Supplement

Figure S-1 Mesh convergence properties

A: Computed sensitivity profiles for a bipolar recording (E12) at 4 mesh sizes, near the center of the large fascicle shown in figures 6-7 (vagus; 42,720 µm², 4.48 µm perineurium). Characteristic mean element lengths are reported based on the mean element length of the generated mesh inside the fascicle, as opposed to the "nominal" mesh length which was passed as an instruction to GMSH. The simulation with a mesh length of 36 µm corresponds to the default settings with which results were generated.

B: Simulated median single-unit field potential (SUFP)magnitudes as a function of mean element length within the small and large fascicles shown in figures 6-7, for typical myelinated and unmyelinated fibers (1.75 and 0.53 µm diameter, respectively). Grey boxes indicate simulations corresponding to our "typical" mesh configuration.

C: Simulated extracellular stimulation thresholds magnitudes as a function of mean element length within the small and large fascicles shown in figures 6-7, for myelinated and unmyelinated fibers. Unmyelinated fiber thresholds in particular show a strong trend with mesh size; our simulations are likely to underestimate the effects of fascicle geometry on unmyelinated axon thresholds.

D: Computed sensitivity profiles for a bipolar recording (E12) for simulation domains ranging in size from 6×6×3 mm to 32×32×16 mm, showing convergence for large model domains. The striped area is the peak region, for which the relative error measurements shown in panel E are calculated.

E: Relative error in computed sensitivity profile in the peak region (striped area in panel D) for different domain sizes, expressed as the RMS difference from the largest domain (32 mm).

Table S-2 Equations for derivation of ultrastructural parameters from fiber diameter and g-ratio

$g_0(d_F) = 0.01876226\, d_F + \ 0.478749 + \ 0.1204\, d_F^{-1}$
$d_{ax,NODE} = \ d_{ax,MYSA} = (0.00630378\, d_F^3 \ + \ 0.207054\, d_F^2 + \ 0.5339) * \frac{g * d_F}{g_0(d_F)}\,(\mu m)$
$d_{ax,STIN} = \ d_{ax,FLUT} = \ d_F * g * \frac{g_0(d_F)}{g_0(d_F)} = d_F * g\,(\mu m)$
$n_{lamella} = \frac{158.4396\, d_F^{1.425488}}{17.281599 + d_F^{1.425488}} + \ 39.5452$
$\ell_{NN} = \frac{1326.7932\, d_F^{3.133103}}{593.404881 + \ d_F^{3.133103}} + 5.1622\, d_F + \ 46.18\ (\mu m)$
$\ell_{FLUT} = 2.5811 * d_F + 19.59\ (\mu m)$

In table S-2, g_0 is the g-ratio predicted from our extrapolation of the relationships in McIntyre et al. (2002). d_F is the (outer) fiber diameter, and $d_{ax,PART}$ where $PART \ \ni \{NODE, MYSA, FLUT, STIN\}$ are the (inner) axon diameters. As we have experimentally-derived distributions for the g-ratios g of individual axons, we scale the ultrastructure relationships by $g/g_0(d_F)$ for each simulated axon to achieve that axon's g-ratio in the STIN compartment. $n_{lamella}$ is the number of myelin lamella (which is not constrained to be integer-valued), and ℓ_{NN} is the length between nodes of Ranvier.

Table S-3 Maximum ion channel conductances for the axon models used. These were not modified from the original sources. For electrotonic properties of the models used, see table 2

