

ACCEPTED MANUSCRIPT • OPEN ACCESS

Overcoming temporal dispersion for measurement of activity-related impedance changes in unmyelinated nerves

To cite this article before publication: Ilya Tarotin *et al* 2022 *J. Neural Eng.* in press <https://doi.org/10.1088/1741-2552/ac669a>

Manuscript version: Accepted Manuscript

Accepted Manuscript is “the version of the article accepted for publication including all changes made as a result of the peer review process, and which may also include the addition to the article by IOP Publishing of a header, an article ID, a cover sheet and/or an ‘Accepted Manuscript’ watermark, but excluding any other editing, typesetting or other changes made by IOP Publishing and/or its licensors”

This Accepted Manuscript is © 2022 The Author(s). Published by IOP Publishing Ltd..

As the Version of Record of this article is going to be / has been published on a gold open access basis under a CC BY 3.0 licence, this Accepted Manuscript is available for reuse under a CC BY 3.0 licence immediately.

Everyone is permitted to use all or part of the original content in this article, provided that they adhere to all the terms of the licence <https://creativecommons.org/licenses/by/3.0>

Although reasonable endeavours have been taken to obtain all necessary permissions from third parties to include their copyrighted content within this article, their full citation and copyright line may not be present in this Accepted Manuscript version. Before using any content from this article, please refer to the Version of Record on IOPscience once published for full citation and copyright details, as permissions may be required. All third party content is fully copyright protected and is not published on a gold open access basis under a CC BY licence, unless that is specifically stated in the figure caption in the Version of Record.

View the [article online](#) for updates and enhancements.

Overcoming temporal dispersion for measurement of activity-related impedance changes in unmyelinated nerves

Ilya Tarotin¹, Svetlana Mastitskaya¹, Enrico Ravagli¹, Justin D Perkins², David Holder¹ and Kirill Aristovich¹

1. Department of Medical Physics and Biomedical Engineering, University College London, Gower Street, London WC1E 6BT, United Kingdom

2. Clinical Science and Services, Royal Veterinary College, Hawkshead Lane, Hatfield, UK.

Email: ilya.tarotin@ucl.ac.uk

Abstract:

Objective: Fast neural Electrical Impedance Tomography (FnEIT) is an imaging technique that has been successful in visualising electrically evoked activity of myelinated fibres in peripheral nerves by measurement of the impedance changes (dZ) accompanying excitation. However, imaging of unmyelinated fibres is challenging due to temporal dispersion (TP) which occurs due to variability in conduction velocities of the fibres and leads to a decrease of the signal below the noise with distance from the stimulus. To overcome TP and allow EIT imaging in unmyelinated nerves, a new experimental and signal processing paradigm is required allowing dZ measurement further from the site of stimulation than compound neural activity is visible. The development of such a paradigm was the main objective of this study.

Approach: A FEM-based statistical model of temporal dispersion in porcine subdiaphragmatic nerve was developed and experimentally validated ex-vivo. Two paradigms for nerve stimulation and processing of the resulting data – continuous stimulation and trains of stimuli, were implemented; the optimal paradigm for recording dispersed dZ in unmyelinated nerves was determined.

Main results: While continuous stimulation and coherent spikes averaging led to higher signal-to-noise ratios (SNR) at close distances from the stimulus, stimulation by trains was more consistent across distances and allowed dZ measurement at up to 15 cm from the stimulus (SNR = 1.8 ± 0.8) if averaged for 30 minutes.

Significance: The study develops a method that for the first time allows measurement of dZ in unmyelinated nerves in simulation and experiment, at the distances where compound action potentials are fully dispersed.

1. Introduction

Electroceuticals or bioelectronic medicines [1] are a novel emerging set of techniques aimed at treating diseases by selective stimulation of nervous tissue and neuromodulation of the internal organs innervated by it. The main nerve of interest is the vagus nerve which is the longest autonomic nerve in the body serving as an interface between the central nervous system and major internal organs. Electrical stimulation of the vagus nerve (VNS) and neuromodulation of the internal organs or areas of the brain supplied by it is a clinically approved technique for treatment of drug-resistant epilepsy [2], [3] and depression [4], and has a great potential for treatment of heart failure [5], rheumatoid arthritis (RA) [6] and a variety of inflammatory diseases via modulation of the cholinergic anti-inflammatory pathway [7]–[9]. However, despite the good clinical efficacy and great potential of VNS, it is prone to adverse effects with an incidence rate of up to 50% [10], [11] originating from non-targeted electrical stimulation of the vagus nerve that induces modulation of all organs supplied by it leading to undesired physiological effects. To reduce side effects, selective stimulation of the specific

1
2
3 fascicle within the nerve leading to a particular organ in the single direction of this organ is
4 required.
5

6 To allow selective stimulation of fascicles in the nerve, it is essential to know their precise
7 location within this nerve. Moreover, organisation of a closed feedback loop neuromodulation
8 of the desired organ relies on the measurement of functional activity of the fascicle supplying
9 this organ. Both capabilities can be achieved with fast neural Electrical Impedance
10 Tomography (EIT), a novel method capable of imaging electrical activity in nerves in their
11 cross-section.
12

13 1) *Fast neural EIT*

14 In fast neural EIT, the same as in conventional EIT, the impedance of the tissue is measured
15 by injecting sinusoidal electric current through a pair of electrodes and recording voltages
16 through other pairs of available electrodes [12]–[15]. Since the applied current amplitude is
17 constant, the measured voltage amplitudes are directly proportional to the tissue impedance
18 according to Ohm's law. Then, the electrode pairs are switched, and this procedure is repeated
19 for all electrode pairs so that numerous voltage measurements with different current injections
20 can be obtained.
21
22

23 EIT recordings usually contain background noise with physiological or hardware-related origin.
24 In fnEIT, physiological noise is primarily caused by neural activity and accounts for up to 95%
25 of total noise [16], with the remaining hardware-related noise including but not limited to
26 thermal effects, current source noise, digitization noise and stray capacitance [17].
27
28

29 Using the recorded voltages, images of the internal electrical impedance of the tissue can be
30 reconstructed by solving an inverse problem with the use of various numerical methods [12]:
31 signal-to-noise ratio exceeding 4 is usually required to obtain reproducible EIT images [18].
32 Previously, fast neural EIT was capable of reconstructing images of functional activity of the
33 brain [13], [14] and peripheral nerves in their cross-section [12], [15], [19] with temporal and
34 spatial resolutions of < 1 ms and < 200 μ m respectively [12], [19]. In addition, EIT imaging in
35 nerves was validated against micro-computed tomography (microCT) and histology [19].
36
37

38 The physiological basis of fast neural EIT relies on a principle of activation of nerve fibres
39 involving voltage-dependent ion channels. When a fibre depolarizes, ion channels switch to
40 an opened state and the impedance of the tissue decreases, so that electric current which is
41 externally applied in EIT, flows through them as the path of the least resistance. The changes
42 usually recorded in fast neural EIT are small and equal up to 0.1-1% of the baseline values in
43 the brain and myelinated nerves [12], [19]–[21], and much smaller ($< 0.01\%$) in mammalian
44 unmyelinated nerves [22]. This requires the noise threshold in the recordings to be $< 0.01\%$
45 which can theoretically be achieved using the existing EIT systems [16] if long-enough
46 averaging of the signal is performed.
47
48

49 2) *Temporal dispersion*

50 Although fast neural EIT is reliable for imaging neural activity in the brain and peripheral nerves,
51 imaging in autonomic nerves is more challenging for two reasons. First, autonomic nerves
52 mainly consist of small unmyelinated C fibres [23], [24] producing lower impedance changes;
53 second, conduction velocities of C fibres are significantly slower and more variable [25], [26].
54 As a result, the amplitude of the compound action potentials (CAPs) being an aggregate sum
55 of action potentials (APs) of individual fibres rapidly decreases along the nerve from the site
56 of its activation. This effect is referred to as temporal dispersion (TD) [26]–[29] which leads to
57 a fall of the CAP below the noise threshold beyond a few centimetres or in some cases even
58 a few millimetres from the stimulus [22], [26], [30].
59
60

1
2
3 The last point is especially important for the achievement of the goals stated for bioelectronic
4 medicines. First, mammalian, including human, vagus nerves are largely unmyelinated [23],
5 [31]. Second, for selective stimulation of the specific fascicles and closed-loop
6 neuromodulation of the internal organ which they supply, the neural activity propagating from
7 the organ must be recorded and imaged at the cervical level. The length of the nerve from the
8 internal organs to the neck in humans is around half a metre [32] that is significantly larger
9 than the theoretical limit of approximately 4 cm allowed by temporal dispersion in unmyelinated
10 fibres [22]. To overcome this limitation, a method capable of measuring dZ further from the
11 onset than allowed by temporal dispersion is required.

12
13
14 The feasibility of this objective can partly be justified by the fact that CAPs in nerves have
15 more pronounced phasic nature than dZs which are mainly monophasic, as was measured
16 experimentally in crabs [33], rats [12], [19] and large animals [34] as well as confirmed in the
17 current study (Figure 6 in Results). Therefore, the expectation is that CAPs will decrease in
18 amplitude much faster than dZ so that dZ will be visible further from the stimulation point than
19 CAPs are.

20
21
22 In addition, study [35] showed that when the stimulation paradigm had been changed from a
23 traditional continuous nerve stimulation to application of a high-frequency series of stimuli
24 separated by resting intervals, it was theoretically possible to record dZ at 20 cm from the
25 stimulus with SNR of 4 if averaging for 30 minutes, while the traditional approach cannot be
26 used at >5 cm [22]. However, this approach was based on a simple statistical model of an
27 arbitrary C fibre nerve which significantly differs from real mammalian autonomic nerves. Also,
28 the experimental parameters such as the noise, geometry of the electrodes and electrical
29 parameters were arbitrarily chosen based on the previously performed experiments in
30 peripheral nerves [12].

31
32 Therefore, in order to provide accurate predictions and determine whether it was realistic to
33 measure dZ in autonomic nerves at distances from the stimulation where CAPs were
34 cancelled out, this approach must have been optimised. It should have accounted for realistic
35 nerve histology and fibre composition as well as included a variety of experimental parameters
36 utilised in impedance measurement experiments performed with mammalian nerves. Then,
37 following the development and optimisation of the method, the predictions must have been
38 verified experimentally.

3) *Purpose*

39
40
41 The main purpose of this study was to develop and optimise a method to overcome temporal
42 dispersion and allow EIT recordings of phasic activity at far distances from the onset along the
43 nerve where compound activity is dispersed. This brings up the opportunity to image fascicles
44 of the autonomic nerves with fast neural EIT at the distances where the CAPs fall below the
45 noise and facilitate the development of bioelectronic medicines for selective stimulation of the
46 vagus nerve, neuromodulation of internal organs and treatment of associated drug-resistant
47 disorders. Specific questions addressed in this study were as follows:

- 48 1. What are the optimal stimulation and signal processing strategies and parameters
49 producing the largest impedance changes at different distances (15, 20 and 50 cm)
50 from the onset?
 - 51 2. How much averaging is required 1) to obtain a measurable signal (SNR > 1) and 2) to
52 image neural activity with EIT (which requires SNR > 4 [18]) at 15, 20 and 50 cm from
53 the site of stimulation?
 - 54 3. Are simulated results confirmed with the experimental data?
- 55
56
57
58
59
60

1
2
3 The first part of the study included the development of the accurate model of dispersion in the
4 porcine subdiaphragmatic nerve which was followed by the development and optimisation of
5 the method for overcoming dispersion and recording dZ at far distances from the stimulation
6 site.
7

8 **2. Methods**

9 **1) Experimental design**

10 The study was divided into the following steps:
11

12 **1. Development of an experimentally driven statistical model of the** 13 **subdiaphragmatic nerve (SN) of the pig.**

14 The model combined a) previously developed accurate FEM model of a C fibre [36] and
15 b) statistical model for simulation of temporal dispersion of CAPs and dZs [22] in a
16 complex nerve, consisting of a composition of unmyelinated and myelinated fibres [37].
17 The parameters of the model were chosen on the basis of ex-vivo experimental
18 recordings (CAP and dZ) obtained using a subdiaphragmatic nerve of the pig subjected
19 to repetitive continuous stimulation. As a result, the model could accurately simulate
20 compound action potentials and impedance changes at ~3 cm from the stimulus and
21 could be therefore utilized for the development and optimisation of a new method for
22 overcoming temporal dispersion and measurement dZ at further distances, where CAPs
23 were cancelled out due to dispersion.
24

25 **2. Development of a method for overcoming dispersion.**

26 Using the developed model, the optimal stimulation and signal processing paradigms to
27 record impedance changes at the distances where compound APs were dispersed were
28 obtained. For this, the modelled nerve fibre was subjected to repetitive stimuli at various
29 frequencies. For dZ extraction, the resultant signals were processed in two ways – 1)
30 averaging and band-pass filtering as single spikes and 2) averaging as trains of spikes
31 allowing band-pass filtering around the whole train thus significantly reducing the
32 bandwidth of the filter. The optimal parameters for dZ measurement at various distances
33 from stimulation were determined, the SNR at these distances was obtained. The final
34 predictions of the model have then been subjected to experimental verification using a
35 preparation of the porcine SN ex vivo (N = 18).
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

