

Endogenous Cortical Oscillations Constrain Neuromodulation by Weak Electric Fields



Stephen L. Schmidt^{a,e}, Apoorva K. Iyengar^b, A. Alban Foulser^g, Michael R. Boyle^{a,e}, Flavio Fröhlich^{a,c,d,e,f,*}

^a Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

^b Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

^c Neurobiology Curriculum, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

^d Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

^e Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

^f Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

^g Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:

Received 11 March 2014

Received in revised form

18 June 2014

Accepted 13 July 2014

Available online 14 August 2014

Keywords:

Transcranial alternating current stimulation

tACS

Optogenetics

Electric field

Multielectrode array

Resonance

ABSTRACT

Background: Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation modality that may modulate cognition by enhancing endogenous neocortical oscillations by application of sine-wave electric fields. Yet, the role of endogenous network activity in enabling and shaping the effects of tACS has remained unclear.

Objective: We combined optogenetic stimulation and multichannel slice electrophysiology to elucidate how the effect of a weak sine-wave electric field depends on the ongoing cortical oscillatory activity. We hypothesized that endogenous cortical oscillations constrain neuromodulation by tACS.

Methods: We studied the effect of weak sine-wave electric fields on oscillatory activity in mouse neocortical slices. Optogenetic control of the network activity enabled the generation of *in vivo*-like cortical oscillations for studying the temporal relationship between network activity and sine-wave electric field stimulation.

Results: Weak electric fields enhanced endogenous oscillations but failed to induce a frequency shift of the ongoing oscillation for stimulation frequencies that were not matched to the endogenous oscillation. This constraint on the effect of electric field stimulation imposed by endogenous network dynamics was limited to the case of weak electric fields targeting *in vivo*-like network dynamics. Together, these results suggest that the key mechanism of tACS may be enhancing, but not overriding, intrinsic network dynamics.

Conclusion: Our results contribute to understanding the inconsistent tACS results from human studies and propose that stimulation precisely adjusted in frequency to the endogenous oscillations is key to rational design of non-invasive brain stimulation paradigms.

© 2014 Elsevier Inc. All rights reserved.

Introduction

Oscillatory activity in neocortex enables temporal organization of information during sensory processing, cognition, and memory

Authorship statement: SS and FF designed the experiments; SS, AI, and AF performed the experiments; SS analyzed the data; MB validated the analysis code, and SS and FF wrote the paper.

The authors declare no competing financial interests.

A small subset of the data included in this manuscript was previously presented in abstract form (6th IEEE Neuroengineering Meeting 2013 and SN 2013, both San Diego).

Research reported in this publication was supported in part by the National Institute of Mental Health of the National Institutes of Health under Award

[1,2]. Perturbing cortical oscillations with brain stimulation has become a key experimental approach to evaluate the functional, frequency-specific roles of cortical oscillations. Targeted enhancement of cortical oscillations may, in the future, also serve as a

Number R01MH101547. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. AAF was funded by the Summer Undergraduate Research Fellowship from the Office of Undergraduate Research at University of North Carolina at Chapel Hill.

* Corresponding author. 115 Mason Farm Rd. NRB 4109F, Chapel Hill, NC 27599, USA.

E-mail address: flavio_frohlich@med.unc.edu (F. Fröhlich).

treatment for psychiatric disorders associated with impaired cortical oscillations such as schizophrenia and autism [3]. Supra-threshold excitation of cortical networks with pulsed transcranial magnetic stimulation (TMS) can alter temporal structure of network activity and mediate changes in associated cognitive processes ([4], for example see Refs. [5,6]). Recently, transcranial alternating current stimulation (tACS) has emerged as an alternative non-invasive brain stimulation approach for targeted modulation of cortical oscillations [7]. TACS applies a weak, sine-wave electric field (EF) that targets neocortical networks. Several studies have demonstrated (1) cognitive enhancement by tACS in humans [8–11] and animals [12] and (2) enhancement of neuronal activity in animal models both *in vitro* [13–15] and *in vivo* [16,17]. However, very little is known about the interaction dynamics between endogenous cortical oscillations and the small, periodic changes in membrane voltage caused by tACS. In fact, differences in endogenous network activity alter the effects of tACS on macroscopic brain activity measured by electroencephalography (EEG) and may explain the variability of behavioral effects for seemingly very similar stimulation paradigms [18]. In the long term, elucidating the role of endogenous activity in shaping the response to non-invasive brain stimulation is crucial for the development of individualized, adaptive stimulation paradigms that take into account ongoing network activity and adjust stimulation accordingly.

Computational simulations suggest that cortical activity is preferentially enhanced by EFs at the frequency of the endogenous oscillation through network resonance [15,17]. Network resonance in this context is defined as the preference of a network to oscillate in a narrow frequency band in response to periodic stimulation (for review of resonance see Ref. [19]), in this case centered on the frequency of the endogenous oscillation. Here, we combine optogenetic network activation with applied EF stimulation *in vitro* to determine how the relationship between the network oscillation and stimulation frequency determines the effect of periodic stimulation on cortical oscillations. Using optogenetic stimulation to generate an *in vivo*-like activity structure provided precise control of the “endogenous” activity [20] that we perturbed with EF stimulation to study the interaction dynamics between endogenous network activity and stimulation. Specifically, we used optogenetic stimulation of layer 5 pyramidal cells in cortical slices of the Thy1-ChR2 mouse [21] to induce a pronounced slow cortical oscillation (1 Hz) that we then targeted with applied EF stimulation. Layer 5 pyramidal cells play a central role in low-frequency cortical oscillations [22] and their optogenetic activation *in vivo* induces network activity that closely mimics spontaneous slow cortical oscillations [20]. Using this approach, we tested the hypothesis that intrinsically oscillating neocortical networks exhibit network resonance by preferentially responding to frequency-matched sine-wave EF stimulation.

Methods

All procedures were approved by The University of North Carolina at Chapel Hill Institute of Animal Use and Care and were in compliance with the National Institute of Health guide for care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Slice preparation and data acquisition

Thy1-ChR2 mice (Jackson Laboratory, Bar Harbor, ME), deeply anesthetized with Euthasol (0.5 mL/kg, Virbac, Fort Worth, TX), were decapitated and their brains extracted and quickly placed in ice-cold sucrose solution (mM: 83.0 NaCl, 2.5 KCl, 0.5 CaCl₂, 3.3 MgSO₄, 1 NaH₂PO₄, 26.2 NaHCO₃, 22.0 Dextrose Anhydrous and 72.0 Sucrose) bubbled with carbogen (95% O₂, 5% CO₂). 200 μ m coronal

sections containing V1 were cut using a vibratome (Leica Microsystems, Wetzlar, Germany) and allowed to recover in incubation solution (mM: 119.0 NaCl, 2.5 KCl, 1.0 NaH₂PO₄, 26.2 NaHCO₃, 22.0 glucose, 2 MgSO₄ and 2.0 CaCl₂) for at least 1 h at 34 °C. After recovery, slices were placed on a MEA 2100 (Multichannel Systems, Reutlingen, Germany) and perfused with artificial cerebrospinal fluid (aCSF, mM: 119.0 NaCl, 4.5 KCl, 1 NaH₂PO₄, 26.2 NaHCO₃, 22.0 glucose, 1.0 MgSO₄, and 1.0 CaCl₂), heated to 36 °C, at a rate of greater than 4 mL per minute as in Ref. [23]. The calcium and magnesium concentrations in the aCSF were modeled after human CSF concentrations [24]. Data were recorded from 59 electrodes (30 μ m diameter) in a 6 by 10 perforated array with 100 μ m spacing between electrodes at a rate of 25 kHz.

Experimental design

Experiments included continuous 1 Hz optogenetic stimulation by blue light (460 nm, 22 mW maximum, approximately 200 μ W delivered at slice surface) from a LED 460 (Prizmatix, Givat Shmuel, Israel). EF was generated by applying a calibrated current from a stimulus isolator (World Precision Instruments, Sarasota, FL) between two AgCl wires in the bath. Slices were arranged on the MEA such that pia was in parallel to the stimulation wires and therefore the applied EF approximately orthogonal to the somato-dendritic axes of the pyramidal cells in the slice preparation. Therefore, the EF was oriented from the wire close to layer 1 of visual cortex to the wire close to the ventral side of the slice such that the positive first half-wave and the negative second half-wave caused a somatic membrane depolarization and hyperpolarization, respectively [25,26]. Experiments consisted of continuous 1 Hz optogenetic stimulation (square-wave, 50% duty cycle) for 64 trials of EF stimulation. Each trial of EF stimulation consisted of 10 s of optogenetic stimulation, 10 s of EF with optogenetic stimulation, followed by an additional 10 s of optogenetic stimulation. The order of trials was randomized for both EF stimulation frequency (0.8, 1.0, 1.2 or 2.0 Hz) and EF stimulation amplitude (1 or 2 mV/mm). The EF was selected to be of similar amplitude to the estimated EF strength during tACS in humans [11,27] and was below action potential threshold for cells at rest [14,26]. For experiments without optogenetic stimulation, the same stimulation paradigm was used except the LED was disconnected from the power supply. In experiments with low intensity optogenetic stimulation, the intensity was decreased to 1/10th of the full intensity (approximately 20 μ W at slice surface). For experiments with temporally varied optogenetic stimulation, we first selected the length of each “UP/DOWN state” from a normal distribution (mean 500 ms, standard deviation 50 ms) for a total of 10 oscillation cycles. “UP” states here refer to the periods when the LED was on. Conversely, “DOWN” states refer to the inactive phase of the LED stimulation cycle.

