Box S1 | **Bioelectronic medicines: the detailed research roadmap**

Creation of a visceral nerve atlas

Structural mapping

Overall objective:
Create organ-centric wiring diagrams in models representative of human anatomy.

Research imperatives:

- Generate tools for high-resolution tracing of the fibre anatomy and taxonomy to and from individual organs
    - Build a library of tracers to visualize the full length of pre-ganglionic axons, ganglionic cell bodies, post-ganglionic axons, and intrinsic neurons[1], with particular focus on tracing that starts at the target organ[2-5]
    - Advance approaches for high-resolution labelling of peripheral neurotransmitters, their receptors and co-receptors, and for imaging of myelination, peri- and epineurium
    - Develop and adapt micrometer-resolution imaging and 3D reconstruction techniques for visceral organs and peripheral nerves (for example, automated tissue slicing, clearing, in situ hybridization, multi-photon imaging)[6-12]
- Explore inter- and intra-species variation in neuroanatomy and establish the optimal animal models for detailed mapping of each organ
    - Update and extend the macro-level innervation map of the major visceral organs in key animal model species and establish the extent to which this map is conserved in humans
    - Characterise the variability in different parts of these maps between individuals
- Build organ-centric high-resolution maps for each visceral organ in their most representative animal model
    - Conduct high-resolution nerve tracing, labelling, and imaging in the animal model of choice, taking the organ as the starting point
    - Standardise and coordinate mapping, data management and 3D visualisation across organs
- Establish methods to image and find nerves in the clinical setting
    - Develop tracers and associated imaging techniques that can be used in human preoperative and intra-operative settings to identify and localize peripheral nerves
    - Identify anatomical landmarks associated with putative intervention points for surgery

Functional mapping

Overall objective:
Map the neural signalling patterns that control individual organ functions.

Research imperatives:

- Generate simultaneous recordings of neural signal pattern and organ function
    - Record both afferent (sensory) and efferent (motor) neural signals and associated end-organ biomarkers at a range of physiological stimuli

    - Combine compound action potential recording from whole nerves and action potential recordings to better sample the signal patterns that may drive organ function[13]
    - Record these data sets in disease models where neural signalling may be implicated
- Establish correlation and causation between neural signal patterns and individual organ functions
    - Establish correlations between spatial and temporal patterns in neural signals and the organ function, leveraging approaches from adaptive neural signal decoding[13,14]
    - Use stimulation and blocking experiments to test whether the identified patterns are necessary and sufficient to control the organ function
    - Investigate molecular, cellular, and physiological mechanisms by which efferent signals translate into changes of organ function and afferent signals arise from changes in organ function
- Iterate as resolution and quality of interfacing technology increase until functional units are determined
    - Use increasing channel count, fibre- and action potential-level recordings, and implanted chronic recording platforms to improve correlations between neural signal patterns and organ functions[15]
    - Establish the degree of redundancy in parallel neural signals and the functional units of fibres; that is, the fibres that convey the same signals controlling the same organ function[16]
- Build data recording standards and central repositories that allow collaborative data mining and re-analysis (analogous to the Allen Brain Atlas[17,18])
    - Develop a standardised online database of visceral nerve activity across organs, species and disease models
    - Provide central tools for signal extraction, annotation and online analysis
    - Enable mining across organs to determine whether and where neural codes are conserved

Advancement of interface technology

Electrodes for visceral nerves

Overall objective:
Adapt and advance electrode-based interfaces towards reliable visceral nerve signal recording and modulation[19].

Research imperatives:

- Miniaturise interfaces for use on small nerves adjacent to organs to enable work in rodent disease models
    - Develop cuffs that optimally interface with nerves at <100μm diameter, recognising the signal-to-noise and charge injection issues associated with recording and stimulation using micron-scale electrode geometries[20-26]
    - Develop high-density arrays with micro-scale features and integrated active electronics which allow stable electrical interfacing with 10-100 channels in small nerves, while minimising axonal damage from displacement[27-30]
- Adapt interface materials and architectures to meet the specific needs of the visceral neuroanatomy

- Develop *in situ* shape-adaptable interfaces that provide intimate contact to maximise signal-to-noise and reliable contact with small diameter nerves, irregular plexi, and ganglia embedded in adipose tissue and ligaments or covering organs and vessels
- Explore approaches that allow reliable interfacing with the same nerve fibres over time[32]