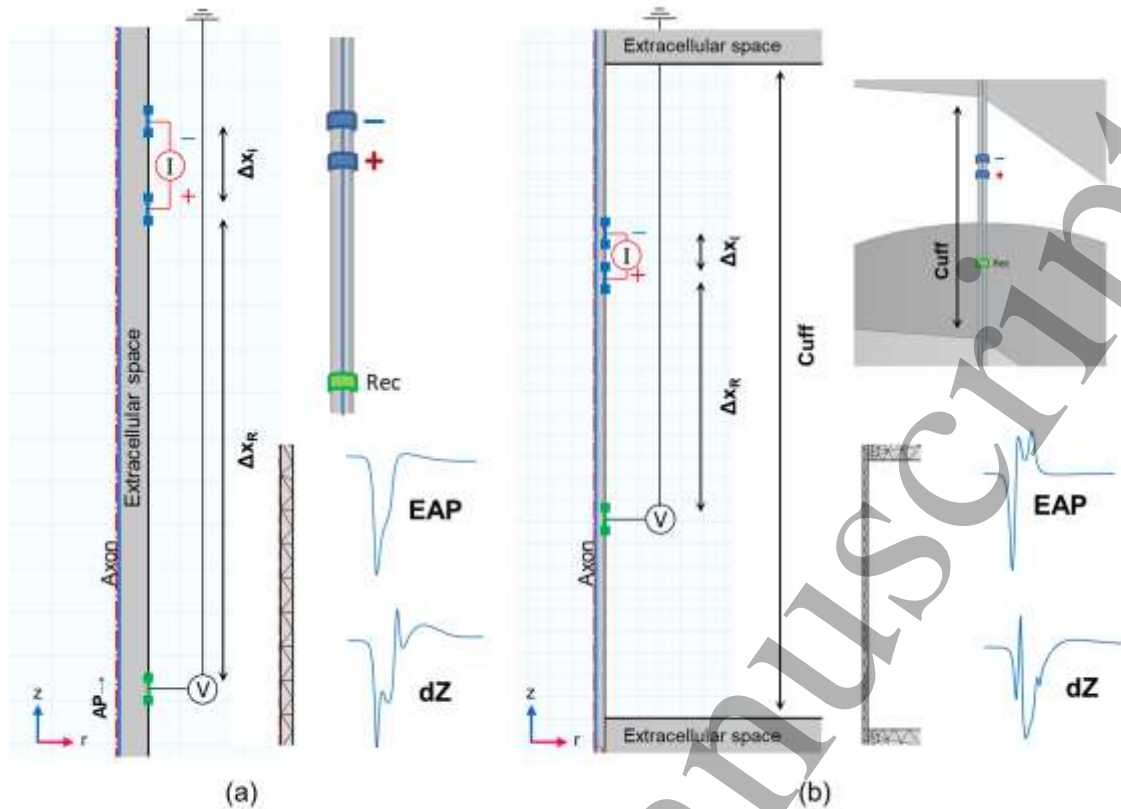


Figure 1 Axisymmetric representation of the model of a mammalian C fibre, based on [36], [38]. The axon is depicted by a blue line, the axis of symmetry is shown by the red dash-dotted line. The AP was induced from the end of the fibre; DC or AC were applied through two external electrodes (blue) placed 7.8 mm and 7.82 mm from the axon's end (distance between injecting electrodes $\Delta x_I = 0.02$ mm); the electric field was recorded by an external electrode (green) placed before the injecting ones, 7.7 mm from the proximal end of the fibre (distance between recording and injecting electrodes $\Delta x_R = 0.1$ mm).

(a) Initial model [36]; (b) The same model with the change in the structure of extracellular space to simulate the condition of ex-vivo experiments with the nerve cuff. For this, the width of the extracellular space was increased 50-fold outside the region along the nerve where electrodes were located ($L_{cuff} = 0.3$ mm).

Shapes of extracellular action potential (EAP) and impedance change (dZ) produced with each model as well as their triangular FEM meshes are depicted on the right.

2) Versatile statistical model of dispersion in nerve

a) FEM model of a single fibre

Impedance changes accompanying neural activity were obtained using the Finite Element (FEM) models of mammalian C fibre ($d = 1 \mu\text{m}$) bi-directionally coupled with the extracellular space (Figure 1) [36]. Two variants of the FEM model were designed in the study. In the first variant (Figure 1 (a)), the C fibre ($d = 1 \mu\text{m}$) was surrounded by a cylinder of extracellular space with the diameter equal to the one of the ring electrodes ($D = 0.01$ mm). The second variant (Figure 1 (b)) was designed to represent the ex-vivo experiments performed using multielectrode cuffs. When the silicone cuff is placed tightly around the nerve, as in the ex-vivo experiment performed in the current study (Figure 2), there is a significantly smaller amount of saline solution inside the cuff (in the electrode-nerve interface) than along other parts of the nerve. The shape of the recorded CAP, in this case, differs from the one recorded using hook electrodes [33], where the saline solution occupies uniform volume along the nerve (as in Figure 1 (a)). Therefore, to simulate the activity of the C fibre in the conditions similar to those when the cuff is present, the width of the extracellular space cylinder was increased 50-fold outside the 0.3 mm region along the nerve where electrodes were located (Figure 1 (b)).

The electric field in the extracellular space was simulated using volume conduction Poisson's equation, and the fibre was modelled using the conductance-based Hodgkin-Huxley type Tigerholm model of a mammalian C nociceptor [38]. The Tigerholm model was chosen as it could accurately represent the active behaviour of mammalian C fibre (porcine subdiaphragmatic nerves mainly consist of C fibres [31]) and was validated experimentally [38]. In addition, the model has previously shown itself capable of optimising experimental parameters for obtaining the largest impedance changes in unmyelinated nerves as well as explaining the biophysical origin of experimental recordings [36].

The Tigerholm model contained 10 ion channels and variable concentrations inside and outside of the fibre; these concentrations, together with other parameters, affected the membrane conductance which, in turn, led to changes in electric potentials inside and outside the membrane (eq. (27)-(28) in the supplementary material of [36]). The impedance change (dZ) signal measured in the model is related to a change in the membrane conductance which is, among other parameters, influenced by variable extra- and intracellular ionic concentrations.

For measurement of impedance change, constant direct current of small amplitude (1.25 μA , or 4 mA/cm^2) was continuously applied to the fibre during stimulation through two external ring electrodes. The reason was that using DC was less computationally intensive than AC, especially at high frequencies, and it was previously shown that dZ obtained with small amplitude DC and AC had the same biophysical origin [36].

Voltage was measured simultaneously using the recording electrode placed before the injecting ones with respect to ground (Figure 1), the same as in [36]. Since the constant current was applied, and because the phase shift $\Delta\phi$ between the injected current and measured voltage is close to zero [34], [39], the measured impedance change dZ may be expressed as follows:

$$dZ = \frac{Z(t) - Z(t_{AP})}{Z(t)} = \frac{|V(t)|e^{j\Delta\phi} - |V(t_{AP})|e^{j\Delta\phi_{AP}}}{|V(t)|e^{j\Delta\phi}} \approx \frac{|V(t)| - |V(t_{AP})|}{|V(t)|} = |dZ| \quad (1)$$

In the equation, impedance change dZ is equal to the relative change of the impedance $Z(t_{AP})$ when AP passes under the electrodes with respect to the baseline impedance of the system $Z = Z(t)$. Using (1), the complex dZ and absolute $|dZ|$ can be expressed in terms of the measured voltages $V = V(t)$ and $V(t_{AP})$.

To extract dZ , DC was applied twice in different polarities with positive and negative electrodes switched [36]. These voltage signals were subtracted from each other so that the identical action potentials were cancelled out, while the dZ which modulates the recorded voltage, doubled, and could be easily extracted. In the experiments performed in the study, this subtraction step was not required as high AC frequencies (≥ 2 kHz) were used and CAPs were removed by bandpass filtering around the carrier frequency (as the characteristic frequency of the CAP is <1 kHz [40]) (Figure 4).

The parameters and geometrical design of the model as well as all the equations describing it can be found in [36].

b) Statistical model of a complex nerve

The action potentials (APs) and impedance changes (dZ s) obtained with the FEM model were incorporated into the statistical model for simulation of the dispersed compound signals of a multi-fibre porcine SN. The modelled nerve included 40000 unmyelinated C fibres and 4000 fast myelinated fibres [37] fibres uniformly distributed in the cross-section of the nerve, with normally distributed conduction velocities (Table 2). Normal distribution of the CV was

considered based on the histological studies in various nerves [23], [41], [42] where the distributions of fibres' diameters (which are directly proportional to conduction velocities [22]) close to normal were obtained; the same assumption was also made in the recent modelling study [22].

The compound dZ was obtained as the sum of single impedance changes of each fibre at the required distance from the site of stimulation:

$$\begin{aligned} CAP(x, t) &= \sum_{i=1}^{N_C=40000} EAP_i^C(x, t) + \sum_{i=1}^{N_M=4000} EAP_i^M(x, t) \\ dZ(x, t) &= \sum_{i=1}^{N_C=40000} dZ_i^C(x, t) + \sum_{i=1}^{N_M=4000} dZ_i^M(x, t) \end{aligned} \quad (2)$$

where CAP and dZ are action potentials and impedance changes of the compound nerve, EAP_i^C , EAP_i^M , dZ_i^C and dZ_i^M represent single extracellular AP and dZ corresponding to C fibres and myelinated fibres respectively (Figure 1).

Due to the difference in geometric and electrical parameters utilised in the single-fibre FEM and the experimentally-based statistical multiple-fibre model, the single APs and dZs simulated with the FEM model (subsection A) were scaled in amplitude before summation, as in [22]. However, compared to [22] where electrode diameter and connective tissue resistivity were only considered, multiple parameters differing between the FEM and statistical models were introduced, so that the predictions of the final model closely agree with experimental data.

For scaling, the obtained extracellular AP and dZ were multiplied by the coefficients $k_{AP} = \prod_i k_{APi}$ and $k_{dZ} = \prod_i k_{dZi}$, where k_{APi} and k_{dZi} accounted for various parameters that differed in the FEM and statistical models and affected AP and dZ respectively:

$$\begin{aligned} EAP_{stat} &= k_{AP} \cdot EAP_{FEM} \\ dZ_{stat} &= k_{dZ} \cdot dZ_{FEM} \end{aligned} \quad (3)$$

The considered parameters affecting the dZ were [36]: the electrodes' diameter (d), extracellular resistivity (ρ), the current density of the applied dZ measuring current (J), the distance between recording and injecting electrodes (Δx_R , Figure 1), the distance between injecting electrodes (Δx_I , Figure 1). The latter three parameters were found to strongly influence the dZ measurements in the previously performed study [36]. For scaling action potentials, the electrodes' diameter (distance between the electrode and the fibre) and conductivity of the extracellular space were considered, as other parameters did not have any effect on its value. The final equations for scaling APs and dZs are presented below:

$$k_{AP} = \left(\frac{d_{FEM}}{(1 - 1/\sqrt{2}) \cdot d_{stat}} \right)^3 \cdot \frac{\rho_{stat}}{\rho_{FEM}} \quad (4)$$

$$k_{dZ} = \left(\frac{d_{FEM}}{(1 - 1/\sqrt{2}) \cdot d_{stat}} \right)^4 \cdot \left(\frac{\rho_{stat}}{\rho_{FEM}} \right)^2 \cdot \frac{J_{stat}}{J_{FEM}} \cdot \frac{dZ_{\Delta x_R stat}}{dZ_{\Delta x_R FEM}} \cdot \frac{dZ_{\Delta x_I stat}}{dZ_{\Delta x_I FEM}} \quad (5)$$

In the equations, index *stat* corresponds to the value used in the experiments and in the statistical model, and *FEM* – in the FEM model. The detailed strategy for evaluation of the scaling coefficients as well as their values are presented in Supplementary Material and in Table 1A.

In addition to the introduced scaling coefficients, conduction velocities (CV) of the fibres in the model were chosen so that simulated CAP and dZ closely match the experimental ones

recorded using the ex vivo preparation of the porcine subdiaphragmatic nerve (SN). The porcine SN mainly consists of unmyelinated C fibres and is a good representation of the subdiaphragmatic branches of human vagus nerves [31].

For the preparation, nerves of 20-25 cm length were sourced from the terminally anaesthetized pigs used in other experimental studies. The nerves were held in an organ bath perfusion chamber filled with oxygenated Krebs-Ringer solution kept at $\sim 30^\circ\text{C}$. Three silicone rubber cuffs with 6 radially arranged electrodes each were placed around the nerve 3, 15 & 20 cm from a cuff for electrical stimulation so that the temporal dispersion of CAPs can be observed (Figure 2 (a), (b)). The stainless-steel electrodes ($0.2 \times 2.3 \text{ mm}^2$, Figure 2 (b)) embedded into a medical-grade silicone rubber base were fabricated using a laser cutter and coated with PEDOT:pTS providing the lowest contact impedance and phase shift ($\sim 300\Omega$ and 1.5° at 1 kHz) among the popular coating electrode materials [34].

The choice of six-electrode design was done 1) for verification purposes – dZ must be equal on electrodes 1 to 4 if the measuring current is applied through electrodes 5 and 6, and 2) to account for possible failures of one or more electrodes on the cuff. These may have happened due to multiple reasons including un-soldered connection, broken wire, increased contact impedance due to initially low-quality or detached PEDOT:pTS coating as well as bad contact of the particular electrode and the nerve.

