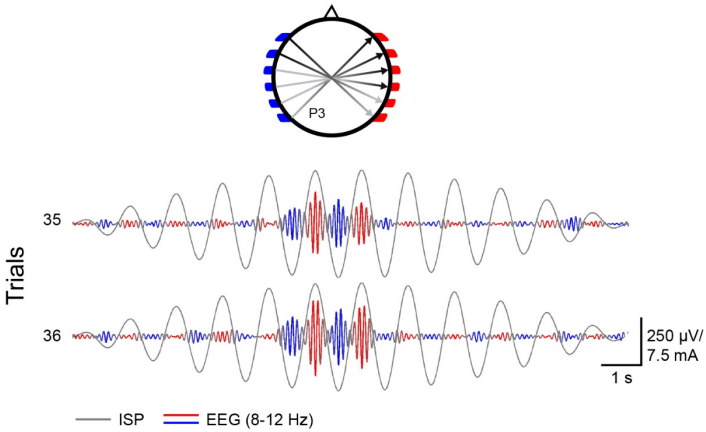
Spatially and temporally targeted neuro-modulation by transcranial electrical stimulation

Summary of the Ph.D. thesis



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Introduction

Electrical stimulation whether invasive or noninvasive is one of the oldest neuromodulatory research tools. In the late 1930s electroconvulsive therapy (ECT) was introduced as the first neurostimulation application to treat several neuropsychological disorders in humans. In parallel, research has been also focused on the delivery of a more localized electrical stimulation. Two techniques emerged, an invasive one called deep brain stimulation (DBS) and a noninvasive one called transcranial electrical stimulation (TES). DBS has been approved as a treatment for numerous neuropsychological disorders, e.g.: Parkinson's disease, essential tremor and dystonia, however, due to its invasive nature the potential for serious complications still exists. That is one of the reasons why researchers' interest has grown exponentially in noninvasive brain stimulation (NIBS) methods like transcranial electrical and magnetic stimulation (TMS). TES became a popular research tool when Nitsche and Paulus (2000) showed that weak electrical current stimulation over the motor cortex was able to induce changes in brain excitability. Since then, the following main methods of TES have been investigated: transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS). TES has been increasingly utilized as a clinical tool for the treatment of neuropsychiatric disorders (e.g.: major depression, migraine, tinnitus and even addiction). Despite the promising results and growing number of publications, the exact mechanisms by which electrical stimulation can affect neural activity has remained unknown.

Recent *in vivo* studies showed that TES can modulate the ongoing neuronal activity in widespread cortical areas if the induced electric field exceeds 1 V/m. In addition, high-intensity, brief gaussian pulses (~6 - 8 V/m electric field in the brain, 50 ms duration) in a closed loop manner can stop ongoing seizure activity and restore normal brain function.

Contrary to animal experiments, clinical studies apply maximum 2 mA stimulation (~0.1 - 0.3 V/m electric fields in the brain). Using realistic head models, *in silico* studies can predict the TES induced electric fields in the human brain, however, further measurements are required to validate these models. A typical clinical session applies stimulation for at least 10 minutes using large (20 - 50 cm^2) stimulating electrodes. Applying currents with these parameters, immediate neuronal effects are unlikely, a cumulative or additive effect can guide the mechanisms in these cases. To date, there is still no consensus about electrical stimulation parameters that can reliably affect ongoing brain activity in humans.

One disadvantage of TES is that it cannot achieve focal deep brain stimulation because currents have to pass through the cortical surface. New engineering solutions are needed to improve TES and to achieve spatial focality.

Aims of the study

The primary goal of my research was to better understand how TES induced electric fields can affect neuronal activity instantaneously. We attempted to identify and validate electrical stimulation

parameters that are necessary to modulate ongoing brain activity both in rodents and humans. The specific aims of my work were:

1. To measure the effect of the presence of soft tissues on electrical stimulation induced electric fields in rats.
2. To determine the electric field necessary to affect single unit activity and membrane potential instantaneously in anaesthetized rats.
3. To translate rodent results into human applications.
4. To design a measurement and stimulation protocol in which TES can alter ongoing neuronal activity in humans. To validate this protocol in rats.