Data analysis

Multiunit traces and stimulation data were recorded using MC_Rack (Multichannel Systems, Reutlingen, Germany). All data analysis was performed by custom-written Matlab (Mathworks, Natick, MA) scripts. Spikes were extracted at the time-points at which the recorded, high-pass filtered trace for each electrode crossed the threshold of -4 times the standard deviation of the trace (1 ms dead time). Application of the EF would occasionally cause a transient, stereotyped voltage spike on all electrodes at the onset, offset or zero-crossing of EF cycles; therefore artifact removal was performed by finding and removing voltage spikes that occurred on 10 or more electrodes within 1 ms. Following artifact removal, only electrodes in V1 were included for subsequent analysis. Single units (SU) were isolated using methods outlined in

[23,28,29]. Spike trains were binned with 1 ms resolution for each SU. The firing rate (FR) effect of EF stimulation was calculated as the FR during EF stimulation divided by the FR of the period without EF stimulation immediately preceding stimulation for each electrode for each trial. The activation ratio was calculated as the number of spikes while the LED was on divided by the total number of spikes in the optogenetic stimulation cycle. Whole-slice spike trains were constructed by summing spike trains across electrodes (1 ms bins). Frequency transforms were calculated by convolution with Morelet wavelets followed by temporal averaging, and subsequent whole-slice spectra were then normalized by dividing by the number of summed SU. Frequency preference was calculated as the mean of the frequency spectra at the frequency ± 0.01 Hz subtracted by the mean of 1.5 times the frequency ± 0.01 Hz. This metric includes not only the enhancement of rhythmic structure at the frequency in question but the suppression of rhythmic structure at frequencies between the fundamental frequency and the first harmonic. Paired data were analyzed using a Wilcoxon signed rank test with a Bonferroni correction for multiple comparisons where appropriate. Confidence intervals were determined from 5% to 95% using bootstrapping with 1000 iterations.

Results

Optogenetic in vitro model of slow cortical oscillation

To probe the interaction dynamics between neocortical network activity and EF stimulation, we developed an experimental approach that enabled precise control of endogenous network dynamics *in vitro*, application of EF stimulation, and simultaneous recording of neuronal activity in all layers of cortical networks. To this end, we first determined if full-field optogenetic stimulation in absence of EF stimulation robustly evoked activity in the entire network and not just pyramidal cells in layer 5 that express ChR2 (Fig. 1A, left). We found that periodic stimulation with blue light (460 nm optogenetic stimulation, 1 Hz, 500 ms of stimulation followed by 500 ms without stimulation for each oscillation cycle) robustly evoked network activity (Fig. 1A, right). Importantly, individual SU in all cortical layers responded to optogenetic stimulation to varying degrees (Fig. 1B, activation ratio, long-tailed distribution). The spread of activation ratios demonstrates that the SU in the stimulated slices did not all exclusively fire in lockstep (activation ratio of 1.0) with the stimulation, but maintained their ability to spike in absence of stimulation (activation ratio of 0.5 if optogenetic stimulation had no effect). The SU maintained sparse firing (activation ratio, median \pm SEM: 0.907 ± 0.003 for all SU, 0.895 ± 0.004 for L1–4, 0.917 ± 0.004 for L5&6; FR: 1.30 ± 0.038 Hz for all SU, 1.33 ± 0.053 Hz for L1–4, 1.29 ± 0.052 Hz for L5&6; $n = 3840, 1536, 2304$ SU*trials, respectively; correlation: $r = 0.125$, $P < 10^{-3}$ for all SU; Fig. 1B). Thus, the optogenetic stimulation of the slices did not cause unphysiological FRs. Despite the cell-to-cell variability, the overall network activity in individual slices exhibited a prominent peak at 1 Hz in most trials (1 Hz peak: 1.05 ± 0.027 spikes²/Hz, $n = 703$ trials, trials sorted by power at 1 Hz, Fig. 1C). These data demonstrate that our optogenetic stimulation paradigm provided a precisely tuned 1 Hz network oscillation but preserved *in vivo*-like sparseness and cell-to-cell variability of the firing patterns of individual neurons.

Neocortex responds to weak EF with network resonance

TACS provides a weak EF that we modeled with an applied EF generated by a voltage-controlled current source. We simultaneously applied optogenetic stimulation to model endogenous network activity (Fig. 2A, Experimental set-up and waveforms for

optogenetic and EF stimulation). We hypothesized that the response to stimulation is guided by network resonance near the frequency of the endogenous oscillation. Accordingly, EF stimulation of equal amplitude at the frequency (or its first harmonic) of the ongoing network oscillation would have a greater effect than stimulation with waveforms at nearby, yet mismatched frequencies. To test this hypothesis, we examined the network response to EF stimulation with two different amplitudes (1 and 2 mV/mm) at stimulation frequencies matched to (1 Hz), twice (2 Hz), and nearby (0.8 and 1.2 Hz) the frequency of the endogenous network oscillation (1 Hz, induced by optogenetic stimulation). We first examined the raster plot of all SU for 1 Hz EF stimulation and determined that EF stimulation did not override the patterned network activity caused by optogenetic stimulation since oscillation cycles with and without EF closely resembled each other (Fig. 2B, raster of all units for 1 Hz stimulation, $n = 3840$ SU*trials); peak activity occurred at the onset of the optogenetic stimulation during each oscillation cycle and low but detectable activity occurred in absence of optogenetic stimulation (second half of stimulation period). Therefore, stimulation with EF amplitudes similar to the ones estimated to occur in human neocortex during tACS [11,27] failed to qualitatively alter the endogenous activity structure. In order to quantify the more subtle effects of EF stimulation on spontaneous cortical oscillations, we assessed the change in frequency structure of the overall network activity within individual slices by computing the change in frequency spectrum (Fig. 2C). In agreement with our hypothesis of frequency-specific modulation, we found that these modulation profiles of oscillation structure exhibited distinct shapes for different stimulation frequencies (0.8, 1.0, 1.2, 2.0 Hz). Independent of the stimulation frequency, the only clear peaks of enhancement were localized at or very close to 1 Hz (and its integer multiples). Therefore, our data reject the alternative hypothesis that EF stimulation enables the selective enhancement of network activity at the stimulation frequency. Rather, enhancement was strongly constrained by the endogenous oscillation, in particular in cases such as this where we induced a pronounced, *in vivo*-like slow oscillation by means of optogenetic stimulation. We quantified the modulation of the oscillatory activity by first determining the amount of enhancement at the EF stimulation frequency. For 1 mV/mm EF amplitude, we exclusively found an increase in frequency preference for EF stimulation frequencies of 1 and 2 Hz. However, for increased stimulation amplitude (2 mV/mm), the EF application significantly enhanced 1, 1.2, and 2 Hz oscillations (Fig. 2D; confidence intervals and statistics Supplementary Table 1). These results show that cortex preferentially responded to EF stimulation at the frequency of endogenous oscillation and its first harmonic. We then examined the effect of the stimulation on the endogenous activity at 1 Hz. Indeed, EF stimulation at the frequency of the network oscillation (1 Hz), its first harmonic (2 Hz), and below the network frequency (0.8 Hz) all enhanced the rhythmic activity caused by optogenetic stimulation at 1 Hz for low amplitude stimulation. EF stimulation amplitude to 2 mV/mm caused an increase of 1 Hz oscillation for all EF stimulation frequencies (Fig. 2E; Supplementary Table 2). Again, we found that perturbations at the frequency of the ongoing oscillation and the first harmonic preferentially enhance the ongoing oscillation. However, EF stimulation at the frequency below that of the ongoing oscillation also enhanced the oscillation. We therefore concluded that cortical networks respond to sine-wave EF stimulation, and likely by extension, tACS, with the properties of network resonance. EF stimulation predominantly enhanced the endogenously present oscillation and shifts in frequency of the endogenous oscillation were unattainable in these experiments. We next probed for outlasting effects by comparing the 10 s after EF enhancement to that of the 10 s preceding stimulation. We did not