With the designed setup, dZ and CAPs were recorded using the 2nd cuff placed at approximately 3 cm from the site of the onset with respect to the electrode on the last cuff using continuous stimulation with frequency $f_{\text{stim}} = 2 \text{ Hz}$, current $I_{\text{stim}} = 20\text{-}40 \text{ mA}$ depending on the thickness of the nerve, pulse width $\text{PW} = 50 \mu\text{s}$, frequency and amplitude of the applied impedance measuring current $f_{\text{AC}} = 1\text{-}6 \text{ kHz}$, $I_{\text{AC}} = 200\text{-}300 \mu\text{A}$ (28 nerves in total).

To obtain satisfactory SNR, averaging was required; for this purpose, CAPs were recorded for 20 seconds, and the dZ – for 10 minutes. Based on the recorded CAP and dZ, the CV (mean and S.D.) of the fibres constituting the statistical model were determined. This was done using times of the negative peaks of the CAPs related to fast (Ab) and slow (C) fibres as well as their widths. Knowing the distances of the recording cuff from the stimulation, the CV could be calculated.

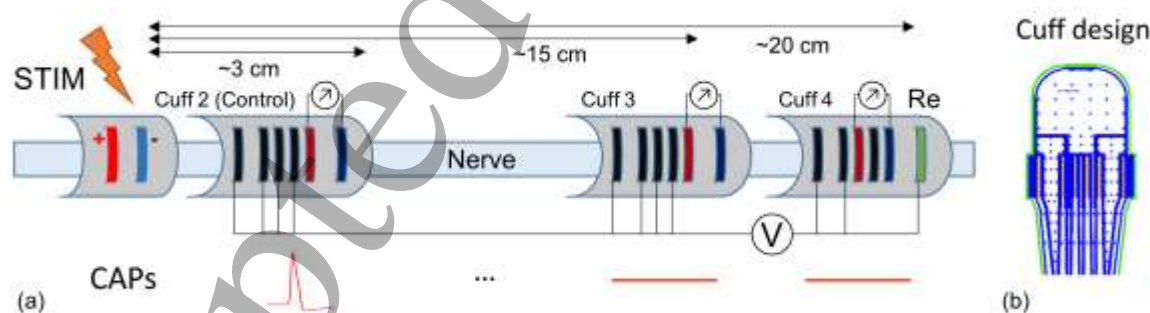


Figure 2 (a) Experimental setup consisted of 4 multi-electrode cuffs placed around the nerve. The first cuff was used for stimulation, the subsequent cuffs were placed at 3, 15 and 20 cm from the first one were used for measurement of CAPs and dZ. For dZ measurement, constant sinusoidal current (1-6 kHz, 200-300 μA) was injected through two last electrodes on each cuff, and voltage was measured on the remaining electrodes in respect to the last electrode on the last cuff. The 2nd cuff which was closest to the stimulation site was used for control and model development as C fibre CAPs were clearly measurable there, cuffs 3 and 4 were far enough so that the action potentials corresponding to C fibres were dispersed, only myelinated fibres' CAPs were visible. (b) Multi-electrode cuff design. Six identical electrodes per cuff with a surface area of 0.46 mm^2 each were used.

The average level of the Gaussian noise present in the ex-vivo experiments ($3.5 \mu\text{V RMS}$ before averaging) was added to the resultant modelled signals as the last step. Addition of

noise allowed to determine the optimal parameters for maximisation of the SNR, defined as the amplitude of the signal divided by the standard deviation of the noise. The optimal parameters to be determined include, among others, the bandwidth of filtering which will be influenced by type and levels of noise present in the recordings.

The resulting finalised model could utilise EAP and dZ produced with the FEM model (Figure 1 (b)) subjected to an arbitrary stimulation paradigm and, on this basis, it could accurately predict values of CAP and dZ produced by the porcine subdiaphragmatic nerve at any distance from the onset of the stimulus. Therefore, the model is versatile and could be used to determine the optimal stimulation paradigm for overcoming dispersion and recording dZ further than CAPs are visible.

The COMSOL and MATLAB model files used for FEM and statistical modelling are provided online in the EIT-lab GitHub repository (<https://github.com/EIT-team/Overcoming-dispersion-trains>).

3) A method for overcoming temporal dispersion in autonomic nerves

a) Model setup

The developed model was utilised to determine the optimal paradigm for dZ measurement at various distances from the stimulation site where CAPs are dispersed (>5 cm from the stimulus in mammalian unmyelinated nerves [22]).

For this, the FEM model of a C fibre (Figure 1 (b)) was subjected to series of repetitive bipolar monophasic stimuli (50 μ s pulse width, 5 nA) which were applied intracellularly across 0.1 mm at the end of the fibre. Two stimulation paradigms were used (Figure 3 (a)): 1) continuous stimulation with the frequency of 1 and 2 Hz (at these frequencies, the fibre never loses the ability to excitation); 2) stimulation with trains of stimuli of 5, 10, 20 and 50 Hz separated by resting intervals to allow the nerve recovery between the consecutive trains (Table 1).

Table 1 Initial stimulation and processing parameters used in the model

Parameter	Value					
Stimulation (train) frequency	1 Hz	2 Hz	5 Hz	10 Hz	20 Hz	50 Hz
$N_{\text{spikes/train}}$	10	20	40	24	14	10
Duration of the train	10 s	10 s	8 s	2.5 s	0.75 s	0.25 s
Time between trains	0 s (continuous)			3 s		
Duration of the simulation	30 minutes					
$N_{\text{trains/30 minutes}}$	180	180	163	327	480	553
Filtering bandwidth (single pulses)	200 Hz					
Theoretical bandwidth (trains)	0.2 Hz	0.2 Hz	0.25 Hz	0.8 Hz	2.7 Hz	8 Hz
Distances of measurement	3, 15, 20, 50 cm					

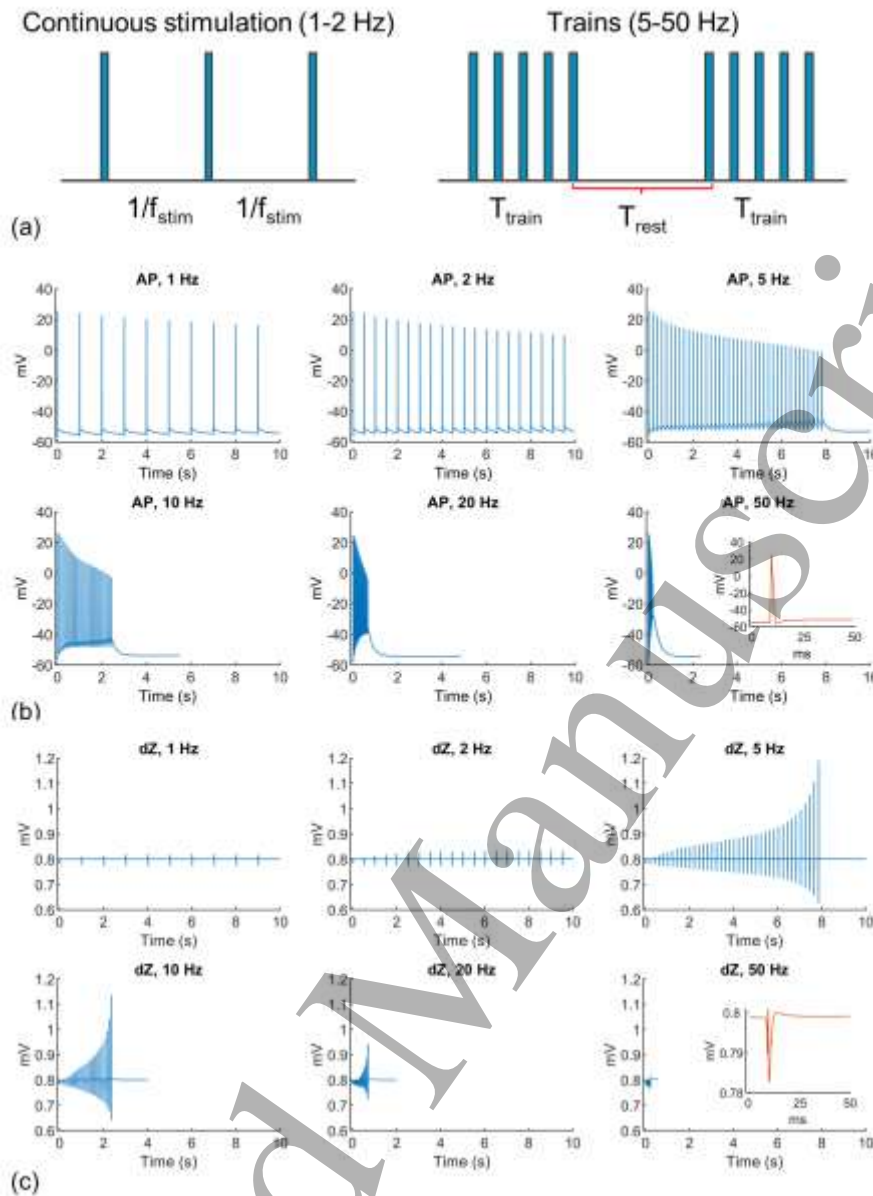


Figure 3 (a) Stimulation paradigms applied to the nerve fibre: 1-2 Hz continuous stimulation and 5, 10, 20, 50 Hz trains. Durations of the trains (T_{train}) and time between trains (T_{rest}) can be found in Table 1.

(b) Trains of action potentials (APs) at 1, 2, 5, 10, 20 and 50 Hz. The amplitudes of consecutive APs are decreasing, the rate of the decrease is larger at higher frequencies. At 1 and 2 Hz, the amplitudes of APs were approximately the same. The shape of a single AP is embedded into the last subplot.

(c) Trains of impedance changes (dZ) of a single C fibre at 1, 2, 5, 10, 20 and 50 Hz, simulated using the updated FEM model of C fibre [36] (Figure 1 (b)). Only a single train is shown at each frequency, although multiple trains were present at >10 Hz during the 10 s interval. In contrast to the APs, the amplitudes of consecutive dZ in the train are increasing. The shape of a single dZ is embedded into the last subplot.

During repetitive stimulation at 5-50 Hz, the amplitudes of the consecutive action potentials were decreasing until the fibre lost the ability to excitation (Figure 3 (b)) that also happens experimentally due to the accumulation of potassium ions in the periaxonal space adjacent to the membrane [43], [44]. Therefore, in case stimulating trains would last longer than the nerve is capable to be activated, the ratio of nerve firing time to the duration of the stimulation (duty cycle) would be reduced.

Thus, durations of the trains were chosen so that 1) the time when the nerve is in the active state (and hence the dZ) is maximised, that includes maximisation of the number of APs per train and the duty cycle, and 2) the nerve survives for the long term (> ~3 hours in the saline

1
2
3 bath) in the ex-vivo experiment (Figure 2 (a)), as increasing SNR to satisfying values may
4 require prolonged averaging. The maximal durations allowing reaching the highest duty cycle
5 were 8 s, 2.5 s, 0.75 s and 0.25 s at 5, 10, 20 and 50 Hz respectively that was equal to 40, 24,
6 14 and 10 spikes per train at these frequencies (Table 1). The resting time T_{rest} between the
7 5-50 Hz trains was chosen to be 3 seconds (Figure 3 (a), Table 1).
8

9
10 In order to achieve long-term survival of the stimulated nerves so that they are stable during
11 the experimental day period (CAPs are not changing for > 6 hours), these parameters were
12 adjusted following testing performed in 3 nerves (Table 3 in Results). For this, CAP amplitudes
13 in the nerves were extracted following their initial stimulation with the maximal train durations
14 provided above and in Table 1. Then, if the CAPs' amplitudes did not sustain for the duration
15 of the experiment, the number of pulses and associated train durations were halved – this was
16 repeated up to two times until the CAPs stable over several hours were achieved. To further
17 improve stability, the resting time was also iteratively increased and its effect on the CAPs
18 were observed.
19

20
21 In contrast to the action potentials, the amplitudes of the single-fibre impedance changes were
22 increasing during stimulation (Figure 3 (c)). The reasons for this behaviour are supposedly
23 similar to the ones for the action potentials (Figure 3 (b)): accumulation of potassium ions in
24 the periaxonal space and sodium ions inside the fibre modifies reversal potentials of these
25 ions and sensitises the associated ion channels thus leading to increased total conductance
26 of the fibre during excitation. This effect is expected to improve the experimental dZ response
27 during repetitive stimulation. However, it is hard to evaluate it experimentally since at least
28 1200 averages are required to reliably detect single spike dZ (10 minutes averaging at 2 Hz
29 stimulation, Methods 2, b), and this would average out the progressively increasing dZ
30 amplitudes. In addition, the number of spikes in the train and the resting time between trains
31 was found to significantly affect nerve survival (6 pulses/train, 5 s between trains, Table 3 in
32 Results) – it was vital for the purposes of the study, and the gradually increasing behaviour of
33 dZ had therefore not been investigated further.
34
35

36
37 The obtained dZ trains (Figure 3 (c)) were incorporated into the statistical model for simulation
38 of the dispersed compound dZ of a multi-fibre nerve described in the previous subsection. The
39 signal-to-noise ratio (SNR, ratio of mean signal to S.D. of the noise) at 3, 15, 20 and 50 cm
40 from the onset of the stimulus was determined using two signal processing paradigms
41 described in the next subsection. For averaging and noise reduction, the total duration of each
42 simulation was chosen to be 30 minutes (Table 1). 50 models were simulated in total to obtain
43 statistics.
44

45 *b) Signal processing*

46
47 To extract compound impedance changes from the dispersed signals obtained in the statistical
48 model, the following signal processing paradigms were performed; signal-to-noise ratios (SNR)
49 using these paradigms were obtained.

