Review

Nerve Stimulation: Immunomodulation and Control of Inflammation

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Neuronal stimulation is an emerging field in modern medicine to control organ function and re-establish physiological homeostasis during illness. Transdermal nerve stimulation with electroacupuncture is currently endorsed by the World Health Organization (WHO) and the National Institutes of Health (NIH), and is used by millions of people to control pain and inflammation. Recent advances in electroacupuncture may permit activation of specific neuronal networks to prevent organ damage in inflammatory and infectious disorders. Experimental studies of nerve stimulation are also providing new information on the functional organization of the nervous system to control inflammation and its clinical implications in infectious and inflammatory disorders. These studies may allow the design of novel non-invasive techniques for nerve stimulation to help to control immune and organ functions.

Clinical Implications of Neuromodulation

Neuronal stimulation is an emerging field in modern medicine for the control of organ function and to re-establish physiological homeostasis during illness. Recent studies indicate that nerve stimulation provides therapeutic benefits in treating inflammation in arthritis, colitis, diabetes, obesity, hemorrhage/resuscitation, pancreatitis, quadriplegia, and infectious disorders such as **endotoxemia** (see Glossary), septic shock, and severe **sepsis** [1–8]. However, most of these studies have been performed with surgical isolation of a given nerve for direct electrical stimulation. Given that surgical anesthetics inhibit neuronal signals to decrease pain, they also interfere with **neuromodulation**; consequently, the need for surgery to achieve direct nerve stimulation precludes repeating the treatment in chronic disorders (e.g., arthritis or colitis) [9]. Thus, recent studies seek alternative non-invasive strategies for nerve stimulation, and have shown that non-surgical transdermal nerve stimulation with electroacupuncture can control inflammation and prevent organ damage in inflammatory and infectious disorders, as in the case of experimental sepsis [9,10]. Neuromodulation with **acupuncture** or **electroacupuncture** is used by millions of people to control pain and inflammation, and to re-establish physiological homeostasis during illness (Box 1). Clinical studies show that acupuncture can improve postoperative recovery, osteoarthritis, migraine, joint pain, stroke, post-traumatic stress disorder, and drug addiction [11]. The NIH estimates that acupuncture has been used by over 15 million Americans [12,13]. The use of electroacupuncture is now endorsed by the American Pain Society, the National Center for Complementary and Alternative Medicine, the NIH, and the WHO [14–16].

Acupuncture was developed by traditional Chinese practitioners to control pain [10,17,18]. The points of stimulation were selected by empirical assays based on the responses of the patients. All but one of 361 **acupoints** in humans are located close to **neuronal networks** [17–19]. Because neuronal networks were evolutionarily selected to achieve physiological homeostasis, it is not surprising that neuromodulation has emerged as one of the first strategies used in

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Box 1. Neuromodulation

Inflammation is crucial for survival, but unregulated inflammation is lethal. One dramatic example is that sepsis accounts for 9% of overall deaths in the USA annually. Sepsis is characterized by detrimental systemic inflammation that causes multiple organ failure. Neuromodulation encompasses efficient systems selected through evolution to control inflammation. Cytokines and neurotransmitters are produced by neurons to modulate immune cells, and vice versa. This bidirectional communication allows the nervous system to sense inflammation, and to activate specific neuronal networks, to control immune cells and avoid the detrimental effects of excessive inflammation. Given that these mechanisms are not specific for single cytokines, they can control inflammation in diverse inflammatory and infectious disorders including complex disorders such as sepsis [9].

medicine to re-establish homeostasis during illness [17]. However, the efficacy of acupuncture or electroacupuncture is very controversial because it relies on the experience and precision of the practitioner with needle insertion, and because of the lack of proper mechanistic studies. Moreover, nerves can be stimulated with different techniques including pressure (acupressure), massage **(Tui na)**, heat (**moxibustion** and fire **cupping**), sound (sonopuncture), or electricity (electroacupuncture) [17,18]. Each technique might activate distinct mechanisms of action with different intensities [17,20] The most typical examples of this medical hypothesis include the differences between acupuncture and electroacupuncture. While acupuncture induces mechanical stimulation in neuromuscular junctions and causes local release of neuromodulators, electroacupuncture represents a transdermal electrical stimulation of the nerves with voltage-dependent effects [17,20]. Many clinical reviews conflate these techniques and create confusion regarding their efficacy, mechanisms, and statistical meta-analyses [21]. Furthermore, the efficacy of acupuncture is also controversial because the lack of proper mechanistic studies in this field prevents an explanation of the clinical limitations of these techniques, and it is not known why electroacupuncture may be effective in some diseases and in some patients, but not in others with similar symptoms. Mechanistic studies will allow the design of more effective techniques for specific disorders and cohorts of patients. Many preliminary studies have been based on the systemic administration of a single drug treatment, a single **denervation** (e.g., **vagotomy**) procedure, or are isolated clinical case reports or trials that have not been replicated. Some of the most reliable results are produced in experimental animal models because, in contrast to clinical trials that can be tainted by the placebo effect and the heterogeneity of patients [21–23], experimental studies are performed in well-characterized models of diseases using homogenous groups of animals [9,24,25]. These recent studies show that electroacupuncture can attenuate inflammation and improve organ function in inflammatory and infectious disorders such as experimental sepsis. We review here recent advances in understanding the neuronal networks and their mechanisms of neuromodulation of the immune system, as well as their clinical implications for controlling inflammation (Box 2). This article intends to connect the empirical clinical information from acupuncture and electroacupuncture with the recent basic cellular and molecular mechanistic studies of neuromodulation. Both fields will benefit from this connection, bringing rigor to clinical and mechanistic studies on electroacupuncture and neuromodulation, and connecting clinical data to recent mechanistic studies in experimental animal models.

Transdermal Stimulation of Sensory Afferent Nerves

Aiming to improve therapeutic efficacy, recent studies have focused on how acupuncture and electroacupuncture activate sensory afferent nerves. Whereas some procedures have not changed much since ancient Chinese medicine, modern techniques of electroacupuncture induce voltage-dependent transdermal nerve stimulation [17,20]. Most studies analyzing how acupuncture modulates sensory afferent nerves have been performed in models of **nociception**; standard experimental models of nociception are based on rodent time-of-response to **noxious stimuli** applied to a tail or paw (**tail-flick** or **paw-withdrawal latency period tests**)

Glossary

Acupoints: specific points on the body for stimulation, where a needle is inserted in acupuncture or pressure is applied in acupressure.

Acupuncture: defined by The National Center for Complementary and Integrative Health at the NIH as 'a technique in which practitioners stimulate specific points (acupoints) on the body — most often by inserting thin needles through the skin.'

Adrenergic receptors: adrenoceptors are G protein-coupled receptors activated by norepinephrine (noradrenaline) and epinephrine (adrenaline).

Allogeneic: used in transplantation biology to refer to material from another genetically different body although belonging to the same species.

Area postrema: a medullar structure in the central nervous system that integrates humoral and neural signals.

Baroreceptors: mechanoreceptors located in the carotid sinus and in the aortic arch. Their function is to sense pressure changes by responding to a change in the tension of the arterial wall. The baroreflex mechanism is a fast response to changes in blood pressure.

Catecholamines: produced mainly by the chromaffin cells of the adrenal medulla and the postganglionic fibers of the sympathetic nervous system. There are three types of catecholamines: epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine.

Celiac ganglia: part of the sympathetic prevertebral chain possessing a great variety of specific receptors and neurotransmitters such as catecholamines, neuropeptides, and nitric oxide. Celiac ganglia contain neurons whose postganglionic axons innervate the stomach, liver, gallbladder, spleen, kidney, small intestine, and colon.

Chromaffin cells: specialized cells of the adrenal medulla that are innervated by the splanchnic nerve and secrete catecholamines into the bloodstream.

Collagenases: enzymes that break the peptide bonds in collagen.

Box 2. Sensory Networks and Central Nervous Processing

Nerve stimulation activates sensory afferent nerves that transmit the information to the somatosensory centers of the CNS across species [17,18] (see Figure 1 in main text). A typical somatosensory system has two neuronal connections: the 'peripheral' first-order neurons and the 'central' second-order neurons [123]. Classically, the first-order neurons innervate around the acupoints and have an axon connecting to the second-order neuron in the central system. The two major populations of sensory neurons are the vagal, originating from the nodose ganglia (NG), and the spinal afferent nerves originating from the dorsal root ganglia (DRG) [123]. The second-order neurons terminate in integrative centers of the somatosensory system, including the reticular system, cerebellum, medulla oblongata, and thalamus [123].

