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SPECIAL FEATURE REVIEW

Inflammation neuroscience: neuro-immune crosstalk and interfaces

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Abstract

Inflammation is a key process in antimicrobial defence and tissue repair, and failure to properly regulate inflammation can result in tissue damage and death. Neural circuits play important roles throughout the course of an inflammatory response, and the neurophysiological and molecular mechanisms are only partly understood. Here, we review key evidence for the neural regulation of inflammation and discuss emerging technologies to further map and harness this neurophysiology, a cornerstone in the rapidly evolving field of *inflammation neuroscience*.

Keywords: devices, homeostatic reflexes, inflammatory diseases, neuro-immune communication, peripheral nerves, translational medicine

INTRODUCTION

The nervous system plays a key role in the regulation of many physiological functions of importance for virtually all organs and tissues. Observations in the 1990s provided convincing support that peripheral nerves are involved in monitoring and regulating the physiological response to injury and infection. A key observation was the absence of a fever response in vagotomised rats injected with a low dose of interleukin (IL)-1 intraperitoneally. This discovery identified the vagus nerve as an important conduit for information on peripheral inflammation to the central nervous system.[1] More evidence of a reflex circuit involved in immune regulation that includes afferent and efferent peripheral nerves emerged with the observation that IL-1 injected into the portal vein in rats resulted in increased motor activity in the splenic nerve, but only if the hepatic branch of the vagus nerve was left intact.[2] In conjunction with these findings, electron microscopy evidence suggested the possibility of a direct neuro-immune junction in spleen.[3]

Subsequently, it was discovered that electrical or pharmacological activation of components in a cytokine-regulating circuit, now designated the 'inflammatory reflex', reduces the release of pro-inflammatory cytokines in inflammation through mechanisms that involve the vagus nerve, cholinergic and adrenergic nerves and receptors, T cells, macrophages and other immune cells.[4–14] Many other neural pathways that regulate inflammation and immunity have subsequently been discovered,[15] and the understanding of their physiological roles is continuously expanding.[16–20] Collectively, these discoveries have initiated the field of 'inflammation neuroscience' focused on the fundamental mechanisms in the neural regulation of immunity and 'bioelectronic medicine',

spearheading translation of this knowledge into novel potential clinical approaches in prevention, monitoring and treatment of a possibly wide range of diseases.[21,22] In this review, we summarise peripheral neuro-immune crosstalk, consider emerging technologies to further map this inflammatory physiology and discuss translational considerations in this field.

NEURO-IMMUNE CROSSTALK

Historically, the immune and nervous systems have been studied independently, but it is now evident that there are a number of overlapping and complex processes that regulate both initiation and resolution of immune responses. Consider the typical clinical course of stroke: Patients often develop systemic immunosuppression[23] and as a result are more susceptible to infections such as pneumonia.[24] Changes in sympathetic activity[25] following a stroke are associated with enhanced antigen-specific T-cell reactivity on the stroke-affected side of the body.[26] Interestingly, blocking adrenergic signals in the liver rescued experimental animals from life-threatening pneumonia following stroke.[19] Furthermore, co-administration of a stimulus and an immunomodulating agent leads to a coupling of the two, so that the stimulus alone ultimately also has an immunomodulatory effect, a phenomenon known as immune conditioning.[27,28]

This connection between the brain, the peripheral nervous system and the immune system provides a structure to tune and coordinate immune responses to challenges. While the specific peripheral neuronal pathways during immune conditioning are yet incompletely understood, more is known about the peripheral autonomic nervous system in the context of neuro-immune crosstalk. The sympathetic and the parasympathetic nervous systems play distinct roles in the regulation of immune activity. The sympathetic nervous system has a capacity to exert both local and systemic control, by direct innervation and systemic neurotransmitter release, respectively. Examples include activation of β2 adrenergic receptors on immune cells with increased cAMP levels that suppresses NF-kB nuclear translocation and leads to inhibition of pro-inflammatory cytokine synthesis[29] and control of T- and B-cell responses.[30] Conversely, activation of α adrenergic receptors on immune cells promotes the production of pro-inflammatory cytokines[31] (Figure 1a).

Similarly, the parasympathetic nervous system, with the primary neurotransmitter acetylcholine (ACh), plays an important role in the control of immune regulation[4,10,32,33] (Figure 1b). The vagus nerve is one of the most extensively studied components of the parasympathetic nervous system, and it plays a central role in the inflammatory reflex. Composed of an efferent and afferent arc, the vagus nerve can sense cytokines in the periphery and respond through an efferent release of ACh that regulates immune responses and inhibit inflammation.[34] Of note, ACh is not exclusively released by neurons, but can also be biosynthesised by a number of immune cells such as B[35] and T cells.[8,33,36] Highlighting the complexity of neuronal control over immune responses, some of the vagal immunomodulatory effects are mediated by adrenergic nerves.[5,6,9,10,37,38] The intricate interplay between adrenergic and cholinergic signalling in the neural regulation of inflammation has been extensively reviewed elsewhere.[30,39–41]

Sensory neurons that innervate barrier tissues such as the skin, gut and lungs participate in the autonomic regulation of inflammation. Many sensory nerve fibres are found in close proximity to immune cells and exert local immunomodulatory effects through the release of neuropeptides[42–45] (Figure 1c). In cutaneous infections, sensory transient receptor potential ion channel-positive ($TRPV1^+$) and/or voltage-gated sodium channel-positive ($Nav1.8^+$) neurons regulate immune responses. Activation of $TRPV1^+$ neurons in the skin elicited a local response type 17 immune response in the absence of tissue damage or pathogen-associated stimuli.[46] Interestingly, this response was also observed in

Box 1. Inflammation neuroscience and bioelectronic medicine

Inflammation neuroscience is the convergence of molecular medicine, neuroscience, engineering and computing to study the fundamental mechanisms of the neural regulation of inflammation and immunity. Bioelectronic medicine is the translational and clinical application of using electronic devices to interface with the peripheral nervous system to target molecular mechanisms for the treatment of disease.

