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Quantitative Comparisons of Block Thresholds and Onset Responses for Charge-Balanced Kilohertz Frequency Waveforms

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Abstract

Objective: There is growing interest in delivering kilohertz frequency (KHF) electrical signals to block conduction in peripheral nerves for treatment of various diseases. Previous studies used different KHF waveforms to achieve block, and it remains unclear how waveform affects nerve block parameters.

Approach: We quantified the effects of waveform on KHF block of the rat tibial nerve in vivo and in computational models. We compared block thresholds and onset responses across current-controlled sinusoids and charge-balanced rectangular waveforms with different asymmetries and duty cycles.

Main Results: Sine waves had higher block thresholds than square waves, but used less power at block threshold. Block threshold had an inverse relationship with duty cycle of rectangular waveforms irrespective of waveform asymmetry. Computational model results were consistent with relationships measured in vivo, although the models underestimated the effect of duty cycle on increasing thresholds. The axonal membrane substantially filtered waveforms, the filter transfer function was strikingly similar across waveforms, and filtering resulted in post-filtered rms block thresholds that were approximately constant across waveforms in silico and in vivo. Onset response was not consistently affected by waveform shape, but onset was smaller at amplitudes well above block threshold. Therefore, waveforms with lower block thresholds (e.g., sine waves or square waves) could be more readily increased to higher amplitudes relative to block threshold to reduce onset response. We also observed a reduction in onset responses across consecutive trials after initial application of supra-block threshold amplitudes.

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Data availability

We deposited datasets for nerves 1 through 14 to the Duke Research Data Repository. Datasets are publicly available (<https://doi.org/10.7924/r4b27wq4m>), and include raw block threshold currents and power consumption at threshold for in vivo and in silico experiments, along with the onset response AUC values at block thresholds from in vivo experiments.

Significance: Waveform had substantial effects on block thresholds, and the amplitude relative to block threshold had substantial effects on onset response. These data inform choice of waveform in subsequent studies and clinical applications, enhance effective use of block in therapeutic applications, and facilitate the design of parameters that achieve block with minimal onset responses.

1. Introduction

Kilohertz frequency (KHF) electrical nerve block temporarily prevents the conduction of action potentials along a nerve. In contrast to pharmacological agents, KHF nerve block allows nearly immediate block or restoration of nerve transmission by turning on or off high frequency electrical signals, typically at or above 10 kHz (Bhadra and Kilgore 2005; Williamson and Andrews 2005; Patel et al. 2018; Pelot and Grill 2020). The ability to toggle between conductive and blocked states presents substantial clinical opportunities for treatment of a wide range of diseases, such as diabetes, heart failure, and urinary incontinence (Gaunt and Prochazka 2009; Chew et al. 2013; Buckley et al. 2017; Horn et al. 2019; Jiman et al. 2019).

Development of therapies that use KHF nerve block requires an understanding of the effects of block signal parameters on threshold and onset response. Blocking with KHF signals requires amplitudes more than double those needed to evoke activity (Bowman and McNeal 1986; Pelot and Grill 2020), and thus applications require consideration of the potential for tissue damage with high charge densities (Cogan et al. 2016) and of reduced battery life with high power consumption (Patel et al. 2017). An additional challenge is the transient nerve excitation evoked when the KHF signal is turned on (Crosby et al. 2017; Bhadra et al. 2019). This onset response may pose an obstacle for clinical translation because undesired, and potentially uncomfortable activation occurs every time the KHF signal is turned on (Bhadra et al. 2018b), and there are currently no methods to eliminate onset response using solely charge-balanced KHF signals. Electrode contact material (Patel et al. 2018), spacing (Ackermann et al. 2009, 2010), surface area (Patel et al. 2017), and placement (Bhadra and Kilgore 2005) can reduce nerve block thresholds, power consumption, or onset responses, but there remains a need to reduce the high thresholds and the onset response of nerve block protocols.

The effect of waveform shape on block threshold, power consumption, and onset response has not been systematically determined. Most studies used sine waves (Bhadra and Kilgore 2005; Williamson and Andrews 2005; Gaunt and Prochazka 2009; Dowden et al. 2010; Chew et al. 2013; Patel and Butera 2015; Jiman et al. 2019), and others used square waves (Boger et al. 2013; Buckley et al. 2017; Yang et al. 2017) or trains of biphasic rectangular pulses (Waataja et al. 2011). Although there are qualitative comparisons between responses to sinusoids, square waves, and rectangular pulse trains (Tai et al. 2004, 2005b), in vivo quantification of block thresholds across waveforms has not been conducted, and the effects of such waveforms on power consumption or onset response have not been characterized. We measured nerve block in vivo and in silico to evaluate systematically the effect of waveform shape on block threshold current and power consumption, as well as on the characteristics of onset response.

2. Methods

We quantified nerve block thresholds across a range of waveforms in vivo using methods adapted from (Bhadra and Kilgore 2005), as well as in computational models that represented our experimental setup. Data analyses and statistics were conducted in MATLAB R2018a (Mathworks; Natick, MA).

2.1 Animal preparation

Acute, non-survival experiments were performed using 16 adult, male Sprague-Dawley rats and 2 adult, female Wistar rats (nerves 5 and 6) (Charles River Laboratories) (median weight: 430 g, IQR: 404–510 g). All procedures were approved by Duke University's Institute for Animal Care and Use Committee and were in accordance with the Guide for Care and Use of Laboratory Animals (8th edition). Rats were housed under USDA- and AAALAC-compliant conditions, with 12 h/12 h light/dark cycle and free access to food, water, and environmental enrichment. On the day of the experiment, rats were briefly (~2–4 min) anesthetized with 3% isoflurane in air, then injected subcutaneously with 1.2 g/kg urethane. Supplementary doses of urethane were delivered through subcutaneous, intramuscular, or intraperitoneal routes to ensure the rat reached and maintained surgical depth, which was assessed using the toe pinch reflex and heart rate. Heart rate and blood oxygenation were monitored continuously using a pulse oximeter (PalmSAT 2500A; Nonin Medical; Plymouth, MN, USA). Body temperature was monitored using a rectal temperature probe (TH-8 Thermalert; Physitemp Instruments, Inc.; Clifton, NJ) and maintained between ~35–38°C with a heated water blanket.

The experimental setup is shown in Figure 1(a). An incision was made on the left hind limb (both hind limbs in one rat (nerves 1 and 2)) from the distal dorsal ankle to 1 cm rostral to the ipsilateral hip joint. The muscle overlying the gastrocnemius was cut parallel to the skin incision to expose the gastrocnemius and the sciatic nerve. The gastrocnemius was dissected from the tibia. The Achilles tendon was dissected and cut at its distal end, and the tendon was tied to a force transducer using umbilical tape. The connective tissue surrounding the sciatic nerve was dissected from ~0.5 cm caudal to the spinal cord to the branching point into the tibial, common peroneal, and sural nerves. The common peroneal and sural nerves were transected, along with the branches of sciatic nerve innervating the hamstring. The tibia was secured at its caudal end by a plastic clamp that was attached to the experimental table.