- 50
51 i. *Averaging of single spikes (coherent spikes averaging)*. This approach has traditionally
52 been used in previous studies involving dZ measurement [12], [15], [19], [33]. In those
53 studies, the recordings were cut into single spikes segments with the time window
54 corresponding to the stimulation frequency around each spike (Figure 4 (a)). Those
55 segments were then 1) band-pass filtered using the bandwidths of 100-3000 Hz
56 depending on the characteristic frequency of these signals (characteristic frequency of A
57 fibres >> C fibres), 2) demodulated using the absolute of Hilbert transform (since the
58 phase shift induced by the membrane is insignificant [34], [36], and the dZ is
59 approximately equal to $|dZ|$, eq. (1)) and 3) averaged together. In the current study, BW
60

was chosen to be 200 Hz to account for the characteristic frequency of the simulated non-dispersed dZ (Figure 1). The resulting signals were averaged across all 50 computed models. SNR was calculated using the formula:

$$SNR_{single} = A_{signal} / \sigma_{Noise} \quad (6)$$

where A_{signal} is the maximal amplitude of the measured signal, σ_{noise} is the standard deviation of the noise after filtering.

- ii. *Processing trains of spikes as a whole.* Instead of cutting the recordings into single spikes, processing was conducted on the entire trains of spikes (Figure 4 (b)). This allowed band-pass filtering with bandwidths around whole trains lasting up to a few seconds. As a result, the theoretical bandwidths for band-pass filtering could be significantly lower at 0.2 – 8 Hz, determined as $BW_2 = 2/T_{train}$ where T_{train} is the duration of the specific train (Table 1). This could allow a significant reduction of the noise without long-continued averaging. However, to differentiate the dZ signal of the C fibres from the signal of myelinated fibres, stimulation artefacts and low-frequency noise present in the recordings, these theoretical filtering bandwidths had to be increased (Table 3 in Results).

SNR at 3, 15, 20 and 50 cm from the onset of the stimulus was computed according to (6), where A_{signal} and σ_{noise} corresponded to a new approach for signal processing. Due to the presence of stimulation artefacts (see the detailed description below) as well as fast myelinated fibres in the experimental recordings (Figure 7, Figure 9), only the ending portions of the dispersed dZ corresponding to the slow C fibres could be recovered. Therefore, the last 100-500 ms in the processed dispersed dZ trains were considered for dZ and SNR calculation (Figure 8 in the Results). Although only the ending portions of the signals were used to measure dZ, the entire spike train signals were filtered: leaving the dZ in the central part of the signal allowed avoiding the filtering edge artefacts. Another artefact appearing due to presence of spikes at the end of the train when filtering the whole signal was of much shorter duration (in the order of tens of milliseconds) than the expected and observed dispersed dZ signal lasting up to 500 ms (Figure 8), so they could be easily differentiated. Based on the obtained results, the optimal stimulation paradigm for recording dZ at far distances from the stimulus was determined.

Stimulation artefacts are inevitable when neural activity is evoked and recorded with the use of external electrodes [12], [19], [33]. Since the membrane of nerve fibres is more resistive than the surrounding connective tissue and physiological solution interface, part of the current applied during stimulation will flow through the conductive pathways along the nerve and an increase in the potential will be therefore measured by the recording electrodes (Figure 5). In addition, the voltage generated by the stimulation current is usually orders of magnitude higher than the recorded physiological voltages, so, the insufficient input range of the amplifier or small charge imbalance between stimulation electrodes may lead to saturation of the recording circuits which can last significantly longer than stimulation pulse itself [45], [46]. To avoid saturation in the current study, charge-balanced biphasic stimulation was used together with an actiCHamp amplifier (Brainproducts GmbH, Gilching, Germany) having a wide input range of ± 400 mV [16].

The influence of stimulation artefact can generally be minimised by placing the recording electrodes as far from the stimulating ones as possible. This is challenging in *ex vivo* conditions: surgically, it was not possible to extract intact undamaged subdiaphragmatic nerves longer than ~20 cm from the anaesthetised pig in the current study. However, *in vivo* approach is much more promising as stimulation and recording can be done in different parts

of the vagus nerve (cervical and subdiaphragmatic); this is an essential part of the future work (see Discussion).

Stimulation artefacts obtained in the current study were nonsaturating [45] so that they could be minimised, and genuine CAP and dZ signals could be extracted through filtering. However, these artefacts could not be completely eliminated that severely affected signal processing in the case when the nerve was stimulated by trains of spikes (Figure 4(b)): only ending parts of the dispersed dZ signals had to be studied and the SNR could have therefore been reduced (see Results and Discussion).

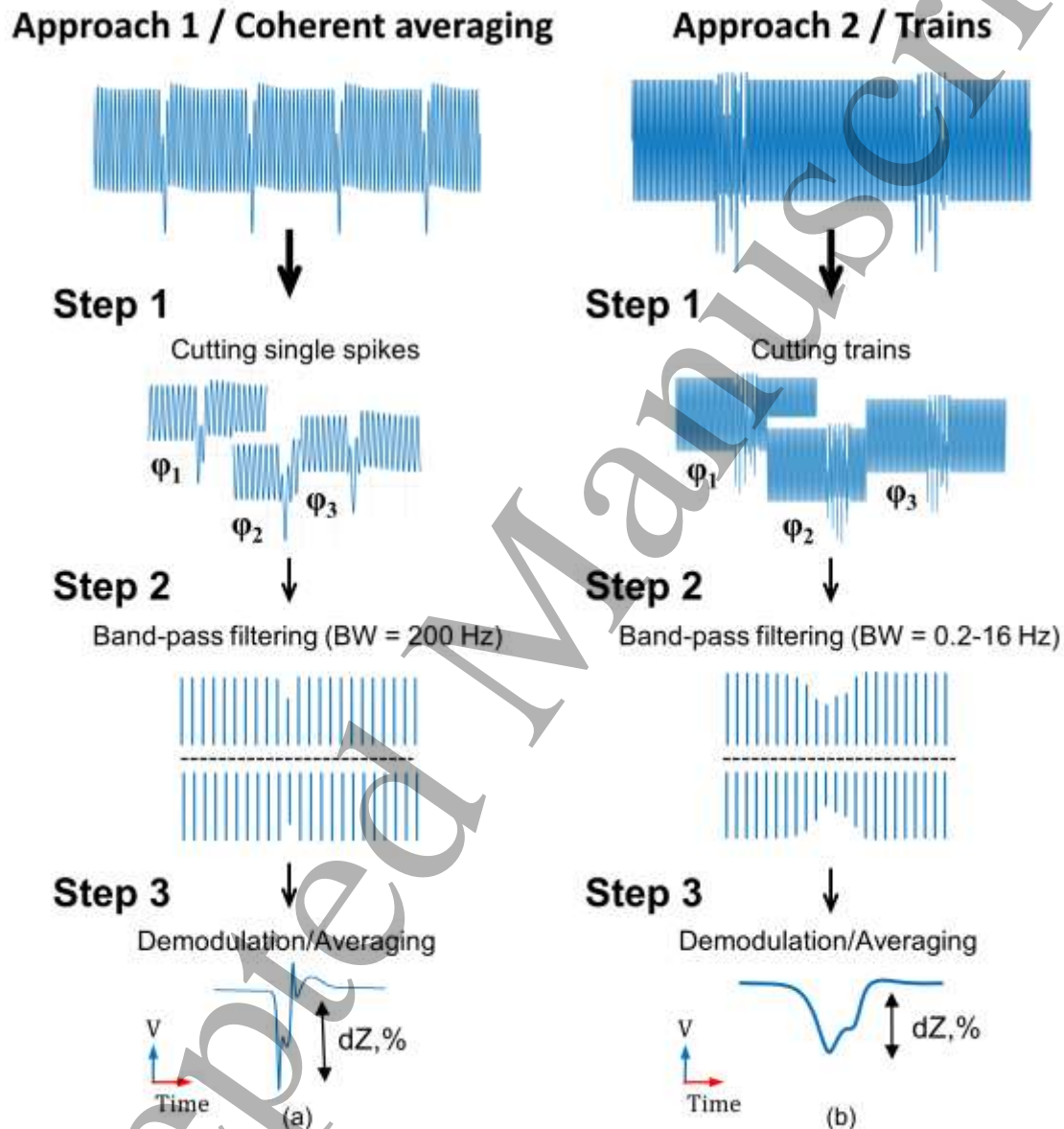


Figure 4 Schematic representation of the signal processing approaches used in the study:

(a) Coherent spikes averaging – single spikes were cut from the recording (Step 1), band pass-filtered with bandwidth (BW) of 200 Hz (Step 2), demodulated and averaged together (Step 3).

(b) Processing as trains – cutting trains as a whole (Step 1), filtering them with low bandwidths (0.2-16 Hz) around the whole trains (Step 2), demodulation and averaging of the resultant signals (Step 3).

AC frequencies shown in the figures do not represent real (higher) frequencies used in the study. In Step 2, the amplitude of the carrier sine wave is very large and is therefore not depicted in full, so the dashed line in the middle was introduced to demonstrate how the impedance change looks following band-pass filtering step on the top and bottom parts of the carrier wave.

1
2
3 c) *Experimental evaluation of the method using porcine subdiaphragmatic nerve ex vivo*
4

5 All experimental procedures complied with regulations in the UK Animal (Scientific Procedures)
6 Act, 1986 and were reviewed and approved by the Animal Welfare and Ethical Review Board.
7 For experimental evaluation of the developed method, an ex-vivo setup with the
8 subdiaphragmatic nerves (SN) of the pig was used. In addition to porcine SNs being similar in
9 fibre composition to the subdiaphragmatic branches of the vagus nerves in humans [31], they
10 are very enduring – the stability of porcine SNs allowed to frequently manipulate them in the
11 saline bath and conduct experiments lasting for more than 8 hours, in accordance with survival
12 times of other mammalian nerves [47], [48]. This is a significant advantage over other
13 unmyelinated nerves commonly used in ex-vivo setting such as a walking leg nerve of the crab
14 [33], [49] (Figure A2 in supplementary material).
15
16

17 The experimental design was the same as in section 2, (b) describing the experimental
18 adjustment of the model. Shortly, porcine SN were held in an organ bath perfusion chamber
19 filled with continuously oxygenated saline solution. Three silicone rubber cuffs each having six
20 radially arranged electrodes made from stainless steel and coated with PEDOT:pTS were
21 placed around the nerve 3, 15 & 20 cm from the same cuff used for electrical stimulation ($f_{stim}=$
22 2 Hz, $I_{stim} = 20-40$ mA, PW = 50 μ s, Figure 2 (a)). Impedance changes were measured by
23 sequential application of the sinusoidal current through two last electrodes on each cuff, and
24 the voltage was recorded on the remaining electrodes on the same cuff in respect to the last
25 electrode on the last cuff (Figure 2 (a)). Then, dZ was obtained by demodulation of the
26 recorded voltage using the absolute of the Hilbert transform (1). Parameters of the applied
27 sinusoidal current were: $f_{AC}= 1-6$ kHz, $I_{AC}= 200-300$ μ A.
28
29

30 The optimal stimulation paradigm for recording dispersed dZ determined with the model was
31 applied to N = 28 nerves to sequentially record dZ using cuffs 3 and 4 at 15 and 20 cm from
32 the onset, where CAPs were dispersed and not measurable (Figure 2 (a)). SNRs at these
33 distances were obtained; implications for imaging unmyelinated nerves with fast neural EIT
34 were investigated.
35

36 Statistical significance of the recorded dZ was verified using a two-sample t-test algorithm by
37 comparison of the measurement amplitude straight after the stimulation artefacts (600-1000
38 ms, Figure 8) with the dZ at all other points following this period (1000-3000 ms).
39

40 The overall design of the study, including the workflow from development of the models to
41 experimental verification, is presented in Figure 5:
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

