Neural regulation of immunity: molecular mechanisms and clinical translation

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Studies bridging neuroscience and immunology have identified neural pathways that regulate immunity and inflammation. Recent research using methodological advances in molecular genetics has improved our understanding of the neural control of immunity. Here we outline mechanistic insights, focusing on translational relevance and conceptual developments. We also summarize findings from recent clinical studies of bioelectronic neuromodulation in inflammatory and autoimmune diseases.

The nervous system and the brain maintain control of homeostasis through bidirectional communication with peripheral physiological systems. Pioneering research at the interface of the nervous system and the immune system identified neural circuits that are triggered by and control inflammation[1–8]. These findings indicated that immunity is under neural control, a new model that bridges neuroscience and immunology.

Reflex pathways mediate neural control of cardiovascular, respiratory, digestive and other functions to facilitate fast adaptation within homeostatic set points. Emerging evidence indicates that many neural circuits controlling immune responses are organized by principles of reflex regulation[2,6,8–11]. Technological advances in molecular genetics and other novel methodologies have allowed selective 'interrogation' of discrete neuronal circuits and led to the discovery of new immunoregulatory pathways[3,4,11–13]. New insights into the anatomical and functional diversity of these neural pathways and the cellular and molecular mechanisms have substantially broadened our understanding of neural regulation of immunity. In parallel, this knowledge has also facilitated conceptual advances for translational development. Cholinergic and catecholaminergic signaling or their functional cooperation importantly mediates neuroimmune regulatory mechanisms in inflammatory and autoimmune conditions, including endotoxemia, sepsis, inflammatory bowel disease and arthritis[8,14–18]. Catecholaminergic signaling has a major role in immune dysregulation in post-stroke conditions and spinal cord injury[4,7,19,20]. This preclinical insight has led to major breakthroughs in the bioelectronic exploration of neuromodulation in treating human inflammatory and autoimmune disease[21,22].

Immune function and its relationship with neurons

The main biological purpose of the immune system is to defend the body against infection and injury by mounting protective responses to pathogens and cell damage. The detection of immunogenic stimuli, generation of protective responses and their resolutions, and formation of immunological memory that facilitates defense upon encountering similar stimuli in the future are characteristic features of immunity and are classified as innate and adaptive[23,24]. Activation of macrophages, dendritic cells, neutrophils, natural killer T (NKT) cells and other immune cells with a role in innate immune function is pivotal in inflammation, a vital biological response to infection or sterile tissue injury aimed at neutralizing the invading pathogen, healing wounds and reestablishing tissue homeostasis[23,25,26]. Macrophages and dendritic cells, functioning as antigen-presenting cells, bridge innate and adaptive immune responses[24]. Upon antigen exposure, T and B lymphocytes undergo cell division and maturation and play key roles in antigen-specific adaptive immunity[27]. Pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) activate pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), and the nucleotide-binding-domain leucine-rich repeat-containing proteins (NLRs) that mediate responses to infection or sterile injury[25,28,29]. TLRs control dendritic cell maturation, subsequent release of costimulatory molecules and increased antigen-presenting capacity[27]. The initial PRR-mediated innate immune cell activation triggers intracellular signaling, leading to stimulation of NF-κB, AP-1 and other transcription factors, and enhanced transcription of targeted genes encoding cytokines, chemokines, defensins and other inflammatory modulators[25,28]. A subgroup of NLRs is linked to the inflammasome (a multimeric structure in the cytosol) with subsequent caspase-1 activation, mediating processing of interleukin (IL)-1β and IL-18 (ref. 29). The release of cytokines, chemokines and other inflammatory mediators further amplifies the inflammatory process by activating other immune cells, facilitating the recruitment of leukocytes and plasma proteins to the sites of injury and infection, and acting on the endothelium to facilitate vasodilation and increased vascular permeability[25,26]. The release of TNF, IL-1β and other proinflammatory mediators is accompanied by the release of anti-inflammatory molecules, including IL-10 and soluble cytokine receptors[2,28,30]. Inflammation is normally resolved in a timely manner by the coordinated action of proresolving mediators, including lipoxins, maresins and resolvins, which restrain leukocyte infiltration

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and increase the phagocytosis of dead cells by neutrophils and macrophages[30,31]. In some conditions, however, this beneficial scenario may not occur. Excessive and nonresolved inflammation can mediate tissue damage and lead to lethal and debilitating inflammatory diseases[2,30,31]. In addition, unbalanced T- and B-cell-mediated processes associated with defects in antigen recognition, effector activity and immunologic memory may result in aberrant immune responses, associated with autoimmunity or allergy[32].

Recent progress in our understanding of the physiological mechanisms controlling immunity and inflammation identified a role for the nervous system[2–4,8,33,34]. Neurons and immune cells communicate with each other. Neuroimmune communication arose early in evolution—in invertebrates, where both the nervous and the immune system have their phylogenetic origins. For instance, the simple nervous system of the soil nematode *Caenorhabditis elegans,* which contains 302 neurons, includes neurons specialized in controlling a noncanonical unfolded protein response pathway required for innate immunity[35]. Moreover, *C. elegans*' resistance against bacterial infections is regulated by neural dopamine signaling[36]. Ongoing research has revealed that PRRs, including TLRs (TLR4) and cytokine receptors, such as type 1 TNF receptor and type 1 IL-1 receptors, are expressed on neurons[37–40], and receptors for acetylcholine and other neurotransmitters are expressed on macrophages, dendritic cells, T cells and other immune cells[8,41]. Peripheral immune cells also synthesize and release acetylcholine, catecholamines and other molecules classically identified as neurotransmitters[8,41,42]. A common 'repertoire' of molecular signals mediates neuroimmune communication and neural control of immune function, governed by principles of reflex regulation.

Neural reflex regulation

The seminal work of Sir Charles Sherrington, Ivan Petrovich Pavlov and others charted the main principles of reflex regulation and the role of sensory, motor and CNS neurocircuitry in this regulation. Sherrington coined the term 'synapse' in the neuron theory related to reflex regulation[43]. In *The Integrative Action of the Nervous System*, he wrote, "the unit reaction in the nervous integration is the reflex," and emphasized the role of neural reflex circuits in maintaining homeostasis in the internal environment ("milieu interieur")[44]. Pavlov discovered conditioned reflex regulation and demonstrated the importance of conditioned reflexes in coordination of viscerosomatic and behavioral responses[45].