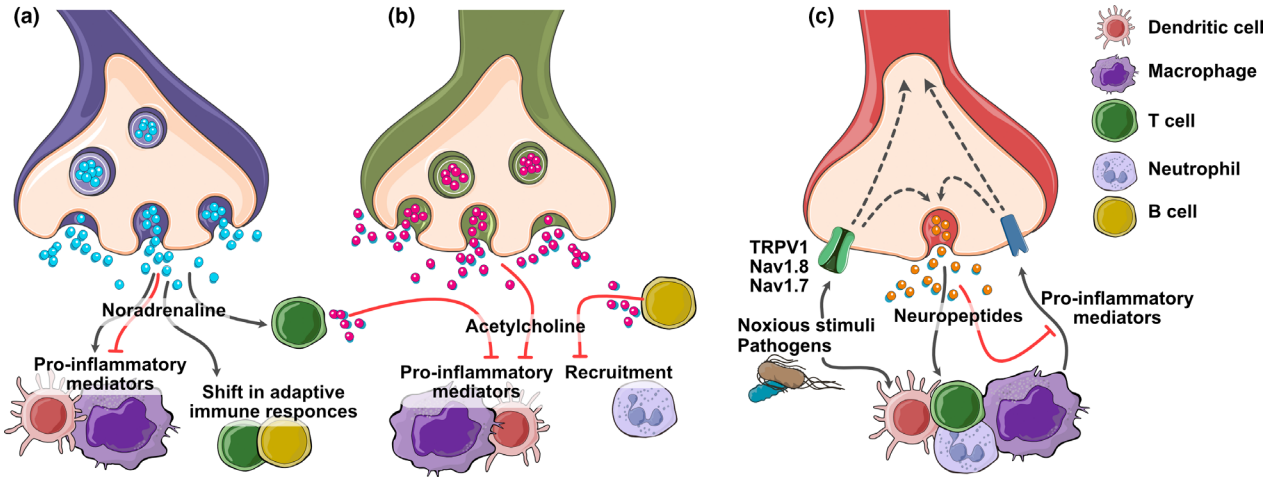

Figure 1. Communication between the nervous system and the immune system. **(a)** Adrenergic innervation reaches the vast majority of the body, including immune organ. Noradrenaline, acting through α- or β-adrenergic receptors on immune cells, has numerous immunomodulatory effects; either promoting or limiting the release of pro-inflammatory mediators and inducing a shift in adaptive immune responses. **(b)** The neurotransmitter acetylcholine, derived from cholinergic nerves or ACh-producing B and T cells, limits the release of pro-inflammatory mediators and immune cell recruitment. **(c)** Sensory neurons can detect pro-inflammatory mediators and noxious stimuli in the periphery. Signals are transmitted to the CNS as well as in an axonal reflex, leading to the release of neuropeptides stimulating extensive local immunomodulatory effects. Parts of this image were modified from SMART (Servier Medical Art), licensed under a Creative Common Attribution 3.0 Generic License.

skin areas anatomically adjacent to the activated area.[46] A similar physiology was also observed in *Candida albicans* skin infections where TRPV1$^+$ neurons, the neuropeptide calcitonin gene-related peptide (CGRP), CD301b$^+$ dermal dendritic cells and dermal γδ T cells interact and promote a local type 17 immune response.[47] Of note, skin in proximity to, but separate from, sites inoculated with heat-*inactivated* *C. albicans* showed enhanced clearance of *C. albicans* upon repeated challenge, and nerve ablation increased susceptibility to *C. albicans* infection.[46] Together, these observations indicate that local interactions between nerves and immune cells can improve and extend barrier defences by initiating an anticipatory immune response in tissues adjacent to the primary challenge.

Importantly, while necessary for effective protection against invading pathogens, activation of sensory reflexes can also promote excessive inflammation. For instance, in an experimental model of allergic airway inflammation, immune cell-derived IL-5 promoted the release of the neuropeptide vasoactive intestinal peptide (VIP) from nociceptive neurons, which in turn induced the production of T helper 2 (Th2)-type cytokines from CD4$^+$ T cells. Additionally, Th2-type cells also have the capacity to secrete VIP upon antigen stimulation,[48] potentially promoting a local type 2 pro-inflammatory loop. However, ablation of Nav1.8$^+$ sensory neurons substantially reduced airway inflammation and bronchial hyperresponsiveness.[49] Likewise, ablation of TRPV1$^+$ and Nav1.8$^+$ neurons in psoriasiform experimental models reversed the disease[17,50] and intradermal botulinum neurotoxin injections, ablating local innervation, reduced local psoriasis in patients.[51,52]

While these observations would indicate that sensory neuron activation promotes and occasionally exacerbates inflammatory responses in barrier tissues, neuropeptides have context-dependent effects, both pro- and anti-inflammatory (Figure 1c). For example, innate lymphoid cells (ILCs), a subset of immune cells abundant in barrier tissues, can respond to CGRP with lower proliferation and reduced IL-13 production, leading to reduced airway inflammation and magnitude of innate type 2 responses after helminth infections.[53,54] Additionally, TRPV1$^+$ neuron signalling through CGRP inhibits the recruitment of neutrophils and γδ T-cell responses on the skin and in the lungs following bacterial infection.[55,56] CGRP also modulates macrophage activity, inhibiting the release of pro-inflammatory cytokines during experimental endotoxaemia[57] and following cutaneous *Staphylococcus aureus* infections.[58]

Amongst the many anti-inflammatory actions of VIP, this immunoregulatory neuropeptide has also been shown to induce tolerogenic dendritic cells and promote T regulatory cell differentiation.[59] It is clear that crosstalk between nerves and immune cells in barrier tissues is extensive and that neuropeptides can act both to promote and to inhibit inflammation, depending on context. Specific immune cells respond to distinct neurotransmitters released by both sensory and motor neurons and other cells. A number of reviews explore this topic in greater detail.[42,48,60] The extent of this bidirectional communication and how the secretion of these neuropeptides is regulated remain to be elucidated.

