Externally induced electric fields alter endogenous cortical activity *in vitro*

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Abstract

Transcranial electrical stimulation (tES) is a novel noninvasive brain stimulation method which has been proposed to interact with and alter endogenous brain oscillations. This stimulation delivers a weak electric current to the brain via sponge electrodes placed on the skull. The tES has behavioral and cognitive effects on healthy populations and improves mood symptoms in several drug-resistant forms of psychiatric disorders including major depression and schizophrenia. The cellular level mechanism of the effect of tES is an active research topic in neuroscience. This study aims to study the effects of tES at the neural population level using mouse cortical slices *in vitro*. We prepared slices of the medial pre-fontal cortex of juvenile mice kept in artificial cerebrospinal fluid (aCSF). Using a 60-channel microelectrode array, we recorded the multiunit neuronal activity of the slices. The slices were perfused with 5 µM carbachol (CCh) aCSF, 5 µM norepinephrine (NE), or control aCSF to induce different spontaneous and persistent neural oscillations. Perfusion of aCSF resulted in slow and sparse neural activity on some individual channels. Perfusion of CCh resulted in continuous firing on most of the individual channels. Perfusion of NE induced bursts of action potentials, which were synchronous across multiple channels. We studied the effect of two types of stimulation waveforms: a high frequency pulse train and a 2Hz sinusoidal wave. To study the stimulation effects, we extracted the median firing rate and the spectral characteristics of population oscillations across the channels for each brain slice. The two stimulation types did not have any significant effect on the neural activity for the slices in the control aCSF group. The high frequency pulse stimulation (HFPS) significantly altered the change in the median firing rate after stimulation offset when compared with pre-stimulus period for slices perfused with CCh, but not with NE. The 2Hz alternating current stimulation (2Hz-ACS) was able to alter the spectral power of NE-induced synchronous oscillations both during the stimulation period and after stimulation offset. Our results provide *in vitro* support for the ability of externally induced electrical fields in altering endogenous brain oscillations. Observations of the outlasting effect with CCh and both online and outlasting effects with NE also demonstrates that different neuromodulatory levels can play important roles in the electric field interaction with endogenous neural activity. This study demonstrates a successful *in vitro* setup as a model for human tES and provides a framework to design more efficient protocols for transcranial brain stimulation.

Introduction

The earliest recorded use of electric current to cure headache symptoms was in 43 AD (Wagner, 2007). The concept has branched far and wide in terms of application, stimulation methodology, and stimulation wave form. One branch led to the development of noninvasive brain stimulation (NIBS), a form of brain stimulation characterized by the use of electrodes located above the skull or on its surface noninvasively. In recent years, NIBS has become a prominent topic in the field of neuroscience for its potential therapeutic application to modulate the symptoms of stroke, pain, and some psychiatric disorders, like depression and bipolar disorder (Wagner, 2007; Fregni, 2007). The noninvasive nature of the NIBS makes it a promising alternative or complement to pharmaceutical interventions. NIBS can be divided into two forms: transcranial magnetic stimulation (tMS) and transcranial electrical stimulation (tES). tMS stimulates through a magnetic coil which can induce an electric field across a brain region with the strength of around 100 V/m inside the brain tissue (Corthout, 2001). The other NIBS

type, tES, involves passing a weak electric current across a brain region through sponge electrodes. Due to limitations of using current in live tissue, the current amplitude has to be low (with resulting electric field strength of about 1 mV/m) and, therefore, the induced electric field is weaker than tMS (Reato, 2015; Fröhlich, 2016). An example of a tES waveform is low field magnetic stimulation (LFMS), a subthreshold waveform characterized by repeated 500 Hz trains of 500 ms duration repeated every 2 seconds (Rohan, 2014). Even though LFMS generates weak fields, it was found to improve mood symptoms in patients with bipolar depression.

tES can be further subdivided into transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS). The difference between tDCS and tACS is the use of either a constant direct current or a sinusoidal waveform, respectively. The weak current of tDCS is not strong enough to directly induce action potential generation in the neurons, but it is has been suggested that the resulting electric field can alter the firing rate of the neurons (Fröhlich, 2016). Studies on tDCS have shown that in addition to acute effects this stimulation also exhibits a persistent, or offline, effect that is believed to be a result in changes of synaptic plasticity (Fröhlich, 2016). In contrast, the tACS form regulates ongoing cortical oscillations and is proposed to work the most effectively if the endogenous oscillation frequency matches the stimulation frequency (Vossen, 2015; Herrmann, 2013; Zaehle, 2010; Boyle, 2013).

From analyzing previous research, it can clearly be seen why NIBS, specifically tES, has become so popular. However, would the same hypotheses and suggestions hold for a study examining the neural effect of tES? Would a tES with pulse waveform and tACS waveform modulate mouse brain medial prefrontal cortical slices at the neuronal level? Additionally, what happens when tES is combined with pharmaceutical drugs, like NE and CCh? An in vitro study would be able to show the changes in neural activity associated with tES combined with NE or CCh on the prefrontal cortex. The in vitro study provides mechanistic insights for the underlying mechanisms of stimulation effect on the neurons. Unlike electroencephalography recordings (EEG), in vitro studies are localized to one area of the brain and analyze a significantly smaller group of neurons at one time. Even though *in vitro* studies use isolated and less active brain tissue, neuromodulators can be directly applied to boost neural activity and to mimic the activity of intact brain tissue. The two most relevant neuromodulators to this study are norepinephrine (NE) and carbachol (CCh, an acetylcholine equivalent). NE has a primary role in activating neurons in the sympathetic system but also increases vigilance. Acetycholine has a similar role in promoting attention and assists with wakefulness (Bear, 2016). Carbochol targets acetylcholine receptors, causing the amplitude of electrical brain activity to increase.