A prototypical reflex arc is comprised of sensory neurons transmitting information to a CNS integrative center (interneurons) and motor neurons to the peripheral site. There are two or more synapses in a reflex arc. Reflexes are initiated when receptors on the sensory neuron respond to a change in the environment. Reflexes can be classified as autonomic or visceral (involuntary) and somatic (voluntary). This classification can be broadened to account for the functional diversity and complexity of many reflexes involving a variety of visceral, somatic or both visceral and somatic components[46]. Another form of reflex is the axon reflex[47], in which receptor activation of peripheral axonal branches of sensory neurons triggers signals transmitted to the bodies (somata) of the neurons and diverted to other peripheral axonal endings with the generation of a response. Thus, peripheral endings of sensory neurons do not just sense changes in the microenvironment but also release substances that actively modulate this microenvironment[47]. Axon reflexes have been described in the regulation of vasomotor responses, including skin blood flow response to rapid local heating and vasodilation[47–49], as well as in pathophysiological conditions such as asthma[50].

Numerous autonomic reflexes have been identified in cardiovascular and metabolic regulation[46,51,52]. The vagus nerve plays a major role in reflex regulation of cardiovascular function (the baroreflex), gastrointestinal motility and secretion, feeding behavior, and glucose homeostasis[46,51,53,54]. The vagus nerve, which is 80% sensory, is classically defined as the main parasympathetic nerve. Afferent (sensory) vagus neurons, with neuronal bodies in the nodose ganglion and the adjacent jugular ganglion, have peripheral axonal endings in thoracic and abdominal organs and glutamatergic axonal terminals in the brainstem nucleus tractus solitarius (NTS). Efferent (motor) vagus preganglionic neurons originate in the brainstem dorsal motor nucleus of the vagus (DMN) and nucleus ambiguus, and innervate visceral organs. Efferent vagus pre- and postganglionic neurons predominantly release acetylcholine. Vagus nerve reflex regulation of visceral function is integrated in the brain via reciprocal neuronal projections between NTS, DMN and area postrema, which together are termed the dorsal vagal complex (DVC). The DVC is linked with other brainstem regions and higher forebrain areas in a brain network that provides multiple points of regulation[51,52,55,56].

Evolving concepts in neural reflex regulation of immunity

Almost 90 years ago Pavlov wrote, "reflexes are the elemental units in the mechanism of perpetual equilibration."[57] Recent insights in neuroscience and immunology have characterized new aspects of this notion of 'perpetual equilibration', in which neural reflex circuits regulate immune homeostasis.

The inflammatory reflex. Sensory neurons in proximity to immune cells are capable of monitoring the immune state in the periphery, responding to its alterations and communicating immune signals to the CNS[58–60]. Afferent (sensory) vagus nerve activity is stimulated by cytokines and other inflammatory products, which interact with cytokine receptors or PRRs (TLR4) expressed on these neurons, or through chemosensory cells in the associated vagal paraganglia[37,38,59,61]. This neuronal activation is communicated to the brainstem NTS[59]. In addition to increased afferent vagus nerve activity, administration of IL-1β into the rat hepatic portal system results in increased efferent vagus nerve and splenic nerve activity[58], suggesting reflexive integration. Studies of electrically stimulating the vagus nerve reveal that signals transmitted through efferent vagus nerve fibers inhibit the release of TNF and other proinflammatory cytokines[1]. This neuronal circuit is termed the 'cholinergic anti-inflammatory pathway'[1,55,62]. Activation of this pathway suppresses excessive inflammation in the liver[1,63], heart[64], pancreas[65] and gastrointestinal tract[66–68]. In a reflex manner, afferent vagus nerve activity is integrated with efferent vagus nerve activity in the brain[56,58], and the inflammatory reflex controls peripheral cytokine levels and inflammation[2,34] (**Fig. 1**). The motor (efferent) arm of the inflammatory reflex relays functional signals from the efferent vagus nerve to the splenic nerve[69], which modulates the release of acetylcholine by a subset of splenic T cells[8,70] (**Fig. 1**). Splenic nerve catecholaminergic endings in the spleen are in proximity to lymphocytes, including T cells, which express choline acetyltransferase (ChAT), the enzyme responsible for acetylcholine biosynthesis[8]. Vagus nerve stimulation increases the release of acetylcholine by these ChAT-expressing T cells through catecholaminergic, β_2-adrenergic receptor signaling[8]. The anti-inflammatory effects of vagus nerve stimulation are abolished in nude mice (which are deviod of T cells), and adoptive transfer of ChAT-expressing T cells into nude mice restores the anti-inflammatory effect, confirming the role of these acetylcholine-producing cells in the circuit[8]. Acetylcholine, released from ChAT-expressing T cells under vagus nerve control,

interacts with a specific receptor on macrophages, the α7 nicotinic acetylcholine receptor (α7nAChR), identified as an essential mediator of cholinergic anti-inflammatory output[71]. Intracellular mechanisms downstream of α7nAChR include inhibition of the nuclear translocation of NF-κB, activation of the JAK2–STAT3 pathway and a role for the inflammasome in suppression of proinflammatory cytokine production[53,63,66,72]. The inflammatory reflex is an example of a non-canonical functional cooperation between the efferent vagus nerve (classically designated as parasympathetic), the splenic nerve (termed sympathetic) and T cells relaying neural signals. In describing this interaction, the use of the classical sympathetic-versus-parasympathetic neuronal designation is imprecise. There is a need for a functionally precise neuronal circuit terminology (**Box 1**).

Vagus nerve–splenic nerve cooperation in controlling B cell and T cell function. In addition to controlling innate immune responses, vagus and splenic nerve signaling is implicated in the regulation of antibody secretion following B cell exposure to blood-borne antigen. Vagus nerve stimulation in a model of *Streptococcus* pneumonia results in retention of B cells in the splenic marginal zone and decreased B cell antibody production. This is due to reduced migration of these cells to the red pulp venous sinuses, where they become antibody-secreting cells. In addition, surgical transection of the splenic nerve abrogates the migration arrest and causes reorganization of lymphoid architecture[5]. These observations suggest that cooperation between vagus nerve function and splenic catecholaminergic signaling regulates the development of humoral immunity by modulating cell trafficking and lymphoid architecture[5]. Vagus nerve–splenic nerve signaling through the celiac ganglia is implicated in T cell activation and egress from the spleen and in regulating experimental hypertension (in response to angiotensin II) in a α7nAChR-dependent manner[73].

Vagus nerve and inflammation resolution. The resolution of inflammation is a critical step in inflammatory responses[31,74]. Netrin-1, an axonal guidance molecule, was identified as an innate immune system proresolving mediator in both lung and abdominal tissues in a murine model of zymosan-induced peritonitis[74]. A dynamic, synergistic relationship between netrin-1 and proresolving lipid mediators, including protectin D1 and RvD5, promotes resolution processes[74]. Vagus nerve signaling controls the expression of netrin-1, indicated by lower netrin-1 levels in mice after vagotomy, in parallel with lower acetylcholine levels and higher TNF, IL-1β, IL-6 and other cytokine and chemokine levels and increased leukocyte presence in inflammatory exudates[74]. These findings suggest that vagus nerve signaling modulates active resolution of inflammation.