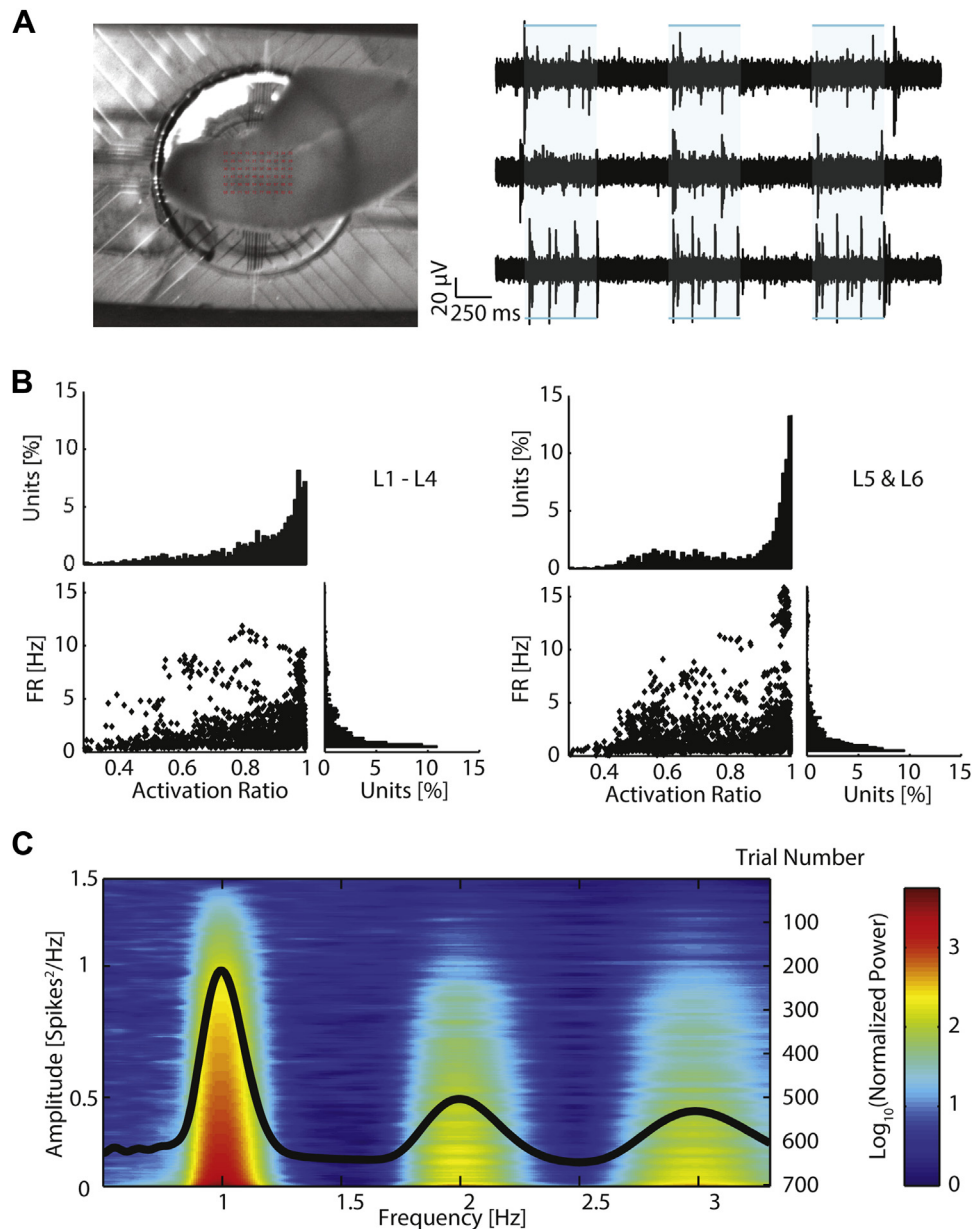


Figure 1. Optogenetic stimulation activates cortical networks *in vitro*. (A) Left: Coronal slice positioned on multi-electrode array with 100 μm spacing between electrodes. Right: Modulation of action potential firing by optogenetic stimulation (3 s trace of multiunit activity for three sample electrodes) (B) FR versus Activation Ratio for all SU in layers 1 through 4 (left) and layers 5 and 6 (right). (C) Frequency response to 10 s of optogenetic stimulation with mean response overlaid (black). Spontaneous activity was modulated by optogenetic stimulation and exhibited pronounced 1 Hz oscillation.

find any outlasting effect for any amplitude or frequency EF stimulation (Fig. S1); this lack of outlasting effect is most likely due to the short duration of the EF stimulation and the randomization of the different stimulation frequency conditions in our study.

EF stimulation increases neuronal activity levels

Does EF stimulation only alter the temporal structure of network activity or does it also alter the net activity levels of individual neurons? To address this question, we next investigated how EF stimulation alters the overall activity levels of individual neurons by measuring FRs of SU. We examined modulation of FR during the UP state (500 ms activity caused by optogenetic stimulation), the DOWN state (500 ms of relative quiescence in absence of optogenetic stimulation), and the entire cycle while EF stimulation was

applied compared to that of the preceding interval of optogenetic stimulation only (Fig. 3). Indeed application of most EF stimulations tested here increased FR during the UP state of the 1 Hz cycle (Fig. 3A left, Supplementary Table 3). During the DOWN state, only EF stimulation that was matched to the frequency of the optogenetic stimulation (1 Hz EF stimulation) caused a FR decrease for both stimulation amplitudes (Fig. 3A center, Supplementary Table 4). When we compared the total FR with EF stimulation to the FR without EF, we observed that 2 mV/mm EF enhanced FR for most stimulation frequencies (Fig. 3A right, Supplementary Table 5). However the total FR was not significantly increased for all low amplitude (1 mV/mm) EF stimulation frequencies except for mismatched frequency (1.2 Hz) stimulation. Thus in agreement with the findings on network oscillation structure, matching the stimulation frequency to the endogenous network frequency

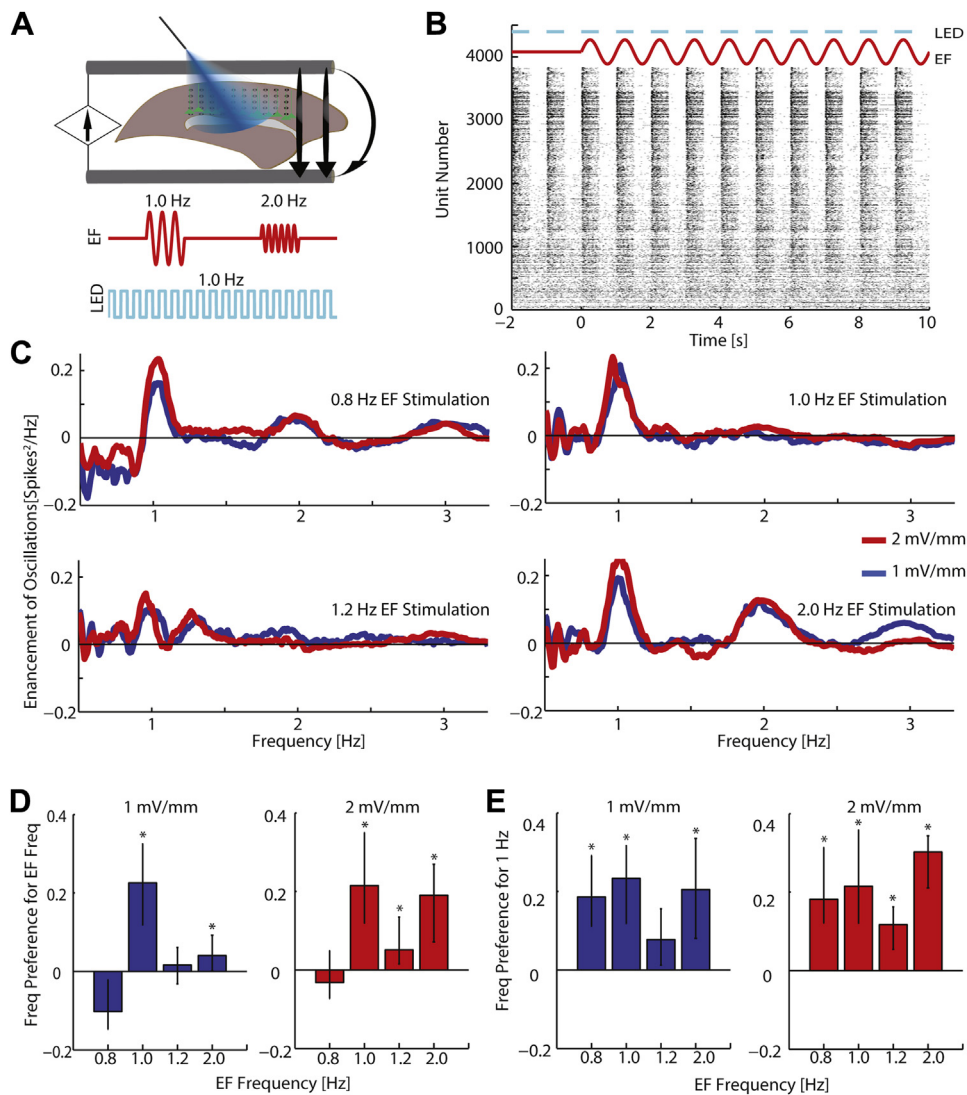


Figure 2. Interaction dynamics between endogenous oscillation and EF stimulation. (A) Top: Optogenetic stimulation (cyan) is applied to L5 pyramidal neurons (green) while EF is applied between two AgCl wires (gray, example field lines in black) with the positive terminal nearest layer 1 of visual cortex. Bottom: Example trials (shortened to 3 s for visualization). (B) Raster plot for all SU for each trial with 1 Hz EF stimulation. (C) Difference spectra between activity during EF stimulation and the preceding 10 s for all EF stimulation frequencies and amplitudes. (D) Frequency preference for the stimulation frequency. (E) Frequency preference for 1 Hz. Error bars indicate 5–95% confidence interval. $*P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

caused differential modulation of the network activity in contrast to non-matching stimulation frequencies.