We used a modified version of the in vitro model (Schmidt, 2013). The model uses a two well 6x10 Microelectrode Array (MEA) system to record brain slice activity. The slices can be electrically and chemically stimulated while they are in the bath. This specific study will use mouse medial prefrontal cortical slices (mPFC) as their layer 5 pyramidal neurons can be easily targeted with an electric field (Radman et al, 2009) and inhibited activity in the prefrontal cortex is often associated with depression symptoms (Bear, 2016).

Materials & Methods

Ethical Statement

All animal surgeries and procedures were approved by the Institute of Animal Use and Care of the University of North Carolina Chapel Hill and were in compliance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH citation). Efforts were made to reduce animal suffering and the number of animals used.

Study Design

We used a sham controlled randomized study design where mouse animal brain slices meeting the study criteria were randomly assigned to sham or stimulation groups. The design criteria restrict the slice tissue by mouse type, age, and brain region. A colony of experimental Thy1-Chr2 YFP black mice was maintained for the experiments. Thy1-Chr2 mice have been genetically modified to express Channelrhodopsin-2 and Enhanced Yellow Fluorescent Protein in layer 5 cortical neurons, pyramidal neurons of the hippocampus, and other brain regions (Jackson Labs). When exposed to blue light (470 nm), the Chr2-YFP expressing neurons become more electrically active and appear bright green when viewed under a microscope. Juvenile mice between 15 and 30 days of age were used as the prefrontal cortex was still developing during the age range. Psychiatric disorders, like depression, have been associated with issues in the prefrontal cortex during development, so, therefore, the cortex was the stimulation target.

Slice Preparation

Slice preparation followed the protocol from Schmidt et. al, 2013 with modifications in the mouse strain, anesthesia dilution, brain region, and slice recovery time. The juvenile Thy1-Chr2 mice were deeply anesthetized with an interperitoneal injection of 10% Euthasol to saline dilution (0.25 mL) and toe pinched to confirm numbness before decapitation. After decapitation, the mouse brain was immediately placed in a cold carbogenated (95% oxygen, 5% carbon dioxide) sucrose slush solution (83.0 mM NaCl, 2.5 mM KCl, 0.5 mM $CaCl_2$, 3.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 22.0 mM Dextrose Anhydrose, 72.0 mM Sucrose) for a few seconds. The importance of the sucrose solution is twofold: 1) it chills the brain, which slows the degradation process and also maintains the brain's shape; and 2) it provides the necessary nutrients the brain needs to conserve its activity. The carbogen was bubbled through the sucrose solution to keep the level of dissolved gases in the solution high so that oxygen and carbon dioxide can diffuse into the brain tissue.

Once the brain was surgically removed, it sat for a minute in fresh, cold carbogenated sucrose solution before slicing. The Leica VT1000S vibratome (Leica Microsystems, Wetzlar, Germany) was used to slice the pre-frontal cortical brain region into 200 µm sections. Only slices with medial prefrontal cortex (mPFC) regions were kept. Extracted slices recovered in incubation solution (stock aCSF modified to contain 2.0mM $MgSO_4$ and 2.0mM $CaCl_2$) for two hours at 34^{o}C using an Isotemp Incubation Water Bath (Fisher Scientific, Pennsylvania, America) before placement on the array. The incubation solution has a high concentration of $MgSO_4$ to inhibit neural activity and allow the slices to recover in an inactive state. The slice was able to remain in the incubation solution for up to 8 hours before recording.

Artificial cerebral spinal fluid (aCSF) Solutions

Three different aCSF solutions were used in experiments to perfuse the slices while recording. The chemicals used to make the solutions were reagent grade from Sigma Aldrich (St. Louis, MO). A stock aCSF solution was made with the following concentrations of solutes: 119.0 mM NaCl, 2.5 mM KCl, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 22.0 mM glucose, 1.0 mM $MgSO_4$, and 1.0 mM $CaCl_2$. Experimental solutions were then created from the stock with the following modifications: control aCSF (aCSF with added KCl to a final concentration of 3.5 mM KCl); NE aCSF (control aCSF with 5 µM of NE added); and CCh aCSF (control aCSF with 5 µM of CCh added).

Microelectrode Array Setup

A two-well Microelectrode Array 2100 (MEA, Multichannel Systems, Reutlingen, Germany) recorded the spontaneous neural activity from two slices at the same time, independent of each other. The slices were placed on perforated microelectrode arrays which have 59 electrodes (30 x 30 µm diameter) organized in a 6 by 10 grid with 200 µm spacing between electrodes and an additional reference electrode. Multi-unit activity was measured across each microelectrode. The array was setup on a vibration-free desk in an electromagnetic noise-free Faraday cage and the metal instruments were grounded to a reference electrode to minimize electrical noise in the recording.