2.2 Electrodes for stimulation and block

A tripolar cuff electrode was placed on the sciatic nerve for stimulation (X-Wide Contact Cuffs, Microprobes; Gaithersburg, MD), and a bipolar cuff electrode was placed on the tibial nerve for the block signal. The tripolar cuff (1 mm inner diameter) contained three Pt(90%)-Ir(10%) ribbon contacts (0.5 mm wide) spaced 1 mm apart edge-to-edge; the cuff was 6.5 mm in length total, including 1.5 mm of silicone beyond the outer edge of each outer contact. The bipolar cuff (1 mm inner diameter) contained two Pt(90%)-Ir(10%) ribbon contacts (0.5 mm wide) spaced 1 mm apart edge-to-edge; the cuff was 5 mm in length total, including 1.5 mm of silicone on each end. The silicone thickness of both cuffs was 0.875

mm. After implantation and before every experiment, we measured the impedance between the middle contact and the shorted outer contacts of the tripolar cuff (median impedance at 10 kHz: 1.2 k Ω , IQR: 1–2 k Ω) and the bipolar cuff (median impedance at 10 kHz: 2.4 k Ω , IQR: 2–2.9 k Ω). After placement, the two cuffs were spaced ~0.5–1 cm edge-to-edge in nerves 1–9, 11, and 13–14. In the remaining seven nerves, both the tripolar stimulating cuff and the bipolar blocking cuff were placed on the sciatic nerve rostral to the nerve branching point, such that the cuffs were spaced ~0.2–0.5 cm edge-to-edge. Measurements from nerves 1–14 were used for results in sections 3.1, 3.2, 3.3, and 3.5, and measurements from nerves 15–18 were used for section 3.4. Measurements from nerve 19 were used to construct the filter for section 3.6.

2.3 Hardware for electrical stimulation and block

A diagram of the electrical stimulation and block hardware is shown in Figure 1(b). We used the PowerLab/4SP (ADInstruments Inc.; Sydney, Australia) to send biphasic symmetric test pulses (1 Hz, 0.2 ms/phase, positive phase first) to an A-M Systems 2200 stimulus isolator (Sequim, WA). As illustrated in Figure 1(b), the isolator's positive output was connected to the middle contact of the tripolar stimulation cuff, while the negative output was connected to two shorted outer contacts. The stimulation amplitude current was set to evoke maximal muscle twitches (~0.7 mA).

We generated KHF waveforms using a Keithley 6221 AC and DC current source (Tektronix, Inc; Beaverton, OR). Arbitrary waveforms were transmitted from a controlling PC to the current source via a National Instruments VISA connection. When applying 2 mA to a load >100 Ω with the Keithley 6221, the amplitudes of high frequency components were substantially attenuated, with greater reduction for larger loads. Since the typical load in this study was ~2–3 k Ω , this limitation resulted in noticeable waveform smoothing and amplitude reduction. To address this issue, we applied the Keithley 6221 output through a 100 Ω resistor, and then used the voltage across the 100 Ω resistor as the input signal to an A-M Systems 4100 Programmable Isolated High-Power Stimulator (Sequim, WA) with 10x gain. The A-M Systems 4100 exhibited a less pronounced reduction in high frequency components with large loads (Supplementary Figure 1). We monitored the KHF signal by recording the voltage across a 100 Ω resistor in series with the bipolar cuff using a battery-powered oscilloscope (Fluke 190-062 ScopeMeter® Test Tool; Fluke Corporation; Everett, WA, USA).

2.4 Removal of dc offset

A high pass filter was applied to both the test pulses and the KHF signal before delivery to the electrodes (Figure 1(b) and (c)). We adapted the two-capacitor and two-inductor design described in (Franke et al., 2014), using 1 μ F capacitors and replacing each of the inductors with 100 k Ω resistors. For the test pulses, we used only one capacitor in series with the load, and one resistor in parallel with the isolator terminals. The resistors provided a sufficiently high impedance to ensure that most of the current entered the load (for our loads of ~2–3 k Ω) while also providing sufficiently low impedance to generate only small changes in isolator output voltage in response to small dc currents. This resistor-based approach was chosen over an inductor-based approach because the frequency response of the inductor can

distort the waveform and confound evaluation of waveform shape effects on block threshold. We validated the use of resistors rather than inductors by comparing block thresholds for a 20 kHz sinusoid with two circuits: (1) using the circuit in (Figure 1(c)), and (2) replacing the resistors with inductors (LHL10TB124J, Taiyo Yuden) totaling 1.2 H on each parallel branch. The resistor circuit yielded a 5% increase in block threshold over the inductor circuit in our single test, and this was within the range of normal variability of block threshold measurements.

2.6 Force recordings

Force recordings were conducted with a transducer containing four strain gauges in a bridge configuration. The output signal was recorded differentially into a bioamplifier (ETH-255; CB Sciences Inc.; Dover, NH) with 10x gain. The amplified signal was digitized at 200 samples per second with a PowerLab/4SP and then digitally low pass filtered below 50 Hz in LabChart, which also displayed the force readings in real-time.

2.7 Experimental protocol

We quantified nerve block across three groups of waveforms. Group 1 consisted of sine waves and square waves (Figure 1(d)). Square waves were symmetric biphasic waveforms consisting of an initial rectangular pulse at one polarity immediately followed by a rectangular pulse of equal amplitude and opposite polarity, with each phase having a 0.5 duty cycle. In Groups 2 and 3, we evaluated the effect of the first phase duty cycle on block with biphasic symmetric (Group 2) and asymmetric (Group 3) waveforms, defining these waveforms as:

$$symD = s(D, \theta) = \begin{cases} 1, 0 \leq \theta < \pi D \\ -1, \pi D \leq \theta < 2\pi D, & \theta \in [0, 2\pi) \\ 0, 2\pi D \leq \theta < 2\pi \end{cases} \quad (1)$$

$$aymD = a(D, \theta) = \begin{cases} 1, 0 \leq \theta < \pi D \\ \frac{-D}{2-D}, \pi D \leq \theta < 2\pi, & \theta \in [0, 2\pi) \end{cases} \quad (2)$$

The variable θ , ranging from 0 to 2π , is the periodic time variable expressed in radians. The value D , ranging from 0 to 1, is the duty cycle of the first phase relative to that of a square wave. We defined three Group 2 waveforms according to Equation 1: sym1, sym0.5, and sym0.25 (Figure 1(d)). All Group 2 waveforms were biphasic pulses in which the primary and secondary phases were of equal duration and amplitude. Shortening the phase duration of Group 2 waveforms produced a gap between pulses. We also defined three Group 3 waveforms according to Equation 2: asym1, asym0.5, asym0.25 (Figure 1(d)). All Group 3 waveforms were biphasic pulses in which the primary and secondary phases could have different durations and there was no gap between consecutive pulses. Therefore, shortening the primary phase duration resulted in a longer secondary phase, with a smaller amplitude applied on the secondary phase to maintain charge balance. Note that sym1 and asym1 are the same as the Group 1 square wave.

