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
  o Build a library of tracers to visualize the full length of pre-ganglionic axons, ganglionic cell bodies, post-ganglionic axons, and intrinsic neurons, with particular focus on tracing that starts at the target organ\(^1\), \(^5\)
  o Advance approaches for high-resolution labelling of peripheral neurotransmitters, their receptors and co-receptors, and for imaging of myelination, peri- and epineurium
  o 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
  o 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
  o 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
  o Conduct high-resolution nerve tracing, labelling, and imaging in the animal model of choice, taking the organ as the starting point
  o Standardise and coordinate mapping, data management and 3D visualisation across organs
• Establish methods to image and find nerves in the clinical setting
  o Develop tracers and associated imaging techniques that can be used in human preoperative and intra-operative settings to identify and localize peripheral nerves
  o 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
  o 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.

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.
  - 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.
  - 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.

- Build data recording standards and central repositories that allow collaborative data mining and re-analysis (analogous to the Allen Brain Atlas)
  - 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.

**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.
  - 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.

- Adapt interface materials and architectures to meet the specific needs of the visceral neuroanatomy
**SUPPLEMENTARY INFORMATION**

- 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

- 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
  - Establish optimal de-coding strategies for action potentials with low signal-to-noise, including optimising the spike detection and sorting algorithms for unmyelinated fibres
  - Develop algorithms for tracking correlated activity over time across multiple fascicles or nerves

**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
  - 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”))
  - Develop methods for delivery, expression, and, distribution of nano-particle and genetically-encoded reporters into peripheral nerve fibres
  - Explore virus serotypes, sub-cellular targeting motifs 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
- 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
  - Apply current-steering and field-shaping techniques for precision activation of fibres within 100µm nerves
SUPPLEMENTARY INFORMATION

- 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

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
- 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

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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)\(^{56,60}\)
  - 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
SUPPLEMENTARY INFORMATION

• 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
    o Sample widely across key visceral organs as experimental procedures can be standardized, streamlined and rapidly conducted
    o Share experimental approaches to, for example, surgical nerve access, sham controls and correlation analysis through publications and workshops
    o 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
  o Focus efforts on a smaller set of organs with clear neural control: parasympathetic (lungs, heart), sympathetic (spleen, adrenal gland), and afferent (carotid sinus, bladder)
  o 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
  o 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
  o Use the neural code, that is, the recorded signalling patterns, as a starting point for stimulation and blocking parameters
  o Seek to apply both block and stimulation at the resolution of functional units
  o Explore the effect of changes to both average variables (for example, frequencies, compound amplitudes) and temporal patterns (for example, spike bursts)
  o 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
  o Underlying neural signals that correlate with disease
  o Neural recording of signal change resulting from stimulation/blocking
  o 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
  o Use immunohistochemistry to understand the immune reaction to neural interfacing over time
  o Conduct within-animal biocompatibility comparisons of surface materials, and establish the degree of scar tissue formation in response to implantation
  o 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
  o Quantify changes to neural impedance, spontaneous activity, and conduction parameters over time in response to chronic stimulation/blocking.62
  o 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
  o Establish if neural signals upon physiological perturbations change over time

• Map long-term adaptations to neuromodulation across connected organs and functions
  o Map any compensatory neural and physiological changes that may lead to resistance of therapy
  o 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
  o Explore whether systemic changes can be modulated for better treatment effect

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