Along with the MEA hardware, the data acquisition setup included PH01 perfusion cannula temperature sensors and controllers (Multichannel Systems, Reutlingen, Germany), Minipuls 3 peristaltic perfusion pump (Gilson, Wisconsin, USA), CVP constant vacuum pump (Multichannel Systems, Reutlingen, Germany), and an Onamo Microscope (Microscope.com, China) with an ultraviolet fluorescent light and filter attachment (470 nm, Nightsea, Massachusetts, USA) and Optix camera Summit adapter (Microscope.com, China). The microscope and fluorescent blue light was used to orient the slices so that YFP expressing pyramidal neurons were directly above the microelectrode grid. The YFP cells fluoresce green and fire action potentials when under blue light which made it possible to validate the slice orientation on the array. This enabled us to keep the slice orientation and recording location the same across all slices. Positive pressure from the vacuum pump held the slice in place through the perforated holes which made it easier to detect neural activity. The camera was used to record images of the slices while shined by blue light (Figure 1a). The temperature controllers kept the aCSF solutions at a temperature of 36^{o}C in the recording wells. Perfusion heads and vacuum tubes were placed across from each other to add and remove recording solution, respectively, from the wells at a constant rate to maintain the liquid level.

Slices on the arrays were perfused with the recording solutions: control, NE, or CCh aCSF bubbled with carbogen. The peristaltic pump pulled liquid from carbogenated aCSF recording solutions into the perfusion tubes. The flow rate of the perfusion was kept at 8 mL/min.

Electrical Stimulation

Before recording, each slice was placed on the array and perfused with recording solution for 10 minutes. The time allowed to slice to reach its endogenous activity after the magnesium block was removed. During the experiment, a slice was recorded for 300 seconds pre-stimulation, 1200 seconds of stimulation, and 600 seconds post-stimulation. During each

experiment, verum slice data which experienced stimulation and control sham slice data which did not experience stimulation were collected simultaneously in separate wells. The verum slices were stimulated with either high frequency pulse stimulation (HFPS, 0.5 kHz pulse train, patterned at 0.5 Hz, 20 mV/mm) or 2-Hz sine wave alternating current stimulation (2Hz-ACS) with a field amplitude of 6 mV/mm. The electric field stimulated the slice for 500 ms every 2 s during the stimulation period. The wave form train was designed to be similar to low field magnetic stimulation (LFMS), a type of magnetic stimulation associated with improved mood symptoms (Rohan et al). A custom C++ code was written to set the times for pre-, during, and post-stimulation and to send the stimulation waveform to the stimulator (Schmidt, 2013) and ultimately to the slice on the MEA through silver chloride wire electrodes in the wells. The wires sat at the bottom of the well and the aCSF current passed across the parallel wires (Figure 1b). The stimulus current across the parallel wires caused an even electric field to be applied to the slice perpendicular to the mPFC region (Figure 1b).

Data Collection and Analysis

The software Multichannel Rack (Multichannel Systems, Reutlingen, Germany) displayed and recorded the data from the MEA. The signal from the hardware went through a high pass Butterworth filter of 300 Hz, which included only the spiking activity of the neurons. A baseline noise activity of 20 μV was considered an acceptable noise level. Electrodes in which the baseline noise level was high were excluded from analysis. After the data was collected, the slices were fixed with formaldehyde and stored at 2^{o}C.

Once recorded, the occurrence time (spike indices) and traces (spike traces) of action potentials were extracted from the raw traces using a custom Matlab (Mathworks, Natick, MA) program. The program recognized action potentials occurred when the recorded voltage was less than -4 times the noise level. All subsequent analyses were performed on spike indices in Matlab. The firing rate time course was extracted for each channel excluding the ones with firing rates less than 0.5 spikes/second. The median firing rate for each slice was extracted and the slices with either a decreasing or fixed firing rate time course were excluded from further analysis as it was assumed to be an inactive slice. In addition to firing rate properties, we also computed the population firing signal for individual channels and the median across multiple channels. The spectral characteristics of the median population-firing signal were also analyzed. The spectral power was calculated by computing power spectral densities obtained from the Fast Fourier Transform of the population-firing signal. The pairwise correlation compared the activity between electrode pairs.

The statistical test used to determine significance was a Wilcoxon Ranked Sum for unpaired data and Wilcoxon Signed Rank for paired data. A p-value of less than 0.05 was considered significant.

