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Measurement of the current-distance relationship using a novel refractory interaction technique

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Abstract

It is important to know the spatial extent of neural activation around the stimulating electrodes when using extracellular electrical stimulation to determine the structure and function of neural circuit connections or for the restoration of function. The current-distance relationship quantifies the relationship between the threshold current for excitation of a neuron, I_{th} , and the distance between the electrode and the neuron, r , with two parameters: the offset, I_o , and the current-distance constant, k , with a quadratic equation, $I_{th}(r) = I_o + k r^2$. We proposed a new method to determine the parameters of the current-distance relationship, and thereby estimate the spatial extent of activation, based on the refractory interaction technique. Refractory interaction is a method that exploits the interaction between the regions of activation produced by two electrodes, when the second stimulus is delivered while neurons activated by the first electrode are in their refractory period. Computer simulations of electrical stimulation of a population of nerve fibers were used to determine the accuracy of the method. The mean relative error in k was 19% and in I_o was 17 %, and the spatial extent of stimulation could be determined with an absolute error of 19 μm and a relative error less than 11%. Subsequently the method was applied to measure the current-distance properties of peripheral motor nerve fibers and indicated that $k = 27 \mu\text{A}/\text{mm}^2$ and $I_o = 49 \mu\text{A}$. This method provided robust estimates of the current-distance properties, and provides a means to determine the spatial extent of activation by extracellular stimulation.

Keywords

electrical stimulation; current-distance relationship; spatial selectivity; refractory period; electrode array

1. Introduction

Extracellular electrical stimulation is used to explore the structure and function of the nervous system (Tehovnik 1996) and to restore sensory and motor function following neurological disease or injury (Grill and Kirsch 2000). To design experiments, interpret responses, or develop neural prostheses, it is important to know the spatial extent of neural excitation in response to an electrical stimulus. The current distance relationship (CDR) quantifies the relationship between the stimulus amplitude and the extent of neural activation around the electrode, although traditionally it was defined based on measurements

from a single neuron, i.e., the threshold current for excitation of a neuron as a function of the distance between the electrode and the neuron (Yeomans 1990). The CDR depends on the stimulus parameters and waveform, the size, shape and orientation of the electrode(s), the electrical properties of the volume conductor, and the size, shape and electrical properties of the neuronal elements (Ranck 1975). Therefore the CDR must be determined separately in each experimental or clinical situation to estimate the extent of neural activation. It is challenging to determine directly the spatial extent over which neurons are activated, since neither the direct neural response nor the position of individual neurons may be accessible. Therefore, a number of empirical methods have been developed to measure the current distance relationship during extracellular stimulation (Mahnam *et al.* 2008). Here we present a novel method, based upon the refractory interaction technique, to estimate the parameters of the current distance relationship and thereby determine the spatial extent of neural activation by extracellular electrical stimulation.

The refractory interaction technique is based on interaction between the regions of activation produced by two neighboring electrodes, with stimulation applied to one electrode while the neurons are refractory due to stimulation through the other electrode. Several methods employing refractory interaction have been proposed to measure the current-distance relationship (Fouriez and Wise 1984, Yeomans *et al.* 1986, Liang *et al.* 1991, Snow *et al.* 2006), but each employs limiting assumptions or is sensitive to measurement noise (Mahnam *et al.* 2008). Since the actual spatial extent of activation cannot be measured directly, it is challenging to validate the results of such methods. Therefore, we first used computer simulations to evaluate the accuracy of the proposed method. Subsequently, we implemented the method to measure the CDR for peripheral motor nerve fibers activated with an intraneural microelectrode array.

2. Methods

2.1. Theory

The refractory interaction technique exploits the interaction between the regions activated by two electrodes, when the interstimulus delay is within the refractory period of the activated neurons (figure 1(a)). Applying stimulus pulse I_a with electrode A activates N_a neurons in a region around the electrode, A_a , and produces response R_a . Similarly, stimulus pulse I_b applied by electrode B activates N_b neurons in region A_b and produces response R_b . If I_b is applied following a delay, when the neurons N_a are in their refractory period and do not respond to stimulus I_b , then the total response to both pulses, R_{ab} , will depend on the spatial overlap between the activated regions A_a and A_b , and the observed response will be less than the arithmetic sum of the individual responses to the two stimuli when delivered separately, $R_a + R_b$.