We next assessed the temporal profile of the FR modulation by applied EF stimulation. In absence of EF stimulation, a pronounced increase in FR occurred at the onset of optogenetic stimulation followed by decay in FR with a relatively abrupt transition to low activity levels when the stimulus was turned off at 500 ms (black traces in Fig. 3B, left). Overall, this activity profile was maintained in presence of EF stimulation (blue and red traces in Fig. 3B left; Top: 1 Hz EF; Bottom: 2 Hz EF). We then calculated the percent change caused by application of EF and fitted a sine-wave to this time-profile of FR modulation (Fig. 3B, right). We found that enhancement of neural activity by EF lagged behind the stimulus waveform for 1 Hz EF stimulation (goodness of fit: $R^2 = 0.71$ for 1 mV/mm and 0.62 for 2 mV/mm; $\varphi = -0.21$ radians for 1 mV/mm at 1 Hz and -0.14 radians for 2 mV/mm at 1 Hz; Fig. 3B, top). Similarly, EF stimulation at 2 Hz caused a temporal modulation profile that followed a 2 Hz sine-wave ($R^2 = 0.45$ for 1 mV/mm and 0.64 for 2 mV/mm, Fig. 3B bottom). Again we observed a phase delay between the

EF stimulation and the change in FR ($\varphi = -0.58$ radians for 1 mV/mm at 1 Hz and -0.74 radians for 2 mV/mm at 2 Hz; for results of 0.8 and 1.2 Hz EF see Fig. S2). We then examined pairwise correlations between SU to determine if the increase in oscillatory structure was caused by an increase in similarity of firing patterns of individual neurons at a finer time-scale. Pairwise correlations on the 50 ms time-scale were virtually unaltered (Supplementary Table 6). The increased oscillatory structure thus did not depend on increased synchronization at faster time-scales. Together, these results demonstrated an overall increase in FR for EF stimulation (with the exception of low amplitude, frequency-matched stimulation) and a temporal modulation of activity levels that closely followed the applied EF waveform.

Conditions that limit the occurrence of network resonance

Our finding of endogenous network dynamics constraining the effect of stimulation is likely most prevalent in the case of pronounced endogenous activity and low amplitude periodic

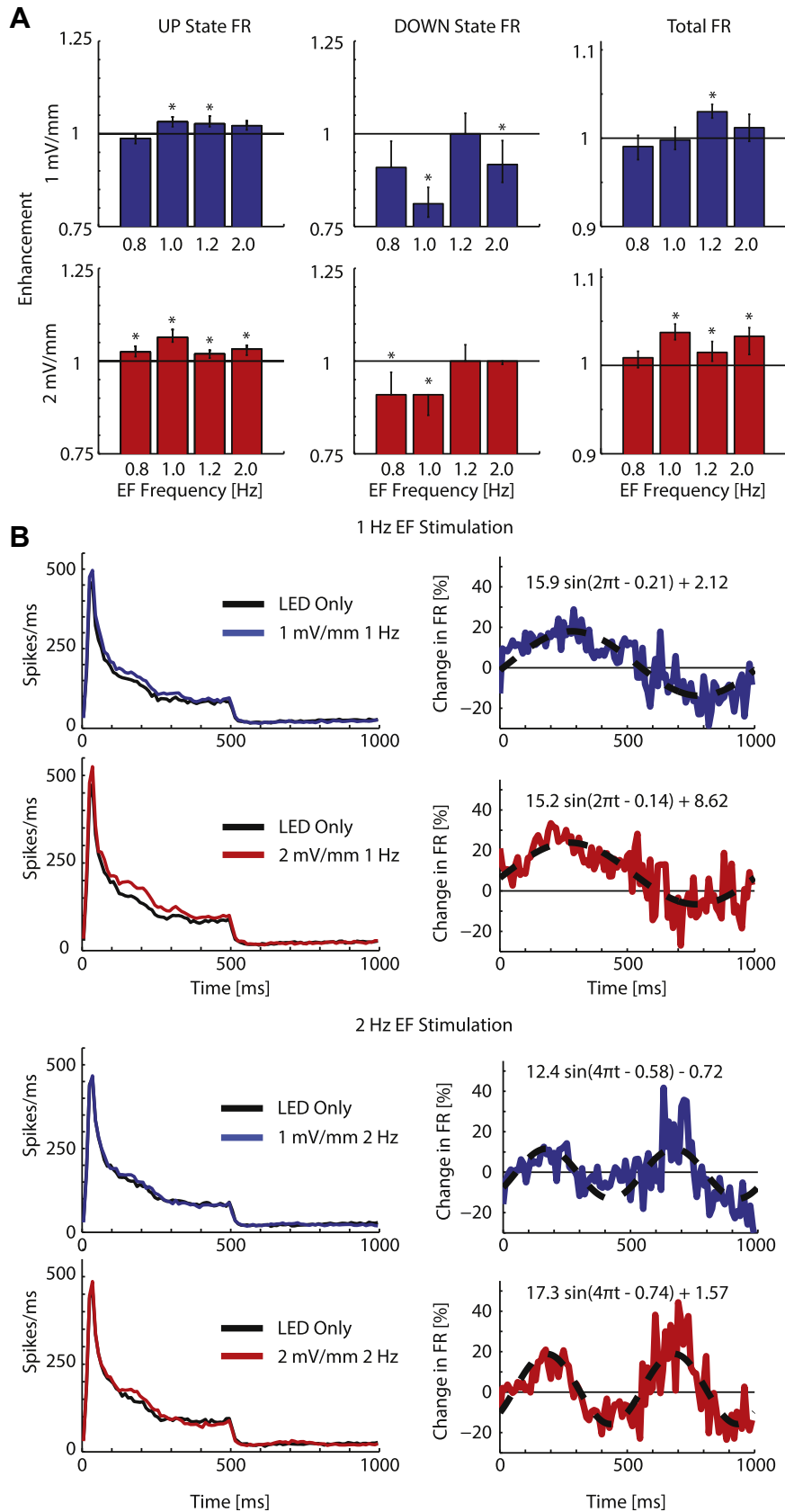


Figure 3. Firing rate modulation by EF stimulation. (A) Left: Enhancement of FR during UP states. FR was enhanced by most EF stimulation amplitudes and frequencies during the UP state. Center: Enhancement of FR during the DOWN state. FR was significantly decreased for 1 Hz EF stimulation across stimulation amplitudes. Right: Total enhancement of FR by EF. In most cases application of 2 mV/mm EF increased total FR while only 1.2 Hz stimulation increased FR for 1 mV/mm EF. SU with an activation ratio of <1 (at least one spike during the DOWN state) shown. (B) Left: Spiking activity as a single cycle of the applied optogenetic stimulation for 1 and 2 Hz EF stimulation (color) with the averaged preceding 10 s of oscillatory activity (black). Right: Percent change by application of EF (color) and fitted sine-waves (black, dashed). Phase delay (shown in radians) between the applied EF signal and resulting change in activity was observed for both conditions. Error bars indicate 5–95% confidence interval. * $P < 0.05$.