Taken together, there are numerous pathways for neuro-immune crosstalk in the periphery. While insights into these neuro-immune interactions provide an improved understanding of immune responses and potentially an entire array of new treatment modalities for diseases characterised by excessive inflammation, there are still major gaps in our understanding of inflammation neuroscience. A more complete understanding of this physiology will require mapping of the neurophysiology of inflammation *in vivo*.

MAPPING PERIPHERAL NEURO-IMMUNE CONNECTIONS

A cornerstone of therapy development in modern medicine is understanding the detailed cellular- and molecular-specific disease-associated mechanisms. Current evidence demonstrates that different subpopulations of neurons play different roles in the inflammatory response, but a comprehensive map of these signals is lacking. Recent data demonstrate that specific combinations of pulse width, pulse amplitude and frequency for electrical activation of the cervical vagus nerve can decrease or increase systemic cytokine levels, also in the absence of inflammation.[61] Pulse width appears to be an important parameter, variation of which was reported to switch between attenuation and augmentation of cytokine release. A plausible explanation of this finding is that different stimulation protocols recruit selective but divergent sets of neurons, activation of which promotes a distinct immune response. Accordingly, it is possible that vagus nerve stimulation may be utilised not only for reducing the release of pro-inflammatory cytokines such as TNF but also for a more detailed fine-tuning of systemic immune responses in a variety of settings in health and disease.

Considering these observations, the electrophysiology and anatomy of immune-regulating neural circuits should be mapped in detail to adequately plan experimental treatments in models of inflammatory diseases and any clinical interventions. Mapping the anatomy and decoding electrical signatures of the vagus nerve – and other conduits that regulate immune system function – to potentially create both an atlas and a dictionary for the neural signals that regulate inflammation are therefore very much of interest. With an atlas of the involved neural reflexes, it is conceivable to use bioelectronic medicine to address one of the common major shortcomings of pharmaceuticals, that is the common lack of anatomical target specificity. While the relatively painstaking traditional methods using mono- and poly-synaptic tracing provide key information on innervation and specifics on potential neuro-immune interactions, this work is still in its infancy. In fact, the details of nerve–immune cell interactions are not established even in the extensively studied inflammatory reflex, let alone in other contexts. However, the development of reporter mice specific for subpopulations of neurons and methods for specific virus-based neural barcoding promise to enable identification and tracing of individual neurons in the body.[62] We postulate that such mapping would permit the development of therapies for regulating immune responses based on interfacing with peripheral nerves that allow for molecular monitoring and better prediction and mitigation of undesirable side effects.

INTERFACING WITH PERIPHERAL NERVES

An interesting prospect is to regulate immunity by the use of electrical nerve stimulation for activation of select nerves. The use of electricity in medicine dates back to year 47 CE in Mesopotamia, where live torpedo fish were used to treat headaches, arthritis and gout.[63] However, it was not until the 19th century that the discoveries by M Faraday enabled the construction of transcutaneous and direct electrical nerve-stimulating devices.[64] Following breakthroughs in neuroscience, Sweet and Wall demonstrated in

1960 the suppression of pain perception by peripheral nerve electrical stimulation – with sustained relief from pain after only two minutes of stimulation.[65] Widespread use of peripheral nerve stimulation was, however, limited both by inadequate knowledge of the human connectome and by lack of commercially available devices.

The early applications of peripheral nerve stimulation were based on adaptations of surgical techniques and stimulators used in the CNS. The paddle-type, multicontact electrodes, used for peripheral nerve stimulation in the 1980s, were developed for CNS use, and the perceived need for electrode optimisation for peripheral nerves was low. However, following the breakthrough of percutaneous electrode placement techniques,[66] interest was sparked in electrode optimisation and precipitated the development of smaller electrodes with decreased implantation risk and improved tissue interface longevity, and without a requirement for transcutaneous wires. Extra-neural interfaces, such as hook or cuff electrodes,[67] have the benefit of leaving nerves intact, but usually target several bundles of axons or fascicles at the same time, significantly limiting selectivity. Higher selectivity can be achieved by penetration of the nerve to access fibres of a single or multiple fascicles,[68,69] but these methods are inherently more invasive and breach the protective epineurium.

A number of technologies and approaches have been considered to develop the localised stimulation and to enable high-quality mechanistic studies of peripheral nerve activation. A list of nerve stimulation strategies and practical/technical considerations can be found in Table 1. Of note, much of the mechanistic understanding of electrical nerve stimulation for the neural regulation of inflammation originates from studies in acute or relatively short-term mouse models. A reason for the widespread use of mice is that genetic mouse models provide informative, well-characterised and convenient platforms to study mechanism, but suitable technology for studies in chronic models is largely lacking, which is a significant barrier to progress with inflammation neuroscience in well-characterised genetic models of chronic disease.