Vagus nerve reflex circuits in kidney injury and sepsis. Vagus nerve stimulation 24 h before renal ischemia–reperfusion injury in mice significantly alleviates the severity of the condition and reduces systemic TNF levels through α7nAChR-mediated signaling in spleen[6] (**Fig. 1**). Isolated efferent vagus nerve signals to spleen are sufficient, and stimulation of afferent vagus fibers is also protective in this model[6]. This result is in line with another study of afferent vagus nerve stimulation in murine endotoxemia[75]. The NTS, where the majority of afferent vagus nerve fibers terminate, is interconnected with the rostral ventrolateral medulla (RVLM) and locus coeruleus (LC), brain regions that control spinal cord-derived catecholaminergic output with immunomodulatory and anti-inflammatory activity[55,56]. Neural reciprocal connectivity also links NTS with hypothalamic nuclei, including the paraventricular nucleus (PVN), a constituent of the hypothalamic–pituitary–adrenal (HPA) axis, with the release of anti-inflammatory glucocorticoids[55,56]. Therefore, there may be a role for other brain-derived pathways activated by afferent vagus nerve signaling.

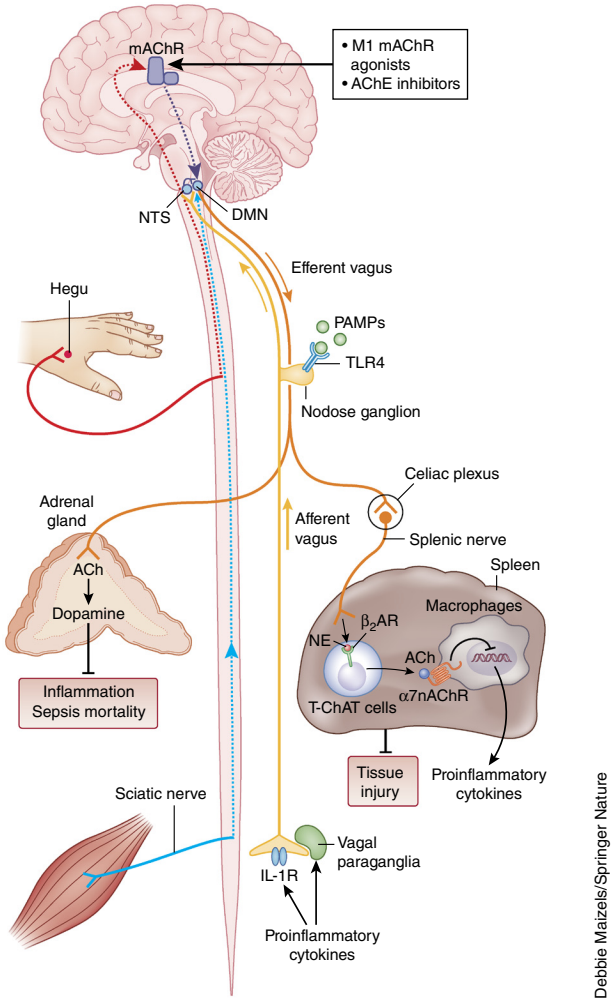

Figure 1 Vagus nerve-mediated reflex circuitry in immunity and inflammation. In the inflammatory reflex, the activity of afferent vagus nerve fibers residing in the nodose ganglion is stimulated by cytokines and PAMPs. The signal is transmitted to the NTS. Reciprocal connections between the NTS and DMN mediate communication with and activation of efferent vagus nerve fibers from the DMN. The signal is propagated to the celiac ganglia and the superior mesenteric ganglion in the celiac plexus, where the splenic nerve originates. Norepinephrine (NE) released from the splenic nerve interacts with β_2-adrenergic receptors (β_2-ARs) and causes the release of acetylcholine (ACh) from T cells containing functional choline acetyltransferase (T-ChAT cells). ACh interacts with α7nAChRs on macrophages and suppresses proinflammatory cytokine release and inflammation. The inflammatory reflex can be activated through brain mAChR-mediated mechanisms by centrally-acting M1 mAChR agonists and acetylcholinesterase (AChE) inhibitors. Somatosensory activation by electroacupuncture at the Hegu point also causes activation of brain mAChR signaling, which then results in activation of efferent vagus and splenic anti-inflammatory signaling. Electroacupuncture at a different acupuncture point activates sciatic nerve signals, which by unknown mechanisms convert to efferent vagus nerve signaling to the adrenal medulla, resulting in dopamine release. Dopamine suppresses inflammation and improves survival in a model of sepsis. Vagus nerve and splenic nerve signaling mediated through α7nAChR on splenocytes controls inflammation in acute kidney injury and alleviates the condition.

Box 1 Nomenclature of reflex circuits and research challenges

Neural circuits organized in a reflex manner regulate immune responses and control inflammation in the context of infection and tissue injury or autoimmune responses. Insight into the inflammatory reflex and other neural immunoregulatory mechanisms indicates that the use of the sympathetic-versus-parasympathetic model of neuron separation to describe these circuits is limiting or imprecise. Some circuits involve complex interaction between somatosensory neurons, sensory autonomic neurons and efferent neurons releasing acetylcholine, catecholamines and other neurotransmitters. We should eliminate convenient but imprecise neuronal designations to accurately describe the diversity of neurons in these complex circuits. It is better to use neuronal terminology based on neurotransmitter release and consistent with advances in molecular and functional neuronal characterization[136–138]. As with all rapidly emerging fields, across disciplines, there are many challenging questions that need to be addressed in the neural regulation of immunity. Further insight into the molecular mechanisms of neuroimmune interactions at peripheral sites is necessary. It is also important to elucidate the mechanisms by which sensory components of reflex circuits are integrated with motor components and to delineate CNS networks and integrative centers. We need to look further into the role of the forebrain and the limbic system in controlling peripheral immune functions[104,105] and vagus[139] and other peripheral nerve activity from the perspective of modern molecular mapping.

Activation of an anti-inflammatory reflex involving the vagus nerve was described in endotoxemic rats using electroacupuncture at a point located at the junction of the first and the second metacarpal bones[76]. In this reflex (**Fig. 1**), somatosensory stimulation via unknown mechanisms reaches the brain and triggers activation of muscarinic acetylcholine receptor (mAChR)-mediated signaling. This activation is linked with efferent vagus nerve activity and catecholaminergic signaling to the spleen, which results in suppression of serum TNF, Il-1β and IL-6 and in improved survival in lethal endotoxemia[76] (**Fig. 1**). Electroacupuncture at another acupuncture point causes stimulation of sciatic nerve activity, which is linked to efferent vagus nerve signaling to the adrenal medulla with subsequently increased dopamine production[10]. Increased release of dopamine in this reflex circuitry ultimately leads to suppression of the systemic inflammatory response through D1-receptor-mediated mechanism and results in improved survival of mice with cecal ligation and puncture-induced polymicrobial sepsis[10] (**Fig. 1**).