We measured block threshold with each waveform using a binary search (Figure 1(e)) (Roldan et al. 2019). During each search, we continuously applied 1 Hz stimulus pulses. After a brief period of only stimulus pulses (median: 3.9 s, IQR: 3.6–4.0 s), we applied an initial KHF signal (0.5–3 mA, 10 s), turned off the KHF signal (median: 10 s, IQR: 5–11 s), then applied another KHF signal (10 s) at a higher or lower amplitude depending on whether conduction block was observed. If nerve conduction block did not occur with the initial KHF signal, we repeated the test with increased amplitude until block occurred. A binary search algorithm then guided the choice of subsequent amplitudes, such that the next amplitude was the average of the highest current that allowed transmission and the lowest current that produced complete block. We defined complete block as the absence of gastrocnemius twitches based on inspection of force recordings in real time. We continued this alternating pattern of brief stimulus-only periods and KHF periods iteratively until our binary search converged (median precision: 0.0625 mA, IQR: 0.0313–0.0625 mA). We defined block threshold as the average between the largest transmission amplitude and the smallest blocking amplitude. After each threshold search—consisting of repeated trials to identify threshold for a given waveform—all stimulus pulses and KHF signals were off before initiating a new threshold search (median inter-search off time: 53 s, IQR: 30–214 s). In a few cases, particularly during the first or second threshold searches of the experiment, we could not achieve nerve conduction block due to the presence of persistent onset response, and such onset responses often dissipated by the third or fourth threshold search of the experiment, after which measurement of block threshold became possible in all subsequent threshold searches. In such cases, we repeated any threshold searches in which we could not initially measure block threshold.

We organized our experiments hierarchically by frequency, waveform groups, threshold searches, and trial amplitudes. First, we randomized the frequency to either 10 kHz or 20 kHz. Next, for the selected frequency, we randomized the order of testing for waveform groups 1, 2, and 3. We then randomized the order of the waveforms in each group and conducted a threshold search for each waveform. Each threshold search consisted of multiple KHF signals at different amplitudes (i.e., trial amplitudes), using the binary search algorithm described above. In the first three nerves (nerves 1–3), we only tested Group 1, running tests at both 10 kHz and 20 kHz. In the next three nerves (nerves 4–6), we tested both Groups 1 and 2, running tests only at 20 kHz. In the subsequent eight nerves (nerves 7–14), we tested Groups 1, 2, and 3 at both 10 kHz and 20 kHz. We achieved nerve conduction block in all nerves tested using this search protocol (sections 3.1, 3.2, 3.3), except at 10 kHz in two nerves (nerves 8 and 14) due to persistent onset responses.

To assess power consumption, we squared each waveform's rms current at block threshold, resulting in power per unit impedance (i.e., mW per k Ω). The rms current reflects the average power present in a signal, in contrast to peak current. We quantified the onset response at block threshold by calculating the area under the curve of the force recording over the 10 s of KHF at the last trial amplitude that caused conduction block.

Rats were euthanized at the termination of experiments with Euthasol (0.5 ml IP; Virbac; Fort Worth, TX, USA) and bilateral thoracotomy within 12 hr of the initial urethane dose.

2.8 Computational model

We implemented a computational model of the in vivo nerve block experiment to quantify the effects of waveform on block threshold current, power consumption, and onset response. We modeled the tissue around the nerve and electrode as a heterogeneous anisotropic volume conductor (Figure 1(f) and (g)). We modeled the contacts of the bipolar cuff as platinum ribbons spanning 270° , embedded in the silicone of the cuff spanning 330° . The model of the nerve was a single, cylindrical fascicle, 0.75 mm in diameter and 50 mm long, consistent with the monofascicular rat tibial nerve (Schmalbruch 1986; Tyler and Durand 2003). The model included a cylindrical muscle region (10 mm diameter) surrounding the nerve and cuff. The space between the inner surface of the cuff and the nerve was modeled as saline. The nerve was placed on the inner cuff surface that was opposite the opening in the cuff, with a $10\text{ }\mu\text{m}$ spacing between the inner cuff wall and the nerve to avoid meshing errors. The material and tissue electrical properties are defined in Table 1. We modeled the perineurium as a thin layer using the contact impedance approach described in (Pelot et al. 2019); the sheet resistance was calculated with the resistivity in Table 1 and a thickness of $22.5\text{ }\mu\text{m}$. We constructed a single finite element model for all frequencies tested, with a single perineurium resistivity defined according to the dc value (Weerasuriya et al. 1984; Pelot et al. 2019).

We used the finite element method in COMSOL Multiphysics v5.3a (Burlington, MA) to solve for electric potentials in the nerve assuming quasistatic conditions. We followed the approach described in (Pelot et al. 2018) to define a point source within the platinum electrodes, with +1 mA applied on the proximal contact and -1 mA applied on the distal contact. The outer surfaces of the muscle layer were set as ground, while the ends of the nerve were defined with zero current boundary conditions. Model elements were tetrahedral with quadratic geometry and solution shape function. We doubled the mesh density until the 10 kHz block threshold of a $5.7\text{ }\mu\text{m}$ axon at the center of the nerve changed $<3\%$ across all waveforms, and the final mesh had 952,587 tetrahedral elements.

We applied the electric potentials from the volume conductor to biophysical models of myelinated axons (McIntyre et al. 2002, 2004) with myelin diameters 5.7, 7.3, 8.7, 10, and $11.5\text{ }\mu\text{m}$ (101, 67, 51, 43, and 41 nodes of Ranvier, respectively) to span the range of efferent fibers reported in the rat tibial nerve (Schmalbruch 1986) that were implemented in NEURON v7.5 (Carnevale and Hines 2006). We made the nodal membrane impedance at the fiber ends linear (passive) and verified that no end-node excitation occurred at our chosen model length (50 mm). Each fiber diameter was simulated using extracellular potentials extracted from the finite element model along the center of the cylindrical fascicle. Simulations were run for 42 ms with a time step of 0.00025 ms. At each time step, we scaled the applied electric potentials by the amplitude of the KHF waveform at that time step. The waveforms simulated were sine, square, symD, and asymD for $D = 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.25, 0.2, 0.15, 0.1$. We performed conduction block tests in the models by applying a suprathreshold monophasic intracellular stimulus pulse at the third node from the proximal end at $t = 32\text{ ms}$, while continuously applying each kilohertz frequency waveform at the block cuff (starting at $t = 2\text{ ms}$), and then monitoring action potentials at the distal 10% nodes of Ranvier (after $t = 32\text{ ms}$ only, allowing onset response to subside). We

conducted binary searches for block threshold until the upper and lower search bounds were within 1% of each other.

3. Results

3.1 Sine waves required higher current but equivalent or lower power to block than square waves

We conducted measurements of block threshold in vivo and in silico (Figure 1), and sine waves had higher block thresholds than square waves (Group 1 waveforms), with in vivo means that were 34% and 28% higher at 10 and 20 kHz, respectively (Table 2; Figure 2(a); paired t-test at 10 kHz: $p = 5e-06$, $t_{stat} = 10.73$, $df = 8$; paired t-test at 20 kHz: $p = 1.94e-05$, $t_{stat} = 6.520$, $df = 13$). Sine waves also had ~30% higher block thresholds than square waves across all model fiber diameters (Figure 2(b)). Although the absolute thresholds differed between models and experiments, the difference between sine and square wave block thresholds was similar when normalized to the square wave threshold (Figure 2(c)).

Sine waves required equivalent (10 kHz) or lower (20 kHz) power than square waves at block threshold, despite requiring higher currents, with in vivo mean power consumption 13% and 18% lower for sine waves at 10 and 20 kHz, respectively (Table 2, Figure 2(d); paired t-test at 10 kHz: $p = 0.113$, $t_{stat} = -1.781$, $df = 8$; paired t-test at 20 kHz: $p = 0.0348$, $t_{stat} = -2.356$, $df = 13$). Sine waves required less power than square waves in all simulations (Figure 2(e)), with differences ranging from -15 to -16% at 10 kHz and -14 to -17% at 20 kHz. Again, although the absolute powers differed, when normalized to the square wave power at block threshold, the difference between sine and square waves was similar in models and experiments (Figure 2(f)). These data collectively show that power consumption of sine waves at block threshold was either the same as or lower than square waves, despite consistently requiring higher current at block threshold.

