Noninvasive Neurograms for Determining Etiology and Severity of Infectious Illness

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EXECUTIVE SUMMARY [FOR PUBLIC DISCOURSE]

Research since the turn of the millennium has revealed, in increasing detail, numerous facets of interaction between the immune and nervous systems. Once thought mutually independent, it is now evident that communication and reciprocal influence between these two systems are critical in maintaining homeostasis. Importantly, this dynamic relationship is prone to disruption by intrinsic or environmental stimuli, forming a heuristic window into disease pathogenesis. For example, it has been demonstrated that in various environments including the gut and respiratory mucosae, lymph nodes, spleen and peritoneum, immune cells and nerve terminals exist in close juxtaposition. Furthermore, numerous 'canonical' receptors of innate immunity, including the pathogen recognition receptors toll-like receptors 3, 4, 7 and 9, formylated peptide receptors, and C-type lectins are expressed on sensory nerve terminals and glial cells, whereas numerous 'canonical' receptors of the nervous system including nicotinic and muscarinic acetylcholine receptors, serotonergic receptors, and neurotrophic growth factor receptors are expressed on immune cells. The significance of these findings has been elevated by recent reports of specific pathogen detection by nociceptors in cutaneous infections with *Staphylococcus aureus* and gastrointestinal infections with *Salmonella typhimurium*, the report of neural control of lymphatic antigen flow, and the description of distinct relay of cytokine presence to the CNS via the vagus nerve in intraperitoneal challenge experiments. Our proposal aims to take advantage of these findings to develop a noninvasive tool, detecting and interpreting neural activity, to shed light on the location, severity, and specific nature of illness of infectious etiology. Specifically, we aim to use noninvasive neurography to distinguish between viral and bacterial infections in a biospecimen-free, point-of-care manner, thus subverting the use of empiric antibiotics in patients with viral illness. In addition, we aim to localize bacterial infections to pave the way for anatomically targeted delivery of antibiotics, limiting the area of exposure to drug. Finally, we aim to decipher pathogen-specific neurogram patterns to direct the optimal selection of antibiotic class where indicated. Altogether, our strategy will limit the use of antibiotics to maximize individual patient outcomes while minimizing public health concerns regarding the emergence of highly virulent, drug-resistant organisms.

DESCRIPTION:

Theory:

Once speculation, it is now evident that the immune system and nervous system are in constant collaboration.[1,2,3] Indeed, axonal reflexes initiate the very earliest stages of inflammation, inducing vasodilation, endothelial permeability, and leukocyte activation and migration to sites of infection or sterile tissue injury, all the while transmitting the sensation of pain.[4] Conversely, immune cells modulate somatosensory signaling thresholds[5], shape neuronal growth[5,6], and share common genetic pathways with neural tissue essential for proper development[7]. Studies in *Caenorhabditis elegans* indicate that common factors involved in both neural and immune cell signaling mediate behavioral immunity, suggesting ancient and conserved pathogen detection/avoidance mechanisms[8].

This interplay has recently been mapped in fine molecular detail. For example, in cutaneous infections with the community-acquired methicillin resistant *Staphylococcus aureus* strain USA300, direct activation of nociceptors by bacterial N-formylated peptides and α-hemolysin trigger action potentials that result in pain sensation and modulation of the immune response[9]. In another example, sessile macrophages in the gastrointestinal myenteric plexus form tight synapses with dense neuronal networks, where these marcophages direct peristalsis in a microbiota-dependent manner[10]. Meanwhile, neuronal products maintain this macrophage population[10] and instruct its phenotype[11] following challenge with *Salmonella typhimurium*.

Seminal work at the Feinstein Institute for Medical Research has demonstrated that vagal efferent activity can attenuate production of tumor necrosis factor-alpha by splenic macrophages[12], an observation that has been carried forward in clinical trials of vagal nerve stimulation for the treatment of Crohn's disease[13] and rheumatoid arthritis[14]. This and related work has stimulated a tremendous translational effort towards the development of electroceuticals for the diagnosis and treatment of a great range of illnesses[15-19] [for a list of clinical trials utilizing invasive and noninvasive vagal nerve stimulation for immune modulation, see reference 30]. The promise of a bioelectronic approach to infectious disease in particular is strengthened by the report of neural control of lymphatic antigen flow[20], and, tantalizingly, the relay of specific cytokine induced signals to the brainstem via the vagus nerve[21]. Taken together, these discoveries evidence the existence of a wealth of information travelling in nerve fibers, offering a previously unexplored avenue for the diagnosis of infectious disease[22, 23].