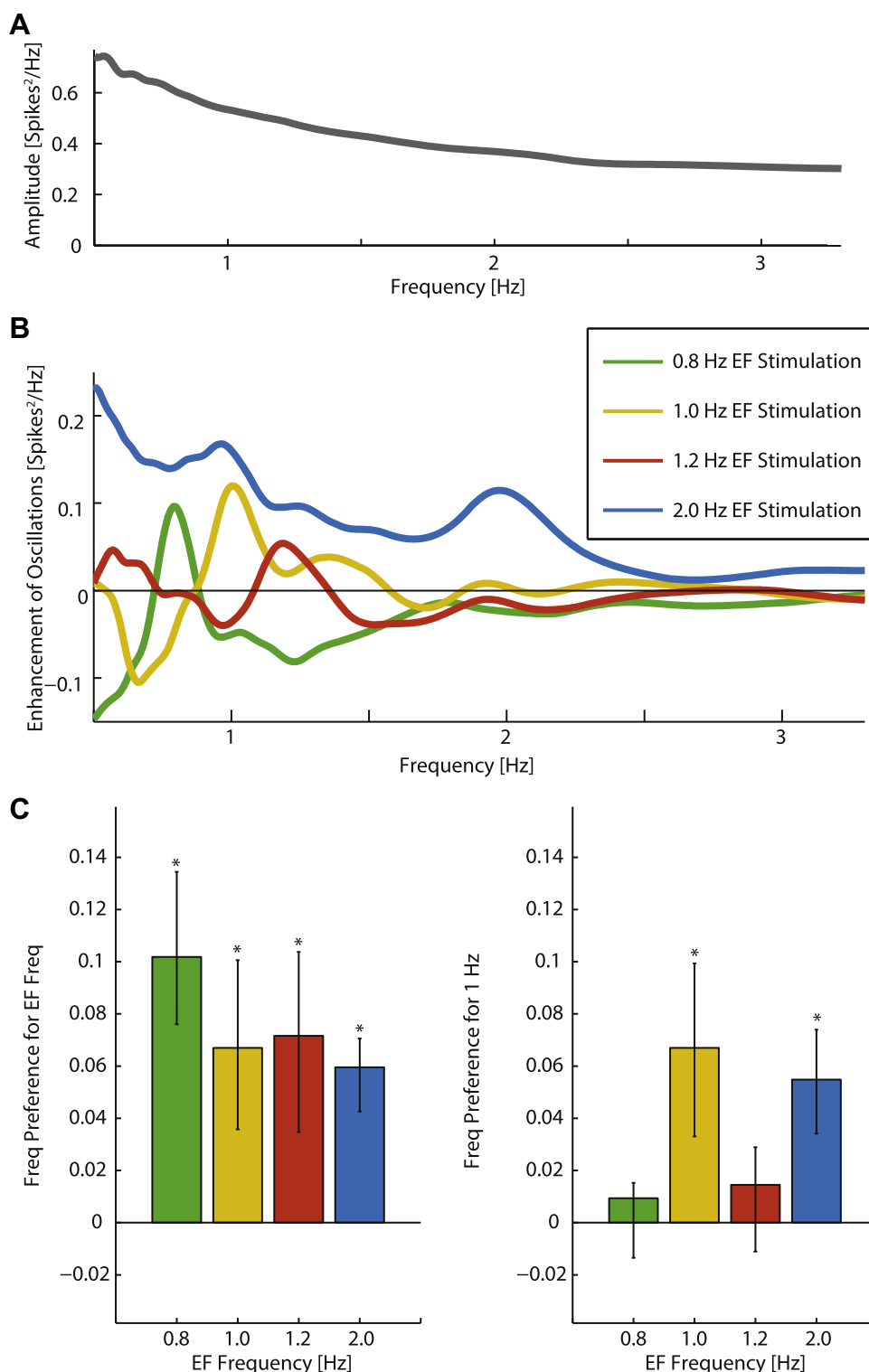


Figure 4. Modulation of frequency structure in absence of an endogenous oscillation. (A) Frequency spectrum of spontaneous activity in cortical slices with neither optogenetic stimulation nor EF stimulation. (B) Enhancement of frequency structure by EF stimulation only. (C) Frequency preference for the EF stimulation frequency (left). Frequency preference for 1 Hz oscillations (right). In absence of strong cortical oscillations, all frequencies were enhanced and only 1 and 2 Hz EF stimulation enhanced preference for 1 Hz oscillations. Error bars indicate 5–95% confidence interval. * $P < 0.05$.

perturbations. To experimentally delineate the conditions in which the above demonstrated interaction dynamics apply, we performed additional control experiments. First, we conducted control experiments with 2 mV/mm EF in the absence of optogenetic stimulation. In these experiments, the network activity without EF

stimulation did not exhibit any peak in the frequency spectrum (Fig. 4A). In contrast to the results presented above, the EF application enhanced the oscillatory structure at the frequency of the EF stimulation (median enhancement at stimulation frequency: 0.102 [0.076 0.132] spikes²/Hz for 0.8 Hz EF, 0.067 [0.032 0.101] spikes²/

Hz for 1.0 Hz EF, 0.073 [0.038 0.104] spikes²/Hz for 1.2 Hz EF, and 0.059 [0.043 0.071] spikes²/Hz for 2.0 Hz EF; $P < 10^{-3}$ for each stimulation frequency; $n = 144$ trials; Fig. 4B and C left). In further contrast to experiments with a strong oscillation evoked by the optogenetic stimulation, only EF stimulation of 1.0 and 2.0 Hz increased the frequency preference for 1.0 Hz oscillations (median enhancement at 1 Hz: 0.009 [−0.015 0.016] spikes²/Hz for 0.8 Hz EF, 0.067 [0.039 0.103] spikes²/Hz for 1.0 Hz EF, 0.015 [−0.010 0.027] spikes²/Hz for 1.2 Hz EF, and 0.055 [0.034 0.075] spikes²/Hz for 2.0 Hz EF; $P = 1$, $P < 10^{-3}$, $P = 1$, $P < 10^{-3}$ respectively; $n = 144$ trials; Fig. 4C, right). Therefore, without an endogenous oscillation, EF stimulation increased oscillatory structure at all stimulation frequencies evaluated. In agreement with our overall hypothesis, these results suggest that the structure of the endogenous activity determines if and to what extent EFs are constrained in their effect on network dynamics. Thus, we next investigated two other scenarios in which we reduced the strength of the endogenous oscillation modeled by optogenetic stimulation. We hypothesized that a reduction in endogenous rhythmic structure would enable even frequency-mismatched stimulation waveforms to enhance oscillations at the EF stimulation frequency.

First, we lowered the intensity of the optogenetic stimulation to 20 μ W. With this lower intensity stimulation, the strength of the evoked oscillation was lower than that caused by the full intensity stimulation (1.15 [1.12 1.24] spikes²/Hz for low intensity optogenetic stimulation, 3.49 [3.35 3.64] spikes²/Hz full intensity, $P < 10^{-3}$ Wilcoxon ranked sum test, $n = 640$ and 703 trials respectively, Fig. 5A). We then applied EF stimulation with 2 mV/mm amplitude. Similar to the experiments with no optogenetic stimulation, EF stimulation was able to increase frequency preference at all stimulation frequencies evaluated (median enhancement for EF stimulation frequency: 0.068 [0.022 0.124] spikes²/Hz for 0.8 Hz EF, 0.173 [0.097 0.260] spikes²/Hz for 1.0 Hz EF, 0.107 [0.075 0.140] spikes²/Hz for 1.2 Hz EF, and 0.047 [0.016 0.083] spikes²/Hz for 2.0 Hz EF; $P < 0.005$ for each stimulation frequency; $n = 160$ trials; Fig. 5B and C left). However, the increase in oscillatory structure at the EF stimulation frequency trended to be larger for 1.0 Hz stimulation (confidence that response to 1 Hz stimulation was different: $P = 0.0898$ for 0.8 Hz stimulation, $P = 0.0595$ for 1.2 Hz stimulation and $P = 0.0029$ for 2 Hz stimulation, Wilcoxon ranked sum test with a Bonferroni correction for 3 comparisons). The frequency preference for the ongoing cortical oscillation was only enhanced for frequency matched (1 Hz) stimulation (median enhancement for optogenetic stimulation frequency: −0.052 [−0.125 −0.009] spikes²/Hz for 0.8 Hz EF, 0.173 [0.101 0.261] spikes²/Hz for 1.0 Hz EF, −0.048 [−0.110 −0.009] spikes²/Hz for 1.2 Hz EF, and 0.039 [−0.015 0.080] spikes²/Hz for 2.0 Hz EF; $P = 0.026$, $P < 10^{-3}$, $P = 0.167$, and $P = 0.383$ respectively; $n = 160$ trials; Fig. 5C right). Thus, with a less pronounced endogenous oscillation, EF stimulation provided the best enhancement when stimulating at the frequency of the ongoing oscillation. However mismatched stimulation also enhanced frequency preference for the EF stimulation frequency.

Second, we reduced the strength of the ongoing oscillation by using an optogenetic stimulation waveform with a broader spectral peak around 1 Hz. Specifically, we varied the length of each “UP” and “DOWN” state by randomly drawing 20 durations from a normal distribution around 500 ms (standard deviation 50 ms). We then compared the multiunit frequency response to optogenetic stimulation of the experiments shown in Figs. 1–3 to the response to the variable duration optogenetic stimulation. The frequency structure of the stimulation waveform still exhibited a peak frequency of 1 Hz. However, the frequency content was spread over a broader band (Fig. 6A). Due to this change in the optogenetic stimulation, the evoked cortical oscillation was also altered but still

exhibited a peak frequency at 1.0 Hz (Fig. 6B). However, the cortical response at 0.8 and 1.2 Hz was relatively enhanced when normalized by the peak (1 Hz) amplitude (Supplementary Table 7, Fig. 6B right). We then applied 2 mV/mm EF stimulation to slices activated with this optogenetic stimulation paradigm. Similar to the results in (Fig. 2C), EF stimulation increased the frequency preference for 1.0 Hz using 1.0, 1.2 and 2.0 Hz stimulation, however EF stimulation at 0.8 Hz did not increase the frequency preference for 1.0 Hz (median enhancement at optogenetic stimulation frequency, 1 Hz: 0.003 [−0.044 0.048] spikes²/Hz for 0.8 Hz EF, 0.199 [0.127 0.289] spikes²/Hz for 1.0 Hz EF, 0.063 [−0.021 0.134] spikes²/Hz for 1.2 Hz EF, and 0.148 [0.053 0.222] spikes²/Hz for 2.0 Hz EF; $P = 1$, $P < 10^{-3}$, $P = 0.014$ and $P = 0.001$ respectively; $n = 176$ trials; Fig. 6C and D left). With increased frequency structure at 0.8 and 1.2 Hz in the optogenetic stimulation, the EF stimulation was able to significantly enhance frequency preference for the applied EF frequency (median enhancement at EF stimulation frequency: 0.446 [0.348 0.532] spikes²/Hz for 0.8 Hz EF, 0.198 [0.127 0.275] spikes²/Hz for 1.0 Hz EF, 0.092 [0.038 0.161] spikes²/Hz for 1.2 Hz EF, and 0.104 [0.040 0.157] spikes²/Hz for 2.0 Hz EF; $P < 10^{-3}$ for all groups; $n = 176$ trials; Fig. 6D right). Therefore, the variable ongoing oscillation also shaped the network response to EF stimulation.

