There is no Biophysical Distinction between Temporal Interference Stimulation and Direct kHz Stimulation for Actuation of Peripheral Nerves

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Abstract: Temporal interference stimulation (TIS) has attracted increasing attention as a promising noninvasive electrical stimulation method. Despite positive results and optimistic expectations, the TIS field has been beset by misunderstandings concerning its mechanism of action and efficacy in safely targeting deep neural structures. Various studies posit that TIS exploits the interference of multiple supraphysiological frequency (kHz range) carriers to essentially deliver low-frequency stimulation at the intersection of the carriers, thereby circumventing limitations associated with tissue impedance and depth penetration. Due to the documented electrophysiological effects of kHz-range electric stimuli, such a picture is an oversimplification. Moreover, recent theoretical modelling work has established that the biophysics of TIS is based on kHz stimulation mechanisms. This paper presents experimental evidence supporting this conclusion, by comparing TIS with direct kHz stimulation on peripheral nerve targets in an invertebrate model (*Locusta migratoria*), and in human subjects. Our findings show that the stimulation effects of TIS are achievable through two-electrode kHz stimulation, without necessitating carrier interference in tissue. By comparing four-electrode TIS with two-electrode stimulation via kHz sine waves for targeting of peripheral nerves, we demonstrate overlapping strength-frequency (s-f) dependence across all stimulation types. Since all stimulation waveforms are governed by the same s-f curve, this implicates a common underlying biophysical mechanism. This equivalence challenges the notion that TIS uniquely facilitates neural engagement via other mechanisms. Furthermore, performing TIS with higher carrier frequencies into the MHz range fails to lead to stimulation. We evaluate the regions of tonic (unmodulated) and phasic (amplitude-modulated) stimulation regions inherent when using TIS, and the associated possibility of off-target effects. Our study further suggests that possible practical advantages of TIS can be achieved in an easier way by simply using amplitude-modulated kHz waveforms.

1. Introduction

Noninvasive electrical stimulation and the use of interferential currents

With electrical neurostimulation of the nervous system there is always the tradeoff between invasiveness and specificity. Applying small electrodes to the exact area of interest is possible with high precision, however at the cost of a complex, invasive, and to some degree damaging surgical procedure.[1] Completely noninvasive electrical stimulation from outside the body is much easier to perform, however is plagued by low spatial specificity, and limited depth of effective penetration. This means only superficial targets can be addressed, for instance nerves located directly below the skin, or brain structures located below the skull. Obtaining suprathreshold neurostimulation, where neurons become sufficiently depolarized and directly

fire action potentials, requires electric fields (> 1V/m) which are difficult to obtain transcutaneously without causing discomfort arising from stimulation of sensory fibers in superficial tissues.[2,3] This greatly limits the diagnostic and therapeutic potential of noninvasive electrical stimulation, and implantable electrodes remain necessary for many applications where direct triggering of action potentials is desired (*i.e.* suprathreshold neurostimulation). The fundamental limitation behind noninvasive central or peripheral electrical stimulation is that electric field decreases inversely with distance from the electrodes for any given conductive medium. The relatively high impedance of skin imposes practical limits to penetration of electrical field.[3] The challenge of how to effectively deliver electrical stimuli through the skin is a fundamental problem for noninvasive bioelectronic medicine. A suggested approach to overcome this basic issue is using high-frequency electric fields, since skin impedance decreases with frequency. The higher the carrier frequency used, the lower is the effective impedance. One method to exploit this, which this paper interrogates, is the concept of mixing multiple high-frequency electrical signals to produce patterns of interference and amplitude-modulated (AM) "beats" in the tissue. This could solve the issue of depth penetration and potentially spatial specificity also. Originally suggested by Nemec in the 1950s under the name "interferential current stimulation", and used extensively in the field of physical therapy and sports medicine,[4–9] this method has regained increasing attention since its demonstration for brain stimulation in mice in the 2017 paper by Grossman *et al.*.[10] While in the older literature the terms "interferential current stimulation" or "interferential therapy" are more common, in recent studies, this type of stimulation using multiple kHz frequencies is termed temporal interference stimulation (TIS).[11–16] TIS has shown efficacy in humans for stimulation of peripheral nerves,[17] and promising examples of its use in brain stimulation have been reported.[16,18]

The principle behind TIS (Figure 1a) is that relatively high-frequency electrical stimulation signals are applied, in a range over 1000 Hz. Neurons can fire action potentials synchronously with low frequencies, that is they can "follow" such frequencies. Stimuli in the range > 1 kHz are at frequency values above the range of neuron firing are sometimes referred to as supraphysiological as they are above the range of electrophysiological activity. For the purpose of TIS, these high frequencies are known as *carriers*. Most papers describing TIS assume *a priori* that the carriers are of too high frequency and too low amplitude to efficiently elicit action potential firing by themselves (we will outline in this paper why this assumption is incorrect).[10,12,15,16,19] Indeed, stimulation current threshold rises with frequency for sinusoidal stimuli of frequency roughly > 50 Hz.[20] To accomplish TIS, multiple carriers of slightly different frequencies (*i.e.* f_1 and f_2) are applied simultaneously. This gives an offset between the two frequencies, Δf (Figure 1a). The two carriers interact inside of the tissue, coming in and out of phase with each other, a phenomenon known as interference. Interference causes amplitude modulation (AM) of the resultant kHz electric fields inside the tissue. The frequency of AM is equal to Δf. The two frequencies f_1 and f_2 thus create an AM "envelope" frequency at Δf. TIS is predicated on the observation that the AM frequency produces neural stimulation effects that are synchronous with this AM frequency. A number of published studies establish that suprathreshold electrical neurostimulation synchronous to the AM frequency is indeed occurring.[10,17,21,22] This has led to two competing interpretations: A) the AM beat waveform produced by the interference is effectively causes phasic stimulation at the target site, while the unmodulated high-frequency carrier has a negligible effect. In other words, the threshold for AM stimulation is significantly lower than for the unmodulated carrier;[10,23] B) TIS leads to phasic stimulation in regions of interference producing AM, while other regions are subject to equal or higher degrees of tonic stimulation by the carrier frequency, since biophysically the origin of the phasic and tonic stimulation is the same kHz stimulation phenomenon.[13,22] Our findings support that interpretation B is correct.

Critically evaluating assumptions behind temporal interference stimulation

In scanning both older and newer literature on TIS (*aka* interferential current stimulation), it is possible to identify three common assumptions or claims about how TIS works and why it is advantageous over standard noninvasive electrical stimulation:

1. The kHz carrier frequencies (*i.e.* f_1 and f_2) at the amplitudes used in TIS **do not** efficiently elicit stimulation themselves but can be regarded as electrophysiologically inert and pass through tissue. Neurons do "not follow" such high frequencies by firing action potentials. The threshold for eventual stimulation by the kHz carrier alone is high enough to be neglected.
2. Neurons **do** respond to the low-frequency AM due to a natural resonant preference for these low frequencies, and can demodulate the beat frequency Δf to afford effective stimulation at the beat frequency. Many papers, without delving into explanations, assume that the high-frequency carrier is inert, and neurons only respond to the low-frequency AM.
3. TIS offers unique targeting of deep neural structures. Since skin impedance decreases with higher frequency, the use of high-frequency carriers can achieve more effective penetration, allowing TIS to effectively stimulate deeper targets than conventional (low-frequency) forms of transcutaneous stimulation. The AM "hotspot" can be targeted to an area of interest without stimulating intervening tissues.

Assumption (1) has been questioned, since supraphysiological frequencies can indeed lead to stimulation, and the associated effect of nerve block.[24–29] The fact that neurons cannot fire at higher frequencies does not mean they are not electrically stimulated by higher frequencies. Experiments in the 1930s-1960s found that kHz frequencies 0.5-50 kHz would lead to evoked action potentials, and that threshold current required for stimulation increased with frequency in this range.[30–33] Stimulation by a periodic symmetric signal (Figure 1b) leads to net depolarization of excitable cells because of the rectification properties of membranes,[34,35] leading to a distinctive strength-frequency (s-f) relationship (Figure 1c). The s-f curve in the supraphysiological frequency kHz range has been confirmed in different excitable tissues and cell types.[20] Over a wider frequency range, it is apparent that the s-f plot is governed by two exponential functions describing increasing thresholds at very low frequencies (< 40 Hz, the so-called accommodation region), and at high frequencies (around 100 kHz and higher), where thermal dissipation effects begin to overtake direct electrical stimulation.[36,37] Nevertheless, stimuli in the range of 1-10 kHz can depolarize cells, with an approximately linear s-f dependence. The reason why a symmetric signal (like shown in Figure 1b) leads to net stimulation is because the depolarizing effect of the first phase of the signal is not fully counteracted by the subsequent opposite-polarity phase, due to nonlinear conductivity across excitable cell membranes.[20,28] This effect can be illustrated by using established computational models. We show the membrane voltage of the model neuron in response to a 1 kHz extracellular stimulation current in Figure 1d. The membrane nonlinearity is a result of asymmetric conduction behavior of sodium and potassium channels. Therefore, the excitable membrane acts to rectify a sinusoidal signal.[34] Rectification causes net depolarization of the membrane because the increase in Na^+ channel conductance is larger than the increase in K^+ channel conductance. Another related effect of kHz stimuli is that nerve cell depolarization can occur via a process of summation of subthreshold depolarizations, since at kHz frequencies successive half-cycles arrive within the absolute refractory period of neurons. This kind of summation effect was first described by Gildemeister,[31] to explain his observations that stimulation efficacy of kHz bursts was strongly dependent on burst duration (aka repetitions of the sinusoidal cycle), with longer bursts resulting in lower apparent current thresholds. This occurs because of the rectification properties of neurons, as each successive kHz sinusoidal cycle pushes the neurons closer to the threshold. This is illustrated in the green suprathreshold membrane voltage trace calculated in Figure 1c. Gildemeister conceptualized a symmetric