- Tailor electrode interfaces to determine the electrical impulse patterns and functional units for each organ
  - Establish if interfaces at near-organ branches provide greater specificity than high-channel arrays on a proximal nerve trunk when isolating electrical units controlling organ functions
  - Apply flexible substrates and signal analysis approaches to minimize motion artefacts from pulsating vasculature and movement of cardiac, diaphragm, intestinal, and somatic muscles[13,33,34]
  - Establish optimal de-coding strategies for action potentials with low signal-to-noise, including optimising the spike detection and sorting algorithms for unmyelinated fibres[13,35-37]
  - Develop algorithms for tracking correlated activity over time across multiple fascicles or nerves[14,38]

Signal imaging and actuation

Overall objective:
Apply biophysical techniques to study action potential patterns and achieve less invasive neuromodulation.

Research imperatives:

- Scale electromagnetic reading, writing and blocking of action potentials to highly-parallel nerve fibres
  - Extend the optogenetic toolbox to the periphery with the aim of imaging a population of individual action potentials and introducing or inhibiting distributed naturalistic signalling patterns[39-41]
  - Establish the *in vivo* reliability, temporal and spatial resolution of micro- and nano-particle reporters and actuators that can be remotely interrogated by optical or electromagnetic imaging approaches (for example, nano-dots, nano-diamonds, magnetic- and piezo-electric particles ("neural dust"))[41-44]
  - Develop methods for delivery, expression, and, distribution of nano-particle and genetically-encoded reporters into peripheral nerve fibres[45,46]
  - Explore virus serotypes, sub-cellular targeting motifs[26] and self-complementary viral vectors for axonal expression
  - Augment imaging techniques to detect action potentials through the highly scattering protective sheaths and membranes, with extended depths of field for simultaneous interrogation of fibre activity through the nerve bundle[47]
- Develop less invasive approaches for recording and modulation of action potentials
  - Extend tomography techniques of various sensing modalities to derive neural signalling patterns
  - Develop ultrasonic and electromagnetic techniques for direct actuation of neural signals in targeted fibre bundles, and radio frequency for signal blocking[48-50]
  - Apply current-steering and field-shaping techniques for precision activation of fibres within 100μm nerves[43,51]

- Move successful technologies towards *in vivo* application
    - Miniaturise and reduce power of electromagnetic sources, waveguides and interfaces for implantable probes
    - Address the biocompatibility and biostability challenges associated with new technology, including pathological immune and neural responses[52-54]

Sensing of organ functions

Overall objective:
Generate functional sensors of physiological variables that support mapping and closed-loop bioelectronic medicines.

Research imperatives:

- Define the physiological timescales and dynamic ranges for the markers of organ function
    - Determine the rate and dynamic range through sampling during physiological perturbations
    - Confirm change in surrogate markers through neurostimulation or blocking experiments
- Advance non-invasive imaging and micro sampling techniques to support functional mapping
    - Develop imaging tracers for neurally-controlled metabolic and endocrine molecules
    - Establish procedures for micro sampling and time-stamped quantification at the arteries and veins of key organs such as the kidney, liver, pancreas, spleen and adrenal gland
    - Explore fluorescent and electrochemical approaches for high temporal and spatial resolution imaging to sense acetylcholine and norepinephrine release[55-57]
- Develop *in vivo* sensors for markers required to close the loop in future bioelectronic medicines
    - Design microscopic, chip-based sensors for monitoring select molecular or physiological markers (for example, blood and airway pressure)
    - Advance long-term sensor reliability during exposure to body fluids with limited biofouling and need for re-calibration over time

Visceral control modules

Overall objective:
Integrate neural interface and power management, signal processing, and data transfer in miniaturised platforms

Research imperatives:

- Develop a research platform that enables chronic neural recording, stimulation and blocking experiments
    - Design wireless, implantable modules that, combined with different neural interfaces, will enable characterisation of functionally specific neural signals to individual organs[58]

  - Prove functionality for a small set of well-characterised organs, and then make the platform broadly available for circuit mapping and exploration of therapeutic hypotheses across visceral organs
  - Develop electronics platforms for control of different neural interface modalities (for example, electrical stimulation, recording and blocking versus optical activation and inhibition)
- Pursue miniaturisation to enable work in key animal models and pave the way for bioelectronic medicines
  - Enable functional mapping and therapeutic feasibility testing in rodent models of chronic diseases
  - Create the building blocks of future microscopic bioelectronic medicines compatible with keyhole surgery and associated broader patient reach
- Increase local signal processing as pattern features are established and interface channel counts increase
  - Initially stream raw neural data from implantable modules to allow unconstrained downstream signal analysis
  - Use identified neural pattern features to inform low-power signal processing and compression and bandwidth management as higher-channel interfaces are developed

Early establishment of therapeutic feasibility

Proof of principle

Overall objective:
Define which visceral neural circuits exert influence over disease progression in representative animal models.