3.2 Duty cycle influenced power at block threshold more in symmetric than asymmetric waveforms

We measured block threshold of symmetric and asymmetric waveforms with varying primary phase duty cycle (Group 2: sym1, sym0.5, sym0.25; Group 3: asym1, asym0.5, asym0.25; Figure 1(d); Equations 1 & 2). Symmetric and charge-balanced asymmetric waveforms had higher block thresholds as duty cycle was decreased in vivo and in silico (Table 2 and Figure 3). We quantified the differences in threshold across duty cycle in vivo by fitting the data using a power function:

$$T_j(D_k) = a_j D^b \quad (3)$$

The block threshold, T , for nerve j was considered proportional to the relative duty cycle, D (1, 0.5, or 0.25), raised to the power of parameter, b , with a parameter of proportionality, a_j , that was specific to nerve j . The natural log of this relationship was solved using a one-way ANCOVA (aoctool in MATLAB 2018a), where b was considered a covariate, and each in vivo data point was grouped based on its nerve index j (e.g., $j = 1$ for nerve 1) to control for inter-subject variability a_j .

$$\ln(T_j(D_k)) = b\ln(D) + \ln(a_j) \quad (4)$$

We fit Equation 4 to raw thresholds for each waveform group (symmetric and asymmetric) and each KHF (10 and 20 kHz) across duty cycles (Supplementary Table 1). For the symmetric (Figure 3(c)) and asymmetric waveforms (Figure 3(f)), parameter b was significantly less than zero and close to -1 at both frequencies (symmetric at 10 kHz, $p = 1.6\text{e-}09$, $b = -1.11$, 95% CI: $[-1.25, -0.98]$; symmetric at 20 kHz, $b = -1.14$, $p = 4.7\text{e-}20$, 95% CI: $[-1.20, -1.07]$; asymmetric at 10 kHz, $b = -1.06$, $p = 2.0\text{e-}09$, 95% CI: $[-1.16, -0.95]$; asymmetric at 20 kHz, $b = -1.03$, $p = 3.0\text{e-}12$, 95% CI: $[-1.12, -0.94]$). These results support an approximately inverse proportional relationship between duty cycle and block threshold (i.e., $T \propto 1/D$) for both symmetric and asymmetric waveforms.

We used a similar approach to quantify the effects of duty cycle on power consumption at block threshold. Duty cycle had a larger effect on power consumption in symmetric waveforms compared to charge-balanced asymmetric waveforms, although the power consumption at block threshold increased as duty cycle increased in all *in silico* data and all *in vivo* data points except one (Figure 4). Using an ANCOVA to fit Equation 4, power consumption at block threshold was inversely related to duty cycle for both symmetric and charge-balanced asymmetric waveforms (Figure 4(c) and (f)). However, in contrast to the fits of block threshold currents, the effects of duty cycle on power consumption were greater for the symmetric waveforms than the asymmetric waveforms, as reflected by b values of larger magnitudes, although all b values were significantly less than zero (symmetric at 10 kHz: $b = -1.23$, $p = 8.7\text{e-}07$, 95% CI: $[-1.51, -0.95]$; asymmetric at 10 kHz: $b = -0.71$, $p = 5.1\text{e-}05$, 95% CI: $[-0.93, -0.48]$; symmetric at 20 kHz: $b = -1.28$, $p = 7.1\text{e-}15$, 95% CI: $[-1.42, -1.14]$; asymmetric at 10 kHz: $b = -0.66$, $p = 5.5\text{e-}07$, 95% CI: $[-0.82, -0.50]$).

3.3 Onset response AUC at block threshold was not influenced by waveform shape

We quantified the onset response at block threshold across waveforms by calculating the area under the curve (AUC) of force during the 10 s KHF signal (Figure 5). Onset response at block threshold did not differ consistently between square and sine waves: 7 of 9 nerves at 10 kHz and 10 of 14 nerves at 20 kHz showed a larger onset response with sines compared to square waves, and there was no group difference detected (paired t-tests, 10 kHz: $p = 0.143$, $t_{\text{stat}} = 1.626$, $df = 8$; 20 kHz: $p = 0.135$, $t_{\text{stat}} = 1.592$, $df = 13$). Furthermore, the sine wave AUC values were generally similar to the square wave AUC values—within a factor of ~ 2 —but much larger in a couple of cases, including one where the sine AUC value was $>20\times$ larger (Figure 5(b)).

Onset responses were approximately constant across duty cycle in symmetric waveforms and trended towards smaller onset with larger duty cycle in asymmetric waveforms (Figure 5(a), (c), (d)). Fitting Equation 4 to AUC in symmetric waveforms showed no significant positive or negative b values, indicating no effect of duty cycle at either frequency (10 kHz: $p = 0.84$, $b = 0.06$, 95% CI: $[-0.53, 0.64]$; 20 kHz: $p = 0.51$, $b = -0.11$, 95% CI: $[-0.45, 0.23]$). In asymmetric waveforms, fitting Equation 4 to AUC also showed no significant positive or negative b values at 20 kHz ($p = 0.16$, $b = -0.44$, 95% CI: $[-1.08, 0.20]$), but at

10 kHz, fits showed a significant negative b value, indicating a decrease in AUC with increased duty cycle ($p = 0.0092$, $b = -0.75$, 95% CI: $[-1.27, -0.24]$).

3.4 Onset response peak was non-monotonically related to normalized stimulation amplitude across all waveform shapes

Given that waveform did not have a clear effect on onset response at block threshold, we evaluated onset response at multiple amplitudes of the KHF signals in four nerves and in silico to obtain onset response profiles (force vs. amplitude) for four waveforms: square, sine, sym0.5, and asym0.5 (Figure 1(d)). We applied amplitudes of 0 to 5 mA in random order for each of the waveforms. In three of the four nerves (15, 16, and 17), we randomized waveforms first, then applied randomized amplitudes (in 0.1 mA increments) of each waveform. In the fourth nerve (18), we randomized amplitudes first (in 0.5 mA increments), then randomized waveform tests for each amplitude. In all trials, we applied KHF for 0.5 s, which was longer than the minimum time for inducing nerve block (Bhadra et al. 2019), and paused for 1–2 s between trials, with an additional 5 s between amplitudes in nerve 18. During each experiment but prior to running any trials, we measured block threshold for all waveforms using the method described in section 2.7. To evaluate the onset response profiles in silico, we ran simulations with each of the four waveforms for 502 ms and applied KHF signals at amplitudes from 0 to 2.5 mA (0.05 mA increments), without any intracellular test pulses, such that any detected action potentials were evoked by the KHF signal. We counted the action potentials due to KHF during the entire simulation to quantify the onset response. This approach to simulating onset response in silico reproduces in vivo characteristics (Bhadra et al. 2007). All other simulation methods were as described in section 2.8.