kHz signal as having the same net effect as applying a DC stimulus, calling it "ambipolar stimulation"[32,33] since each electrode causes the same net depolarization (there is no "anode" or "cathode", as in DC stimulation). He observed that cells below both electrodes were depolarized to the same level, as if both were acting like DC cathodes. This depolarization response of excitable cells to kHz signals was modeled in two recent theoretical papers,[13,38] which further conclude that this same rectification effect is occurring during TIS. These papers point out the importance of considering the effect of sustained application of kHz carriers on tissues superficial to the region being targeted by TIS. When considering peripheral nerve targets, if the kHz stimulation signal is maintained for long periods (> 1s), so-called tonic stimulation, various outcomes are possible: 1) Motor neurons subjected to kHz tonic stimulation can cause sustained downstream muscle contraction (tetanus), if the kHz is of sufficient amplitude to cause depolarization via the summation effect. Tetanic contraction occurs because motorneurons remain depolarized and can continue to fire action potentials, albeit not synchronously with the stimulation signal; 2) a refractory period is enforced by the sustained kHz stimulation signal, a phenomenon known as kHz nerve block. In many reported cases of peripheral nerves, application of tonic kHz stimulation causes initial downstream motor activity for a period of time, which is subsequently followed by nerve block.[24,27,39] Such a nerve no longer responds to electrical stimulation, and also afferent pain signal may be blocked from propagating. This phenomenon is an area of active research, especially in the context of pain management. The kHz stimulation and kHz nerve block are linked, and the transition from tonic stimulation to refractory effects is complex and depends on many factors.[39] In any case, the simple assumption that kHz frequencies being inert from the point of view of depolarizing neuronal cell membranes during a TIS experiment should not be taken for granted. However, it has been postulated theoretically[13] and reported experimentally,[22] that due to resonant coupling of the kHz signal, a given beat frequency can result in an optimally low stimulation threshold. Thus, it is possible to have a situation where a given unmodulated carrier is subthreshold, while the region of AM gives suprathreshold stimulation at the beat frequency. Therefore, in this paper we set out to experimentally verify the stimulation thresholds for unmodulated kHz stimuli and compare them with AM kHz and AM delivered by TIS (Figure 1e). We found that TIS shows the same s-f relation as kHz stimulation alone, however indeed it is possible to observe cases where an optimal AM frequency gives a lower threshold for stimulation.

The assumption (2) essentially comes from experimental observation, where TIS produces evoked activity synchronous with the beat frequency.[17,21,22] It is important to recall that in TIS, in a frequency/power spectrum of the stimulation energy, there is no energy at low frequency. There is no low frequency component inherent in the electrical signal and extraction of a low-frequency (aka demodulation) is possible only if the cells have suitable nonlinear properties. This has indeed been implied by some published studies of TIS. If neurons only respond to low-frequencies and filter high frequencies, then the evoked activity observed to be synchronous with the AM beat frequency at Δf can be regarded as evidence for some biophysical effect that generates stimulation at this Δf. However, if assumption (1) is incorrect, and the kHz frequency itself is the origin of stimulation, activity synchronous with at Δf can be explained in a simple way, without invoking another biophysical mechanism. Namely, where there is maximal interference producing regions of AM, the kHz stimulation becomes suprathreshold at the frequency of Δf. In simpler words, the kHz frequency stimulus is effectively turning on and off with frequency at Δf. We show that this is indeed the case by comparing TIS stimulation thresholds with a single premodulated or unmodulated kHz sine waves: In all cases, the s-f relation for downstream motor activity identical or very similar. Moreover, it is possible to observe smooth and reversible transitions between tetanic and phasic muscle contractions by turning modulation on/off while maintaining a fixed kHz stimulation current amplitude.

The final assumption (3) is based on the well-documented properties of impedance of skin and other tissues, [20,40] however upon closer examination it seems to be a fallacy. The claim is that TIS using higher frequencies

(kHz) will encounter lower attenuation of the signal than conventional low-frequency (Hz) stimulation. This would be true when comparing two periodic signals, but the argument does not hold for the standard neurostimulation pulse methods that are used. Essentially, higher frequencies will pass through tissues with lower loss. It is important to remember that an alternating current signal is capacitively-coupled through skin: the encountered impedance is proportional to phase duration, and not the frequency of repetition of the signal. That is, a monophasic rectangular pulse of 0.2 ms will encounter approximately equivalent impedance as a 5000 Hz sine wave, as the phase duration of each type of signal is the same. In terms of impedance, and thus the depth of penetration of electric field into the tissue, a pulse of 0.2 ms repeated every 1 s has an equal "penetrating ability" as a 5000 Hz sine wave. The frequency of repetition (1 Hz in this example) does not have any bearing on impedance. Typical neurostimulation impulses (monophasic and biphasic) used in common practice have a phase duration in the range of 0.1 ms – 2 ms. In the frequency domain, this is equal to 10 kHz – 500 Hz. To the best of our knowledge, all the published reports on TIS use carrier frequencies within this frequency range, therefore the relevant tissue impedance values do not differ from those encountered by commonly-used neurostimulation impulses in transcutaneous electrical nerve stimulation (TENS). We therefore have endeavored to perform TIS stimulation using carrier frequencies up to 1 MHz, where skin/tissue impedance falls even more. If TIS worked with such high frequency carriers, this would make claim (3) quite compelling. However, we were not able to observe any stimulation effect using low-frequency AM envelopes constructed using 0.1 – 1 MHz carrier frequencies. These high frequencies alone are known to have a much higher current threshold for stimulation.[36,37] This negative result provides evidence that the TIS stimulation effect is linked to the kHz frequency itself. Since the kHz carrier can elicit stimulation in its own right (tonic, unmodulated stimulation), the notion that TIS can selectively target deep structures is, at best, an oversimplification. The latter part of this paper will cover the question of depth and tonic/phasic stimulation regions, concluding that in terms of depth of practical stimulation, there is likely no inherent advantage to using TIS over a simple pre-modulated kHz waveform.

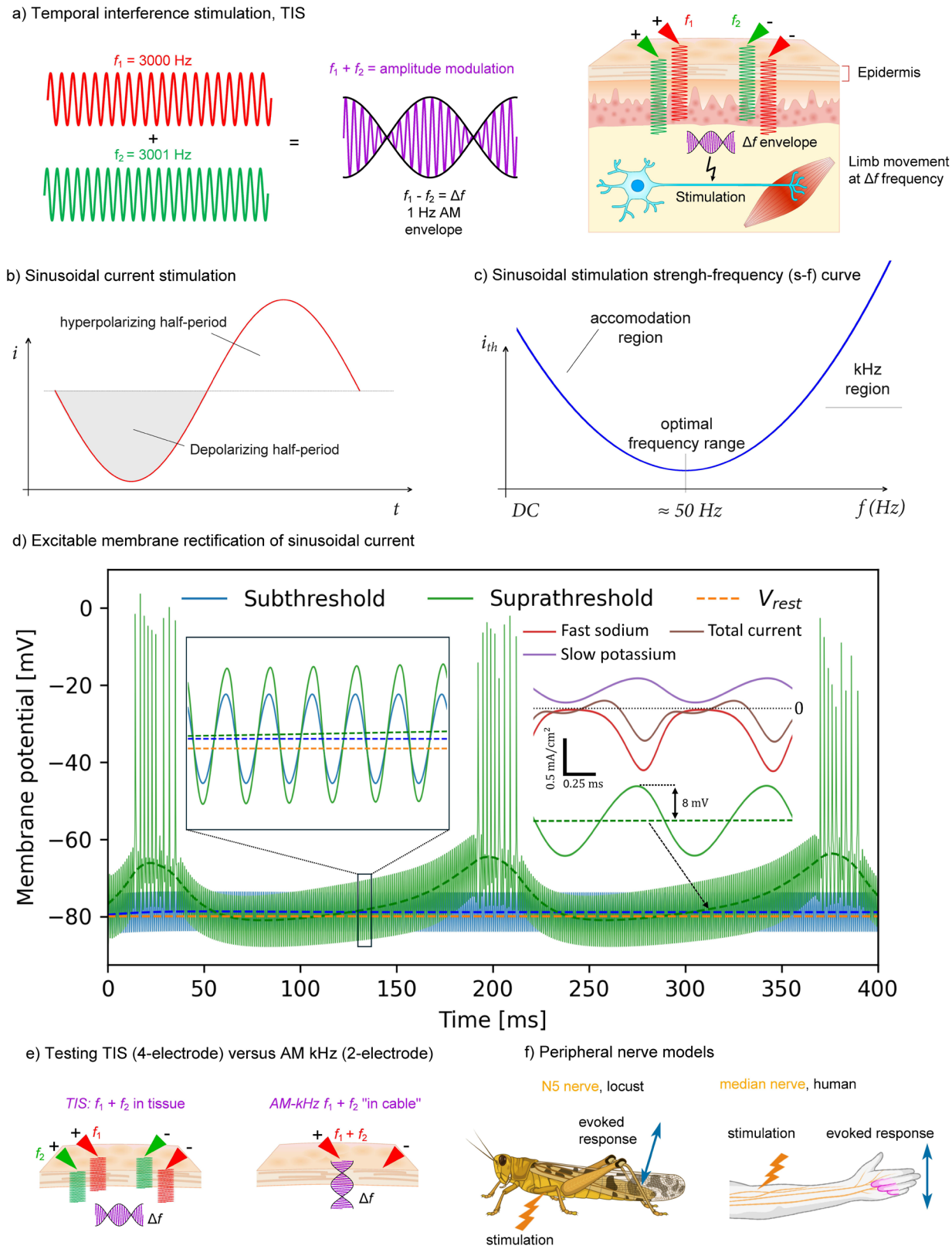

Figure 1. Testing the assumptions of TIS with respect to kHz electrical stimulation. a) The basic principle of TIS involves two kHz carriers at two different frequencies, f_1 and f_2, which interact in a given