Research imperatives:
- Leverage the rich body of rodent disease models for rapid proof of effect, provided the target circuit bears resemblance to human innervation
  - Use rats where feasible from a disease model point of view as this has higher feasibility and simpler scalability in terms of neural interface dimensions, instrumentation and surgical robustness as compared to mice
  - Utilise disease models representative of the organ pathophysiology (as opposed to only cellular or molecular pathophysiology)[59,60]
  - Revisit the validity of a rodent-derived proof of principle as data emerge on innervation and signal pattern variability between species
- Record neuronal and biomarker patterns to identify functional relationships during disease progression
  - Record chronic time series in individual animals during disease onset and progression
  - Use implantable, wireless platforms for less variability than repeat terminal measures, no confounding of neural signals by anaesthesia, lower animal use, and improved animal welfare
  - Conduct correlation and causation analyses with control for drift in the recording platforms
- Test the effect of acute and chronic blocking or stimulation at a stage of established disease
  - Study the effect on established disease to get a more reliable, early indication of therapeutic feasibility

    - Test partial/intermittent as well as full signal blocking and stimulation; as interfaces and stimulation equipment advance, test the effect of introducing more naturalistic patterns of neural activity
- Seek rapid read-outs across visceral organs and functions, whilst sharing findings with the community
    - Sample widely across key visceral organs as experimental procedures can be standardized, streamlined and rapidly conducted
    - Share experimental approaches to, for example, surgical nerve access, sham controls and correlation analysis through publications and workshops
    - Focus on proving the principle of nerve intervention, using available data on neurotransmitter agonist/antagonist and direct organ stimulation to inform the hypotheses to test

Treatment codes

Overall objective:
Identify specific neural patterns for targeted effects in disease in a set of model organs.

Research imperatives:

- Establish optimal points for modulation of the disease-associated function in a focused set of model organs
    - Focus efforts on a smaller set of organs with clear neural control: parasympathetic (lungs, heart), sympathetic (spleen[61], adrenal gland), and afferent (carotid sinus, bladder)
    - Use the anatomical mapping and functional decoding to identify putative points for intervention – near the organ on small nerve branches or farther from the organ on the mixed, pre-ganglionic bundles of nerves
    - Compare the effect of stimulation/blocking on different nodal points, utilising interface arrays to gain functionally-specific effect in multi-function bundles
- Create the equivalent of dose-response curves in the multi-dimensional space of neural signalling patterns
    - Use the neural code, that is, the recorded signalling patterns, as a starting point for stimulation and blocking parameters
    - Seek to apply both block and stimulation at the resolution of functional units
    - Explore the effect of changes to both average variables (for example, frequencies, compound amplitudes) and temporal patterns (for example, spike bursts)
    - Use response variables that are on a similar timescale as the neural signalling patterns, including intermediary/surrogate biomarkers
- Test the efficacy and side-effect profile of closed-loop control in response to
    - Underlying neural signals that correlate with disease
    - Neural recording of signal change resulting from stimulation/blocking
    - Molecular or physiological markers of the disease-associated organ function

Long-term responses

Overall objective:
Investigate pathological immune and neural responses over time to disease-modifying neuromodulation.