A typical example of sensory signal processing is the **baroreceptor** reflex. When blood pressure falls, baroreceptor firing decreases to restore blood pressure. The nucleus tractus solitarius (NTS) receives sensory signals from the carotid sinus baroreceptors via the glossopharyngeal nerve (CN-IX) and the aortic arch baroreceptors via the vagal (CN-X) branches [124]. Then, the NTS activates efferent pathways inducing heartbeat acceleration and blood vessel constriction. Electroacupuncture activates similar networks depending on the acupoint and the type of electrical stimulation. For instance, ST36 stimulation activates the sciatic nerve, which in turn activates the NTS via the paratrigeminal nucleus (Pa5) region of the medulla oblongata [125,126]. The NTS also integrates endocrine signals through the **area postrema** (AP) because this region lacks the blood–brain barrier and therefore it can receive endocrine signals from the peripheral bloodstream [127]. The NTS then coordinates efferent pathways either directly or indirectly. Direct innervations from the NTS to the paraventricular nucleus (PVN) and the vagal dorsal motor nucleus (DMN) allow specific activation of the HPA axis and the parasympathetic nervous system, respectively [128]. However, parasympathetic innervation of the heart originates mostly from the nucleus ambiguus. Indirect innervations allow the NTS to coordinate the sympathetic nervous system (via the rostral ventrolateral medulla, RVLM). The NTS sends excitatory (glutamatergic) fibers to activate the caudal ventrolateral medulla, which sends inhibitory (GABAergic) fibers to inhibit C1 neurons of the RVLM [127].

The RVLM is the primary regulator of the sympathetic nervous system, also sending excitatory (glutamatergic) fibers to preganglionic neurons of the spinal cord [127]. In addition, the RVLM sends neurons to the locus coeruleus in the pons, the principal source of norepinephrine innervations of the hypothalamic paraventricular nucleus [129]. The NTS also modulates the parasympathetic system via the dorsal motor nucleus. Fibers from the NTS converge with vagal afferent fibers from the nodose ganglion in the vagal dorsal motor nucleus of the medulla oblongata, which generates the preganglionic vagal efferent fibers, whereas the medullar nucleus ambiguous generates the cardiovascular vagal efferent nerves [127]. After processing, these centers coordinate the effector pathways that control physiological homeostasis.

[17,27]. For instance, acupuncture on ST36 (3 mm below the knee joint) reduces pain nociception to a noxious stimulus, thereby increasing the tail-flick and paw-withdrawal latency periods or time of response. Preliminary results suggest that acupuncture on ST36 induces analgesia by activating the **sciatic nerve** through mechanical stimulation of the connective tissue [28]. In addition, these effects can be prevented by disrupting the connective tissue around the acupoint with **collagenase** [28]. Conversely, the rotation and surface roughness of acupuncture needles can increase the mechanical stimulation of connective tissue, and thereby can enhance the effects of acupuncture [28–30]. Although these mechanisms are not well known, preliminary experimental studies in mice suggest that acupuncture induces mechanical stimulation of fibroblasts (the most common cells of connective tissue) through mechanical tension of the actinomyosin cytoskeleton [29,31,32]. Future studies will be necessary to determine the precise mechanisms of activation and whether this type of mechanical stimulation is similar to that of endothelial cells in arteries mediated by the stress-activated protein kinase (SAPK)/Jun amino-terminal kinase (JNK) pathways [33,34]. The SAPK and JNK pathways can activate mammalian immune and endothelial cells to produce inflammatory factors such as TNF and IL-6 that can cause antinociception [33,35,36]. Indeed, preliminary studies indicate that acupuncture induces mechanical stimulation of rat **mast cell degranulation**, which causes antinociception by releasing TNF and IL-6 [28]. The potential of these factors to prevent nociception has been demonstrated in several studies showing that **intraplantar** injection of either TNF or IL-6 prevents nociception in rodent paws [35,36]. It is not well known how these factors affect sensory nerves, but recent studies suggest that this effect may

Cupping: the technique of applying heated cups to the skin, creating suction, thereby gently drawing the skin upwards into the cup. It is used, often in combination with acupuncture, to treat pain in muscles and connective tissues, such as neck, back, and shoulder pain, muscle knots, and swelling.

Degranulation: a cellular process that releases molecules from secretory vesicles called granules found inside some cells. It is used by immune cells including granulocytes (neutrophils, basophils, and eosinophils) and mast cells.

Denervation: loss of nerve supply with chemical toxicity, physical injury, or intentional surgical interruption of a nerve.

Electroacupuncture: a permutation of acupuncture, in which the needles are charged with low-voltage electric currents to stimulate the acupuncture points.

Endotoxemia: indicates the presence of endotoxins in the blood. Endotoxins are derived from gram-negative bacteria and can cause hemorrhages, necrosis of the kidneys, and cardiovascular shock.

Gastroparesis: a clinical condition that decreases the normal spontaneous movement of the muscles (motility) in your stomach to propel food through your digestive tract.

Glycogenolysis: a process by which glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, is broken down into glucose to provide immediate energy and to maintain blood glucose levels during fasting.

Humoral responses: those physiological responses mediated by molecules released into the humors or extracellular body fluids, such as secreted antibodies and complement proteins.

Hypothalamic–pituitary–adrenal (HPA) axis: a complex set of direct influences and feedback interactions among three endocrine glands: the hypothalamus, the pituitary gland (a pea-shaped structure located below the thalamus), and the adrenal (also called 'suprarenal') glands (small, conical organs on top of the kidneys).

Immunomodulation: regulatory adjustment of the immune system. It

be mediated by T lymphocytes because it was prevented by inhibiting T lymphocytes; conversely, it was restored by transferring **allogeneic** lymphocytes into immunosuppressed mice [36,37]. The potential of TNF and IL-6 to affect nociception can also be prevented by opioid inhibitors such as **naloxone**, neutralizing antibodies, or μ-opioid receptor antagonists [38–41]. Together, these results suggest that acupuncture can induce mast cells (and perhaps other degranulating immune cells) to release inflammatory cytokines. These cytokines can activate T lymphocytes to produce opioids, which in turn can activate μ-opioid receptors of sensory nerves to prevent nociception [42]. Indeed, these mechanisms do not appear to be specific for TNF and IL-6 because preliminary studies in rats show that interferon (IFN)-α can also affect neuronal networks through **opioid receptors** [42].

Detailed and rigorous mechanistic studies are needed to design more effective and specific techniques for non-invasive neuronal stimulation. A classic mechanistic study on acupuncture in mice shows that acupuncture on ST36 causes antinociception by inducing extracellular adenosine [43]. Specifically, adenosine-1 receptor (A1R) knockout ($Adora1^{tm1Jgsc}$) mice are refractory to ST36-induced antinociception [43]. A1Rs inhibit adenylyl cyclase, activate potassium channels, and block transient calcium channels, and thus inhibit neuronal sensory nerve activity. Accordingly, deoxycoformycin – a selective A1R agonist – can replicate the analgesic effects of acupuncture on ST36 in mice, and thus might be potentially considered as a novel therapeutic treatment against pain [44].

Electroacupuncture induces antinociception through mechanisms similar to those described for acupuncture [17,45]. For instance, mechanical stimulation contributes to the effects of electroacupuncture based on the frequency of electrical stimulation. Mechanical stimulation at high frequency induces stress fibers in fibroblasts to orient away from the direction of stretch, but the fibers remain randomly oriented when subjected to stretches at low frequencies [46]. Similarly to acupuncture, electroacupuncture on GB30 (acupoints innervated by the sciatic and gluteal nerves) induces antinociception through a mechanism prevented by opioid antagonists such as naloxone [47]. However, these mechanisms appear to vary depending on the immune status of the animal. For instance in healthy-uninjured control rats, electroacupuncture on GB30 could prevent nociception via μ- and δ-opioid receptors at low frequency (<10 Hz), but via κ-opioid receptors at high frequency (>100 Hz) [47]. In injured rats (treated with a plantar injection of complete Freund's adjuvant, CFA), both low- and high-frequency electroacupuncture on GB30 prevented nociception via μ- and δ-opioid receptors but not by κ-opioid receptors [47]. Thus, persistent inflammation or pain might diminish the role of κ-opioid receptors in high-frequency electroacupuncture. Similarly to the effects described in acupuncture, the effects of electroacupuncture are also mediated by the inhibition of adenylyl cyclase and blockade of calcium channels to abrogate pain nociception in sensory nerves [48–50]. Moreover, a subthreshold dose of morphine (2.5 mg/kg) was shown to enhance electroacupuncture-induced analgesia additively at low frequency (10 Hz) but synergistically at high frequency (100 Hz) in this model [47]. Thus, these mechanistic studies are important for the design of novel candidate non-invasive techniques for nerve stimulation, as well as for determining the proper combination approach of electroacupuncture with conventional pharmacological treatments.