medium via interference, creating areas of amplitude modulation (AM). At midpoints between stimulation electrodes, maximum AM depth is achieved. The AM occurs at a frequency which will be equal to the frequency difference between the two carriers, Δf. TIS can be used *in vivo* to deliver transcutaneous stimulation, where AM occurs at an area of interest to deliver phasic stimulation at a target region. The experiments in this paper explore this effect on peripheral nerve targets containing motor neurons which enervate muscles. b) Periodic symmetric stimulation signals like a sinusoid do lead to net depolarization of cells, because of membrane rectification. The depolarizing half-period leads to more net Na^+ influx than K^+ outflux. c) Sinusoidal stimulation is governed by a characteristic strength-frequency (s-f) curve, where the threshold minimum is around 50 Hz. Higher frequencies result in larger stimulation threshold currents. d) 1 kHz sinusoidal stimulation demonstrated on a mammalian myelinated axon model,[13] demonstrating the effect of rectification leading to net depolarization. The model shows the calculated membrane potential during application of an extracellular stimulating current. Three test cases are shown: no stimulation, (orange line V_{rest}), subthreshold stimulation (blue line), and suprathreshold stimulation (green line).All dashed lines are low-pass filtered, solid lines are not filtered. Subthreshold stimulation gives a net membrane depolarization, however when the stimulation current becomes suprathreshold, one can see the summation effect at work as the membrane depolarizes more with each successive sinusoidal period, until threshold is reached and action potentials fire at the preferred resonance frequency of the neuron (5 Hz in this case). The net depolarization caused by sub- and supra-threshold stimuli is due to larger contribution of Na^+ inward currents versus outward K^+ currents, broken down in the inset on the right which shows the total transmembrane current density calculated for this model neuron. The application of a kHz stimulus can be conceptualized as analogous to a cathodic DC stimulation. e) In this work, we compare TIS, which is delivered by two electrode pairs, causing interference and AM in the tissue, with premodulated kHz stimulation, which is delivered using a single pair of electrodes. We find that biophysically, the two stimulation modalities are equivalent. f) The peripheral nerve stimulation models tested in this work. Motorneuron (insect) / motor fiber (human) recruitment and downstream evoked movement is the evaluated biomarker.

We have compared various kHz noninvasive stimulation protocols, including AM-sine and unmodulated sine waves, with TIS, on two peripheral nerve targets, shown in Figure 1f: a) The N5 nerve of *Locusta migratoria*, responsible for hind leg motion; and b) the median nerve in human volunteers. Stimulation is quantified via evoked downstream motor response (leg or hand, respectively). In all cases, we have compared threshold currents to achieve a given motor response as a function of stimulation type, and of the electrical stimulation signal frequency. Next, we have evaluated the effect of changing AM frequency while keeping a fixed carrier frequency. These experiments lead to the conclusion that, at least in the case of peripheral nerves, TIS is biophysically equivalent to kHz stimulation and that some other, parallel, mechanism of demodulation is not present. Finally, with the knowledge that TIS = kHz stimulation, we address the issue that TIS will necessarily produce regions of phasic and tonic stimulation, which introduces caveats and unique opportunities.

2. *Results*

TIS has the same strength-frequency dependence as 2-electrode kHz stimulation

To provide rapid prototyping of the TIS protocol versus kHz stimulation, we suggest the use of an invertebrate such as the locust (*Locusta migratoria*). Insects can provide a useful model for noninvasive electrical stimulation, as despite obvious structural and physiological differences to mammals/humans, their cuticle has comparable electrical characteristics to human skin.[41] Moreover, the locust has a relatively simple and well-understood nervous system, including a pair of large symmetric motorsensory nerves known as the N5 nerves, projecting from the metathoracic ganglion (Figure 2a).[42] The N5 nerves control

movement of the remarkable hind legs of the animal, yet contain only about 70 axons.[42,43] Stimulation of the N5 nerve leads to a highly-reproducible biomarker in the form of evoked leg motion, which can be finely tracked with video image analysis.[44] The reason for this reproducible evoked biomarker is that the largest muscle in the leg, the extensor tibiae, responsible for jumping, is controlled by only three neurons. Of the three, the fast extensor tibiae motoneuron (FETi) has an axonal cross section three times larger than any other neuron inside the N5 nerve, thus it is preferentially stimulated and the downstream actuation of the extensor tibiae muscle is a reliable biomarker. The stimulation of the FETi motoroneuron was described by Zurita et al.[44,45] using nerve-cuff stimulation and tracking protocols for the N5 nerve in the locust, which inspired our method to stimulate the N5 target noninvasively using TIS. We applied two electrode pairs aligned with the N5 nerves so that opposite electrode pairs are oriented obliquely with respect to the axon direction. This way, the midway regions between the two electrode pairs where modulation index is maximal will be aligned over the N5. Using TIS in this configuration, we were able to evoke robust actuation of leg motion, using envelope frequencies between 0.1 – 2 Hz. We next compared TIS using this optimized 4-electrode placement for stimulating the N5 nerve, versus a 2-electrode arrangement where the stimulation electrode was placed directly above the N5 nerve (Figure 2a). The tested frequency range of the applied kHz signal, or carrier signal, was 500-12500 Hz. As the biomarker for stimulation, we evaluated the motion of the leg, using video to track movement. In the 2-electrode arrangement, the following types of electrical signals were tested: 1) An amplitude-modulated sine wave (AM-SW), or 2) a burst-modulated sine wave (SW burst) (Figure 2b). The Δf, or modulation frequency, f_{mod}, in the case of AM-SW and SW burst, was set to 1 Hz for all experiments. In comparing the three stimulation types, TIS, AM-SW, and SW burst, we qualitatively found that each type produced the same type of motor response in the locust leg. We next quantified the threshold stimulation current in order to achieve a full leg extension for each stimulation pattern, and testing frequencies in the range from 500 Hz to 12500 Hz. With increasing frequency, higher current was required to reach threshold (Figure 2b). All three stimulation types demonstrated the same current threshold/frequency dependence (s-f curve). The essentially identical qualitative and quantitative features between all three stimulation types indicates that, at the mechanistic level, TIS works by delivering kHz stimulation. The observation that TIS and kHz stimulation have the same s-f dependence indicates that both are the same type of kHz stimulation. It is important to underscore that the current threshold values we report are for one carrier only, that is, to achieve the same stimulation effect with TIS versus the 2-electrode modalities, twice as much total current must be used.