Long-term electrical stimulation of peripheral nerves in mice is not trivial. The development of peripheral nerve interfaces has to consider the physiological and anatomical aspects of nerve size, shape, complexity and composition. For example, the mouse cervical vagus nerve, a relatively large nerve in the mouse, only has a diameter of approximately 100 micrometres and it is difficult to attach an electrode and wires that will stay functional as the mice move around and exhibit their normal, social behaviour.[70] Successful chronic stimulation requires electrodes that are biocompatible, flexible, anatomically fixed and maintain device functional integrity and sufficiently low interface impedance to perform reliably over time.[71] Recent reports indicate that using wired electrodes for interfacing with the cervical vagus nerve in chronic experimental models in mice is possible,[72] but the technology is not yet widespread or established. A particular concern is maintaining lead connections over time in freely moving and interacting mice. One solution to this latter problem is to use untethered devices, which also reduces the risk of infection.[73] Furthermore, interfacing with more peripheral smaller nerve branches puts further requirements on electrodes for chronic use in terms of biocompatibility and long-term device and interface integrity.

Several approaches have been tested in order to achieve non-invasive stimulation of select peripheral nerves. Successful activation of the splenic nerve using focused ultrasound was reported, for example in a mouse model of inflammatory arthritis, but this method is technically challenging for use in experimental models of chronic inflammation.[74–76] Ultrasound in clinical practice is often highly operator-dependent for performance and precision. This shortcoming might potentially be mitigated by instead using ultrasound as an energy source for activation of previously implanted electrodes at specific target nerves, but this approach requires prior surgery.[77] Another method for nerve stimulation involves optogenetics, which is very attractive for its potential specificity in targeting subpopulations of nerve fibres in genetic mouse models. It is widely used and has contributed significantly to furthering our understanding of reflex neurophysiology, including in the regulation of immunity.[20,78,79] However, because of the complicated nature of the technology involved in optogenetic stimulation, it is not yet well suited for studies of chronic conditions with repeated stimulation over longer periods of time. Furthermore, strategies involving magnetic materials are interesting and appear promising, including the possibility of 'magnetogenetics' – a

Table 1. Nerve stimulation strategies and the advantages and disadvantages associated with each method

Reference	Activation method		Stimulation type	Advantages	Disadvantages
67	Hook or cuff electrodes	Wrap/attach to epineurium	Electrical	• Minimal nerve damage • Activates groups of nerves • User-operated	• Low signal-to-noise ratio • Low resolution of stimulation • Low selectivity • Surgical
68,69	Micro-needle	Intraneural electrode	Electrical	• Good resolution of stimulation • Good signal-to-noise ratio • Low stimulation intensity • Selective	• Breach of the perineurium • Difficult to position • Surgical
85,99	Temporally interfering electric fields		Electrical	• Non-invasive • Deep tissue penetration • Precise orientation of the electric field	• Limited experience
74–76	Ultrasound		Mechanical	• Non-invasive • Low cost	• Potential low selectivity • Operator-dependent
20,100,101	Optogenetic		Biochemical	• Highly selective and cell type-specific • Cellular action potential morphology • Minimally-invasive • High spatiotemporal resolution	• Currently restricted to animal models • Gene therapy-based • Maintenance of the transgene over time
80	Magnetic		Electromagnetic field	• Non-invasive • Deep tissue penetration • No skin contact is necessary	• Heat increase • Not compatible with other electronic implants • Low selectivity and diffuse stimulation
			Magnetogenetics	• Similar as to the optogenetic, but without the need for implanted optical waveguides or light-emitting devices	• Currently restricted to animal models • Gene therapy-based • Limited experience
81,102	DREADDs		Chemogenetic	• Non-invasive • Highly selective and cell type-specific • High spatiotemporal resolution • Selective suppression or activation of nerve signals • Reversible • Titratable	• Currently restricted to animal models • Gene therapy-based

concept in which a single protein responsive to a magnetic field is introduced/expressed and in that way may offer similar specificity as optogenetics.[80] In yet another approach, specificity in nerve activation is achieved by the use of artificially designer receptors exclusively activated by designer drugs (DREADDs) – introduced proteins or enzymes that respond to exogenous drugs and cause activation in only a defined subset of nerves.[81,82]

An interesting development uses a hybrid approach with surgical implantation of photocapacitors on target nerves and transcutaneous transmission of far-red light for subsequent nerve activation. This technology may provide a path forward to realise reproducible nerve stimulation sustainable in genetic models of chronic diseases.[83,84] In addition, refinement of a non-invasive nerve activation method based on temporally interfering electrical fields hitherto exclusively used in brain[85] might be an interesting option for peripheral nerve stimulation, and enable the study of the mechanistically very informative genetic mouse models. These methods, perhaps in combination,

are promising tools for improving our insights on the neural regulation of inflammation.

TRANSLATIONAL CONSIDERATIONS

Pre-clinical disease models have shown that vagus nerve stimulation (VNS) is potentially a supplementary, if not alternative, treatment modality in conditions and diseases such as sepsis, ischaemia/reperfusion injury, rheumatoid arthritis (RA) and inflammatory bowel disease.[4,11,86–89] VNS has been utilised in human clinical trials for the treatment of RA, and VNS was reported to improve the 28-joint disease activity score (DAS) and decrease serum TNF in RA patients over the course of 3 months.[90] Similar results were observed in a patient cohort with multidrug refractory RA.[91] In patients with Crohn's disease, VNS has also been reported to contribute to reduced symptoms, and in some cases clinical remission over the course of 6 months.[92,93] Thus, the available clinical data are encouraging, but well-controlled larger studies will be needed for the evaluation of the clinical efficacy in human autoimmune diseases.

As the technology of peripheral nerve stimulation develops and the understanding of the underlying molecular mechanisms are mapped, new treatment modalities may become available for autoimmune and other human diseases. A fascinating prospect is the possibility to measure and potentially decode electrical activity in peripheral nerves as a measurement of local inflammation. There are already reports that suggest that it is possible,[70,94–96] but much work remains in improving interface technology, signal processing, and mathematical modelling and analysis before this approach may become a useful tool for monitoring inflammation intensity and characteristics.