We propose a method to determine the current distance relationship using measurements from two special cases (minimum overlap and full overlap) to calculate the parameters I_o and k of the quadratic relationship between the threshold current, I_{th} , and the electrode to fiber distance, r ,

$$I_{th}(r) = I_o + kr^2 \quad (1)$$

where I_o , the absolute threshold, determines the minimum stimulus amplitude for a detectable response and k , the current distance constant, determines the rate of change of stimulus threshold at different distances from the electrode. Despite some evidence that the current-distance relationship may be linear or cubic for certain ranges of electrode to neuron

distance (Bean 1974; Marcus *et al.* 1979; Hentall *et al.* 1984), a quadratic relationship was used in this study because previous theoretical (e.g., BeMent and Ranck 1969b, Bean 1974, Rattay 2008), computational (e.g., Grill and Mortimer 1996), and experimental studies (e.g., Stoney *et al.* 1968, BeMent and Ranck 1969a, Rank 1975, Bagshaw and Evans, 1976, Yeomans *et al.* 1986) on activation of myelinated axons support the use of the quadratic form of the current-distance curve over the range of distances considered in this study (several hundred microns).

An arbitrary amplitude stimulus, I_a , is applied to electrode A that results in activated region A_a . The stimulus amplitudes that when applied by electrode B generate minimum and full overlap are determined and designated I_1 and I_2 , respectively (figure 1(b)). The resulting current distance equations for these two cases are

$$I_1 = I_0 + k(L - r_a)^2 \quad (2)$$

$$I_2 = I_0 + k(L + r_a)^2 \quad (3)$$

where r_a and r_b are the radii of activation around electrode A and B respectively, and L is the distance between electrodes. Expressions for I_0 and k are then obtained by combining these two equations.

$$I_0 = I_a - \frac{(I_2 - I_1)^2}{8(I_1 + I_2 - 2I_a)} \quad (4)$$

$$k = \frac{I_1 + I_2 - 2I_a}{2L^2} \quad (5)$$

2.2. Computer Simulations

A computational model of electrical stimulation of a population of nerve fibers was used to evaluate the proposed method of measuring the current-distance relationship. The geometric characteristics of the model were based on anatomical data for the branch of sciatic nerve innervating the medial gastrocnemius in the cat. Four hundred alpha motoneuron axons were uniformly distributed within a cylindrical nerve trunk, 400 μm in diameter (Eccles and Sherrington 1930). The volume conductor was assumed infinite and homogeneous, but anisotropic with longitudinal resistivity $\rho_l = 200 \Omega\text{cm}$ and transverse resistivity $\rho_t = 1250 \Omega\text{cm}$ (Deurloo *et al.* 1998). For simplicity, inhomogeneities including intraneural blood vessels and the perineurium, were not included in the model. The proposed method relies on comparing the responses evoked by electrodes A and B individually to the response to stimulation with both electrodes. Inhomogeneities will have equivalent effects when stimulating one or both electrodes and therefore will not effect estimation of the current distance relationship. The potential resulting from application of current with a point source electrode at the origin was calculated analytically at a point (x, y, z) as (Nicholson, 1967; Wait, 1990):

$$V(x, y, z) = \frac{\rho_l I}{4\pi \sqrt{\frac{\rho_l}{\rho_i} ((y - y_{elec})^2 + z^2) + x^2}} \quad (6)$$

A previously developed and validated cable model of a mammalian myelinated nerve fiber (McIntyre *et al.* 2002) was implemented in NEURON V6.0 (Hines and Carnevale 1997) and solved using a variable-step DASPK (Differential Algebraic Solver with the Preconditioned Krylov method) integrator with an absolute tolerance of 0.001. The original model reproduces the temporal dynamics of excitability, strength-duration properties, and current-distance properties of mammalian nerve fibers, and the present implementation was validated by repeating the previously reported simulations (McIntyre *et al.* 2002). Fiber diameters were selected randomly from a normal distribution with mean 13.6 μm and variance 1.55 μm^2 (Eccles and Sherrington 1930). The dimensions of different sections of the model fibers were calculated by interpolation of the data presented in Table 1 of McIntyre *et al.* (2002). Each fiber had 20 nodes of Ranvier and the electrodes were placed near the 10th node. The longitudinal position of the node nearest to the electrode was selected randomly from a uniform distribution. Three separate populations were generated with different randomly selected nerve fiber diameters and positions. Cathodic, monophasic 100 μs duration pulses or cathodic-first 100 μs per phase symmetric biphasic pulses were used for stimulation. All the results presented in this study are from simulations with monophasic pulses, except when stated otherwise. Action potentials were detected at the end of each nerve fiber as an increase of the transmembrane potential to 0 mV. The percentage of activated fibers was measured as the response of the population to stimulation.