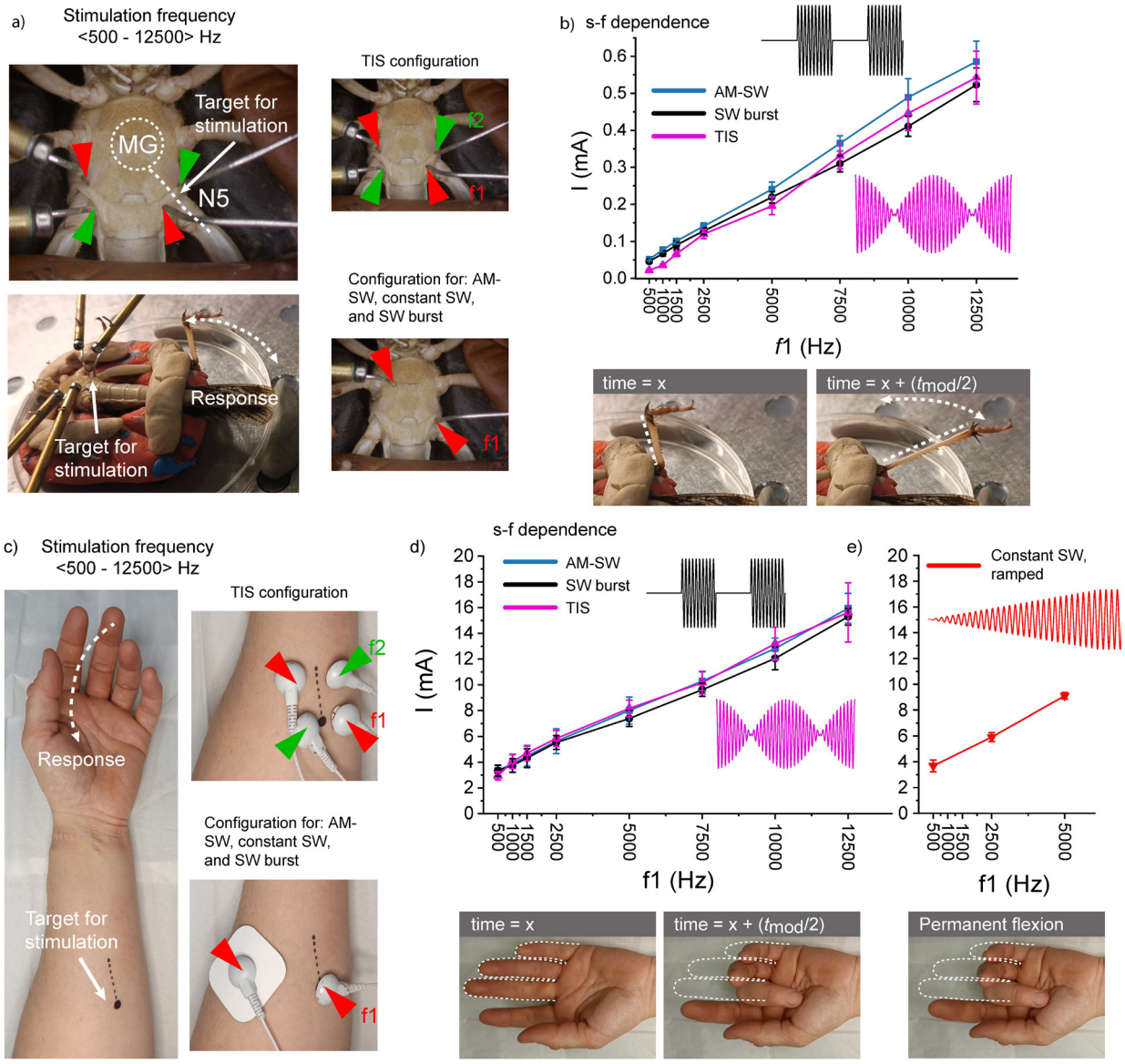

Figure 2. Comparison of strength-frequency (s-f) for TIS versus AM-SW and SW burst for stimulation in insect and human model. for N=3 locusts. a) Experimental setup for stimulation and quantifying response using video recording of evoked leg extension (*extensor tibiae* muscle) caused by stimulation of the N5 nerve, which is the chosen biomarker for stimulation. The stimulation electrode configurations for AM-SW and SW burst are shown, together with the 4-electrode TIS configuration. b) Current threshold/frequency dependence for N5 nerve stimulation. Three stimulation waveforms were tested: AM-SW, SW burst, and TIS, with the modulation frequency set to 1 Hz. The current value applies to one current source only, *i.e.* one carrier in the case of TIS. The inset image shows the evaluated biomarker: leg extension, which occurs at one-half the modulation period. c) Stimulation of the median nerve, comparing TIS with AM-SW and SW. Stimulation of the median nerve in the forearm results in reproducible motor response in the form of finger flexion. As a biomarker for stimulation, we track flexion of the middle finger. For tests shown in a-c, we arbitrarily define threshold response as the middle finger flexing until it contacts the palm. Two electrode placement configurations were used for median nerve stimulation. For AM-SW, constant SW, and SW burst, two-electrode arrangement, 10 mm ⌀ primary

electrode gel-assisted AgCl, versus large ground 4×4 cm pad. For TIS, two-pairs of 10 mm ⌀ gel-assisted AgCl electrodes. d) s-f dependence experiment: Current threshold value for obtaining finger flexion to the palm of the hand (evoked flexion motion shown in inset photograph) as a function of stimulation current frequency in the range 500-12500 Hz, N=3 participants. e) Stimulation current threshold at 500, 2500, and 5000 Hz for sustained tetanic flexion evoked by a constant SW stimulus. The current magnitude is ramped from 0 over 30 seconds. Here the current threshold values overlap with the s-f dependence found for the other kHz waveforms.

We next extrapolated the experiment of comparing TIS with direct kHz stimulation to human subjects. As a convenient stimulation target, we chose the median nerve in the forearm. The biomarker evaluated to quantify stimulation is flexion and movement of the middle finger, where threshold is defined as the current needed to achieve complete flexion and contact of the finger to the palm of the hand (Figure 2c). For TIS, the median nerve was optimally targeted by placing opposite stimulation pairs such as to have the nerve lying along the midline between the electrodes, analogous to the electrode placement used for the locust N5. For AM-SW, SW burst, or constant SW stimulation, the electrode was placed directly over the median nerve, and stimulation was applied versus a relatively large ground electrode. The electrode placements and stimulation conditions are shown in Figure 2c. We performed stimulation using carrier frequencies between 500-12500 Hz, first with a f_{mod} (=Δf) of 0.25 Hz, increasing stimulation current from 1 mA until threshold motor activation was reached (except for constant SW, where stimulation was ramped from 0). The s-f dependance for all stimulation modes was found to be the same (Figure 2d). As before with the locust model, qualitatively the movement evoked by each stimulation was the same. Participants reported that the sensation accompanying AM-SW and TIS was essentially identical, with minimal or no skin sensation at the site of stimulation. The SW burst was accompanied by obvious skin sensation, and was subjectively less comfortable than the AM-SW and TIS stimulation patterns. Nevertheless, these results evidence that TIS operates mechanistically via kHz stimulation. It is important to note that once again, as with the locust model, the current thresholds are reported with respect to one current source only, thus to achieve the same stimulation outcome, TIS must use ×2 the current as the two-electrode configuration of AW-SW or SW burst. We wanted to interrogate the stimulation effect of applying a single unmodulated sine wave. We applied 500, 2500, 5000 Hz stimulation, which was slowly amplitude-ramped from zero amplitude over 30 seconds, to prevent any contamination of the experiment from a low-frequency on-off artefact (Figure 2e). We observed a sustained tetanic contraction of the fingers in all participants, finding similar current threshold values as for the amplitude-modulated waveforms. It should be noted that for the single sine wave ramp experiment, there was high variability between participants in terms of subjective feeling. All participants reliably had a sustained contraction lasting at least tens of seconds. It is known from the literature that sustained kHz tonic stimulation leads to tetanus, and eventually some form of nerve block, and that there are many factors which affect the transition from stimulation to block. An in-depth consideration of nerve block processes is beyond the scope of this study. The point of this sustained sine wave stimulation experiment is here to illustrate that the current threshold required for phasic stimulation via AM-SW/SW-burst/TIS has the same s-f relation as for tonic stimulation. The difference between the phasic/tonic (and eventual nerve block) is only in terms of how long the stimulation signal is applied. This question of phasic/tonic threshold will be elaborated further in upcoming sections of this article.

AM envelopes constructed with carrier frequencies in the range 0.1 – 1 MHz do not lead to phasic stimulation

To test the hypothesis that TIS is in principle a type of kHz stimulation and no other envelope demodulation mechanism is occurring we performed an additional experiment to simply extrapolate the s-f curve to even

higher frequencies. Using high-frequency voltage sources (see experimental methods) we attempted to apply TIS using carriers in the range of hundreds of kHz and even up to 1 MHz. Using such high frequency carriers, it was possible to easily measure low-frequency AM envelopes inside of the mesothorax using a recording electrode. However, we found that as frequency was increased, more and more current had to be applied to get the same stimulation (as expected from the s-f relation). By 100 kHz, response was only visible using > 3 mA, and driving voltages in excess of 10V (with this range of power dissipation we began to damage the insect tissue). The fading of neural response at higher frequencies, and transition into a thermal dissipation regime, is reported in several studies of kHz neurostimulation.[29,36] This disappearance of response, while the AM envelope remained at frequencies of 0.25-2 Hz, is evidence pointing towards the conclusion that TIS acts via "conventional" kHz stimulation and there is not another mechanism of low-frequency envelope demodulation.

Varying amplitude modulation frequency Δf has limited impact on the suprathreshold stimulation current threshold

We next performed a stimulation experiment in locusts, with varying the beat frequency Δf (0.1 – 1 Hz) while keeping the carrier frequency fixed at 2500 Hz. At $\Delta f = 1$ Hz, we fixed the stimulation amplitude to the lowest threshold for detectable movement, which was found to be 90-100 μA. We monitored the deflection angle, θ, of the femoral-tibial joint, defining Δθ as the difference between the unstimulated resting angle and the maximum deflection angle evoked by stimulation (Figure 3a). Stimulation at the minimum threshold current at $\Delta f = 1$ Hz gives a Δθ of 5°, and a phasic movement with a period of 1 second. As Δf is lowered, the value of Δθ increases. By $\Delta f = 0.1$ Hz, the Δθ has increased to 34°, with a phasic movement every 10 seconds. What is apparent is that the lower the beat frequency is, the longer the N5 nerve is subject to kHz stimulation, thus causing the *extensor tibiae* muscle contraction to be sustained for a longer period, giving a larger Δθ. This point is made more evident by decreasing the $\Delta f = 0$ Hz, that is, stimulation with unmodulated 2500 Hz. The leg then deflects to a maximum Δθ of 57° and remains at this position in a tetanic contraction. This experiment aligns with the original observation of Gildemeister,[31] that the stimulation effect is proportional to the duration of application of kHz pulses, known as the temporal summation effect. The effect of having the stimulation set to the threshold for suprathreshold kHz stimulation and the transition between tonic and phasic stimulation can be neatly demonstrated as shown in Figure 3b: For two-electrode stimulation, the output currents of f_1 and f_2 with $\Delta f > 0$ Hz are set to be equal (the signals are added up to create a premodulated kHz signal), and to the threshold for evoked phasic movement of the leg. If one of the two frequencies is turned off, tonic stimulation will result, and the leg remains extended in tetanic contraction. This shows that, for the FETi motorneuron, the threshold current for the phasic stimulation and the tonic stimulation are exactly equal. The same effect can be recreated in 4-electrode TIS by setting of f_1 and f_2 to threshold, and then manually moving one of the stimulation electrodes over the N5 nerve, instead of having the N5 nerve optimally at the midpoint between the electrode pairs. With the electrode over the N5, the stimulation is tonic, as the electrode is moved to the side by just a millimeter, the stimulation becomes phasic. This balance between zones of tonic/phasic stimulation will be discussed in detail in the following section of this article.

