients [136]. As we think about the relationship between pathological brain states and abnormal network connectivity, this begs the question of whether or not functional connectivity can be modulated on a frequency basis using tACS. Understanding how network connectivity may or may not change as a function of tACS frequency may help guide our frequency-specific stimulation in treating these disorders.

Application of tES to Sleep Oscillations

A complete understanding of the effects of tES on human brain activity and behavior will require linking the findings of the microscopic domains (cellular recordings, computational models) to the discoveries from the macroscopic domains (human studies with EEG, MEG, and fMRI). Sleep is a promising frontier in terms of bringing these different levels of analysis together. More specifically, the slow oscillation (< 1 Hz) represents a strong candidate for such an undertaking for several reasons. First, we have an advanced understanding of the cellular and synaptic mechanisms underlying slow oscillations (SO). Second, weak electrical fields with frequencies mimicking the frequency of cortical SO have been applied in brain slices in vitro, in rats in vivo, and humans, and also studied in computational models. Third, SO can be artificially induced in vivo with anesthetic agents. We will discuss these three points in more detail.

Mechanisms of Slow Oscillations

In order to understand the effects of DC, oscillatory DC (rhythmic stimulation with a DC offset), or AC stimulation, we need to understand the mechanisms underlying different endogenous brain rhythms. SO are prevalent during slow-wave sleep and can be observed under anesthesia in vivo and in vitro, when the medium mimics in vivo conditions of the cerebrospinal fluid [10]. Mechanistically, SO have been very well studied and have been suggested to be generated and sustained in the neocortex [137–139] although thalamic circuits may also contribute [140]. This allows for investigating these rhythms in cortical slices [10]. The SO represents a low-frequency oscillation (~1 Hz) in the membrane potential of cortical neurons [141, 142] with the neurons alternating between so-called UP and DOWN states [139, 143]. The UP state is associated with the depolarized, i.e. active, phase of cortical neurons and most cortical neurons fire action potentials during this state [144]. During the DOWN state, neurons are silent and do not fire action potentials. These DOWN states can last for several hundreds of milliseconds and represent the prolonged hyperpolarizing phases of cortical neurons [144]. The synchronization of the slow oscillation of many neurons leads to the characteristic slow waves (< 4 Hz) seen in depth and surface EEG [142, 143, 145]. Of note, the prolonged silent or hyperpolarized phase, synchronized across many neurons, is unique to the slow oscillation during natural sleep and anesthesia [146, 147].

Internal dynamics need to be taken into account to understand which aspects of the slow oscillation can be modulated by weak electrical fields [148]. Specifically, for SO, the transition to the DOWN state is associated with activity-dependent reduction in synaptic strength that is maximal at the end of the UP state [148–151]. Thus, modulating the termination of UP states that are intrinsically determined may be difficult. In contrast, the transition from DOWN to UP state is driven by slight depolarizations that shorten the down-state [148]. This idea of differential susceptibility of different phases of the SO cycle has been supported by an in vitro study of ferret slices [18] and a computational model [152].

Modulating the Slow Oscillation with Weak Electric Fields

Modulation of SO using AC, DC, and oscillatory DC waveforms has gained significant interest in the last decade for the following reasons. First, SO has been implicated in coordinating other sleep rhythms (e.g. sleep spindles), providing a restorative function and promoting memory consolidation [155]. Thus, applying electrical stimulation to further boost SO will help to prove their causal role in the proposed processes [153]. Second, SO induces very pronounced endogenous electric fields and is therefore ideally suited to study the importance of those extracellular fields in entraining physiological neocortical network activity [18]. Thus, manipulation of SO with weak electrical stimulation has been probed in slices, in vivo in rats and ferrets, in humans, and in computational models.

