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Kilohertz Transcranial Magnetic Perturbation (kTMP): A New Non-invasive Method to Modulate Cortical Excitability

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Abstract

Non-invasive brain stimulation (NIBS) provides a method for safely perturbing brain activity, and has been employed in basic research to test hypotheses concerning brainbehavior relationships with increasing translational applications. We introduce and evaluate a novel subthreshold NIBS method: kilohertz transcranial magnetic perturbation (kTMP). kTMP is a magnetic induction method that delivers continuous kHz-frequency cortical electric fields (E-fields) which may be amplitude-modulated to potentially mimic electrical activity at endogenous frequencies. We used TMS to compare the amplitude of motor-evoked potentials (MEPs) in a hand muscle before and after kTMP. In Experiment 1, we applied kTMP for 10 min over motor cortex to induce an E-field amplitude of approximately 2.0 V/m, comparing the effects of waveforms at frequencies of 2.0, 3.5, or 5.0 kHz. In Experiments 2 and 3 we used two forms of amplitude-modulated kTMP with a carrier frequency at 3.5 kHz and modulation frequencies of either 20 or 140 Hz. The only percept associated with kTMP was an auditory tone, making kTMP amenable for doubleblind experimentation. Relative to sham stimulation, non-modulated kTMP at 2.0 and 3.5 kHz resulted in an increase in cortical excitability, with Experiments 2 and 3 providing a replication of this effect for the 3.5 kHz condition. Although amplitude-modulated kTMP increased MEP amplitude compared to sham, no enhancement was found compared to non-modulated kTMP. kTMP opens a new experimental NIBS space inducing relatively large amplitude subthreshold E-fields able to increase cortical excitability with minimal sensation.

eLife assessment

This **important** study introduces and evaluates the efficacy of a novel form of non-invasive brain stimulation in humans: kilohertz transcranial magnetic perturbation (kTMP). The evidence provided for the ability of kTMP to increase cortical excitability with minimal sensation is **compelling**, with two separate replication experiments. Although exploratory in nature, this work represents new avenues for non-invasive brain stimulation research that has potential long-term appeal for both clinical and research applications. This paper will be of significant interest to neuroscientists interested in brain stimulation.

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Introduction

Electromagnetic non-invasive brain stimulation (NIBS) refers to a group of methods which perturb brain function by coupling an applied electric field (E-field) to neurons without the need to introduce electrodes into brain tissue. The NIBS E-field can safely manipulate neural excitability, providing neuroscientists with a powerful tool to advance our understanding of brain function. Evidence that NIBS can promote brain plasticity [1] has prompted clinicians to pursue NIBS interventions in the treatment of psychiatric and neurologic disorders [2–7].

The NIBS E-field amplitude can be categorized as subthreshold or suprathreshold. Suprathreshold fields are sufficient to elicit immediate action potentials in neurons initially at resting membrane potential. Subthreshold E-fields are insufficient to directly cause action potentials but are employed to alter the state of the targeted neurons on time scales ranging from immediate entrainment effects to plasticity effects that extend well past the stimulation epoch [8,9]. As such, subthreshold and suprathreshold methods have different experimental and clinical utilities.

Two broad categories of NIBS methods exist, delivering the E-field to the brain via injection of current through electrodes in contact with the scalp or magnetic induction from a coil placed over the scalp. In electrical methods, such as transcranial electrical stimulation (tES), the current and E-field can be constant as in transcranial direct current stimulation (tDCS) or time varying as in transcranial alternating current stimulation (tACS). Magnetic induction methods, such as transcranial magnetic stimulation (TMS), deliver a time-varying current to the coil, generating a changing magnetic field and consequently an induced E-field in superficial brain regions. TMS is delivered as a brief pulse (typically 200–300 μs) and is referred to as repetitive TMS (rTMS) when delivered as a train of pulses.

The E-fields of tES and TMS differ in important ways. First, for TMS the E-field amplitude is linearly proportional to the frequency of the current source, whereas the tES E-field amplitude is independent of this frequency. Second, the E-fields for the two methods exist in orthogonal subspaces [10]. As such, the E-fields between TMS and tES cannot be matched exactly; for example, the TMS E-field does not have a radial component. Thus, even when the E-fields for tES and TMS are similar in focality, they may target different populations of neurons.

Third, for spatially similar cortical E-fields, the scalp E-field amplitude for tES is much greater than the scalp E-field for TMS because a large fraction of tES current travels along the path of least resistance and is, thus, shunted across the scalp [9]. Although dependent on a number of factors, this property can be captured by the ratio of scalp to cortical E-field, which is

approximately 18 times larger with tES compared to TMS [10]. Thus, the scalp E-field amplitude ultimately places a severe constraint on the focality and amplitude of tES cortical E-fields due to scalp peripheral nerve stimulation and muscle stimulation. Estimates of the tES cortical E-fields in the physiological frequency range suggest that the maximum for most human participants is around 0.5 V/m [11,12]; beyond this value scalp stimulation becomes detectable and soon intolerable. TMS systems are far less burdened by constraints imposed by the scalp E-field amplitude, allowing the method to be used to produce both subthreshold and suprathreshold cortical E-fields.

Motivated by these considerations, we developed a new magnetic induction NIBS approach, in which the subthreshold modulation of neural excitability is brought about via oscillating magnetic fields at kHz frequencies. We refer to this system as kilohertz transcranial magnetic perturbation (kTMP). In our new method, we drive a TMS coil using a high-current large-bandwidth amplifier operating in the kHz range. In this way, the E-field magnitude constraints imposed by scalp stimulation associated with tES are greatly diminished (**Fig 1**). The energetic requirements for a magnetic induction system would be prohibitive at low frequencies such as those measured with EEG. However, this cost is reduced to a manageable level by operating at higher frequencies. While the prototype kTMP system used in the present experiments produced cortical E-fields of 2 V/m at 2 – 5 kHz, kTMP has the potential to significantly increase the range of subthreshold E-field induction with the focality typical of TMS systems. In addition, the kTMP waveform can be amplitude modulated at frequencies matching endogenous neural rhythms [13].

Since neurons near resting membrane potential act as low-pass filters, a critical question centers on whether narrow band kHz E-fields can effectively couple to the transmembrane potential to influence neural activity. This question has been explored in invasive and non-invasive empirical studies (reviewed in [14]) and in model simulations [15]. Experimentally, suprathreshold kHz fields have been used to produce nerve blocking [16] and temporal interference effects [17]. Converging evidence also indicates that subthreshold kHz fields are effective in modulating neural excitability. In particular, kHz tACS can induce an increase in post-stimulation motor-evoked potentials approximately equal to that observed following standard, low frequency tES or rTMS [18]. Intriguingly, low frequency magnetic stimulation (LFMS), a kHz method that mimics the magnetic field waveforms used in MRI, applies weak kHz E-fields across most of the brain and has been reported to have mood-altering effects [19–22]. In addition, kHz E-fields, again applied by MRI gradients, have been shown to alter brain glucose metabolism in a manner that scales with the field amplitude [23].

We report here the results of three experiments with healthy human participants that evaluate the efficacy of kTMP in modulating cortical excitability. Adopting a conventional approach for evaluating the efficacy of NIBS methods [24–27], we used suprathreshold TMS to measure motor evoked potentials (MEPs) elicited in a hand muscle, comparing the amplitude of the MEPs before and after kTMP stimulation. For all the experiments, the kTMP amplitude was set to a produce a peak cortical E-field amplitude of approximately 2.0 V/m at the targeted primary motor cortex. In Experiment 1, we tested nonamplitude modulated kTMP at three different carrier frequencies (2 kHz, 3.5 kHz, and 5 kHz), comparing these conditions to a sham condition (0.01 V/m at 3.5 kHz). In Experiment 2 and 3, the carrier frequency was fixed at 3.5 kHz and we investigated two forms of amplitude-modulated kTMP (AM kTMP) with modulation frequencies of either 20 Hz or 140 Hz.

Methods

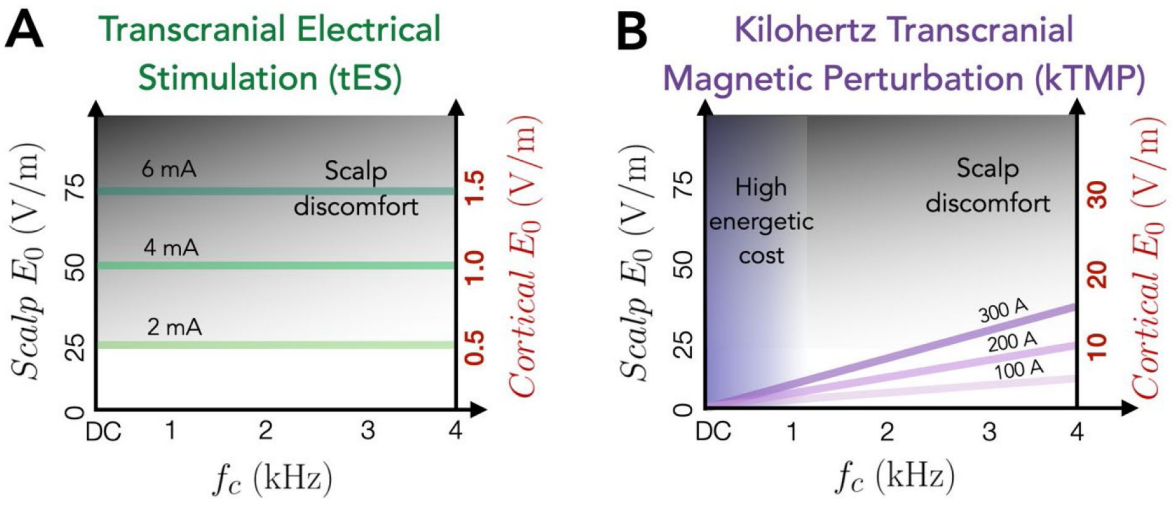


Figure 1.

An illustration of the practical constraints of tES and kTMP in frequency and amplitude space.

Solid lines represent the dependency of the E-field amplitude upon frequency and amplitude of the electric current supplied to the electrodes (A) or coil (B). Calculations are based on for a typical 5 × 7 cm tES electrode montage or figure-of-eight TMS/kTMP coil. Left vertical axis represents estimated scalp E-field amplitude while the right vertical axis represents the estimated motor cortex E-field amplitude. Shaded zones represent regions of the perturbation space constrained by practical considerations. Both methods are constrained by the scalp E-field magnitude, which at high values may result in discomfort due to peripheral nerve stimulation (gray shading). Note the substantial difference in the cortical E-field range that can be delivered tolerably for tES and kTMP. Illustrated here are approximate levels of discomfort for sustained waveforms (tES and kTMP) as opposed to pulsed methods (e.g., TMS). Note that magnetic induction methods such as kTMP (or TMS) are additionally constrained by high energetic costs (purple shading) required to generate E-fields of sufficient magnitude to influence neuronal states at low frequencies.

Apparatus

The **kilohertz transcranial magnetic perturbation (kTMP)** system consists of a highamplitude current source, a TMS coil, and a control system. The same TMS coil may be connected to either the kTMP current source or to a commercial TMS pulse generator (MagVenture MagPro R30 with MagOption), permitting interleaved kTMP-TMS experiments and ensuring identical kTMP and TMS E-field distributions up to an amplitude scaling factor. The kTMP amplifier (AE Techron Model 7794) is a voltage-controlled current source capable of delivering up to 200 A to the coil. We used an actively-liquid-cooled figure-of-eight coil (MagVenture Cool-B65; inner and outer loop diameter of 35 mm and 75 mm, respectively).

The kTMP control system consists of a personal computer (PC), input/output PCI card, and a custom interface to read the coil's built-in temperature sensor. Using a data acquisition toolbox (Mathworks R2018a), the PCI card was programmed to deliver analog input to the amplifier, thus specifying the temporal waveform of the E-field. The input waveform can either be at a fixed-amplitude sinusoidal frequency (non-modulated) or amplitude modulated.