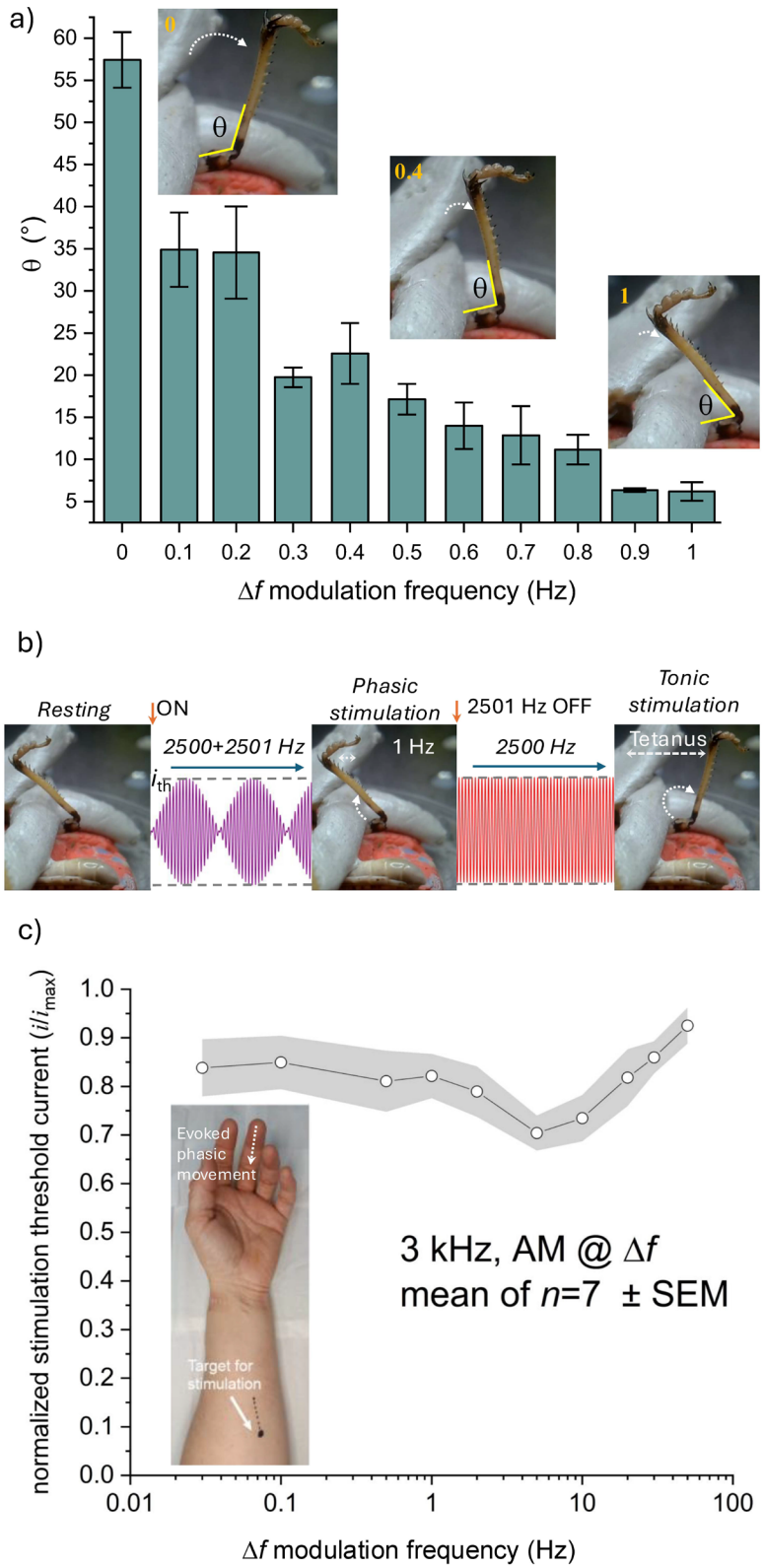

Figure 3. Stimulation thresholds as a function of amplitude modulation frequency Δ*f*. *a*) Leg deflection angle, $\Delta\theta$, caused by *extensor tibiae* actuation, as a function of modulation frequency Δf. Different Δf give

phasic contraction, while $\Delta f = 0$ causes tetanic contraction. Experiment is performed in 2-electrode AM-SW mode with a fixed frequency of 2500 Hz and fixed max current amplitude of 98 μA, which is the minimal threshold for movement at $\Delta f = 1$ Hz. b) This experiment involves applying an at-threshold stimulus via AM-kHz, and then turning off the modulation, while keeping the current setpoint constant. This test shows that threshold current for the phasic stimulation and the tonic stimulation are equal. c) Effect of amplitude modulation frequency Δf on median nerve stimulation. The graph shows the dependence of current threshold of evoked muscle contraction on the envelope frequency, for a fixed stimulation frequency of 3000 Hz. The characteristic U-shaped response predicted by simulations is demonstrated.

The experiment shown in Figure 3a can be regarded as a good demonstration of the Gildemeister effect. The model of nonlinear membrane conduction properties which result in rectification of sinusoidal signals also predicts that this rectifier circuit responds to an optimum modulation frequency, aka the "preferred frequency" of the neuron.[13] That is, a TIS frequency that gives the lowest activation current threshold.[13] For myelinated axons, the model predicts a U-shape of current threshold versus AM frequency, where an optimal minimum of current threshold will exist at a given AM frequency, and very low or very high AM frequency will lead to higher current thresholds. This optimal AM frequency is predicted to be in the range of 5-40 Hz. Experimental data, however, suggest that this effect may be weak in peripheral nerve stimulation. In his 1939 paper, Katz reported no threshold difference between rapid and slow amplitude ramps on kHz stimulation of the frog sciatic nerve.[30] Budde et al.[22] recently demonstrated this U-curve with AM-kHz stimulation of the sciatic nerve, with dependence roughly ±10 – 20% lower thresholds for $\Delta f =$ 10-50 Hz than for very slow or fast AM. In the stimulation experiments on the locust nerve, we did not observe a U-shaped curve, but rather stimulation threshold tended to decline as AM frequency approached zero (=unmodulated sine wave, Figure 3a). However, unlike the axons considered in the model, the locust neurons are unmyelinated. We performed an experiment to investigate the effect of AM frequency on stimulation threshold on the human median nerve, interrogating the lowest current amplitude needed to evoke the smallest perceptible motion (verified visually), and we did indeed observe a shallow U-shaped dependence on current threshold with an apparent optimal beat frequency of $\Delta f = 5$ Hz (Figure 3b), where threshold was lower by roughly 20% compared to low or high AM frequencies.

TIS intrinsically gives bimodal stimulation with tonic and phasic regions, while two-electrode kHz stimulation is monomodal

In TIS stimulation, the kHz carriers are unmodulated, and amplitude modulation arises due to interference in the tissue. As a general rule, maximum depth of AM will occur at midpoints between electrode pairs. This configuration means, however, that regions of tissue superficial to the point of maximum AM will be exposed to kHz carrier stimulation with a lower index of modulation. Moreover, since amplitude will fall with distance from the stimulation electrode, these areas will be exposed to relatively high unmodulated kHz carrier. This will thus cause regions of phasic simulation (AM region where interference is optimal), and regions of tonic stimulation (regions closer to the stimulation electrodes). This intrinsic issue has been pointed out by other researchers.[13,22,38] The electric field magnitudes of these unmodulated regions versus areas of high AM corresponding to phasic stimulation can be visualized using finite element models. The relevant activating function A (second-order spatial derivative of the electric potential along the nerve)[46] generated by stimulation currents can be calculated for a given geometry. In the case of TIS for the median nerve in the forearm, we can calculate activating function and plot its amplitude (Figure 4a-c). To quantify and visualize the stimulation response mode (tonic, phasic, or subthreshold) in the various locations in the tissue we introduce the stimulation mode index (SMI) defined as:

$$SMI = \begin{cases} 0 & \text{if} \quad A_{MAX} < A_T \\ \dfrac{A_{MAX} - A_T}{A_{MAX} - A_{MIN}} & \text{if} \quad A_{MIN} \leq A_T \leq A_{MAX} \\ 1 & \text{if} \quad A_{MIN} > A_T \end{cases} \tag{1}$$

where A_{MIN} and A_{MAX} are the minimum and maximum values of the activating function envelope and A_T is the threshold activation function value for stimulation. We obtained the value for the stimulation threshold A_T in the FEM simulation by using experimental current threshold measurements from Figure 2d. For 1 kHz carrier frequency calculated stimulation threshold A_T is 2000 V/m^2. SMI value 0 represents unstimulated region, value 1 tonic region and values between 0 and 1 define the phasic response region. SMI for different values of stimulation currents I_1 and I_2 is shown in Figure 4d). It reveals regions nearby electrodes, which have a high unmodulated activating function (SMI=1). Nervous tissue in these regions would be subject to a tonic stimulation effect. In between the electrodes, there are regions of maximum modulation index of the activating function. Here, phasic stimulation will be observed at the beat frequency Δf. These calculations align with what is observed experimentally, as shown in Figure 2c. The tonic/phasic transition can be observed in a simple way by shifting the position of the electrodes on the skin such that one of the electrodes is positioned over the median nerve directly, which results in tonic stimulation and sustained contraction.