Recruitment curves of the percentage of activated fibers as a function of the stimulation amplitude were obtained by applying different pulse amplitudes through an electrode positioned at the middle of the nerve bundle, and were used to select appropriate stimulation amplitudes in subsequent simulations (figure 2(a)). Refractory curves of the percentage of activated fibers as a function of the interpulse interval were obtained by applying two equal amplitude pulses through the same electrode at amplitudes that, when applied as single pulses, activated 25%, 50%, or 75% of the fibers, and determining the percentage of nerve fibers activated by the second pulse (figure 2(b)).

Simulations employing two electrodes were conducted to evaluate the proposed method. Electrodes were placed perpendicular to the nerve fibers, and when not stated otherwise, at equal distances from the center of the nerve. The responses (i.e., percentage of fibers activated), R_a and R_b , to stimuli, I_a and I_b , applied to electrodes *A* and *B* were obtained. Subsequently, the response R_{ab} to paired stimulation with I_a followed by I_b at an appropriate interpulse interval was measured for a range of stimulus amplitudes, I_b .

2.3. Experimental Measurements

Acute experiments were conducted on three cats to measure the current-distance relationship of intraneural stimulation of peripheral nerves using a microelectrode array. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of Duke University. Adult male cats (3.7–5.0 kg) were anesthetized with ketamine HCl (Ketaset, 35 mg/kg, intramuscular, supplemented as required during surgical preparation) and anesthesia was maintained with α -chloralose (Sigma, 65 mg/kg, intravenous, supplemented at 15 mg/kg). Depth of anesthesia was monitored via blood pressure, heart rate, and blink and withdrawal reflexes. A catheter was placed in the carotid artery to measure blood pressure. Body temperature was maintained between 38 °C and 39 °C with a heating blanket. Saline 0.9% with 8.4 mg/ml sodium bicarbonate and 5% dextrose

was administered intravenously (10–15 ml/kg/h). Animals were intubated and end tidal CO₂ was maintained between 3% and 4% with artificial respiration.

The sciatic nerve was exposed via an incision and blunt dissection, and a cuff electrode was implanted around the compound nerve trunk. Stimulation was applied through the cuff electrode to evaluate the overall excitability of the nerve and fidelity of the electromyographic response prior to insertion of the intraneural microelectrode. The branch that innervates the medial gastrocnemius was isolated in the popliteal fossa and supported on a pair of plastic hooks. Under a surgical microscope, a longitudinal incision was made in the perineurium, and a micromanipulator was used to insert a seven-contact silicon-based microelectrode array (kindly provided by the University of Michigan) into the medial gastrocnemius branch, transverse to the long axis of the nerve. The electrode contacts were arranged in linear array, oriented perpendicular to the nerve fibers with the central five contacts spaced 100 μ m apart and the flanking contacts spaced 200 μ m their adjacent contacts. Two stainless steel wires, insulated except at their tips, were inserted into the belly of the medial gastrocnemius to record the electromyogram (EMG).

A multi-function input-output board (MIO-16E, National Instruments) was used to generate stimulation pulses and record the EMG response. Voltage pulses generated by the analog outputs of the MIO board were input to two independent linear voltage-to-current converters, which generated regulated current stimuli with amplitudes proportional to the input voltages. Stimuli were either cathodic, monophasic 100 μ s duration pulses or cathodic-first 100 μ s per phase symmetric biphasic pulses, delivered at 0.33 Hz. Large needles inserted in the proximal hindlimb served as the counter electrodes. The applied currents were monitored by measuring the voltages across series 1 k Ω resistors with isolated differential amplifiers. The EMG was amplified, filtered (1 Hz–10 kHz), and sampled (100 kHz) for 15 ms after each stimulus. The integral of absolute value of the EMG was computed for each trial as a measure of the response.

Recruitment curves of the EMG response as a function of the stimulation current amplitude were recorded for individual electrodes by applying different amplitude stimuli in random order. The stimulus amplitudes to evoke a response equal to half of the maximum response were used subsequently to generate refractory curves of the EMG response to the second pulse of paired-pulse stimulations as a function of the interpulse interval for single electrodes. The refractory curves were computed as the integral of the absolute difference between the responses to single and paired-pulse stimulation. The delay between the pulses that resulted in neither temporal summation nor recovery from refractoriness was used for the subsequent two-electrode studies.

For each stimulus amplitude I_a applied to one electrode, A , a range of stimulus amplitudes I_b , which spanned the full range of recruitment, was applied to the other electrode, B . For each pair of currents, I_a and I_b , the EMG responses R_a and R_b to single pulses I_a and I_b , and the EMG response, R_{ab} to two-electrode paired pulse stimulation, I_a followed by I_b , were recorded.