Figure 6 shows the onset response versus KHF signal amplitude across all nerves, waveforms, and frequencies. Current amplitude had a non-monotonic effect on the onset response: onset responses close to block threshold were larger than the onset responses at amplitudes that were appreciably higher or lower than block threshold, resulting in “mound”-shaped profiles of onset response versus amplitude (e.g., nerve 15 at 20 kHz sym0.5 and asym0.5; nerve 17 at 40 kHz square and sine). Although the effects of waveform on onset response were not consistent (Figure 6(e)), normalizing the currents by block threshold for each waveform, each frequency, and each nerve generated onset profiles that generally overlapped across waveforms (Figure 6(f)). Block threshold was generally localized near the peak of the “mound”-shaped onset profiles, indicating that onset responses close to block threshold were generally higher than onset responses at amplitudes substantially above ($>200\%$) or below ($<25\%$) block threshold. However, some datasets had onset responses that increased and then plateaued with increasing current amplitude (e.g., nerve 17 at 20 kHz square, sine, and sym0.5; nerve 16 at 20 kHz square). Non-monotonic onset response profiles were also present in silico (Figure 6, 20 kHz model), with more evoked action potentials near block thresholds and fewer at lower and higher amplitudes. While the raw profiles of in silico onset responses peaked at different amplitudes across waveforms (Figure 6(e), bottom row), normalizing by the block threshold of the 5.7 μm fiber generated profiles that nearly overlapped across waveforms (Figure 6(f), bottom row), as observed in vivo.

3.5 Onset response was lower after first supra-block threshold trial within each threshold search

Since trends in onset response at block threshold across waveforms were variable, we also analyzed the onset response over the course of the threshold search to quantify history-dependent changes in onset responses. In the majority of datasets, onset response was smaller after the first supra-block threshold amplitude trial within each threshold search, irrespective of frequency or waveform. The onset response AUC (Figure 7(a)) was first normalized to the first trial in each threshold search (Figure 7(b)), revealing that subsequent trials had a weaker onset response than the first trial in the search. However, when normalizing instead to the first supra-block threshold amplitude trial (i.e., the first trial in the threshold search that produced conduction block) (Figure 7(c)), an even larger proportion of subsequent trials exhibited a lower AUC. These results indicate a history effect on onset response due to application of supra-block threshold KHF.

3.6 Axonal filtering of kilohertz-frequency waveforms accounts for block threshold differences across waveforms

To probe the mechanisms underlying waveform effects on block threshold, we measured the time series of transmembrane potential in silico produced by sine, square, sym0.5, sym0.25, asym0.5, and asym0.25 at each waveform's block threshold, revealing that the input waveforms were substantially filtered by the membrane (Figure 8(a)). Sine waveforms produced sinusoidal time series of transmembrane potential that were simply scaled and time-shifted compared to the input extracellular potential, while all other waveforms produced time series of transmembrane potential with a different shape than the input extracellular potential. We quantified filtering by computing the ratio between the Fourier series coefficients of the filtered waveforms (i.e., transmembrane potentials) and those of the corresponding input waveforms (i.e., extracellular potentials). These ratios indicated prominent low pass filtering (Figure 8(b)), and the substantial consistency across waveforms reflected a single transfer function at block threshold across all waveforms. While metrics such as peak amplitude, rms amplitude, and charge per phase differed across the extracellular potential waveforms, i.e., the pre-filter waveforms, these same metrics were notably more similar for the transmembrane potential waveforms, i.e., the post-filter waveforms (Figure 8(c)). Further, rms amplitudes of the filtered signals were particularly consistent across all waveforms after filtering (maximum deviation relative to symm 10 kHz: 4.4%, for asym0.25 at 10 kHz).

While in vivo measurement of transmembrane potentials to investigate filtering properties was not possible in our experiments, we probed the relationship between axonal filtering shown in Figure 8(b) and the well-established frequency-dependent increase in block thresholds (Bhadra and Kilgore 2005) to identify alternative methods to quantify axonal filtering in vivo. We measured block threshold of sine waves across frequencies in silico from 2 to 8 kHz in 2 kHz increments and from 10 to 140 kHz in 10 kHz increments (Figure 9(a)), and we then calculated the transfer function at block threshold for each frequency. The transfer function for individual sine waves across frequencies was highly consistent with the transfer function observed for arbitrary waveforms (Figure 9(b)). Meanwhile, inverting the transfer function amplitude produced a curve that mirrored the frequency-dependent

increases in block thresholds (Figure 9(c)). These results indicated that the frequency dependence of block thresholds could be derived from axonal filtering properties.

We therefore used the reverse strategy to apply filtering to our in vivo data by measuring the frequency dependence of block threshold in vivo and inverting those measurements to construct a transfer function. We measured block thresholds at 10, 20, 40, 60, 80, and 100 kHz in a single nerve in vivo (nerve 19). We constructed a frequency domain filter by inverting those block thresholds and normalizing the inverse to 10 kHz (Supplementary Figure 2). We then applied the resulting filter to our in vivo block thresholds from nerves 1–14 and quantified the rms of the filtered waveforms at block threshold. Quantification of filtered signal rms rather than unfiltered signal amplitude resulted in a reduction or elimination of differences across all waveforms at both 10 kHz and 20 kHz (Figure 10), and mapped all measurements onto a similar range of values. Square and sine wave thresholds were no longer different (paired t-test at 10 kHz: $p = 0.303$, $t_{\text{stat}} = 1.102$, $df = 8$; paired t-test at 20 kHz: $p = 0.947$, $t_{\text{stat}} = -0.067$, $df = 13$), and all symD and asymD relationships were smaller, with only symD at 10 kHz maintaining a significant reduction with increasing duty cycle (symmetric at 10 kHz, $b = -0.14$, $p = 0.017$, 95% CI: $[-0.25, -0.03]$; symmetric at 20 kHz, $b = -0.04$, $p = 0.13$, 95% CI: $[-0.09, 0.01]$; asymmetric at 10 kHz, $b = -0.10$, $p = 0.055$, 95% CI: $[-0.20, 0.00]$; asymmetric at 20 kHz, $b = -0.02$, $p = 0.58$, 95% CI: $[-0.12, 0.07]$).

4. Discussion

Kilohertz frequency electrical signals enable reversible block of nerve activity, although high currents, power consumption, and onset responses pose potential obstacles to translation of nerve block interventions. We quantified the effects of kilohertz frequency waveform parameters on block threshold, power consumption at block threshold, and onset response. Our key findings based on both in vivo experiments and computational models were that sine waves required higher currents to block than square waves but trended toward lower power, that duty cycle reductions in symmetric and asymmetric waveforms resulted in higher current and power consumption to block, and that axonal filtering of waveforms had a substantial effect on waveform shape at the level of the transmembrane potential and thus also on block threshold. We further showed that the onset response was smaller at amplitudes higher than block threshold irrespective of waveform shape.

Symmetric square waves at full duty cycle had the lowest block current thresholds among the tested waveforms, while sine waves had the lowest power consumption. Previous work in vivo (Tai et al. 2005b) and in unmyelinated axon models (Tai et al. 2005a) provided examples of square waves blocking at lower thresholds than sinusoids. Our work extends those findings by quantifying this comparison in vivo and in silico and showing that sinusoids required ~30% higher currents to block than square waves at both 10 and 20 kHz. An intuitive interpretation suggests that this difference results from the square wave spending more time at maximal amplitude than sine waves, thus generating greater membrane depolarization at a given frequency and amplitude. However, square waves have ~57% more charge per phase compared to sine waves of equal amplitude, which is not consistent with the differences in block threshold reflected in our measurements. Rather than

the charge per phase, we observed that steady-state depolarization during the KHF signal is heavily influenced by the spectral content of the waveforms (i.e., their Fourier series representation). The fundamental frequency component of a square wave's Fourier series has an amplitude of $(4/\pi) \approx 1.27$, which is consistent with the ~30% difference we measured in vivo and in silico. Temporal and spectral analyses of the transmembrane potentials in silico during square and sine KHF signals support this explanation, showing strong similarities between the amplitudes of the fundamental frequencies of both the applied signals and the resulting transmembrane potential response at block threshold (Supplementary Figure 3).