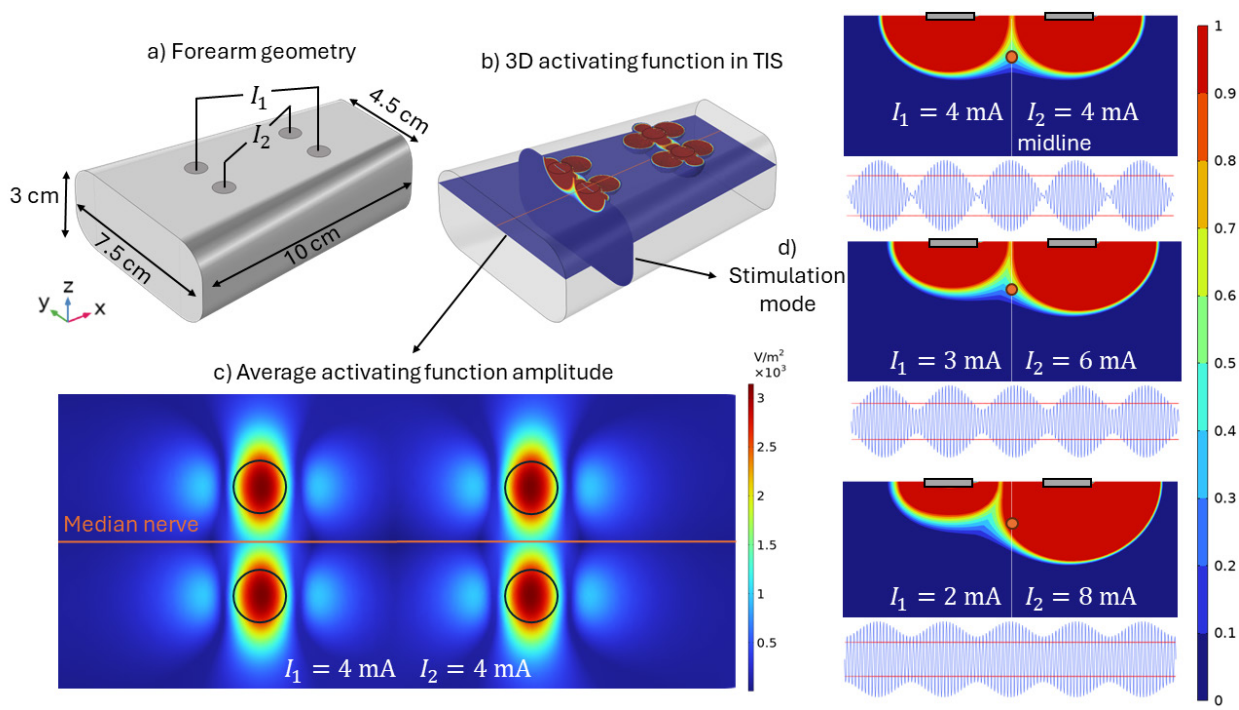

Figure 4. Simulated tonic versus phasic stimulation regions in the model human forearm. a) Geometry used for simulation, reflecting the experiment with the human median nerve. Electrodes placed on the forearm are depicted in gray, the nerve is orange. b) 3D image of the calculated activating function (second-order spatial derivative of the electric potential) in x direction. c) Horizontal cross section showing the average activating function at the nerve depth. Electrode positions are outlined in black. d) Stimulation mode index (SMI, Eq. 1) in vertical cross section for three different stimulation current ratios. The tonic

region (SMI = 1) is shown in red, unstimulated region in blue (SMI = 0), and phasic regions (0 < SMI < 1) are found at between electrode pairs. Corresponding time dependance of the activation function is plotted at the median nerve location depicted as an orange circle. The horizontal red line is the stimulation threshold of 2000 V/m^2. In the red region the activating function is never below the stimulation threshold. In the blue region the activating function is never above the threshold, while in the in-between the regions the activation function goes above or below the threshold at the TIS beat frequency Δf.

Since we have observed the essential equivalence of stimulation effects with TIS and AM-kHz, it can be useful to apply finite element modeling to visualize key similarities and differences between the two stimulation approaches (Figure 5). We make a simple geometry to illustrate some general points: Assuming a simple uniformly-conductive medium with circular symmetry, one can see that performing 4-electrode TIS or 2-electrode AM-kHz will lead to equivalent stimulation in the center of the model: a phasic stimulus with the same amplitude and modulation index. The critical difference is that TIS will require 4 electrodes and thus twice as much total current injection, and will create regions of relatively high-intensity tonic stimulation below the respective electrodes (bimodal stimulation). In 2-electrode AM-kHz, the regions below the stimulation electrodes are exposed instead to phasic stimulation (or in any case: necessarily monomodal). It should be noted that in practical experiments, like we have done in Figure 2, one of the two electrodes in AM-kHz can be much larger than the primary stimulation electrode, thus limiting unwanted stimulation at that electrode site by decreasing current density.

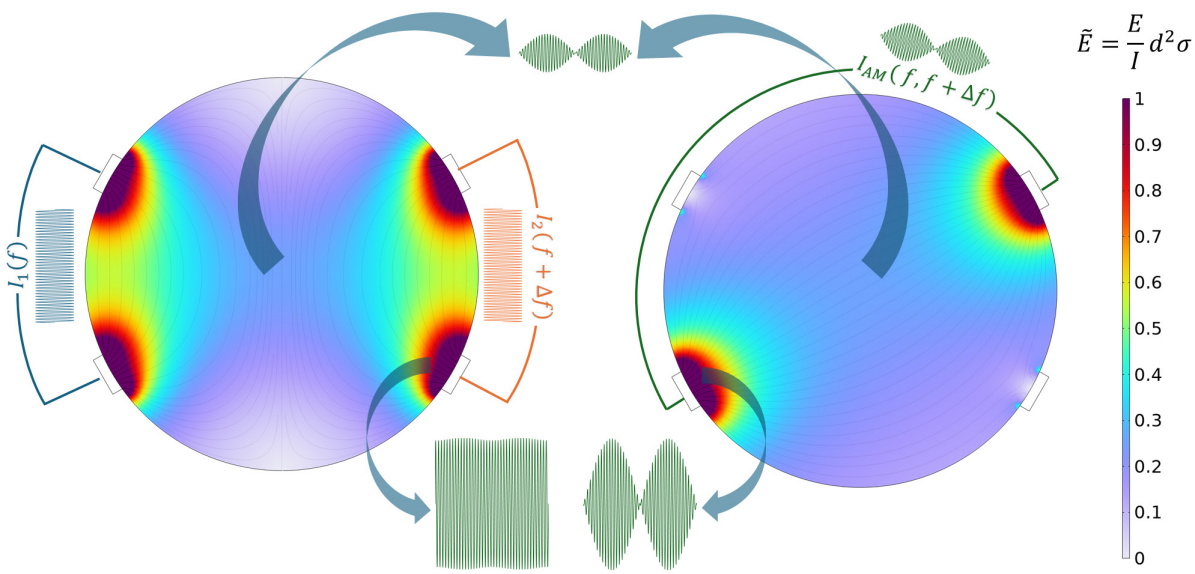

Figure 5. Generalized calculation of the scaled electric field Ē, comparing TIS with a 2-electrode AM-kHz stimulation in a circular medium of diameter *d*, conductivity σ, stimulation current *I* and electric field amplitude *E*. The value of the scaled electric field Ē is only a function of the geometry and is independent on the chosen values of *I*, *σ,* or spatial scale *d*. This picture explains why TIS will always result in higher amplitudes of unmodulated carriers superficial to the region of maximum amplitude modulation where phasic stimulation is delivered. Both stimulation methods lead to the same amplitude of phasic stimulation in the middle of the medium.

3. Discussion and conclusions

The TIS method has been proposed as a noninvasive electrical stimulation technique which can yield focused stimulation at a target region at-depth. In many recent papers describing the use of this method, it is assumed at the outset that the kHz frequencies used as carriers are not able to elicit stimulation on their own, at least not at the amplitudes being used in the experiments. The assumption is often worded that kHz frequencies used in TIS are "too high to drive effective neural firing" or that neurons "cannot follow high frequencies". However, such a formulation can be misleading. Symmetric periodic signals in the kHz range will lead to net depolarization of neurons and can in fact stimulate. Moreover, the time of application of the signal matters, due to the principle known as "temporal summation", the "summation effect", or "Gildemeister effect", where multiple successive subthreshold stimuli result in net suprathreshold stimulation.[33,47] While the notion that kHz frequencies cannot lead to net stimulation is not correct, it is possible that under the conditions used for TIS, the kHz carrier is indeed subthreshold for direct stimulation of action potentials, and suprathreshold amplitude is only reached in regions AM caused by interference of multiple carriers. Our experiments show, however, that for peripheral nerve targets this does not appear to be correct. We find, in two different peripheral nerve models (in an insect model and in humans), that direct neurostimulation is driven directly by the kHz stimulus itself, and that the threshold currents for TIS versus a single sine wave appear to be the same, *i.e.* the characteristic s-f curve which is established in the literature. Since TIS operates by delivering a kHz stimulus, this also implies that the idea behind higher "depth" possible with TIS versus "conventional" biphasic stimulus waveforms (which have similar phase duration) is not correct. The stimulus phase duration of a biphasic pulse lasting 200 μs is equal to that of a periodic signal of 5 kHz: with respect to tissue impedance these two stimuli encounter the same impedance. At least in the case of the simple evoked motor responses from stimulating peripheral nerve models, we see that TIS is essentially equivalent to kHz stimulation. This encourages the idea to construct AM via interference of much higher frequency carriers, as such frequencies are less likely to stimulate themselves in will in fact benefit from lower skin impedance. By using carrier frequencies into the 0.1-1 MHz range, we found that low-frequency AM envelopes generated from such signals do not in fact stimulate peripheral nerves, which provides further evidence that TIS is biophysically equivalent to kHz nerve stimulation.