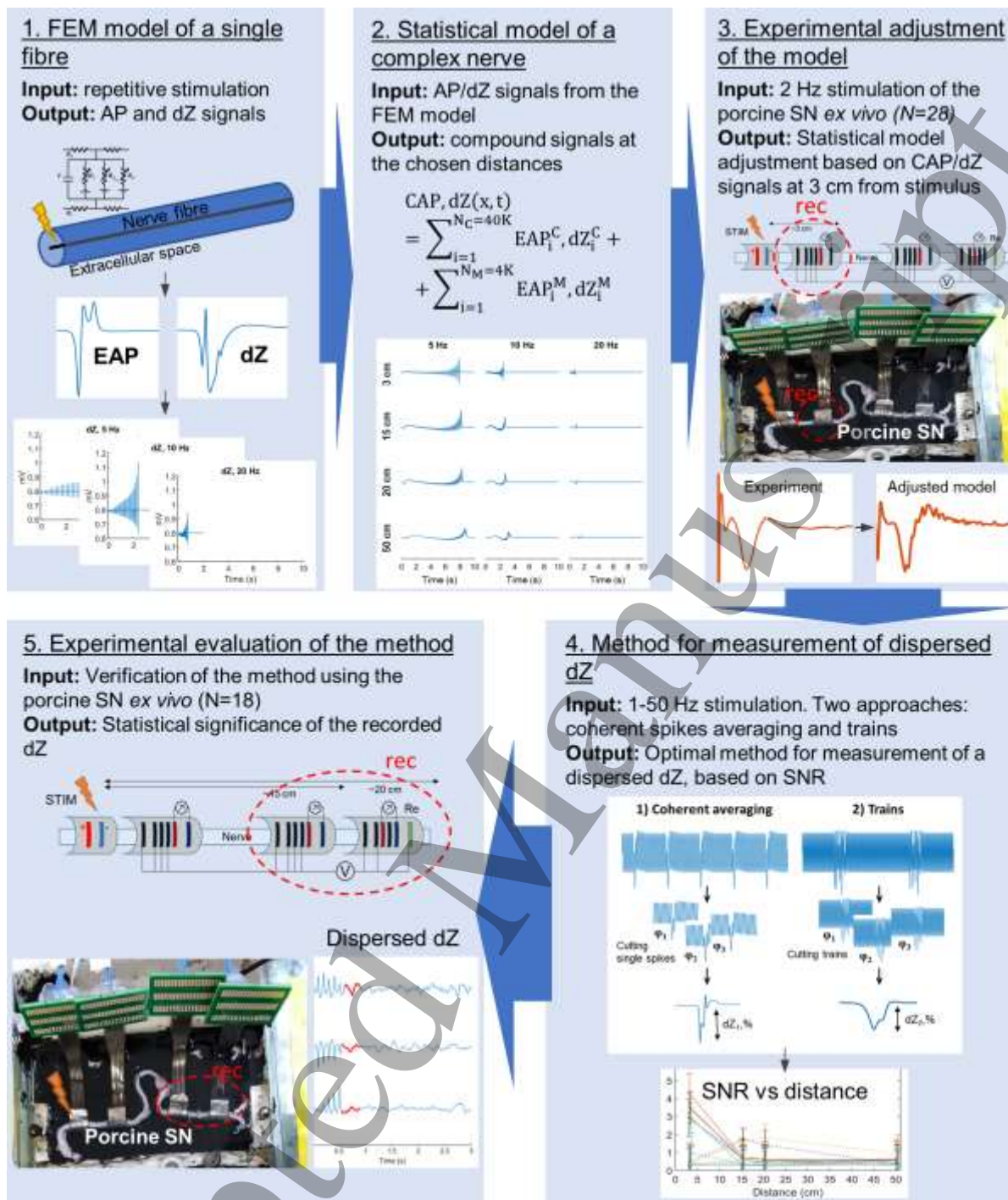


Figure 5 Overall design of the study. The study could be divided into the five main steps:

- 1) FEM model of a single C fibre was developed to simulate AP and dZ signals resulting from repetitive stimulation (Figure 1).
- 2) Based on the simulated signals, statistical model of the complex nerve with 40K unmyelinated and 4K myelinated fibres was developed, and compound dispersed signals were simulated at the chosen distances.
- 3) The developed statistical model was adjusted to correspond with the amplitudes, durations, and latencies of CAP and dZ signals recorded experimentally (Figure 6). For this, porcine subdiaphragmatic nerves (SN, *ex vivo*) were subjected to continuous 2 Hz stimulation, AC was applied and the CAP/dZ signals were measured at ~3 cm from the stimulus.
- 4) Experimentally adjusted model was utilised for optimising the method for measurement of a dispersed dZ at up to 50 cm from stimulation. Two stimulation and signal processing approaches were used: a) continuous stimulation with averaging of coherent spikes and b) stimulation with short trains of stimuli with processing the whole trains (Figure 4). The method providing the highest SNR was determined.
- 5) The optimised method was evaluated experimentally using *ex vivo* preparation of porcine subdiaphragmatic nerves (porcine SN, N = 18).

The MATLAB code written for data processing is provided in the EIT-lab GitHub repository (<https://github.com/EIT-team/Overcoming-dispersion-trains>); all the recorded unprocessed data will be available online in the SPARC portal (<https://sparc.science/>) after approval.

3. Results

1) *Experimental adjustment of the statistical model of the porcine SN*

Initial recordings of compound action potentials and impedance changes at 3 cm from the stimulation site (cuff 2, N = 28 nerves, Figure 2 (a)) allowed adjusting the statistical model to correspond to experimental data (Figure 6, Table 2).

The CAPs recorded at cuff 2 were equal to $34 \pm 17 \mu\text{V}$ for myelinated fibres and $69 \pm 44 \mu\text{V}$ for unmyelinated fibres (mean \pm s.d., Figure 6 (a)). The peak of myelinated fibres CAP was observed on average at 5 ms from the stimulus, peak of C fibres CAP – at 30 ms from the stimulus that allowed to determine their conduction velocities (CV): $v_{\text{fast}} = 8 \pm 3 \text{ m/s}$, $v_{\text{C}} = 0.8 \pm 0.3 \text{ m/s}$ (mean \pm s.d., Table 2). The amplitude of the CAP of fast myelinated fibres was lower in the experiment than in the model because part of it was covered by stimulation artefact thus decreasing its amplitude (Figure 6 (a)). The dZ were equal to $(1.42 \pm 1.11) \cdot 10^{-4}\%$ ($0.16 \pm 0.19 \mu\text{V}$) and $(6.96 \pm 4.61) \cdot 10^{-4}\%$ ($0.74 \pm 0.58 \mu\text{V}$) in fast myelinated fibres and unmyelinated C fibres respectively.

Table 2 Main parameters of the statistical models

Parameter	Modified statistical model
C fibres number	40000
C fibres CV	$0.8 \pm 0.3 \text{ m/s}$
Myelinated fibres number	4000
Myelinated fibres CV	$8 \pm 3 \text{ m/s}$
Noise, RMS before averaging	$3.5 \mu\text{V}$

2) *Optimisation and experimental evaluation of the method for measurement of the dispersed dZ*

a) *Method development and optimisation*

Using the designed statistical model of dispersion in the porcine SN, the method for recording dZ further from the site of the onset than CAPs are measurable was developed and optimised.

Images of the dispersed dZ with and without fast myelinated fibres (the real porcine SN case and artificial unmyelinated case) show that the C fibre activity is more dispersed and constitute a low-frequency component in the resultant signal, while myelinated fibres correspond to a high-frequency component (Figure 7, blue and red lines). Therefore, to measure dZ of the C fibres in the mixed porcine SN nerve, the ending parts of the signals were considered.

By stimulation of 3 nerves with trains of pulses of 5, 10, 20 and 50 Hz with 3-second intervals between trains, and following the approach described in the Methods section (Methods 3, a), it was found that to achieve long-term (>6 hours) survival of the nerves, the number of pulses in each train must not exceed six and the interval between trains must be increased to 5 seconds, independent of the applied train frequency. The model was modified accordingly (Table 3).

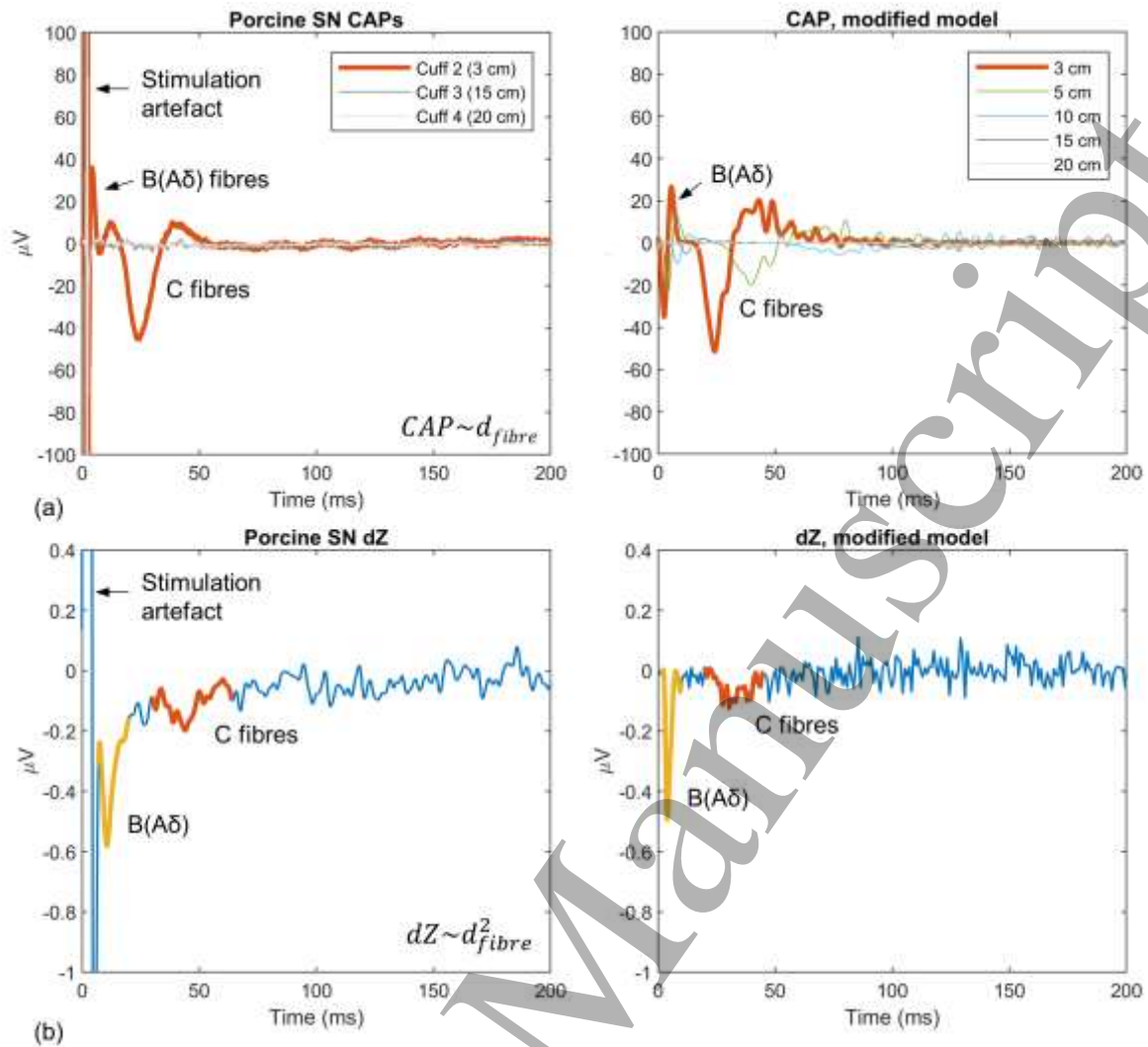


Figure 6 (a) Compound action potentials (CAPs) obtained experimentally (left) and with the statistical model (right), averaged for 20 seconds. Measurements performed using cuff 2 (Figure 2 (a)) at ~ 3 cm from the site of stimulation are shown in red; measurements at 15 and 20 cm from stimulation (cuffs 3 and 4) are shown in grey. Additional CAP simulations at 5 and 10 cm are presented in green and blue to demonstrate the decreasing amplitude of the CAP with distance; at further distances starting from 15 cm, C fibres' CAPs are fully dispersed. (b) Impedance changes (dZs) obtained experimentally (left) and with the statistical model (right), averaged for 10 minutes. dZs of B(A δ) and C fibres are highlighted with yellow and red respectively. The number and types of fibres as well as their conduction velocities were adjusted in the modified model to closely match CAP and dZ with the experimental recordings.

Table 3 Optimised stimulation and processing parameters used in the model

Parameter	Value						
Stimulation (train) frequency	1 Hz	2 Hz	5 Hz	10 Hz	20 Hz	50 Hz	
$N_{\text{spikes/train}}$	10	20	6	6	6	6	
Duration of the train	10 s	10 s	1.2 s	0.6 s	0.3 s	0.15 s	
Time between trains	0 s (continuous)				5 s		
Duration of the simulation	30 minutes						
$N_{\text{trains/30 minutes}}$	180	180	163	327	480	553	
Filtering bandwidth (single pulses)	200 Hz						
Corrected bandwidth (trains)	10 Hz						
Distances of measurement	3, 15, 20, 50 cm						

The developed model has shown that the SNR obtained using the coherent spike averaging approach for signal processing (Table 1, Figure 4 (a)) were larger at short distances from the onset while falling exponentially at longer distances (solid lines in Figure 8, Table 4). The second approach involving processing of whole trains (Table 1, Figure 4 (b)) led to smaller SNR at 3 cm from the stimulus, but increased to > 1 at 15 and 20 cm from the site of the stimulus after 30 minutes of averaging (Figure 8, Table 4).

Therefore, the optimal parameters for measurement of dZ at far distances from the site stimulation predicted with the model were: stimulation with 5 or 10 Hz trains, 6 pulses per train, 5-second interval between trains (Table 4). Filtering bandwidth was increased to 10 Hz to differentiate the dZ signal of the C fibres from the signal corresponding to myelinated fibres, stimulation artefacts and low-frequency noise present in the recordings (Figure 7, Figure 9).