Finally, we asked if increasing the strength of the EF stimulation would enable the EF stimulation to override the endogenous network dynamics. To answer this question, we performed experiments with 8 mV/mm field strength. At such high amplitude, the EF stimulation was indeed able to increase frequency preference for the EF frequency despite the ongoing oscillation at 1 Hz (median enhancement at EF stimulation frequency: 0.382 [0.258 0.560] spikes²/Hz for 0.8 Hz EF, 0.471 [0.221 0.661] spikes²/Hz for 1.0 Hz EF, 0.319 [0.173 0.441] spikes²/Hz for 1.2 Hz EF, and of 0.185 [0.130 0.290] spikes²/Hz for 2.0 Hz EF; $P < 10^{-3}$; $n = 208$ trials; Fig. 7A and B left). Only EF stimulation at 1.0 Hz increased the frequency preference for 1.0 Hz while EF stimulation at 0.8 Hz suppressed the frequency preference for 1.0 Hz (median enhancement at optogenetic stimulation frequency: −0.142 [−0.209 −0.049] spikes²/Hz for 0.8 Hz EF, 0.483 [0.223 0.661] spikes²/Hz for 1.0 Hz EF, 0.0444 [0.000 0.126] spikes²/Hz for 1.2 Hz EF, and 0.100 [−0.018 0.245] spikes²/Hz for 2.0 Hz EF; $P = 0.017$, $P < 10^{-3}$, $P = 0.915$ and $P = 0.054$ respectively; $n = 208$ trials; Fig. 7B right). From these data, we concluded that indeed a comparatively strong EF may override the response-dependence of EF stimulation on the ongoing cortical oscillation.

Discussion

Targeted modulation of cortical oscillations with non-invasive brain stimulation has the potential to enable significant progress in (1) the study of the causal role of oscillations in cognition and behavior and (2) the development of novel treatments for neurological and psychiatric disorders associated with deficits in the macroscopic cortical activity patterns. However, in absence of a direct demonstration of the underlying mechanism of action by which such stimulation interacts with ongoing network dynamics, this progress will be severely hampered.

Application of weak EFs by transcranial current stimulation represents a promising approach to non-invasive brain stimulation [30–32]. The presence of endogenous activity is crucial for EFs to have an effect on network dynamics since the depolarization caused by the EF is too small to activate neurons at rest [14,17,33]. In essence, neurons need to be close to firing threshold for the resulting sub-millivolt perturbation of the membrane voltage to be an effective modulator of endogenous network activity. Previous *in vitro* studies have shown enhancing effects of sine-wave EF stimulation on ongoing network activity [13–15]. However, the

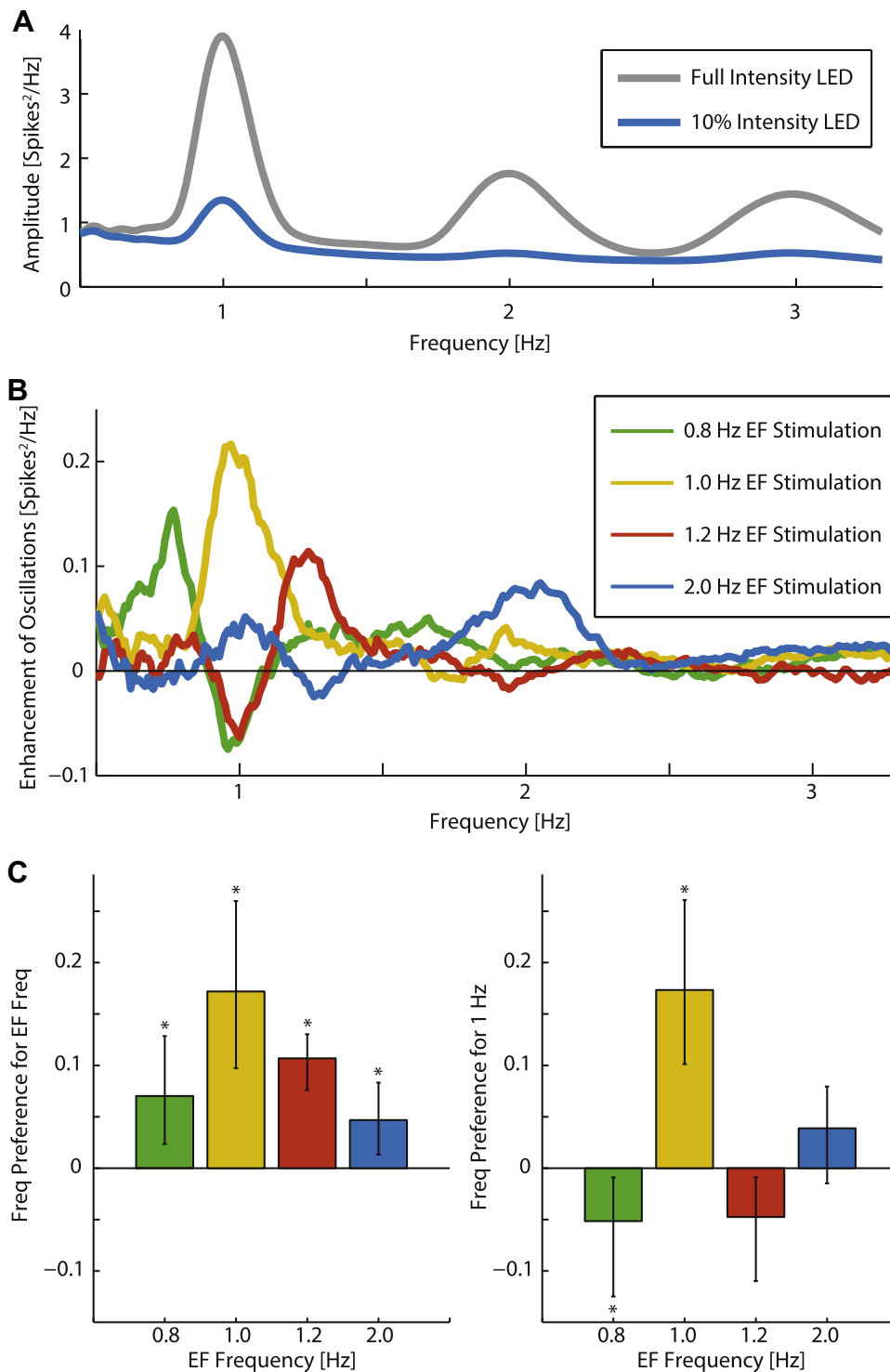


Figure 5. EF stimulation of networks exhibiting weak cortical oscillations. (A) Spectra of the response to full intensity optogenetic stimulation (gray) and 10% intensity optogenetic stimulation (blue). (B) Enhancement of cortical oscillations by EF stimulation. (C) Change in frequency preference for the EF stimulation frequency (left) and for the 1 Hz oscillation (right). Error bars indicate 5–95% confidence interval. * $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

direct assessment of the interaction dynamics has been limited to observational descriptions of the effects of weak EF due to limited control of spontaneous or pharmacologically induced oscillations. Here, we used optogenetic tools to directly assess these interaction dynamics by exercising precise temporal control of both the endogenous activity (induced by optogenetic stimulation) and the EF stimulation. We established that sine-wave EF patterns with

very low amplitude can indeed enhance network dynamics even in presence of very pronounced, rhythmic neocortical network activity that resembles *in vivo* activity patterns. In agreement with predictions from previous experimental [15,34] and computational work [17], we show that the choice of stimulation frequency relative to the endogenous oscillation frequency plays a key role in determining the effect of stimulation. Specifically, we found that a