Thus, there is strong impetus for the development of a device capable of interception and decoding of peripheral neural activity to aid the diagnostician in the differentiation of illness

secondary to microbial infection. We hypothesize that distinct patterns of neural activity will correspond to distinct categories of infection such that it may be possible to distinguish between infections that are due to, for example:

- Viral vs. bacterial vs. fungal organisms
- Intracellular vs. extracellular organisms
- Gram (-) vs. gram (+) organisms
- Any microbe vs. flare of underlying inflammatory disease (ex., RA, SLE, FMF)

In addition, neural signatures may help localize the site of infection, provide an index of disease severity, and warn against impending sepsis or other form of clinical deterioration. This information will ultimately aid clinicians in the more selective use of antibiotics.

Challenges:

There are two major developmental challenges towards our approach. The first is technical, concerning the ability to detect and decode high quality data from peripheral nerves. The second is biological, concerning the sensitivity, specificity, and overall clinical utility of the derived data.

a) Technical. For practical purposes, including ease of use, cost-effectiveness, safety and wide applicability, the proposed device must be noninvasive and provide real-time readout. Currently in clinical diagnostic use for noninvasive detection of bioelectrical activity are techniques such as electrocardiography, electroencephalography, and galvanometry utilizing surface electrodes. These methods offer the advantage of rapid data generation and signal detection in real-time, requiring only basic training for technicians. Resolution is largely dependent on the number of electrodes used. Other approaches for noninvasive neural recording with single fiber resolution have been developed and tested in non-anesthetized behaving animals, however, each of these has the significant drawback of requiring either an *a priori* implant[24] or the presence of genetically encoded reporters[25-27]. Therefore, noninvasive detection of neural activity is currently best accomplished with surface electrodes, although this approach is limited by the low voltage nature of the peripheral nervous system as well as interference in awake subjects secondary to movement, surrounding tissue impedance and physiological perturbations such as breathing and myocardial depolarization. To overcome these limitations, we propose the development of a novel neural activity detection device,

consisting of an ultrasound/photoacoustic probe hybridized with a curved, high-density electrode array [Figures 1 and 2].

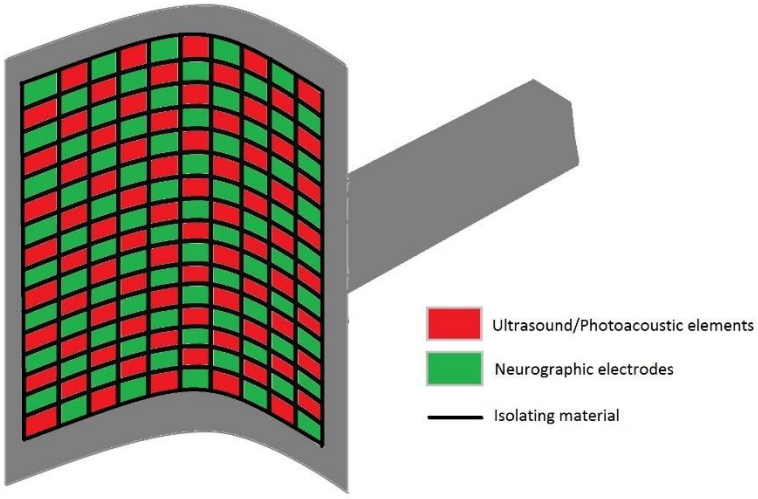


Figure 1 – Probe diagram.

