Chapter 4

Gateway Reflex: A Neuro-Immune Crosstalk for Organ-Specific Disease Development

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Abstract

Homeostasis of the central nervous system (CNS) is strictly regulated by a unique structure of blood vessels, the blood-brain barrier (BBB). Experimental and clinical evidence has revealed that abnormalities in the BBB in chronic inflammatory diseases such as multiple sclerosis (MS). By using an animal model of MS, we identified novel neuro-immune crosstalk to explain how pathogenic immune cells enter the CNS to disrupt its homeostasis, a phenomenon we named the gateway reflex. Regional neural inputs such as gravity, electricity, pain or chronic stress cause specific neural activation to create a gateway of immune cells, particularly pathogenic ones, at specific blood vessels. Moreover, the recently discovered stress-induced gateway reflex uncovered a stress-induced neural link between the brain, gastrointestine, and heart. Thus, the gateway reflex is critical for the homeostasis of various organs, and aberrant activation of neural pathways by the gateway reflex disrupts normal organ homeostasis. The inflammatory reflex is another mechanism for local neuro-immune interactions. It potently exerts a cholinergic anti-inflammatory effect on various disease conditions. In this section, we discuss emerging roles for local neuro-immune interactions, with a special focus on the gateway reflex.

Keywords: gateway reflex, experimental autoimmune encephalomyelitis, central nervous system, chemokines, pathogenic CD4+ T cells

1. Introduction

A variety of environmental stimulations such as light, temperature, sound, and so on activates specific neurons to trigger biological responses. Gravity is another stimulus on land animals. Without sufficient gravity stimulation, physical functions including bone mass and

muscle strength change, as observed in astronauts who stayed in the International Space Station [1, 2]. In addition to these physical stimulations, various events in daily social interactions associated with psychological alterations including anxiety, mental stresses or positive emotions are also stimulatory factors that trigger specific neural pathways to affect body functions. A well-studied mechanism to cope with these stimulations includes the release of glucocorticoids via the hypothalamus-pituitary-adrenal grand (HPA) axis, which systemically changes various physiological functions including the immune system [3, 4]. Besides this systemic regulation, we and other groups have identified local regulations of the inflammatory status by various environmental cues that cause specific neural activations. Here we summarize examples of specific neuro-immune interactions in organ homeostasis.

2. Main text

2.1. Gravity gateway reflex

The central nervous system (CNS) is considered an immune privileged site due to structural protections by the blood-brain barrier (BBB) [5]. The BBB is formed by tight cell-cell interactions between blood endothelial cells and tight liner sheets, a structure that lies outside the basement membrane and consists of pericytes, neurons, microglias, and astrocytes. Tight junctions by the interactions of tight-junction molecules including claudins and occludins are critical for the cell-cell interactions in the BBB that sequester cerebrospinal fluid from circulating blood components [6]. However, despite the BBB, there exist a certain number of immune cells in the CNS to prevent brain tumors and viral infections. In pathological conditions of the CNS, excessive immune cells from the blood accumulate from the breached BBB to cause chronic inflammatory diseases including multiple sclerosis (MS). It is also known that brain micro-inflammation is associated with neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease [7]. Inflammatory cytokines including IL-1β, IL-17A, IFNγ and TNFα increase the BBB permeability, while chemokines recruit immune cells from the blood to cause inflammation [8–10]. However, where and how local CNS inflammation is induced in the BBB is poorly understood.

MS is a chronic inflammatory disease of the CNS. It is characterized by impairments in sensory, motor, autonomic, and neurocognitive functions due to autoimmune-mediated demyelination in CNS nerves [11]. Genetic factors strongly contribute to the MS pathogenesis, and genome-wide association studies (GWAS) have revealed that certain alleles of major histocompatibility complex (MHC) class II genes including HLA-DRB1*15:01 and HLA-DRB1*13:03 and genes involved in CD4+ T cell activation and homeostasis such as IL-2Rα and IL-7R are genetically associated with MS development [12–15]. These genetic results strongly suggest that autoreactive CD4+ T cells play a vital role in the MS pathogenesis and are supported by animal models of MS including experimental autoimmune encephalomyelitis (EAE) [16–19]. Using an adaptive transfer model of EAE [20], we investigated how and where myelin-autoreactive pathogenic CD4+ T cells initially invade the CNS. Whole-mount sections of adult mice with pathogenic CD4+ T cell transfer sacrificed just before the onset of

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EAE symptoms revealed that pathogenic CD4+ T cells mainly accumulated at the L5 spinal cord, around the dorsal vessels in particular [18]. On the other hand, no accumulation was observed in the brain or upper spinal levels at the preclinical time point of EAE. Various chemokines including CCL20 attract type-17 CD4+ T (Th17) cells, which are known to have a key pathogenic role in EAE [21, 22], to accumulate more in the dorsal vessels of L5 than L1 cord. Indeed, CCL20 neutralization or deficiency of its receptor, CCR6, on pathogenic CD4+ T cells abrogated the cell accumulation in the L5 spinal cord [18].