Axon reflex-like neuroimmune control. *Staphylococcus aureus* is a major cause of wound and surgical infections. In an axon reflex-like fashion, sensory neurons specialized in pain perception (nociceptors) modulate the local immune response to *S. aureus*[3] (**Fig. 2**). *S. aureus* activates sensory neurons in the mouse hindpaw in a direct manner, and genetic ablation of TLR2 and MyD88 does not alter this effect. In this case, the pathogen activates nociceptors by releasing N-formyl peptides and the pore-forming toxin α-hemolysin, inducing calcium flux and action potentials (**Fig. 2**). In turn, these sensory neurons release immunomodulatory peptides, including calcitonin gene-related peptide, galanin and somatostatin at the site of infection (**Fig. 2**). These neuropeptides inhibit innate immune activation via interactions with their receptors expressed on neutrophils, monocytes and macrophages[3] (**Fig. 2**). Pain in *S. aureus* infection is abrogated in mice with Nav1.8-lineage neurons, which include nociceptors, ablated using a Cre-loxP-based approach (**Box 2**). This genetic ablation also results in increased local inflammation, tissue TNF levels and lymphadenopathy during infection, indicating a tonic anti-inflammatory role of nociceptors[3]. This role for nociceptors is newly identified; nociceptor activation has been previously associated with neurogenic inflammation, including vasodilation and capillary permeability[77]. Nociceptor inhibition of inflammation occurring at later stages, atfer the acute vascular phase of inflammation[3], may provide adaptive advantages by limiting inflammatory damage. Further insight into the role of nociceptors and other sensory neurons in the modulation of pathogen-induced inflammation may identify new therapeutic targets.

Recent advances in catecholaminergic regulation of immunity

Neural catecholaminergic circuitries regulate immune function[78]. In addition to their role in the inflammatory reflex[8,16,79] and brain reward circuitry regulation of immune function[12] (discussed in the next section), catecholaminergic mechanisms have been recently implicated in other neuro-immunomodulatory circuits.

Lymphocyte trafficking. Neural catecholaminergic control was implicated in regulating the access of autoreactive T cells to the CNS in mice with autoimmune encephalomyelitis, an animal model of multiple sclerosis[9] (**Fig. 3**). Pathogenic $CD4^+$ T cells enter the CNS via the dorsal blood vessels of the fifth lumbar cord of the spine. This process is under the complex control of sensory dorsal root ganglia neurons (with nerve endings in the soleus muscles) communicating with catecholaminergic neurons[9]. This neural circuitry, a 'gateway reflex'[80], is activated by soleus muscle contractions (**Fig. 3**). Sensory neuronal activity then stimulates catecholaminergic output, resulting in IL-6 amplifier-dependent upregulation of the chemokine CCL20 in the L5 dorsal blood vessels and in facilitation of the pathogenic process[9] (**Fig. 3**). Impairment of this neuronal circuit by suspending mice by their tails to limit gravity stimulation of the soleus muscle reduces localized chemokine expression and suppresses the entry of pathogenic T cells[9]. Further characterization of this reflex control of blood brain barrier permeability to pathogenic T cells suggests new possibilities for therapeutic intervention[80].

Catecholaminergic signaling plays a role in retaining lymphocytes within lymph nodes and directing their distribution among lymphoid tissues[81]. This is mediated via lymphocyte β_2-adrenergic receptor signaling, which is associated with chemokine receptors CXCR4 in T cells and CCR7 in B cells[81]. This neural circuit, which reduces lymphocyte recruitment into peripheral tissues, may provide an important physiological mechanism in preventing T cell-mediated tissue damage[81].

Immunosuppression following stroke and spinal cord injury. CNS injuries, including stroke and spinal cord injury, can result in immunosuppression[82,83]. While post-stroke immunosuppression may serve to protect the brain from inflammatory damage, it mediates increased susceptibility to infection and increased mortality[83]. Therefore, it is important to understand the mechanisms of post-stroke immunosuppression. Experimental evidence points to unbalanced neural catecholaminergic control of immune function as an underlying event in post-stroke sequelae. In a mouse stroke model, the impaired antibacterial immune response was primarily related to a catecholamine-mediated defect in early lymphocyte activation[20]. A causative role of catecholaminergic overactivation in post-stroke mortality, mediated via β-adrenoreceptor mechanisms, was subsequently

supported by observations that pharmacological blockade of these receptors by propranolol significantly reduced the mortality rate[20]. A regulatory role for catecholaminergic innervations of the liver was recently demonstrated in a mouse stroke model. Catecholaminergic signaling acting on hepatic-invariant NKT cells causes a shift from proinflammatory T helper type 1 (T_H1)-type cytokines to anti-inflammatory T_H2-type cytokines, promotes systemic immunosuppression through IL-10 and results in increased bacterial infection[7,84] (**Fig. 4**).

Catecholaminergic signaling also plays a major role in immune regulation following spinal cord injury, which is associated with increased susceptibility to infection[4,19,85]. In this context, activation in catecholaminergic signaling is triggered as part of the exaggerated and potentially life-threatening autonomic reflexes elicited when the brainstem control over spinal autonomic circuitry is lost (in case of high-level spinal cord injury above the thoracic level 5)[4,19,86]. High-level spinal cord injury at thoracic level 3 reportedly results in increased catecholaminergic output to the spleen, which is associated with impaired antibody production, lymphocyte apoptosis and concomitant immune suppression[85,87]. A recent study using pseudorabies virus expressing GFP to perform retrograde labeling from spleen to spinal cord, Cre-*loxP* genetic labeling of specific neuronal populations and designer receptors exclusively activated by designer drugs (DREADDs) chemogenetics (**Box 2**) provided important insight into this regulation[4]. Spinal cord injury at thoracic level 3 results in significant neuronal plasticity within spinal autonomic networks below the levels of the injury and activation of an anti-inflammatory reflex circuitry[4]. This anti-inflammatory reflex comprises primary sensory afferents, interneurons and preganglionic neurons in the spinal cord, with resultant increased catecholaminergic output to the spleen[4]. Inhibition of this reflex by DREADD chemogenetic silencing of glutamatergic excitatory neurons within an aberrant intraspinal circuitry alleviates post-spinal cord injury immune suppression[4]. This approach prevents the characteristic splenic atrophy and restores the total number of splenocytes, including helper $CD4^+$ and cytotoxic $CD8^+$ T cells and $B220^+$ B cells[4]. Excessive spinally generated sympathetic catecholaminergic activation originating from below the lesion in high-level thoracic spinal cord injury was shown to play a causative role in immunosuppression and increased bacterial load in lungs of mice with inducible murine pneumonia[19]. Spinal cord injury-level-dependent immune suppression and increased infectious complications (indicated by occurrence of pneumonia) have also been observed in humans[19]. Further mechanistic insight into the role of catecholaminergic neurocircuitries in post-stroke and post-spinal cord injury may guide new therapeutic strategies[4,7,82].