Research imperatives:

- Expand the set of electrochemical, electrophysiological, and immunohistochemical tools for evaluating the effect of chronic electrode implantation on visceral peripheral nerves[52,54]
- Examine the immune reaction at visceral nerves when interfacing with different architectures and materials
    - Use immunohistochemistry to understand the immune reaction to neural interfacing over time
    - Conduct within-animal biocompatibility comparisons of surface materials, and establish the degree of scar tissue formation in response to implantation
    - Explore differences across model species given differences in rodent and human immune systems
- Determine the extent of neurological damage and plasticity (anatomical and physiological) in response to chronic neuromodulation
    - Quantify changes to neural impedance, spontaneous activity, and conduction parameters over time in response to chronic stimulation/blocking[62]
    - Use imaging and high-resolution anatomical mapping to determine whether axonal numbers and morphology change over time upon chronic stimulation or blocking (for example, axonal loss)[52,53,63,64]
    - Establish if neural signals upon physiological perturbations change over time
- Map long-term adaptations to neuromodulation across connected organs and functions
    - Map any compensatory neural and physiological changes that may lead to resistance of therapy
    - Assess potential changes both locally in the targeted organ and distally in other organs and functions in the same system (for example, hemodynamics and metabolism); where multi-organ systems are affected, ensure animal models are representative of the human system and its pathology
    - Explore whether systemic changes can be modulated for better treatment effect

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References

1. Cai, D. *et al.* Improved tools for the Brainbow toolbox. *Nature Meth.* **10**, 540–547 (2013).
2. Callaway, E. M. Transneuronal circuit tracing with neurotropic viruses. *Curr. Opin Neurobiol.* **18**, 617–623 (2008).
3. Sun, Y. *et al.* Cell-type-specific circuit connectivity of hippocampal CA1 revealed through Cre-dependent rabies tracing. *Cell Rep.* **7**, 269–280 (2014).
4. Gautron, L. *et al.* Identifying the efferent projections of leptin-responsive neurons in the dorsomedial hypothalamus using a novel conditional tracing approach. *J. Compar. Neurol.* **518**, 2090–2108 (2010).
5. Harris, J. A. *et al.* Adeno-associated viral vectors for anterograde axonal tracing with fluorescent proteins in nontransgenic and Cre driver mice. *Curr. Protocol. Neurosci.* Unit 1.20 (2012).
6. Yoo, P. B. *et al.* Somatic innervation of the feline lower urinary tract. *Brain Res.* **1246**, 80–87 (2008).
7. Johnson, G. A. *et al.* A multidimensional magnetic resonance histology atlas of the Wistar rat brain. *NeuroImage* **62**, 1848–1856 (2012)
8. Ertürk, A. *et al.* Three-dimensional imaging of solvent-cleared organs using 3DISCO. *Nature Protocol.* **7**, 1983–1995 (2012).
9. Ragan, T. *et al.* Serial two-photon tomography for automated ex vivo mouse brain imaging. *Nature Meth.* **9**, 255–258 (2012).
10. Ragan, T. *et al.* High-resolution whole organ imaging using two-photon tissue cytometry. *J. Biomed. Opt.* **12**, 014015 (2007).
11. Osten, P. & Margrie, T. W. Mapping brain circuitry with a light microscope. *Nature Meth.* **10**, 515–523 (2013).
12. Gonga, H. *et al.* Continuously tracing brain-wide long-distance axonal projections in mice at a one-micron voxel resolution. *NeuroImage* **74**, 87–98 (2013).