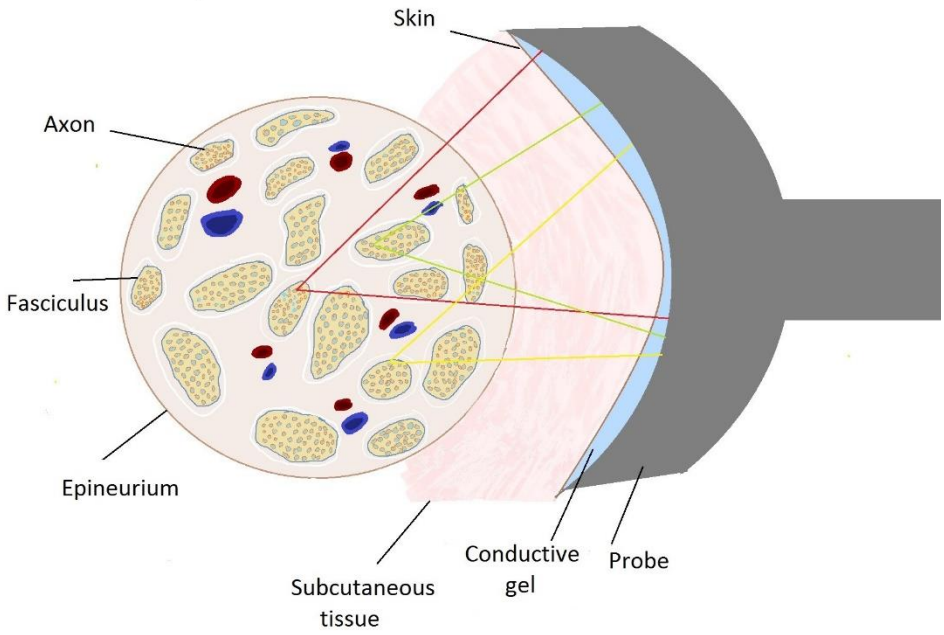


Figure 2 – Nerve fiber cross section with apposed probe for detection of neural activity.

The use of ultrasound and photoacoustic elements together will enable anatomical validation of probe placement, normalizing for structural variability between individuals. The horizontal curvature of the probe will aid in focused detection of nerve bundle activity, while the multitude of neurographic electrodes may aid in higher resolution signal isolation of fascicular activity via blind source separation. In the longitudinal dimension, the probe will be able to determine impulse velocity, decay, and frequency. This multidimensional data will be broadcast to a receiver for signal amplification, filtering and deconvolution [proposed sequence in Figure 3]. Initial experiments for device validation may include readily accessible nerves with minimal overlying tissue, such as the ulnar or sural nerves. Once a functional device has been developed, the study of electrical activity as related to infectious disease would ideally focus on the vagus nerve initially. The anatomic location of the vagus is conducive to noninvasive identification. In addition, about 80% of the human vagus nerve is thought to consist of sensory afferent fibers, which encompass an enormous footprint in the thorax and abdomen, thus relaying on a constant basis the *milieu intérieur* to the CNS. Future development may enable the more targeted detection of bioelectric activity at other sites of interest [Figure 4]. The spleen is an attractive alternative, considering its role in blood sampling, closeness to the body surface, and lack of interference from signaling units involved in orchestrating organ function. Parallel studies in large animals will yield lessons in appropriate signal dissection and signal dynamics over the natural history of controlled microbial challenge experiments.