2.4. Calculation of the parameters of the current-distance relationship

The results from the simulations and experiments were processed using MATLAB (The Mathworks, Inc., Natick, MA) to determine the parameters, I_0 and k , of the current-distance relationship. A relative refractory response, R_r , was defined to quantify the changes of R_{ab} relative to R_a and R_b :

$$R_r = 100 \frac{R_a + R_b - R_{ab}}{R_a} \quad (7)$$

where R_b , R_{ab} and R_r are functions of I_b . For small values of I_b , there is no overlap between the regions of activation, $R_{ab} = R_a + R_b$, and R_r is equal to 0. For larger values of I_b , $R_{ab} < R_a + R_b$, and R_r increases with the amount of overlap, converging to 100 for full overlap. The relationship between R_r and I_b was sigmoidal (see figure 3(b) for simulations and figure 5(a) for experiments), and the I_b values for which R_r diverged from 0 and converged to 100 corresponded to minimum and full overlap, respectively. To find these I_b values despite fluctuations in R_r , a sigmoid function was fit to the relative refractory response data, R_r vs. I_b

$$s(i) = \frac{a \cdot e^{c(i-b)}}{1 + e^{c(i-b)}} - d \quad (8)$$

where a determines the range, b the point of inflection, c the slope, and d , the offset of the curve. The critical current values, I_1 and I_2 , were determined from the curve fit as the points where R_r was equal to 5% and 95%, respectively. By equating s to 5% and 95% of its maximum in equation 8, I_1 and I_2 were found as:

$$I_1 = b + \frac{1}{c} \ln \frac{5}{95} \quad (9)$$

$$I_2 = b + \frac{1}{c} \ln \frac{95}{5} \quad (10)$$

These values were then used to calculate the parameters of the current-distance relationship, I_0 and k , using equations 4 and 5.

In the experiments, different stimulus amplitudes were usually necessary for electrodes A and B to produce the same response. To compensate for these differences in the calculation of the current-distance parameters, the recruitment data for electrodes A and B were also fit using a sigmoid function (equation 8), and the ratio of the b parameters from each the sigmoid fits, which represent the positions of the fits along the stimulus current axis, was used as a scaling factor for the stimulus amplitude of electrode A . In cases that the coefficient of variation of the parameters b for two recruitment curves was larger than 0.5, the data from that experiment were discarded (17 % of the total number of measurements).

3. Results

We developed a method to estimate the parameters of the current-distance relationship using the refractory interaction technique. Computer simulations of stimulation of a population of nerve fibers were used to assess the accuracy of the method, and the method was then applied to measure experimentally the current-distance properties of electrical stimulation of peripheral motor nerve fibers.

3.1. Computer Simulations

Input-output curves of the percent of axons activated as a function of the stimulation intensity were obtained for three different random distributions of modeled nerve fibers and were used to determine the current amplitudes required to activate 25%, 50%, or 75% of the population (figure 2(a)). There were no appreciable differences between recruitment curves for the three populations. Refractory curves of the percentage of nerve fibers activated by the second pulse in single-electrode paired-pulse stimulation as a function of the interpulse interval (IPI) were obtained with stimulus amplitudes that activated 25%, 50%, or 75% of the fibers (figure 2(b)). When the IPI was ≤ 0.5 ms, temporal summation was observed, when $0.6 \text{ ms} \leq \text{IPI} \leq 0.9$ ms, the fibers were refractory and did not respond to the second pulse, and when the IPI ≥ 1.0 ms, fibers emerged from the refractory period and responded to the second pulse. The effect of IPI was similar for different stimulus amplitudes, and an IPI = 0.75 ms was used for subsequent two-electrode simulations.

Simulations of refractory interaction were conducted for I_a equal to 4, 6 and 8 μA with two electrodes positioned straddling the center of the nerve trunk 200 μm apart along a line perpendicular to the direction of the nerve fibers in three different randomized populations. The values of I_a were selected such that the region of activation included at least a few fibers (3, 5 and 6 fibers in the three distributions for I_a equal to 4 μA) and did not extend beyond the nerve dimensions. The single- and paired-pulse responses to a range of I_b values from 1 to 35 μA , with $I_a = 8 \mu\text{A}$, are shown in figure 3(a). For small values of I_b , when no overlap was expected, the paired pulse response, R_{ab} , was close to the sum of single-pulse responses, $R_a + R_b$. For larger values of I_b , R_{ab} decreased relative to $R_a + R_b$ and converged to R_b when A_b fully overlapped A_a . The resulting relative refractory responses, R_r (equation 7; figure 3(b)), were fit with a sigmoid function (equation 8) and I_1 and I_2 were estimated from the parameters of the sigmoid using equations 9 and 10. These values, and the estimated parameters of the current-distance relationship, I_0 and k , determined using equations 4 and 5 are listed in Table 1.