The fundamental challenge with TIS lies in the rectification mechanism that demodulates the TIS signal. This rectification occurs not only in the targeted region of high amplitude modulation (AM) between the electrode pairs but also in areas close to the electrodes where the AM is low. Crucially, the demodulation of the unmodulated signal does not result in zero transmembrane current; instead, it produces a constant cathodic DC transmembrane current. This constant cathodic current can activate nerves in various modalities, including phasic, tonic, or even cause nerve block effects. Consequently, it becomes impossible to avoid off-target effects near the electrodes using the TIS principle. The issue is not about neurons responding to high-frequency stimuli—such responses are necessary for TIS to function, as the TIS signal comprises only high-frequency components. Rather, the problem arises from the neurons' response to the resultant cathodic DC current near the stimulation electrodes, which poses a challenge for precise targeting.

Therefore, the picture that emerges from our experiments is that TIS is a form of kHz stimulation. The region of AM formed by interference does indeed lead to phasic stimulation at the offset frequency Δf, as the kHz stimulus signal is being modulated between a subthreshold and suprathreshold amplitude. However, the important corollary to this is that regions superficial to that AM hotspot will necessarily be exposed to higher amplitudes of kHz carrier, which could result in a tonic stimulation effect in this region. The confounding effects of TIS resulting in tonic versus phasic stimulation regions has been discussed in the context of brain stimulation and peripheral nerves.[13,22,38] However, via comparing different modulated waveforms and unmodulated SW with TIS, our work shows definitively for the first time that the phasic

and tonic stimulation are equivalent in terms of biophysical mechanism of kHz stimulation, with overlapping s-f curves.

Therefore, TIS can be useful in delivering bimodal tonic and phasic stimulation, and two competing effects should be considered: 1. The optimal AM frequency effect giving lower thresholds than for the unmodulated carrier, the so-called U-curve, and 2. The geometry of the electrodes and tissue, where electric fields will decline inversely with distance. An advantage of TIS over a 2-electrode kHz stimulation configuration is the opportunity to use current ratios to steer the position of AM hotspots, and/or exploiting the 180-degree phase shift that will exist between hotspot regions perpendicular to electrode pairs. These features allow interesting degrees of control of phasic stimulation. These advantages have been nicely documented by Budde et al.[22] However, this has to be considered in the context of the intervening tissue, which is under such conditions necessarily exposed higher amplitudes of kHz signal with a lower index of modulation. If the target is a peripheral nerve below the skin, with no intervening nervous structures, the TIS method could be advantageous for noninvasive neurostimulation. However, in situations where intervening nervous structure do exist superficially to the target region, TIS may be problematic to apply with obtaining suprathreshold tonic stimulation as a byproduct. These results indicate that there is always a tradeoff between phasic stimulation at-depth and tonic stimulation at superficial areas. This tradeoff is unavoidable due to the inverse distance governing electric field. In Figure 5, we show a generalized scalable plot which shows how relative electric field ratios between maximum-modulated phasic regions and unmodulated tonic regions will always hold, regardless of the diameter, conductivity, or stimulation current. It should be noted, this tradeoff may be partially offset by the beat-frequency resonance effect giving the U-shape of stimulation threshold, and this effect should merit further study.

In conclusion, while TIS can be a powerful tool to direct stimulation to a region of interest, using electrode geometry and current steering techniques, it is not simply a surrogate for delivering low-frequency stimulation. In the literature, there has been controversy as to the mechanism of how TIS works. Many studies have oversimplified the picture of TIS, maintaining that the high-frequency carriers are inert. Our experiments provide evidence that this picture is incorrect. Our experiments show that the stimulation observed when using TIS can be explained by conventional kHz stimulation models. This mechanistic basis must be considered in planning safe and effective TIS applications. Moreover, in many cases, we would argue that a premodulated kHz stimulation arrangement may provide some key benefits of TIS but in a more straightforward way.

4. Experimental section

Ethics: Experiments on locusts, as invertebrates, do not fall under legislation in the Czech Republic as animal experiments, therefore no special ethical permission is required (Act No. 246/1992 Sb.). Experiments on human healthy volunteers were performed in accordance with approval from the CEITEC Ethical committee for research on human subjects. Stimulation is delivered using the Digitimer DS5 device which is clinically-approved in the EU.

Electrical stimulation and recording hardware: The kHz stimulation experiments were conducted using the Digitimer DS4 (for *in vitro* prototyping and locust experiments), and the Digitimer DS5 (for experiments on human participants). For high-frequency experiments in the range of 15 kHz to 1 MHz, we used A-301HS amplifiers from A.A. Lab systems Ltd. Stimulation electrodes for locust experiments were Ag wires (0.6 mm diameter) with chloridated tips (made via immersion in commercial chlorine bleach solution). For human transcutaneous stimulation, the primary stimulation electrodes always consisted of commercial ECG Ag/AgCl gel-assisted adhesive electrodes. For modulated kHz experiments, we used larger return

electrodes consisting of gel-assisted carbon TENS pads. Stimulation waveforms are generated by a 2-channel function generator (Keysight Technologies EDU33212A). Synchronization of clock-circuit for each of the channels should be performed via the internal software prior to each experimental session. For premodulation of a sine wave stimulus (AM-sine), two protocols were used: either internal AM modulation provided on one channel of the function generator (AM by multiplication), or by having each channel output a carrier and then mixing the carriers in a BNC T-spitter, which was then fed into the DS5 or DS4 (AM by addition, essentially mimicking the AM created in TIS). While we noted no differences in results from the two methods of AM generation, most experiments were carried out by the carrier mixing for convenience and to more faithfully compare with TIS conditions. Both current simulators (DS4 and DS5) suffer from current amplitude roll-off with increasing frequency > 1 kHz, as can be found in the documentation for each device. This effect was described in detail by FallahRad et al.[26] To correct for this roll-off, the actual output stimulation current was always monitored in all experiments by registering voltage drop over a calibrated shunt resistor, using a digital oscilloscope (Picoscope 2206D, input impedance 1 MΩ). All currents reported in this paper are these measured current values.

Stimulation experiments on *Locusta migratoria*: Animals were obtained at a local pet shop and kept in the laboratory in a terrarium with heat lamp with grass and oats *ad libitum*. The only inclusion criteria for performing the experiment were adult individuals with fully-intact anatomy and lack of any obvious superficial injuries. Prior to the stimulation experiment, we anesthetized the locust by cooling to 5 °C for around 30 min. Next, the animal was immobilized with the ventral side facing upward, in a bed made of modelling clay, and allowed to warm up to room temperature prior to starting the experiment. Stimulation was delivered via 2 or 4 Ag/AgCl wires placed onto the cuticle using *xyz* micromanipulators (S-725CRM, Signatone USA). Contact between the wire and the cuticle was enhanced by adding a drop of EKG gel onto the tip of the wire prior to making contact with the cuticle. Video of the leg was recorded using a digital camera (Logitech), deflection angles were calculated using ImageJ. Data were obtained from naïve animals, and individuals were not reused for subsequent experiments with data collection. We would note, however, that the animals survive this experimental sequence without a problem, and can be returned to the terrarium after marking with a colored permanent marker.

Stimulation experiments on human subjects: Subjects were healthy volunteers who expressed written informed consent. The forearm was placed in a standard position on a table, allowing easy observation by the experimenter and comfort for the subject. Electrodes were placed onto the skin and adhered in place using tape. The primary stimulation electrodes were always gel-assisted AgCl EKG electrodes, (Ceracarta), in two-electrode stimulation protocols the ground electrode was a larger TENS pad electrode 4 × 4 cm. Subjects were not told of the specific sequence of stimulation or variable changes, but were not otherwise blinded. In all experimental protocols, stimulation was applied with increasing amplitude until the given threshold movement was achieved.

Computational modeling:

NEURON model (used for Figure 1d) was taken from Mirzakhalili et al.,[13] the model files are published and freely available online at https://github.com/mirzakhalili/Mirzakhalili-et-al–CellSystems-2020. The computation for Figure 1d was made by simulating 1 kHz extracellular axonal stimulation for three different current amplitudes 0 mA (V_{rest}), 2 mA (subthreshold) and 3 mA (suprathreshold). The results were evaluated at node site 45.

Finite-element modeling (Figures 4 and 5): Calculations were done in COMSOL Multiphysics 6.2 using the AC/DC Module and Electric currents physics interface. The study was solved in frequency domain for carrier frequencies of 1000 Hz and 1001 Hz with different amplitudes of stimulation currents I_1 and I_2.

Activating functions are calculated as a second-order spatial derivative of the electric potential along the nerve. The modulation maximum and minimum of the activation function is calculated as $A_{MAX} = |A_1 + A_2|$ and $A_{MIN} = |A_1 - A_2|$ where A_1 and A_2 are activation functions created only by currents I_1 and I_2 respectively. The modulation depth is calculated as $A_{MAX} - A_{MIN}$ and the modulation index is given by $m = (A_{MAX} - A_{MIN})/(A_{MAX} + A_{MIN})$. Forearm tissue is modeled as an anisotropic conductor with the conductivity and the dielectric constant parallel to the muscle fibers (x direction) $\sigma_x = 0.6\ \mathrm{S/m}$ and $\epsilon_{r,x} = 1.3 \cdot 10^6$ and across the muscle fibers $\sigma_y = \sigma_z = 0.35\ \mathrm{S/m}$ and $\epsilon_{r,y} = \epsilon_{r,z} = 6 \cdot 10^5$. Values are taken and adapted from Gabriel et al.[48]

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Author contributions:

A.O. conducted all computational modeling (Figs 1, 4, and 5), with support from V. Đ. P.O., supported by J.T., performed all experiments and data analysis for Figure 2. P.O. made the first observation that thresholds of kHz stimulation appear similar for modulated and unmodulated waveforms. E.D.G. and J.T. performed experiments and data analysis for Figure 3. V.Đ. and E.D.G. supervised the work and obtained funding. A.O., V.Đ., and E.D.G. conceived the project idea and performed conceptualization. The manuscript was written by E.D.G. with input from all authors.