Interestingly, even without EAE induction, chemokine levels were higher in the L5 dorsal vessels than in the L1 cord. These results suggest a distinct property of L5 dorsal vessels both in the presence and absence of pathogenic CD4+ T cells. The L5 spinal level has the largest dorsal root ganglion (DRG) in both human and mice, and it is known that sensory neurons in the L5 DRG distribute to the soleus muscles, the main anti-gravity muscles of the body [23, 24]. These facts led us to hypothesize a link between gravity, L5 vessels and local inflammation. We examined this possibility using a ground experiment employed by the National Aeronautics and Space Administration (NASA) and Japan Aerospace Exploration Agency (JAXA) [25, 26]. The tail suspension method puts mice in a handstand position, such that the hind limbs are released from gravity stimulation. As hypothesized, after the tail suspension, chemokine expressions of the L5 dorsal vessels were reduced and pathogenic CD4+ T cells hardly invaded the L5 cord. Chemokines were instead upregulated at the cervical cords as though another gateway for immune cells was formed by the greater gravity stimulation on the arm muscles imposed by the tail suspension [18]. Consistently, the tail suspension significantly reduced the expression of a neural activation marker, c-Fos, in the L5 DRG, suggesting a correlation between regional neural activation and local inflammation in the CNS. Consistently, stimulation of the soleus muscles by weak electric pulses, which mimic gravity-mediated sensory activation, during tail suspension restored chemokine expression, pathogenic CD4+ T cell accumulation, and c-Fos levels at the L5 dorsal vessels [18]. That study identified L5 dorsal vessels as a blood vessel gateway for immune cells including pathogenic CD4+ T cells to the CNS, and revealed that regional sensory neural activation by gravity affects CNS homeostasis and causes local inflammation at L5 dorsal blood vessels when CNS-autoreactive pathogenic CD4+ T cells are present. This phenomenon, which represents a novel neuro-immune interaction, was termed the "gateway reflex" [27–34]. Since then, we have found various types of gateway reflexes depending on the neural stimulation. Thus, the abovementioned example is known as the gravity gateway reflex (**Figure 1**).

How the regional neural pathway by gravity stimulation regulates chemokine expressions in L5 blood vessels is an important question. Contribution of the autonomic nervous system was suggested. In fact, neural activation examined by c-Fos expression was higher in sympathetic ganglions at the L5 cord than at the L1 cord. In addition, the blood flow speed of the L5 dorsal vessels but not of other blood vessels including the L1 dorsal vessels, femoral artery or portal vein, slowed after tail suspension. Functionally, the treatment of mice with β-adrenergic receptor antagonists or chemical sympathectomy inhibited chemokine expressions, the accumulation of pathogenic CD4+ T cells at the L5 dorsal vessels, and the clinical scores of EAE [18]. Norepinephrine is one of the major neurotransmitters of sympathetic nerves. Indeed, in vitro experiments suggest that norepinephrine enhances chemokine expressions from