Bench testing indicated that the system when running in kTMP mode did not produce marked changes in coil temperature. As an added safeguard, the PCI card was set up to receive an analog input from the coil's temperature sensor and create an automatic shutdown if the coil temperature exceeded 41° C, which is within the guidelines established by the International Electrotechnical Commission (IEC). In practice, for E-fields up to 2 V/m used in the present experiments, the coil temperature never rose above 32° C during system operation.

Participants

Forty-nine young adults were recruited through various advertisements posted to the Berkeley community. The number of participants in Experiments 1, 2, and 3 was 16 (12 female), 16 (10 female), and 15 (11 female), respectively. Seven of the participants in Exp 2 had also been tested in Exp 1 (minimum 3 weeks between the last session of Exp 1 and first session of Exp 2). All participants were naive to the purpose of the study, provided informed consent, and were financially compensated. Given the novelty of kTMP as a NIBS method, the IRB at UC Berkeley enlisted an outside expert and members of the campus Environmental and Health Safety Committee to evaluate the system. Following their reports, the protocol was approved by the IRB at UC Berkeley with the kTMP system deemed a non-significant risk device for E-fields up to 8 V/m for up to 20 min of stimulation.

Procedure

To evaluate the kTMP system as a new tool to modulate neuronal excitability, we measured the impact of kTMP on corticospinal excitability using suprathreshold TMS stimulation over motor cortex. **Figure 2** depicts an overview of the experimental hardware and protocol. In brief, kTMP stimulation was preceded by two 4–7 min probe blocks and followed by three such blocks. In Exps 1 and 2, each block consisted of single-pulse TMS and two paired-pulse protocols, short intracortical inhibition and intracortical facilitation. In Exp 3, only the single-pulse TMS protocol was employed (see below for details).

Each experiment consisted of five 2-hr test sessions, with each session separated by a minimum of 2 days. The first test session was used to determine the optimal coil position ("hotspot") and threshold intensity for eliciting MEPs with suprathreshold single-pulse TMS (see below). The position of the hotspot was recorded by a neuronavigation system (Brainsight, Rogue Research, Montreal, Canada). This allowed the experimenter to return to the same position for each TMS block (see below), as well as during the application of kTMP. The other four sessions were used to

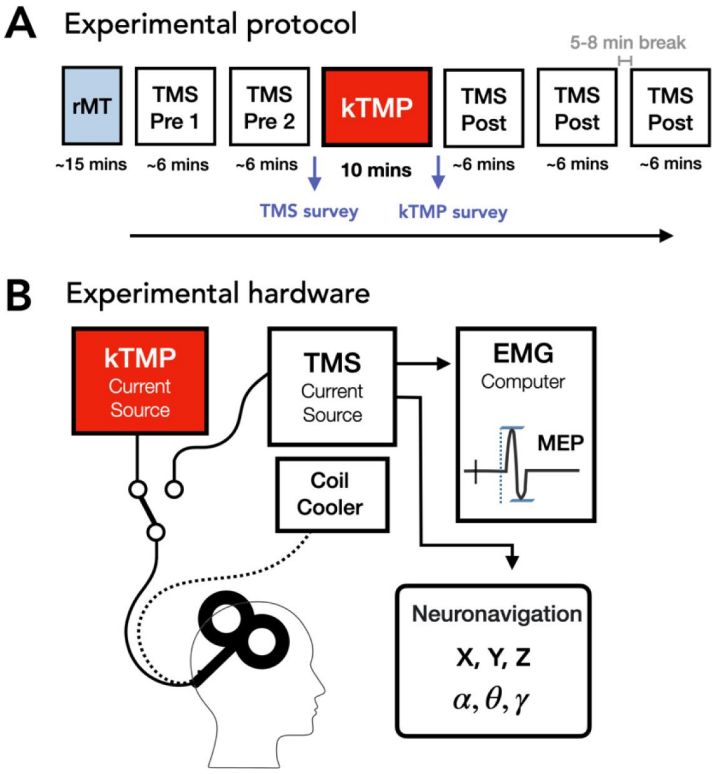


Figure 2.

Experimental protocol and hardware.

A. Timing of each experimental session. After determining the participant's resting motor threshold (rMT), TMS assessment blocks were conducted before (Pre) and after (Post) kTMP stimulation (active or sham). For Exps 1 and 2 each TMS block assessed singlepulse, SICI and ICF; only the single-pulse protocol was used in Exp 3. **B.** The same coil was driven by either the TMS current source or the kTMP current source. TMS pulses were recorded in an auxiliary channel of the EMG and triggered the neuronavigation system to record the coordinates of the coil in 3-D space.

test the efficacy of different kTMP parameters on cortical excitability, with a focus on variation of the carrier frequency for non-modulated kTMP in Exp 1 and both non-modulated and amplitude-modulated kTMP in Exp 2 and Exp 3.

Two steps were taken to create a double-blinding protocol. First, we created a coding system such that the experimenter typed in a number that was paired to the desired stimulation condition in an arbitrary and random manner, one that varied across participants in a manner unknown to the experimenter. Second, we played a tone at 3.5 kHz using the Tone Generator Application Sonic^{TM} (VonBruno 2015) to create a constant background sound during kTMP stimulation in all conditions, including sham, effectively masking the amplifier sound.

kTMP

For each carrier frequency, the current amplitude was selected to achieve a peak E-field amplitude of 2.0 V/m at a distance of 14 mm perpendicular to the coil surface, a distance we took to represent the approximate depth of the motor cortex from the overlying scalp [28]. The current amplitude needed to obtain the required E-field amplitude is estimated as follows. The E-field amplitude, E_0 (units of V/m), is proportional to the frequency, f_c (units of Hz), and amplitude I (units of A) of the current source according to:

$$E_0 = kf_cI$$

For the MagVenture Cool-B65 coil used in the kTMP system E_0 = 185 V/m when f_c = 3570 Hz (280 µs biphasic TMS pulse) and I = 6950 A [29,30]. From these reference values we estimate k:

$$k = 7.875 \times 10^{-6} \frac{\text{Vs}}{\text{Am}}$$

where V, s, A, and m, correspond to volts, seconds, amperes, and meters, respectively. We verified the accuracy of our estimates within a 5% error using E-field measurements obtained from a triangular probe following the method of Nieminen [31].

Experiment 1. Non-Modulated kTMP

We used a within-subject design, testing each participant on each of four kTMP stimulation conditions, with the order counter-balanced across participants. For three of the conditions, the carrier frequency (2 kHz, 3.5 kHz, 5 kHz) was paired with an intensity to create an E-field at the superficial aspect of the hand area of the motor cortex of E_0 = 2 V/m. We set the non-modulated E-field to be a sinewave with frequency f_c (see **Fig 3**):

$$E_{NM}(t) = E_o \cos(2\pi f_c t)$$

Note that we did not adjust for individual differences in scalp-to-cortex distance. For the sham condition, we used a 3.5 kHz carrier frequency producing a 0.01 V/m E-field at the approximate distance of the cortical surface.

Experiments 2 and 3. Amplitude-Modulated kTMP (AM kTMP)

For Exps 2 and 3 we again used a within-subject design, testing each participant in four sessions. Two of the conditions were repeated from Exp 1: the 3.5 kHz non-modulated kTMP condition at 2 V/m and the sham condition. For the other two conditions, the carrier frequency was set at 3.5 kHz and the waveform was amplitude modulated (AM) at modulation frequencies of either 20 Hz or 140 Hz. The 3.5 kHz carrier frequency was chosen since we had obtained the largest effect size compared to sham at this frequency in Exp 1. We selected the 20 Hz modulation frequency given the relevance of beta to motor function [32–34] and the 140 Hz modulation frequency based on literature concerning ripple effects at this frequency [35–37]. The peak cortical E-field

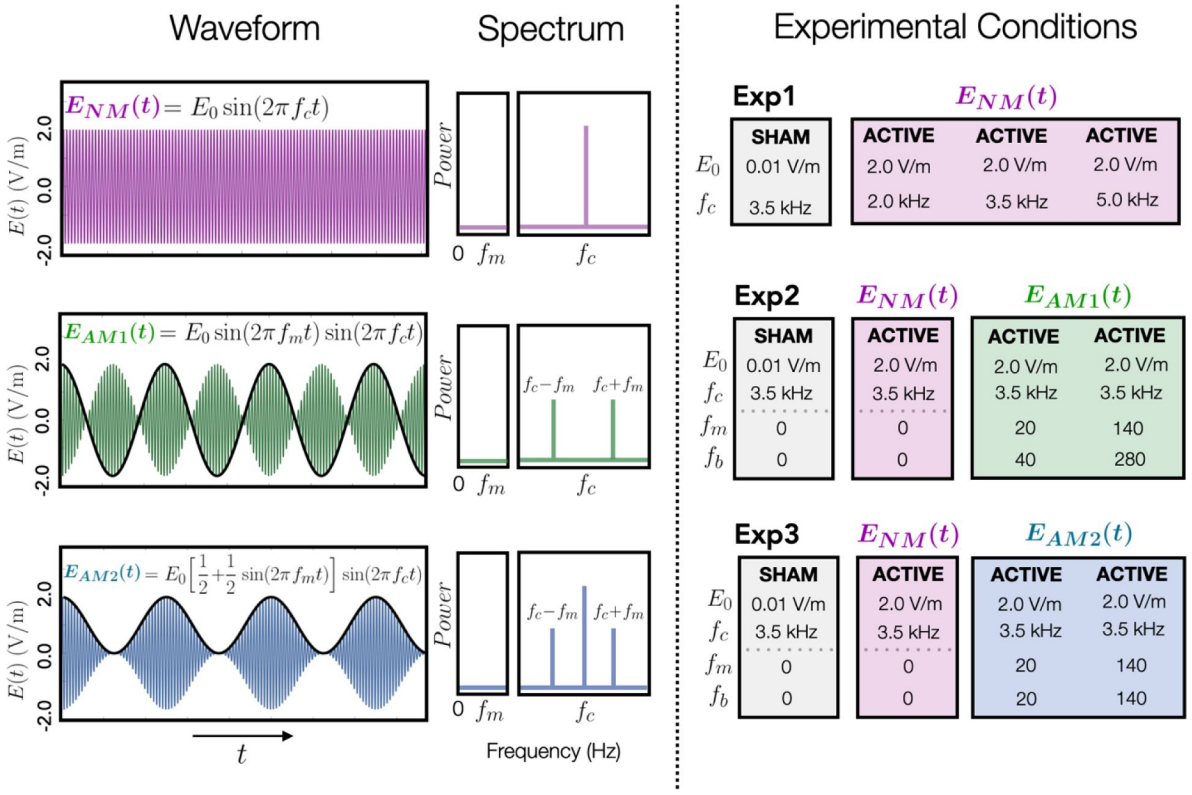


Figure 3.

Waveforms, spectra and conditions for the three kTMP experiments.

A commonly used form of amplitude modulation is $E(t) = E_0[a + m \sin(2\pi f_m t)] \sin(2\pi f_c t)$. The constants f_c, f_m, and E_0 refer to the carrier frequency, modulation frequency and the E-field amplitude (cortical E-field) respectively. Left column, Waveforms: E_{NM}, E_{AM1} and E_{AM2} refer to the non-modulated waveform and the two forms of amplitude modulation tested. Black lines indicate the modulation frequency. Center column, Spectrum: Carrier frequency and sidebands for the corresponding waveforms. Note the absence of power at the modulated frequency (f_m). Right column, Waveform parameters and characteristics for each experiment. f_b refers to the burst repetition frequency.

amplitude for the AM conditions was 2 V/m, identical to the non-modulated condition. Note that the inclusion of the 3.5 kHz non-modulated condition and sham for both experiments provide two replications of these conditions from Exp 1 as well as points of comparison for the AM kTMP conditions.