Tissue protection. Enhancing tissue-protective functions in resident macrophages is an important component of immunity[88]. Advanced transcriptional profiling and imaging systems (**Box 2**) have been used to characterize neural circuitry that regulates gut immunity and potentiates tissue-protective macrophage programming[13] (**Fig. 4**). Lamina propria-zone macrophages possess a characteristic proinflammatory phenotype that allows them to respond rapidly to damage to the epithelial barrier[13]. In contrast, muscularis macrophages, localized deeper in the gut wall, have an anti-inflammatory, tissue-protective phenotype[13]. Tissue-protective macrophage programming is further activated in the presence of pathogenic *Salmonella* in the bowel. Importantly, the regulatory mechanism underlying this enhancement is provided by activation of extrinsic catecholaminergic neuronal output, mediated by β_2-adrenergic receptors on muscularis macrophages. These findings reveal a neural mechanism that potentiates the tissue-protective macrophage function in the face of inflammatory damage caused by environmental perturbations in the intestinal muscularis. They also provide further insight into the

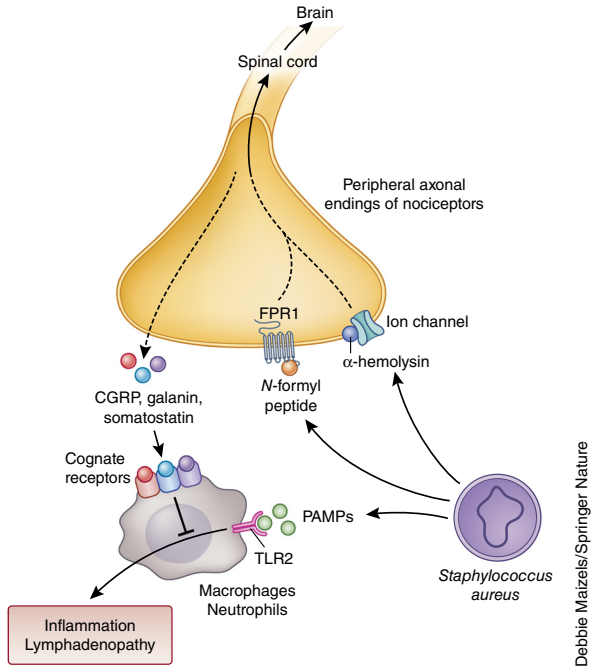

Figure 2 Axon reflex-like regulation of inflammation in bacterial infection. *S. aureus* activates nociceptors transmitting signals in an orthodromic manner to the CNS (to the spinal cord and then the signals are transmitted to the brain). The pathogen activates nociceptors by secreting *N*-formyl peptide and α-hemolysin. *N*-formyl peptide interacts with formyl peptide receptor 1 (FPR1) on the neuronal membrane, mediating hyperalgesia. α-hemolysin binds to the neuronal membrane and oligomerizes to form pores, allowing ionic entry and depolarization. This nociceptor activation by *S. aureus* occurs in parallel with the immune response and inflammation involving monocytes, macrophages and neutrophils, which sense PAMPs via a TLR2-dependent mechanism. Nociceptors release neuropeptides, including calcitonin gene-related peptide (CGRP), galanin and somatostatin, and this release is likely triggered by neural impulses propagating in an antidromic manner. These neuropeptides suppress inflammation and alleviate lymphadenopathy. Interaction between neuropeptides and their receptors on macrophages, neutrophils and T cells provides a mediating mechanism.

reciprocal and dynamic neuroimmune interactions with relevance to disease tolerance in response to pathogens[13,88]. Afferent vagus nerve fibers sense immune and metabolic alterations in the viscera, including the liver and the gut[53,54]. In addition, afferent vagus neurons express functional β_2-adrenergic receptors[89,90]. The gut and the liver are interconnected with the brain[54]. It is intriguing to hypothesize a role for afferent vagus nerve projections in reflex integration of catecholaminergic signaling to the liver and the gut (**Fig. 4**).

Pavlovian conditioning and reward system regulation of immunity

Pavlov's discoveries highlighted the importance of conditioned reflexes in the autonomic regulation of physiological functions[45,57]. The study of Pavlovian conditioning has advanced[91], and one important new focus is the reflex conditioning of immunity. Regulation of immune function in the Pavlovian conditioning model represents another line of evidence for CNS–immune system interaction and regulation[92]. This 'learned immune response' may be a key mechanism preparing the organism for immunogenic threats and in adaptive strategies[93]. Immune conditioning was demonstrated by pairing antigen (egg albumin) administration and activating mucosal mast cell secretion (unconditioned stimulus) with an audiovisual cue (conditioned

Box 2 Techniques and tools

Studies on neural regulation of immune function have combined approaches from neuroscience and immunology. Nerve transections (such as selective vagotomies), electrical nerve stimulation, pharmacological modalities (including receptor agonist and antagonists) and knockout and transgenic mice in models of inflammatory and autoimmune conditions are basic components of this methodology. Relatively new approaches based on advances in molecular genetics, including the Cre-*loxP* system[3,11,13], DREADDs[4,12] and optogenetics[108], have also been used. The Cre-*loxP* conditional system of DNA recombination is a powerful tool for tissue- and cell-specific gene inactivation, *de novo* induction of select gene-encoding sequences and other types of gene manipulation[140,141]. DREADDs are chemogenetic tools that have been used to modulate neuronal activity in a cell- and projection-specific manner in freely moving animals based on activation of chemogenetically engineered G-protein-coupled receptors by chemical actuators such as clozapine-N-oxide (CNO)[142]. Optogenetics provide an elegant approach for defining specific neuronal function and evaluating neuronal circuitry[143]. In optogenetics, light-sensitive proteins (opsins) expressed on specific neurons by injecting a Cre-dependent viral vector or in transgenic mice can be targeted by light exposure to selectively activate or silence neuronal activity[143,144]. In addition to studying the brain, optogenetics can be used in examining spinal cord and peripheral neural circuitry[145]. Combining Cre-recombinant and optogenetic methods provides a selective, genetically guided approach for visualization and functional characterization of the molecular diversity of afferent vagus neurons[137,138]. Selective methodologies based on single-cell transcriptomics allow precise characterization of the complex brain cellular architecture and circuitry, as well as peripheral somatosensory and autonomic neurons[136,146]. Other new approaches and technology remain to be implemented, including CRISPR/Cas9 tools for genome editing, CLARITY and imaging systems for studying brain circuitry[147], miniaturized electrodes and devices suitable for recording and modulation of discrete neuronal circuits in freely moving mice, and computerized systems for processing and decoding a large amount of data. These new technologies will facilitate research on anatomical and functional characterization of reflex circuitry in immunity.