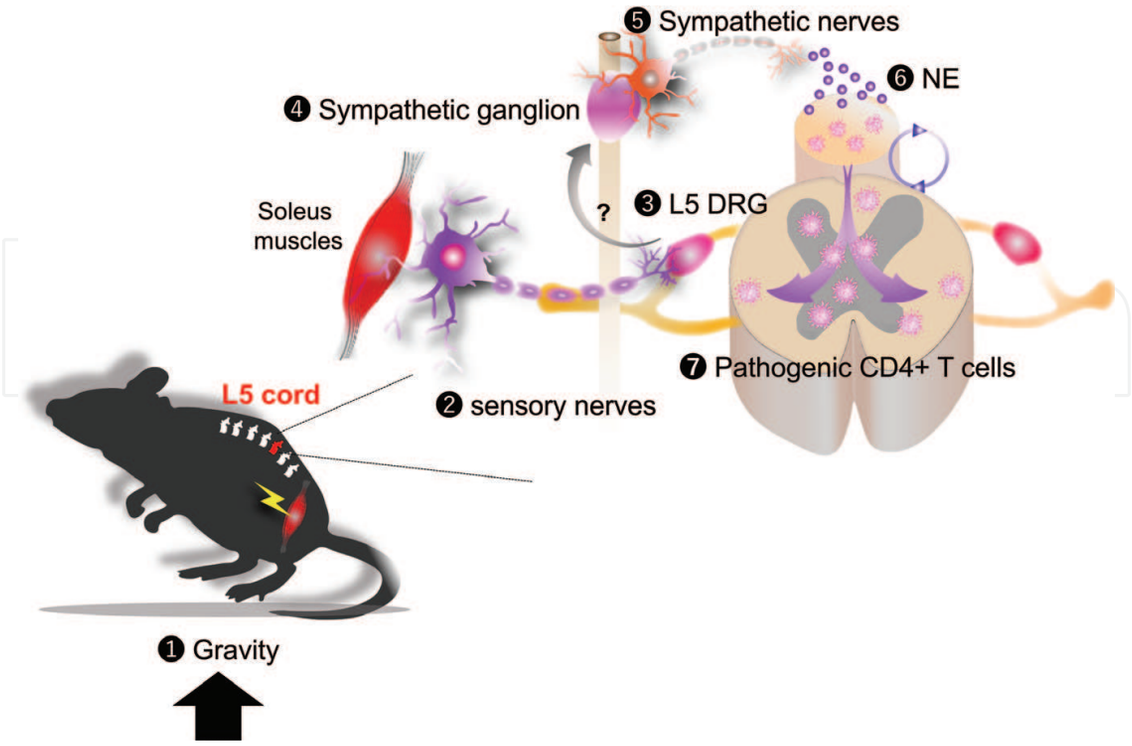

Figure 1. Gravity gateway reflex. Stimulation of the soleus muscles by gravity (1) induces the activation of specific sensory nerves (2). The cell bodies of these sensory neurons are located at the dorsal root ganglion (DRG) of the fifth lumbar (L5) spinal cord (3). Neural activation via the L5 DRG neurons travels to the L5 sympathetic ganglion (4) and induces the activation of sympathetic nerves (5), which results in norepinephrine (NE) secretion (6) at the L5 dorsal vessels. NE enhances the inflammation amplifier in the L5 dorsal vessels, causing an upregulation of chemokines and recruiting pathogenic CD4+ T cells from the vessels (7).

endothelial cell lines with activation of the inflammation amplifier [18]. The inflammation amplifier is a molecular mechanism that operates in nonimmune cells including endothelial cells to produce a large amount of pro-inflammatory mediators including chemokines, cytokines and growth factors upon the concomitant activation of two transcription factors, NF-κB and STAT3 [35–37]. The co-activation of NF-κB and STAT3 by cytokines such as IL-17, IL-6, and TNFα augments NF-κB activity and upregulates NF-κB-target genes including chemokines, which can be further enhanced by neurotransmitters including norepinephrine and ATP (**Figure 2**) [18, 38]. The contribution of nonimmune cells to chronic inflammation via the inflammation amplifier has been found significant in the development of various disease models and evidence of inflammation amplifier activation has been observed in patients with MS, rheumatoid arthritis, atherosclerosis, and chronic rejection after lung transplantation [39–45]. Thus, the gateway reflex represents novel local neuro-immune communication involving the activation of specific sensory and sympathetic neurons for the formation of blood vessel gateways (**Figure 1**). Because chemokine levels are constantly higher at the L5 dorsal vessels under normal conditions without EAE induction, the gateway reflex could have a physiological role as well. Indeed, it is reported in mice that after a learning task, CD4+ T cells, but not CD8+ T cells, accumulated in the CNS, and mice devoid of CD4+ T cells showed impaired learning performance and neurogenesis in the hippocampus [46–48]. The gravity gateway reflex may be utilized by these cells to enter the CNS to maintain and/or control the homeostasis and function of the CNS.

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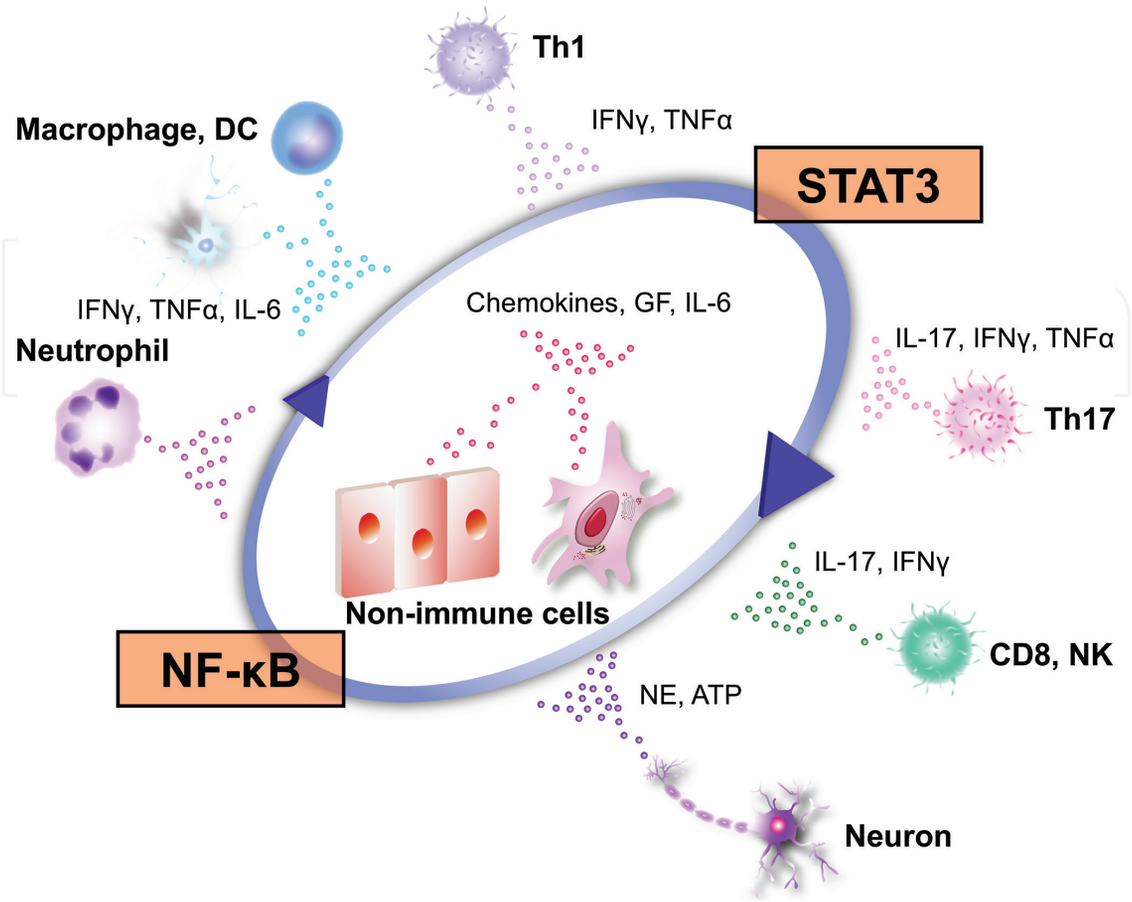