Exps 2 and 3 differed in the form used for amplitude modulation. In general, an amplitude modulated current can be written as

$$E(t) = A(t)\sin(2\pi f_c t).$$

where $A(t)$ is a time dependent amplitude modulation factor. A popular form used in communications systems is:

$$A(t) = E_o[a + mf(t)]$$

where $f(t)$ is the signal one wishes to convey between two components of a system and the ratio m/a is the modulation index. The modulation index is typically chosen to suit the proposed means of demodulation.

In *Experiment 2*, we used $f(t) = \sin(2\pi f_m t)$ and an infinite modulation index. Correspondingly the E-field has time dependence of the form:

$$E_{AM1}(t) = E_o \sin(2\pi f_m t)\sin(2\pi f_c t)$$

where f_m is the modulation frequency. In Experiment 3, we used $f(t) = \sin(2\pi f_m t)$ and a modulation index of one (see **Fig 3**).

$$E_{AM2}(t) = E_o\left[\frac{1}{2} + \frac{1}{2}\sin(2\pi f_m t)\right]\sin(2\pi f_c t).$$

Although the same modulation frequencies are used to calculate E_{AM1} and E_{AM2}they differ with respect to the spectrum of their upper envelope, the frequency of which we refer to as the burst repetition frequency (f_b). For E_{AM1} the burst repetition frequency is double that of the modulation frequency, whereas for $E_{AM2}$the burst repetition frequency and the modulation frequency are matched. The burst repetition frequency may be an important parameter in determining the neural effects of kTMP.

TMS: Hotspot and Threshold Procedure (Session 1)

Single-pulse TMS was applied over left hemisphere primary motor cortex to determine the resting motor threshold (rMT) for the first dorsal interosseous muscle (FDI) in the right hand. We focused on FDI since it is relatively easy to isolate in all individuals and threshold values are stable across test sessions [e.g., 38–40].

The TMS coil was placed tangentially on the scalp with the handle pointing backward and laterally at 45° from the midline. TMS was administered with a biphasic pulse waveform with a posterior–anterior direction of the second, dominant phase of the induced current. The stimulator intensity was initially set to 30% of maximal stimulator output and single pulses were generated at 5 s intervals, with the experimenter visually monitoring the electromyography (EMG) output for MEPs. If no MEPs were detected after 2 or 3 pulses, the experimenter moved the coil a few mm. If a search over the candidate area failed to produce any MEPs, the stimulator output was increased (step size of 3%), with the location search repeated. Once MEPs were detected, a more focal search was conducted to identify the optimal location for eliciting MEPs. This location was registered in three-dimensional space relative to the subject's head using the Brainsight neuronavigation

system to ensure consistent coil position during and between experimental sessions. rMT was defined as the minimum TMS intensity required to evoke MEPs of at least 50 μV peak-topeak amplitude on 5 of 10 consecutive trials.

The mean threshold was 58% (SD = 11.3%), 63% (SD = 11.1%) and 56% (SD = 9.2%) of maximum stimulator output in Experiments 1, 2 and 3 respectively. We repeated the threshold procedure in each of the kTMP sessions to capture possible intra-individual baseline changes in the cortico-excitability of the participants. In practice, the individual's threshold values remained very stable across days (SD = 2.4%).

TMS Assays of Corticospinal Excitability (Sessions 2-5)

For Exps 1 and 2, each of the five probe blocks (two pre-kTMP and three post-kTMP) included single-pulse TMS (SP), and two paired-pulse protocols: short intracortical inhibition (SICI) and intracortical facilitation (ICF) [41,42]. Similar to SP, paired pulse protocols were administered with biphasic pulse waveforms with a posterior–anterior direction of the second, dominant phase of the induced current. These three protocols have been widely used in prior studies designed to evaluate the efficacy of tES and rTMS methods in altering neural excitability [43–45]. For SP, the stimulation level was set at 120% of rMT. For the paired-pulse assays, the suprathreshold pulse was preceded by a subthreshold conditioning stimulus set at 80% of rMT, with an interstimulus interval (ISI) of 3 ms or 10 ms for SICI and ICF, respectively. The probe block consisted of 90 trials, 30 for each of the three assays, with the order randomized within the block. For Exp 3, the five probe blocks consisted only of the single-pulse TMS assay. We made this decision after finding that kTMP had no effect on SICI or ICF in Exps 1 and 2. We lengthened the breaks between blocks in Exp 3 to match the timing of the three post blocks relative to kTMP stimulation in the first two experiments.

We developed a system to read and record the spatial and angular position of the coil with respect to the hotspot in real time from Brainsight. This information was recorded at the time of each TMS pulse and used to exclude trials in which the coil was distant from the hotspot or the angle had changed from the optimal hotspot orientation.

EMG

EMG activity was recorded (Bagnoli-8 EMG System, Delsys Inc.) from surface electrodes placed over the right FDI muscles, with a reference electrode over the right elbow. The experimenter visually inspected the EMG traces on a monitor to ensure that the participant remained relaxed (i.e., negligible EMG background activity in FDI), to detect the presence or absence of MEPs in response to the TMS pulses, and, since kTMP is a novel brain stimulation modality, to monitor for safety by checking for after discharges or other features suggesting excessive increase in excitability that could evolve into a seizure.

The EMG signal was amplified and bandpass filtered on-line between 20 and 450 Hz. The signals were digitized at 2000 Hz for offline analysis. All EMG measures were analyzed with custom scripts written in Matlab 2018a. EMG was recorded continuously during the experiment. Offline, data were segmented based on a TTL pulse from the TMS system recorded by the EMG amplifier on an auxiliary channel.

Subjective Reports

We informally assessed the participants' subjective experience in Exp 1, focusing on reports concerning the perception of sound emitted from the system, tactile sensation, and discomfort. This process was formalized in Exps 2 and 3. In these studies, we administered a short survey in which participants answered three questions concerning 1) annoyance, 2) pain, and 3) subjective experience of muscle activation/movement. Participants responded to each question using a

keyboard to type in a number using an 11-point scale (0 = not at all; 10 = extremely). This survey was based on a systematic rating system that has been employed to characterize the degree of disturbance caused by TMS [46]. We administered the survey twice. The first time was after the second TMS probe block, providing ratings on the subjective experience of suprathreshold TMS. The second time was after kTMP, providing ratings on the subjective experience of kTMP stimulation.

Data Analysis

MEP Data

For each trial, the peak-to-peak amplitude of the MEP was calculated over a window 15– 45 ms after the suprathreshold TMS pulse. Trials were excluded from the analysis based on the following criteria: 1) If the MEP amplitude was 2.5 standard deviations above or below the mean, with the mean and standard deviation calculated separately for each TMS assay (SP, ICF, SICI) for each probe block. 2) If the Brainsight recording indicated that the coil was more than 3 mm (Euclidian distance) from the optimal hotspot location or had an angular or twist error more than 5° from the optimal trajectory angle. 3) If noise in the EMG signal 100 ms before the TMS pulse exceeded 2.5 standard deviations of the mean EMG signal. On average 10% (SD = 3%) of the trials were excluded per participant with a range of 4.8% to 20%. After cleaning the MEP data, there were a minimum of 20 MEP measures per protocol in each assessment block for each individual, a sufficient number for performing the MEP analyses [44,47–49].

Raw MEP amplitudes were log-transformed to normalize the distribution of MEP amplitudes [50–53]. The average log-transformed MEP amplitude was then calculated for each of the three TMS protocols in each probe block on an individual basis. After averages were calculated, the data were exponentiated to get the MEPs back to an easily interpreted scale (i.e., mV). SICI and ICF measures were calculated by computing a ratio of the paired-pulse MEP average over the single pulse MEP average for each block. For all three TMS assays the effect of kTMP stimulation was operationalized as the average percent change post kTMP relative to the two baseline blocks (averaged). For example, a value of 0% would indicate no change in single-pulse MEP amplitude from preto poststimulation, whereas a value of 100% would indicate the single-pulse MEP amplitude doubled from preto post-stimulation. Thus, the main analysis focuses on the three postkTMP stimulation probe blocks for each of the three TMS assays (SP, SICI, ICF).

Missing Data

Although we aimed for a fully within-subject design, a subset of the participants only had data for three of the four sessions. Missing data were due to 1) technical issues with the Brainsight neuronavigation system (n = 2/47), 2) university suspension of testing with human participants in March 2020 due to the onset of the COVID-19 pandemic (n = 5/47), or 3) determination that the results from a session were a statistical outlier (n = 3/47). The latter three had an increase in corticospinal excitability three standard deviations above the mean in one condition. All of these were active kTMP conditions and, if included would have inflated the effect of kTMP on corticospinal excitability. To account for missing data and subject variability, all analyses used a linear mixed-effects model with a random factor of participant.

Linear Mixed Effects Models

Linear mixed effects models were implemented in RStudio using the software package lme4 [54]. Each mixed effects model used Participant as a random effect, Experiment as a fixed effect, and experimental variables (e.g., active vs. sham, post-stimulation block) as fixed effects. Likelihood ratio tests were used to obtain *p*-values in evaluating experimental fixed effects and interaction effects. Cohen's *d* was calculated based on the methods outlined in Brysbaert & Stevens

[55] for mixed effects models. Cohen's d can be interpreted as a standardized mean difference between conditions; according to convention, $d \cong 0.2$ is considered a small effect, $d \cong 0.5$ is considered a medium effect and $d > 0.8$ considered a large effect [56].

Results

Across the three experiments, participants found kTMP stimulation to be tolerable and, indeed, as described below, essentially imperceptible. No adverse events occurred, and no discharges were observed in the EMG after kTMP stimulation.

In each experiment, five TMS probe blocks were sandwiched around active or sham kTMP stimulation (**Fig 2A**). We pooled the data across the two pre-kTMP probe blocks to establish a baseline measure. The post stimulation blocks assessed changes in neural excitability at three time points after kTMP stimulation, operationalized as average percent change post kTMP relative to baseline.

Single Pulse Assay

Repeated kTMP Conditions (3.5 kHz and Sham) across Experiments

In Exp 1, three carrier frequencies of kTMP stimulation were tested (2 kHz, 3.5 kHz and 5 kHz) along with a sham kTMP condition (0.01 V/m, 3.5 kHz). Exps 2 and 3 included the same non-modulated 3.5 kHz condition (and sham), providing an opportunity for evaluating reproducibility and a reference point for assessing the effect of AM kTMP (see below). We first tested if the change in corticospinal excitability for the 3.5 kHz condition varied across the three experiments. Using a mixed effect model with fixed factor of Experiment and random effect of Participant we found no difference across the three experiments for the non-modulated 3.5 kHz condition [$\chi^2(2) = 0.41$ $p = 0.813$]. Similarly, we found no difference across experiments for the sham condition [$\chi^2(2) = 2.77$ $p = 0.251$; **Fig 4A**]. **Figure 4B** displays change in MEP amplitude across the three post kTMP blocks for sham and the 3.5 kHz condition for the three experiments. Based on these results, we combined the data across the three experiments for these two conditions in subsequent analyses. Note that, although the factor of Experiment was not significant, we include Experiment as a fixed factor in subsequent models that include data from the three experiments.

Non-Modulated kTMP Increases Cortical Excitability

We next compared sham vs. active non-modulated kTMP (2 kHz, 3.5 kHz and 5 kHz) and found that active kTMP produced a significant increase in corticospinal excitability [$\chi^2(1) = 23.83$ $p < 0.001$; $d = 0.51$]. Pairwise comparisons of each active condition to sham showed that an increase was observed following both 2 kHz [$\chi^2(1) = 6.90$, $p = 0.009$; $d = 0.49$] and 3.5 kHz kTMP [$\chi^2(1) = 37.57$, $p < 0.001$; $d = 0.70$; **Fig 5**: Non-Modulated conditions]. The 5 kHz condition failed to reach significance [$\chi^2(1) = 1.43$, $p = 0.232$; $d = 0.21$]. A comparison of the three active conditions showed that the effect on MEP amplitude was influenced by carrier frequency [$\chi^2(1) = 9.60$ $p = 0.008$]. Specifically, the 3.5 kHz condition produced a larger increase in MEP amplitude compared to the 5 kHz condition [$\chi^2(1) = 8.64$ $p = 0.003$; $d = 0.50$]. No difference was observed between the 2 kHz and 3.5 kHz conditions [$\chi^2(1) = 3.25$ $p = 0.071$; $d = 0.30$] or between the 2 kHz and 5 kHz conditions [$\chi^2(1) = 2.44$ $p = 0.118$; $d = 0.28$].