The errors of the method were determined by comparing the estimated current-distance parameters with the actual values. The position of each nerve fiber and its threshold were accessible in the simulations, and these data were used to obtain the true current-distance relationship (figure 4). Since the excitation threshold depends on the fiber diameter and the position of the node of Ranvier nearest to the electrode, as well as the electrode to fiber distance, the thresholds for fibers at the same distance from the electrode were not unique but formed a band. The lower boundary of this band represents the furthest fibers activated by each stimulus amplitude and therefore defined the boundary of the spatial extent of activation. The points representing the lower boundary were obtained by dividing the distance range into 10 μm divisions and finding the minimum threshold point in each division. The current-distance relationship (equation 1) was fit to these points (bold line in figure 4) and used as the standard to which the results of the proposed method were compared ("optimal values" in Tables 1 and 2). The mean relative error in k was 19 % (range 0.92 – 39 %) and in I_0 was 17 % (range 3.8 – 41 %) across the three values of I_a in each of the three populations.

The errors estimating the spatial extent of neural activation were determined by comparing the distances predicted with the estimated current-distance curve with the distances obtained from the actual current-distance curve for stimulus amplitudes from 7 to 40 μA . The mean relative error was less than 11 % (Table 1), and the absolute errors were less than 53 μm (mean = 19 μm).

Simulations were repeated with electrode B positioned on the border of the nerve bundle and electrode A either 200 μm or 300 μm toward the center of the bundle (Table 2). The mean

relative errors in k were 8.7 % and 3.1 %, and the mean relative errors in I_0 were 46 % and 11 %, respectively, for the 200 μm and 300 μm interelectrode spacings. The absolute errors in estimating the spatial extent of activation ranged from 0 to 65 μm (mean = 13 μm) for interelectrode spacing of 200 μm and from 3.6 to 33 μm (mean = 7.3 μm) for interelectrode spacing of 300 μm . Further, the method was tested using symmetric biphasic stimulus pulses with similar results (Table 2). The mean relative error in k was 7.6 %, the mean relative error in I_0 was 16 %, and the absolute errors in estimating the spatial extent of activation were less than 35 μm (mean = 9.0 μm).

3.2. Experimental Measurements

The refractory interaction method was implemented to measure the current-distance properties of intraneural stimulation using a silicon microelectrode array inserted into the branch of the sciatic nerve innervating the medial gastrocnemius in three cats. Input-output curves of the evoked electromyogram (EMG) as a function of the stimulation amplitude were obtained for each electrode (figure 6(a)), and were used to select appropriate stimulus amplitudes in subsequent paired-pulse and two-electrode studies. Paired-pulse stimuli were applied through a single electrode to obtain refractory curves of the difference between responses to single- and paired-pulse stimuli as a function of the IPI (figure 6(b)). Temporal summation was observed for IPIs up to 0.5–0.8 ms, while refractoriness was observed up to 1.5–2.5 ms, and an IPI of 1 ms was used for the subsequent two-electrode experiments.

In five two-electrode experiments, EMG responses to stimulation at electrode *A* alone, electrode *B* alone, and electrode *A* followed by electrode *B* were recorded. The stimulus amplitude at electrode *A* was constant while a range of stimulus amplitudes spanning the recruitment range was applied through electrode *B* (figure 7(a)). The resulting relative refractory responses, R_r (equation 7), were fit with a sigmoid function (equation 8; figure 7(b)), and I_1 and I_2 , the stimulus amplitudes that when delivered to electrode *B* yielded minimum and full overlap, respectively, were estimated from the parameters of the sigmoid using equations 9 and 10. These values were used to calculate the parameters of the current-distance relationship, $k = 27 \pm 11 \mu\text{A}/\text{mm}^2$ (mean $\pm$ s.d, $n=5$) and $I_0 = 49 \pm 13 \mu\text{A}$, using equations 4 and 5 (Table 3).