It is tempting to speculate that closed-loop systems, proof of concept of which has already been reported in adjacent areas of medicine,[97,98] can help personalise the treatment of the notoriously variable clinical symptoms that are common in chronic inflammatory diseases. Continuous recording of key physiological indicators, such as a cytokine level, and activity in inflammation-sensing nerves might provide information that enables frequent adjustments of therapeutic intensity, that is activity of, for example, a vagus nerve stimulator. Analogous to the way insulin is dosed based on physiological readouts such as blood glucose in diabetes, it might be possible to continuously adjust the dose of anti-inflammatory nerve stimulation based on physiological measurements, be it nerve activity, cytokine levels, pain or other relevant variables. These enticing prospects certainly warrant intense work on mapping mechanisms and physiology in inflammation neuroscience.

CONCLUSIONS

Decades of work in *inflammation neuroscience* has provided compelling evidence that crosstalk between the nervous and immune system plays important roles in the regulation of inflammation, from initiation to resolution. Mechanistic insights on the peripheral nerve regulation of immunity have laid the groundwork for clinical studies using nerve stimulation to reduce the intensity of chronic inflammatory diseases. Clinical observations are encouraging and suggest that it may be possible to replace some anti-inflammatory drugs with electric current and nerve stimulation to control and improve chronic inflammatory diseases.

While there is ample support for the neural regulation of inflammation, detailed mechanistic studies of the neurophysiology and molecular mechanisms that are key in the crosstalk between nerves and immune cells are somewhat lacking. Several interesting efforts to overcome these gaps are ongoing using a wide range of approaches, including optogenetics, magnetogenetics, DREADDs, photocapacitors and temporal interference. Progress is also being made in the development of recording electrodes and signal processing aimed at decoding inflammation-associated sensory information in peripheral nerves, despite that the understanding of both the functional anatomy and physiology of the different reflex circuits is limited.

Going forward, the emerging technologies will be important to improve specificity in nerve interfaces *in vivo*, better delineate the functional anatomy and the electrophysiology of immune-regulating neural circuits, and provide an 'atlas and dictionary' of neuro-immune interactions. We postulate that future work in *inflammation neuroscience* will shed important light on the pathophysiology of excessive inflammation and inflammatory diseases, and likely provide novel

insights on signals that promote resolution and healing. This new knowledge will be useful for developing and improving novel therapeutic approaches and perhaps enable the use of electrons to supplement or even substitute drugs.

CONFLICT OF INTEREST

LT has no conflicts of interest to declare relative to this manuscript. PSO is a co-founder and shareholder of Emune AB and ChAT Therapeutics.

AUTHOR CONTRIBUTION

Laura Tarnawski: Conceptualization; Project administration; Supervision; Writing-original draft; Writing-review & editing. **Peder S Olofsson:** Conceptualization; Resources; Writing-original draft; Writing-review & editing.

REFERENCES

1. Watkins LR, Goehler LE, Relton JK *et al*. Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 1995; **183**: 27–31.
2. Niijima A. The afferent discharges from sensors for interleukin 1β in the hepatoportal system in the anesthetized rat. *J Auton Nerv Syst* 1996; **61**: 287–291.
3. Felten SY, Olschowka J. Noradrenergic sympathetic innervation of the spleen: II. Tyrosine hydroxylase (TH)-positive nerve terminals form synapticlike contacts on lymphocytes in the splenic white pulp. *J Neurosci Res* 1987; **18**: 37–48.