	Myelinated Afferent (Gaines et al., 2018)	Myelinated Efferent (Gaines et al., 2018; McIntyre et al., 2002)	Unmyelinated Axon (Sundt et al., 2015)
NODE	$\overline{g_{\mathrm{Na}_f}} = 3.0\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{Na}_p}} = 0.01\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_f}} = 0.02737\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.04106\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.006005\ \mathrm{S/cm^2}$	$\overline{g_{\mathrm{Na}_f}} = 3.0\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{Na}_p}} = 0.01\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_f}} = 0.02568\mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.08\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.007\ \mathrm{S/cm^2}$	$\overline{g_{\mathrm{Na}_f}} = 0.04\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.04\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.0001\ \mathrm{S/cm^2}$
MYSA	$\overline{g_{\mathrm{K}_f}} = 0.1642\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.001324\ \mathrm{S/cm^2}$ $\overline{g_q} = 0.003102\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.001716\ \mathrm{S/cm^2}$	$\overline{g_{\mathrm{K}_f}} = 0.15074\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.002581\ \mathrm{S/cm^2}$ $\overline{g_q} = 0.002232\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.002\ \mathrm{S/cm^2}$	
FLUT	$\overline{g_{\mathrm{K}_f}} = 0.02737\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.001324\ \mathrm{S/cm^2}$ $\overline{g_q} = 0.003102\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.0001716\ \mathrm{S/cm^2}$	$\overline{g_{\mathrm{K}_f}} = 0.02568\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.002581\ \mathrm{S/cm^2}$ $\overline{g_q} = 0.002232\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.0002\ \mathrm{S/cm^2}$	
STIN	$\overline{g_{\mathrm{K}_f}} = 0.02737\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.001324\ \mathrm{S/cm^2}$ $\overline{g_q} = 0.003102\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.0001716\ \mathrm{S/cm^2}$	$\overline{g_{\mathrm{K}_f}} = 0.02568\ \mathrm{S/cm^2}$ $\overline{g_{\mathrm{K}_s}} = 0.002581\ \mathrm{S/cm^2}$ $\overline{g_q} = 0.002232\ \mathrm{S/cm^2}$ $\overline{g_{leak}} = 0.0002\ \mathrm{S/cm^2}$	

Conductances: g_{Na_f} fast sodium; g_{Na_p} persistent sodium; g_{K_f} fast potassium; g_{K_s} slow (delayed-rectifier) potassium; g_q HCN channel; g_{leak} leakage

Figure S-4. Parameter sweeps for NEURON model properties

A: Single-unit field potentials (SUFPs, left) and evoked compound action potentials (ECAPs, right) for an example unmyelinated axon (0.53 µm diameter) for a range of segment discretization lengths ranging from 2 to 50 µm per segment. The simulation with a segment discretization length of 20 µm corresponds to the default settings with which results were generated, and is indicated with a grey box. ECAP shows the summation of 4000 unmyelinated axons to a 3.95 mA stimulus, as per figure 10D.

B: RMS error (RMSE) relative to the 2 µm simulation, across all SFAPs (left, n=4000) and ECAPs (right). Grey boxes indicate simulations corresponding to the default settings of ViNERS.

C: SUFPs for an example myelinated axon (1.75 µm diameter, top) and an example unmyelinated axon (0.53 µm diameter, bottom) for a range of fiber lengths (4 mm – 12 mm), and corresponding ECAPs (3.95 mA and 3.95 mA, respectively). Note high-frequency interpolation artifact for small model fiber lengths; this high-frequency artifact does not have a visible effect on the simulated ECAPs. Asterisk indicates finite-simulation-domain artifact for a 6 mm unmyelinated axon model. Grey boxes indicate simulations corresponding to the default settings of ViNERS.

D: RMS error (RMSE) relative to the 12 mm simulation, across all SFAPs (left, n=4000) and ECAPs (right). Grey boxes indicate simulations corresponding to the default settings of ViNERS.

Table S-5 Simulated axon properties.