Results

Comparison of trans- and subcutaneous electric stimulation in rats

The presence of soft tissues can reduce the amount of currents that enter the brain, and the question arise; whether currents of given magnitude even reach the cortex to alter neural activity. Therefore, we examined how skin and head musculature surrounding the skull can influence the magnitude of induced electric fields in the rodent brain. Using a 32-channel silicon probe we measured the intracerebral voltage gradients and applied current to the shaved scalp (transcutaneous TES) then to the parietal bone (subcutaneous TES). Transcutaneous TES through the same size of electrodes resulted in an $80 \pm 5\%$ current loss, independent of the stimulus intensity. In a more direct physiological comparison, we tested the effects of

externally applied direct currents on the intracellularly recorded transmembrane potential (V_m) and spiking of neurons in the deep layers of the visual cortex. Subcutaneous (skull) stimulation exerted clear and predictable effects on V_m. Anodal stimulation induced depolarization of V_m, increased firing rate and reduced V_m power in delta frequency band. Using the same current intensities, transcutaneous (scalp) stimulation produced much smaller and more variable effects. Only the highest current intensity (800 μA or 2 V/m) had significant effects on V_m and firing rate (delta power was unaltered).

Measuring current spread through scalp, skull and brain in human cadavers

Currently, *in silico* models are offering the best estimates about the currents needed to induce electric fields of sufficient magnitude intracranially in the human brain. However, there are many uncertainties in such modeling. As an alternative to modeling, we carried out high spatial density, 3-D intracerebral measurements in cadaver brains in situ ($n = 11$). Thirty-six custom-made multisite electrodes (three to seven sites per electrode, 198 in total) were inserted into the brain. Applying tACS, the highest electric fields occurred in the neocortex near the stimulation electrodes. The relationship between applied current or voltage and the measured electric fields was linear. The frequency of stimulation had only a small effect on the magnitude of the induced fields. As expected, larger size electrodes induced larger electric fields.

We also measured the transcutaneous (scalp), subcutaneous (skull with scalp removed), and direct epidural stimulation induced electric fields and found that 58 ± 7% of the current applied through the scalp diffused through the soft tissue surrounding the head. Another 16 ± 8% of the current was attenuated by the resistance of the skull. Extrapolation of transcutaneous stimulation results suggested that approximately 6 mA current applied to the scalp would be needed to reach 1 V/m voltage gradient in the living brain.

Affecting human brain network activity by Intersectional Short Pulse stimulation

To deliver stronger currents to the brain we need to minimize peripheral effects; and we should be able to record the brain activity and stimulate simultaneously. Our proposed solution uses spatio-temporally rotating Intersectional Short Pulse (ISP) stimulation in which current is delivered through multiple pairs of stimulation electrodes by rapidly switching (μs scale) currents between them. An added advantage of fast pulse stimulation is that the transients of high frequency pulses do not saturate recording amplifiers even at relatively high intensities.

To test ISP in humans, a circular array of 12 stimulation electrodes was placed around the head and ISP stimulation was applied in healthy human subjects (n=18). Scalp EEG was monitored by a 2-site montage (P3 and P4 against reference Pz). To avoid onset and offset effects, ISP stimulation consisted of a train of 1-Hz sinusoids with increasing and decreasing intensity (0, 1.5, 3, 4.5, 6, 7.5, 6, 4.5, 3, 1.5, 0 mA per cycle) for 12 s, repeated 60 times for each subject. The low frequency

(1 Hz) stimulation allowed us to investigate the anodal–cathodal phase modulation of the amplitude of the spontaneous EEG. The alpha-band modulation was present in both hemispheres and alternated in phase, due to the shifting of the anodal–cathodal current direction. Significant modulation of the LFP amplitude by the TES phase was observed at current intensities of 4.5, 6, and 7.5 mA at each hemisphere. As a control for potential arousal effects, 3 subjects were also tested with the same ISP protocol but with the stimulation electrodes placed on the abdominal wall. No hemisphere-specific, stimulation phase-induced modulation of alpha waves was observed.

Focused TES effect by ISP stimulation

For many clinical applications, it would be desirable to apply TES in a spatially targeted manner and to monitor the ongoing brain activity simultaneously in order to verify online effects. During ISP stimulation, each electrode pair will generate an electric field which will polarize the cellular membrane of neurons in the brain. Because of the short integration time constant of the neuronal membrane (20 ms), neurons can temporally integrate multiple electric fields with similar vector directions.