Neuronal Efferent Networks of Neuromodulation

The central nervous system (CNS) coordinates three effector pathways to control organ function: the **hypothalamic–pituitary–adrenal (HPA) axis** and the sympathetic and parasympathetic pathways (Figure 1 and Box 2). The HPA is a chemically stable **humoral** pathway that allows lasting systemic signaling through the bloodstream. The parasympathetic system is

has natural (in homeostasis in the immune system) and human-induced forms (in which immune responses are induced, amplified, attenuated, or prevented according to therapeutic goals).

Intraplantar: within the sole of the foot.

Mast cell: type of granulocyte derived from the myeloid stem cell that is a part of the immune and neuroimmune systems. When activated, a mast cell can either selectively release (piecemeal degranulation) or rapidly release (anaphylactic degranulation) 'mediators', or compounds that induce inflammation, from storage granules into the local microenvironment.

Moxibustion: a traditional Japanese practice, a form of heat therapy in which dried herbal agents called 'moxa' are burned near the skin as a counter-irritant in the treatment of disease. This practice has evolved to use warming needles inserted in the skin similar to acupuncture.

Naloxone: a synthetic drug similar to morphine that blocks opiate receptors in the nervous system.

Natural killer (NK) cells: a type of lymphocyte (white blood cell) and a component of the innate immune system that plays a major role in the host-rejection of both tumors and virally infected cells.

Neuromodulation: a physiological process by which a given neuron uses one or more chemicals to regulate diverse populations of cells.

Nociception: the sensory nervous system's response to certain harmful or potentially harmful stimuli. Intense chemical, mechanical, or thermal stimulation of sensory nerve cells called nociceptors produces a signal that travels along a chain of nerve fibers via the spinal cord to the brain and triggers a variety of physiological and behavioral responses that include experience of pain.

Noxious stimuli: external or internal physical changes (such as a heat) that induce afferent input in the nervous system, with or without sensory experience or a behavioral response. They are normally perceived as potentially tissue-damaging events that can cause pain. Algogenic stimuli are those that

a neuronal pathway that produces acetylcholine, an unstable neurotransmitter with a short lifespan that produces transient and local effects. The **sympathetic system** is a hybrid mechanism with both neuronal innervations and humoral responses. It innervates most of the organs with pre-ganglionic cholinergic fibers (producing acetylcholine) or post-ganglionic **adrenergic** fibers (producing **catecholamines**). The sympathetic system also induces systemic release of catecholamines from the adrenal glands.

The HPA Axis

The HPA axis governs the systemic release of glucocorticoids from the adrenal glands into the bloodstream (Figure 2A, Key Figure). A typical example of its role is that acupuncture on GB30 inhibits CFA-induced paw edema in mice through a mechanism that is prevented by adrenalectomy and glucocorticoid inhibitors [51–53]. Afferent sensory networks activate the paraventricular nucleus of the hypothalamus to secrete vasopressin and corticotrophin releasing hormone [53]. Both compounds activate the anterior lobe of the pituitary gland to release adrenocorticotrophic hormone (ACTH) into the bloodstream (Table S1 in the supplemental information online). ACTH then activates the zona fasciculate of the cortex of the adrenal glands to release glucocorticoids; mainly corticosterone in rodents and hydrocortisone (cortisol) in humans [51,54]. Glucocorticoids have a half-life of 8–10 h, and their effects are broadly classified into metabolic (inducing glucogenic enzymes in the liver to increase blood glucose) and immune (to reduce inflammation) mechanisms. At the cellular level, mammalian glucocorticoid receptors (GRs) are expressed in almost all cells and regulate development, metabolism, and inflammation [55]. Dormant GRs reside in cytosolic complexes with multiple proteins such as FKBP52 (FK506-binding protein 52) and heat shock proteins (HSP70 and HSP90) [56,57]. Glucocorticoids diffuse through cell membranes and bind to intracellular GRs to release them from dormant complexes (Figure 2B). Active GRs can inhibit inflammation through two mechanisms: transactivation of anti-inflammatory genes and transrepression of inflammatory genes. During transactivation, activated GRs homodimerize, translocate into the nucleus, and bind to specific DNA elements to induce the expression of anti-inflammatory genes coding for proteins such as IL-4 and IL-10 [56,57]. During transrepression, activated GRs bind to other transcriptional factors such as NF-κB to inhibit their ability to induce inflammatory cytokines [56,58]. Thus, HPA stimulation can be a successful strategy to induce the production and systemic distribution of glucocorticoids to modulate metabolic and immune responses.

The Sympathetic Nervous System

The sympathetic neuronal network runs through the spinal cord and innervates most of the viscera inducing both local neurogenic or systemic release of catecholamines from the adrenal glands (Figure 3A). The typical mechanism of sympathetic modulation is via activation of the adrenal glands to produce systemic release of catecholamines [42,59,60]. Pre-ganglionic sympathetic branches innervate the adrenal medulla and activate **chromaffin cells** to release catecholamines into the bloodstream. The systemic release of catecholamines can cause adverse effects such as systemic metabolic lipolysis and immunosuppression [42,59,60]. Thus, more recent studies have focused on the potential to induce local release of neurogenic catecholamines through sympathetic innervation in different tissues.

Catecholamines have a short half-life (1–4 minutes) and produce local effects such as hepatic **glycogenolysis**, cardiac contraction, and bronchial relaxation [26]. Sympathetic stimulation can induce either local or systemic catecholamine secretion depending on the electrical frequency [61,62]. Specifically, high-frequency electroacupuncture on ST36 inhibits carrageenan-induced paw inflammation through pre-ganglionic innervation of the adrenal glands in rodents [61,62]. By contrast, low-frequency electroacupuncture on ST36 in rodents inhibits

cause pain and are commonly noxious.

Opioid receptors: a group of inhibitory G protein-coupled receptors activated by opioids. Opioid receptors have analgesic and neuroprotective effects.

Parasympathetic nervous system: the part of the autonomic nervous system that contains cholinergic fibers, conserves energy, slows the heart rate, increases intestinal and gland activity, and relaxes sphincter muscles in the gastrointestinal tract.

Paw-withdrawal latency period: a nociceptive assay that measures the time of an animal, usually a rodent, to detect a noxious stimulus in the paw such as the feeling of pain. These assays measure the existence of pain through behaviors such as withdrawal, licking, immobility, and vocalization.

Sciatic nerve: longest and widest single nerve in the human body, going from the top of the leg to the foot on the posterior aspect. The sciatic nerve provides the connection to the nervous system for nearly the whole of the skin of the leg, the muscles of the back of the thigh, and those of the leg and foot.

Sepsis: when an infection reaches the bloodstream and causes systemic inflammation in the body. Sepsis can develop into septic shock when the inflammatory responses cause a significant drop in arterial blood pressure that can lead to respiratory or heart failure, stroke, failure of other organs, and death. Severe sepsis is when the infection/inflammation is severe enough to affect the function of your organs.

Sympathetic nervous system: the part of the autonomic nervous system that runs through the spinal cord, contains adrenergic fibers and induces vasoconstriction and increased heart rate.

Tail-flick latency test: a nociceptive assay that measures the time of an animal, usually a rodent, to detect a noxious stimulus in flick the tail.

Tui na: a technique of acupressure based on the deep manual massage of muscles and specific body parts improve blood flow and nerve stimulation similar to acupuncture to facilitate healing and treat pain and inflammation.

inflammation in experimental models of paw swelling, lung leukocyte migration, surgical trauma, sepsis, and arthritis through local sympathetic post-ganglionic innervations, independently of the adrenal glands [63–66]. Thus, high-frequency electroacupuncture appears to activate pre-ganglionic innervation of the adrenal medulla to induce systemic catecholamines, whereas low-frequency electroacupuncture appears to activate specific sympathetic post-ganglionic innervation to induce local release of neurogenic norepinephrine [61]. Recent studies in rodents suggest that local sympathetic regulation of the immune system can provide clinical advantages for treating inflammatory disorders such as arthritis by preventing systemic immunosuppression and susceptibility to secondary infections [67,68]. It has been postulated that many of the local effects of sympathetic innervation are mediated by a direct interaction of the post-ganglionic nerves with macrophages. For instance, sympathetic fibers of the gut muscularis in mice release norepinephrine, which binds to α2-adrenergic receptors on muscularis macrophages to activate a tissue-protective phenotype [69] and to regulate gastrointestinal