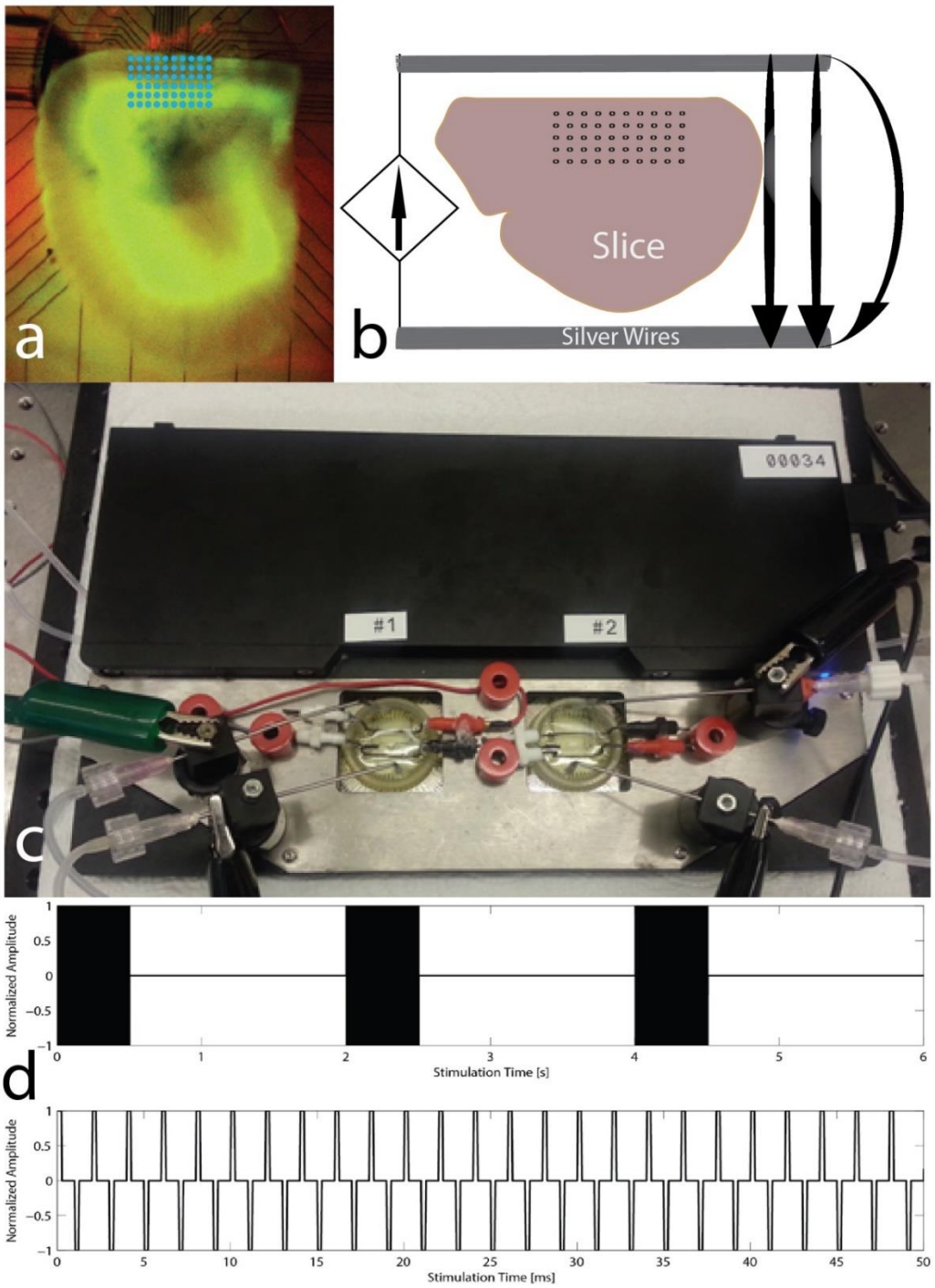

Figure 1 mPFC slices and the recording/stimulation system. (a) Electrodes superimposed over a fluorescence image of a mPFC slice on the MEA. (b) mPFC slice, microelectrodes, and stimulation wires. The stimulation was applied across the two Ag/AgCl wires, shown by 2 parallel gray cylinders. The slice is placed in the electric field so that positive currents depolarize cells in mPFC layer 5. The up black arrow is the electric current generated by the stimulator, while the down arrows is the induced electric field in the bath. (c) The recording apparatus for simultaneous recording of two slices. (d) The graphical representation of the HFPS stimulation waveform. During the stimulation, electric field was applied to the network for 500 ms of every 2 s. The stimulation consisted of 240 μs pulses separated by 760 μs, resulting in a 500 Hz charge-balanced pulsed waveform.

Results

We first present the results for the effect of high frequency pulse stimulation on slices bathed in control, CCh, and NE ACSF. Then we will discuss the effect of 2Hz sinusoidal current stimulation on slices bathed in control and NE stimulation. Both verum and sham groups are considered in these two series of experiments.

1. Interaction of neural activity with high frequency pulse stimulation (HFPS)

We first analyzed the median firing rates (FR) of pre-stimulus period for all groups to make sure that there is no bias between verum and sham population. The FR did not significantly differ between sham and verum groups for all three aCSF types pre-stimulation (n = 18 aCSF verum, 13 aCSF sham, 20 CCh verum, 26 CCh sham, 31 NE verum, 39 NE sham slices). However, sham control aCSF slices trended towards lower FRs than the matched verum slices ($p = 0.0576$ for aCSF, 0.5403 for CCh and 1 for NE). The results also show that the median FR in pre-stimulation was greater in the presence of carbachol (CCh) or norepinephrine (NE) than for control aCSF as is expected for these two neuromodulators (Figure 2a).

Next we extracted the percent increase in median FR during the stimulation compared to pre stimulus period for all three groups (Figure 2b). The results show an increase for verum and sham populations regardless of aCSF type. The increase in FR was most likely due to the experiment time since the sham stimulated slices were not significantly different from verum slices when matched by aCSF type ($p = 1$ for aCSF, 0.1659 for CCh and 0.2403 for NE). Taken together, these results indicate that application of HFPS stimulation did not immediately modulate FR.