Figure 2. Inflammation amplifier. Co-activation of the transcription factors NF-κB and STAT3 in nonimmune cells such as endothelial cells and fibroblasts induces a synergistic effect on the production of inflammation factors such as chemokines, growth factors, and IL-6. Various factors activating NF-κB and STAT3 including IL-17, TNFα, and IL-6 can drive the amplifier. Secreted IL-6 is thought to act on nonimmune cells to form a positive feedback loop for this synergistic effect. Massive production of chemokines and growth factors by the inflammation amplifier play an essential role in the pathogenesis of many inflammatory diseases. DC, dendritic cells; GF, growth factors; NK, natural killer cells; NE, norepinephrine; Th, helper T cells.

2.2. Electric gateway reflex

The gateway reflex is not specific to the soleus-L5 axis because electrical stimulation of regional sensory neurons in different muscles can induce the formation of gateways in the dorsal vessels of different spinal cord levels. For instance, electric stimulation of the quadriceps, which are controlled by the L3 DRG neurons, induces chemokine expressions at the L3 dorsal vessels. Similarly, electric stimulations of the triceps upregulate chemokines at the dorsal vessels of the cervical to thoracic spinal cords (**Figure 3**) [18]. These results suggest that the electric gateway reflex can be artificially controlled, raising a possibility for a therapeutic application of the gateway reflex to various CNS diseases like MS and brain tumors.

2.3. Pain gateway reflex

We have examined whether other sensory stimulations can also generate the gateway reflex. We focused on pain because it is a tonic sensory stimulation [49, 50] and associated with various diseases that significantly compromise quality of life [51]. Some studies suggest that

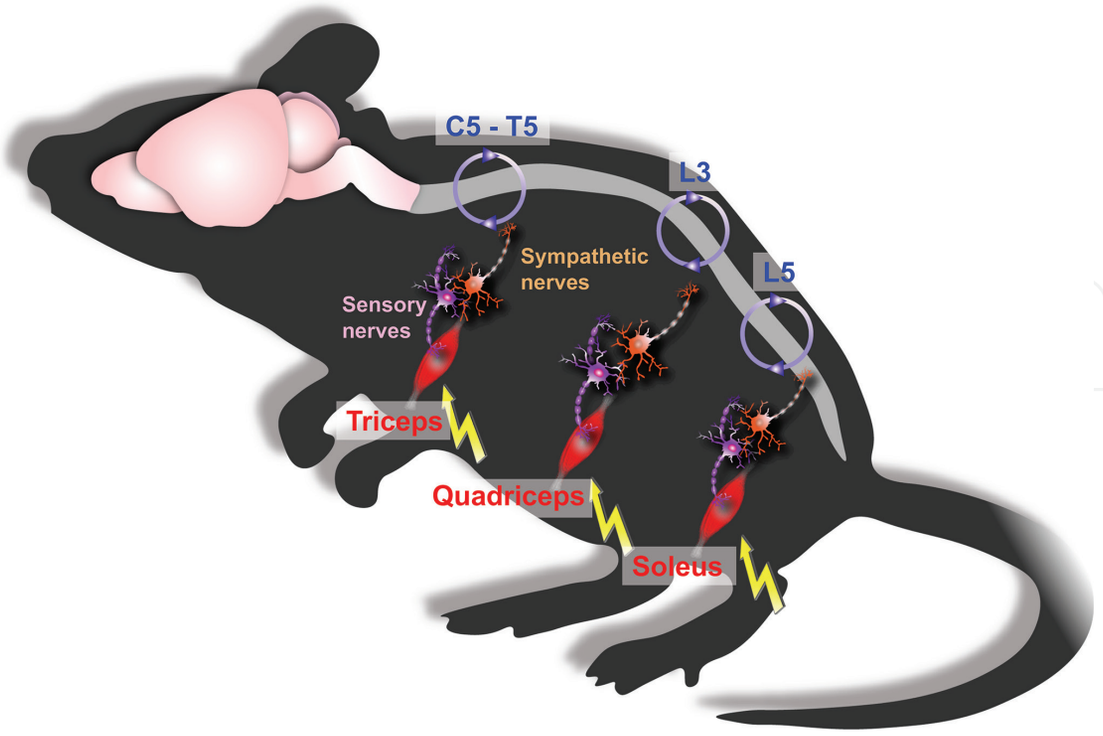

Figure 3. Electric gateway reflex. Electric stimulation to the triceps induces chemokine upregulation at the dorsal vessels of the fifth cervical (C5) to fifth thoracic (T5) spinal cord through sensory-sympathetic neural activation, which leads to activation of the inflammation amplifier at the C5-T5 dorsal vessels. In a similar fashion, electric stimulation of the quadriceps and soleus muscles induces a gateway at the L3 and L5 dorsal vessels, respectively.