> **Vagotomy:** a surgical procedure sectioning the vagus nerve. The most typical procedures include cervical or subdiaphragmatic vagotomy to study the role of the vagus nerve.
> **Vagus nerve:** the longest and principal nerve of the parasympathetic nervous system connecting the brain with most of the viscera.
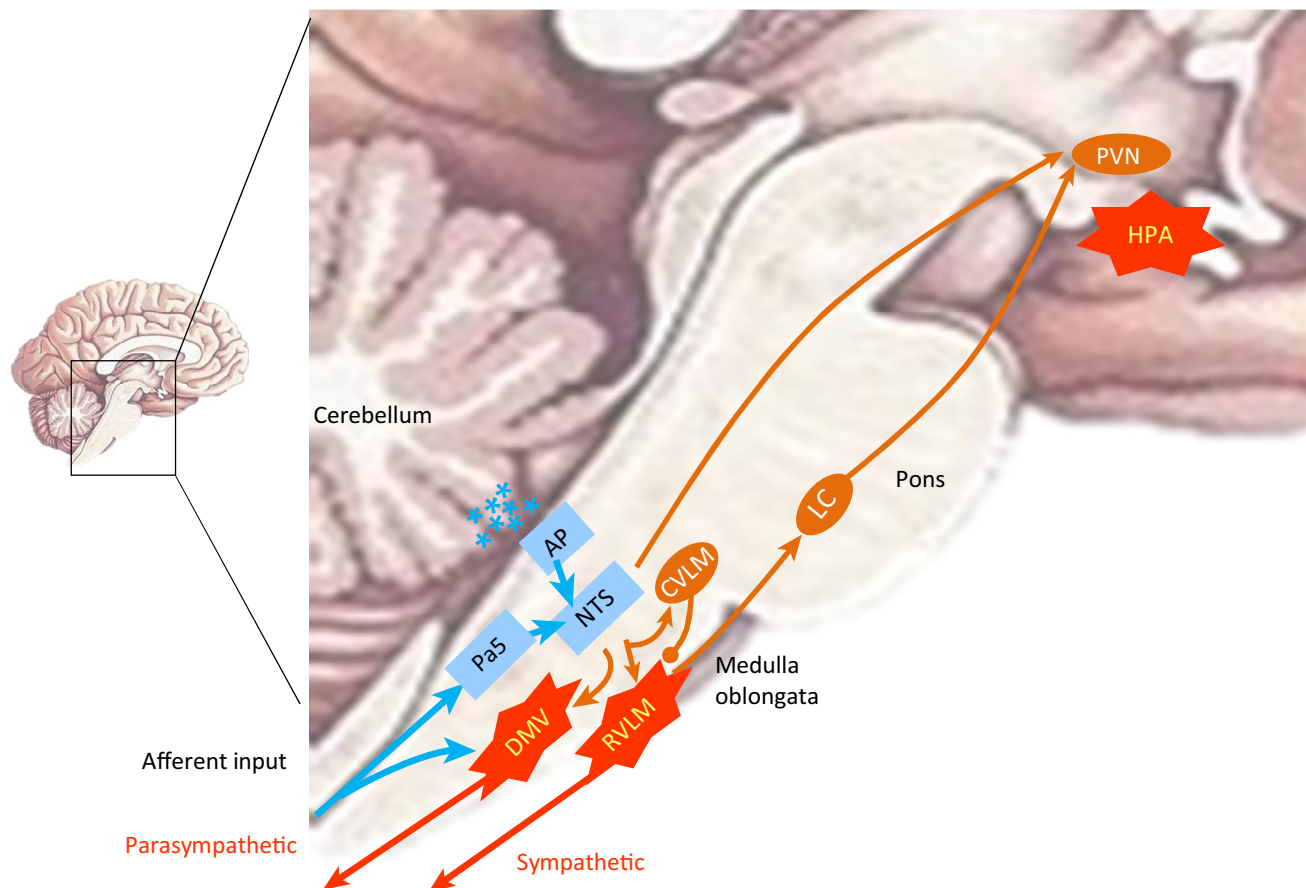

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Figure 1. Central Processing of Mammalian Sensory Information. Neuronal stimulation activates the nucleus tractus solitarius (NTS) via the paratrigeminal nucleus (Pa5) of the medulla oblongata. The NTS also integrates endocrine signals (*) through the area postrema (AP), which lacks a blood–brain barrier. Direct innervations from the NTS to the paraventricular nucleus (PVN) and the vagal dorsal motor nucleus (DMN) allow specific activation of the hypothalamic–pituitary–adrenal (HPA) axis and the parasympathetic nervous system, respectively. However, parasympathetic innervation of the heart originates mostly from nucleus ambiguus. Indirect innervation allows the NTS to coordinate both the HPA axis and the sympathetic nervous system. The RVLM also sends neurons to the locus coeruleus (LC) in the pons, the principal source of norepinephrine innervation of the hypothalamic paraventricular nucleus (PVN). NTS innervates the caudal ventrolateral medulla (CVLM), which in turn can modulate the RVLM.

Key Figure

The Hypothalamic–Pituitary–Adrenal (HPA) Axis

(A)

(B)
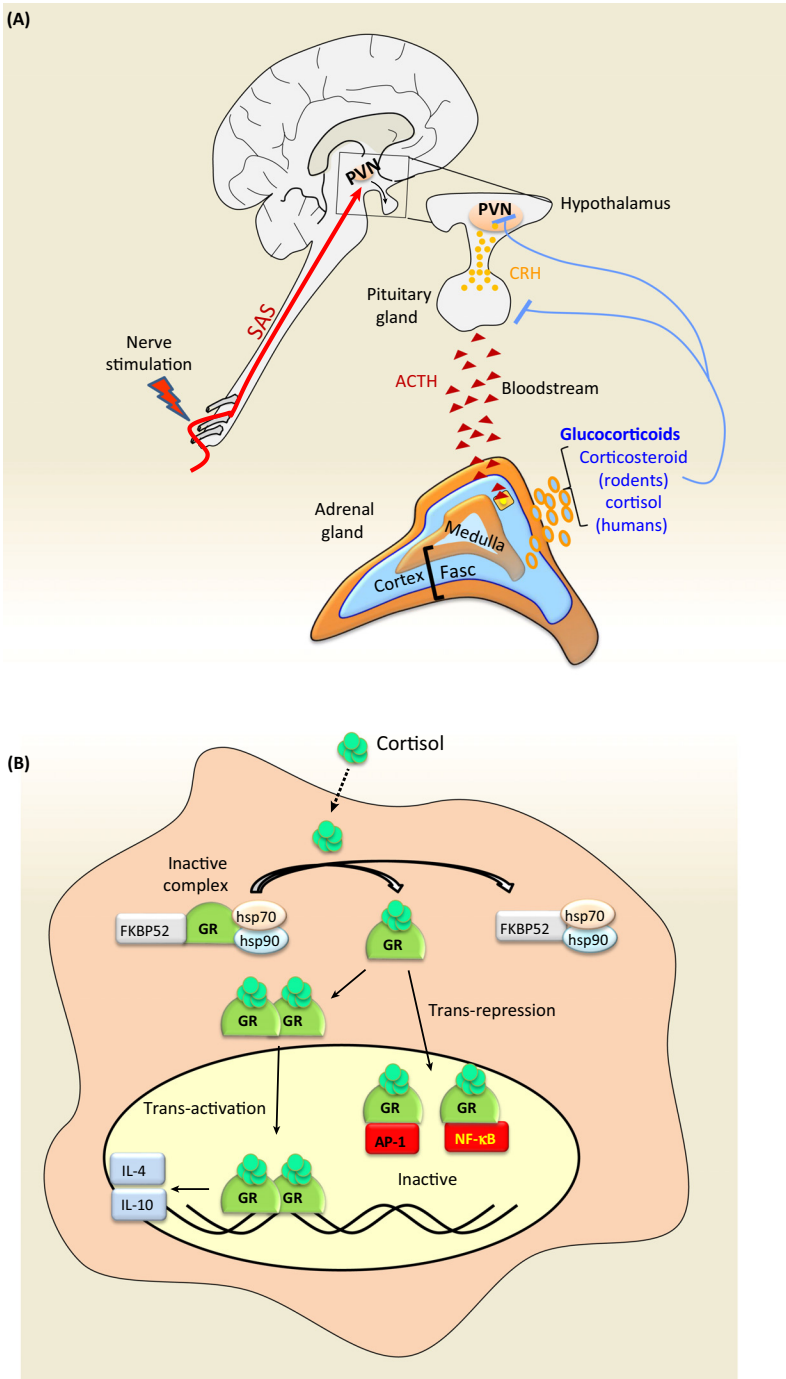
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motility [70]. Recent studies in mice also indicate that sympathetic neuron-associated macrophages contribute to obesity by importing and metabolizing norepinephrine [71]. By contrast, sympathetic splenic nerves can control inflammation in experimental sepsis by activating T lymphocytes and not by interacting directly with macrophages [72,73]. The splenic nerves release norepinephrine which can activate T lymphocytes via α2 adrenergic receptors to produce acetylcholine [72–75]. Acetylcholine and other cholinergic agonists such as nicotine bind to α7 acetylcholine receptors (α7nAChRs) on murine splenic macrophages to inhibit the production of inflammatory factors such as TNF [74,75]. Similar rodent studies have reported that sympathetic stimulation can also prevent lymphocyte apoptosis induced by surgical trauma by inhibiting the expression of proapoptotic proteins such as Fas [64,65,76]. Given that TNF induces apoptosis, future studies will be necessary to determine whether this is a direct effect of the splenic nerve on lymphocytes or a consequence of inhibiting TNF production in macrophages [10,77,78].