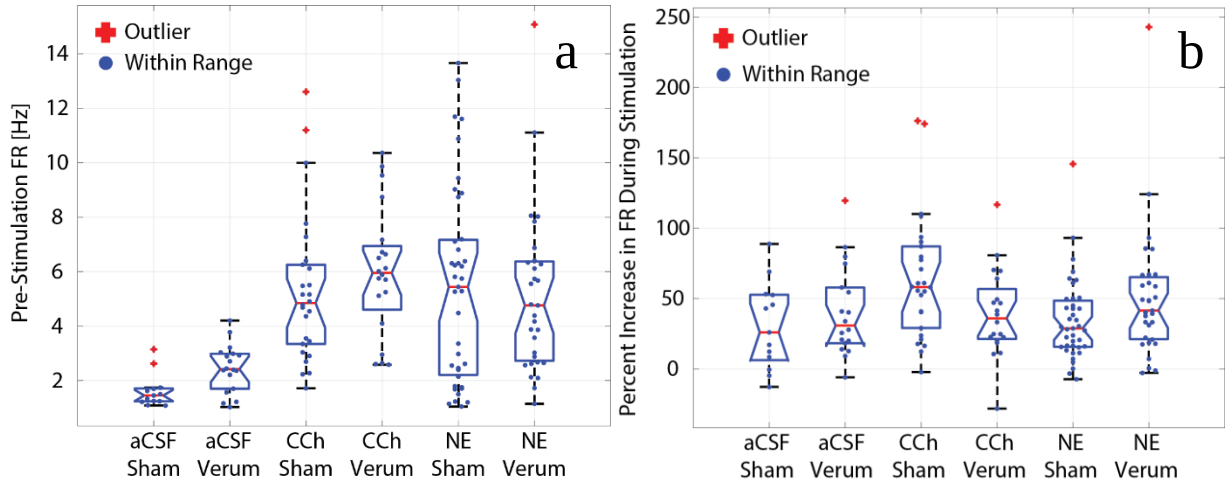

Figure 2 pertains to the results for comparison of all experimental types. (a) The pre-stimulation FR for all slices which were not significantly different from each other. (b) The percent increase in raw FR during the stimulation period. There were no significant changes between any of the experimental types comparing during- and pre-stimulation.

To analyze the possible long-lasting effects of stimulation, we extracted the percentage increase of median FR during the first 10 minutes post-stimulation compared to pre-stimulus period. The results show that for networks bathed in CCh aCSF, the increase in FR was significantly reduced by application of HFPS stimulation compared to sham stimulation (Figure 3, p = 1 for aCSF, 0.0198 for CCh and 0.1902 for NE). Since the verum stimulation only reduced the increase in FR for CCh treated slices, it implies the pharmaceutical treatment influenced the neural response to stimulation.

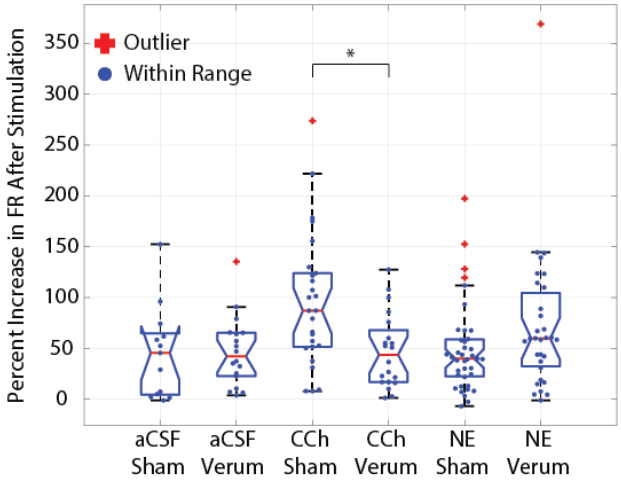

Figure 3 The long-lasting effect of high frequency pulse stimulation (HFPS) on neural activity. The percent increase in median firing rate (FR) during the post-stimulus period compared to pre-stimulus period for all groups. The FR keeps increasing for all groups but the HFPS reduces the increase in firing rate for CCh when compared to sham (p=0.0198).

NE induced the emergence of synchronous oscillations across multiple channels

Slices bathed in NE aCSF exhibited synchronized activity across multiple channels. These oscillations appeared in the form of waxing and waning burst of neural activity on raw traces synchronized across multiple channels (Figure 4a). The rate of these bursts were around 2 burst per second. These synchronized oscillations were not observed under pure ACSF or CCh experiments and were exclusive to the NE treated group. After observation of the synchronized activity, we considered the NE slices as an in vitro model for ongoing brain oscillations and further analyzed their response to HFPS to reveal its possible interactions with ongoing oscillations. We were interested to see if HFPS paired with NE would alter the frequency or amplitude of the emerged oscillations.

We first studied how the spectral power of these oscillations changed during the course of experiment in sham group. To do this we first extracted the average MU activity signal (red trace in Fig. 4.a), then calculated its power spectrum for the 5 minutes of the pre-stimulation epoch and the first 5 minutes of the post-stimulation period. The median power spectra (with one standard deviation interval in dashed lines) are shown in Fig. 4.b. We calculated the area under the power spectrum curve for pre and post-stimulus periods as an indicator of the total spectral power. The results showed that the total median power of oscillation increased over the time significantly in sham NE slices (median total power [95% confidence interval]: 11.1 [9.39 13.0]

au pre-stimulation and 18.0 [13.4 23.0] au post-stimulation, $p < 10\text{-}4$ Wilcoxon signed rank test, n = 39 NE sham slices).

We next looked at the effect of HFPS on the frequency of NE-induced oscillations. The results are shown in Figure 4.c. The spontaneous oscillation occurred between 0.5 to 4.5 Hz across networks with a median frequency of 2.02 Hz before stimulation. The frequency of the oscillation decreased in the post-stimulation period for sham and verum (frequency: 1.83 [1.67 1.98] Hz for verum and 1.98 [1.75 2.13] Hz for sham $p = 0.0077$ for verum and 0.0334 for sham Wilcoxon signed rank test, n = 31 for NE verum and 39 for NE sham). The change in oscillation frequency was not different between sham and verum stimulation (Figure 4c, frequency change: -0.453 [-0.679 -0.226] Hz for verum and -0.377 [-0.603 -0.000] Hz for sham; $p = 0.7317$).

As a next step, we analyzed the HFPS effect on the total spectral power of NE-induced oscillations. The change in total power spectra between pre- and post- stimulation were not significantly different between verum and sham (Figure 4d, change in total power: 7.49 [4.60 14.5] au verum and 4.36 [2.98 10.3] au for verum, $p = 0.2664$, n = 31 NE verum and 39 NE sham slices). The analysis showed the HFPS had no significant effect on changing the oscillation power, though there was a trend showing that in some slices the HFPS increased the power (Figure 4.d, upper dashed red trace).