pain occurrence is positively correlated with disease severity in MS [52–54], and mechanical allodynia and thermal hyperalgesia are induced in mice during EAE [55, 56]. In the adoptive transfer EAE model we used, recipient mice that received myelin-specific pathogenic CD4+ T cells developed transient paralysis, but then recovered and hardly relapsed even 100 days later. To examine how pain affects EAE symptoms, we induced pain sensation in mice by partial ligation of the middle branch of the trigeminal nerves, which are composed of sensory nerves alone [57]. Pain induction with pathogenic CD4+ T cell transfer significantly worsened the paralysis. By contrast, pain medicines improved EAE development [19]. Thus, pain is not simply an alert of the disease or injury status, but it also has a pathogenic role, triggering EAE relapse. Since a large part of MS patients show relapse and remission and pain is more frequently claimed by MS patients with higher disease scores [52, 53], we examined the effects of pain induction in mice that had recovered from EAE (EAE-recovered mice). As expected, EAE-recovered mice showed a clear sign of relapse after the partial ligation of the trigeminal nerve or injection of pain-inducing chemicals such as substance P and capsaicin [19]. As described above, under normal conditions, the blood vessel gateway for immune cells is the L5 dorsal vessels due to the effect of gravity [18]. To identify specific blood vessels that act as a gateway for immune cells that cause the pain-induced relapse, immunohistological examination of the CNS from EAE-recovered mice was performed. Although the motility of EAE-recovered mice was not significantly different from normal healthy mice, the meningeal region of the L5 cord of EAE-recovered mice contained a high number of periphery-derived

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monocytes that expressed high levels of MHC class II [18]. These MHC class II high monocytes distributed around the meningeal region of the L5 cord in EAE-recovered mice. After pain induction, however, these cells accumulated at the ventral vessels of the L5 cord bilaterally. Experimental evidence that (1) norepinephrine signaling could be detected around the L5 ventral vessels, (2) MHC class II high monocytes expressed chemokine receptor CX3CR1, and (3) MHC class II high monocytes secreted chemokine CX3CL1, a ligand for CX3CR1, after norepinephrine stimulation suggested an auto/paracrine loop is responsible for the accumulation of MHC class II high monocytes around the L5 ventral vessels via norepinephrine regulation of the CX3CL1-CX3CR1 axis. Because freshly isolated MHC class II high monocytes from EAE-recovered mice have autoantigen (MOG)-presenting capacity without additional peptide loading, it can be suggested that MHC class II high monocytes accumulated around the L5 ventral vessels would activate pathogenic CD4+ T cells through autoantigen presentation, followed by regional inflammation and disease relapse. Suppression of norepinephrine signaling by a β1 blocker or sympathetic nerve ablation by chemical sympathectomy inhibited the pain-mediated accumulation of MHC class II high monocytes around the L5 ventral vessels [19]. These results identified the L5 ventral vessels as the gateway for pain-induced relapse in the EAE model (**Figure 4**). This pain gateway reflex is the third example of the gateway reflex following the aforementioned gravity and electric examples.

2.4. Stress gateway reflex

Chronic stresses deteriorate illness, an effect attributed to the proverbs "Illness starts in the mind" and "Care killed the cat." Chronic stress conditions often cause gastric and intestinal diseases via the brain-gut axis. Although these diseases are well known and often experienced, the underlying molecular mechanisms remain to be elucidated. Stresses generate neural activations involving multiple brain areas such as the paraventricular nucleus (PVN), dorsomedial nucleus of hypothalamus (DMH), dorsal motor nucleus of the vagal nerve (DMX), and vagal nerve pathway [58]. We therefore hypothesized that chronic stresses might induce a specific gateway reflex. Indeed, experiments confirmed the stress gateway reflex. We serendipitously found that micro-inflammation induced by the stress gateway reflex activates an otherwise resting neural circuit to enhance a stress response that causes fatal gastrointestinal and heart failure in mice [38]. Therefore, the stress gateway reflex may explain the mechanisms for the abovementioned proverbs, as described below.

The gravity gateway reflex directs donor pathogenic CD4+ T cells to the dorsal vessels of the L5 spinal cord under normal conditions (**Figure 1**). However, under chronic stress conditions, the cells instead invade specific blood vessels of the boundary area of the third ventricle (3V), thalamus, and part of hippocampus [dentate gyrus, (DG)] to establish micro-inflammation. Therefore, chronic stresses can alter the location of the immune cell gateway from the L5 dorsal vessels to specific vessels in the brain. Immunohistochemistry of c-Fos revealed that the resulting micro-inflammation led to enhanced neural activations in the PVN and DMH, which are stress sensing areas of the hypothalamus. Neural tracing experiments identified direct connections via noradrenergic neurons between the PVN and the specific vessels of the boundary area of 3V, thalamus and DG. Moreover, these experiments showed direct