Fiber Diameter (µm)	g-ratio	conduction velocity (m/s)	SUFP magnitude (nV_{PP})	axon count
0.68	0.442	1.16	26.5	14
0.99	0.548	1.87	76.9	50
1.15	0.529	2.10	90.5	65
1.34	0.479	2.28	149.3	45
2.26	0.316	3.06	102.0	2
1.27	0.633	2.56	219.1	49
1.51	0.533	2.72	150.2	50
1.71	0.550	3.14	201.6	70
1.50	0.671	3.09	274.4	83
1.95	0.569	3.69	248.1	55
1.75	0.669	3.60	288.9	65
1.99	0.679	4.18	290.9	57
2.24	0.561	4.30	379.4	40
2.57	0.622	5.45	112.0	46
2.24	0.674	4.79	484.6	67
2.90	0.648	6.64	339.7	31
3.61	0.584	8.76	430.9	5
2.46	0.746	5.73	516.0	18
3.18	0.705	8.03	443.2	23
3.63	0.677	9.54	537.6	16
7.19	0.665	28.53	722.9	1
4.89	0.707	16.03	703.2	8
4.15	0.706	12.39	759.5	7
5.72	0.712	20.75	580.4	3

Conduction velocity and SUFP magnitudes were derived from the NEURON-based simulation as described in the methods, "Modelling Axon Populations in NEURON", using the single-fascicle geometry shown in Figure 9(B)

Figure S-6. Convergence analysis for number of membrane current profiles.
A: comparison of ECAPs simulated using 8, 12, 24, 36, 48, and 72 membrane current profiles, for 8 different arrangements of axons within the fascicle pattern shown in figure 10A. from left to right: myelinated axon response, sub-maximal stimulus (21% ± 1% of axons recruited); myelinated axon response, maximal stimulus (99.5% ± 0.1% of axons recruited); unmyelinated axon response, maximal stimulus (4.8 ± 0.1% of axons recruited).

B: Scatterplots of ECAP magnitudes (peak-peak voltages) for the ECAPs shown in panel A. The effect of changing the number of membrane current profiles is similar in magnitude to the variation in response between different axon arrangements within the nerve.

Table S-7 Equations of trajectory for example tortuous fascicle

$$x = \text{range}(-6\ mm, 6mm)$$
$$y = 0.075\cos(3\pi\ x)/\left(1 + e^{-\frac{x}{0.5\ mm}}\right)$$
$$z = 0.075\sin(3\pi\ x)/\left(1 + e^{-\frac{x}{0.5\ mm}}\right) + 0.22\ mm$$

Table S-8 Indicative runtime for each of the modules of ViNERS

Module	Runtime (minutes)	Details	Parallel execution
Meshing & Extracellular Field generation (models.nerve_anatomy)	14.78	Mesh formation and extracellular potential	No
Axon population generation (models.axon_population)	0.37 ± 0.05 (n=3)	pelvic; other axon population runtimes may vary.	No
Sensitivity field calculation (models.nerve_anatomy)[1]	112.9	Time for one fascicle at standard mesh resolution; proportional to number of mesh points.	No
Computation of membrane current profiles (models.membrane_currents)	126.0	Standard selection of n=24 groups.	Yes (12 cores)
Computation of SUFPs (models.axon_sfap)	109.3 ± 4.91 (n=8)	Grid of median axons of each type	Yes (12 cores)
Computation of axon thresholds (models.axon_thresholds)	10.26 ± 1.05 (n=14)	Reduced sample of 15 axons, as described in methods	Yes (12 cores)
Computation of extracellular stimulus responses (models.nerve_stimulation)	1,860 ± 22 (n=3) (30.5 hours)	31 stimulation levels; runtime is linear in number of stimulation levels.	Yes (12 cores)
Assembly of responses into ECAPs (models.ECAP_response)	83.5 ± 48.6 (n=3)	31 stimulation levels	Yes (12 cores)

All module execution times were run on an x64-based PC based on an Intel Xeon Gold 6128 (12 cores) with 96 GiB RAM running Windows 10, MATLAB R2019b, and NEURON 7.7.2. Where multiple runs have been sampled, data are presented as mean ± standard deviation.

[1] Assuming sensitivity field is not derived from extracellular potential via reciprocity theorem.