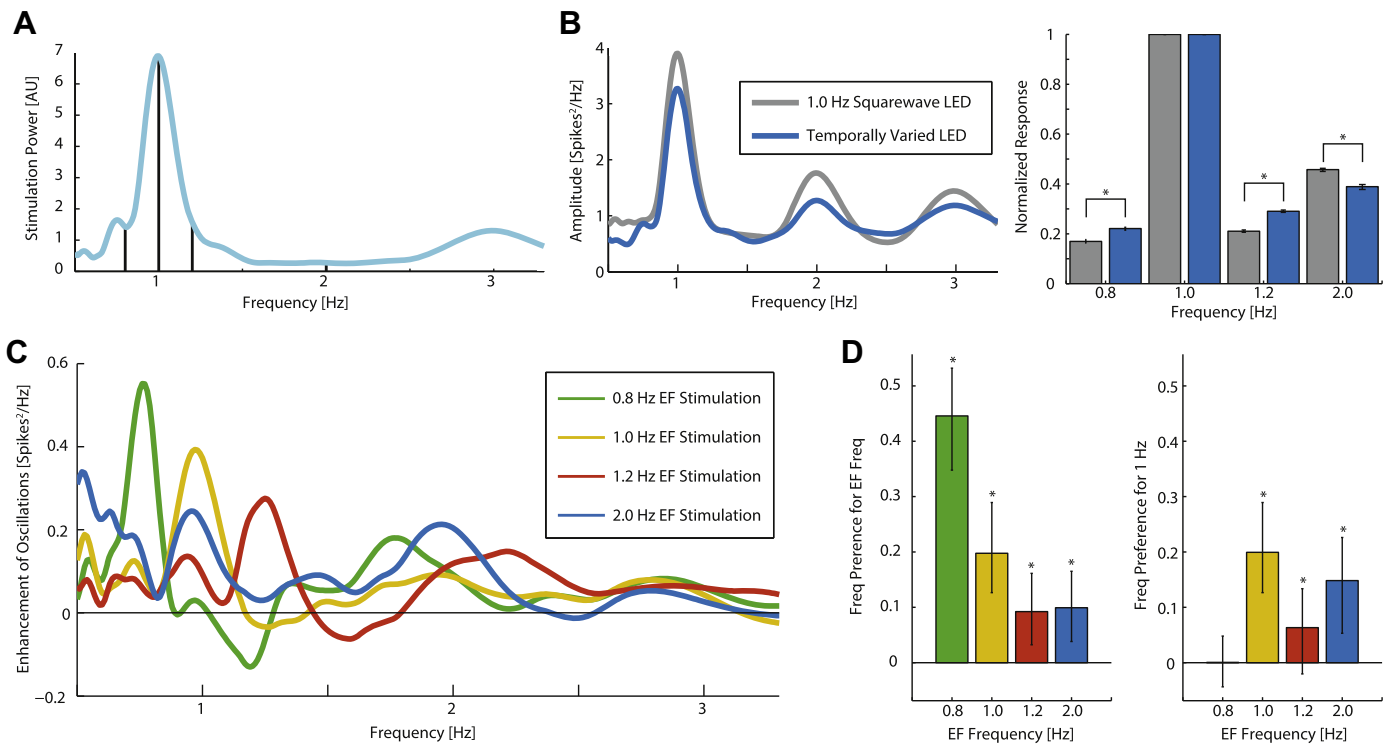


Figure 6. EF stimulation of networks exhibiting cortical oscillations with variable UP and DOWN state duration. (A) Spectrum of the 10 s optogenetic stimulation waveform (cyan). EF stimulation frequencies are indicated in black. (B) Left: Spectra of the response to 1 Hz square-wave optogenetic stimulation (gray) and response to temporally variant optogenetic stimulation (blue). Right: Response optogenetic stimulation, normalized by the oscillation amplitude at 1 Hz. (C) Spectra of the enhancement of oscillations by EF stimulation for 0.8 (green), 1.0 (yellow), 1.2 (red), 2.0 (blue) Hz stimulation. (D) Left: Frequency preference for oscillations at the frequency of the EF stimulation. Right: Frequency preference for 1 Hz oscillations. Error bars indicate 5–95% confidence interval. * $P < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pronounced endogenous oscillation precluded the generation of activity patterns at other (nearby) frequencies and that the enhancement of the endogenous oscillation depended on the EF stimulation frequency. We then tested the apparent constraint endogenous network dynamics imposed on modulatory effects of stimulation; indeed, with no endogenous oscillation present or with application of higher amplitude EF stimulation, oscillations at the EF stimulation frequency could be enhanced. We also reduced the strength of the endogenous oscillation (by reduced optogenetic stimulation) and found that these dynamics still constrained the effects of stimulation at trend level. Finally, with a temporally varied endogenous oscillation, we again found that mismatched stimulation could increase the endogenous oscillation but also increased oscillatory structure at the EF stimulation frequency.

The field has yet to converge on whether application of EF increases the net average FR of networks. In our hands, the average

FR over 10 s was increased for most of the 2 mV/mm EF stimulation frequencies. This result agrees with the previously reported increase in multiunit activity evoked by field strengths as low as 1 mV/mm in [14]. However other studies have observed that while EF stimulation increased FR during the positive-going phase of the stimulation, during the negative-going phase of the stimulation the FR was decreased, thus resulting in no net change [15]. This distinction may be important for future studies aimed at determining if tACS acts by synchronizing neural oscillators or evoking more activity as a function of the temporal structure of the stimulation waveform.

Our findings demonstrate that the state of the targeted networks plays an important role in determining the response to EF stimulation. Such state-dependent response of neuronal circuits is well known for sensory input that reaches sensory areas in cortex. For example, the neuronal representation of visual [35] and

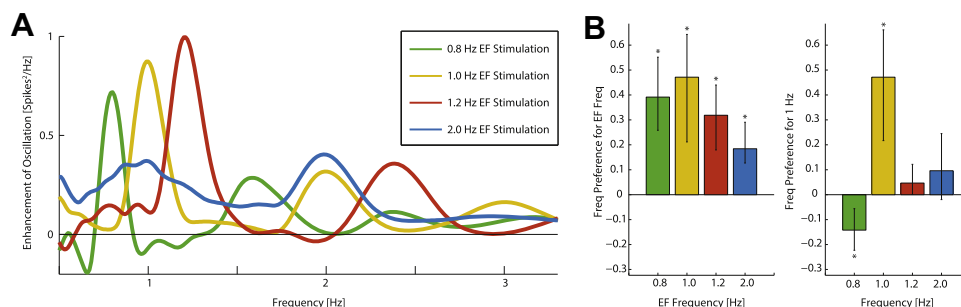


Figure 7. High amplitude EF stimulation. (A) Difference spectra between 10 s of application of 8 mV/mm EF stimulation and the preceding 10 s. (B) Left: Frequency preference for the EF stimulation frequency. Right: Frequency preference for 1 Hz oscillations. Error bars indicate 5–95% confidence interval. * $P < 0.05$.

auditory [36] inputs depends on overall brain state. Our results suggest that this “gating” by brain state (defined by the presence of specific network-level activity patterns) also applies to external stimuli directly provided to neuronal networks by brain stimulation. Matching the brain stimulation to the specific endogenous network dynamics of an individual at a given time-point may therefore be crucial for effectiveness and thus an appealing opportunity for individualized treatment. Indeed, in human motor cortex, the presence of a pronounced beta oscillation was found to determine whether beta (20 Hz) or theta (5 Hz) frequency tACS best facilitated motor-evoked potentials by transcranial magnetic stimulation [34]. Here we have performed EF stimulation in the presence and absence of a cortical oscillation and likewise observed different cortical response-dependent on the cortical oscillation. We used EF stimulation frequencies within the same frequency band as the ongoing cortical oscillation since oscillations in different frequency bands are likely generated by different mechanisms and presently cannot be simultaneously modeled in a slice preparation. Importantly, a similar phenomenon has also been reported for alpha oscillations where the effect of tACS on macroscopic network dynamics depended on the strength of the ongoing oscillation [37]. However, in this case, the presence of strong alpha oscillations precluded further enhancement by frequency-matched tACS. Yet, enhancement was successful for the case of weak endogenous alpha oscillations in the same participants. This difference in the effect of frequency-matched stimulation can have several causes, including the presence of ceiling effects. Taken together with the results presented here, the effect of EF stimulation may vary from enhancement at any frequency, to enhancement only of the endogenous oscillation, to no enhancement at the stimulation frequency, depending on the strength of the ongoing cortical oscillation.

Importantly, further studies will be needed to fully elucidate the mechanism of action of tACS for other cortical oscillations with different frequency structure such as the gamma oscillation (>30 Hz). Since cortical oscillations at different frequencies are likely generated by distinct single cell and network mechanisms, it is as of now unclear if the work presented here will generalize to other cortical oscillations. For example, the gamma oscillation likely arises from the dynamic interaction of pyramidal cells and fast-spiking inhibitory interneurons that exhibit their own resonance profile [38]. Nevertheless, the overall approach taken in this study will likely be equally elucidating when applied to the study of tACS that targets other frequency bands and therefore lays the groundwork for a comprehensive understanding of tACS and its interaction with cortical network dynamics [39].

Our study focused on unraveling the interaction dynamics between periodic, weak EF and oscillatory network activity. Understanding tACS will require the study of other important aspects of stimulation delivery. In particular, we used a model system of tACS where we directly applied calibrated EF to cortical slices *in vitro* and recorded microscopic network dynamics measured by SU activity. Thus, our study does not provide any insight into the spatial patterning of the stimulation (i.e. EF distribution) as a result of the current application to the scalp in human tACS. Only the intersection of the mechanisms of temporal targeting (demonstrated here), spatial targeting [40], and functional targeting [34] will provide the tools for developing highly specific stimulation paradigms. We also cannot, with in one slice, examine the mechanisms of applying anti-phase tACS to different brain regions. This type of stimulation has shown interesting behavioral results [8,41], but cannot be probed with the methods used here. Likely, distinct mechanisms such as switching between multiple stable states may mediate such effects [42]. Nevertheless, our *in vitro* approach provides essential advantages that can help build an important bridge

between computational studies (that inherently lack biological plausibility) and *in vivo* studies in animals and humans that often do not provide the level of experimental access and control required to elucidate the interaction dynamics. Using optogenetic stimulation of layer 5 pyramidal cells causes naturalistic slow rhythmic activity *in vivo*; here we adapted this approach for *in vitro* stimulation. We found such stimulation to cause a clear network rhythm although individual cells continued to exhibit low, naturalistic firing rates in presence of stimulation. Given the known role of layer 5 pyramidal cell to greatly contribute to the genesis of slow (cortical) rhythms, our approach provided the opportunity to maintain *in vivo*-like activity patterns *in vitro*.

Together, these results provide a mechanism of action of tACS at the network level and point toward next-generation tACS that provides individualized, state-dependent feedback tACS for the enhancement of cortical oscillations [43]. The most important next step toward this goal will be the successful recording of brain activity during stimulation as recently demonstrated for EEG combined with tACS [44].

Acknowledgments

The authors thank the Fröhlich lab and the anonymous reviewers for their valuable input.

Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.brs.2014.07.033>.