While square waves had the lowest block current amplitude, sine waves had the lowest power consumption among the waveforms evaluated, blocking at ~10–20% lower power than square waves. While the difference in power consumption was too small to achieve statistical significance at 10 kHz, the finding otherwise generalized across our in vivo and in silico data. In terms of spectral components, this finding reflects that the higher frequency harmonics of the square wave did not contribute substantively to nerve block (Supplementary Figure 3) because these higher frequency harmonics were more heavily filtered by the axon (Figure 8(b)). These results are important for the design of nerve block devices, identifying a clear tradeoff between the two most commonly used waveforms for KHF nerve block. Further, it is important to ensure that the hardware can generate the intended waveforms without distortion (e.g., attenuation, rounding of squares). Generating KHF waveforms remains a challenge even in laboratory settings (Patel and Butera 2018; FallahRad et al. 2019; Rapeaux and Constandinou 2020), and it is necessary to measure output waveforms at the desired frequencies, output loads, and amplitudes. In this study, we rigorously evaluated our stimulators (Supplementary Figure 1) to confirm the validity of our findings.

We used power functions to quantify current amplitude at block threshold as a function of primary phase duty cycle in symmetric and charge-balanced asymmetric waveforms, revealing an inverse relationship between duty cycle and block threshold. Surprisingly, this relationship had a similar magnitude across waveform groups despite the appreciable differences in second phase amplitude and duration in sym0.5 and sym0.25 compared to asym0.5 and asym0.25 (Figure 3(c) vs. (f)). Raw threshold currents were also similar between symmetric and asymmetric waveforms of a given duty cycle both in vivo and in silico (Table 2; Figure 3), although we observed minor differences that suggest a more complex effect of the second phase of the pulses. Specifically, it is unclear why asymmetric waveforms had slightly higher block thresholds than the symmetric waveforms at intermediate duty cycles (e.g., $D = 0.5$, Table 2 in vivo and Figure 3(e) in silico) while also having somewhat lower block thresholds at the lowest duty cycles (e.g., $D = 0.25$, 20 kHz, Table 2 in vivo; $D = 0.1$, 20 kHz, Figure 3(e) in silico). While symmetric and asymmetric waveforms with similar threshold had the same charge per phase, charge per phase at block threshold did not account for differences in current thresholds across waveforms (Supplementary Figure 4). Our power function fits also revealed that duty cycle had a smaller impact on power consumption in charge-balanced asymmetric waveforms than in symmetric waveforms. This effect was also evident in the raw data, where both in vivo (Table 2) and in silico (Figure 4(e)) power consumption was markedly larger in symmetric waveforms for $D < 0.5$, but not at longer duty cycles. The waveform can partially account

for these results, as the power in asymD is always less than symD of a given amplitude for $D < 1$, approaching 0.5 as D approaches 0. Further, asym0.25 had a somewhat smaller threshold than sym0.25, and squaring the thresholds to calculate power amplified the difference. Notably, spectral analysis and axonal filtering also help account for these phenomena, since symmetric waveforms have slightly more spectral content at or close to the fundamental frequency compared to asymmetric waveforms at $D = 0.5$, but this spectral content gradually shifts away from the fundamental frequency more quickly in symmetric waveforms as D increases (Supplementary Figure 5). These differences in spectral content explain how thresholds of symmetric waveforms could be lower at intermediate duty cycles while being higher at the lowest duty cycles. Although our findings indicate that charge-balanced asymmetry can be leveraged to improve the power-efficiency of nerve block compared to symmetric waveforms of a given duty cycle, the waveforms with smaller primary phase duty cycles were all less power-efficient than the square and sine waves and had no clear advantage in terms of onset response. Therefore, these data emphasize that full duty cycle waveforms should be used when the goal is to block with low current and power consumption.

Filtering of the KHF waveform by the axonal membrane was substantial, and amplitude, charge per phase, and particularly rms amplitude at block threshold were more consistent across waveforms after accounting for the filtering. The axonal membrane acted as a low-pass filter of the extracellular voltage. As the frequency of the extracellular voltage at the node of Ranvier increased, the nodal membrane capacitance reduced the membrane impedance relative to the predominantly resistive longitudinal axonal pathways, thus reducing the relative voltage drop across the membrane. We showed that this low pass filtering was quantitatively very similar across tested waveforms and across sinusoids at block threshold (Figure 9(b)). This result was also consistent with the finding that average transmembrane potential was similar across waveforms at block threshold since this determines the steady-state conductance of the nodal ion channels (Figure 8(a)). Most strikingly, the inverse of the low-pass filter closely followed the changes in block threshold across frequency (Figure 9(c)). This finding not only suggests that membrane filtering underlies frequency-dependent effects on nerve block, but also demonstrates that measurable frequency-dependent block thresholds can be exploited to acquire information about less readily measurable membrane properties. These findings provide significant insights on the nature of nerve block across waveforms and frequencies and suggest that axonal filtering is important in understanding nerve block with KHF signals.

We did not observe a clear effect of waveform shape on onset response at block threshold, but onset response across waveforms was smallest at current amplitudes well below or well above block threshold. While a majority of nerves had larger onset response with sine than with square waves at block threshold (Figure 5(b)), and 10 kHz asymmetric waveforms had smaller onset response with larger primary phase duty cycles (Figure 5(d)), onset responses at block threshold were overall variable across nerves, and the influence of waveform shape was inconsistent. Nevertheless, peak onset response vs. normalized amplitude profiles revealed that onset response was generally smallest at amplitudes well below or well above block threshold irrespective of waveform, with profiles overlapped across waveforms for a given animal and frequency and for the modeled data at 20 kHz (Figure 6(f)). The results are

consistent with previous studies in which higher KHF amplitudes reduced onset response (Bowman and McNeal 1986; Bhadra and Kilgore 2005; Tai et al. 2005b; Bhadra et al. 2019).

Our data are the first to quantify onset response profiles across waveform shapes, highlighting the potential of waveform shape to reduce onset response (Yi and Grill 2020). Since square waveforms had the lowest threshold among those tested, our results indicate that applying square waveforms for block may help reduce onset response simply because amplitudes can be increased well above block threshold while using lower current amplitudes than required of other waveforms. However, use of a square wave to reduce onset response should be weighed against its relatively higher power consumption (Figure 2(a)) and higher charge per phase (Supplementary Figure 4) compared to a sine wave, the latter of which may increase the risk of tissue damage. Some of our data showed onset responses that increased and then plateaued across amplitudes (e.g., Figure 6, nerve 16 at 20 kHz (square), nerve 17 at 10 and 20 kHz)—rather than exhibiting a clear mound-shaped onset response profile—and those profiles generally had smaller onset responses than the others. Onset response in rat sciatic nerve block was described in terms of an initial summated muscle twitch that was always present (Phase I) followed by asynchronous muscle contraction that was not always present (Phase II) (Bhadra et al. 2019). Examples in which onset response plateaued may contain only Phase I onset responses. Under this interpretation, square waves usually had the least prominent Phase II (e.g., individual grey profiles for nerve 15 at 20 kHz in Figure 6). Alternative sources of variability in onset response remain to be identified and quantified. Notably, charge per phase at block threshold did not account for differences in onset response (Supplementary Figure 6). Further, we used a fixed 1 mm nerve cuff inner diameter for all experiments, and we did not quantify nerve diameters or the effect of electrode fit on blocking parameters. Nerve diameters vary across animals (Kilgore and Bhadra 2014; Ling et al., 2019), and our use of a fixed cuff inner diameter across all nerves may underlie some of the variability in block threshold and onset responses across animals. This effect remains to be quantified systematically.