Frohlich and McCormick [18] used the in vitro neocortical SO from acute slices of ferret visual cortex to demonstrate that externally applied weak electrical fields (physiological amplitudes that are found in vivo) and endogenous electric fields can directly modulate neuronal dynamics. Recorded oscillations are therefore not only a mere epiphenomenon of the underlying neuronal activity but rather actively modulate neuronal activity. The application of constant depolarizing currents (corresponding to anodal tDCS in humans) accelerated the slow oscillation frequency by shortening the duration of the down states (with no concurrent modulation of the up-state duration). Frohlich and McCormick [18] further highlighted the importance of ongoing network activity for weak electrical fields to have an effect. They applied sine-wave electrical fields that approximately matched the frequency of the spontaneous network oscillation and found that the SO became more periodic and entrained to the applied field. Importantly, weak external electrical fields preferentially enhanced the slow oscillation when their frequency was matching the intrinsic frequency. Along this line, Schmidt et al. [15] used an optogenetic approach to further confirm that weak alternating electric fields only enhanced endogenous oscillations when the stimulation frequencies were matched to the endogenous oscillations. In addition, ongoing network activity is necessary to amplify the effect of weak electrical fields by bringing the membrane voltage of neurons close to the threshold [18]. These important *in vitro* results hint at the fact that the amplification of network-wide weak perturbations by synaptic interaction may be an important aspect of the mechanism of tES.

Frohlich and McCormick [18] provided further support for this hypothesis with a computational network model showing that neuronal activity modulations by weak electric fields can be explained by small but simultaneous somatic depolarization of all neurons in the network. In a multi-scale computational model, Reato et al. [152] demonstrated that that intrinsic network dynamics of slow oscillatory activity can rectify mixed polarizations leading to an unidirectional increase of firing rates in case a monophasic alternating current is used (ON/OFF periods with ramp-up ramp down properties). Due to the cortical folding of the cortex, the applied electric fields show bi-directional polarities throughout the cortex, thus some regions might receive anodal stimulation while others experience cathodal stimulation. Thus, applying a constant DC would lead to both an increase and decrease of firing rates. In contrast when using monophasic alternating DC, the computational model predicts that entrainment occurs regardless of polarity (this applies for monophasic stimulation) via a modulation of the duration of the endogenous up- and down-state. Specifically, UP states will align with the ON phase of the anodal stimulation and the down-states with the ON phases of the cathodal stimulation and therefore only a rectified increase but no decrease in firing rate will be obtained [152]. However, this model only holds true if the OFF period of the alternating current field has a current strength of 0. Collectively, the findings from *in vitro* and computational studies emphasize that if and how tES affects neuronal activity depends on the intrinsic network activity (and on the applied field parameters).

To fully understand how tES affects SO in humans, we need a comprehensive physiological understanding of tES-induced effects on neuronal activity in the intact brain. This issue has been investigated by applying tES at frequencies of

cortical SO to multiple cortical regions in anesthetized and behaving rats [154], and anesthetized ferrets [45]. Ozen et al. [154] placed the stimulation electrodes on the surface of the skull or on the dura. Extra- and intracellular recordings showed an entrainment (phase-locking) of neurons to the externally applied sinusoidal electrical field. This effect was more pronounced if the network already exhibited intrinsic SO (anesthesia), further emphasizing that effectiveness of tES rests upon the internal network dynamics. Considering that rodents have lissencephalic brains and the human cortex exhibits pronounced folding which leads to uncontrolled and mixed field orientations, it is difficult to directly interpolate *in vivo* findings in rodents to humans. The ferret represents a model species with a gyrencephalic brain that helps overcome this limitation. Applying tACS at different slow oscillatory frequencies (0.5–3.5 Hz), Ali et al. [45] showed that multi-unit activity in anesthetized ferrets is entrained to the specific applied frequency. Whether this effect is restricted to a stimulated network that already exhibits intrinsic slow oscillatory activity remains unknown because only anesthetized ferrets were investigated.