4. Borovikova LV, Ivanova S, Zhang M *et al*. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000; **405**: 458–462.
5. Huston JM, Ochani M, Rosas-Ballina M *et al*. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med* 2006; **203**: 1623–1628.
6. Wang HH, Yu M, Ochani M *et al*. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature* 2003; **421**: 384–388.
7. Rosas-Ballina M, Ochani M, Parrish WR *et al*. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc Natl Acad Sci USA* 2008; **105**: 11008–11013.
8. Rosas-Ballina M, Olofsson PS, Ochani M *et al*. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 2011; **334**: 98–101.
9. Olofsson PS, Katz DA, Rosas-Ballina M *et al*. α7 nicotinic acetylcholine receptor (α7nAChR) expression in bone marrow-derived non-T cells is required for the inflammatory reflex. *Mol Med* 2012; **18**: 539–543.
10. Tarnawski L, Reardon C, Caravaca AS *et al*. Adenylyl cyclase 6 mediates inhibition of TNF in the inflammatory reflex. *Front Immunol* 2018; **9**: 2648.
11. Inoue T, Abe C, Sung SJ *et al*. Vagus nerve stimulation mediates protection from kidney ischemia-reperfusion injury through α7nAChR$^+$ splenocytes. *J Clin Invest* 2016; **126**: 1939–1952.
12. Vida G, Peña G, Kanashiro A *et al*. β2-Adrenoreceptors of regulatory lymphocytes are essential for vagal neuromodulation of the innate immune system. *FASEB J* 2011; **25**: 4476–4485.
13. Ulloa L. The vagus nerve and the nicotinic anti-inflammatory pathway. *Nat Rev Drug Discov* 2005; **4**: 673–684.
14. Tracey KJ. Understanding immunity requires more than immunology. *Nat Immunol* 2010; **11**: 561–564.
15. Pavlov VA, Chavan SS, Tracey KJ. Molecular and functional neuroscience in immunity. *Annu Rev Immunol* 2018; **36**: 783–812.
16. Mina-Osorio P, Rosas-Ballina M, Valdes-Ferrer SI, Al-Abed Y, Tracey KJ, Diamond B. Neural signaling in the spleen controls B-cell responses to blood-borne antigen. *Mol Med* 2012; **18**: 618–627.
17. Riol-Blanco L, Ordovas-Montanes J, Perro M *et al*. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature* 2014; **510**: 157–161.
18. Hanes WM, Olofsson PS, Talbot S *et al*. Neuronal circuits modulate antigen flow through lymph nodes. *Bioelectron Med* 2016; **3**: 18–28.
19. Wong CHY, Jenne CN, Lee W-Y, Léger C, Kubes P. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. *Science* 2011; **334**: 101–105.
20. Huang S, Ziegler CGK, Austin J *et al*. Lymph nodes are innervated by a unique population of sensory neurons with immunomodulatory potential. *Cell* 2021; **184**: 441–459.e25.
21. Olofsson PS, Bouton C. Bioelectronic medicine: an unexpected path to new therapies. *J Intern Med* 2019; **286**: 237–239.
22. Datta-Chaudhuri T, Zanos T, Chang EH *et al*. The Fourth bioelectronic medicine summit 'technology targeting molecular mechanisms': current progress, challenges, and charting the future. *Bioelectron Med* 2021; **7**: 7.
23. Shi K, Wood K, Shi FD, Wang X, Liu Q. Stroke-induced immunosuppression and poststroke infection. *Stroke Vasc. Neurol* 2018; **3**: 34–41.
24. Sellars C, Bowie L, Bagg J *et al*. Risk factors for chest infection in acute stroke: a prospective cohort study. *Stroke* 2007; **38**: 2284–2291.
25. Tarkowski E, Naver H, Wallin BG, Blomstrand C, Tarkowski A. Lateralization of T-lymphocyte responses in patients with stroke: Effect of sympathetic dysfunction? *Stroke* 1995; **26**: 57–62.
26. Tarkowski E, Ekelund P, Tarkowski A. Enhancement of antigen-specific T-cell reactivity on the affected side in stroke patients. *J Neuroimmunol* 1991; **34**: 61–67.
27. Ader R, Cohen N. Behaviorally conditioned immunosuppression. *Psychosom Med* 1975; **37**: 333.
28. Pacheco-López G, Niemi M-B, Kou W, Härting M, Fandrey J, Schedlowski M. Neural substrates for behaviorally conditioned immunosuppression in the rat. *J Neurosci* 2005; **25**: 2330–2337.

29. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol* 2006; **6**: 318–328.
30. Sharma D, Farrar JD. Adrenergic regulation of immune cell function and inflammation. *Semin Immunopathol* 2020; **42**: 709–717.
31. Grisanti LA, Woster AP, Dahlman J, Sauter ER, Combs CK, Porter JE. α1-adrenergic receptors positively regulate toll-like receptor cytokine production from human monocytes and macrophages. *J Pharmacol Exp Ther* 2011; **338**: 648–657.
32. Malin SG, Shavva VS, Tarnawski L, Olofsson PS. Functions of acetylcholine-producing lymphocytes in immunobiology. *Curr Opin Neurobiol* 2020; **62**: 115–121.
33. Cox MA, Duncan GS, Lin GHY *et al*. Choline acetyltransferase–expressing T cells are required to control chronic viral infection. *Science* 2019; **363**: 639–644.
34. Tracey KJ. Reflex control of immunity. *Nat Rev Immunol* 2009; **9**: 418–428.
35. Reardon C, Duncan GS, Brüstle A *et al*. Lymphocyte-derived ACh regulates local innate but not adaptive immunity. *Proc Natl Acad Sci USA* 2013; **110**: 1410–1415.
36. Olofsson PS, Steinberg BE, Sobbi R *et al*. Blood pressure regulation by $CD4^+$ lymphocytes expressing choline acetyltransferase. *Nat Biotechnol* 2016; **34**: 1066–1071.
37. Parrish WR, Rosas-Ballina M, Gallowitsch-Puerta M *et al*. Modulation of TNF release by choline requires α7 subunit nicotinic acetylcholine receptor-mediated signaling. *Mol Med* 2008; **14**: 567–574.
38. Rosas-Ballina M, Goldstein RS, Gallowitsch-Puerta M *et al*. The selective α7 agonist GTS-21 attenuates cytokine production in human whole blood and human monocytes activated by ligands for TLR2, TLR3, TLR4, TLR9, and RAGE. *Mol Med* 2009; **15**: 195–202.
39. Olofsson PS, Metz CN, Pavlov VA. The neuroimmune communicatome in inflammation. In: *Inflammation - From Molecular and Cellular Mechanisms to the Clinic*. Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2017, pp 1485–1516.
40. Cox MA, Bassi C, Saunders ME *et al*. Beyond neurotransmission: acetylcholine in immunity and inflammation. *J Intern Med* 2020; **287**: 120–133.
41. Wessler I, Kirkpatrick CJ. Cholinergic signaling controls immune functions and promotes homeostasis. *Int Immunopharmacol* 2020; **83**: 106345.
42. Chu C, Artis D, Chiu IM. Neuro-immune interactions in the tissues. *Immunity* 2020; **52**: 464–474.
43. Ishida-Yamamoto A, Senba E, Tohyama M. Distribution and fine structure of calcitonin gene-related peptide-like immunoreactive nerve fibers in the rat skin. *Brain Res* 1989; **491**: 93–101.
44. Navarro X, Verdú E, Wendelschafer-Crabb G, Kennedy WR. Innervation of cutaneous structures in the mouse hind paw: a confocal microscopy immunohistochemical study. *J Neurosc Res* 1995; **41**: 111–120
45. Talbot S, Foster SL, Woolf CJ. Neuroimmunity: physiology and pathology. *Annu Rev Immunol* 2016; **34**: 421–447.
46. Cohen JA, Edwards TN, Liu AW *et al*. Cutaneous $TRPV1^+$ neurons trigger protective innate type 17 anticipatory immunity. *Cell* 2019; **178**: 919–932.e14.
47. Kashem SW, Riedl MS, Yao C, Honda CN, Vulchanova L, Kaplan DH. Nociceptive sensory fibers drive interleukin-23 production from $CD301b^+$ dermal dendritic cells and drive protective cutaneous immunity. *Immunity* 2015; **43**: 515–526.
48. Delgado M, Pozo D, Ganea D. The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacol Rev* 2004; **56**: 249–290.
49. Talbot S, Abdulnour R-EE, Burkett PR *et al*. Silencing nociceptor neurons reduces allergic airway inflammation. *Neuron* 2015; **87**: 341–354.
50. Ostrowski SM, Belkadi A, Loyd CM, Diaconu D, Ward NL. Cutaneous denervation of psoriasiform mouse skin improves acanthosis and inflammation in a sensory neuropeptide-dependent manner. *J Invest Dermatol* 2011; **131**: 1530–1538.
51. Kim J, Krueger JG. Highly effective new treatments for psoriasis target the IL-23/type 17 T cell autoimmune axis. *Annu Rev Med* 2017; **68**: 255–269.
52. González C, Franco M, Londoño A, Valenzuela F. Breaking paradigms in the treatment of psoriasis: use of botulinum toxin for the treatment of plaque psoriasis. *Dermatol Ther* 2020; **33**: e14319.
53. Nagashima H, Mahlakõiv T, Shih HY *et al*. Neuropeptide CGRP Limits Group 2 Innate Lymphoid Cell Responses and Constrains Type 2 Inflammation. *Immunity* 2019; **51**: 682–695.e6.
54. Wallrapp A, Burkett PR, Riesenfeld SJ *et al*. Calcitonin gene-related peptide negatively regulates alarmin-driven type 2 innate lymphoid cell responses. *Immunity* 2019; **51**: 709–723.e6.
55. Baral P, Umans BD, Li L *et al*. Nociceptor sensory neurons suppress neutrophil and γδ T cell responses in bacterial lung infections and lethal pneumonia. *Nat Med* 2018; **24**: 417–426.
56. Pinho-Ribeiro FA, Baddal B, Haarsma R *et al*. blocking neuronal signaling to immune cells treats streptococcal invasive infection. *Cell* 2018; **173**: 1083–1097.e22.
57. Gomes RN, Castro-Faria-Neto HC, Bozza PT *et al*. Calcitonin gene-related peptide inhibits local acute inflammation and protects mice against lethal endotoxemia. *Shock* 2006; **24**: 590–594.
58. Chiu IM, Heesters BA, Ghasemlou N *et al*. Bacteria activate sensory neurons that modulate pain and inflammation. *Nature* 2013; **501**: 52–57.
59. Chorny A, Gonzalez-Rey E, Fernandez-Martin A, Pozo D, Ganea D, Delgado M. Vasoactive intestinal peptide induces regulatory dendritic cells with therapeutic effects on autoimmune disorders. *Proc Natl Acad Sci USA* 2005; **102**: 13562–13567.
60. Reichmann F, Holzer P. Neuropeptide Y: a stressful review. *Neuropeptides* 2016; **55**: 99–109.
61. Tsaava T, Datta-Chaudhuri T, Addorisio ME *et al*. Specific vagus nerve stimulation parameters alter serum cytokine levels in the absence of inflammation. *Bioelectron Med* 2020; **6**: 8.
62. Kebschull JM, Garcia da Silva P, Reid AP, Peikon ID, Albeanu DF, Zador AM. High-throughput mapping of single-neuron projections by sequencing of barcoded RNA. *Neuron* 2016; **91**: 975–987.