The effect of active non-modulated kTMP stimulation persisted across 36 min of the poststimulation probe blocks. There was no significant effect of Time (i.e., Post Block) on MEP amplitude [$\chi^2(2) = 5.778$ $p = 0.056$] nor was there a significant interaction between Time and

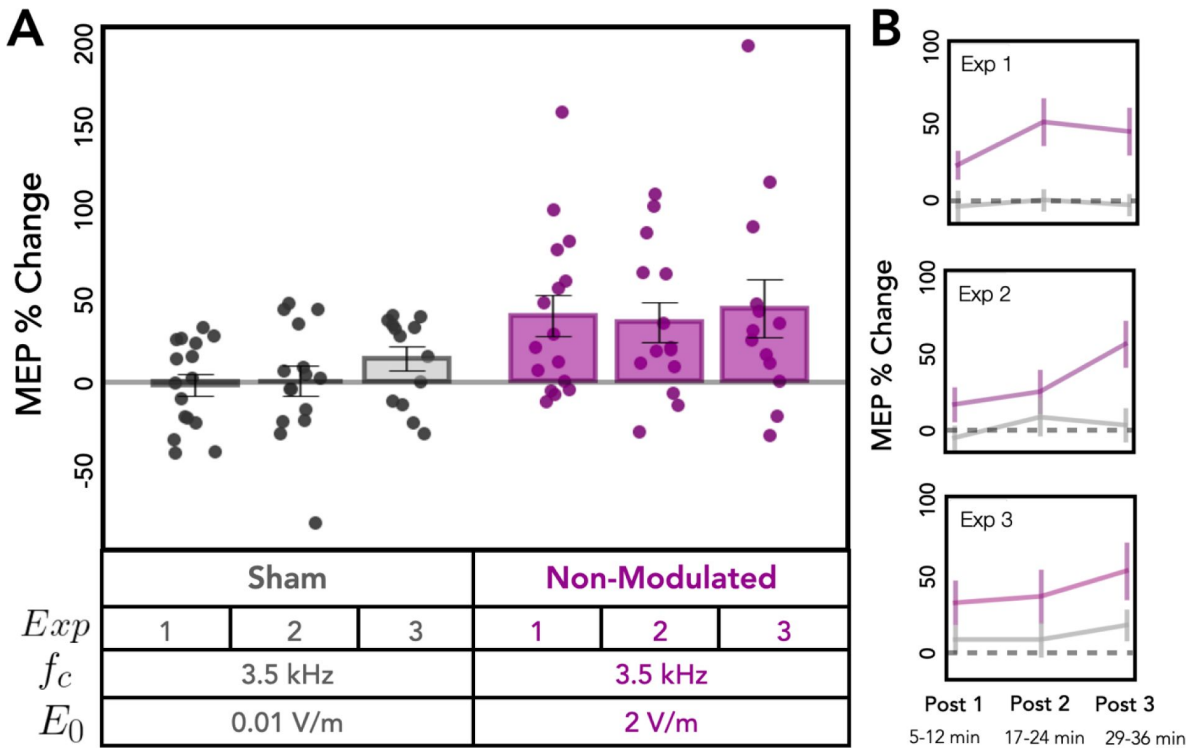
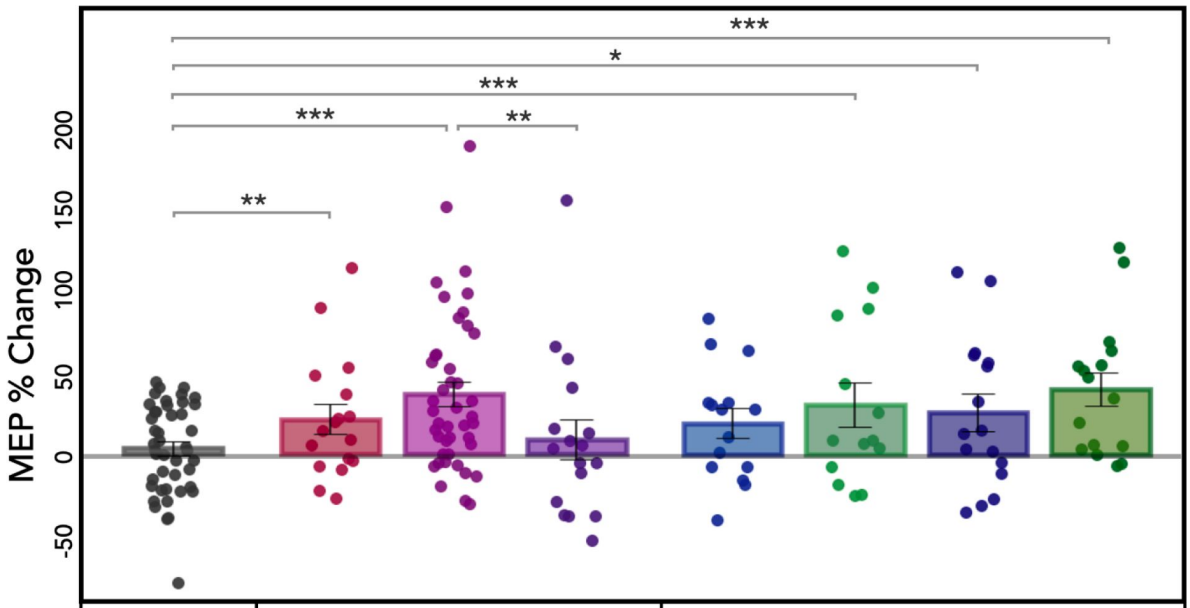


Figure 4.

Replication of post-stimulation change in cortical excitability for sham and non-modulated 3.5 kHz kTMP stimulation.

A. Change in MEP amplitude measured with single pulse TMS following sham stimulation (left) and kTMP stimulation at 3.5 kHz (right). Dots denote values for individual subjects, bars— mean values, and whiskers—standard error. MEP change post-intervention did not significantly differ across experiments for sham and 3.5 kHz. **B**. Change in MEP amplitude for the three post kTMP blocks for the 3.5 kHz condition (mean ± standard error).



	Sham	Non-Modulated			Amplitude-Modulated			
Exp	1,2,3	1	1,2,3	1	3	2	3	2
f_c	3.5 kHz	2 kHz	3.5 kHz	5 kHz	3.5 kHz			
f_m	0	0	0	0	20	20	140	140
f_b	0	0	0	0	20	40	140	280

Figure 5.

Post-stimulation changes in cortical excitability as measured by single-pulse TMS for all conditions.

Percent change in MEP amplitude following sham and active kHz stimulation, relative to baseline. Dots denote values for individual subjects, bars—mean values, and whiskers—standard error. Note that the data for the Sham and non-modulated 3.5 kHz conditions are combined across the three experiments. * $p < .05$, ** $p < .01$, *** $p < .001$.

Stimulation Condition for non-modulated kTMP [$\chi^2(6) = 109.0696$ $p = 0.1226$; **Fig 6**: Non-Modulated conditions].

Amplitude-Modulated kTMP Produces Similar Increase in Cortical Excitability

We tested the effect of AM kTMP in Exps 2 and 3, with the two experiments using different forms of amplitude modulation (E_{AM1} and E_{AM2}; see **Fig 3**). For these experiments, we used a carrier frequency of 3.5 kHz given that this condition had produced the largest effect in Exp 1.

Similar to what we observed from the single-pulse probe for non-modulated 3.5 kHz stimulation, AM kTMP resulted in an increase in MEP amplitude compared to sham [$\chi^2(1) = 23.59$ $p < 0.001$; $d = 0.54$; **Fig 5**, AM conditions]. Pairwise comparisons showed that this effect was observed in three of the four AM conditions: E_{AM1} [$f_m = 20$, $f_b = 40$; $\chi^2(1) = 13.72$ $p < 0.001$; $d = 0.74$], E_{AM2} [$f_m = 140$, $f_b = 140$;$\chi^2(1) = 5.61$ $p = 0.018$; $d = 0.62$] and E_{AM1} [$f_m = 140$, $f_b = 280$; $\chi^2(1) = 22.69$ $p < 0.001$; $d = 1.00$]. The E_{AM2}con-dition [$f_m = 20$, $f_b = 20$] showed a numeric increase, but this comparison was not significant [$\chi^2(1) = 2.75$ $p = 0.097$; $d = 0.427$].

Limiting our analysis to the four active AM conditions, we found that MEP amplitude was not dependent on modulating frequency [f_m: $\chi^2(1) = 2.33$ $p = 0.127$] or burst repetition frequency [f_b : $\chi^2(3) = 3.92$ $p = 0.27$]. We found an effect of Time (i.e., Post Block) on MEP amplitude across all AM conditions [$\chi^2(2) = 7.45$ p = 0.024], but no significant interaction between Post Block and Stimulation condition [$\chi^2(6) = 3.14$ $p = 0.791$]. Pairwise comparisons showed that MEP amplitude was greater in Post Block 3 compared to Post Block 1 [$\chi^2(1) = 7.75$ $p = 0.005$; **Fig 6**: AM conditions]. No other comparisons were significant [all χ^2's < 2.30, all p's > 0.129].

Our main interest in the AM conditions is to determine if amplitude modulation at physiologically relevant frequencies produces a change on cortical excitability beyond that produced by the kHz carrier frequency. To this end, we compared the effect of AM kTMP to non-modulated kTMP, with the latter limited to the 3.5 kHz condition (pooled data). Using a mixed effect model, this contrast was not significant [$\chi^2(1) = 0.05$ $p = 0.833$; $d = 0.03$]. Thus, the AM conditions provide another demonstration that kHz stimulation can increase cortical excitability. However, at least with a 2 V/m E-field, we did not observe an effect from the AM component that was above and beyond non-modulated kTMP.

Paired-Pulse Assays

We included two paired-pulse assays that provide probes of intracortical inhibition (SICI) or facilitation (ICF). To confirm that we observed an effect from the paired-pulse protocols, we focused on the pre-kTMP data, thus avoiding any influence from kTMP stimulation. We pooled the data across the two pre-kTMP blocks for each individual and performed an analysis on the data from the participants from Exps 1 and 2. This analysis showed a strong SICI effect, with a mean decrease of 36% on the paired-pulse trials relative to the single pulse trials [$t(125) = -24.47$, $p < .001$; **Fig 7**, top]. In addition, we saw a facilitatory effect for the ICF protocol with a mean increase of 22% on the paired pulse trials relative to the single pulse trials [$t(125) = 6.86$, $p < .001$; **Fig 7**, bottom].