SO have been proposed to play a key role in sleep-dependent memory consolidation [155]. Marshall et al. [153] were the first to demonstrate causality in this memory process by applying monophasic, slow-oscillatory tDCS (0.75 Hz, also compare [152]) during the first half hour of NREM sleep in healthy sleeping subjects. They found a significant increase in declarative memory along with increased slow-oscillatory and slow spindle activity (8–12 Hz) in stimulation-free EEG intervals (1 min intervals without stimulation in alternation with five 5 min stimulation periods). As mentioned in previous parts of this book chapter, the pronounced stimulation artifacts in the EEG prevent an accurate analysis of the EEG during tES application. Along this line, Reato et al. [152] predicted with their computational model (approximating the stimulation settings from [153]) that the rectified increase in firing rate leads to a faster downscaling of synaptic strength. Convincing evidence exists that SO are involved in downscaling synaptic connections to ensure the synaptic homeostasis of the brain [156] with high firing rates favoring synaptic depression [157, 158]. In addition, this downscaling process might lead to an increased synaptic signal-to-noise ratio that could explain the beneficial effect of sleep on memory consolidation [156, 159, 160]. Assuming that stimulation accelerates synaptic downscaling by increasing the firing rate, the rate of downscaling should be decelerated after the stimulation has stopped [152]. Their assumption was confirmed in the human dataset recorded by Marshall et al. [153]. Marshall et al. were further able to replicate the behavioral and EEG findings in rats [161, 162]. In addition, some studies were able to replicate, at least partially, the findings from Marshall et al. [155] in humans [163–166]. However, other groups found contradicting results on EEG and memory consolidation when applying monophasic slow-oscillatory tDCS [167, 168]. One of the differences between the studies was the waveform of the used tDCS pulse, e.g. Marshall et al. [155] were using ramp-up, ramp-down shaped pulses, and Sahlem et al. [167] were applying square-waves. Whether and how the tDCS pulse shape is critical for the effectiveness of oscillatory tDCS needs to be further investigated with the interdisciplinary toolkit discussed in the previous sections of this chapter. In addition, whether pure tACS (non-monophasic) in the slow-oscillatory frequency range has a similar effect on human brain network activity has so far not been studied and remains to be determined.

Anesthesia as a Tool to Study Slow Oscillations

Certain anesthetic agents (e.g. ketamine-xylazine, urethane, propofol) allow for the induction of SO that resemble the SO of natural sleep [169]. The main features of the slow oscillation (high amplitude waves generated by an alteration of UP and DOWN states) found during sleep can be mimicked by anesthesia [146, 147, 170]. Thus, anesthesia is used to model SO. In contrast to humans, it is very difficult to predict and schedule natural sleep, and more specifically slow wave sleep, in

rodents or ferrets for studying tES effect on SO. Thus, anesthesia was used to approximate slow wave sleep *in vivo* [45, 154] for testing the effects of tES on slow brain rhythms. Both studies, discussed in more details above, demonstrated enhancement of oscillatory activity in response to approximately frequency-matched stimulation. Thus, anesthesia can indeed serve as a model system to understand the neurophysiological effects of tES. Furthermore, the depth of sleep-like states and therefore the level of synchronization of SO can more easily be modulated by different anesthetic doses. Consequently, the role of the internal network state in the effectiveness of tES to enhance SO can more specifically be investigated. Nevertheless, future studies should further investigate effects of tES on SO in naturally sleeping animals because some features of the SO differ between anesthesia and natural slow-wave sleep, e.g. the rhythmicity and synchrony across the cortex [146, 147, 170].

Outlook

In this book chapter, we have attempted to pull together results from a vast set of different neuroscience methods to delineate how tES engages network targets in the brain. We have first introduced basic results on changes in excitability of individual neurons, followed by a discussion of modulation of network dynamics *in vitro* and *in vivo*. We then considered computational models as a complementary strategy to investigate the spatial targeting (forward models) and the targeting of neuronal dynamics (neural models). Next, we reviewed studies in humans that used noninvasive monitoring of brain activity (EEG, MEG, and fMRI) to demonstrate targeting of brain network dynamics by tES. In particular, we focused on the underlying dynamic principles that guide the interaction between tES and endogenous network dynamics. We then provide two unique perspectives that we believe will be central to furthering our understanding of targeting brain networks with tES. First, we look at functional connectivity and discuss how such analysis strategies that focus on dynamic interaction between activities at different locations within the brain will be vital for understanding global effects of brain stimulation. Second, we consider low-frequency rhythms during sleep and anesthesia as a case study for how the different methods discussed in earlier sections of the chapter can come together not only for understanding the mechanisms of tES and but also for the design of effective tES strategies to modulate memory consolidation. We hope that this review provides an integrated overview of today's research on how tES targets network dynamics and inspires a new area of rational design of brain stimulation to target physiological and pathological network states.

Given the noninvasive and low-cost nature of tES combined with the promising behavioral results, it is imperative to understand the underlying mechanisms of tES. The various levels of investigation described in this chapter, from microscopic to macroscopic and from in silico to in vivo domains, are essential to arrive at a holistic understanding of the mechanisms of tES. Once this is achieved, rational design of tES paradigms to target specific network dynamics will become the norm. Ultimately, this will help to usher in a new area of neuroscience in which tES serves as a broadly used, effective research tool for probing and understanding functional networks of the human brain as well as a transformative therapeutic tool for treating disorders of brain networks.

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