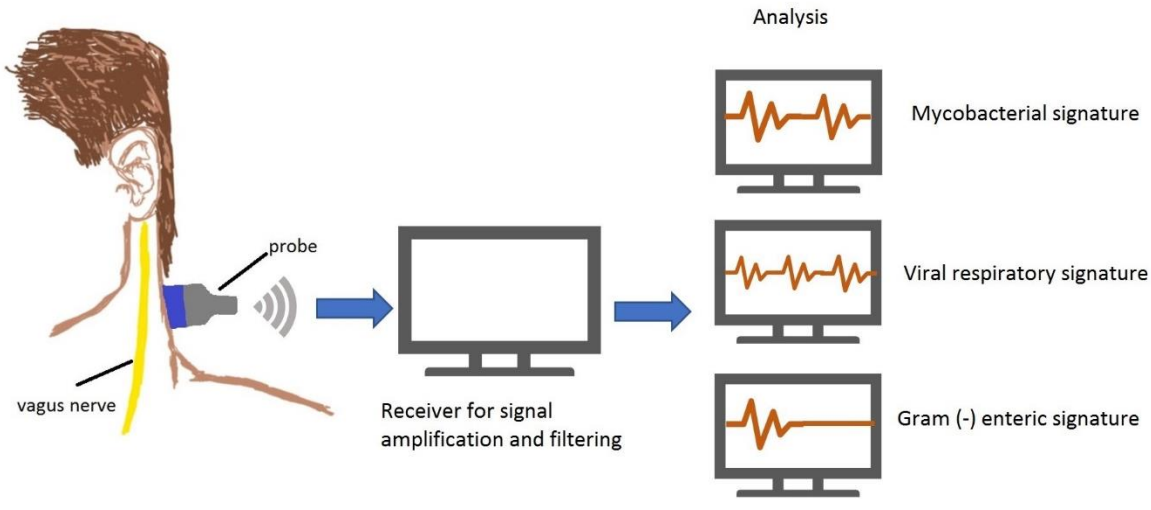


Figure 3 – Proposed utility of the noninvasive neurographic device.

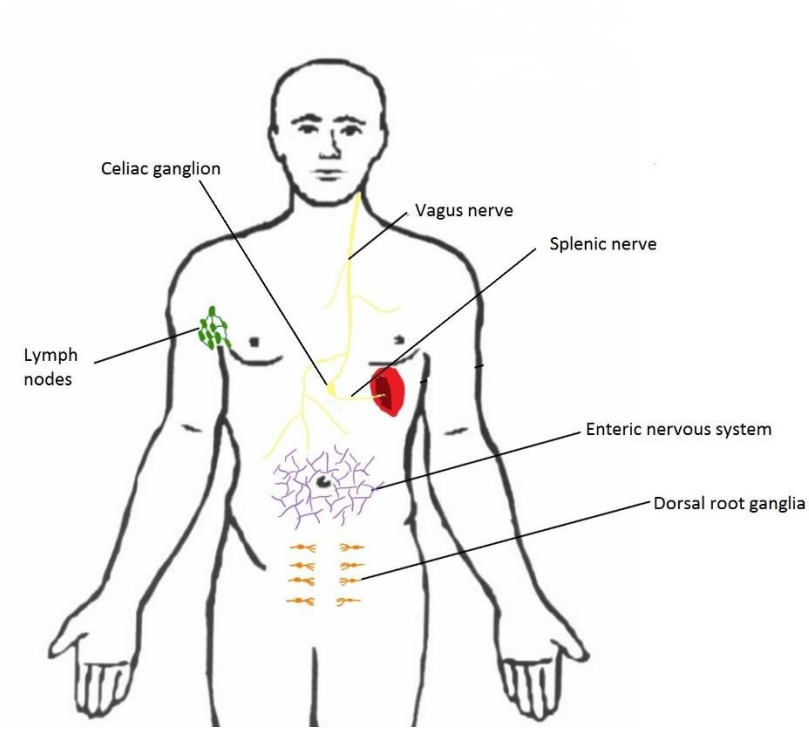
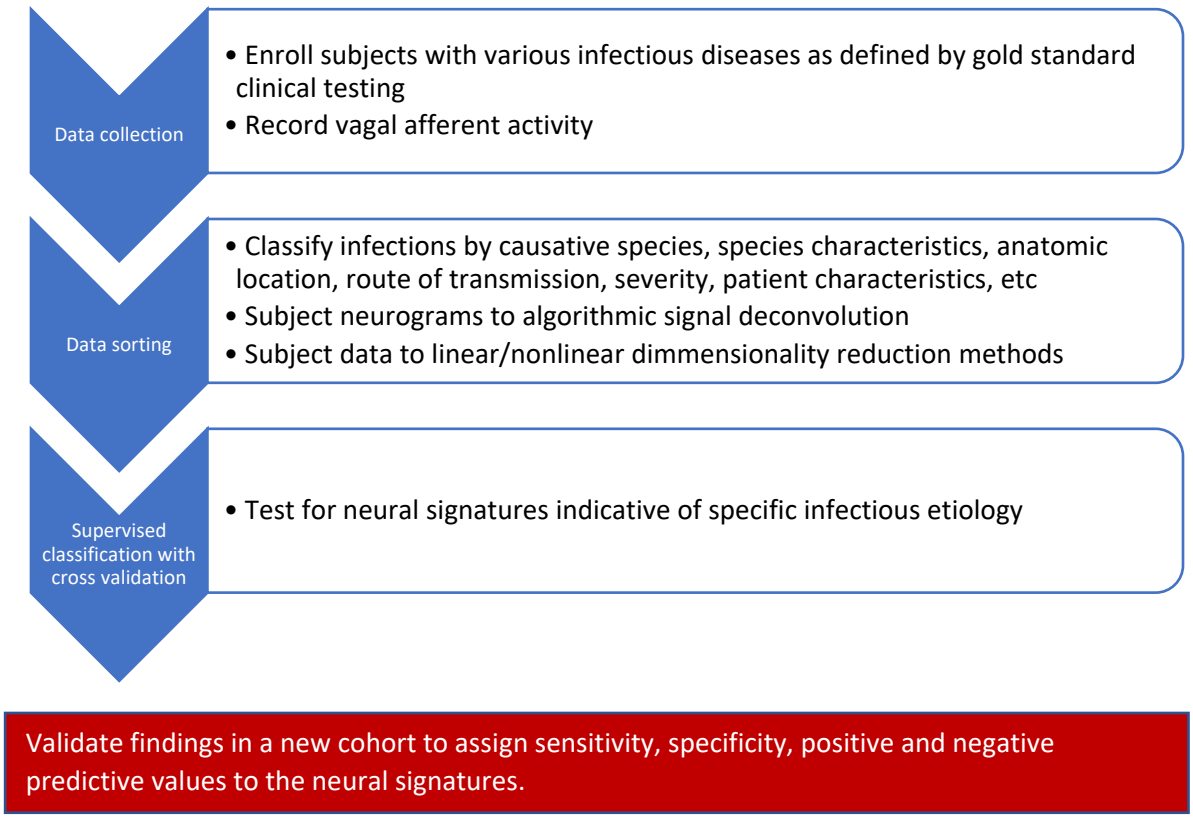