We also extracted the peak oscillatory power in verum and sham groups and the logarithm value of the results are shown in Figure 4.e. The peak oscillatory power increased as the experiment progressed ($p < 10\text{-}4$ for both verum and sham Wilcoxon signed rank test). The change from pre-stimulation peak power to post-stimulation peak power trended to be larger for networks which received verum stimulation (peak power: 0.432 [0.0930 1.30] au for verum and 0.0592 [0.0397 0.190] au for sham; $p = 0.1214$), but it was not significant.

As the last step we asked if there was any correlation between the pre-stimulation spectral power with change in the FR from the pre-stimulation to the post-stimulation period. The results are shown in figure 4.f. We found that the power of the oscillation in the pre-stimulation was not correlated with the change in FR the pre- to post-stimulation periods ($r = -0.284$ for verum and -0.115 for sham, $p = 0.2428$ and 0.9710 respectively). Because the correlation was not significant, the result simply state that the starting oscillatory power did not determine the change in FR due to stimulation.

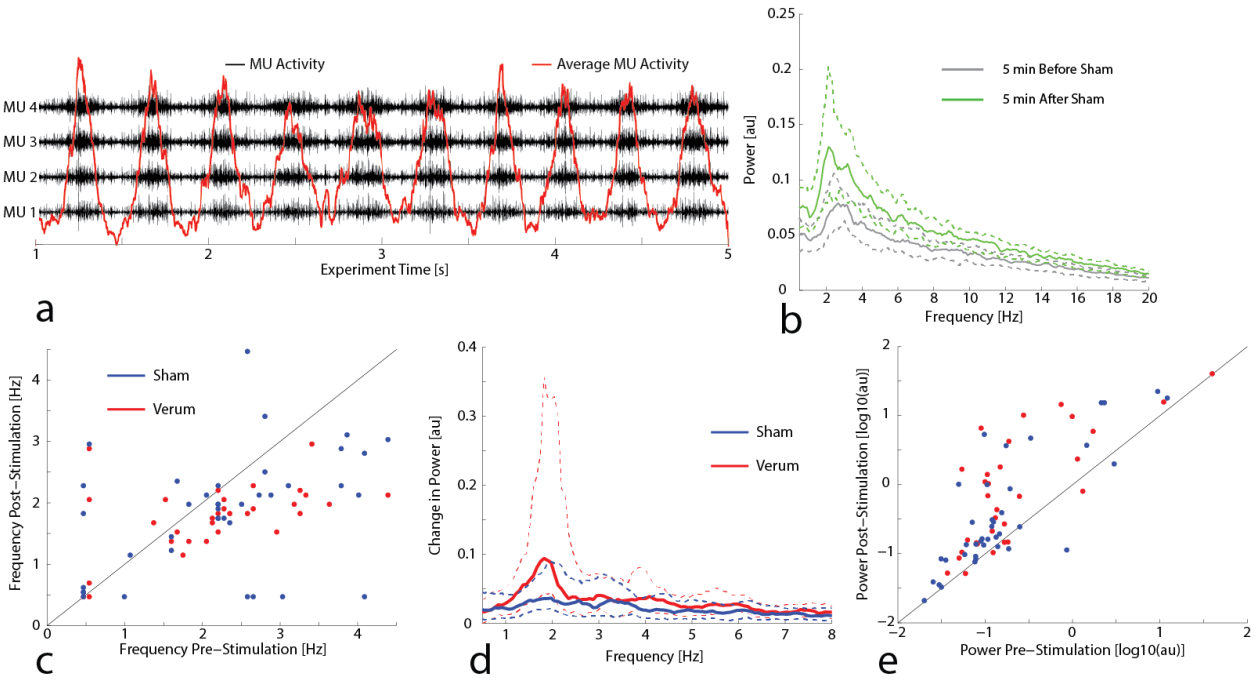

Figure 4 The NE-induced synchronized oscillations and the HFPS effect on these oscillations. (a) An example of the synchronous activity recorded in 4 electrodes of a NE-treated slice. The red line is the average activity across the channels superimposed over the recording. (b) Comparison of the power spectrum of the sham NE slices in the last 5 minutes of pre and post stimulation periods. The dashed lines are within one standard deviation of the median solid line. The total spectral power (area under the spectral curve) is higher for post-stimulation period especially within the 2-4 Hz frequency range. (c) is a plot of points with the pre- and post-stimulation frequencies as the x and y-axis, respectively. (d) shows the change in spectral power from pre- to post-stimulation for verum and sham, with the median as the solid line and standard deviation as dashed lines. (e) is a plot of the pre- and post-stimulation power as points. The majority of points are above the line x=y. (f) is a plot comparing the pre-stimulation power to the change in FR from pre- to post-stimulation.

2. Interaction of neural activity with 2 Hz alternating current stimulation (2Hz-ACS)

The 2Hz-ACS is composed of a 2 Hz sinusoidal waveform. Our measurements inside the ACSF solution indicated that this stimulation induces an alternating electric field in the slice with a strength of 6 mV/mm (peak to peak voltage). These experiments include verum (19) and sham (20) NE-treated slices since the synchronous neural activity (Figure 4.a) was not observed under pure or CCh ACSF records. In these new series of experiments we used the NE-induced synchronous oscillations as a model of synchronous neural population oscillations in vivo or human and asked if a sinusoidal waveform (as a common waveform in noninvasive brain stimulation protocol) can interact with slice oscillations or not.