Table S-9 ANCOVA tables of results: thresholds

	Myelinated Axon Model					Unmyelinated Axon Model				
Effect	SS	DF	Mean Sq.	F	p	SS	DF	Mean Sq.	F	p
PW	17227	1	17227	9.32	0.003	403354	1	403354	1.59	0.212
FD	4.2	1	4.2	0	0.962	323511	1	323511	1.27	0.264
Sep	1043625	[1]	1043625	564	< 0.0001	17294502	[1]	17294502	68.0	< 0.0001
PW*FD	137944	1	137944	74.6	< 0.0001	1796157	1	1796157	7.07	0.001
PW*sep	191516	1	191516	104	< 0.0001	419586	1	419586	1.65	0.204
FD*sep	2908	1	2909	1.57	0.214	81323	1	81323	0.32	0.574
Error	116485	63	1829			16006291	63	254155		
Total	6909157	69				107231820	69			

Abbreviations: PW, perineurium width (continuous). FD, fascicle diameter (continuous). sep, fascicle-electrode separation (categorical).

Table S-10 ANCOVA tables of results: SUFP magnitudes

	Myelinated Axon Model					Unmyelinated Axon Model				
Effect	SS	DF	Mean Sq.	F	p	SS	DF	Mean Sq.	F	p
PW	19214	1	19214	41.2	< 0.0001	954	1	954	51.1	< 0.0001
FD	56266	1	56266	121	< 0.0001	2003	1	2003	107	< 0.0001
sep	521718	[1]	521718	1118	< 0.0001	13127	[1]	13127	702	< 0.0001
PW*FD	524	1	524	1.12	0.293	214	1	214	11.5	0.001
PW*sep	12860	1	12860	27.6	< 0.0001	784	1	784	41.9	< 0.0001
FD*sep	47335	1	47335	101	< 0.0001	1789	1	1789	95.7	< 0.0001
3-way	1980	1	1980	4.2	0.0436	248	1	248	13.3	0.0005
Error	28925	62	467			1159	62	19		
Total	1800772	69				38181	69			

Abbreviations: PW, perineurium width (continuous). FD, fascicle diameter (continuous). sep, fascicle-electrode separation (categorical, co-variate).