To test our prediction of focal effect in rats, current pulses were delivered sequentially and in a spatially asymmetric manner. Single unit activity was recorded bilaterally in the CA1 region of both hippocampi with 32-channel silicon probes (7 anesthetized rats and 1 chronically implanted rat). The hemisphere target of the bipolar stimulation configuration was alternated. The artifacts of the short duration stimulation pulses did not affect the recording quality. Of the

127 isolated single units, 55 were significantly affected by at least one configuration of the stimulation protocol (32 increased and 23 decreased its firing rate). To quantify the focusing effect of ISP, we calculated the fold-change of unit discharge in the targeted and non-targeted hemispheres (1.8 ± 2.35-fold vs. 1.017 ± 0.63-fold; mean ± SD; $n = 55$ units).

Discussion

Using *in vivo* experiments in rodents, we determined the minimum electric field that is necessary to alter the ongoing brain activity – approximately 1 V/m. Although this is a very weak field, there were reasons to believe that the currently used stimulation parameters in humans do not reach this threshold. Therefore, we measured the 3-dimensional distribution of the TES induced electric fields in human cadavers, and we found that up to 80 percent of the current applied to the scalp is lost due to the shunting effects of the soft tissue. To reduce the peripheral side effects and enable simultaneous recording and stimulation, we designed an 'Intersectional Short Pulse' stimulation method that allowed injection of higher current intensities into the brain. We recorded EEG activity in healthy subjects while applying ISP to the scalp. These experiments demonstrated clearly that at higher current intensities it is indeed possible to modulate the ongoing brain activity. Finally, we demonstrated the spatial specificity of the ISP method in rats.

TES-induced physiological effects

Despite thousands of publications on NIBS techniques in the past decade, we lack of understanding how TES can affect neurons and can lead to behavioral and/or therapeutical outcomes *in vivo*. In principle, the efficacy of TES depends on a variety of factors including neuronal geometry, alignment of neurons relative to the electric field, type and distribution of ion channels in the neurons and degree of myelination. Neurotransmitter-induced postsynaptic potentials and ephaptic coupling can affect neuronal excitability. Both mechanisms can influence subthreshold membrane potentials and spiking. When a neuron is about to elicit an action potential, even a weak electric field can bias spike threshold. *In vitro* experiments have shown that <1 V/m oscillatory field can be coupled to intracellularly generated oscillations. Whether such weak electric field (<1 V/m) could result in clinical changes must be measured *in vivo*. Especially considering that hippocampal theta oscillations across the CA1 pyramidal layer can elicit endogenous voltage gradients of > 4 V/m. Yet, the requirements of affecting spike threshold of some neurons occasionally in wide areas of the brain versus consistently biasing activity of neuronal circuits are different. Our *in vivo* intracellular recordings have revealed that > 1 V/m intracerebral electric fields were needed to exert measurable effects on spikes and subthreshold V_m, but several times larger currents were required to measurably affect the associated network rhythms. This difference may be explained by the competition between the applied fields and the strong influence of endogenous network patterns.

Current flow through the scalp, skull and brain

In rodent experiments, TES induced electric fields are typically ten-fold stronger compared to human studies and stimulating electrodes are often placed on the skull, the dura mater, or directly on the brain surface. Translation of animal experiments are extremely difficult. On the other hand, modeling has become increasingly sophisticated over the years, experimental data are needed to justify the modeling assumptions. Using scalp, cranial, and epidural stimulation electrodes and multiple recording electrodes, we quantified the 3-D spread of electric fields in both rodents and human cadavers. Our findings confirm that the scalp, subcutaneous tissue, and muscles function as an effective shunt, resulting in at least 50% reduction of applied current intensity. The serial resistance of the skull further reduces the current flow by another 10 - 25%, depending on the thickness of the skull. Given the importance of these attenuating factors, the amount of soft tissue, hair, and skull thickness should be taken into account in estimating the magnitude of TES induced intracerebral electric fields, and variation of these factors may explain the large individual variability in humans in response to TES.