13. Plachta, D. T. T. *et al.* Blood pressure control with selective vagal nerve stimulation and minimal side effects. *J. Neural Eng.* **11**, 036011 (2014).
14. Horn, C. C. & Friedman, I. I. Separation of hepatic and gastrointestinal signals from the common "hepatic" branch of the vagus. *Am. J. Physiol.* **287**, R120–R126 (2004).
15. Berényi, A. *et al.* Large-scale, high-density (up to 512 channels) recording of local circuits in behaving animals. *J. Neurophysiol.* **111**, 1132–1149 (2014).
16. Su, C.-K. *et al.* Biophysical and histological determinants underlying natural firing behaviors of splanchnic sympathetic preganglionic neurons in neonatal rats. *Neuroscience* **150**, 926–937 (2007).
17. Kandel, E.R. *et al*. Neuroscience thinks big (and collaboratively). *Nature Rev*. *Neurosci*. **14**, 659–664 (2013).
18. Sobolev, A. *et al*. Integrated platform and API for electrophysiological data. *Front*. *Neuroinform*. **8**, 32 (2014).
19. Durand, D. M., Ghovanloo, M. & Krames, E. Time to address the problems at the neural interface. *J. Neural Eng.* **11**, 020201 (2014).
20. Khan, S. *et al.* in *Thin Films and Coatings in Biology: Biological and Medical Physics, Biomedical Engineering* Ch. 9 (ed. Nazapour, S.) 301–330 (Springer, 2013).
21. Takashi, D. Y. *et al.* in *Nanotechnology and Neuroscience: Nano-electronic, Photonic and Mechanical Neuronal Interfacing* Ch. 4 (eds De Vattorio, M. *et al.*) 71–134 (Springer, 2014).
22. Ghane-Motlagh, B. *et al.* Design and implementation challenges of microelectrode arrays: a review. *MSA* **4**, 483–495 (2013).
23. Castagnola, E. *et al.* Smaller, softer, lower-impedance electrodes for human neuroprosthesis: a pragmatic approach. *Front. Neuroeng.* 16 Apr 2014 (doi:10.3389/fneng.2014.00008).
24. Cogan, S. F. Neural stimulation and recording electrodes. *Annu*. *Rev*. *Biomed*. *Eng*. **10**, 275–309 (2008).
25. Loeb, G. E. and Peck, R. A. Cuff electrodes for chronic stimulation and recording of peripheral nerve activity. *J*. *Neurosci*. *Meth*. **64**, 95–103 (1996).
26. Gradinaru, V. *et al*. Molecular and cellular approaches for diversifying and extending optogenetics. *Cell* **141**, 154–165 (2010).
27. Wark, H. A. C. *et al*. A new high-density (25 electrodes/mm^2) penetrating microelectrode array for recording and stimulating sub-millimeter neuroanatomical structures *J*. *Neural Eng*. **10,** 045003 (2013).
28. Viventi, J. *et al*. Flexible, foldable, actively multiplexed, high-density electrode array for mapping brain activity in vivo. *Nature Neurosci*. **14**, 1599–1605 (2011).
29. Delivopoulos, E. *et al*. Concurrent recordings of bladder afferents from multiple nerves using a microfabricated PDMS microchannel electrode array. *Lab*. *Chip* **12**, 2540–2551 (2012).
30. Chew, D. J. *et al*. A microchannel neuroprosthesis for bladder control after spinal cord injury in rat. *Sci*. *Transl*. *Med*. **5**, 210ra155 (2013).
31. Schuettler, M. *et al.* Multichannel neural cuff electrodes with integrated multiplexer circuit. *1st Ann. Internat. Conf. Microtechnol. Med. Biol.* (2000).
32. Malmstrom, J. A., McNaughton, T. G. & Horch K.W. Recording properties and biocompatibility of chronically implanted polymer-based intrafascicular electrodes. *Ann. Biomed. Eng.* **26**, 1055–1064 (1998).
33. Hassler, C. *et al.* Polymers for neural implants. *J. Polymer Sci. Part B* **49**, 18–33 (2011).
34. Schuettler, M. *et al.* Fabrication of implantable microelectrode arrays by laser cutting of silicone rubber and platinum foil. *J. Neural Eng.* **2**, S121 (2005).
35. Yang, Z. *et al.* A new EC–PC threshold estimation method for *in vivo* neural spike detection. *J. Neural Eng*. **9**, 046017 (2012).