In the majority of threshold searches, onset response was smaller after application of the first trial that exceeded block threshold (Figure 7(c)). These are the first results to demonstrate a carryover effect on onset response. Indeed, normalizing to the first supra-block threshold trial (Figure 7(c)) accounted for more variability in onset response data than normalizing to simply the first trial (Figure 7(b)), suggesting that strong initial muscle contractions—and therefore muscle fatigue—are less relevant than achieving a blocked state for initiating these carryover effects. Rather, our observations are indicative of an onset response carryover effect that may be related to but distinct from block carryover effects reported by others (Bhadra et al. 2018a; Wang et al. 2019; Pelot and Grill 2020), and we did not observe prominent examples of such block carryover effects in the majority of our trials (i.e., force twitch recovery was almost immediate following KHF block). A time-dependent reduction in neural onset response as we observed has been reported previously in single fiber recordings after a long application of KHF (Bowman and McNeal 1986), albeit at sub-threshold amplitudes and at much longer durations than our trials (20 min). The causes of KHF carryover effects are unknown, but are thought to involve changes in ion concentrations due to a net influx of sodium and efflux of potassium (Ackermann et al. 2011b). Studies reporting block carryover provide evidence that such effects are likely to be

intensified at larger amplitudes, longer stimulus durations, and lower frequencies (Bhadra et al. 2018a; Wang et al. 2019; Pelot and Grill 2020). Future studies using biophysical axon models that track ion concentration (e.g., (Tigerholm et al. 2014)) may provide additional insight into the time-dependent effects of block.

In addition to the order of trials, additional studies investigating the dynamics of carryover must consider the timing between trials, as this may also affect the onset response (Supplementary Figure 7). Notably, whereas the onset responses had prominent carryover effects during the threshold search (Figure 7), where 10 s of KHF signal were delivered for each trial, we did not observe onset response carryover effects in our amplitude sweep tests where only 0.5 s of KHF signal was delivered per trial (Supplementary Figure 8). Further, the inter-trial pauses were longer in the binary searches (median: 10 s) than in the amplitude sweep tests (<5 s), and the longer duration of KHF signals used in our threshold searches may have contributed to the persistent effects on onset response. While we calculated different metrics to quantify onset response (i.e., AUC in threshold searches, peak onset response in amplitude sweeps), the strong correlation between these metrics and onset response duration (Supplementary Figure 9) validate their interchangeability in interpreting results and in comparing to literature (Bhadra and Kilgore 2005; Patel et al. 2018; Bhadra et al. 2019). In this study, we used muscle onset response as representative of neural onset response, which is a sound approach for our conclusions because the time scales differ by only tens of milliseconds (Patel et al. 2018). Nevertheless, future work performed using direct nerve recordings (Patel and Butera 2015; Pelot and Grill 2020) will further clarify the effects of waveform across fiber types and diameters.

We limited the focus of our study to a small frequency range and relatively simple variations in waveform shape. Within that range, waveform shape had similar effects at 10 and 20 kHz, despite 10 kHz having smaller average threshold currents, lower power consumption, and generally larger onset responses (Table 2). While our computational models exhibited trends strikingly similar to our in vivo data in terms of relative block threshold current and power consumption trends, duty cycle effects were less pronounced in silico (consistent with a previous report (Dali et al. 2019)), and this discrepancy points to a need for models that can capture the dynamics of lower duty cycles. Further, our spectral analyses were conducted with a bipolar cuff electrode, a myelinated fiber model, and a monofascicular nerve morphology with 0.75 mm diameter, and it remains to be determined how the filtering properties observed translate across alternative model specifications. Finally, we did not observe any noticeable differences between our two female Wistar rat experiments and the rest of the experiments in male Sprague-Dawley rats.

Conclusion

The data presented here are the first to quantify the impact of waveform shape across block threshold, power consumption, and onset response. We found that sine waves blocked at higher currents but same or lower power than square waves, that reducing the duty cycle increased currents and power consumption for block, that filtering by the axonal membrane had substantial effects on block properties of waveforms, and that onset response decreased at amplitudes well above block thresholds across all waveforms tested. Our findings inform

the choice of waveform in future clinical and scientific studies and motivate new investigations into the nature of axonal filtering and onset responses that can clarify mechanisms and facilitate translation of KHF nerve block as a novel and potent therapeutic modality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1:

Setup of in vivo experiments and in silico models of nerve block. (a) Urethane-anesthetized rats were instrumented with a stimulation cuff on the sciatic nerve and a blocking cuff on either the tibial branch (shown) or the sciatic nerve (not shown). Common peroneal and sural branches were transected (red crosses). The Achilles tendon was dissected from the tibia, and attached to a force transducer. (b) Block diagram of stimulation and recording hardware. Red lines represent the positive output terminals. See the text for additional details. (c) Schematic of “DC Filter Circuit 1” adapted from (Franke et al., 2014). “DC Filter Circuit 2” used only R1 and C1, such that R2 was open circuit and C2 was short circuit. (d) Waveforms evaluated at 10 and 20 kHz. (e) Schematic illustration of a binary search for block threshold across amplitudes. The height and width of the blue area show the amplitude and duration of the KHF signal (right-hand y axis). (f) Finite element model of tibial nerve and blocking cuff. Full extent of nerve (50 mm) not shown. (g) Materials and dimensions of the finite element model.

Figure 2:

Ladder plots of in vivo and in silico measurements of current (a–c) and power (d–f) at block thresholds for sine and square waves at 10 and 20 kHz. (a & d) In vivo current (a) and power (d) thresholds where each line is an individual nerve. (b & e) In silico current (b) and power (e) thresholds across fiber diameters noted in plot in microns (color-coded). (c & f) Overlaid thresholds for the in vivo (blue dots) and in silico (colored lines) studies, normalized to the square wave threshold. For in vivo data, the mean and 95% confidence interval of the mean are plotted as a black dot with whiskers.

Figure 3:

Block thresholds across duty cycles in symmetric (a–c) and charge-balanced asymmetric (d–f) waveforms. Lines depict within-nerve (a & d; in vivo) or within-fiber diameter (b & e; in silico) recordings. Color-coded numbers (b) indicate model nerve fiber diameters in microns. The 5.7 μm data from (b; blue triangles and dotted line) are also shown in (e). Current thresholds (c & f) were normalized either by nerve-specific coefficients a_i obtained from fitting Equation 4 (in vivo data) or to thresholds at $D = 1$ (in silico data). The normalized plots of Equation 3 based on Equation 4 fits are shown as red dotted curves (mean) and red shaded areas (95% confidence interval), with exponents annotated at the top of the plots.