No Effect of kTMP on Measures of Intracortical Inhibition or Facilitation

We then asked if the magnitude of the SICI effect was modulated by kTMP (**Fig 7**, top). Compared to sham, we observed no effect of active non-modulated kTMP [$\chi^2(1) = 0.81$, $p = 0.367$], no effect of Time (i.e., Post Block) [$\chi^2(2) = 0.53$, $p = 0.768$] and no interaction between these two factors [$\chi^2(6) = 5.47$, $p = 0.486$]. Similarly, there was no effect of active AM kTMP on SICI (relative to

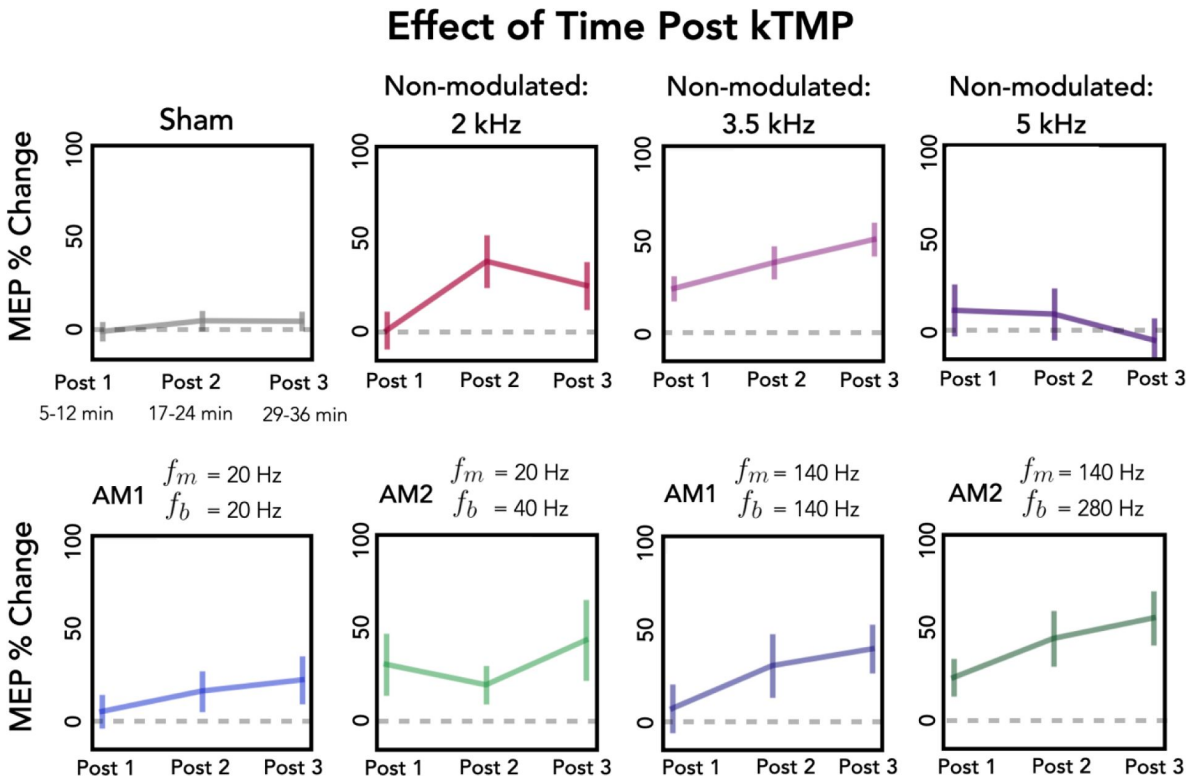
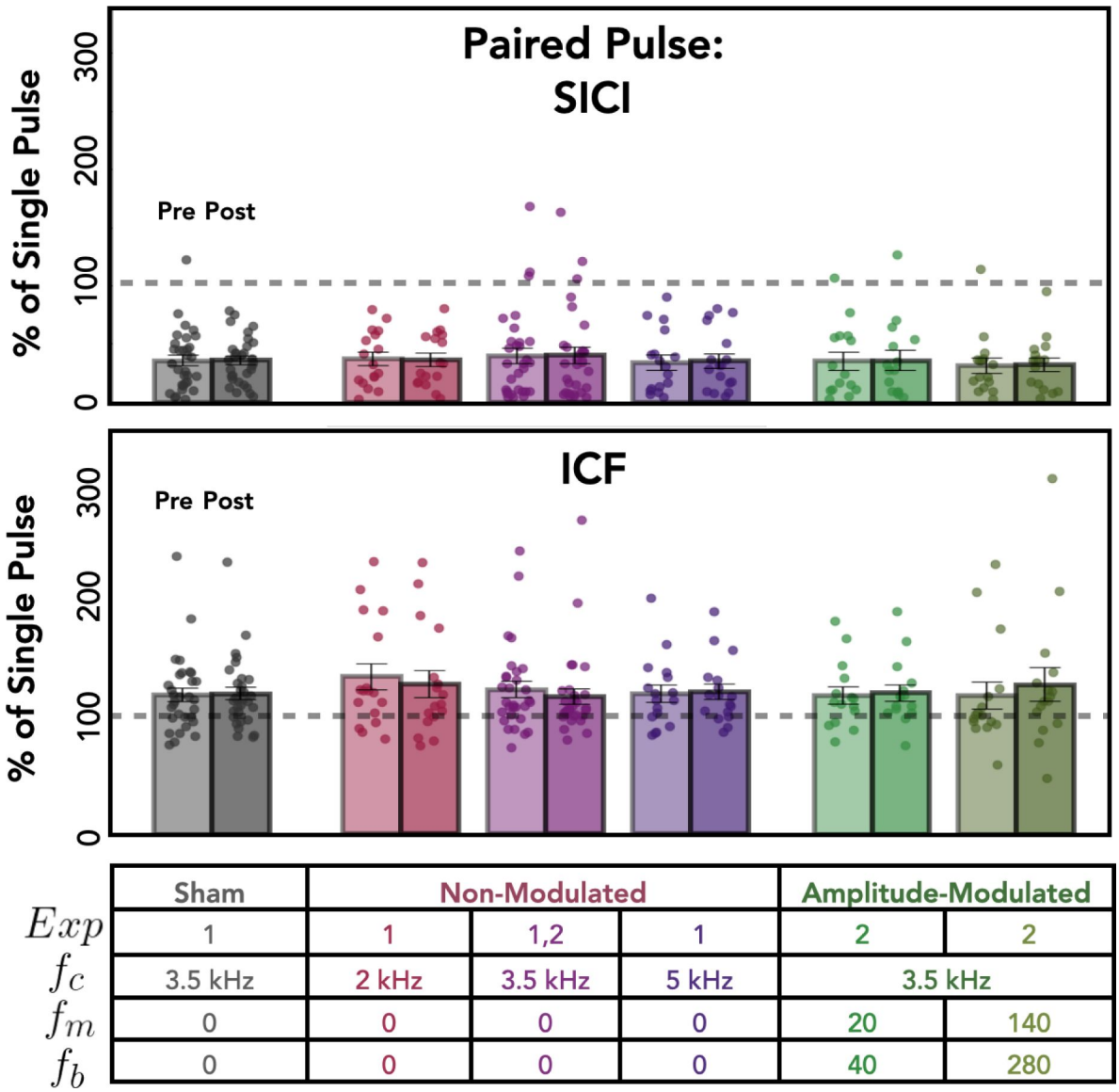


Figure 6.

Post-stimulation change in cortical excitability as measured by single-pulse TMS across the three post-kTMP blocks.

Percent change in MEP amplitude following sham and active kHz stimulation, relative to baseline for the three post blocks (error bars represent standard error). The data for the Sham and non-modulated 3.5 kHz conditions are combined across the three experiments.



	Sham	Non-Modulated			Amplitude-Modulated	
Exp	1	1	1,2	1	2	2
f_c	3.5 kHz	2 kHz	3.5 kHz	5 kHz	3.5 kHz	
f_m	0	0	0	0	20	140
f_b	0	0	0	0	40	280

Figure 7.

kTMP did not produce any change in measures of short intracortical inhibition or intracortical facilitation.

Data are plotted as the ratio of the paired-pulse MEP amplitude over the single pulse MEP amplitude, with an inter-pulse interval of 3 ms for SICI and 10 ms for ICF. Each pair of bars shows this ratio for pre-kTMP (averaged over two probe blocks) and post-kTMP (averaged over three probe blocks).

sham) [$\chi^2(1) = 0.56$, $p = 0.455$], no effect of Time [$\chi^2(2) = 0.39$, $p = 0.822$] and no interaction between the two factors [$\chi^2(4) = 4.17$, $p = 0.384$]. In sum, kTMP does not appear to influence the magnitude of intracortical inhibition measured by SICI.

As with SICI, there was no evidence that kTMP influenced ICF (**Fig 7**, bottom): We found no effect of active non-modulated kTMP compared to sham [$\chi^2(1) = 0.318$, $p = 0.572$], no effect of Time (i.e., Post Block) [$\chi^2(2) = 1.48$, $p = 0.478$], and no interaction between the two factors [$\chi^2(6) = 5.07$, $p = 0.534$]. Similarly, we found no effect of active AM kTMP compared to sham [$\chi^2(1) = 0.11$, $p = 0.745$], no effect of Time [$\chi^2(2) = 0.71$, $p = 0.702$] and no interaction between the two factors [$\chi^2(4) = 2.43$, $p = 0.657$].

In summary, kTMP, either non-modulated or amplitude modulated at 2 V/m, did not produce any measurable effect on the paired-pulse assays of intracortical inhibition or facilitation, SICI and ICF.

Subjective Experience during kTMP Stimulation

Informal observations from the participants in Exp 1 indicated that the coil did not produce any detectable tactile sensation. The amplifier produces a sound, but one that was effectively masked by playing a louder, pre-recorded sound (3.5 kHz tone) in all conditions. We opted to not ask participants to judge if they were in an active or sham condition. Given that each participant returned for multiple sessions, we did not want to alert them to the presence of a sham condition, preferring to simply describe the study as one testing a new method of non-invasive brain stimulation. As such, we focused on their subjective ratings. Participants provided three ratings using an 11-point scale (0 = not at all; 10 = extremely) in response to questions on annoyance, pain, and subjective experience of muscle activation/movement. **Figure 8** presents the data for each measure following TMS, active kTMP, and sham kTMP. In line with expectations for TMS [46], the participants were aware of muscle twitches, but the stimulation was well-tolerated in terms of annoyance and pain given that the coil was positioned over primary motor cortex.

Of primary interest for the present report, the modal rating was 0 for all three ratings following active kTMP (**Fig 8**). Using a mixed effects model, with a fixed factor of Stimulation type (i.e., active vs. sham) and Participant as a random effect, we found no difference between active and sham kTMP for Annoyance [$\chi^2(1) = 0.18$, $p = 0.672$], Pain [$\chi^2(1) = 0.29$, $p = 0.591$] and the subjective experience of Muscle Twitches [$\chi^2(1) = 0.01$, $p = 0.972$; **Fig 8**]. Some participants rated active and sham kTMP high in terms of annoyance. Based on open ended survey responses these scores appear to be related to general features of the experiment such as tension from the neuronavigation headband and the requirement to maintain a sitting posture for an extended period. In summary, the survey data and subjective reports suggest that kTMP is well suited for double-blind experimentation.

Discussion

In this paper, we present a new method, kTMP, for exploring the subthreshold NIBS experimental space. The kTMP E-field has the waveform flexibility of kilohertz tES along with the potential amplitude range and focality of TMS. Moreover, amplitude modulation of the kTMP E-field yields a waveform which could potentially introduce stimulation dynamics at frequencies matching endogenous neural rhythms (e.g., alpha, beta).

Summary of Experimental Findings and Limitations

Across three experiments, we assessed the ability of non-modulated and amplitude-modulated kTMP to alter cortical excitability, using suprathreshold TMS over motor cortex to elicit MEPs in a finger muscle. In Experiment 1, non-modulated kTMP with a targeted cortical E-field of 2 V/m,

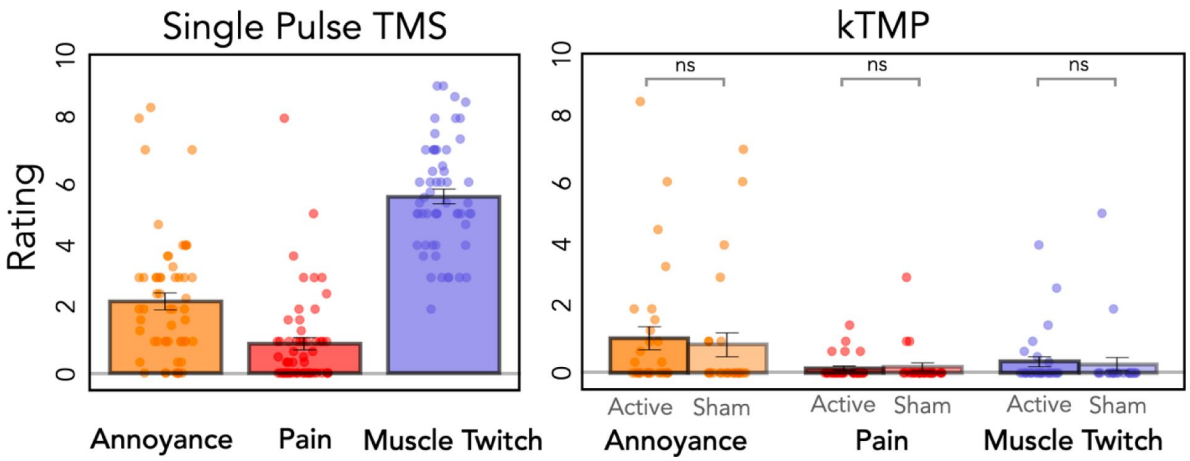


Figure 8.

kTMP stimulation is not associated with any subjective experience.