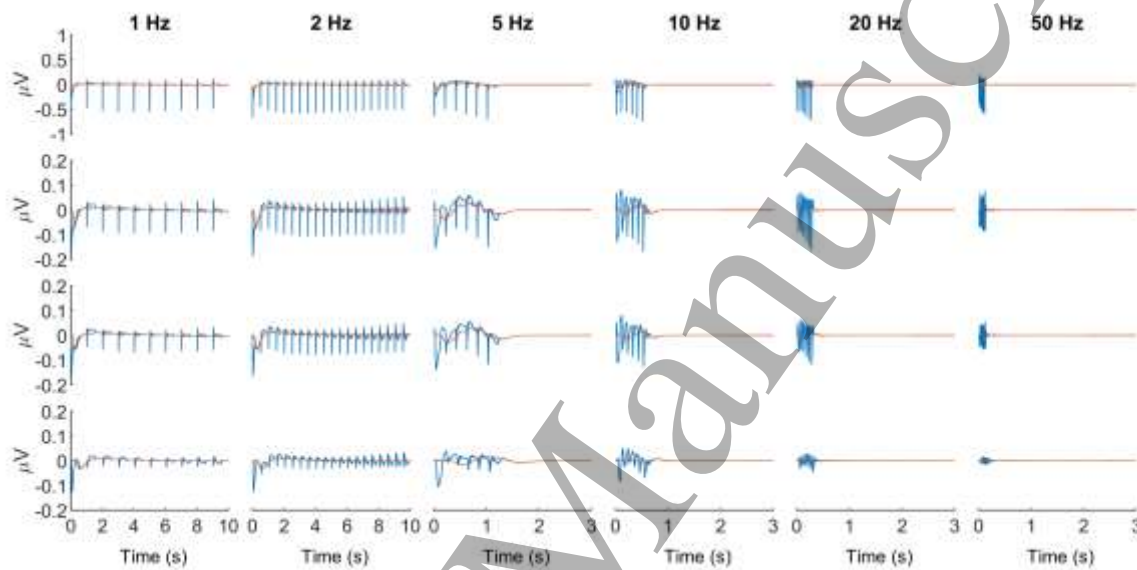


Figure 7 (Red lines) Dispersed dZs of an artificial unmyelinated nerve obtained with a statistical model containing 40000 C-fibres, with EAP shape based on the cuff electrode design depicted on Figure 1 (b). The stimulation frequencies were 1, 2, 5, 10, 20 and 50 Hz (columns), and readings were made at 3, 15, 20 and 50 cm from the stimulus (rows). Resultant dZs were detrended after summation; noise was omitted for readability.

(Blue lines) Dispersed dZs of a mixed nerve obtained with an experimentally adjusted statistical model containing 40000 C-fibres and 4000 fast myelinated fibres.

Table 4 SNR simulated with the experimentally adjusted model after 30 minutes averaging

Signal processing	Stimulation frequency	SNR (mean \pm s.d.) *			
		3 cm	15 cm	20 cm	50 cm
Coherent spikes averaging	1 Hz	2.9 \pm 1.1	0.7 \pm 1.0	0.6 \pm 0.9	0.6 \pm 1.1
	2 Hz	4.3 \pm 1.1	0.7 \pm 1.0	0.6 \pm 1.0	0.6 \pm 0.8
	5 Hz	3.4 \pm 1.0	0.8 \pm 0.8	0.6 \pm 1.1	0.4 \pm 0.8
	10 Hz	3.5 \pm 0.9	0.6 \pm 0.8	0.4 \pm 0.9	0.5 \pm 0.9
	20 Hz	2.9 \pm 0.9	0.3 \pm 1.2	0.5 \pm 0.9	0.3 \pm 1.1
	50 Hz	1.0 \pm 1.1	0.3 \pm 1.0	0.2 \pm 1.0	0.4 \pm 0.9
Processing as trains	1 Hz	0.3 \pm 1.0	0.3 \pm 1.1	0.3 \pm 1.0	0.4 \pm 0.7
	2 Hz	0.4 \pm 1.1	0.4 \pm 1.0	0.3 \pm 1.1	0.7 \pm 1.1
	5 Hz	0.4 \pm 1.0	1.6 \pm 0.7	1.8 \pm 0.8	0.9 \pm 1.0
	10 Hz	0.5 \pm 0.9	1.7 \pm 0.6	1.4 \pm 0.8	0.4 \pm 0.8
	20 Hz	0.4 \pm 1.1	0.6 \pm 0.8	0.4 \pm 1.0	0.3 \pm 0.8
	50 Hz	0.3 \pm 1.0	0.9 \pm 0.3	0.3 \pm 1.1	0.3 \pm 0.9

* The stimulation frequencies where SNR ≥ 1 and the optimal train frequencies are highlighted in blue

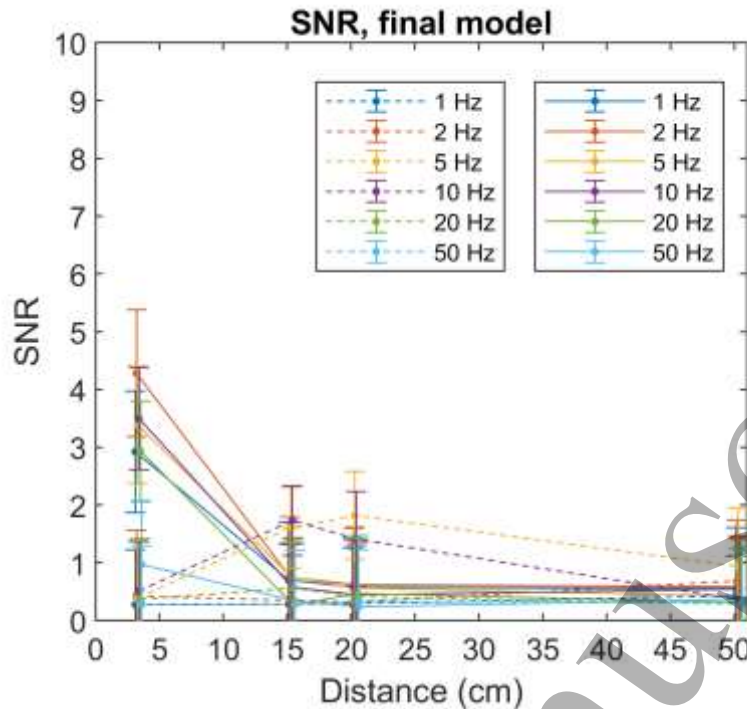


Figure 8 SNR computed using the finalised statistical model based on the recorded experimental data on the subdiaphragmatic nerve of the pig. Two signal processing approaches were used (Figure 4): coherent spikes averaging (depicted with solid lines) and processing trains of spikes as a whole (dashed lines). The minimal used filtering bandwidth was equal to 10 Hz. The resulting numbers are presented in Table 4.

Even with the optimal parameters, averaging for 30 minutes only produces SNR marginally higher than the limit of detectability at 15 and 20 cm from the stimulus (1.8 ± 0.8 at 10 Hz, 15 cm, Table 4). Therefore, to measure dZ at longer distances as well as to obtain an SNR of 4 which is minimally required for reproducible imaging of fast impedance changes with EIT [18], longer averaging will be required (see Discussion for details).

b) Experimental evaluation of the developed approach

The optimal stimulation and signal processing paradigm for recording dispersed dZ in unmyelinated fibres determined in the modelling study (10 Hz trains, 6 pulses/train, 5 seconds interval between trains, filtering bandwidth 10 Hz) were applied to 28 porcine subdiaphragmatic nerves *ex-vivo*, out of which $N = 18$ recordings had a satisfactory level of noise smaller than $4 \mu\text{V}$ RMS before averaging, in agreement with the developed modified model.

For each nerve, the dZ measurement was sequentially performed using cuff 3 and cuff 4, located at 15 and 20 cm from the site of stimulation respectively (Figure 2). Due to the presence of fast myelinated fibres and stimulation artefacts in the recordings (Figure 7, Figure 9), measurements were done at the ending stages of the dispersed dZ, from 0.6 s to 1.1 s and from 0.65 to 1.15 s for the used 10 Hz trains at cuff 3 and cuff 4 respectively (Figure 7 (b)).

The resulting dZ were equal to $(1.11 \pm 1.03) \cdot 10^{-4}\%$ ($0.11 \pm 0.10 \mu\text{V}$) at 15 cm (cuff 3, Figure 2 (a)), and $(1.17 \pm 1.21) \cdot 10^{-4}\%$ ($0.12 \pm 0.10 \mu\text{V}$) at 20 cm (cuff 4, Figure 2 (a)) from the site of stimulation. The SNR at 15 cm after 30 minutes of averaging was 1.8 ± 0.7 , decreasing to 1.7 ± 0.6 at 20 cm that is in agreement with the predictions of the developed model (Figure 8 (b), Table 4). The mean absolute value of the determined dZ across all nerves was found to be significantly larger than the mean at every other point in the recording ($P < 0.01$, $N = 18$).

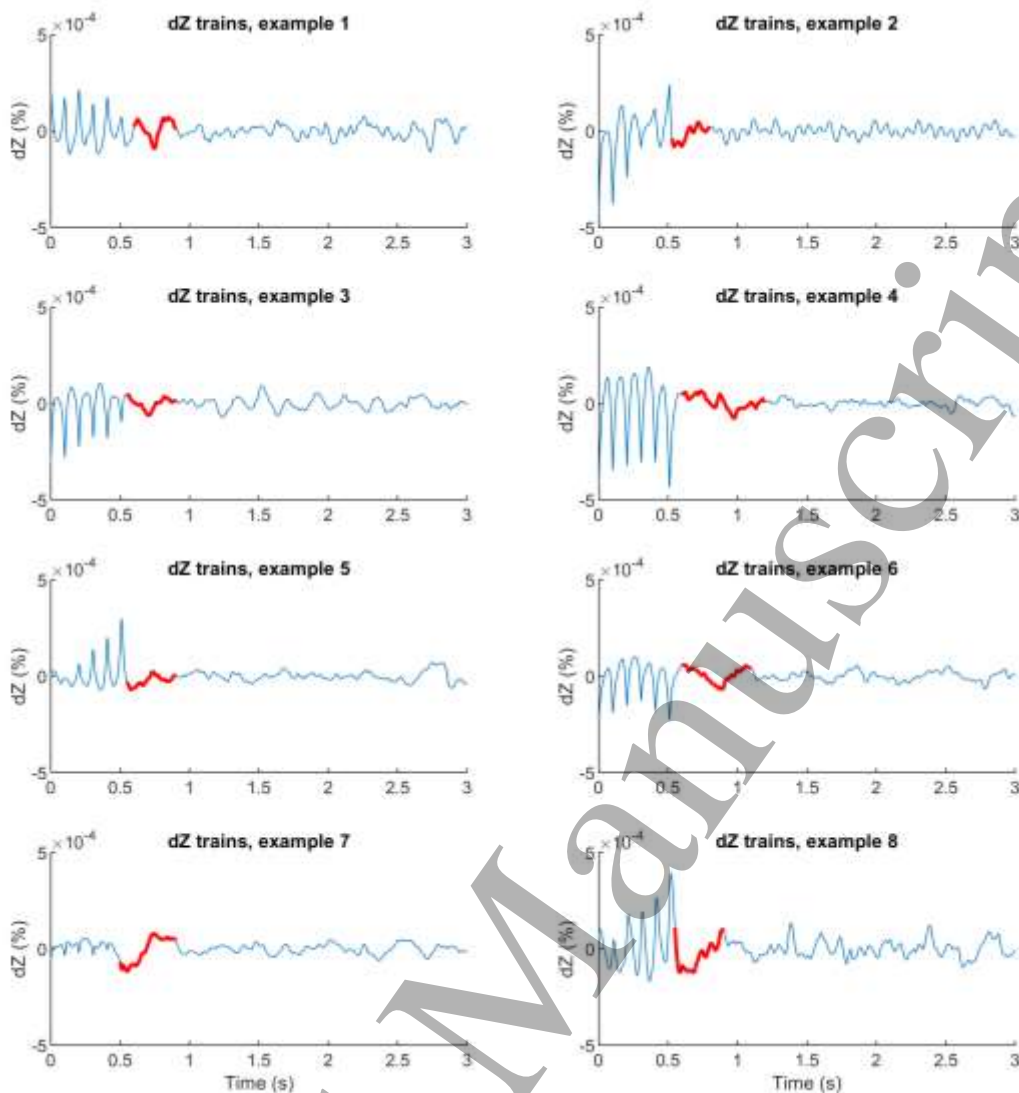


Figure 9 Selected examples of dZ traces (filtered, demodulated and averaged voltage recordings, Figure 4) of the selected experimental recordings performed with the optimal stimulation paradigm obtained in the modelling study (10 Hz trains, BW = 10 Hz). Noisy spikes in the beginnings of the recordings are stimulation artefacts. Locations where dZ was expected and where it was measured are highlighted in red.