stimulus). Re-exposing animals to only the cue generates a response (mast cell release of proteases), with a similar magnitude compared to that elicited by exposure to both the cue and the antigen[92]. Immune conditioning with a mediating role for catecholaminergic signaling and the HPA axis has been described in animal models[93]. Immune conditioning in humans has also been reported[94,95]. For instance, after repeated pairing of a flavored drink (conditioned stimulus) and cyclosporin A (unconditioned stimulus), the drink alone during evocation mimics the effects of cyclosporin A and results in suppression of immune function, including impaired T_H1 cytokine production and reduced T cell proliferation[94]. Interestingly, a role for catecholaminergic signaling and the HPA axis in immune conditioning indicated in rodent models[93] has not been shown in humans[94]. It has been suggested that the inflammatory reflex may be involved in neuroimmune communication in Pavlovian conditioning of immunity[93].

Behavioral conditioning has also been implicated in the placebo effect[96,97]. Another important component is an expectation of a certain positive outcome, with suggested activation of the brain reward system[96,98]. Recently, direct stimulation of dopamine receptors using DREADDs (**Box 2**) in a brain reward region (the ventral tegmental area) was shown to enhance peripheral immune responses to bacterial (*E. coli*) exposure[12]. This is indicated by increased phagocytic activity of dendritic cells and macrophages, activation of monocyte and macrophage bactericidal properties, and reduced bacterial load in the liver. Brain stimulation also results in increased T-cell responses in a model of delayed-type hypersensitivity[12]. Chemical ablation of peripheral catecholaminergic neurons abolishes these alterations, implicating catecholaminergic signaling in mediating the effects of reward circuit activation on peripheral immune antibacterial defense[12]. Although the use of DREADDs for brain reward circuit stimulation may differ from reward stimuli generated by expectations, these findings may suggest a pathway by which placebo modulates peripheral immune function[12]. Antimicrobial immune activation as a result of reward circuit stimulation may be beneficial in feeding, associated with the possibility of pathogen exposure[12]. The vagus nerve has important roles in feeding behavior: it transmits signals for satiety and for metabolic and inflammatory status to the brain[99]; these signals reach the NTS and then spread to other brain areas, including reward centers[53,100]. Activation of immune function, which is important for protection against pathogens, needs to be balanced to avoid deleterious effects on commensal flora and dietary components in the intestine[101]. In this broader context it is intriguing to consider and to further study whether afferent vagus nerve signaling to the brain reward system plays a role in brain-integrated fine-tuning and balancing antimicrobial defenses. Satiety ('stop eating') signals through afferent vagus nerve fibers reaching brain reward centers may serve to mitigate excessive activation of immune responses. Cholecystokinin and leptin play important roles in mediation of satiety by the afferent vagus nerve[99]. Cholecystokinin, acting both via vagus nerve afferents and directly in the brain, triggers activation of efferent vagus nerve signaling, which in turn suppresses the release of proinflammatory cytokines[101].

Brain integration and coordination of neuroimmune pathways

Sherrington postulated that "the simple reflex is…probably a purely abstract conception," pointing to the CNS reflex organization and that a reflex does not function in isolation[44]. Given the importance of neural regulation of immunity and inflammation, it is interesting to consider brain mechanisms that integrate this regulation. Afferent and efferent vagus nerve signals are linked through neural interactions between the NTS and the DMN in the DVC (refs. 55,56; **Fig. 1**). These interactions are of interest in the brainstem integration of the inflammatory reflex. Neuronal activation in the DVC is further propagated through ascending projections to the RVLM and then to LC, associated with brain control of catecholaminergic regulation[55,56] (**Fig. 4**). Considering the involvement of catecholaminergic signaling in several neuro-immunomodulatory pathways[7,9,13,81] (**Figs. 3** and **4**), this brain circuitry warrants further exploration to gain insight into their integration with the inflammatory reflex. Bidirectional neuronal projections between the DVC and the hypothalamic PVN provide a neuroanatomical substrate for communication between the inflammatory reflex and the HPA axis[54,55]. Direct projections or multisynaptic connectivity between the DVC, RVLM, LC, insular cortex, hypothalamus, hippocampus and other constituents of the limbic system form an extended brain network controlling peripheral autonomic function[51,102,103]. The limbic system and other forebrain regions have been associated with regulation of immune function[104,105]. Experimental evidence has been gathered using brain lesions and other methodologies that do not allow precise examinations. Using selective tools (**Box 2**) will facilitate mapping of brain networks integrating immunoregulatory pathways (**Box 1**).

Cholinergic neurons in the brain constitute a major neuromodulatory system[106]. Basal forebrain cholinergic neurons project to the hippocampus and other components of the limbic system. M1 and other mAChRs play a major role in mediating cholinergic signaling in these and other brain areas. Experimental evidence

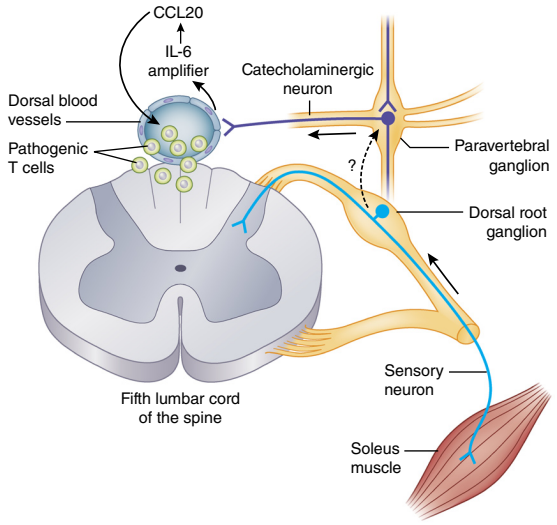

Figure 3 Neural modulation of T cell access to CNS. Sensory neurons activation by soleus muscle stimulation causes (by unknown mechanism) activation of catecholaminergic signaling to dorsal blood vessels of the fifth lumbar cord. This is functionally related to increased expression of CCL20 and other chemokines through a mechanism involving an IL-6 amplifier. The increased production of CCL20 leads to increased accumulation of pathogenic $CD4^+$ T cells in the fifth lumbar cord and facilitates their entry through the blood brain barrier. (Adapted from ref. 80, Oxford University Press.)