Mean ratings (SE in parenthesis) combined across Exp 2 and Exp 3 on a 0-to-10 scale in response to questions assessing annoyance, pain, and awareness of finger movement. For TMS, the survey was administered after the second baseline probe block; for kTMP, the survey was administered after kTMP stimulation.

produced an increase in corticospinal excitability at 2 and 3.5 kHz. The latter condition also resulted in a larger increase in MEP amplitude compared to stimulation at 5 kHz, suggesting a possible effect of carrier frequency. Experiments 2 and 3 provided replications of the efficacy of non-modulated kTMP at 3.5 kHz to increase corticospinal excitability. Importantly, participants experienced no tactile sensation or discomfort from the procedure. Indeed, the only percept of kTMP at 2.0 V/m is a tone at the carrier frequency emanating from the amplifier. This sound was masked in our experiments by a background tone played during active and sham kTMP stimulation.

Due to pandemic related university regulations, we were limited to a two-hour experimental session. As such, we were only able to assess the impact of kTMP on neural excitability in three probe blocks, spanning a 36 min post-stimulation epoch. We did not observe a reduction of the kTMP effect across this window. Future testing with extended post-stimulation assays will be required to establish the duration of the effect of kTMP on cortical excitability.

We also tested two forms of AM, using modulation frequencies of 20 Hz and 140 Hz. An increase in corticospinal excitability was observed in three of the four conditions, associated with high burst repetition frequencies. Critically, none of the AM kTMP conditions produced an increase above that observed with non-modulated 3.5 kHz kTMP, suggesting that at least for an E-field amplitude of 2 V/m, amplitude modulation at physiologicallyrelevant frequencies does not induce an additional lasting change in corticospinal excitability over that arising from the kHz carrier frequency alone.

Despite the absence of an effect from amplitude modulation, the present results are encouraging in that, across a range of conditions, we consistently observed an increase in cortical excitability in response to single-pulse TMS following the application of a 2 V/m E-field for 10 min. On average the increase in MEP amplitude following 3.5 kHz nonmodulated kTMP with a cortical E-field of 2 V/m, the condition in which we have the largest sample size, was 37%. This value is in the range observed from studies using a variety of NIBS methods and protocols [18,57,58].

Potential Mechanisms of kTMP

At present, we can only tentatively speculate about how kTMP modulates cortical excitability. As with other NIBS modalities, a mechanistic understanding would address (1) what cellular element is directly perturbed, (2) which cortical neurons are modulated, and (3) how modulation confers lasting cortical excitability. We discuss these questions below.

Regarding acute effects, suprathreshold TMS activates neurons by inducing a brief E-field pulse with dominant frequency component in the kilohertz range (2–6 kHz) [59], demonstrating that such frequencies can acutely affect neuronal polarization. While the slower time constants of the soma and dendrite membranes mostly filter out kilohertz frequencies, the faster time constants of the axonal membrane allow kilohertz waveforms to produce transient polarization in the axons, especially axonal (presynaptic) terminals [60–64]. Whereas suprathreshold TMS pulses induce action potentials in the axons, kTMP would only produce subthreshold perturbation of the axonal membrane potential. Thus, the effects of kTMP may be mediated by acute subthreshold polarization perturbation of presynaptic terminals.

Regarding the modulation of specific cortical circuits, the single-pulse and paired-pulse TMS measurements can provide some insights; it has to be recognized, however, that these results depend on both the intervention (kTMP) as well as the TMS probe characteristics. The shape of the TMS pulse and coil orientation impact how a suprathreshold TMS pulse elicits an MEP: The MEP can come about via direct excitation of the axons of the corticospinal neurons (D-wave), via indirect effects on neurons that provide synaptic input to corticospinal neurons (I-waves) or a combination of both. We applied the TMS probes using suprathreshold biphasic TMS pulses with a posterior–anterior dominant induced current. At the probing stimulation level (120% rMT), this

configuration has been shown to preferentially recruit I-waves [65]. Moreover, since kTMP used the same coil configuration, it can be inferred that the kTMP E-field coupled most strongly to the same neural elements as the TMS probing pulses. Assuming that we are unable to probe the impact of kTMP on D-waves, we can speculate that the plasticity induced by kTMP predominantly affects excitatory synaptic inputs to pyramidal tract neurons. This mechanistic hypothesis is similar to that proposed for intermittent theta burst stimulation (iTBS), another sub-threshold magnetic induction protocol that produces a temporally extended increase in single-pulse MEPs [66]. However, it will be important to employ alternative coil configurations in future research to assess if kTMP can modulate the excitability of D-waves, representing direct activation of the axons of pyramidal tract neurons.

We did not observe any effect of kTMP on the two paired-pulse protocols, SICI and ICF. They appear to probe, respectively, GABA-mediated inhibitory and glutamate-mediated excitatory cortical circuits [42]. Although we observed the classic signatures of SICI and ICF, kTMP did not modulate either of these measures of local intracortical neural dynamics. This pattern matches that observed with iTBS: A meta-analysis of iTBS studies failed to find consistent changes in SICI or ICF despite a consistent increase in single-pulse MEPs [44]. In contrast, a meta-analysis of anodal tDCS revealed an increase in singlepulse MEP amplitude, a decrease in SICI, and an increase in ICF [67]. Interestingly, tACS at 20 Hz also produced a similar pattern on all three measures in a phase-dependent manner during stimulation [68]. It is notable that kTMP and iTBS share the same spatial distribution of the E-field (determined by the TMS coil configuration), which is very different from that of the electrical stimulation with tDCS and tACS. Moreover, both TMS and kTMP involve stimuli in the kHz spectrum whereas tDCS and 20 Hz tACS use E-fields that vary on slower time scales. This variation can result in a different pattern of polarization across neural elements. The overlapping characteristics shared by kTMP and iTBS may account for the similarity in coupling to specific cortical circuits which differ from those affected by tDCS and low frequency tACS.

Finally, we can consider different hypotheses concerning how kHz stimulation produces changes in neuronal activity lasting beyond the stimulus train. One possibility is that the continuous acute perturbation of the presynaptic terminals interacts with the endogenous axonal signaling, for example via stochastic resonance, ultimately causing synaptic potentiation [69]. Another option is that the nonlinear properties of the neuronal membrane lead to temporal summation of successive kilohertz subthreshold cycles and, consequently, a facilitation of synaptic transmission [14]. The repeated perturbation of the neuronal membrane could also result in accumulation of calcium in presynaptic terminals, yielding synaptic plasticity effects [18]. Presently, these possibilities are speculative and need to be assessed by further research into the cellular mechanisms of subthreshold kilohertz neuromodulation.

Methodological Advantages of kTMP

A limiting factor for tES studies is that these methods are restricted to the very low end of subthreshold space in terms of E-field amplitude. Thus, there is a limited range over which one could obtain dose-dependent effects; indeed, efforts to establish dose-dependent response functions with NIBS protocols have provided mixed results [39,40]. In contrast, kTMP has the potential to open a large subthreshold experimental space, one that has considerable range in terms of E-field amplitude, carrier frequency, AM waveform, and stimulation duration. We should be able to achieve at least a 4-fold increase in the kTMP cortical E-field amplitude (e.g., up to 8 V/m in the kHz range) [10,15] without uncomfortable scalp stimulation. This expanded parameter space, especially in terms of E-field amplitude should prove beneficial in deriving dose-dependent response functions. Theoretically, kTMP could reach suprathreshold E-field amplitudes, although moving into that space will require careful assessment of issues related to power requirements, coil heating, acoustic noise, and participant safety.

In addition to offering a large subthreshold experimental space, there are other noteworthy features of kTMP. First, kTMP is ideally suited for double-blind experimentation. As verified in the subjective reports of our participants, the only percept associated with 2.0 V/m kTMP is a tone at the carrier frequency emanating from the amplifier, one that can be masked. Informally, we have placed the coil at different locations on the scalp and found that, even when positioned over inferior prefrontal or occipital cortex, there is no scalp stimulation or tactile percept from kTMP stimulation, issues that can impact TMS and tES protocols [46,70].

Second, for studies using TMS as a probe of NIBS efficacy, the E-fields of the perturbation (e.g., kTMP) and probe (e.g., suprathreshold single-pulse TMS) have matched spatial distribution and only differ in terms of their waveforms and strengths. In contrast, the E-fields of tES and TMS cannot be matched [10] and, as such, likely impact different neural populations even when the targeted region is ostensibly the same. Beyond the experimental convenience of using the same TMS coil for both perturbation and probe as in our prototype, the E-field alignment may increase experimental robustness.

Third, the experimenter can create a kTMP waveform of unlimited flexibility. For example, using AM kTMP to introduce perturbations at physiologically relevant frequencies could provide a method to enhance plasticity or even induce neural entrainment. Although we failed to observe an additional effect of AM kTMP, research using non-human models suggests that entrainment effects from NIBS requires strong E-fields since the exogenous stimulation pattern must compete with endogenous brain rhythms [9,15,71]. As such, kTMP offers an approach that combines an expanded range of subthreshold E-field amplitudes with waveform flexibility in the kHz range.

Fourth, E-fields produced through magnetic induction, unlike tES E-fields, depend weakly upon the distribution of tissue conductivity - estimates of which vary widely in the literature [72]. Indeed, for spherical shell head models the E-field produced through magnetic induction is independent of conductivity—something that does not hold for tES E-fields. This should reduce variability when modeling the E-field based on individualized head geometry.

Fifth, as with other NIBS methods [73–76] we anticipate that simultaneous EEG and kTMP will enable the investigation of the evolution of neural effects in real-time. For example, with amplitude modulated kTMP the artifact from the kHz carrier frequency can be removed, albeit with possible technical hurdles connected to nonlinearities associated with presently available recording and stimulation hardware [77]. This approach could be used to tailor the stimulation waveform on an individual basis or for closed-loop control, promising avenues for translational applications of NIBS.

Conclusion

In conclusion kTMP offers an opportunity to explore a new experimental space, one with a relatively large range of subthreshold E-field induction, the focality of TMS, and the potential to impose E-fields at physiological relevant frequencies, with significantly less tolerability issues than tES or suprathreshold TMS.

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Author contributions

D.S., L.L., R.B.I. and B.I. conceived the project. D.S. designed and developed kTMP. L.L. and R.B.I designed the TMS experiments. L.L. and C.M. coordinated the data collection and C.M. performed the data and statistical analyses. A.V.P. contributed to the experiment design and data analysis and interpretation. L.L., D.S., and C.M. drafted the paper, and all authors participated in the editing process. All authors approve the final version of the manuscript and are accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Conflict of Interest

The authors declare the following competing interests: LL, CM, BI, RBI and DS have stock ownership and AVP is a paid consultant of Magnetic Tides, a non-publicly traded company created to develop new methods of non-invasive brain stimulation. UC Berkeley holds the patent rights related to the kTMP technology and has provided Magnetic Tides with an exclusive licensing agreement.