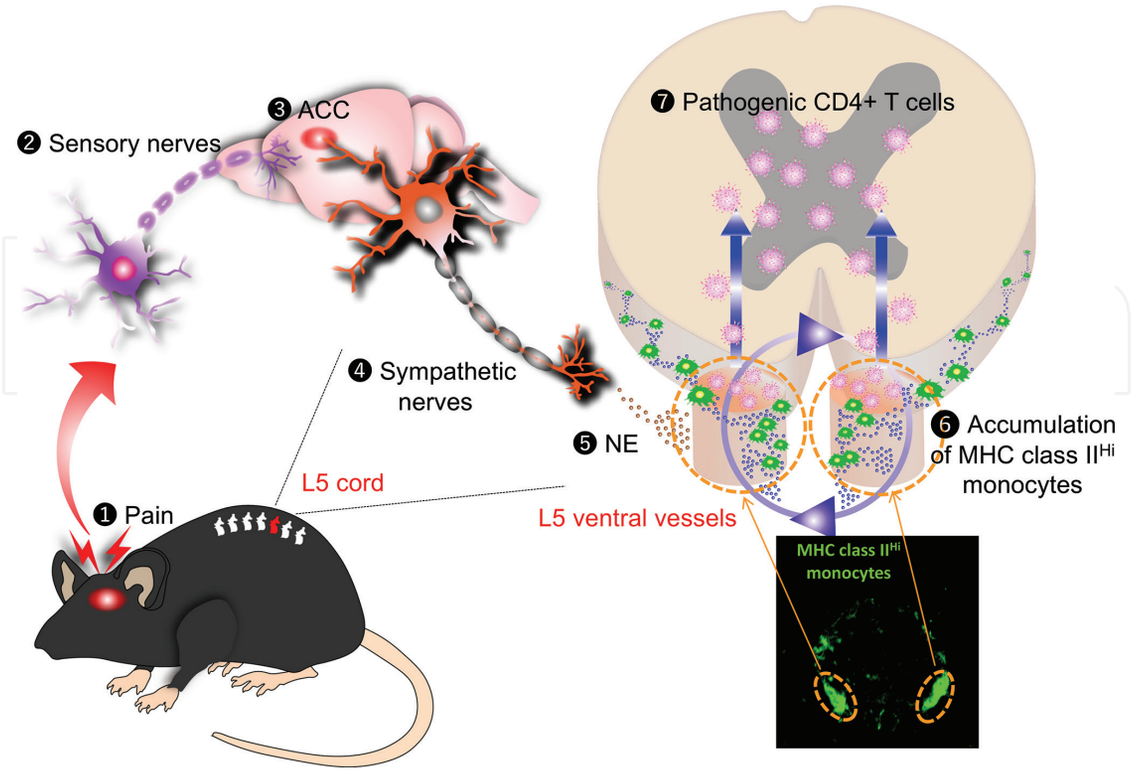

Figure 4. Pain gateway reflex. Pain induces nociceptive sensory nerve stimulation (1, 2), leading to activation of the anterior cingulate cortex (ACC), a pain-processing area of the brain (3). Specific sympathetic nerves are then activated (4) to induce norepinephrine (NE) release around the ventral vessels of the spinal cords (5). NE around the L5 ventral vessels induces the production of chemokine CX3CL1 from MHC class II high (MHC class II^{HI}) monocytes, further recruiting these cells in an auto/paracrine manner (6). MHC class II high monocytes are able to present myelin autoantigens to activate pathogenic CD4+ T cells, leading to disease relapse (7).

connections via nonnoradrenergic neurons between the specific vessels and the DMH. Since the PVN is a key orchestrator of stress signals, its activation is expected to affect specific blood vessels via a new noradrenergic neural pathway. Indeed, the expression of chemokines such as CCL5 was upregulated at specific vessels in mice with chronic stress alone. CNS-reactive pathogenic CD4+ T cells in the blood circulation of the stressed mice sensed the upregulation of CCL5, caused micro-inflammation with peripheral derived MHC class II monocytes at specific vessels of the boundary area of the 3V, thalamus, and DG [38]. ATP is released during an inflammatory response, but it also acts as a neurotransmitter [59, 60]. The injection of an ATP receptor antagonist at specific blood vessels of the boundary area of the 3V, thalamus and DG suppressed both neural activation in the DMH and the mortality rate of EAE mice with chronic stress. Importantly, the direct injection of ATP or cytokines at these specific vessels, which mimicked the micro-inflammation, induced fatal gastrointestinal dysfunction in stressed mice without EAE induction. These results suggest that brain micro-inflammation at specific vessels of the boundary area of the 3V, thalamus, and DG activates a resting neural pathway through ATP production, thus strongly enhancing the stress response to cause fatal gastrointestinal damage via the DMX and vagal nerve activation. These results uncovered the stress gateway reflex acting as a direct link between micro-inflammation at a particular site in the brain and gastrointestinal homeostasis (**Figure 5**) [38].