4. Discussion and Conclusions

A new method to measure the current-distance relationship using the refractory interaction technique was developed based on two specific cases of minimum and full overlap between regions of neural activation by a pair of electrodes. Model-based evaluation indicated that the method provided robust estimates of the current-distance relationship and the spatial extent of neural activation (mean relative error less than 11 %). The results across different current amplitudes and different populations were consistent, and the standard deviations of the estimated parameters were only 4–30 % of the mean values. Although the maximum errors in the current distance parameters appeared to be relatively large (e.g., up to 46 % error in I_0), the errors are substantially less than the maximum errors in estimates of the current distance parameters using previous refractory interaction methods (Mahnam *et al.* 2008). The method was used to measure the current-distance parameters of peripheral motor fibers with an intrafascicular microelectrode array. The results were consistent across experiments, demonstrating the reliability of the method in measuring the CDR parameters. The estimated absolute thresholds, I_0 , were close to the minimum stimulus amplitudes obtained from measurements of recruitment curves (Table 3). The estimated current-distance constant was $27 \mu\text{A}/\text{mm}^2$, which is in the lower end of the range reported for stimulation of neural fibers (Ranck 1975). The proposed method can be used to estimate the spatial extent of neural activation or to evaluate the spatial selectivity of electrical stimulation with electrode arrays.

In comparison to previous methods used to estimate the current-distance relationship, the proposed method has the advantage of estimating both parameters of the quadratic CDR (equation 1) using only two electrodes. In contrast, the methods proposed by Yeomans *et al.* (1986) and Snow *et al.* (2006) do not provide the absolute threshold of the CDR, I_0 , and the methods proposed by Fouriez and Wise (1984) and Liang *et al.* (1991) require repeated measurements with different interelectrode distances to determine both the absolute threshold and the current-distance constant. Further, the methods proposed by Yeomans *et al.* (1986) and Snow *et al.* (2006) use the amount of overlap directly in computation of the CDR parameters, and therefore the results may be sensitive to violation of the underlying assumptions, including a uniform distribution of neurons in the medium and a linear relationship between the response and the number of activated neurons. The proposed method enables direct calculation of the parameters of the CDR, using two specific cases of minimum and full overlap, which are determined based on changes in the response (instead of absolute values). The specific cases of minimum and full overlap correspond to I_a values for which the defined relative refractory response (equation 7) starts to saturate. Therefore, the quantitative estimates of the CDR parameters are obtained based on specific qualitative changes in the relative response, which results in good estimates of the CDR parameters despite the nonlinear relationship between the response (EMG in this study) and the extent of neural activation. This makes the method reliable for other behavioral studies with CNS stimulation where the relationship between the recorded response and the extent of activation may be more complicated. Finally, in comparison to direct measurement of the CDR using the response of a single neuron, the proposed method estimates the CDR based on the response of an actual population of activated neurons. Therefore, variability in activation of neurons due to excitation of different neural elements, and differences in their orientation and excitability are not expected to have significant effect on the estimate of the extent of activation. For a quantitative comparison of these methods, please refer to Mahnam *et al.* (2008).

As noted in the Introduction, the current distance relationship is sensitive to stimulation and electrode parameters, as well as the properties of the neurons and tissue. Previous estimates of current distance parameters exhibited substantial variation even within experiments (Fouriez and Wise 1984, Yeomans *et al.* 1986, Liang *et al.* 1991). Therefore, it is not surprising that we observed differences in the current distance parameters estimated with the computational model and from the experimental measurements. For example, the model represented the electrode as a point source, whereas the finite size of the stimulating electrode in the experiments may have influenced the current distance properties, especially for the small electrode to fiber distances considered here. Further, the larger absolute thresholds in the experiments may reflect damage to nerve fibers immediately adjacent to the electrode that occurred during insertion and rendered those fibers unavailable for stimulation. The model did not include the inhomogeneities present in the nerve, including the surrounding perineurium. The presence of the perineurium could clearly impact the absolute thresholds for stimulation, and may be one factor contributing to the differences in I_0 and k in the model-based and experimental measurements of the current distance relationship. These limitations, however, reinforce the utility of the model-based approach to development and validation of the proposed method. The accuracy and reliability of the proposed method was evaluated by comparing the estimated current distance relationships to the true current distance relationships in the same simulations (Tables 1 and 2).

The estimation of the parameters of the CDR was more vulnerable to errors with small current amplitudes. For small I_a , the extent of activation for electrode A is small and the two radii of activation for electrode B (i.e., minimum and full overlap) will be similar. In addition, the error associated with representing the activated region by a limited number of nerve fibers increases for small regions of activation, corresponding to small I_a . Indeed, the

absolute threshold was underestimated in most cases, but increased as I_a increased. However, for other methods based on refractory interaction (Fouriez and Wise 1984, Yeomans *et al.* 1986, Liang *et al.* 1991, Snow *et al.* 2006), the results are more sensitive to measurement errors, since only a single point is used to estimate the CDR (Mahnam *et al.* 2008). For large I_a , the activated region R_a may extend beyond the border of the nerve. In this case the quadratic CDR is violated, equation (1) cannot be applied, and the current-distance constant, k , is underestimated. However, a uniform distribution of fibers in the region of electrode B is not required, and the proposed method provided accurate and consistent results even when electrode B was placed on the border of the nerve (Table 2).