36. Carlson, D. E. *et al.* Multichannel electrophysiological spike sorting via joint dictionary learning and mixture modelling. *IEEE Trans. Biomed. Eng.* **61**, 41–54 (2014).
37. Schaub, M. T. & Schultz, S. R. The Ising decoder: reading out the activity of large neural ensembles. *J. Comput. Neurosci.* **32**, 101–118 (2012).
38. Su , C.-K. *et al.* Computational solution of spike overlapping using data-based subtraction algorithms to resolve synchronous sympathetic nerve discharge. *Front. Comput. Neurosci.* 31 Oct 2013 (doi: 10.3389/fncom.2013.00149).
39. Peterka, D. S., Takahashi, H. & Yuste, R. Imaging voltage in neurons. *Neuron* **69**, 9–21 (2010).
40. Cohen, A. E. & Venkatachalam, V. Bringing bioelectricity to light. *Annu. Rev. Biophys.* 24 Apr 2014
(doi: 10.1146/annurev-biophys-051013-022717).
41. Knöpfel T. Genetically encoded optical indicators for the analysis of neuronal circuits. *Nature Rev. Neurosci.* **13**, 687–700 (2012).
42. Dolde, F., Fedder, H. & Doherty, M. W. Electric-field sensing using single diamond spins. *Nature Phys.* **7**, 459–463 (2011).
43. Sweeney, J. D., Ksienski, D. A. & Mortimer, J. T. A nerve cuff technique for selective excitation of peripheral nerve trunk regions. *IEEE Trans. Biomed. Eng.* **37**, 7 (1990).
44. Dongjin, S. *et al.* Neural dust: an ultrasonic, low power solution for chronic brain-machine interfaces. arXiv:1307.2196 [q-bio.NC] (Submitted on 8 Jul 2013).
45. Mutoh, H. *et al.* Genetically engineered fluorescent voltage reporters. *ACS Chem. Neurosci.* **3**, 585–592 (2012).
46. Kralj, J. M. *et al*. Optical recording of action potentials in mammalian neurons using a microbial rhodopsin. *Nature Meth*. **9**, 90–95 (2012).
47. Swoger, J., Pamaloni, F. & Stelzer, E. H. K. Light-sheet-based fluorescence microscopy for three-dimensional imaging of biological samples. *Cold. Spring Harb. Protoc.* pdb.top080168 (2014).
48. Legon, W. *et al.* Transcranial focused ultrasound modulates the activity of primary somatosensory cortex in humans. *Nature Neurosci.* **17**, 322–329 (2014).
49. Babbs, C. F. *et al.* A compact theory of magnetic nerve stimulation: predicting how to aim. *Biomed. Eng.* **13**, 53 (2014).
50. Liu, C. *et al.* Functional magnetic stimulation system and pulsed magnetic-field effect on peripheral nerve. *IEEE Trans. Magnetics* **49**, 1853–1856 (2013).
51. Grill, W.M. Jr. & Mortimer, J.T. Quantification of recruitment properties of multiple contact cuff electrodes. *IEEE Trans. Rehab. Eng*. **4**, 2 (1996).
52. Thila, M-. T. *et al.* Time course of tissue remodelling and electrophysiology in the rat sciatic nerve after spiral cuff electrode implantation. *J. Neuroimmunol.* **185**, 103–114 (2007).
53. Urbanchek, M. G. *et al.* Microscale electrode implantation during nerve repair: effects on nerve morphology, electromyography, and recovery of muscle contractile function. *Plastic Reconstructive Surg.***128**, 270e–278e (2011).
54. El Tahrya, R. *et al.* Repeated assessment of larynx compound muscle action potentials using a self-sizing cuff electrode around the vagus nerve in experimental rats. *J. Neurosci. Meth.* **198**, 287–293 (2011).
55. Klockow, J. L. *et al.* ExoSensor 517: a dual-analyte fluorescent chemosensor for visualizing neurotransmitter exocytosis. *ACS Chem. Neurosci.* **4**, 1334–1338 (2013).
56. Yakushenko, A. *et al.* On-chip fast scan cyclic voltammetry for selective detection of redox active neurotransmitters. *Phys. Status Solidi A* 3 Apr 2014 (doi: 10.1002/pssa.201330276).
57. Lemaître, F. *et al.* Recent advances in electrochemical detection of exocytosis. *Electrochimica Acta* 26 Feb 2014 (doi: 10.1016/j.electacta.2014.02.059).
58. Marblestone, A. H. *et al*. Physical principles for scalable neural recording. *Front*. *Comput*. *Neurosci*. **21**, October 2013 (doi: 10.3389/fncom.2013.00137).
59. Conde, S. V. &, Peers C. Carotid body chemotransduction gets the human touch. *J*. *Physiol*. **591**, 6131–6132 (2013).
60. Ribeiro, M. J. *et al*. Carotid body denervation prevents the development of insulin resistance and hypertension induced by hypercaloric diets. *Diabetes* **62**, 2905–2916 (2013).
61. Rosas-Ballina, M. *et al*. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc*. *Natl Acad*. *Sci*. *USA* **105**, 11008–11013 (2008).
62. Ackermann, D. M. Jr *et al.* Separated interface nerve electrode prevents direct current induced nerve damage. *J. Neurosci. Meth.* **201**, 173–176 (2011).
63. McCreery, D. B. *et al.* Relationship between stimulus amplitude, stimulus frequency and neural damage during electrical stimulation of sciatic nerve of cat. *Med. Biol. Eng. Comput.* ***33***, 426–429 (1995).
64. Agnew, W. F. *et al*. Evolution and resolution of stimulation-induced axonal injury in peripheral nerve. *Muscle Nerve* **22**, 1393–1402 (1999).