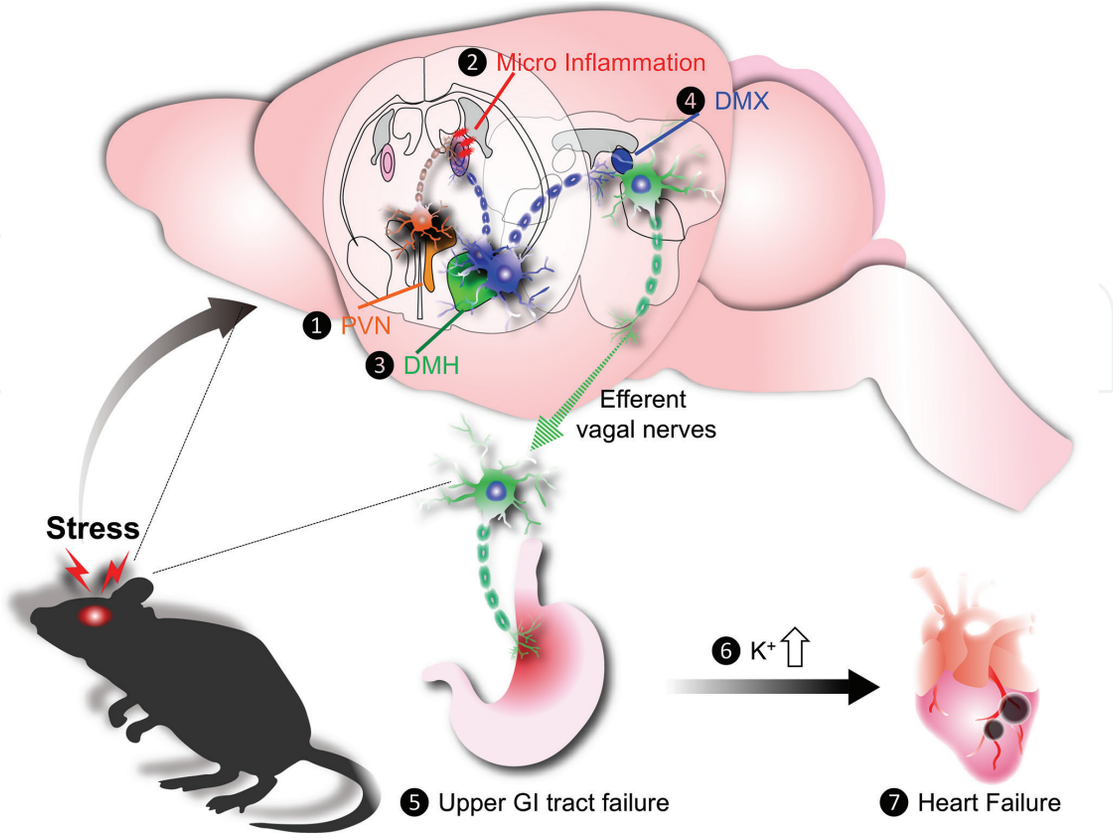

Figure 5. Stress gateway reflex. Chronic stress induces activation of the paraventricular nucleus (PVN) (1). We identified neural connections from the PVN to the specific vessels of the boundary area of the third ventricle, thalamus and dentate gyrus, which induces micro-inflammation around the specific vessels (2). The resulting micro-inflammation induces activation of a neural pathway that connects to the dorsomedial nucleus of hypothalamus (DMH) (3) and dorsal motor nucleus of the vagal nerve (DMX) (4), resulting in severe upper gastrointestinal (GI) tract failure via efferent vagal nerves (5). The increase of potassium ions (K^+) in blood circulation by the upper GI tract failure (6) explains at least in part heart failure associated with cardiac myocyte necrosis (7).

Several reports have shown the concurrence of inflammatory bowel diseases and MS [61–65]. Moreover, brain micro-inflammations were observed in patients with neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [66, 67], epilepsy [68], and psychological disorders [69]. We therefore suggest that brain micro-inflammations could regulate the homeostasis of organ functions including the brain itself by acting as a switch to stimulate resting neural pathways, which could account for the comorbidities observed in many diseases.

2.5. Cholinergic anti-inflammatory pathway

The Tracey laboratory has demonstrated using a mouse model of sepsis that the activation of vagal nerves, which mainly consist of parasympathetic nerves, suppresses an inflammatory response [70]. They revealed that lipopolysaccharide induces norepinephrine release in the spleen via vagal and splenic nerves. The norepinephrine stimulates a novel subset of CD4+ T cells that express β1/2 adrenergic receptor and produce acetylcholine. The resulting acetylcholine then acts on activated macrophages expressing α7 nicotinic receptor to suppress the