The estimated current-distance constant, k , was smaller and the absolute threshold, I_0 , was larger when larger currents I_a were applied to electrode A (Table 1). This may be due in part to an inhibitory effect of the first pulse preventing some fibers on the border of the activated region of the second pulse from being activated. The effect of this behavior is to increase the apparent overlap, which results in smaller I_1 and I_2 . Usually there was a slight increase in the number of such fibers for higher I_a values, which resulted in smaller k and larger I_0 estimates. However, very few fibers exhibited this behavior and did not have a substantial effect on the estimated current-distance parameters. For the first distribution of fibers, the mean number of fibers across 17 values of I_b from 3 to 35 μA was 1.4, 1.9 and 2.1, respectively, when I_a was equal to 4, 6 and 8 μA .

Large variability in the excitability of fibers may also affect the accuracy of the methods using refractory interaction, including the proposed method. Nerve fibers that are activated by electrode A but not by electrode B , when there is full (geometric) overlap between the regions activated by A and B (figure 1(b)), will result in an overestimation of I_2 (equations 2–3). However, in our baseline simulations no fibers were identified in the overlap region that were activated by electrode A , but not by electrode B , and additional simulations of fiber populations with higher variability in excitability (created by increasing the variance of fiber diameters to $4 \mu\text{m}^2$), indicated that such behavior was rare (only one fiber in one population out of 6 simulated populations exhibited this behavior). Thus, while a theoretical possibility, it appears unlikely that such fibers will influence estimation of I_0 and k using the proposed method.

The results of the proposed method were also accurate when charge-balanced biphasic pulses were used instead of monophasic pulses. The anodic phase of the stimulus activated additional fibers at the virtual cathodes, increased the apparent density of fibers in the region of activation, and improved the accuracy of the method (Table 2). Further, the relative errors in estimating the spatial extent of activation were smaller when the interelectrode distance was increased from 200 μm to 300 μm (Table 2), again due to the lower sensitivity of the estimated current-distance parameters when more fibers were activated.

Although we applied the novel refractory interaction method to intraneural stimulation of peripheral nerve, the proposed method can be readily applied to predict the spatial extent of neural activation other applications of electrical stimulation or to evaluate the spatial selectivity of activation with electrode arrays. The method requires a monotonic relationship between the number of activated neurons and the observed response (EMG in our application), but does not rely on a linear input output relationship. Before application in the central nervous system, which contains a diversity of neural elements (Grill and McIntyre 2001), model-based studies are required to assess the accuracy of the method in the presence of variable density and excitability of the different neural elements as well as potential indirect effects on excitation resulting from stimulation of presynaptic terminal axons (Baldissera *et al.* 1972; Gustafson and Jankowska 1976).

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Figure 1.

Geometric model of refractory interaction. **a.** Geometry of the overlap of the regions of activation of two electrodes positioned distance L apart. **b.** Regions of activation for electrodes A and B, in the cases of minimum and full overlap. r , r_1 and r_2 are the radii of the activated regions for I_a on electrode A, $I_b = I_1$ on electrode B for minimum overlap, and $I_b = I_2$ for full overlap, respectively.

Figure 2.

Excitation properties of the computational model of stimulation of a population of peripheral motor nerve fibers. **a.** Mean $\pm$ standard deviation of the input-output (recruitment) curve across three different random populations activated by cathodic monophasic stimuli delivered with a point source electrode. **b.** Refractory curves for current amplitudes that when applied as single pulses activated 25%, 50%, and 75% of the fibers.

Figure 3. Responses to two-electrode paired pulse stimulation in the model of a population of peripheral motor nerve fibers. **a.** Responses to individual stimuli applied separately to each electrode, R_a and R_b , responses to paired pulses delivered to both electrodes, R_{ab} , and the arithmetic sum of the individual responses, $R_b + R_a$, for $I_a = 8$ μA. **b.** Relative paired-pulse responses, R_r , and the sigmoid function fit to the data.

Figure 4.

True and estimated current-distance relationships for the population model. Excitation thresholds for individual fibers (points), nine current-distance curves estimated using the refractory interaction method with $I_a = 4, 6$ and $8 \mu\text{A}$ across three different populations (thin lines), and the fit of the current-distance equation to the points at the lower boundary of the threshold band (thick line) as the optimal current-distance relationship.