Data availability

The data used to support the results are available upon request from the corresponding author.

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Editors

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Carnegie Mellon University, Pittsburgh, United States of America

Reviewer #1 (Public review):

Summary:

This paper reports the first results on the effects of a novel waveform for weak transcranial magnetic stimulation, which is refered to as "perturbation" (kTMP). The waveform is sinusoidal at kHz frequency with subthreshold intensities of 2V/m, instead of the suprathreshold pulses used in conventional TMS (~100V/m). The effect reported here concerns motor-evoked potentials (MEPs) elicited on the hand with single-pulse TMS. These MEPs are considered a marker of "corotico-spinal excitability". The manuscripts report that kTMP at 3.5kHz enhances MEPs with a medium effect size, with independent replication of this finding on 3 separate cohorts of subjects (N=16, 15, 16). This result is important for the field of non-invasive brain stimulation. The evidence in support of this claim is compelling. Despite the replications, this remains an exploratory study that will require replication with adequately powered planned comparisons.

Strengths:

• This is a novel modality for non-invasive brain stimulation.
• Knowing the history in this field, this is likely to lead to a large number of follow-up studies in basic and clinical research.
• The modality causes practically no sensation, which makes it perfectly suitable for control conditions. Indeed, the study itself used a persuasive double-blinding procedure.
• The replication of the main result in two subsequent experiments is very compelling.
• The effect size of Cohen's d=0.5 is very promising.
• It is nice the E-fields were measured on a phantom, in addition to modeling.

Weakness:

• Statistical analysis combining Experiments 1, 2, 3 after inspecting the data is inappropriate.
• Post-hoc definition of outliers that were removed is unfortunate.
• While sensation has been documented, blinding was not directly assessed.
• Despite the replications, this remains an exploratory study as it lacks power analysis and planned comparisons.

Other comments from an earlier review were adequately addressed.

https://doi.org/10.7554/eLife.92088.2.sa2

Reviewer #2 (Public review):

Summary:

kTMP is a novel method of stimulating the brain using electromagnetic fields. It has potential benefits over existing technology because it is a safe and easy technology. It explores a range of brain frequencies that has not been explored in depth before (2-5kHz) and thus offers new opportunities.

Strengths:

This work relied on standard methods and was carefully and conservatively performed.

Weaknesses:

There were few weaknesses. The sham condition was prepared as well as could be done, but sham is always challenging in a treatment with sound and sensation, and with knowledgeable operators. New technology, also, is very exciting to subjects and it is difficult to achieve a natural experiment. These difficulties are related to the technology, however, and not to the execution of these experiments..

https://doi.org/10.7554/eLife.92088.2.sa1

Author response:

The following is the authors' response to the original reviews.

Response to Public Comments

(1) BioRxiv version history.

Reviewer 1 correctly noted that we have posted different versions of the paper on bioRxiv and that there were significant changes between the initial version and the one posted as part of the eLife preprint process. Here we provide a summary of that history.

We initially posted a bioRxiv preprint in November, 2021 (Version I) that included the results of two experiments. In Experiment 1, we compared conditions in which the stimulation frequency was at 2 kHz, 3.5 kHz, or 5.0 kHz. In Experiment 2, we replicated the 3.5 kHz condition of Experiment 1 and included two amplitude-modulated (AM) conditions, with a 3.5 kHz carrier signal modulated at 20 Hz or 140 Hz. Relative to the sham stimulation, non-modulated kTMP at 2 kHz and 3.5 kHz resulted in an increase in cortical excitability in Experiment 1. This effect was replicated in Experiment 2.

In the original posting, we reported that there was an additional boost in excitability in the 20 Hz AM condition above that of the non-modulated condition. However, in re-examining the results, we recognized that the 20 Hz AM condition included an outlier that was pulling the group mean higher. We should have caught this outlier in the initial submission given that the resultant percent change for this individual is 3 standard deviations above the mean. Given the skew in the distribution, we also performed a log transform on the MEPs (which improves the normality and homoscedasticity of MEP distributions) and repeated the analysis. However, even here the participant's results remained well outside the distribution. As such, we removed this participant and repeated all analyses. In this new analysis, there was no longer a significant difference between the 20 Hz AM and non-modulated conditions in Experiment 2. Indeed, all three true stimulation conditions (non-modulated, AM 20 Hz, AM 140 Hz) produced a similar boost in cortical excitability compared to sham. Thus, the results of Experiment 2 are consistent with those of Experiment 1, showing, in three new conditions,

the efficacy of kHz stimulation on cortical excitability. But the results fail to provide evidence of an additional boost from amplitude modulation.

We posted a second bioRxiv preprint in May, 2023 (Version 2) with the corrected results for Experiment 2, along with changes throughout the manuscript given the new analyses.

Given the null results for the AM conditions, we decided to run a third experiment prior to submitting the work for publication. Here we used an alternative form of amplitude modulation (see Kasten et. al., NeuroImage 2018). In brief, we again observed a boost in cortical excitability in from non-modulated kTMP at 3.5 kHz, but no additional effect of amplitude modulation. This work is included in the third bioRrxiv preprint (Version 3), the paper that was submitted and reviewed at eLife.

(2) Statistical analysis.

Reviewer 1 raised a concern with the statistical analyses performed on aggregate data across experiments. We recognize that this is atypical and was certainly not part of an *a priori* plan. Here we describe our goal with the analyses and the thought process that led us to combine the data across the experiments.

Our overarching aim is to examine the effect of corticospinal excitability of different kTMP waveforms (carrier frequency and amplitude modulated frequency) matched at the same estimated cortical E-field (2 V/m). Our core comparison was of the active conditions relative to a sham condition (E-field = 0.01 V/m). We included the non-modulated 3.5 kHz condition in Experiments 2 and 3 to provide a baseline from which we could assess whether amplitude modulation produced a measurable difference from that observed with non-modulated stimulation. Thus, this non-modulated condition as well as the sham condition was repeated in all three experiments. This provided an opportunity to examine the effect of kTMP with a relatively large sample, as well as assess how well the effects replicate, and resulted in the strategy we have taken in reporting the results.

As a first step, we present the data from the 3.5 kHz non-modulated and sham conditions (including the individual participant data) for all three experiments in 4. We used a linear mixed effect model to examine if there was an effect of Experiment (Exps 1, 2, 3) and observed no significant difference within each condition. Given this, we opted to pool the data for the sham and 3.5 kHz non-modulated conditions across the three experiments. Once data were pooled, we examined the effect of the carrier frequency and amplitude modulated frequency of the kTMP waveform.

(3) Carry-over effects

As suggested by Reviewer 1, we will examine in the revision if there is a carry-over effect across sessions (for the most part, 2-day intervals between sessions). For this, we will compare MEP amplitude in baseline blocks (pre-kTMP) across the four experimental sessions.

Reviewer 1 also commented that mixing the single- and paired-pulse protocols might have impacted the results. While our *a priori* focus was on the single-pulse results, we wanted to include multiple probes given the novelty of our stimulation method. Mixing single- and different paired-pulse protocols has been relatively common in the non-invasive brain stimulation literature (e.g., Nitsche 2005, Huang et al, 2005, López-Alonso 2014, Batsikadze et al 2013) and we are unaware of any reports suggested that mixed designs (single and paired) distort the picture compared to pure designs (single only).

(4) Sensation and Blinding

Reviewer 2 bought up concerns about the sham condition and blinding of kTMP stimulation. We do think that kTMP is nearly ideal for blinding. The amplifier does emit an audible tone

(at least for individuals with normal hearing) when set to an intensity to produce a 2 V/m E-field. For this reason, the participants and the experimenter wore ear plugs. Moreover, we played a 3.5 kHz tone in all conditions, including the sham condition, which effectively masked the amplifier sound. We measured the participant's subjective rating of annoyance, pain, and muscle twitches after each kTMP session (active and sham). Using a linear mixed effect model, we found no difference between active and sham for each of these ratings suggesting that sensation was similar for active and sham (Fig 8). This matches our experience that kHz stimulation in the range used here has no perceptible sensation induced by the coil. To blind the experimenters (and participants) we used a coding system in which the experimenter typed in a number that had been randomly paired to a stimulation condition that varied across participants in a manner unknown to the experimenter.

Reviewer 1 asked why we did not explicitly ask participants if they thought they were in an active or sham condition. This would certainly be a useful question. However, we did not want to alert them of the presence of a sham condition, preferring to simply describe the study as one testing a new method of non-invasive brain stimulation. Thus, we opted to focus on their subjective ratings of annoyance, pain, and finger twitches after kTMP stimulation for each experimental session.

Response to Recommendations for the Authors

Reviewer #1:

Reviewer # 1 in the public review noted the possibility of carry-over effects and suggested that we compare the amplitude of the MEPS in the pre blocks across the four sessions.

Although we did not anticipate carry-over effects lasting 2 or more days, we have now conducted an analysis in which we use a linear mixed effect model with a fixed factor of Session and a random factor of Participant. The results show that there is not an effect of session [$\chi2(3) = 4.51$, $p = 0.211$].

Author response table 1.

Session #	MEP (Mean)	MEP (SE)
Session 1	1.12	0.12
Session 2	1.43	0.18
Session 3	1.27	0.17
Session 4	1.36	0.16

> *Detailed comments and some suggestions to maybe improve the writing and figures:*
>
> *Abstract:*
>
> *BioRxiv Version 1: "We replicated this effect in Experiment 2 and found that amplitude-modulation at 20 Hz produced an additional boost in cortical excitability. "*
>
> *BioRxiv Version 2, 3 and current manuscript: "Although amplitude-modulated kTMP increased MEP amplitude compared to sham, no enhancement was found compared to non-modulated kTMP."*

> *I am a little concerned about this history because the conclusions seem to have changed. It looks like the new data has a larger number of subjects, which could explain the divergence. Although it is generally not good practice to analyze the data at interim time points, without accounting for alpha spending. It appears that data analysis methods may have also changed, as some of the extreme points in version 1 seem to be no longer in the new manuscript (Figure 4 Sham Experiment 1).*

In the public review above we explain in detail the different versions of the bioRxiv preprint and how the results changed from the first version to the current manuscript.

> *Introduction:*
> *"Second, the E-fields for the two methods exist in orthogonal subspaces" Can you explain what this means?*

Thank you for this suggestion, we have updated the paper (pg. 4, line 78-81) by adding two sentences to explain what we mean by orthogonal subspaces and describe the consequences of this with respect to the E-fields resulting from tES and TMS. Specifically, we now comment that even if the E-fields of tES and TMS are similar in focality, they may target different populations of neurons.

> *"In addition, the kTMP waveform can be amplitude modulated to potentially mimic E-fields at frequencies matching endogenous neural rhythms [15]." That may be so, but reference [15] makes the exact opposite point, namely, that kHz stimulation has little effect on neuronal firing until you get to very strong fields. The paper that makes that claim is by Nir Grossman, but in my view, it is flawed as responses are most likely due to peripheral nerve (axon) stimulation there given the excessive currents used in that study. The reference to Wang and Peterchev [17] is in agreement with that by showing that you need 2 orders of magnitude stronger fields to activate neurons.*

The reviewers are correct that that Ref 15 (Esmaeilpour et al, 2021), as well as Wang et al, 2023 use much higher E-fields than we target in our present study. However, our point here is that, while we cannot use our approach to apply E-fields at endogenous frequencies, we can do amplitude modulation of the kHz carrier frequency at these lower frequencies. We cited Esmaeilpour et al., (2021) because they show that high frequency stimulation with amplitude-modulated waveforms resulted in dynamic modulation at the “beating” frequency. Given we are well in subthreshold space in this paper, and well below the E-field levels in Esmaeilpour et al (2021), the open question is whether amplitude modulation at this level will be able to perturb neural activity (e.g., increase power of endogenous oscillations at the targeted frequency).

To address this concern, we modified the sentence (pg.6, lines 120-121) to now read "In addition, the kTMP waveform can be amplitude modulated at frequencies matching endogenous neural rhythms." In this way, we are describing a general property of kTMP (as well as other methods that can use high frequency signals).