Other work indicates that splenic nerve activation, with either IFN-α or electrical splanchnic stimulation in mice, can also regulate other immune cells such as inhibiting **natural killer cells** [42,79]. Together, these results suggest that sympathetic stimulation can induce local release of neurogenic norepinephrine, and this may provide clinical advantages by inducing local control of inflammation and thus avoiding collateral effects in non-targeted tissues. Future studies will be necessary to determine the specific neuronal networks and organ innervations activated by each acupoint of stimulation.

Catecholamines control almost all cellular functions depending on the receptor targeted. Adrenergic (norepinephrine or epinephrine) or dopaminergic receptors (which are G protein-coupled seven-transmembrane domain receptors) are expressed in most mammalian cells (Figure 3B). These receptors are classified according to their functions into three major groups: (i) α1-adrenoceptors coupled to Gq proteins, which are G protein complexes that activate phospholipase C to cleave phosphatidylinositol 4,5-bisphosphate into diacylglycerol (DAG) and inositol trisphosphate (IP3, induces intracellular release of Ca^{2+} from the endoplasmic reticulum) [80,81]. IP3 and DAG both act as second messengers that activate protein kinase C [82], which subsequently activates IκB kinase (IKK) to phosphorylate the inhibitory IκB protein, leading to NF-κB activation [80,81], an important signaling pathway inducing inflammatory cytokines such as TNF. (ii) α2-adrenoceptors and D2-like dopaminergic (D2, D3, D4) receptors are coupled to Gi proteins which inhibit adenylyl cyclase and prevent the formation of cAMP. (iii) Conversely, β-adrenoceptors and D1-like dopaminergic (D1, D5) receptors are coupled to Gs proteins which activate adenylyl cyclase and increase the production of cAMP, leading to the activation of protein kinase A and cAMP response element-binding protein (CREB) [83]. The anti-inflammatory effects of this pathway rely on the potential of protein kinase A and CREB to inhibit NF-κB by modulating common transcriptional cofactors, in other words the CREB

Figure 2. (A) Electroacupuncture on GB30 induces sciatic sensorial afferent signals (SAS) activating the paraventricular nucleus (PVN) to secrete corticotrophin-releasing hormone (CRH). CRH activates the anterior lobe of the pituitary gland to release adrenocorticotrophic hormone (ACTH) into the bloodstream. ACTH activates the zona fasciculata (Fasc) of the adrenal glands to produce glucocorticoids, which induce a negative feedback inhibiting the PVN and the pituitary gland. (B) At the cellular level, inactive glucocorticoid receptors (GRs) reside in the cytosol complexed with other proteins including heat shock proteins (HSP70 and HSP90) and FKBP52. Glucocorticoids diffuse into the cells and release the receptor from the inactive complex. The GRs homodimerize and transactivate anti-inflammatory cytokines such as IL-4 and IL-10. During transrepression, GRs bind to transcription factors such as AP-1 and NF-κB, and prevent them from inducing inflammatory factors.
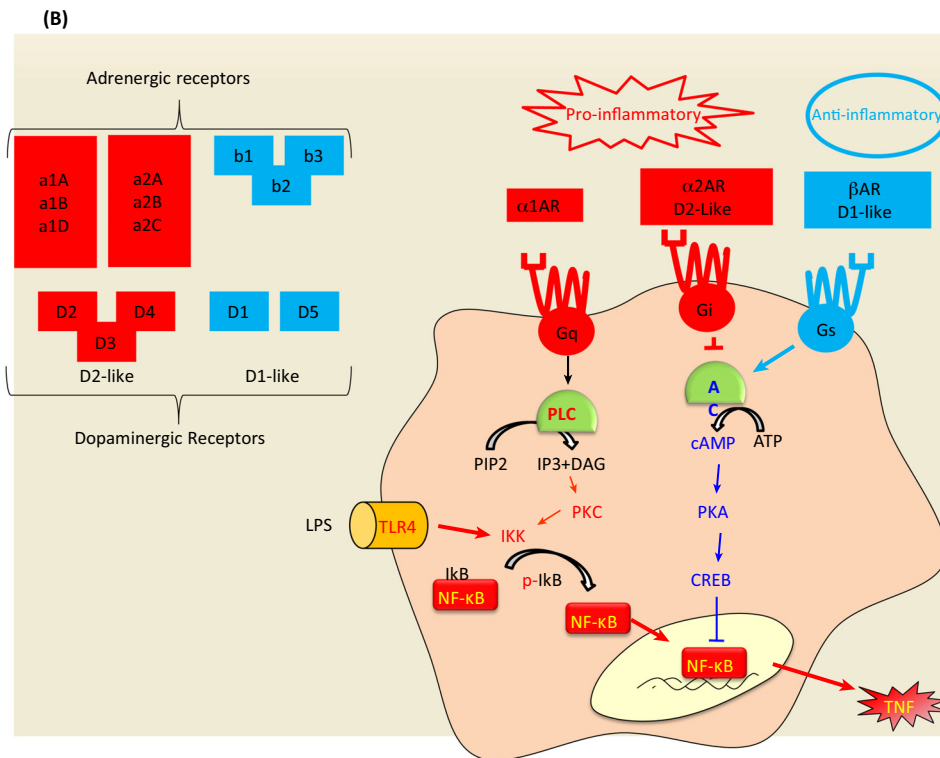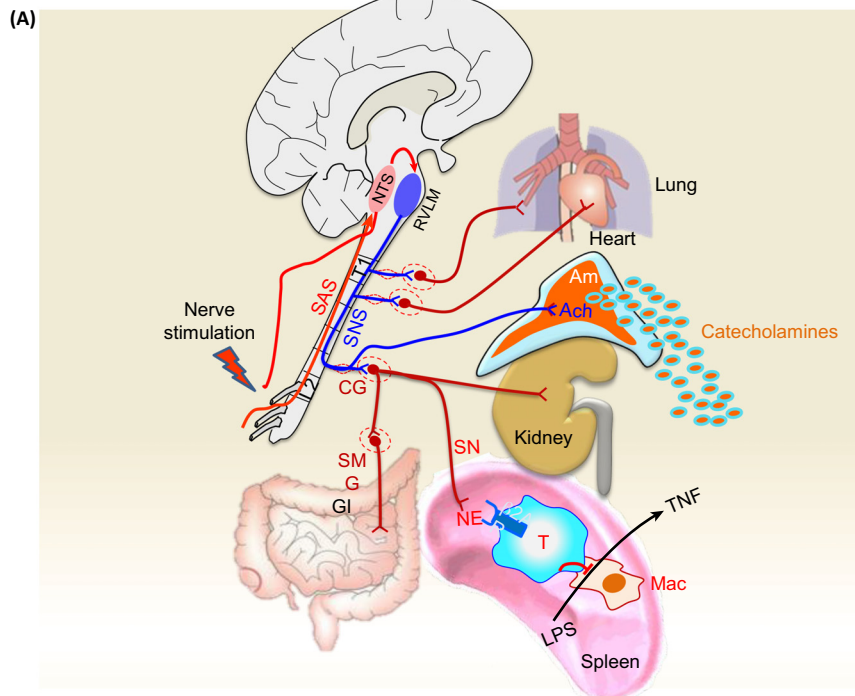
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binding protein [84]. These receptors have different affinities for catecholamines; norepinephrine has a higher affinity for α1- and α3-adrenoceptors, whereas epinephrine has a higher affinity for β2- and α2-adrenoceptors [85]. Moreover, tissue-specific receptor expression allows epinephrine to induce different cellular responses such as constricting minute blood vessels (via α-receptors), but dilates blood vessels in skeletal muscles and liver (via β-receptors) [86]. The study of these mechanisms have allowed the design of selective β-adrenergic blockers for hypertension and arrhythmia, as well as to protect the heart from a second heart attack. Similar studies are warranted to determine the precise molecular mechanisms induced by sympathetic stimulation in inflammatory disorders.