We first examined if the baseline (pre-stimulus) properties of verum and sham slices are the same or not. We calculated the total spectrum power for two groups (Figure 5.a). The total spectral power for verum and sham slices during the pre-stimulation period were not significantly different (p=0.565), indicating there was not a bias between verum and sham.

As the next step, we analyzed the change in the total spectral power for verum and sham groups during the entire course of the experiment. The change in total spectral power for sham slices over recording time trended to increase (Figure 5.b) but the differences were not significant. The power of verum slices significantly increased from pre- to during stimulation, but it decreased between during to post stimulation (Figure 5.c).

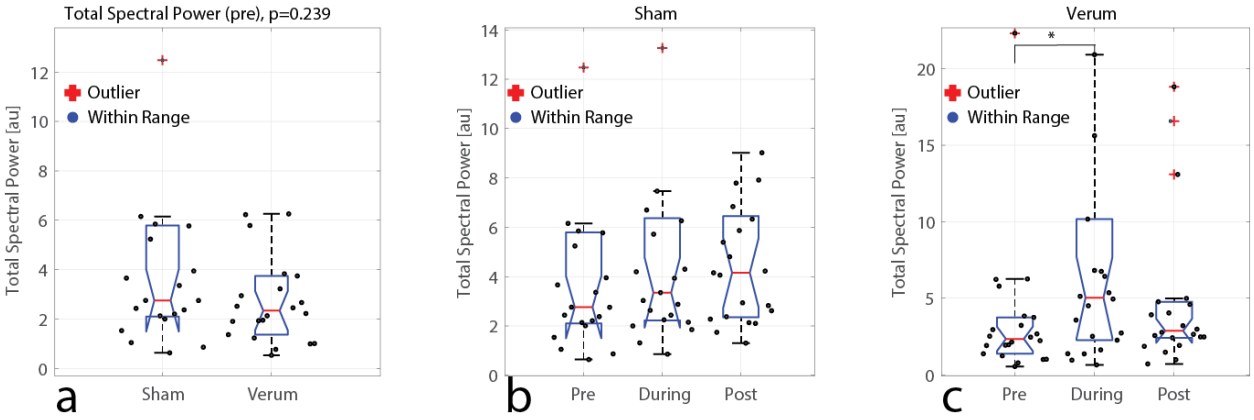

Figure 5 Spectral power analysis of NE-treated slices in response to 2Hz-ACS. (a) There is not a significant baseline difference in spectral power between sham and verum slices before stimulation (p=0.565). (b) The spectral power of the sham slices over the recording periods. The sham slices show an increase in power over time but not statistically significant. (c) The spectral power of the verum slices over the recording. The verum slices show a significant change in power between pre- and during stimulation periods (online effect), but not between pre- and post-stimulation periods.

We further analyzed the data by comparing the change from pre to during periods and the change from pre to post-stimulation periods for verum and sham groups. We found a significant difference between verum and sham groups when looking at the change in total power from pre-stimulation to during-stimulation periods (Figure 6.a). We did not find a significant difference between verum and sham groups when comparing the change in the total power from pre-stimulation to post-stimulation epochs (Figure 6.b).

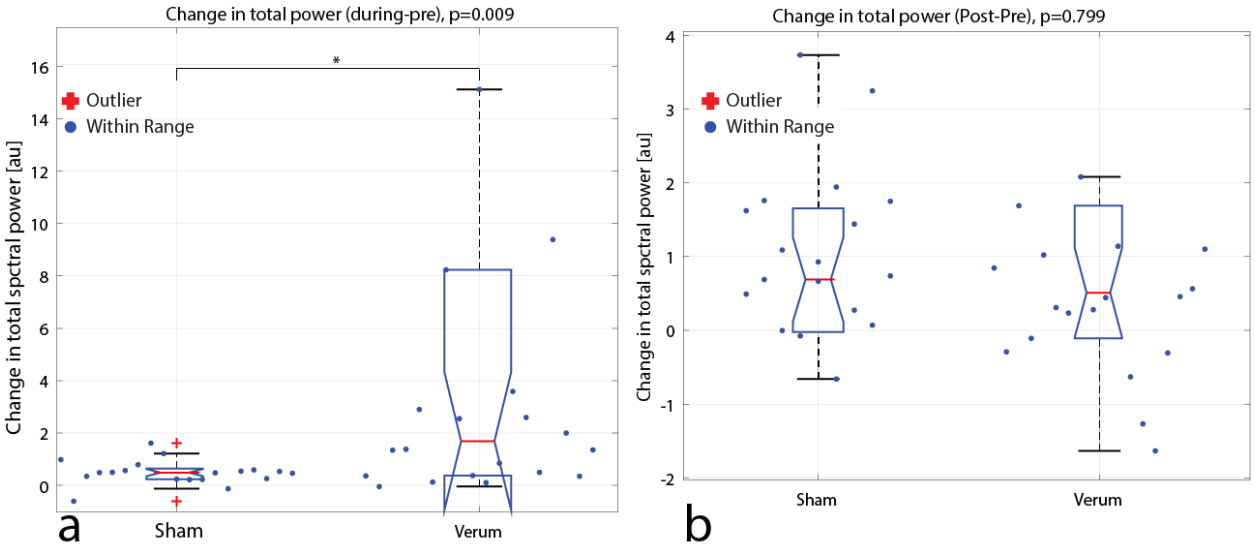

Figure 6 Acute effect of 2Hz-ACS on spectral power of NE treated slices. (a) There is a significant difference between the change in total spectral power of the sham and verum slices when comparing during minus pre-stimulation. (b) There is no significant difference between the change in total spectral power of the sham and verum slices when comparing post minus pre-stimulation.