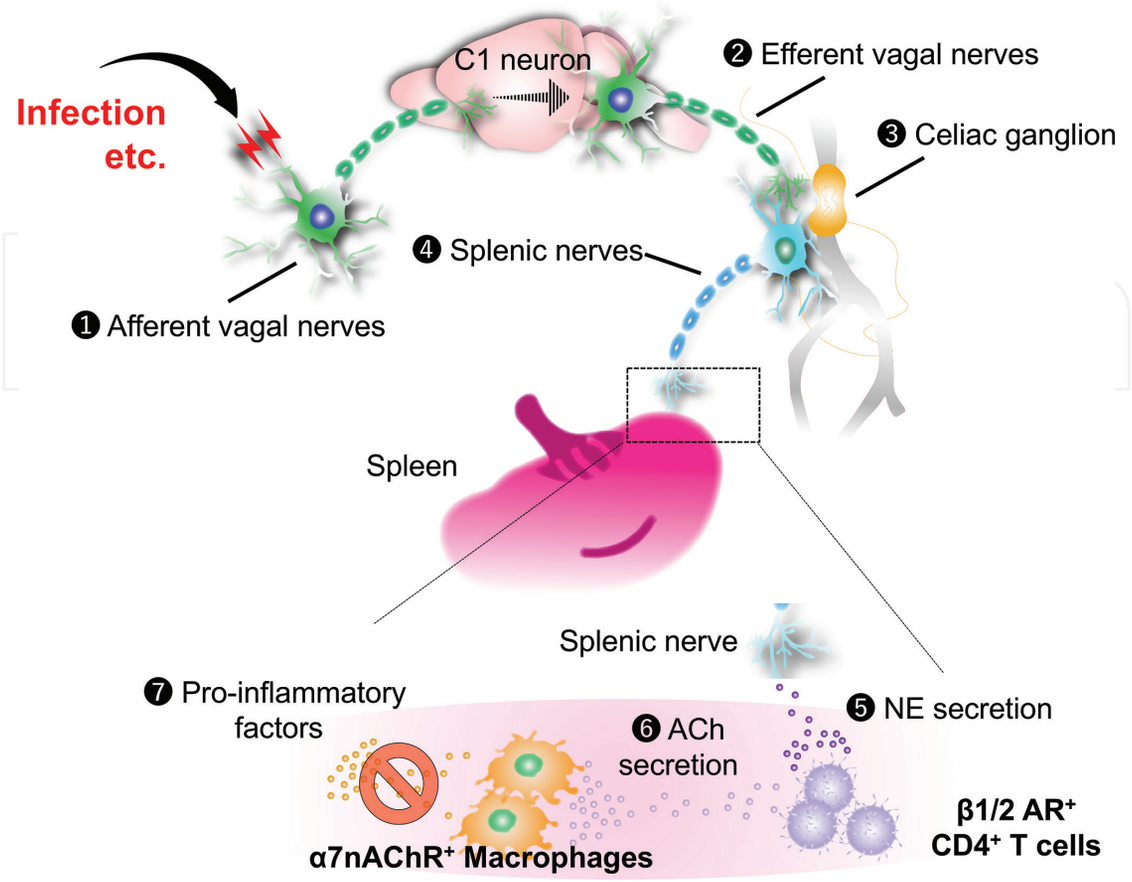

Figure 6. Inflammatory reflex. Afferent (1) and efferent vagal nerve (2) activation by infection, and so on induces neural activation in the celiac ganglion (3), followed by the production of norepinephrine (NE) by the splenic nerves (4). NE stimulates the release of acetylcholine (ACh) from a subset of CD4+ T cells expressing β1/2 adrenaline receptor (AR) (5). Then, ACh (6) acts on macrophages expressing $\alpha 7$ nicotinic acetylcholine receptor (α7nAChR) to suppress the expression of pro-inflammatory factors such as HMGB1 and TNFα. It is suggested that C1 neurons in the brain mediate the cholinergic anti-inflammatory effect.

expression of pro-inflammatory factors such as TNFα and HMGB1 (**Figure 6**) [71–73]. The stimulation of macrophages with nicotine inhibits NF-κB activation, but not MAP kinase activation in response to endotoxin [72]. This cholinergic anti-inflammatory pathway is called the "inflammatory reflex" [27–29, 31, 32, 34, 74–76]. In addition to infection, this pathway exerts anti-inflammatory effects in various disease models including renal ischemia–reperfusion injury, acute kidney injury, pressure overload-induced cardiac hypertrophy and neointimal hyperplasia [77–81]. The direct stimulation of C1 neurons in the medullary reticular formation induced this anti-inflammatory effect in mice [79], suggesting the involvement of C1 neurons between afferent and efferent vagal nerves. Activation of the cholinergic anti-inflammatory pathway can be induced by acupuncture and ultrasound, thus inhibiting the pathology of animal models [82, 83]. These findings establish a scientific basis for acupuncture and physical therapy. In addition, activation of the inflammatory reflex through vagal nerve stimulation by an implantable device has been tested in humans for chronic inflammatory diseases including rheumatoid arthritis with promising results [84, 85].

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2.6. Future directions

Accumulating evidence has demonstrated the significant effects of regional neuro-immune interactions on organ homeostasis, particularly during inflammation and diseases. The gateway reflex and inflammatory reflex can be induced by various stimulations to activate these interactions. Stimulation of the vagal nerves by an implantable device has already shown an anti-inflammatory effect in humans [84, 85]. The stimulation of the neurons responsible for the gateway reflex and inflammatory reflex at the body surface, as is the case with acupuncture, could lead to therapies that are less invasive and less costly, although elucidation of the precise neural circuits activated by the reflexes is required. Neural mapping is still challenging, but recent techniques and tools including the tissue clearing method CUBIC [86–89], optogenetics and chemogenetics [90, 91], and transgenic mice reporting neural circuitry and activations [92–94] will greatly contribute. Because neural circuits run throughout the body and because nonimmune cells as well as immune cells [18, 19, 71, 95–97] are able to secrete and respond to neurotransmitters, specific regional neuro-immune interactions such as the gateway reflex and inflammatory reflex bear potential as a significant therapeutic strategy to recover organ homeostasis.

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Conflict of interest

The authors have no conflict of interest for the article.

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