The Parasympathetic Nervous System

The **vagus nerve** is the principal parasympathetic nerve connecting the CNS with the viscera in mammals (Figure 4A). Vagal stimulation modulates multiple physiological functions from digestion to inflammation [87,88]. Either auricular ST (stomach), SI (small intestine) or somatic (ST36) acupuncture in humans increases gastrointestinal motility via vagal stimulation [89]. Similarly, electroacupuncture on PC6 and ST36 has been reported to increase gastric motility in patients with **gastroparesis** via vagal stimulation [90]. Our laboratory group reported that electroacupuncture on ST36 reduces serum levels of inflammatory cytokines and prevents organ damage in mice with polymicrobial peritonitis induced by cecal ligation and puncture [9,91]. These effects were mediated by the vagus nerve and therefore prevented by surgical vagotomy. Electroacupuncture on ST36 has also been found to prevent burn-induced inflammation and lung tissue injury via vagal stimulation [92]. Vagal stimulation is also achieved by stimulating other acupoints. Indeed, a report indicated that electroacupuncture on GV20 and GV14 in mice with experimental ischemic stroke could improve cerebral blood perfusion and reduce brain damage, apoptosis, oxidative stress, and inflammation via vagal stimulation [93].

The vagus nerve is a cholinergic nerve that produces acetylcholine, a neurotransmitter with a short half-life that induces local effects, but that is too chemically unstable to produce systemic effects. Acetylcholine has been mostly studied as a neurotransmitter but its effects on immune cells are not well known [2]. Acetylcholine and other cholinergic agonists such as nicotine inhibit TNF production in murine macrophages by inhibiting the NF-κB pathway via α7 nicotinic cholinergic receptors (α7nAChRs) [2,72–74,94,95] (Figure 4B). Experimental, epidemiological, and clinical trials show that nicotine inhibits inflammation in multiple disorders including ulcerative colitis, arthritis, sepsis, schizophrenia, and Alzheimer's disease [96–99]. However, nicotine has many detrimental side effects that limit its clinical use [100,101]. Thus, current studies focus on specific α7nAChR-agonists to treat inflammation and prevent the side-effects

Figure 3. The Sympathetic Nervous System (SNS). (A) In mammals, neuronal stimulation converges in the activation of the sensorial afferent signal (SAS) that activates the nucleus tractus solitarius (NTS). The NTS activates directly or indirectly the rostral ventrolateral medulla (RVLM), the primary regulator of the sympathetic nervous system (SNS). The SNS runs through the spinal cord and innervates most of the viscera including the lungs, heart, kidneys, and gastrointestinal tract (GI) through post-ganglionic innervations after the activation of the specific ganglia. Neuronal post-ganglionic innervation induces neurogenic norepinephrine (NE). Splenic norepinephrine inhibits the production of inflammatory cytokines in splenic macrophages by activating β2-adrenergic receptors (β2ARs) on T 'modulatory' lymphocytes. The SNS can also induce the systemic production of catecholamines by activating chromaffin cells via pre-ganglionic innervations to release acetylcholine (ACh) in the adrenal medulla (Am). (B) At the cellular level, the receptors are classified into three major groups. α1-Adrenoceptors (α1ARs) signal through Gq, which activates phospholipase C (PLC) to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) to diacyl glycerol (DAG) and inositol trisphosphate (IP3). Both IP3 and DAG activate PKC to phosphorylate the IκB kinase (IKK) and induce phosphorylated (p-) IκB. α2-Adrenoceptors (α2ARs) and dopaminergic D2-like receptors are coupled to Gi proteins, which inhibit adenylyl cyclase (AC) and prevent the formation of cAMP. β-Adrenoceptors (βARs) and dopaminergic D1-like receptors are coupled to Gs proteins, which activate AC to induce cAMP and activates PKA and CREB. The anti-inflammatory potential of this pathway relies on the ability of CREB to inhibit the NF-κB pathway.
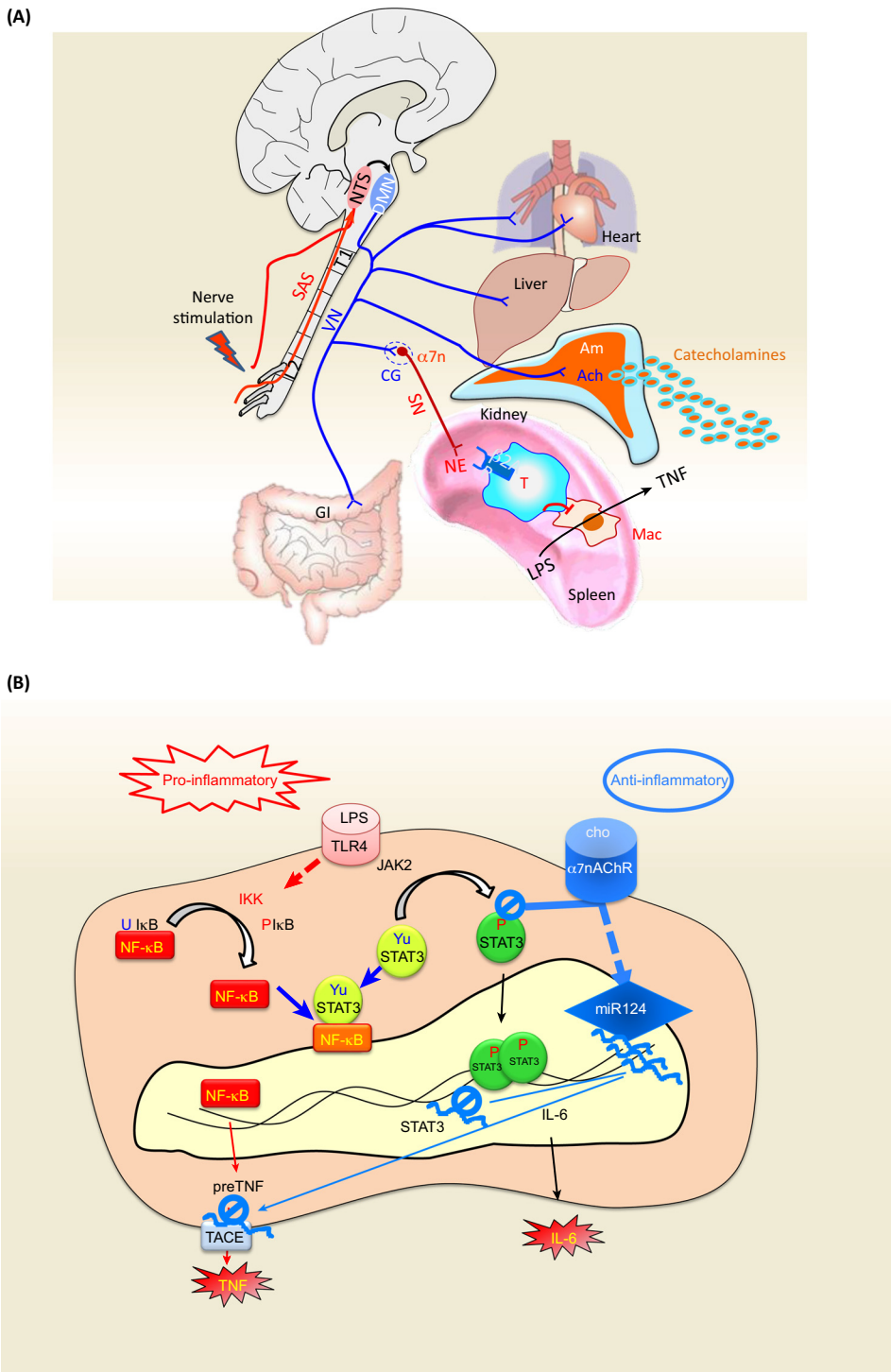
Figure 4. The Parasympathetic Nervous System. (A) In mammals, sensorial afferent signal (SAS) activates the NTS, which modulates the vagal dorsal motor nucleus (DMN). The vagus nerve (VN) is the principal parasympathetic nerve innervating most of the viscera including the heart, lungs, liver, adrenal glands, and the gastrointestinal tract (GI). The vagus

(See figure legend on the bottom of the next page.)

of nicotine [1]. Lipopolysaccharide (LPS) activates TLR4 receptors, which activate the NF-κB and JAK2–STAT3 pathways to induce TNF and IL-6, respectively [102–105]. α7nAChR-agonists can inhibit LPS-induced activation of NF-κB and also STAT3 tyrosine phosphorylation by JAK2, without affecting its serine phosphorylation [102–105]. Thus, α7nAChR-agonists can induce tyrosine-unphosphorylated STAT3 (Yu-STAT3), which could bind to NF-κB, leading to a reciprocal transrepression similar to that described for glucocorticoids [106]. Recent rodent studies also reported that α7nAChR agonists could induce the expression of noncoding microRNA-124 (miR-124) which inhibits the translation of TNF-converting enzyme (TACE), a protease required for the processing of the membrane-anchored precursor pre-TNF [107,108]. Collectively, these data suggest that, similarly to α- and β-adrenergic blockers, selective α7nAChR-agonists may provide pharmacological advantages for treating inflammation. Future tests should also aim to determine how well these findings can be translated to humans.