indicates that the inflammatory reflex is activated in the brain through cholinergic, M1 mAChR-mediated stimulation[14–16] (**Fig. 1**). This mAChR-mediated control of the inflammatory reflex in brain has been defined in murine models of inflammatory conditions, including endotoxemia, hemorrhagic shock and inflammatory bowel disease[14–16,107] (**Fig. 1**). Ongoing research using optogenetic stimulation and selective genetic ablation has indicated a role for forebrain cholinergic signaling in the regulation of the inflammatory reflex[108]. In addition to optogenetics, which provide a very selective approach for gaining mechanistic insight into the functional role of specific neuronal populations (**Box 2**), electrical stimulation has also been explored. Electrical deep brain stimulation, transcranial direct current stimulation and transcranial magnetic stimulation are in clinical or exploratory use for various conditions[109,110]. Further delineation of brain regions and networks integrating and coordinating immunoregulatory pathways (**Box 1**) may suggest new approaches to modulate immune responses and inflammation[62].

Pavlov pointed to the importance of brain—specifically cortex—integration of conditioned reflexes[57]. In Pavlovian conditioning of immune function, the complex brain mechanisms of integration and coordination remain poorly understood[93]. In a rat model of immune conditioning using saccharine (as a conditioned stimulus) and cyclosporin A (as an unconditioned stimulus), lesions of the insular cortex performed before and after acquisition disrupt the behavioral component of the conditioned response (taste aversion)[111]. In this model, the insular cortex and amygdala play a role in processing and consolidating visceral information required at acquisition time[93,111]. Subsequently, at evocation time, a neural circuit between the insular cortex, hypothalamus and brain regions associated with catecholaminergic regulation seems to play a role in modulating immunosupression[93,111]. Forebrain cholinergic signaling has a major involvement in cortical regulation, learning and memory[112,113]. It is interesting to consider a possible role for cholinergic signaling in regulating learning and memory with cortical involvement in Pavlovian conditioning of immune responses.

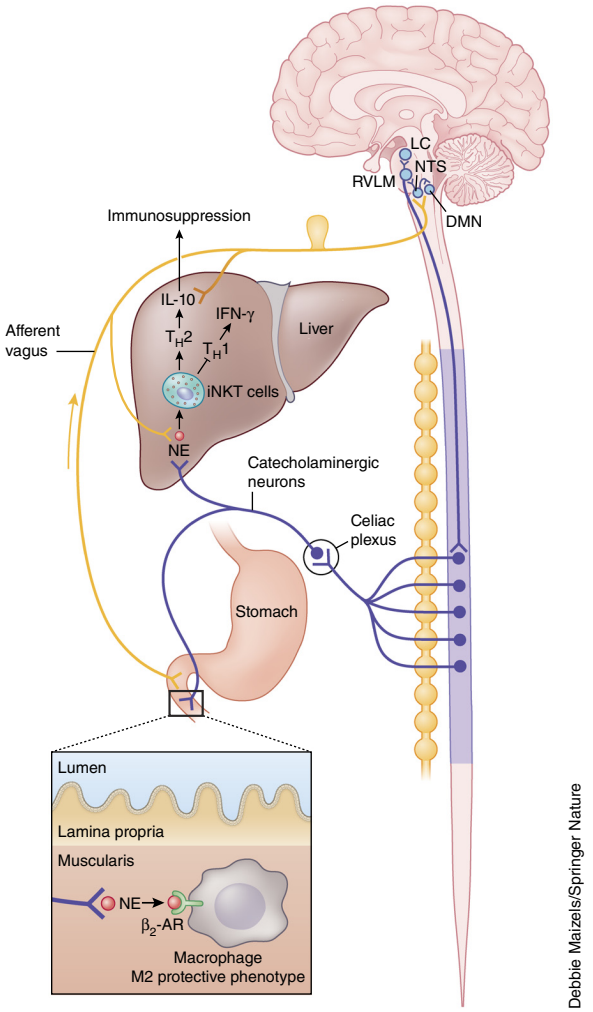

Figure 4 Catecholaminergic circuits in the neural regulation of immune responses. Catecholaminergic nerves reaching the liver and the gut control immune function following stroke and in response to pathogenic *Salmonella*, respectively. Following stroke, the release of norepinephrine (NE) from catecholaminergic nerve endings in the liver modulate the activity of invariant NKT (iNKT) cells. This modulation includes suppression of T_H1-type release of cytokines such as interferon (IFN)-γ and enhancement of T_H2-type cytokine release, including IL-10. This functional shift and IL-10 release promote immunosuppression and increased susceptibility to bacterial infection. During infection with pathogenic *Salmonella*, catecholaminergic signaling to the small intestine reinforces a tissue-protective M2 gene-expression programming in muscularis macrophages. This is mediated via the release of NE interacting with β_2-AR expressed on these macrophages. Afferent vagus neurons transmit signals for alterations in immune and metabolic homeostasis to the brainstem NTS. Reciprocal neural projections connect NTS with DMN, RVLM and LC. LC and RVLM, through descending projections to the spinal cord, are involved in brain regulation of catecholaminergic output. Therefore, afferent vagus neurons may provide a sensory circuit mediating a closed-loop, brain-integrated neural regulation. Efferent vagus nerve fibers from DMN (not shown) also innervated ganglia in the celiac plexus, the likely origin of these postganglionic catecholaminergic fibers. Therefore, a role for efferent vagus nerve fibers to the celiac ganglia in such closed-loop regulation also is interesting to consider.

Neural reflex regulation of obesity-associated inflammation

Early studies of the vagus nerve established a role in the reflex regulation of gastrointestinal motility and secretion, feeding behavior, and other physiological, metabolic and behavioral functions[46,52–54]. Importantly, dysregulation of vagus nerve reflex circuits controlling metabolic homeostasis due to high-fat diets and voluntary overeating has been suggested as a factor in the onset of weight gain and obesity, hepatic insulin resistance and type 2 diabetes[114,115]. A characteristic feature of obesity is chronic low-grade inflammation, driven by dysregulated release of cytokines and adipokines, including leptin, adiponectin and resistin from metabolically transformed adipocytes and macrophages, and from other immune cells infiltrating expanded abdominal adipose tissue[116,117]. This inflammation is implicated in insulin resistance and other metabolic and cardiovascular derangements and represents a promising therapeutic target[116,118].