FIGURE S1

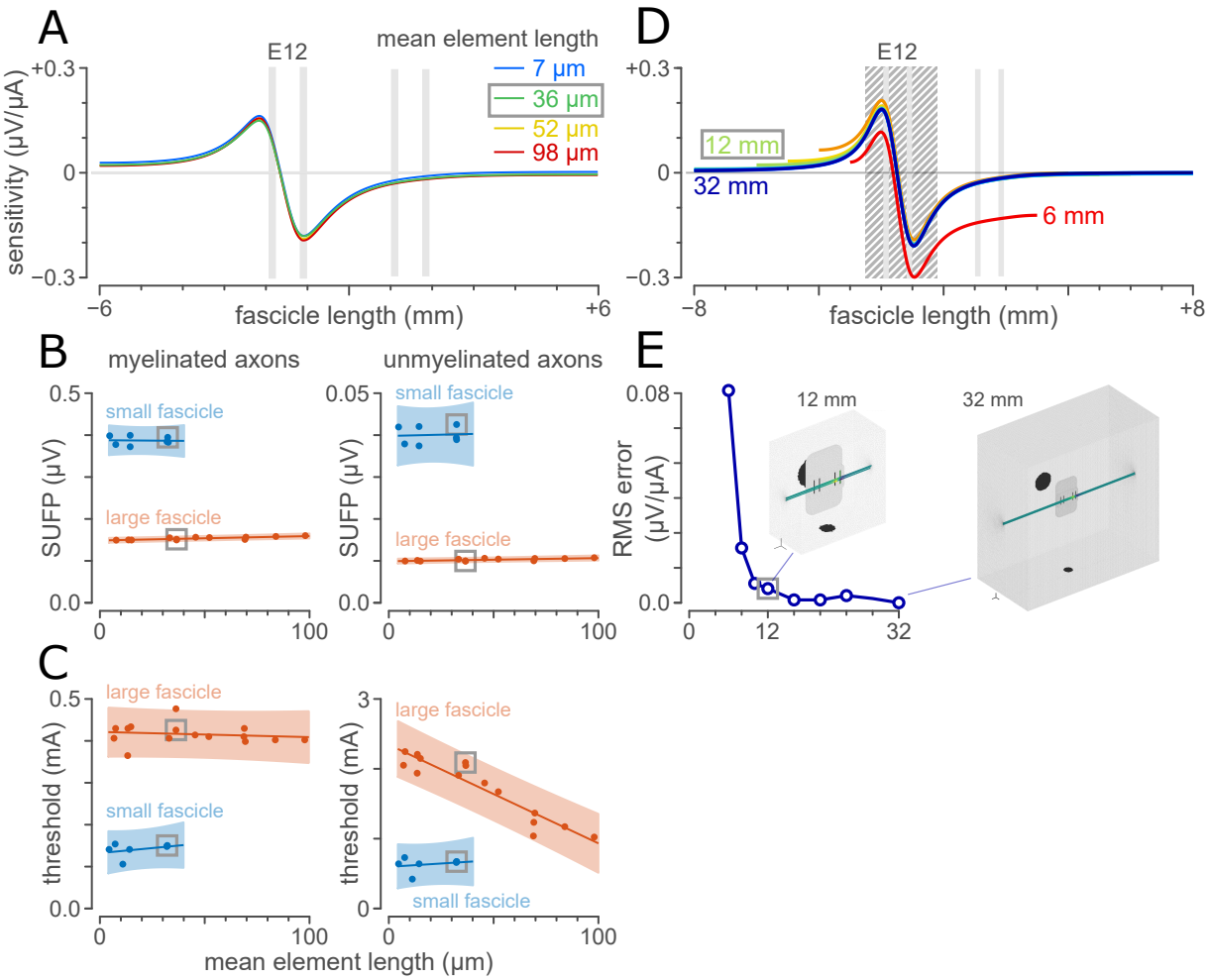

A
E12
mean element length
7 µm
36 µm
52 µm
98 µm
sensitivity (µV/µA)
+0.3
0
−0.3
−6
+6
fascicle length (mm)
D
E12
12 mm
32 mm
6 mm
+0.3
0
−0.3
−8
+8
fascicle length (mm)
B
myelinated axons
unmyelinated axons
small fascicle
large fascicle
SUFP (µV)
0.5
0.0
0.05
0
100
E
RMS error (µV/µA)
0.08
0.0
12 mm
32 mm
0
12
32
C
large fascicle
small fascicle
threshold (mA)
0.5
0.0
3
0
100
mean element length (µm)