Figure 4 – Sites of bioelectric activity implicated in infectious disease process.

b) Biological. Given the successful development of a neural activity monitoring device, the relevance of this activity to the differential diagnosis of infectious disease will need to be established. Given that efferent neural activity directs the classical symptomatology of infection, including emesis, diarrhea, coryza, cough, as well as elevations in heart rate, blood pressure, body temperature and serum cortisol; it would be expected that afferent disease-sensitive mechanisms provide stimulus and constant feedback. To associate neural activity with specific infectious entities, studies with human subjects will be necessary. Specifically, subjects with various ongoing infectious diseases as defined by gold standard clinical testing will be enrolled and the activity of their afferent vagal signals recorded. The study will proceed per the following chart:



Risks:

We do not anticipate any major risk to subjects from our noninvasive, noninterventional approach. Given the ubiquitous use of ultrasound and surface electrography in the clinical setting, minimizing harm to patients will center on the principles of good clinical practice including pathogen containment and hand hygiene. Critically ill patients, patients with carotid bruits or cerebrovascular disease, and patients with cervical pathology will not be enrolled.

STATE OF THE ART:

Current diagnosis of infectious disease requires costly and time consuming methods such as culture, nucleic acid amplification, biomarker assays, and microscopic analyses. Our proposed approach bears several significant advantages over these methods:

a) Speed. Neurograms collected in real-time and aided by computer interpretation of infectious signatures may provide clinicians with diagnostic answers with the same speed and efficiency that electrocardiograms may reveal an ongoing myocardial infarction. Depending on the periodicity of these signatures, it may be possible to reach a diagnosis or narrow the differential in less time than it takes to draw blood for culture.