4. Discussion

1) Summary of results

- a) The statistical model of temporal dispersion in porcine subdiaphragmatic nerve based on the FEM model of C nociceptor and morphometric data on this type of nerve was developed (Figure 5, steps 1-3). The model was matched to the experimental recordings of compound action potentials and impedance changes measured in pig subdiaphragmatic nerves at 3 cm from the stimulation ex vivo. The designed versatile model could accurately predict the shape and amplitude of the dispersed signals observed in porcine SN subjected to various stimulation paradigms.
- b) The designed model was used for the development and optimisation of a novel stimulation and signal processing paradigm to record impedance changes in unmyelinated nerves at the distances from the stimulus where CAPs are dispersed (Figure 5, step 4). This determined optimal paradigm was the following: 5 or 10 Hz trains with 6 pulses/train and 5-second interval between trains, and subsequent signal

1
2
3 processing using 10 Hz band-pass filter to account for the presence of myelinated
4 fibres, low-frequency noise and stimulation artefacts (Table 1). The resulting SNR
5 predicted with the model with stimulation by 10 Hz trains were 1.8 ± 0.8 and 1.4 ± 1.1 at
6 15 and 20 cm respectively (Figure 8, Table 4). Thus, more than 30 minutes of
7 averaging would be required to record dZ further than 20 cm from the stimulus.
8

- 9 c) The model's predictions were evaluated experimentally by stimulation of pig
10 subdiaphragmatic nerves by trains of stimuli ex vivo (Figure 9, Figure 5, step 5). The
11 predictions were in good agreement with the simulations: although the levels of noise
12 were significantly reduced compared to the previous dZ measurement experiments
13 [12], SNR obtained after 30 minutes of averaging (Results, subsection 3) was at the
14 edge of detectability at 15 and 20 cm from the onset of the stimulus. This was partly
15 due to low-frequency noise and stimulation artefacts observed in the recordings that
16 required increasing the bandwidth of filtering to a minimum of 10 Hz.
17

18 2) *Answers to the stated questions*

- 19
20 a) What are the optimal stimulation and signal processing strategies producing the largest
21 impedance changes at different distances (15, 20 and 50 cm) from the onset?
22

23 The optimal stimulation strategies producing the largest impedance change signal are
24 stimulation with 10 Hz trains, with 6 pulses per train and 5 seconds between trains, for the
25 long-term survival of the nerve necessary for the desired chronic implantation. Other
26 parameters were: AC current – 200-300 μ A amplitude, 1-1.5 kHz frequency, stimulation
27 current – 20-30 mA amplitude, 50 μ s pulse width.
28

- 29 b) How much averaging is required 1) to obtain a measurable signal ($\text{SNR} > 1$) and 2) to
30 image neural activity with EIT (which requires $\text{SNR} > 4$ [18]) at 15, 20 and 50 cm from
31 the site of stimulation?
32

33 Given the very low experimentally achieved noise of 3.5 μ V RMS before averaging, the dZ in
34 the porcine SN at 15-20 cm from the stimulus was at the edge of detectability ($\text{SNR} < 2$) if
35 averaged for 30 minutes. To obtain larger SNR, longer averaging would be required. At 50 cm
36 from the stimulus, to obtain $\text{SNR} \geq 2$ one would need to average for approximately 1.5 hours,
37 given the noise decreases with the square root of the number of averages (Table 4).
38

39 In order to image fast neural activity in fascicles of unmyelinated nerves in their cross-section,
40 a minimal SNR of 4 obtained by sequential switching between 14 electrode pairs is required
41 [18], [19]. Therefore, to achieve imaging with the obtained optimal paradigm, and considering
42 that the noise decreases with the square root of the duration of averaging, the minimal
43 averaging time would equal to 30 minutes $\cdot (4/1.8)^2 \cdot 14$ electrode pairs ≈ 34 hours at 15 cm
44 from the site of stimulation. This time would increase significantly for imaging at further
45 distances in accordance with the Table 4.
46

47 However, even such a long duration of averaging may be clinically feasible, for example, in
48 implantable neuromodulation devices [2]–[4] that may run the specified stimulation paradigm
49 for days or even weeks without any adverse effects to patients. In addition, if the internal
50 organisation of the nerve being stimulated is known, for example, using the feedback system
51 based on the physiological responses (such as respiratory breath rate or heart rate) following
52 stimulation with multi-electrode cuff arrays [50], there is no need in the execution of a full
53 imaging paradigm with 14 electrode pairs. Instead, one could concentrate on specific electrode
54 pairs corresponding to the required fascicles that may help to decrease the duration of
55 averaging by two- or three-fold.
56
57
58
59
60

1
2
3 In addition, future plans include transitioning to an in vivo experimental paradigm which has
4 several potential advantages over the ex vivo one used in the current study. Although the level
5 of noise is expected to increase in vivo (to 8-10 μV before averaging [12], [19]), the SNR may
6 be improved (given enough averaging) for the following reasons:

- 7 • The contact between the cuff and the nerve should improve due to the absence of
8 saline solution in and around the cuffs, and this will lead to stronger signals.
- 9 • The whole length of the vagus nerve can be utilised in vivo that will significantly reduce
10 stimulation artefacts strongly affecting the recorded dZ signals.

11 In the anaesthetized animal, stimulation can be done in the cervical part of the nerve while the
12 recording cuff can be placed in the subdiaphragmatic part. This was not possible to perform
13 ex vivo due to the limited size of the nerve bath and complex surgical procedures required to
14 remove longer parts of the nerve from the body. Removal of stimulation artefacts will enable
15 measurement of the whole duration of the compound dZ signals, and not just the ending parts
16 which follow stimulation artefacts (Figure 9), and this is expected to strongly improve the SNR.

17 Even with such a long averaging expected to be required for EIT imaging, the new
18 experimental paradigm developed in the study demonstrates the feasibility of recording
19 impedance changes far from the site of stimulation, which cannot be currently achieved using
20 other existing methods.

21 c) Are simulated results confirmed with experimental data?

22 The SNR measured in the performed ex-vivo experiments were in agreement with the values
23 obtained with the developed model of dispersion (Figure 8, Figure 9, Results, 3)). With the
24 average level of noise achieved in the developed experimental setup (3.5 μV RMS), the
25 maximal distance from the onset where significant reproducible dZ could be measured in 30
26 minutes was 15 cm.

27 3) *Limitations*

28 The current study had several limitations. First, it was the statistical model and not the FEM
29 model which was used for simulation of dispersion in unmyelinated nerves and for obtaining
30 the optimal parameters for dZ measurement. Since computational time and the amount of
31 required resources rise exponentially with the number of fibres in the FEM model, FEM
32 simulation of 40 thousand-fibre nerve would be very computationally intensive and will demand
33 unrealistic time and resources. For instance, it takes about an hour for a 40 ms simulation of
34 the single C fibre FEM model described by a system of 20 differential equations, while a model
35 with 50 fibres of the same type required a whole week on a double-CPU machine with 128 Gb
36 RAM [22]. However, the statistical model was based on the signals simulated with the realistic
37 mammalian C fibre FEM model that brought them into accordance with real experimental data.

38 Second, the FEM model was not solved for each fibre location in the cross-section of the
39 cylindrical external space, so that the simulations were performed only for the fibre placed in
40 the centre. This was done for two main reasons. First, the system was symmetric (1 μm fibre
41 in the centre of 10 μm external space), so that placement of the fibre in other positions inside
42 the cylinder could be assumed to not significantly alter the shape of the measured signal but
43 to mainly affect its amplitude. Also, during the transition to the multi-fibre statistical model, the
44 diameter of the cuff was re-adjusted so that the uniformly distributed fibres were on average
45 equidistant from the electrodes (supplementary material, eq. (3A)), and the brought by small
46 variations in the shapes of single APs is not expected to significantly contribute into the error
47 of the resultant CAP. In addition, this simplification allowed to significantly reduce
48 computational time: in the current study, the fibre was stimulated with trains of pulses lasting
49 up to 10 seconds (Table 1) that took up to 40 hours per single simulation.

50
51
52
53
54
55
56
57
58
59
60

1
2
3 Third, the porcine subdiaphragmatic nerves used in the ex-vivo experimental study were short,
4 of up to 20 cm long, that led to large stimulation artefacts overlapping with the dZ measured
5 at these distances. In addition, since around 10 per cent of the porcine SN consists of fast and
6 large myelinated fibres producing larger dZ, they produce the artefactual signal of the same
7 kind as stimulation appearing during the initial phase of the dispersed signal (Figure 7).
8 Therefore, only the ending part of the dispersed dZ not covered under these artefacts had to
9 be studied (Figure 9), and the SNR may have thus been reduced. One way to significantly
10 reduce these artefacts is to use longer nerves, and this can be achieved in the *in vivo*
11 experiments which are planned as the next step of the presented study.
12
13

14 4) *Future work*

15
16 In order to remove or significantly reduce the stimulation artefacts and the artefacts caused
17 by the presence of the myelinated fibres, the future work will be to perform an experimental
18 study in paralysed pigs *in vivo* with stimulation on the cervical part of the vagus nerve and
19 recording on the subdiaphragmatic part which is around one metre apart. This will allow
20 measurement of the pure dZ signal not contaminated by this type of artefact. In addition, *in*
21 *vivo* setup may provide better contact between the cuff electrodes and the nerve due to the
22 absence of a highly conductive saline interface present in the ex vivo bath; this will potentially
23 lead to larger signals and the SNR.
24
25

26 5. Conclusion

27
28 It is challenging to measure compound action potentials and impedance changes in
29 unmyelinated nerves starting from a few centimetres from the site of stimulation. The
30 developed experimentally adjusted computational model of temporal dispersion in nerve
31 allowed simulation of compound APs and dZs at various distances from the variable stimulus.
32 With the model, optimal stimulation and signal processing parameters for dZ measurement
33 were determined. It was shown that stimulation of the nerve by trains of stimuli allows
34 recording impedance changes further from the onset than it is possible with a traditional
35 continuous stimulation and averaging of consecutive spikes. The findings were evaluated
36 experimentally using the porcine subdiaphragmatic nerve *ex vivo*. This work enables a new
37 way for measurement of impedance changes accompanying excitation at distances from the
38 stimulation where standard approaches are not feasible.
39
40

41 The models of dispersion in complex-fibre nerves designed for optimisation of the
42 experimental parameters in the current study can be used by research community for studying
43 the dispersion-related properties in any types of nerves and the development of novel
44 techniques for sensing neural activity in unmyelinated nerves. The latter can be particularly
45 useful for facilitation of the novel field of bioelectronic medicines aimed at neuromodulation of
46 internal organs via stimulation of the vagus nerve: development of novel closed-loop solutions
47 involving stimulation of the nerve in response to the recorded physiological signals will
48 enhance the treatment outcomes for a variate of drug-resistant disorders including, among
49 others, epilepsy, depression, and cardiovascular diseases.
50
51

52 Acknowledgements

53
54 The COMSOL and MATLAB model files used for FEM and statistical modelling, as well as the
55 MATLAB code written for data processing are provided online in the EIT-lab GitHub repository
56 (<https://github.com/EIT-team/Overcoming-dispersion-trains>). All the recorded unprocessed
57 data will be available online in the SPARC portal (<https://sparc.science/>) after approval.
58
59