References

- [1] Buzsáki G. Rhythms of the brain. Oxford; New York: Oxford University Press; 2006.
- [2] Wang XJ. Neurophysiological and computational principles of cortical rhythms in cognition. *Physiol Rev* 2010 Jul;90(3):1195–268 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review].
- [3] Uhlhaas PJ, Singer W. Neuronal dynamics and neuropsychiatric disorders: toward a translational paradigm for dysfunctional large-scale networks. *Neuron* 2012 Sep 20;75(6):963–80 [Research Support, Non-U.S. Gov't Review].
- [4] Romei V, Gross J, Thut G. On the role of prestimulus alpha rhythms over occipito-parietal areas in visual input regulation: correlation or causation? *J Neurosci* 2010 Jun 23;30(25):8692–7.
- [5] Sauseng P, Klimesch W, Heise KF, et al. Brain oscillatory substrates of visual short-term memory capacity. *Curr Biol* 2009 Nov 17;19(21):1846–52 [Research Support, Non-U.S. Gov't].
- [6] Thut G, Veniero D, Romei V, Miniussi C, Schyns P, Gross J. Rhythmic TMS causes local entrainment of natural oscillatory signatures. *Curr Biol* 2011 Jul 26;21(14):1176–85 [Research Support, Non-U.S. Gov't].
- [7] Herrmann CS, Rach S, Neuling T, Struber D. Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. *Front Hum Neurosci* 2013;7:279.
- [8] Polania R, Nitsche MA, Korman C, Batsikadze G, Paulus W. The importance of timing in segregated theta phase-coupling for cognitive performance. *Curr Biol* 2012 Jul 24;22(14):1314–8 [Research Support, Non-U.S. Gov't].
- [9] Santarnecchi E, Polizzotto NR, Godone M, et al. Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. *Curr Biol* 2013 Aug 5;23(15):1449–53.
- [10] Kirov R, Weiss C, Siebner HR, Born J, Marshall L. Slow oscillation electrical brain stimulation during waking promotes EEG theta activity and memory encoding. *Proc Natl Acad Sci U S A* 2009 Sep 8;106(36):15460–5 [Research Support, Non-U.S. Gov't].
- [11] Marshall L, Helgadottir H, Molle M, Born J. Boosting slow oscillations during sleep potentiates memory. *Nature* 2006 Nov 30;444(7119):610–3 [Clinical Trial Research Support, Non-U.S. Gov't].
- [12] Binder S, Rawohl J, Born J, Marshall L. Transcranial slow oscillation stimulation during NREM sleep enhances acquisition of the radial maze task and modulates cortical network activity in rats. *Front Behav Neurosci* 2014;7:220.
- [13] Deans JK, Powell AD, Jefferys JG. Sensitivity of coherent oscillations in rat hippocampus to AC electric fields. *J Physiol* 2007 Sep 1;583(Pt 2):555–65 [In Vitro Research Support, Non-U.S. Gov't].
- [14] Fröhlich F, McCormick DA. Endogenous electric fields may guide neocortical network activity. *Neuron* 2010 Jul 15;67(1):129–43 [In Vitro Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].

- [15] Reato D, Rahman A, Bikson M, Parra LC. Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. *J Neurosci* 2010 Nov 10;30(45):15067–79 [Research Support, N.I.H., Extramural].
- [16] Ozen S, Sirota A, Belluscio MA, et al. Transcranial electric stimulation entrains cortical neuronal populations in rats. *J Neurosci* 2010 Aug 25;30(34):11476–85 [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].
- [17] Ali MM, Sellers KK, Frohlich F. Transcranial alternating current stimulation modulates large-scale cortical network activity by network resonance. *J Neurosci* 2013 Jul 3;33(27):11262–75.
- [18] Neuling T, Rach S, Herrmann CS. Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states. *Front Hum Neurosci* 2013;7:161.
- [19] Hutcheon B, Yarom Y. Resonance, oscillation and the intrinsic frequency preferences of neurons. *Trends Neurosci* 2000 May;23(5):216–22 [Research Support, Non-U.S. Gov't Review].
- [20] Beltramo R, D'Urso G, Dal Maschio M, et al. Layer-specific excitatory circuits differentially control recurrent network dynamics in the neocortex. *Nat Neurosci* 2013 Feb;16(2):227–34 [In Vitro Research Support, Non-U.S. Gov't].
- [21] Arenkiel BR, Peca J, Davison IG, et al. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron* 2007 Apr 19;54(2):205–18 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].
- [22] Chauvette S, Volgushev M, Timofeev I. Origin of active states in local neocortical networks during slow sleep oscillation. *Cereb Cortex* 2010 Nov;20(11):2660–74 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].
- [23] Schmidt SL, Chew EY, Bennett DV, Hammad MA, Frohlich F. Differential effects of cholinergic and noradrenergic neuromodulation on spontaneous cortical network dynamics. *Neuropharmacology* 2013 May 3;72C:259–73.
- [24] Sanchez-Vives MV, McCormick DA. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat Neurosci* 2000 Oct;3(10):1027–34 [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.].
- [25] Tranchina D, Nicholson C. A model for the polarization of neurons by extrinsically applied electric fields. *Biophys J* 1986 Dec;50(6):1139–56 [Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.].
- [26] Bikson M, Inoue M, Akiyama H, et al. Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *J Physiol* 2004 May 15;557(Pt 1):175–90 [In Vitro Research Support, Non-U.S. Gov't].
- [27] Datta A, Bansal V, Diaz J, Patel J, Reato D, Bikson M. Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimul* 2009 Oct;2(4):201–207.e1 [Evaluation Studies Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].
- [28] Fee MS, Mitra PP, Kleinfeld D. Automatic sorting of multiple unit neuronal signals in the presence of anisotropic and non-Gaussian variability. *J Neurosci Methods* 1996 Nov;69(2):175–88.
- [29] Hill DN, Mehta SB, Kleinfeld D. Quality metrics to accompany spike sorting of extracellular signals. *J Neurosci* 2011 Jun 15;31(24):8699–705 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].
- [30] Brunoni AR, Shiozawa P, Truong D, et al. Understanding tDCS effects in schizophrenia: a systematic review of clinical data and an integrated computation modeling analysis. *Expert Rev Med Devices* 2014 Jul;11(4):383–94.
- [31] Reato D, Rahman A, Bikson M, Parra LC. Effects of weak transcranial alternating current stimulation on brain activity—a review of known mechanisms from animal studies. *Front Hum Neurosci* 2013;7:687 [Review].
- [32] Kuo MF, Paulus W, Nitsche MA. Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *Neuroimage* 2014 Jan 15;85 Pt 3:948–60 [Research Support, Non-U.S. Gov't].
- [33] Radman T, Ramos RL, Brumberg JC, Bikson M. Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. *Brain Stimul* 2009 Oct;2(4):215–28. 28 e1–3 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].
- [34] Feurra M, Pasqualetti P, Bianco G, Santarnecchi E, Rossi A, Rossi S. State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. *J Neurosci* 2013 Oct 30;33(44):17483–9 [Randomized Controlled Trial Research Support, Non-U.S. Gov't].
- [35] Niell CM, Stryker MP. Modulation of visual responses by behavioral state in mouse visual cortex. *Neuron* 2010 Feb 25;65(4):472–9 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].
- [36] Curto C, Sakata S, Marguet S, Itskov V, Harris KD. A simple model of cortical dynamics explains variability and state dependence of sensory responses in urethane-anesthetized auditory cortex. *J Neurosci* 2009 Aug 26;29(34):10600–12 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't].
- [37] Neuling T, Wagner S, Wolters CH, Zaehle T, Herrmann CS. Finite-element model predicts current density distribution for clinical applications of tDCS and tACS. *Front Psychiatry* 2012;3:83.
- [38] Cardin JA, Carlen M, Meletis K, et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 2009 Jun 4;459(7247):663–7 [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.].
- [39] Frohlich F, Schmidt SL. Rational design of transcranial current stimulation (TCS) through mechanistic insights into cortical network dynamics. *Front Hum Neurosci* 2013;7:804 [Review].
- [40] Kuo HI, Bikson M, Datta A, et al. Comparing cortical plasticity induced by conventional and high-definition 4 x 1 ring tDCS: a neurophysiological study. *Brain Stimul* 2013 Jul;6(4):644–8.
- [41] Struber D, Rach S, Trautmann-Lengsfeld SA, Engel AK, Herrmann CS. Anti-phasic 40 Hz oscillatory current stimulation affects bistable motion perception. *Brain Topogr* 2014 Jan;27(1):158–71 [Research Support, Non-U.S. Gov't].
- [42] Kutchko KM, Frohlich F. Emergence of metastable state dynamics in interconnected cortical networks with propagation delays. *PLoS Comput Biol* 2013 Oct;9(10):e1003304 [Research Support, Non-U.S. Gov't].
- [43] Boyle MR, Frohlich F. EEG feedback-controlled transcranial alternating current stimulation. *I IEEE Embs C Neur E* 2013:140–3.
- [44] Helfrich RF, Schneider TR, Rach S, Trautmann-Lengsfeld SA, Engel AK, Herrmann CS. Entrainment of brain oscillations by transcranial alternating current stimulation. *Curr Biol* 2014 Feb 3;24(3):333–9 [Research Support, Non-U.S. Gov't].