Obesity-associated inflammation and related metabolic dysregulation are under neural reflex control[11,53]. The use of a Cre-*loxP* approach (**Box 2**) allowed insulin-2 promoter (Rip)-mediated conditional deletion of *Pten*, a gene encoding a negative regulator of phosphatidylinositol 3-kinase (PI3K) signaling in neurons[11]. Mice with this deletion exhibit higher PI3K activation in the hypothalamus, including the PVN and increased (c-Fos) neuronal activation in the NTS and DMN (ref. 11). They also have increased splenic and peritoneal macrophages exhibiting an M2-like phenotype, associated with lower TNF, higher IL-10 and adiponectin circulatory levels, and improved peripheral insulin sensitivity[11]. Pharmacological cholinergic blockade and genetic α7nAChR deletion demonstrate the dependency of increased insulin sensitivity in Rip-Cre^{+} *Pten*$^{loxP/loxP}$ mice on cholinergic signaling. In the context of high-fat-diet-induced obesity and type 2 diabetes, these mice also have high insulin sensitivity and predominant M2 macrophage infiltration in visceral adipose tissue. This protective phenotype is abolished after vagotomy, implicating the inflammatory reflex coupled with hypothalamic signaling in controlling inflammation in obesity[11,119]. Importantly, they also indicate a role for cholinergic signaling within the inflammatory reflex in promoting M2 macrophage differentiation[11]. Obesity and obesity-associated disorders, including metabolic syndrome, type 2 diabetes, nonalcoholic steatohepatitis and cardiovascular disease, represent an enormous health burden[53,118,120,121]. There is a growing interest in vagus nerve anti-inflammatory circuitry and the brain in the treatment of obesity and obesity-related disorders[53,122–124].

Translational developments

The efficacy of electrical stimulation of the inflammatory reflex, α7nAChR agonists, β_2-adrenergic agonists, D1 dopamine receptor agonists and centrally acting M1 mAChR agonists and acetylcholinesterase inhibitors in inflammatory and autoimmune conditions has been shown in preclinical settings[1,10,15,16,62,63,66,125–127]. Insights into the reflex regulation of immunity and inflammation also generated a paradigm-switching field of bioelectronic medicine[128]. This field explores selective modulation of discrete neuronal circuits to target molecular mechanisms in disease pathogenesis[128]. Preclinical research paved the way for ongoing clinical trials of neuromodulation in inflammatory and autoimmune diseases. Results from two clinical studies of electrical stimulation of the inflammatory reflex in Crohn's disease[21] and rheumatoid arthritis[22] were recently published.

Crohn's disease is a disabling chronic inflammatory disease of the bowel, affecting young people and greatly decreasing quality of life[21]. Treatment options for Crohn's are limited, expensive and accompanied by significant side effects[129]. Six months of vagus nerve stimulation by an implanted electronic device results in significant clinical remission, measured by lower Crohn's disease activity index and improved endoscopic findings in 5 of 7 patients with active disease[21]. These beneficial effects are associated with improved vagus nerve activity determined by heart rate variability analysis[21].

Rheumatoid arthritis is a debilitating chronic inflammatory and autoimmune disease, and many patients with this disease do not respond to available treatment options[129]. A recent study using an implanted electronic device to stimulate the inflammatory reflex (up to 4 times per day) in patients with rheumatoid arthritis demonstrated the efficacy of this approach in alleviating the disease for up to 84 d (ref. 22). Two cohorts of patients were studied: 7 early-disease stage patients who had not responded to methotrexate and 10 patients in later disease stages who failed biological therapies. Stimulation of the inflammatory reflex significantly improved disease scores in both cohorts[22]. Withdrawal of the neurostimulation significantly worsened disease severity and its reactivation restored benefit. The improvements in the clinical score were accompanied by significant anti-inflammatory effects indicated by decreases in TNF and other cytokines[22].

Metabolic syndrome is an obesity-driven condition that increases the risk of type 2 diabetes and other debilitating disorders[130]. The centrally acting acetylcholinesterase inhibitor galantamine, an FDA-approved drug for Alzheimer's disease, is also a pharmacological activator of the inflammatory reflex (**Fig. 1**). Galantamine is anti-inflammatory in animal models of obesity and metabolic syndrome[123]. The efficacy of galantamine in patients with metabolic syndrome is being studied.

Future directions

Neural regulation of immunity and inflammation is a rapidly evolving field, with significant opportunities for new discoveries. Future research will better characterize existing pathways and map new neural circuitry in the periphery and in the CNS. It is plausible that the future will bring new modes of neuromodulation therapy to target diseases currently treated with drugs. The clinical study of neuromodulation has advanced. For instance, a recent study successfully restored muscle activation using intracortically recorded signals in a paralyzed human[131]. This study provides a revolutionary approach in advancing brain neuromodulation in the treatment of paralysis[131]. Millions of people worldwide live with the debilitating consequences of rheumatoid arthritis, inflammatory bowel disease, diabetes, post-stroke conditions, spinal cord injuries and other types of chronic illness characterized by immune derangements and inflammation. In addition to electrical stimulation of the inflammatory reflex, new approaches for treating dysregulated immune responses and aberrant inflammation have already migrated from the drawing boards to preclinical exploration. Recording brain neuronal electrical activity in the context of inflammatory and autoimmune conditions, decoding this information and then using it to modulate brain activity to 'correct' immune derangements is under exploration[62]. Recording and decoding the characteristic activity patterns of sensory neurons in the periphery in response to specific pathogens, cytokines or other immune molecules would allow researchers to define selective 'immune neurograms'. This information can be used to develop a new class of biomarkers with potential implications for infectious and inflammatory disease diagnosis and progression monitoring[132]. Deciphering these neural signatures using computerized learning and other strategies may enable new therapeutic approaches based on neural footprints of immunity[132]. Establishing brain and peripheral neural closed-loop systems that monitor and modulate immune homeostasis will be explored in disease diagnoses and treatment. Knowledge defining the depth and the selectivity of regulatory

control of the nervous system on immune function will maximize efforts to implement principles of personalized or precision medicine in the treatment of inflammatory and autoimmune illness[133,134].

Summary

Preclinical research using newly available tools and methodologies is broadening and improving our understanding of neural regulation of immunity and contributing to conceptual advances in the field. Here we have presented an overview of multiple aspects of immunity and inflammation regulation by neural circuitries, many of which operate on principles of reflex regulation. In 1929 Pavlov wrote, "fresh (new) reflexes are continually being discovered." Did he envision a time when the targets would be reflexes in immunity? Maybe not, but he would undoubtedly flash a reflexive smile[135]. Neural pathways regulating immunity can involve multiple and heterogeneous neuronal types. Some circuits modulating various immune responses share common circuitry, for example, the vagus nerve in pathways controlling innate and adaptive immune responses and inflammation resolution. This can be viewed as an advantage in coordinated immune regulation in response to immunogenic threats. Mechanistic insights from preclinical research on neurocircuitry in inflammatory and autoimmune conditions have been recently translated to clinical studies with promising results.

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The authors declare competing financial interests: details are available in the online version of the paper.

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