References:

1. Krames, E. S., Peckham, P. H. & Rezai, A. R. *(eds) Neuromodulation (Academic Press, 2009); https://doi.org/10.1023/B:MYCO.0000003704.30293.b6*

2. Peterchev, A. V. *et al.* Fundamentals of transcranial electric and magnetic stimulation dose: Definition, selection, and reporting practices. *Brain Stimul.* **5**, 435–453 (2012).

3. Huang, Y. *et al.* Measurements and models of electric fields in the in vivo human brain during transcranial electric stimulation. *Elife* **6**, 1–26 (2017).

4. Ganne, J. M. Interferential Therapy. *Aust. J. Physiother.* **22**, 101–110 (1976).

5. Goats, G. C. Interferential current therapy. *Br. J. Sports Med.* **24**, 87–92 (1990).

6. Stone, J. A. 'Russian' Electrical Stimulation: The early experiments. *Athl. Ther. Today* **2**, 1019–1030 (1997).

7. Ward, A. R. Electrical stimulation using kilohertz-frequency alternating current. *Phys. Ther.* **89**, 181–190 (2009).

8. Petrofsky, J., Laymon, M., Prowse, M., Gunda, S. & Batt, J. The transfer of current through skin and muscle during electrical stimulation with sine, square, Russian and interferential waveforms. *J. Med. Eng. Technol.* **33**, 170–181 (2009).

9. Almeida, N., Paladini, L. H., Korelo, R. I. G., Liebano, R. E. & de Macedo, A. C. B. Immediate Effects of the Combination of Interferential Therapy Parameters on Chronic Low Back Pain: A Randomized Controlled Trial. *Pain Pract.* **20**, 615–625 (2020).

10. Grossman, N. *et al.* Noninvasive Deep Brain Stimulation via Temporally Interfering Electric Fields. *Cell* **169**, 1029-1041.e16 (2017).

11. Cao, J. & Grover, P. STIMULUS: Noninvasive Dynamic Patterns of Neurostimulation Using Spatio-Temporal Interference. *IEEE Trans. Biomed. Eng.* **67**, 726–737 (2020).

12. Lee, S., Lee, C., Park, J. & Im, C. H. Individually customized transcranial temporal interference stimulation for focused modulation of deep brain structures: a simulation study with different head models. *Sci. Rep.* **10**, 11730 (2020).

13. Mirzakhalili, E., Barra, B., Capogrosso, M. & Lempka, S. F. Biophysics of Temporal Interference Stimulation. *Cell Syst.* **11**, 557-572.e5 (2020).

14. Missey, F., Rusina, E., Acerbo, E. & Botzanowski, B. Orientation of Temporal Interference for Non-invasive Deep Brain Stimulation in Epilepsy. *Front. Neurosci.* **15**, 633988 (2021).

15. Zhu, Z. & Yin, L. A mini-review: recent advancements in temporal interference stimulation in modulating brain function and behavior. *Front. Hum. Neurosci.* **17**, 1–6 (2023).

16. Violante, I. R. *et al.* Non-invasive temporal interference electrical stimulation of the human hippocampus. *Nat. Neurosci.* **26**, 1994–2004 (2023).

17. Missey, F. *et al.* Obstructive Sleep Apnea Improves with Non-invasive Hypoglossal Nerve Stimulation using Temporal Interference. *Bioelectron. Med.* **9**, 18 (2023).

18. Violante, I. *et al.* Non-invasive temporal interference electrical stimulation of the human hippocampus. *Brain Stimul.* **16**, 408 (2023).

19. Acerbo, E. *et al.* Focal non-invasive deep-brain stimulation with temporal interference for the suppression of epileptic biomarkers. *Front. Neurosci.* **16**, 945221 (2022).

20. Reilly, J. P. *Applied Bioelectricity*. *Applied Bioelectricity* (1998). doi:10.1007/978-1-4612-1664-3.

21. Botzanowski, B. *et al.* Noninvasive Stimulation of Peripheral Nerves using Temporally-Interfering Electrical Fields. *Adv. Healthc. Mater.* **2200075**, 2200075 (2022).

22. Budde, R. B., Williams, M. T. & Irazoqui, P. P. Temporal interference current stimulation in peripheral nerves is not driven by envelope extraction. *J. Neural Eng.* **20**, (2023).

23. Vieira, P. G., Krause, M. R. & Pack, C. C. Temporal interference stimulation disrupts spike timing in the primate brain. *Nat. Commun.* **15**, 11–17 (2024).

24. Zhang, X., Roppolo, J. R., De Groat, W. C. & Tai, C. Mechanism of nerve conduction block induced by high-frequency biphasic electrical currents. *IEEE Trans. Biomed. Eng.* **53**, 2445–2454 (2006).

25. Joseph, L. & Butera, R. J. High-frequency stimulation selectively blocks different types of fibers in frog sciatic nerve. *IEEE Trans. Neural Syst. Rehabil. Eng.* **19**, 550–557 (2011).

26. FallahRad, M. *et al.* Electrophysiology equipment for reliable study of kHz electrical stimulation. *J. Physiol.* **597**, 2131–2137 (2019).

27. Kilgore, K. L. & Bhadra, N. Reversible nerve conduction block using kilohertz frequency alternating current. *Neuromodulation* **17**, 242–255 (2014).

28. Neudorfer, C. *et al.* Kilohertz-frequency stimulation of the nervous system: A review of underlying mechanisms. *Brain Stimul.* **14**, 513–530 (2021).

29. Ward, A. R. & Robertson, V. J. Variation in motor threshold with frequency using kHz frequency alternating current. *Muscle and Nerve* **24**, 1303–1311 (2001).

30. Katz, B. Nerve Excitation By High-Frequency Alternating Current. *J. Physiol.* **96**, 202–224 (1939).

31. Gildemeister, M. Untersuchungen über die Wirkung der Mittelfrequenzströme auf den Menschen. *Pflugers Arch. Gesamte Physiol. Menschen Tiere* **247**, 366–404 (1944).

32. Wyss, O. A. M. Mittelfrequenz-Impulsreizung als Prinzip der Interferenzreizung mit mittelfrequenten Sinusströmen. *Biomed. Tech.* **11**, 144–150 (1966).

33. Bromm, B. & Lullies, H. Über den Mechanismus der Reizwirkung mittelfrequenter Wechselströme auf die Nervenmembran. *Pflugers Arch. Gesamte Physiol. Menschen Tiere* **289**, 215–226 (1966).

34. Sabah, N. H. Rectification in biological membranes. *IEEE Eng. Med. Biol.* **19**, 106–113 (2000).

35. Kaniusas, E. *Biomedical Signals and Sensors I - Linking Physiological Phenomena and Biosignals*. (Springer, 2008).

36. Dalziel, C. F. Electric shock hazard. *IEEE Spectr.* **9**, 41–50 (1972).

37. Patrick Reilly, J., Freeman, V. T. & Larkin, W. D. Sensory Effects of Transient Electrical Stimulation—Evaluation with a Neuroelectric Model. *IEEE Trans. Biomed. Eng.* **BME-32**, 1001–1011 (1985).

38. Wang, B., Aberra, A. S., Grill, W. M. & Peterchev, A. V. Responses of model cortical neurons to temporal interference stimulation and related transcranial alternating current stimulation modalities. *J. Neural Eng.* **19**, 066047 (2022).

39. Patel, Y. A. & Butera, R. J. Challenges associated with nerve conduction block using kilohertz electrical stimulation. *J. Neural Eng.* **15**, (2018).

40. Grimnes, S. Impedance measurement of individual skin surface electrodes. *Med. Biol. Eng. Comput.* **21**, 750–755 (1983).

41. Lin, Q. *et al.* Resilient conductive membrane synthesized by in-situ polymerisation for wearable non-invasive electronics on moving appendages of cyborg insect. *npj Flex. Electron.* **7**, 1–10 (2023).

42. Burrows, M. *The Neurobiology of an Insect Brain*. (Oxford University Press, 1996).

43. Field, L. H. & Burrows, M. Reflex Effects of the Femoral Chordotonal Organ Upon Leg Motor Neurones of the Locust. *J. Exp. Biol.* **101**, 265–285 (1982).

44. Zurita, F. *et al.* In vivo closed-loop control of a locust's leg using nerve stimulation. *Sci. Rep.* **12**, 1–9 (2022).

45. Zurita, F. *et al.* Thermoformed Parylene-C Cuff Electrodes for Small Nerve Interfacing. *Adv. NanoBiomed Res.* **4**, (2024).

46. Rattay, F. Analysis of Models for External Stimulation of Axons. *IEEE Trans. Biomed. Eng.* **BME-33**, 974–977 (1986).

47. Schoen, I. & Fromherz, P. Extracellular Stimulation of Mammalian Neurons Through Repetitive Activation of Na+ Channels by Weak Capacitive Currents on a Silicon Chip. *J. Neurophysiol.* **100**, 346–357 (2008).

48. Gabriel, S., Lau, R. W. & Gabriel, C. The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz. *Phys. Med. Biol.* **41**, 2251–2269 (1996).