63. Cambiaghi M, Sconocchia S. Scribonius largus (probably before 1CE–after 48CE). *J Neurol* 2018; **265**: 2466–2468.
64. Althaus J. A treatise on medical electricity, theoretical and practical; and its use in the treatment of paralysis, neuralgia, and other diseases. *Br Foreign Med Chir Rev* 1859; **24**: 157–158.
65. Wall PD, Swert WH. Temporary abolition of pain in man. *Science* 1967; **155**: 108–109.
66. Weiner RL, Reed KL. Peripheral neurostimulation for control of intractable occipital neuralgia. *Neuromodulation* 1999; **2**: 217–221.
67. Caravaca AS, Gallina AL, Tarnawski L *et al*. An effective method for acute vagus nerve stimulation in experimental inflammation. *Front Neurosci* 2019; **13**: 877.
68. Badia J, Boretius T, Andreu D, Azevedo-Coste C, Stieglitz T, Navarro X. Comparative analysis of transverse intrafascicular multichannel, longitudinal intrafascicular and multipolar cuff electrodes for the selective stimulation of nerve fascicles. *J Neural Eng* 2011; **8**: 036023.
69. Branner A, Stein RB, Normann RA. Selective stimulation of cat sciatic nerve using an array of varying-length microelectrodes. *J Neurophysiol* 2001; **85**: 1585–1594.
70. Caravaca AS, Tsaava T, Goldman L *et al*. A novel flexible cuff-like microelectrode for dual purpose, acute and chronic electrical interfacing with the mouse cervical vagus nerve. *J Neural Eng* 2017; **14**: 066005.
71. Loeb GE, Peck RA. Cuff electrodes for chronic stimulation and recording of peripheral nerve activity. *J Neurosci Methods* 1996; **64**: 95–103.
72. Falcone JD, Liu T, Goldman L *et al*. A novel microwire interface for small diameter peripheral nerves in a chronic, awake murine model. *J Neural Eng* 2020; **17**: 046003.
73. Pena AE, Kuntaegowdanahalli SS, Abbas JJ, Patrick J, Horch KW, Jung R. Mechanical fatigue resistance of an implantable branched lead system for a distributed set of longitudinal intrafascicular electrodes. *J Neural Eng* 2017; **14**: 066014.
74. Zachs DP, Offutt SJ, Graham RS *et al*. Noninvasive ultrasound stimulation of the spleen to treat inflammatory arthritis. *Nat Commun* 2019; **10**: 951.
75. Cotero V, Fan Y, Tsaava T *et al*. Noninvasive sub-organ ultrasound stimulation for targeted neuromodulation. *Nat Commun* 2019; **10**: 952.
76. Downs ME, Lee SA, Yang G, Kim S, Wang Q, Konofagou EE. Non-invasive peripheral nerve stimulation via focused ultrasound *in vivo*. *Phys Med Biol* 2018; **63**: 35011.
77. Piech DK, Johnson BC, Shen K *et al*. A wireless millimetre-scale implantable neural stimulator with ultrasonically powered bidirectional communication. *Nat Biomed Eng* 2020; **4**: 207–222.
78. Lehner KR, Silverman HA, Addorisio ME *et al*. Forebrain cholinergic signaling regulates innate immune responses and inflammation. *Front Immunol* 2019; **10**: 585.
79. Deisseroth K. Optogenetics: 10 years of microbial opsins in neuroscience. *Nat Neurosci* 2015; **18**: 1213–1225.
80. Christiansen MG, Senko AW, Anikeeva P. Magnetic strategies for nervous system control. *Annu Rev Neurosci* 2019; **42**: 271–293.
81. Roth BL. DREADDs for neuroscientists. *Neuron* 2016; **89**: 683–694.
82. Shields BC, Kahuno E, Kim C *et al*. Deconstructing behavioral neuropharmacology with cellular specificity. *Science* 2017; **356**: eaaj2161.
83. Rand D, Jakešová M, Lubin G *et al*. Direct electrical neurostimulation with organic pigment photocapacitors. *Adv Mater* 2018; **30**: 1707292.
84. Caravaca AS. Neural control of inflammation [Doctoral dissertation, Karolinska Institute, Stockholm]. 2021; ISBN 978-91-8016-184-8
85. Missey F, Rusina E, Acerbo E *et al*. Orientation of temporal interference for non-invasive deep brain stimulation in epilepsy. *Front Neurosci* 2021; **15**: 633988.
86. Levine YA, Koopman FA, Faltys M *et al*. Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. *PLoS One* 2014; **9**: e104530.
87. Wang H, Liao H, Ochani M *et al*. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med* 2004; **10**: 1216–1221.
88. Bonaz B, Sinniger V, Pellissier S. Vagus nerve stimulation: a new promising therapeutic tool in inflammatory bowel disease. *J Intern Med* 2017; **282**: 46–63.
89. Eberhardson M, Levine YA, Tarnawski L, Olofsson PS. The brain-gut axis, inflammatory bowel disease and bioelectronic medicine. *Int Immunol* 2021; **33**: 349–356.
90. Koopman FA, Chavan SS, Miljko S *et al*. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc Natl Acad Sci USA* 2016; **113**: 8284–8289.
91. Genovese MC, Gaylis NB, Sikes D *et al*. Safety and efficacy of neurostimulation with a miniaturised vagus nerve stimulation device in patients with multidrug-refractory rheumatoid arthritis: a two-stage multicentre, randomised pilot study. *Lancet Rheumatol* 2020; **2**: e527–e538.
92. Sinniger V, Pellissier S, Fauvelle F *et al*. A 12-month pilot study outcomes of vagus nerve stimulation in Crohn's disease. *Neurogastroenterol Motil* 2020; **32**: e13911.
93. Bonaz B, Sinniger V, Pellissier S. Therapeutic potential of vagus nerve stimulation for inflammatory bowel diseases. *Front Neurosci* 2021; **15**: 650971.
94. Steinberg BE, Silverman HA, Robbiati S *et al*. Cytokine-specific neurograms in the sensory vagus nerve. *Bioelectron Med* 2016; **3**: 7–17.
95. Silverman HA, Stiegler A, Tsaava T *et al*. Standardization of methods to record Vagus nerve activity in mice. *Bioelectron Med* 2018; **4**: 3.
96. Zanos TP, Silverman HA, Levy T *et al*. Identification of cytokine-specific sensory neural signals by decoding murine vagus nerve activity. *Proc Natl Acad Sci USA* 2018; **115**: e4843–e4852.
97. Bouton CE, Shaikhouni A, Annetta NV *et al*. Restoring cortical control of functional movement in a human with quadriplegia. *Nature* 2016; **533**: 247–250.
98. Kovatchev B. Automated closed-loop control of diabetes: the artificial pancreas. *Bioelectron Med* 2018; **4**: 14.

99. Grossman N, Bono D, Dedic N *et al*. Noninvasive deep brain stimulation via temporally interfering electric fields. *Cell* 2017; **169**: 1029–1041.e16.
100. Shen Y, Campbell RE, Côté DC, Paquet M-E. Challenges for therapeutic applications of opsin-based optogenetic tools in humans. *Front Neural Circuits* 2020; **14**: 41.
101. Williams JC, Entcheva E. Optogenetic versus Electrical Stimulation of Human Cardiomyocytes: Modeling Insights. *Biophys J* 2015; **108**: 1934–1945.
102. Smith KS, Bucci DJ, Luikart BW, Mahler SV. DREADDS: use and application in behavioral neuroscience. *Behav Neurosci* 2016; **130**: 137–155.