Neuro-Immunomodulation and the Functional Organization of the Nervous System

Neuronal **immunomodulation** represents a collection of mechanisms selected through evolution to restrain the immune system and prevent deleterious inflammation [109,110]. These systems can control various inflammatory factors, and may bring clinical advantages in treating complex pathologies including infectious disorders such as sepsis. Data indicate that auricular electrostimulation [111], acupuncture [112,113], or electroacupuncture [114] on ST36 can attenuate serum concentrations of inflammatory cytokines TNF, IL-1β, and IL-6, as well as improving kidney and lung injury in septic rodents. These mechanisms are not specific to sepsis, and electroacupuncture on ST36 can also prevent intestinal injury in ischemic and hemorrhagic rodents [115]. Mechanistically, these effects were reported to occur via the vagus nerve because they were prevented by surgical vagotomy [2,116]. Our studies have shown that electroacupuncture on ST36 decreases serum TNF, MCP1, IL-6, and IFN-γ, and improved mouse survival in polymicrobial peritonitis [9]. These effects were mediated by the vagus nerve because they were prevented by surgical vagotomy. However, these mechanisms appeared to be independent of neuronal modulation of the spleen because ST36 electrostimulation still inhibited systemic inflammation in splenectomized animals [2]. By contrast, electroacupuncture on ST36 activated the production of DOPA decarboxylase in the adrenal glands, leading to systemic release of dopamine that inhibits the production of inflammatory factors in macrophages [9]. These results were recently confirmed by other investigators showing that acupuncture on ST36, but not electrostimulation, also inhibited serum TNF levels in endotoxemic mice [117]. Similar studies on other acupoints appear to suggest that different neuronal networks can contribute to control inflammation in experimental sepsis. Thus, electroacupuncture on LI4 also reduces serum TNF, IL-6, and IL-1β levels in septic mice without inducing glucocorticoids [116]. These effects were attributed to both the sympathetic and parasympathetic systems because the effects were prevented by parasympathetic nicotinic antagonists as well as by chemical sympathectomy and the administration of sympathetic β-blockers such as propranolol [116]. Although specific mechanistic studies will be necessary to delineate the

nerve inhibits the production of inflammatory cytokines in the spleen by activating the sympathetic adrenergic splenic nerve (SN) via α7-nicotinic acetylcholine receptors (α7nAChRs) at the **celiac ganglion** (CG). Norepinephrine from the splenic nerve activates T 'modulatory' lymphocytes to inhibit cytokine production in splenic macrophages. Furthermore, efferent vagal fibers induce the systemic release of catecholamines from the adrenal medulla (Am). (B) At the cellular level, choline (cho), a selective agonist of α7nAChRs, inhibits lipopolysaccharide (LPS)-induced STAT3 tyrosine phosphorylation (P-STAT3), preventing STAT3-mediated transcriptional activation of IL-6. LPS also induces the phosphorylation of IκB, leading to the release of NF-κB which can interact with tyrosine unphosphorylated STAT3 (Yu-STAT3) to produce mutual transregulation. α7nAChRs also induce the production of noncoding microRNA-124 (miR124) which inhibits the translation of the STAT3 and TACE (TNF-converting enzyme) mRNAs.

neuronal networks, these studies are valuable by suggesting the clinical potential of different neuronal networks and different levels of functional organization of the nervous system in controlling inflammation.

Taken together, the emerging studies on neuronal stimulation suggest three models of functional organization of the nervous system in inducing physiological homeostasis [118]. Classically, the sympathetic and parasympathetic systems are described as 'antagonistic' mechanisms opposing one another to balance physiological homeostasis (Figure 5). A
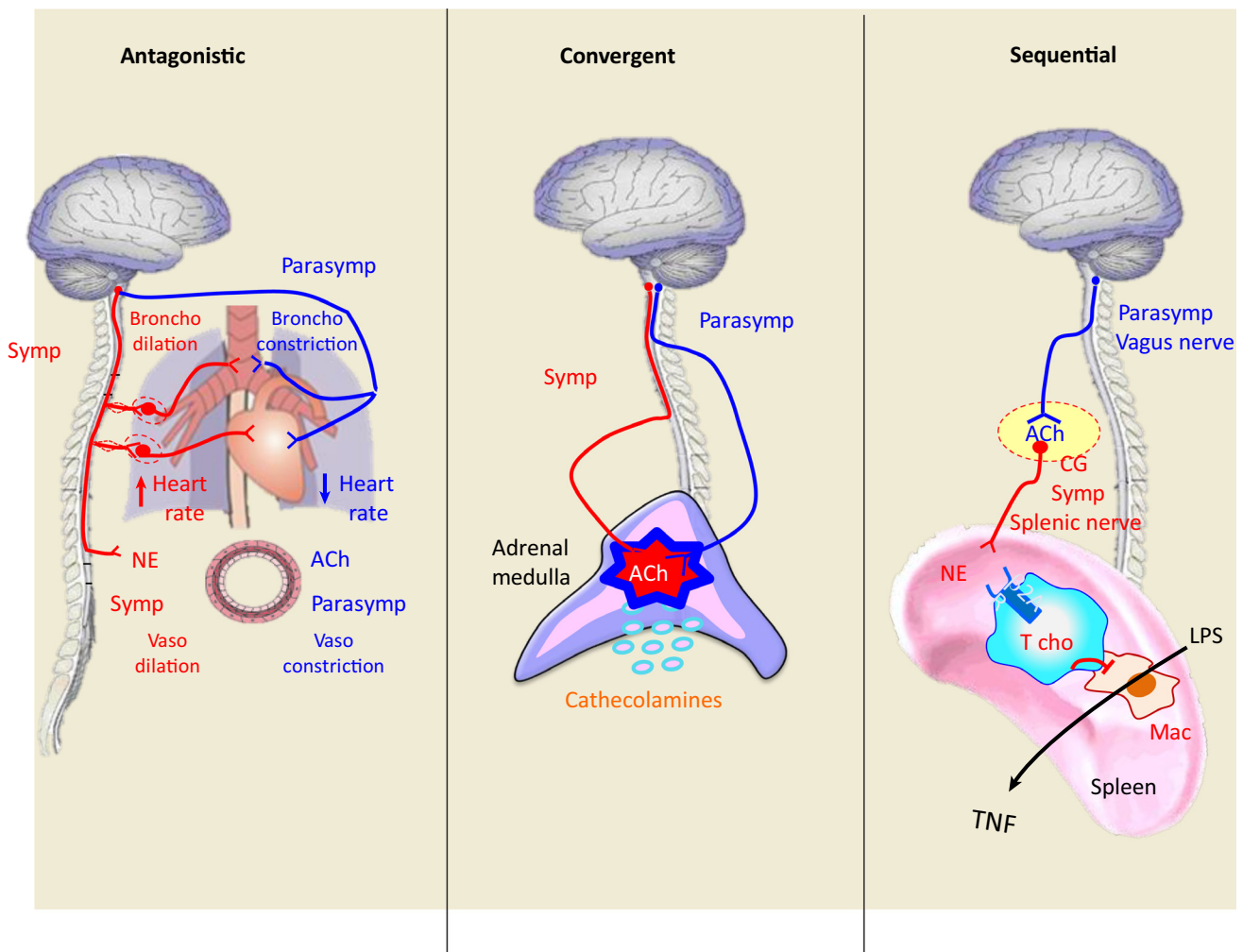
Trends in Molecular Medicine

Figure 5. A Model of the Functional Organization of the Mammalian Autonomic Nervous System. Classically, the sympathetic (Symp) and parasympathetic (Parasymp) systems are described as 'antagonistic' systems with opposing signals to maintain physiological homeostasis. A typical example is the baroreceptor reflex, where the sympathetic and parasympathetic systems secrete norepinephrine (NE) or acetylcholine (ACh) to increase and decrease heart rate and blood pressure, respectively. Although acetylcholine produces vasodilation, there is no evidence for parasympathetic innervation of blood vessels. Recent studies on electroacupuncture reveal a second model of 'convergent' functional organization of the nervous system. In this model, both the preganglionic sympathetic and parasympathetic vagus nerves converge on the release of acetylcholine (ACh) in the adrenal medulla to activate chromaffin cells to secrete catecholamines. A third model of organization is the 'sequential' connection between the parasympathetic vagus nerve (VN) that activates the sympathetic splenic nerve (SN). The parasympathetic vagus nerve (VN) inhibits TNF production in splenic macrophages (Mac) by activating the sympathetic splenic nerve (SN) at the celiac ganglion (CG). The splenic nerve releases norepinephrine (NE) that activates T cholinergic (Tcho) lymphocytes via α2-adrenoceptors (α2ARs) [72,73].