ISP: injecting high current intensities and targeting brain regions

Our *in vivo* rat data suggest that in order to alter the ongoing firing rate of neurons, one must generate at least 1 V/m electric field in the vicinity of those cells. In addition, we estimated from our cadaver experiments that scalp-applied currents should exceed 4 - 6 mA to achieve this voltage gradients. Is it possible to reach 1 V/m electric field in the human brain using TES? According to modeling, primate

and human studies; the answer is no, if we apply a 'standard' TES protocol (2 mA current intensity, 20 cm^2 electrode surface). Even though there is a linear relationship between applied current and induced electric field, one cannot simply increase the current intensity above 2 mA, because 1) >2 mA currents will induce increased risk of side effects and 2) the recording amplifiers will be saturated during TES. To reduce scalp sensation or other side effects, to increase the direct effects of TES on brain activity and to prevent the amplifier saturation, we developed a new method called 'intersectional short-pulse' stimulation.

ISP uses brief and rapidly rotating current pulses via multiple stimulation electrode pairs. In theory, the more stimulation electrode pairs are used, the smaller the adverse effects are on the periphery and other brain regions. In our human measurements, we used six pairs of stimulation electrodes which reduced the required local momentary current by six-fold. ISP was tolerable even at 7 mA current intensities; however, we could not eliminate all the adverse skin effects and vestibular reactions. In addition, the high frequency pulses during ISP stimulation did not saturate the recording amplifiers; therefore, we were able to measure the ongoing EEG activity during ISP. Applying 1Hz modulated ISP, we found that >4.5 mA currents were required to reliably bias the amplitude of occipital alpha waves.

TES might be an alternative treatment option to DBS in several clinical conditions if we could recruit deep-lying neurons without affecting superficial ones. It is generally thought that TES cannot limit the high-intensity region to a small target volume; however, a recent

study achieved 'non-invasive DBS' using multiple interfering waveforms. Our modeling showed that ISP can exploit the time-integrating property of the neuronal membrane (~20 ms). Using just three rotating dipoles in rats, we demonstrated a proof of principle for the spatial focusing effect of ISP by confining the ISP effect to largely one hemisphere.

In conclusion, we demonstrate that affecting neuronal circuits directly and instantaneously in the human brain requires higher intensity currents than used in conventional TES experiments.

Materials and methods

Comparison of transcutaneous and subcutaneous TES in vivo

Long-Evans rats (350–450 g) were implanted with custom-made stimulating electrodes under urethane anesthesia. For transcutaneous electrical stimulations, a pair of silicon single-pocket electrodes (2-by-2-by-1 mm, 4 mm^2 surface area) filled with conductive EEG gel was glued on both sides of the head of the rats. Small incision was made on the scalp and a 1.2 diameter craniotomy was drilled and a 32-channel silicon probe was inserted in the CA1 region of the hippocampus (location: 3 mm posterior from bregma and 2 mm lateral of the midline). Varying frequencies (10, 100, and 1000 Hz) at varying amplitudes (10, 20, 50, 100, and 200 μA) were used. After the transcutaneous condition, the simulating electrodes were attached to the skull (transcranial stimulation) and the above-mentioned protocol was repeated. We calculated the first spatial derivative of the recorded potential values and estimated the shunting effect of the skin.

Effect of TES on membrane potential and single unit activity

Similarly, to extracellular experiments; trans- and subcutaneous stimulation were performed in anesthetized Wistar rats. Blind in vivo whole-cell recordings were carried out from cortical neurons meanwhile direct current stimulation was applied (0.2 - 0.8 mA). After the whole-cell transmembrane potential recordings, the recorded neurons were detached from the pipette and the same set of electrical stimuli were recorded extracellularly. To obtain the transmembrane voltage, artifacts were subtracted from intracellularly recorded potentials. Power spectra of the stimulated and control epochs were calculated on a trial-by-trial basis, using fast Fourier transform, before averaging. Spectra were whitened by the 1/f method.