FIGURE S4

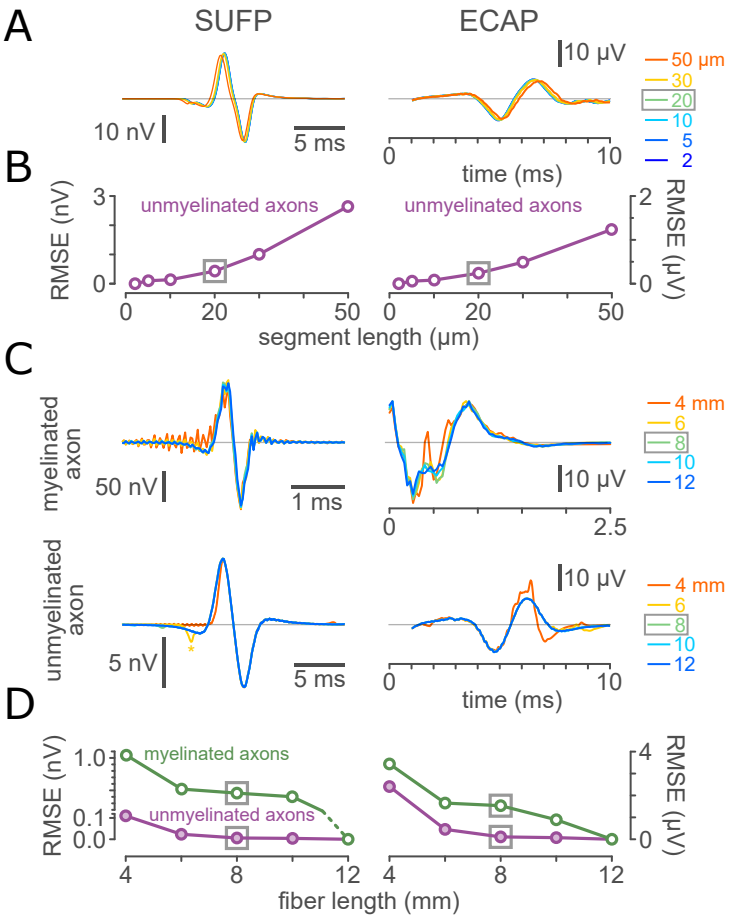
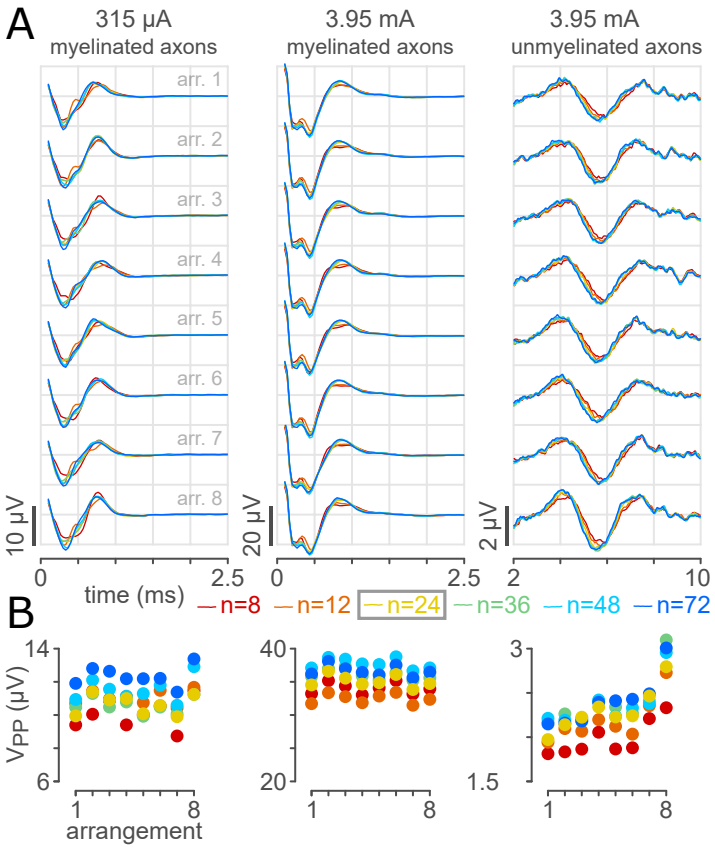

A
SUFP
ECAP
10 nV
5 ms
10 µV
50 µm
30
20
10
5
2
0
10
time (ms)
B
RMSE (nV)
3
0
unmyelinated axons
unmyelinated axons
2
0
RMSE (µV)
0
20
50
segment length (µm)
C
myelinated axon
50 nV
1 ms
10 µV
4 mm
6
8
10
12
0
2.5
unmyelinated axon
5 nV
5 ms
10 µV
time (ms)
D
RMSE (nV)
1.0
0.1
0.0
myelinated axons
unmyelinated axons
4
8
12
RMSE (µV)
4
0
fiber length (mm)

FIGURE S6


A
315 µA
myelinated axons
3.95 mA
myelinated axons
3.95 mA
unmyelinated axons
arr. 1
arr. 2
arr. 3
arr. 4
arr. 5
arr. 6
arr. 7
arr. 8
10 µV
20 µV
2 µV
0
2.5
0
2.5
2
10
time (ms)
n=8
n=12
n=24
n=36
n=48
n=72
B
VPP (µV)
14
6
40
20
3
1.5
1
8
1
8
1
8
arrangement