characteristic example is the baroreceptor reflex system (Box 2). Arterial baroreceptors are stretch receptors stimulated by distension of the arterial wall to control blood pressure. If blood pressure falls, baroreceptor firing rate decreases, and the CNS activates the sympathetic system to produce norepinephrine and increase heart rate and blood pressure. Conversely, when blood pressure rises, the baroreceptor reflex activates the **parasympathetic nervous system** to release acetylcholine and decrease heart rate and blood pressure [1]. The sympathetic and parasympathetic nervous systems produce antagonistic signals with norepinephrine and acetylcholine to balance both heart rate and blood pressure [8]. Although acetylcholine triggers vasodilation, there is no evidence for parasympathetic innervation of blood vessels. As previously mentioned, recent work from our laboratory has suggested a second level of organization in which the sympathetic and parasympathetic nervous systems might 'converge' and induce an additive production of acetylcholine in the adrenal medulla [9] (Figure 5). In this scenario, electroacupuncture on ST36 has been shown to inhibit systemic inflammation in experimental murine sepsis by inducing the production of catecholamines from the adrenal medulla [9]. In contrast to the lack of vagal innervations in many organs, there is strong evidence showing both vagal and sympathetic preganglionic innervation of the adrenal glands. Both, Kollmann (1860) and Teitelbaum (1933) traced nerve fibers from the posterior vagus trunk in the subdiaphragmatic region of the left adrenal gland. In addition, retrograde tracers injected at the center of the adrenal medulla have demonstrated staining of the ipsi- and contralateral vagal sensory (nodose) ganglia, as well as the ipsi- and contralateral dorsal motor nucleus of the vagus nerve, with labeling extending from the cranial to the caudal limits of the nucleus [119]. These results suggest that there are direct afferent (sensory) and efferent (motor) vagal innervations in the adrenal glands. We posit that both parasympathetic vagal innervation and cholinergic preganglionic sympathetic neurons converge to modulate the production of acetylcholine in the adrenal medulla [120]. Moreover, ST36 stimulation has been shown to induce catecholamines and inhibit serum TNF in α7nAChR-knockout mice, suggesting that acetylcholine-induced production of catecholamines might be independent of α7nAChRs [9]. These results concur with recent studies indicating that α6β4nAChRs modulate exocytosis, and therefore that these receptors may regulate catecholamine release in human chromaffin cells [121]. Lastly, recent findings from different laboratories suggest a third level of functional organization in which a 'sequential' connection between the parasympathetic and sympathetic systems inhibits splenic TNF production in septic mice. In this case, the parasympathetic and sympathetic systems are connected as a sequential part of the same mechanism. The parasympathetic vagus nerve (which does not innervate the spleen) activates the sympathetic splenic nerve to release norepinephrine, which in turn inhibits splenic TNF production by activating cholinergic T lymphocytes [72,73,95,122]. The vagus nerve can activate the splenic nerve by inducing acetylcholine in the mesenteric ganglia [95]. Collectively, such studies on neuromodulation are beginning to suggest new models of functional organization of the nervous system in controlling inflammation and immunity. The models of functional organization of the nervous system might help in designing novel candidate therapeutic strategies to costimulate different neuronal networks and achieve the most effective control of inflammation.

Concluding Remarks

Neuronal stimulation is a promising emerging field in modern medicine for the control of organ function and re-establishing physiological homeostasis during illness. Multiple recent studies show the potential of nerve stimulation to control inflammation and improve organ function in multiple experimental disorders from arthritis and colitis to diabetes and sepsis. Conversely, multiple clinical studies show that neuromodulation with acupuncture or electroacupuncture can control pain and inflammation in multiple disorders such as postoperative recovery,

Outstanding Questions

Why have clinical trials provided controversial results regarding the efficacy of acupuncture and electroacupuncture? Three major factors taint the results of these clinical trials. The placebo effect, the heterogeneous practices of nerve stimulation, and the lack of mechanistic explanations. In contrast to clinical trials that are tainted by the placebo effect and the heterogeneity of patients, experimental studies with well-characterized models of diseases in homogenous groups of animals will be necessary to define the therapeutic efficacy of these mechanisms. Elucidating these mechanisms will be essential in understanding why acupuncture or electroacupuncture may provide clinical advantages in specific diseases and cohorts of patients, but not in others with similar diagnoses. Nevertheless, neuromodulation with acupuncture or electroacupuncture is endorsed by the WHO and the NIH, and is used by millions of people to control pain and inflammation, and to re-establish physiological homeostasis during illness.

Can neuromodulation provide clinical advantages in treating different ailments? Recent studies indicate that neuromodulation with nerve stimulation may provide significant clinical advantages in controlling pain, inflammation, and organ function without inducing side effects. For instance, nerve stimulation can activate specific networks to induce local control of inflammation in inflammatory disorders, such as rheumatoid arthritis, without inducing general immunosuppression or affecting other regions and organs. To achieve this, many additional questions, robust mechanistic insight, and further validation in humans remain to be addressed.

Box 3. Clinician's Corner

Vagal stimulation has been approved by the FDA for treating refractory epilepsy and depression [129]. Right cervical vagal nerve stimulation (VNS) is effective for treating heart failure in preclinical studies and in a Phase II clinical trial [130]. Small reports have described the use of VNS for bipolar disorder, treatment-resistant anxiety disorders, Alzheimer's disease, chronic refractory headaches, and obesity, although none of these uses has received FDA approval [131]. VNS with an implantable electrical generator improved rheumatoid arthritis [132]. Nerve stimulation can provide clinical advantages over conventional interventions such as small-molecule medications and monoclonal antibodies. Biological anti-rheumatic drug therapies are expensive and increase the risk of systemic immunosuppression, infections, and malignancies. By contrast, nerve stimulation may provide therapeutic advantages by inducing 'local' regulation of inflammation and avoiding the 'systemic' side-effects of pharmacological anti-inflammatory therapies. Non-invasive transdermal nerve stimulation prevents organ damage in inflammatory and infectious disorders. Acupuncture or electroacupuncture are used worldwide by millions of people in multiple disorders. Current studies focus on how these techniques activate neuronal networks and their mechanisms of neuromodulation. Proper mechanistic studies will be crucial to determine why acupuncture and electroacupuncture are effective in some patients and diseases, but not in others with similar symptoms. For instance, ST36 electroacupuncture can control inflammation in experimental sepsis, but not in septic patients with adrenal insufficiency [9].

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osteoarthritis, migraine, stroke, post-traumatic stress disorder, and drug addiction. Electro-acupuncture is now endorsed by the American Pain Society, the National Center for Complementary and Alternative Medicine, the NIH, and the WHO, and is used by millions of people to control pain and inflammation. However, as discussed, the efficacy of these techniques remains controversial. For instance, it is not clear why electroacupuncture on ST36 can control inflammation and improve organ function in experimental sepsis, but not in septic patients with adrenal insufficiency. These studies have allowed the design of selective dopaminergic agonists for treating inflammation and renal function in experimental sepsis [9]. Investigating the molecular mechanisms of neuromodulation may allow the design of novel pharmacological treatments. In line with this, studies on the HPA axis have allowed the design of glucocorticoids for treating inflammation, while studies on sympathetic regulation of the baroreflex system have allowed the design of selective β-adrenergic blockers for treating hypertension and arrhythmia. Accordingly, studies on vagal neuromodulation seem to support the design of specific α7nAChR-agonists to control inflammation in infectious disorders such as sepsis. Mechanistic studies on acupuncture and electroacupuncture are also providing vital information about the functional organization of the nervous system in the control the immune system and inflammation. These models of functional organization may help in designing novel therapeutic strategies where co-stimulating of different neuronal networks is intended to achieve the most effective control of inflammation. As such, mechanistic studies of transdermal stimulation of sensory afferent nerves are important in the development of new non-invasive techniques for nerve stimulation. Consequently, although many unanswered questions remain (see Outstanding Questions and Box 3), nerve stimulation is emerging as a promising medical area that may provide clinical advantages for treating inflammatory disorders such as arthritis or sepsis, and to prevent systemic immunosuppression and susceptibility to secondary infections.

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at https://doi.org/10.1016/j.molmed.2017.10.006.

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