Figure 5. Microelectrode placement in the nerve fascicle for experiments. The overlapped regions of activation of electrode A (left) and B (right) is also demonstrated, The position of nerve fibers and their excitations in response to electrode A (squared), electrode B (circled) and two-electrode stimulation (black) are borrowed from a sample simulation.

Figure 6.

Excitation properties of intraneural microstimulation of peripheral motor nerve fibers. **a.** Sample input-output (recruitment) curve of the electromyogram (EMG) evoked in the medial gastrocnemius as a function of the stimulation amplitude from one experiment and the sigmoid fit to the data. **b.** Refractory curves of the EMG evoked in response to single and paired-pulse stimulations (dotted and thin lines respectively) with different inter-pulse intervals, and the refractory curve of the integral of absolute difference between these single- and paired pulse EMG responses (thick line).

Figure 7. EMG responses to two-electrode paired-pulse intraneural microstimulation of peripheral motor nerve fibers. **a.** Responses to individual stimuli applied separately to each electrode, R_a and R_b , responses to paired pulses delivered to both electrodes, R_{ab} , and the arithmetic sum of the individual responses, $R_b + R_a$. **b.** Relative refractory paired-pulse responses, R_r , and the sigmoid function fit to the data.

Estimated and actual parameters of the current-distance relationship in computer simulations of extracellular stimulation in three different populations of motor nerve fibers.

Table 1

Population number	I _a	I ₁	I ₂	k (μA/mm ²)		I ₀ (μA)	Relative Error (%)	
				mean±s.d.			mean±s.d.	mean±s.d.
1	4	9.15	20.8	275		3.22		7.1 ± 5.6
	6	8.73	22.5	240	233±46	4.77	4.37±1.01	3.2 ± 1.4
	8	6.12	24.6	183		5.11		10 ± 1.2
2	4	14.5	15.5	274		3.99		7.3 ± 3.1
	6	9.08	21.2	228	227±48	5.00	4.89±0.855	1.5 ± 1.0
	8	7.01	23.2	178		5.69		8.6 ± 2.0
3	4	11.8	20.5	304		3.61		11 ± 3.6
	6	9.46	24.3	273	266±43	4.73	4.88±1.35	8.3 ± 2.1
	8	9.07	24.5	220		6.30		5.7 ± 6.8
Average across three populations	4			284±17		3.61±0.39		
	6			247±23		4.83±0.15		
	8			194±23		5.70±0.60		
Optimal values				218		5.33		

Estimated and actual parameters of the current-distance relationship in computer simulations with different electrode positions, different interelectrode distances (IED), and biphasic stimulus pulses.

Table 2

	I_a	I_I	I_2	k ($\mu A/mm^2$)		I_0 (μA)	Relative Error (%)	
				mean $\pm$ s.d.			mean $\pm$ s.d.	mean $\pm$ s.d.
Electrode B on border IED: 200 μm	4	3.67	23.6	241	1.42		11 $\pm$ 15	
	6	4.98	27.1	251	2.95	2.88 $\pm$ 1.43	5.8 $\pm$ 9.1	
	8	5.33	28.2	219	4.27		4.8 $\pm$ 5.5	
Electrode B on border IED: 300 μm	4	19.5	28.8	224	3.73		5.9 $\pm$ 7.7	
	6	16.2	33.0	207	215 $\pm$ 8.50	5.05 4.70 $\pm$ 0.851	3.6 $\pm$ 1.1	
	8	12.9	41.7	215	5.32		0.75 $\pm$ 0.00	
Biphasic Stimulation IED: 300 μm	4	23.5	30.0	253	3.88		4.8 $\pm$ 4.4	
	6	19.5	33.5	228	231 $\pm$ 20.2	5.41 5.24 $\pm$ 1.28	2.6 $\pm$ 0.46	
	8	16.2	38.1	213	6.43		4.8 $\pm$ 7.8	
Optimal values				218	5.33			

Table 3

Parameters of the current-distance relationship of peripheral motor fibers measured with an intrafascicular microelectrode array. IPI is the inter-spike interval used in two-electrode experiments, L is the distance between electrodes and $I_{50\%}$ is the stimulus amplitude for 50% response obtained from recruitment curve fit.

Experiment	IPI (ms)	L (mm)	$I_{50\%}$ (μ A)	I_a (μ A)	I_1 (μ A)	I_2 (μ A)	I_0 (μ A)	k (μ A/mm ²)
1	1	0.6	53	60	43	57	36	36
2	1	0.4	73	53	70	78	67	42
2	1	0.6	49	40	44	54	42	17
3	1	0.4	75	80	40	47	59	19
3	1	0.6	40	60	62	70	39	23
Mean							49	27
Std							13	11