Figure 4:

Power consumption at block threshold across duty cycles in symmetric (a–c) and charge-balanced asymmetric (d–f) waveforms. Lines depict within-nerve (a & d; in vivo) or within-fiber diameter (b & e; in silico) recordings. Color-coded numbers (b) indicate model nerve fiber diameters in microns for all in silico data. The 5.7 μm data from (b; blue triangles and dotted lines) are also shown in (e). Normalized power consumption values (c & f) were normalized either by nerve-specific coefficients a_j obtained from fitting Equation 3 (in vivo data) or to power consumption at $D = 1$ (in silico data). The normalized plots of Equation 3 based on Equation 4 fits are shown as red dotted curves (mean) and red shaded areas (95% confidence interval), with exponents annotated at the top of the plots.

Figure 5:

Onset responses at block threshold for all nerves, frequencies, and waveforms. (a) Raw onset responses. Plots were truncated either after 3 seconds or after the response dropped below 0.12 N, whichever occurred sooner. Nerve number is annotated below each response. (b, c, d) Raw and normalized area under the curve (AUC) of the onset response comparing sine and square waves (b), symmetric waveforms with varying duty cycle (c), and charge-balanced asymmetric waveforms with varying duty cycle (d). Plots of Equation 3 based on Equation 4 fits are shown as red dotted lines (mean) and red shaded areas (95% confidence interval) (c, d), with exponents annotated at the top of the plots. Normalized AUC values were normalized by square (b) or by normalizing factors a_j obtained from fitting Equation 3 (c, d).

Figure 6:

Onset responses evoked by 0.5 s of KHF signal at different randomized amplitudes across waveforms for nerves 15–18. Raw force recordings are shown in gray, sorted by stimulation amplitude. Envelopes of the peak onset responses highlight the overall profile of onset versus amplitude. Block threshold is indicated by either a red 'x' (columns (a)–(d)) or a dashed red vertical line (column (f)). Waveforms tested were square (a), sine (b), sym0.5 (c), and asym0.5 (d). Profiles for all waveforms are overlaid in columns (e) and (f), where the x axes are normalized to block threshold in column (f). The row with in silico data (bottom) displays the number of action potentials (APs) generated in each fiber diameter during the 0.5 s KHF signal across amplitudes. Fiber diameters are color-coded and annotated in

microns. In silico data in column (f) was normalized by the block threshold of the 5.7 μm fiber.

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Figure 7:

Onset responses as functions of trial number within a threshold search for all waveforms, frequencies, and nerves. (a) Raw onset response AUC. (b) Normalized onset response AUC, normalized to the first trial in each threshold search. (c) Normalized onset response AUC, normalized to the first supra-block threshold trial in each threshold search. AUC was calculated over the 10 seconds during which KHF was applied.

Figure 8:

Effects of axonal filtering on waveforms at block threshold in 5.7 μm model fiber. (a) Time series of extracellular potential (V_{ext}) and steady-state transmembrane potential (V_m) at the proximal node with highest peak transmembrane potential (node 49). V_{ext} and V_m are shown at each waveform's respective block threshold. Y-axis for V_{ext} is inverted. Dashed red line denotes mean transmembrane potential. (b) Transfer function of axonal membrane filtering at block threshold for data shown in (a). Magnitude in decibels (dB) was calculated as $20 \cdot \log_{10}(|V_m/V_{ext}|)$. (c) Comparison of peak amplitude, rms amplitude, and charge per phase in unfiltered (top) and filtered (bottom) signals. Unfiltered signals are V_{ext} from (a). Filtered signals are V_m from (a). Charge per phase for the filtered signal was calculated as the area between one of the phases and the mean line. All metrics in (c) are normalized to square at 10 kHz.

Figure 9:

Relationship of axonal filtering to frequency-dependent block thresholds. (a) Block thresholds of sine waves from 2 kHz to 140 kHz in silico of a single 5.7 μm fiber. (b) Transfer function amplitude across sine waves at different frequencies (black line with diamond symbols) superimposed by transfer functions of sine, square, sym0.5, sym0.25, asym0.5, and asym0.25 (colored circles and triangles from Figure 8(b)). Magnitude in decibels (dB) was calculated as $20 \cdot \log_{10}(|V_m/V_{ext}|)$. All transfer functions were computed at block threshold for each respective waveform. (c) Overlay of block thresholds (yellow line from (a)) and multiplicative inverse of transfer function amplitude (inverse of black line from (b)).

Figure 10:

Reduction or elimination of waveform effects in data from nerves 1–14 quantified by filtered waveform rms at block threshold. Filtering and rms calculations were performed in the frequency domain by obtaining each waveform's Fourier series coefficients from 10 to 100 kHz (in 10 kHz increments), scaling the components according to the derived filter (Supplementary Figure 2), and then multiplying the Euclidean norm of the Fourier series coefficients by 0.7071. In the square and sine plots (a), the mean and 95% confidence interval of the mean are plotted as a black dot with whiskers. In the symD (b) and asymD (c) plots, the normalized plots of Equation 3 based on Equation 4 fits are shown as red dotted curves (mean) and red shaded areas (95% confidence interval), with exponents annotated at the top of the plots.

Table 1.

Material properties of the finite element model.

Material	Resistivity (Ω m)	References
Platinum	1.06e-7	(De Podesta 2001)
Silicone	1e12	(Callister and Rethwisch 2012)
Saline	0.568	(Horch and Kipke 2017)
Endoneurium (Nerve)	1.75 longitudinally; 6 radially	(Ranck Jr. and BeMent 1965; Pelot et al. 2017)
Perineurium	1149	(Weerasuriya et al. 1984; Pelot et al. 2019)
Muscle	2.86 longitudinally; 11.6 radially	(Gielen et al. 1984)

Table 2.

Summary of current, power, and onset response area across waveforms at block threshold for in vivo data. The onset response at block threshold across waveforms was quantified by calculating the area under the curve during the 10 s KHF signal. The number of animals per group were: Group 1 (n = 9 at 10 kHz, n = 14 at 20 kHz), Group 2 (n = 6 at 10 kHz, n = 11 at 20 kHz), and Group 3 (n = 5 at 10 kHz, n = 7 at 20 kHz). Data are expressed as mean(SD).

Group	Waveform	10 kHz			20 kHz		
		Current (mA)	Power (mW/k Ω)	Onset (N*sec)	Current (mA)	Power (mW/k Ω)	Onset (N*sec)
1	square	0.71(0.16)	0.54(0.23)	0.70(0.77)	1.06(0.30)	1.21(0.74)	0.19(0.15)
	sine	0.95(0.14)	0.47(0.13)	1.30(1.17)	1.36(0.38)	0.99(0.59)	0.27(0.27)
2	Sym1 (square)	0.71(0.15)	0.53(0.22)	0.63(0.96)	1.15(0.45)	1.50(1.28)	0.31(0.24)
	sym0.5	1.34(0.33)	0.95(0.49)	0.39(0.42)	2.21(0.86)	2.77(2.46)	0.44(0.53)
	sym0.25	3.35(0.84)	2.97(1.52)	0.37(0.26)	5.45(1.66)	8.12(5.08)	0.58(0.94)
3	asym1 (square)	0.78(0.10)	0.61(0.15)	1.07(1.41)	1.13(0.38)	1.40(0.96)	0.31(0.19)
	asym0.5	1.60(0.30)	0.88(0.31)	1.36(1.15)	2.61(1.09)	2.61(2.15)	0.99(0.73)
	asym0.25	3.39(0.71)	1.70(0.70)	2.00(1.54)	4.67(1.36)	3.35(1.92)	0.73(0.61)