> *I am not aware of any in-vitro study showing the effects of kHz stimulation at 2V/m. The review paper by Neudorfer et al is very good. But if I got it correctly in a quick read it is not clear that there is experimental evidence for subthreshold effects. They do talk about facilitation, but the two experimental papers cited there on the auditory nerve don't quantify field magnitudes. I would really love it if you could point me to a relevant empirical study showing the effects of kHz stimulation at 2 V/m.*
>
> *Perhaps all this is a moot point as you are interested in lasting (plastic) effects on MEP. For this, you cite one study with 11 subjects showing the effects of kHz tACS on MEPs [20].*

> *I guess that is a start. The reference [21] is only a safety study, so it is probably not a good reference for that. Reference [22] also seems out of place as it is a modeling study. The effects on depression of low-intensity magnetic stimulation in references [23-26] are intriguing.*

We agree with the reviewer that Ref 20 (now Ref 18: Chaieb, Antal & Paulus; 2011) is the most relevant one to cite here since it provides empirical evidence for changes in neural excitability from kHz stimulation, and in fact, serves as the model for the current study. We have retained Refs 23-26 (now Ref 19-22: Rohan et al., 2014; Carlezon et al., 2005; Rohan et al., 2004 & Dublin et al., 2019) since they also do show kHz effects on mood and removed Refs 21 (Chaieb et al., 2014) and 22 (Wang et al., 2018) for the reasons cited by the Reviewer.

> *Figure 1: "The gray dashed function depicts the dependence of scalp stimulation threshold upon frequency [14]." It's hard to tell from that reference what the exact shape is, but the frequency dependence is likely steeper than what is shown here, i.e. 2 mA at 10 Hz can be really quite unpleasant.*

We have removed the gray dashed line given that this might be taken to suggest a discrete transition. We now just have a graded transition to reflect that the tolerance of tES is subjective. We start the shading at 2 mA for the lowest frequencies given that there is general agreement that 2 mA is well-tolerated and decrease the shading intensity as frequency increases. The general aim of the figure is not to make strong claims about the threshold of scalp discomfort for tES, but to show that kTMP can target much higher cortical E-fields within the tolerable range.

> *Methods:*
> *Procedures:*
> *It does not seem like double-blinding has been directly assessed.*

We did not assess double blinding by directly assessing whether the participant was in a sham or active condition. We did not want to alert the participants of the presence of a sham condition after the first session of the 4-session study, preferring to simply describe the study as a test of a new method of non-invasive brain stimulation. For this reason, we opted to focus on their subjective ratings of annoyance, pain, and finger twitches after kTMP stimulation for each experimental session. These ratings did not differ between active and sham kTMP, which suggests kTMP has good potential for double blinding.

> *MEP data analysis: Taking the mean of log power is unusual, but I suppose the reference provided gives a good justification. Does this explain the deviation from the biorxiv v1 results?*

We opted to perform a logarithmic transformation of MEP amplitudes to improve the normality and homoscedasticity of the MEP distribution. We cite three papers (Refs 50-52: Peterchev et al., 2013, Nielsen 1996a, & Nielsen 1996b) that have applied a similar approach in handling MEP data. We had not done the transformation in the first bioRxiv but opted to do so in the eLife submission based on further review of the literature. We note that the two analyses produce similar statistical outcomes once we removed the outlier discussed in the Public Review.

> *"Interactions were tested by comparing a model in which the fixed effects were restricted to be additive against a second model that could have multiplicative and additive effects." Not sure what this means. Why not run a full model with interactions included and read off the stats from that single model for the various factors? Should one not*

> *avoid running multiple models as one would have to correct p-values for multiple comparisons for every new test?*

We used the lme4 package in R to fit our linear mixed effect models (Ref 54: Bates, Mächler, Bolker & Walker, 2015). In this package they intentionally leave out *p*-values for individual models or factors because they note there is a lack of convergence in the field about how to calculate parameter estimates in complex situations for linear mixed effect models (e.g., unbalanced designs). They suggest model comparison using the likelihood-ratio test to obtain and report *p*-values, which is what we report in the current manuscript.

We revised the text in the section *Linear Mixed Effects Models* to state that likelihood ratio tests were used to obtain *p*-values to remove any confusion.

> *Procedures:*
> *kTPM: Nice that fields were measured. Would be nice to see the data that established the empirical constant k.*

We have expanded our discussion of how we established *k* in the Methods section. We first derived *k* using the equation E0 = kfcI based on previously published reports of the current (I) and frequency (fc) of the MagVenture Cool-B65 coil (now Refs 29-30: Deng, Lisanby & Peterchev, 2013; Drakaki, Mathiesen, Siebner, Madsen & Thielscher, 2022). We then verified this value using the triangular E-field probe to within 5% error.

> *Figure 3, spectrum. The placement of the fm label on the left panel is confusing. It suggests that fm was at the edge of the spectrum shown, which would not be the best way to show that there is nothing there - obviously, there isn't, but the figure could be more didactic.*

Thanks for pointing this out. We modified the figure, moving the ‘fm’ label to the center of the first panel. This change makes it clear that there is no peak at the amplitude modulated frequency.

> *"a trio of TMS assays of cortical excitability" Can you clarify what this means?*

Sorry for the confusion. The trio of TMS assays refers to the single pulse and two paired-pulse protocols (SICI - ICF). We edited the Procedure section to clarify this (pg 9, line 195-197).

> *Figure 2A: it would be nice to indicate which TMS blocks were single pulse and which were the two paired-pulse protocols. It is hard to keep track of it all for the three different experiments.*

We have now clarified in the text (see above) that all three probes were used in each block for Experiments 1 and 2, and only the single-pulse probe in Experiment 3. We have modified the legend for Figure 2 to also provide this information.

> *Results:*
> *"Based on these results, we combined the data across the three experiments for these two conditions in subsequent analyses." This strikes me as inappropriate. Should not a single model have been used with a fixed effect of experiment and fixed effect of stimulation condition?*

We recognize that pooling data across experiments may be atypical. Indeed, our initial plan was to simply analyze each experiment on its own (completely within-subject analysis). However, after completing the three experiments, we realized that since the sham and non-

modulated 3.5 kHz conditions were included in each experiment, we had an opportunity to examine the effect of kTMP in a relatively large N study (for NIBS research). Before pooling the data, we wanted to make sure that the factor of experiment did not impact the results and our analysis showed there was no effect of experiment. Note that we did not include the factor of stimulation condition in this model because we did not want to do multiple comparisons of the same contrast (3.5 kHz compared to sham). By pooling the data before analysis of the stimulation conditions we could then focus on our two key independent variables: 1) kTMP carrier frequency and 2) kTMP amplitude modulated frequency, doing fewer significance tests to minimize multiple comparisons. The linear mixed effect (LME) model allows us to include a random effect of participant. In this way, we account for the fact that some comparisons are within subjects and some comparisons are between subjects.

The reviewer is correct that after pooling the data, we could have continued to include the factor of experiment in the LME models. This factor could still account for variance even though it was not significant in the initial test. Given this, we have now reanalyzed the data including the fixed factor of experiment in all the comparisons that contain data from multiple experiments. This has led us to modify the text in the Methods section under *Linear Mixed Effects Models* and in the Results section under *Repeated kTMP Conditions (3.5 kHz and Sham) across Experiments*. In addition, the results of the LME models have been updated throughout the Results section. We note that the pattern of results was unchanged with this modification of our analyses.

> *"Pairwise comparisons of each active condition to sham showed that an increase was observed following both 2 kHz ..." I suppose this is all for Experiment 1? It is a little confusing to go back and forth between combining experiments and then separate analyses per experiment without some guiding text, aside from being a bit messy from the statistical point of view.*

We did not go back to performing separate analyses of the experiments after pooling the data. Once we ran the test to justify pooling the data, subsequent tests were done with the pooled data to evaluate the effects of carrier frequency and amplitude modulation.

> *Figure 5 is confusing because the horizontal lines with ** on top seem to refer to the same set of sham subjects, but the subjects of Experiments 2 and 3 are different from Experiment 1, so in these pairwise comparisons there is a mix of between-subject and within subject-comparison going on here. Did I get that right?*

Yes – that is correct. As noted above we pooled the data after showing that there was no effect of experiment. Thus, the data for the sham and 3.5 kHz non-modulated conditions are from three different experiments. There was some overlap of subjects in Experiments 1 and Experiment 2 (Experiment 3 was all new participants). We used a linear mixed effect model so that we could account for this mixed design. Participant was always included as a random factor, which allows us to account for the fact that some comparisons are within, and some are between. Based on a previous comment, we now include Experiment as a fixed factor (see above) which provides a way to evaluate variance across the different experiments.

> *"We next compared sham vs. active non-modulated kTMP and found that active kTMP produced a significant increase in corticospinal excitability [$\chi 2(1) = 23.46$ $p < 0.001$" Is this for the 3.5Hz condition?*

No, that is for an omnibus comparison of non-modulated kTMP (including 2 kHz, 3.5 kHz and 5 kHz conditions) vs. sham. We have edited the paper to include the three conditions that are included as the active non-modulated kTMP conditions for clarity (pg. 22, line 463). Having observed a significant omnibus result, we continued with paired comparisons: “Pairwise

comparisons of each active condition to sham showed that an increase was observed following both 2 kHz [$\chi2(1) = 6.90$, $p = 0.009$; $d = 0.49$] and 3.5 kHz kTMP [$\chi2(1) = 37.75$, $p < 0.001$; $d = 0.70$; Fig 5: Non-Modulated conditions]. The 5 kHz condition failed to reach significance [$\chi2(1) = 1.43$, $p = 0.232$; $d = 0.21$]."

> *Paired-Pulse Assays: There are a number of results here without pointing to a figure, and at one point there is a reference to Figure 6, which may be in error. It would help to point the reader to some visual corresponding the the stats.*

Thank you. This was an error on line 542. It should have read Figure 7. We have added two other pointers to Figure 7 where we discuss the absence of an effect of kTMP on SICI.

> ***Reviewer #2 (Recommendations For The Authors):***
>
> *I would recommend a couple of changes to the background.*
>
> *"Orthogonal subspaces" line 78. This is a fairly formal term that has little relevance here, although the difference between scalar and vector potential-based fields is interesting to think about. If it stays, it should be mathematically supported, but it's easily rewritten to deliver the gist of it.*

We have updated the paper by adding text that we hope will clarify what we mean by orthogonal subspaces (pg. 4, line 78-81). We note that we developed the math behind this statement in a previous paper (Ref # 10: Sheltraw et al., 2021). We have changed the location of the citation so that it directly follows these sentences and will provide a pointer to readers interested in the physics and math concerning orthogonal subspaces.

> *The statement that the scalp e-field for TES is greater than the e-field for TMS for similar cortical fields needs a little more clarification, since historically they have operated orders of magnitude apart, and it is easy to misread and trip over this statement (although it is factually true). Presenting a couple of numbers at cortical and scalp positions would help illustrate the point. That you are not considering applying TES at traditional TMS levels but rather TMS at TES values is what is initially easy to miss.*

We appreciate the feedback and have updated this section to provide the reader with a better intuition of this point. We now specify that the scalp to cortical E-field ratio is approximately 18 times larger for tES compared to TMS and cite our previous paper which has much more detail about how this was calculated.

> *A note that the figures show scalp sensation around 1.0 V/m while the text states 0.5; cortical depths are an important thing for the reader to keep in mind.*

This comment, when considered in tandem with one of the comments of Reviewer 1 led us to revise Figure 1. We removed the dashed gray line which might be taken to suggest a strict cutoff in terms of tolerability (which we did not intend). We now use shading that fades away to make the point of continuity. We have extended this down to a cortical E-field of 0.5 V/m to correspond with the text.

> *This is a nicely done and carefully reported experiment and I look forward to seeing more.*

Thank you for your kind note!

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