Measurements on human cadavers

Cadavers without known brain disorder were selected for measurements. After the skull was cleaned, 36 penetration holes were drilled (1.2 mm diameter) and custom-made, multi-contact recording electrodes were inserted into the brain (n = 198 channels). Four or seven pairs of stimulation electrodes (Ag/AgCl EEG electrodes, 10 mm diameter) were attached to the skull surface with conductive paste. Sinusoid stimuli with varying intensities (1, 2, 3, 4, 5, and 6 V) at 10 Hz and varying frequencies (5, 20, 50, 100, 200, 500, 1000, and 2000 Hz) at 5 V were used. In order to measure the shunting effect of the skin and skull, instead of retracting the skin, four or six 5-mm long incisions (15 mm apart from each other) were made in the coronal plane, connecting one mastoid with the other. Four or six custom-made 7 contact site recording electrodes were inserted into the brain

and transcutaneous AC stimulation was performed, as described above. After the skin measurements, the skin incisions were carefully connected, and the scalp was removed while the recording electrodes were kept in place. The stimulating electrodes were attached to the skull surface and the same stimulation protocol was applied. In separate experiments, to compare the effect of subcutaneous stimulation to intracranial stimulations, additional stimulating electrodes were placed intracranially in three cases. Sponge electrodes with the encapsulated Ag/AgCl plates were placed on the surface of the brain. At the end of the measurements, anthropometric data of the skull was measured. In each condition, the first spatial derivative of the recorded voltage signals was calculated.

Intersectional short pulse stimulation (ISP) on humans

Human transcutaneous ISP stimulation and EEG recording were performed on healthy subjects (all males, age = 21 - 66 years). 6 pairs of stimulating sponge electrodes were attached to the skin bilaterally. The ISP stimulation consisted of 6 x 10 μs pulses repeated at 16.66 kHz (100% duty cycle). The amplitude of the pulses was modulated by a 1-Hz sine wave, linearly ramping up from zero to maximum in 6 seconds, then ramping down to zero in 6 seconds (0 - 10 mA). EEG scalp recordings were performed during stimulation (P3 and P4 locations [10/20 system], occipital region). Frequency-amplitude and phase-amplitude analysis of the recorded EEG signals were performed.

Intersectional short pulse stimulation in rats

Two custom-designed stimulation strips were 3-D printed and glued bilaterally on the surfaces of the temporal bones of the rats. During ISP stimulation, the neuronal activity was recorded in the CA1 region of the hippocampus with two 32-channel silicon probes. Each stimulation trial consisted of 3 x 2.5 μs pulses repeated at 133 kHz (100% duty cycle) for 500 ms and followed by 1 s pause. Neuronal spikes were detected from the digitally high-pass filtered signal (1–3 kHz) by Spikedetekt2. Detected spikes were automatically sorted using KlustaKwik2, followed by manual adjustment of the clusters using KlustaViewa software to get well-isolated single units (multi-unit and noise clusters were discarded).

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Publications related to the subject of this thesis

1. *Mihály Vöröslakos*, Yuichi Takeuchi, Kitti Brinyiczki, Tamás Zombori, Azahara Oliva, Antonio Fernández-Ruiz, Gábor Kozák, Zsigmond Tamás Kincses, Béla Iványi, György Buzsáki, Antal Berényi (2018) **Direct effects of transcranial electric stimulation on brain circuits in rats and humans.** Nature Communications
   DOI: 10.1038/s41467-018-02928-3

2. Anli Liu, *Mihály Vöröslakos*, Greg Kronberg, Simon Henin, Matthew Krause, Yu Huang, Alexander Opitz, Ashesh Mehta, Christopher C. Pack, Bart Krekelberg, Antal Berényi, Lucas Parra, Lucia Melloni, Orrin Devinsky, György Buzsáki (2018) **Immediate neurophysiological effects of transcranial electrical stimulation.**
   Nature Communications
   DOI: 10.1038/s41467-018-07233-7

Other publications

1. Komal Kampasi, Daniel F. English, John Seymour, Eran Stark, Sam McKenzie, *Mihály Vöröslakos*, György Buzsáki, Kensall D. Wise, Euisik Yoon (2018) **Dual color optogenetic control of neural populations using low-noise, multishank optoelectrodes.** Microsystems & Nanoengineering. DOI: 10.1038/s41378-018-0009-2

2. Kyounghwan Na, Zachariah J Sperry, Jiaao Lu, *Mihály Vöröslakos*, Saman S Parizi, Tim M Bruns, Euisik Yoon, John P Seymour (2018) **Novel diamond shuttle to deliver flexible bioelectronics with reduced tissue compression**. bioRxiv, DOI: 10.1101/435800

Cumulative impact factor of the publications related to the thesis

23.76

Cumulative impact factor of all publications

29.376