Looking for possible signs of the long-lasting effects of 2Hz- ACS, we further extended the analysis by limiting the power spectral analysis over a narrow frequency range of 1.75 to 2.25 Hz centered on the simulation frequency. The pre-stimulation narrow-band power for verum and sham were not significantly different ($p>0.5$, Figure 7.a), showing there was no baseline bias between the groups. Similar to the results obtained for total power, the narrow-band spectral power of the verum slices changed significantly when comparing during to pre-stimulation ($p<0.05$, Figure 7.C). We also found that the narrow-band power was significantly different between verum and sham when comparing pre- and post-stimulation periods ($p<0.05$, Figure 7).

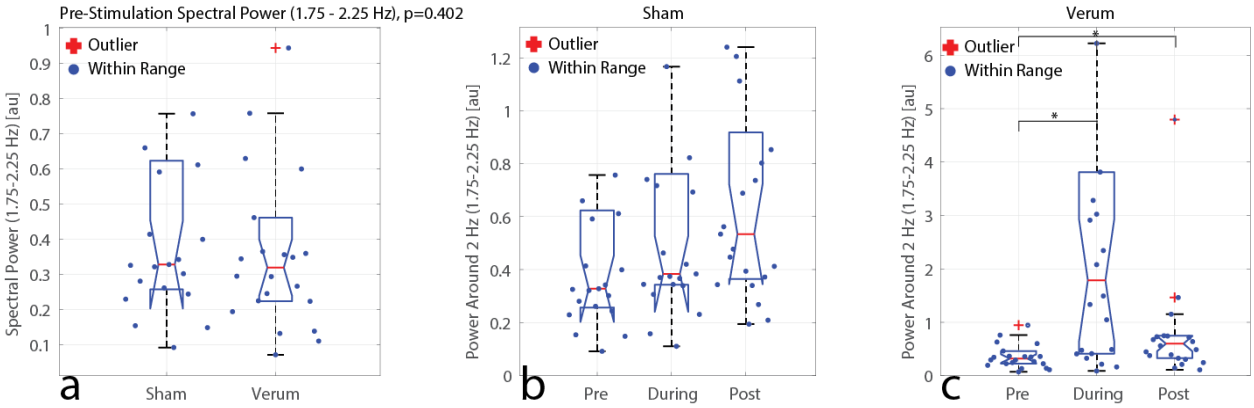

Figure 7 Acute and outlasting effect of 2Hz-ACS stimulation on narrowband spectral power of the NE-treated slices. The box plots compare the narrowband spectral power between sham and verum slices in the frequency range of 1.75-2.25 Hz. (a) The pre-stimulation baseline narrowband spectral power is not significantly different between sham and verum slices. (b) Sham slices show an increasing trend in their narrowband power over the course of the experiment, but the differences are not significant. (c) The narrowband spectral power is significantly higher during the stimulation when compared to pre-stimulation period (acute effect). The narrowband spectral power is significantly higher during the post-stimulation period when compared to pre-stimulation power (outlasting effect), p=0.0003, p=0.012 respectively.

Discussion

Our in vitro slice model allowed us to examine the neural effects of HFPS and 2Hz-ACS while paired with NE, CCh, or pure aCSF solutions. The results showed that HFPS did not induce any acute effect but had an outlasting effect. This type of stimulation significantly reduced the increase in after-stimulation firing rate in CCh perfused slices. However, HFPS did not modulate firing rates of slices bathed in aCSF or NE aCSF. We also found that perfusion of 5 µM NE caused the spontaneous activity within the network to organize into a range of 0.5 to 4.5 Hz synchronous oscillations with a median of 2 Hz. While not significant, HFPS trended to increase the power of these ongoing oscillations compared to sham stimulation. The second stimulation type, 2Hz-ACS, showed both online and outlasting effects. The total spectral power of the population neural activity showed only online change due to stimulation. Further spectral analysis revealed the outlasting effect of the 2Hz-ACS. The narrowband spectral power (centered on the stimulation) was changed both during and after stimulation.

This study shows that the weak electrical field generated by 2Hz-ACS can alter the activity of cortical neurons with acute and long-lasting outcomes. Our findings also highlight the

importance of 2Hz-ACS in the context of the neuromodulatory level. CCh treated slices showed outlasting effect in response to HFPS, and NE treated slices showed significant acute and outlasting responses to the 2Hz-ACS. In addition, exposure of mPFC slices to NE and the resulting ongoing oscillations has provided us with the framework to create a promising in vitro neural oscillation model and furthered our understanding of stimulation interaction with brain oscillations. Even though our study focuses on the in vitro level, the results have greater implications for the study of the interaction between transcranial electric stimulation and brain oscillations in in vivo animal and human studies.

Although the results showed many promising trends in terms of capturing the interaction between the applied electric field and the neural activity, not all of them were significant. These trends might grow and become significant if we increase the number of samples or perform analysis on the channel level instead of the slice level. Our preliminary investigation showed that not all 59 recorded channels from a slice have reliable recordings during the entire course of the experiment. Some of the channels have a partially successful recording rather than a complete one during the 35-minute course of the experiment. We propose that the next series of analysis should be performed by excluding the affected channels. An issue specific to the 2Hz-ACS experiments was that while the endogenous frequency was different between slices (0.5 – 4 Hz), we always stimulated them with 2Hz. The stimulation frequency could be customized to the endogenous frequency of the slice over the pre-stimulation period.

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