b) Ease of use. The existence of infectious signatures would allow for computer aided diagnoses, thus minimally requiring that technicians with basic training obtain the neurograms. It is commonplace in the clinical setting for non-physicians to collect electrocardiography and ultrasound data. Furthermore, the rapid and noninvasive nature of this approach may be utilized at the point-of-care, in community clinics or emergency room triage.
c) Biospecimen-free. Neurograms are directly obtained from the patient without the need for the collection of blood, urine, stool, or tissue. This spares the time and resources necessary to collect these specimens and the possibility of a false-negative test due to misrepresentative sampling.
d) Global applicability. Such devices, when successfully developed and validated, need only be purchased once, enabling their use for extended periods. Because there is no need for lab space or highly specialized facilities, they can be deployed globally in resource poor settings, an essential component of any solution to combatting antibiotic resistance.
e) Novelty. While bioelectric activity has been harnessed in the diagnosis of intrinsic neurological and cardiologic maladies, noninvasive neurograms would represent a new dimension for the diagnosis of infectious disease, in addition to inflammatory diseases without an infectious source. Neurograms would also add to the armamentarium of tools available to the clinician faced with a common illness that does not merit thorough workup, such as the pediatrician who often resorts a ‘watch and wait’ approach for many young patients.

In summary, here we lay the conceptual groundwork for the development of a noninvasive bioelectronic device for the diagnostic differentiation of illness of infectious origin. The detection of specific neurographic signatures may reveal in real-time the source and nature of an infectious process such that timely and anatomically targeted use of antibiotics may be initiated. Likewise, the empiric use of antibiotics pending conventional lab results may be subverted. While we do not expect these neurograms to reveal the antibiotic sensitivity profiles of disease causing organisms, the ability to rapidly and accurately distinguish between bacterial and nonbacterial infections on the basis of a neurogram would be transformative in limiting superfluous antibiotic use. Furthermore, it is conceivable that during local or global outbreaks of resistant organisms, the ability to rapidly identify patients with communicable organisms for contact precaution would aid in control efforts. Finally, we predict that as the technology advances, higher resolution detection of sensory afferent activity down to the single-fiber level

would open doors not only to better-defined diagnoses, but also interventions in the form of electronic stimulation to orchestrate immune responses, augmenting the body's natural defenses—thus supplanting the use of antibiotics altogether.

EXECUTION:

We at the Feinstein Institute for Medical Research are uniquely equipped to pursue the design and validation of the proposed method. In addition to being home to many of the discoveries in neuroimmune interactions over the past two decades, the Institute has recently launched the Center for Bioelectronic Medicine[28], which brings together leading experts in biomedical engineering, neural interfaces and software development. The Institute is also involved in several strategic national and international partnerships with the common goal of advancing electroceuticals in academia and industry, most recently convening in conjunction with the Karolinska Institutet a gathering of the minds[29] as this new discipline takes root. The key obstacle towards implementation will be the ability to detect, amplify and stratify neural signals noninvasively. While the proposition is challenging, we believe that the device modelled above is an important first step. The facilities available at the Institute, including a class 100 microfabrication suite and a rapid prototyping core with 3D printing capability will be indispensable as we move forward. Northwell Health, our parent organization, boasts 21 hospitals and numerous community practices as well as a robust clinical research program that will be crucial to providing a broad patient base and research oversight. We expect the device development phase to span several months to one year.

Studies with human subjects will be contingent upon IRB approval and HIPAA compliance. Subjects will be enrolled given informed consent, which will include a discussion of risk as described in the 'Risk' section of this proposal, as well as a discussion of the potential benefits to the subjects and the general population. Data collected will be encrypted, deidentified and only available to the investigators. Recruiters and investigators will be trained in techniques for pathogen containment when working with subjects with infectious disease. Subjects will be enrolled without preference for race, ethnicity, gender, religion, sexual orientation or socioeconomic status. We expect that the enrolled cohorts will be inclusive of women, children and minorities. Where appropriate, consent will be provided by a parent/guardian or healthcare proxy. Regular review will identify, disclose and rectify any harms posed to patients that were previously unforeseen.

Studies in animals will be subject to approval by our Institutional Animal Care and Use Committee and will be guided by the principles of good animal husbandry. Animals will be housed in a facility staffed around the clock with trained technicians and veterinary oversight. Animals will not be subject to undue harm or distress for which they will be routinely monitored.

To our knowledge, there exists no prior art in using noninvasive neurograms for the diagnosis of infectious disease.

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