60 The work was supported by NIH SPARC grant no: 1OT2OD026545-01.

References

- [1] E. Waltz, "A spark at the periphery," *Nat. Biotechnol.*, vol. 34, no. 9, pp. 904–908, Sep. 2016, doi: 10.1038/nbt.3667.
- [2] H. F. J. González, A. Yengo-Kahn, and D. J. Englot, "Vagus Nerve Stimulation for the Treatment of Epilepsy," *Neurosurg. Clin. N. Am.*, vol. 30, no. 2, pp. 219–230, Apr. 2019, doi: 10.1016/j.nec.2018.12.005.
- [3] D. M. Mulders, C. C. de Vos, I. Vosman, and M. J. A. M. van Putten, "The effect of vagus nerve stimulation on cardiorespiratory parameters during rest and exercise," *Seizure*, vol. 33, pp. 24–28, Dec. 2015, doi: 10.1016/j.seizure.2015.10.004.
- [4] C. R. Conway, A. Kumar, W. Xiong, M. Bunker, S. T. Aaronson, and A. J. Rush, "Chronic Vagus Nerve Stimulation Significantly Improves Quality of Life in Treatment-Resistant Major Depression," *J. Clin. Psychiatry*, vol. 79, no. 5, Aug. 2018, doi: 10.4088/JCP.18m12178.
- [5] H. N. Sabbah, I. Ihsar, A. Zaretsky, S. Rastogi, M. Wang, and R. C. Gupta, "Vagus nerve stimulation in experimental heart failure," *Heart Fail. Rev.*, vol. 16, no. 2, pp. 171–178, Mar. 2011, doi: 10.1007/s10741-010-9209-z.
- [6] F. A. Koopman *et al.*, "Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis," *Proc. Natl. Acad. Sci.*, vol. 113, no. 29, pp. 8284–8289, Jul. 2016, doi: 10.1073/pnas.1605635113.
- [7] B. Bonaz, V. Sinniger, and S. Pellissier, "Anti-inflammatory properties of the vagus nerve: potential therapeutic implications of vagus nerve stimulation," *J. Physiol.*, vol. 594, no. 20, pp. 5781–5790, 2016, doi: 10.1111/JP271539.
- [8] M. Rosas-Ballina *et al.*, "Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, no. 31, pp. 11008–11013, 2008, doi: 10.1073/pnas.0803237105.
- [9] L. V. Borovikova *et al.*, "Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin," *Nature*, vol. 405, no. 6785, pp. 458–462, May 2000, doi: 10.1038/35013070.
- [10] E. Ben-Menachem, "Vagus-nerve stimulation for the treatment of epilepsy," *Lancet Neurol.*, vol. 1, no. 8, pp. 477–482, Dec. 2002, doi: 10.1016/S1474-4422(02)00220-X.
- [11] S. Klinkenberg *et al.*, "Vagus nerve stimulation in children with intractable epilepsy: A randomized controlled trial," *Dev. Med. Child Neurol.*, vol. 54, no. 9, pp. 855–861, 2012, doi: 10.1111/j.1469-8749.2012.04305.x.
- [12] K. Aristovich *et al.*, "Imaging fast neural traffic at fascicular level with electrical impedance tomography: proof of principle in rat sciatic nerve," *J. Neural Eng.*, vol. 15, no. 5, p. 056025, Oct. 2018, doi: 10.1088/1741-2552/aad78e.
- [13] K. Y. Aristovich, B. C. Packham, H. Koo, G. S. dos Santos, A. McEvoy, and D. S. Holder, "Imaging fast electrical activity in the brain with electrical impedance tomography," *Neuroimage*, vol. 124, pp. 204–213, 2016, doi: 10.1016/j.neuroimage.2015.08.071.
- [14] S. Hannan, M. Faulkner, K. Aristovich, J. Avery, M. C. Walker, and D. S. Holder, "In vivo imaging of deep neural activity from the cortical surface during hippocampal epileptiform events in the rat brain using electrical impedance tomography," *Neuroimage*, vol. 209, no. October 2019, 2020, doi: 10.1016/j.neuroimage.2020.116525.
- [15] E. Ravagli, S. Mastitskaya, N. Thompson, K. Aristovich, and D. Holder, "Optimization of the electrode drive pattern for imaging fascicular compound action potentials in peripheral nerve with fast neural electrical impedance tomography," *Physiol. Meas.*, vol. 40, no. 11, p. 115007, Dec. 2019, doi: 10.1088/1361-6579/ab54eb.
- [16] J. Avery, T. Dowrick, M. Faulkner, N. Goren, and D. Holder, "A versatile and reproducible multi-frequency electrical impedance tomography system," *Sensors (Switzerland)*, vol. 17, no. 2, p. 280, Jan. 2017, doi: 10.3390/s17020280.
- [17] L. Fabrizi, A. McEwan, E. Woo, and D. S. Holder, "Analysis of resting noise characteristics of three EIT systems in order to compare suitability for time difference imaging with scalp electrodes during epileptic seizures," *Physiol. Meas.*, vol. 28, no. 7, pp. S217–S236, Jul. 2007, doi: 10.1088/0967-3334/28/7/S16.
- [18] O. Gilad and D. S. Holder, "Impedance changes recorded with scalp electrodes during visual evoked responses: Implications for Electrical Impedance Tomography of fast neural activity," *Neuroimage*, vol. 47, no. 2, pp. 514–522, 2009, doi: 10.1016/j.neuroimage.2009.04.085.
- [19] E. Ravagli *et al.*, "Imaging fascicular organization of rat sciatic nerves with fast neural electrical impedance tomography," *Nat. Commun.*, vol. 11, no. 1, 2020, doi: 10.1038/s41467-020-20127-x.
- [20] M. Faulkner, S. Hannan, K. Aristovich, J. Avery, and D. Holder, "Feasibility of imaging evoked activity throughout the rat brain using electrical impedance tomography," *Neuroimage*, vol. 178, no. May, pp. 1–10, Sep. 2018, doi: 10.1016/j.neuroimage.2018.05.022.
- [21] J. Hope, Z. Aqrave, M. Lim, F. Vanholsbeeck, and A. McDaid, "Increasing signal amplitude in electrical impedance tomography of neural activity using a parallel resistor inductor capacitor (RLC) circuit," *J. Neural Eng.*, vol. 16, no. 6, 2019, doi: 10.1088/1741-2552/ab462b.
- [22] I. Tarotin, K. Aristovich, and D. Holder, "Effect of dispersion in nerve on compound action potential and impedance change: a modelling study," *Physiol. Meas.*, vol. 40, no. 3, p. 034001, Mar. 2019, doi: 10.1088/1361-6579/ab08ce.
- [23] T. Shimizu, M. Hayashi, A. Kawata, T. Mizutani, K. Watabe, and S. Matsubara, "A morphometric study of the vagus nerve in amyotrophic lateral sclerosis with circulatory collapse," *Amyotroph. Lateral Scler.*, vol.

- 12, no. 5, pp. 356–362, 2011, doi: 10.3109/17482968.2011.566342.
- [24] J. C. Precht and T. L. Powley, "A light and electron microscopic examination of the vagal hepatic branch of the rat," *Anat. Embryol. (Berl.)*, vol. 176, no. 1, pp. 115–126, 1987, doi: 10.1007/BF00309759.
- [25] J. C. G. Coleridge and H. M. Coleridge, "Afferent vagal C fibre innervation of the lungs and airways and its functional significance," in *Reviews of physiology, biochemistry and pharmacology*, vol. 99, 1984, pp. 1–110.
- [26] W. J. J. Freeman, "Spatial Divergence and Temporal Dispersion in Primary Olfactory Nerve of Cat," *J. Neurophysiol.*, vol. 35, no. 6, pp. 733–744, 1972, doi: 10.1152/jn.1972.35.6.733.
- [27] R. K. Olney, H. J. Budingen, and R. G. Miller, "The effect of temporal dispersion on compound action potential area in human peripheral nerve.," *Muscle Nerve*, vol. 10, no. 8, pp. 728–33, 1987, doi: 10.1002/mus.880100809.
- [28] L. J. Dorfman, "The distribution of conduction velocities (DCV) in peripheral nerves: A review," *Muscle Nerve*, vol. 7, no. 1, pp. 2–11, 1984, doi: 10.1002/mus.880070103.
- [29] W. J. Schulte-Mattler, T. Müller, D. Georgiadis, M. E. Kornhuber, and S. Zierz, "Length dependence of variables associated with temporal dispersion in human motor nerves," *Muscle and Nerve*, vol. 24, no. 4, pp. 527–533, 2001, doi: 10.1002/mus.1036.
- [30] R. B. Chang, D. E. Strochlic, E. K. Williams, B. D. Umans, and S. D. Liberles, "Vagal Sensory Neuron Subtypes that Differentially Control Breathing," *Cell*, vol. 161, no. 3, pp. 622–633, Apr. 2015, doi: 10.1016/j.cell.2015.03.022.
- [31] M. L. Settell *et al.*, "Functional vagotomy in the cervical vagus nerve of the domestic pig: implications for the study of vagus nerve stimulation," *J. Neural Eng.*, vol. 17, no. 2, p. 026022, 2020, doi: 10.1088/1741-2552/ab7ad4.
- [32] N. Thompson, S. Mastitskaya, and D. Holder, "Avoiding off-target effects in electrical stimulation of the cervical vagus nerve: Neuroanatomical tracing techniques to study fascicular anatomy of the vagus nerve," *J. Neurosci. Methods*, vol. 325, no. June, 2019, doi: 10.1016/j.jneumeth.2019.108325.
- [33] C. A. R. Chapman *et al.*, "Optimisation of bioimpedance measurements of neuronal activity with an ex vivo preparation of Cancer pagurus peripheral nerves," *J. Neurosci. Methods*, vol. 327, no. February, p. 108322, Nov. 2019, doi: 10.1016/j.jneumeth.2019.108322.
- [34] C. A. R. Chapman *et al.*, "Electrode fabrication and interface optimization for imaging of evoked peripheral nervous system activity with electrical impedance tomography (EIT)," *J. Neural Eng.*, vol. 16, no. 1, p. 016001, Feb. 2019, doi: 10.1088/1741-2552/aae868.
- [35] I. Tarotin, S. Mastitskaya, S. Hannan, E. Ravagli, K. Aristovich, and D. Holder, "SPARC: Method for Overcoming Temporal Dispersion in Unmyelinated Nerves for Imaging C Fibres with Electrical Impedance Tomography (EIT)," *FASEB J.*, vol. 34, no. S1, pp. 1–1, Apr. 2020, doi: 10.1096/fasebj.2020.34.s1.05498.
- [36] I. Tarotin, K. Aristovich, and D. Holder, "Model of Impedance Changes in Unmyelinated Nerve Fibers," *IEEE Trans. Biomed. Eng.*, vol. 66, no. 2, pp. 471–484, Feb. 2019, doi: 10.1109/TBME.2018.2849220.
- [37] N. Stakenborg *et al.*, "Comparison between the cervical and abdominal vagus nerves in mice, pigs, and humans," *Neurogastroenterol. Motil.*, vol. 32, no. 9, pp. 1–8, Sep. 2020, doi: 10.1111/nmo.13889.
- [38] J. Tigerholm *et al.*, "Modeling activity-dependent changes of axonal spike conduction in primary afferent C-nociceptors.," *J. Neurophysiol.*, vol. 111, no. 9, pp. 1721–35, 2014, doi: 10.1152/jn.00777.2012.
- [39] K. S. Cole and H. J. Curtis, "Electric Impedance of the Squid Giant Axon During Activity.," *J. Gen. Physiol.*, vol. 22, no. 5, pp. 649–70, 1939, doi: 10.1085/jgp.22.5.649.
- [40] I. Tarotin, K. Aristovich, and D. Holder, "Simulation of impedance changes with a FEM model of a myelinated nerve fibre," *J. Neural Eng.*, vol. 16, no. 5, p. 056026, Sep. 2019, doi: 10.1088/1741-2552/ab2d1c.
- [41] I. A. Boyd and K. U. Kalu, "Scaling factor relating conduction velocity and diameter for myelinated afferent nerve fibres in the cat hind limb.," *J. Physiol.*, vol. 289, no. 1, pp. 277–297, Apr. 1979, doi: 10.1113/jphysiol.1979.sp012737.
- [42] N. Soltanpour and R. M. Santer, "Preservation of the cervical vagus nerve in aged rats: morphometric and enzyme histochemical evidence," *J. Auton. Nerv. Syst.*, vol. 60, no. 1–2, pp. 93–101, Aug. 1996, doi: 10.1016/0165-1838(96)00038-0.
- [43] D. R. Scriven, "Modeling repetitive firing and bursting in a small unmyelinated nerve fiber," *Biophys. J.*, vol. 35, no. 3, pp. 715–730, Sep. 1981, doi: 10.1016/S0006-3495(81)84823-0.
- [44] Y. Zhong, J. Wang, J. Beckel, W. De Groat, and C. Tai, "Model Analysis of Post-Stimulation Effect on Axonal Conduction and Block," *IEEE Trans. Biomed. Eng.*, vol. 9294, no. c, pp. 1–1, 2021, doi: 10.1109/TBME.2021.3057522.
- [45] E. A. Brown, J. D. Ross, R. A. Blum, Y. Nam, B. C. Wheeler, and S. P. DeWeerth, "Stimulus-Artifact Elimination in a Multi-Electrode System," *IEEE Trans. Biomed. Circuits Syst.*, vol. 2, no. 1, pp. 10–21, Mar. 2008, doi: 10.1109/TBCAS.2008.918285.
- [46] A. Shadmani *et al.*, "Stimulation and Artifact-Suppression Techniques for In Vitro High-Density Microelectrode Array Systems," *IEEE Trans. Biomed. Eng.*, vol. 66, no. 9, pp. 2481–2490, Sep. 2019, doi: 10.1109/TBME.2018.2890530.
- [47] A. Kagiava and G. Theophilidis, "High concentrations of dichloroacetate have minor effects on the vitality of the mammalian nerve fibers," *Anticancer. Drugs*, vol. 22, no. 3, pp. 273–276, Mar. 2011, doi: 10.1097/CAD.0b013e3283425888.
- [48] C. Bastian, S. Brunet, and S. Baltan, "Ex vivo studies of optic nerve axon electrophysiology," *Methods Mol. Biol.*, vol. 2143, pp. 169–177, 2020, doi: 10.1007/978-1-0716-0585-1_13.

- 1
2
3 [49] K. Y. Aristovich, G. S. Dos Santos, and D. S. Holder, "Investigation of potential artefactual changes in
4 measurements of impedance changes during evoked activity: implications to electrical impedance
5 tomography of brain function.," *Physiol. Meas.*, vol. 36, no. 6, pp. 1245–59, 2015, doi: 10.1088/0967-
6 3334/36/6/1245.
- 7 [50] K. Aristovich *et al.*, "Model-based geometrical optimisation and in vivo validation of a spatially selective
8 multielectrode cuff array for vagus nerve neuromodulation," *J. Neurosci. Methods*, vol. 352, p. 109079, Mar.
9 2021, doi: 10.1016/j.jneumeth.2021.109079.
- 10 [51] F. Mechler and J. D. Victor, "Dipole characterization of single neurons from their extracellular action
11 potentials," *J. Comput. Neurosci.*, vol. 32, no. 1, pp. 73–100, Feb. 2012, doi: 10.1007/s10827-011-0341-0.
- 12 [52] J. N. Milstein and C. Koch, "Dynamic Moment Analysis of the Extracellular Electric Field of a Biologically
13 Realistic Spiking Neuron," *Neural Comput.*, vol. 20, no. 8, pp. 2070–2084, Aug. 2008, doi:
14 10.1162/neco.2008.06-07-537.
- 15 [53] A. L. Hodgkin and A. F. Huxley, "A quantitative description of membrane current and its application to
16 conduction and excitation in nerve," *J. Physiol.*, vol. 117, no. 4, pp. 500–544, Aug. 1952, doi:
17 10.1113/jphysiol.1952.sp004764.
- 18 [54] A. Q. Q. Choi, J. K. K. Cavanaugh, and D. M. M. Durand, "Selectivity of multiple-contact nerve cuff
19 electrodes: a simulation analysis," *IEEE Trans. Biomed. Eng.*, vol. 48, no. 2, pp. 165–172, 2001, doi:
20 10.1109/10.909637.
- 21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60