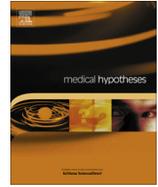




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## Chronic fatigue syndrome from vagus nerve infection: A psychoneuroimmunological hypothesis

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### ABSTRACT

Chronic fatigue syndrome (CFS) is an often-debilitating condition of unknown origin. There is a general consensus among CFS researchers that the symptoms seem to reflect an ongoing immune response, perhaps due to viral infection. Thus, most CFS research has focused upon trying to uncover that putative immune system dysfunction or specific pathogenic agent. However, no single causative agent has been found. In this speculative article, I describe a new hypothesis for the etiology of CFS: infection of the vagus nerve. When immune cells of otherwise healthy individuals detect any peripheral infection, they release proinflammatory cytokines. Chemoreceptors of the sensory vagus nerve detect these localized proinflammatory cytokines, and send a signal to the brain to initiate sickness behavior. Sickness behavior is an involuntary response that includes fatigue, fever, myalgia, depression, and other symptoms that overlap with CFS. The vagus nerve infection hypothesis of CFS contends that CFS symptoms are a pathologically exaggerated version of normal sickness behavior that can occur when sensory vagal ganglia or paragan-glia are themselves infected with any virus or bacteria. Drawing upon relevant findings from the neuropathic pain literature, I explain how pathogen-activated glial cells can bombard the sensory vagus nerve with proinflammatory cytokines and other neuroexcitatory substances, initiating an exaggerated and intractable sickness behavior signal. According to this hypothesis, any pathogenic infection of the vagus nerve can cause CFS, which resolves the ongoing controversy about finding a single pathogen. The vagus nerve infection hypothesis offers testable hypotheses for researchers, animal models, and specific treatment strategies.

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### Introduction

Chronic fatigue syndrome (CFS) is an often-debilitating state of constant intense exhaustion that is unmitigated by rest or sleep. A diagnosis of CFS is given in the absence of alternative diagnoses, and the United States Center for Disease Control definition of this syndrome is based entirely upon subjective symptom self-report [1,2]. Prognosis is poor [3]. The cause of CFS is unknown and is the source of considerable contentious debate. Previous studies of CFS patients have reported a diverse array of viral and even bacterial agents (e.g. [4–11]), as well as many immune system abnormalities (e.g. [12,13]). These findings have led most researchers to assume a role for pathogen-induced immune system activation in CFS. However, inconsistent and contradictory results between (and even within) studies have left the field at a loss to explain the causal mechanisms. No single pathogen has emerged as the common etiological agent.

In this article, I describe a hypothesis that integrates many of the general observations in CFS and explains some of the conflicting observations. Rather than continuing the search for one specific virus or bacteria as the root cause of CFS, this hypothesis focuses on the *location* of an infection, along the sensory (afferent) vagus nerve. The Vagus Nerve Infection Hypothesis (VNIH) of CFS is as follows: **While the sensory vagus nerve normally signals the body to rest when it senses a peripheral infection, that fatigue signal is pathologically exaggerated when an infection is located on the vagus nerve itself.** More specifically: Immune cells, including neuroimmune cells called glial cells, sense infection and launch the same basic neuroexcitatory response regardless of infection type. When the glial cells that envelop the sensitive vagus nerve are activated by any viral or bacterial infection, their neuroexcitatory secretions escalate afferent vagus nerve signaling, which is misinterpreted by the brain as evidence of a severe peripheral infection. The brain then initiates *sickness behavior*, which includes fatigue and many other CFS symptoms (see Key Terms Table). Because of the way that glial cell activation may persist in a pathological positive feedback loop (as it does in neuropathic pain conditions), these CFS symptoms can persist for many years.

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 Key Terms Table
 

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**Glial cell:** Neuroimmune cells that include astrocytes and oligodendrocytes in the central nervous system or satellite glial cells, Schwann cells, and enteric glial cells in the peripheral nervous system. Glial cells are in close proximity to nerve cells and release neuroexcitatory substances when they encounter a foreign pathogen. These substances include proinflammatory cytokines, glutamate, nerve growth factor, prostaglandin, nitric oxide, and reactive oxygen species

**Neurotropic virus:** A virus that has particular affinity for nerve tissue. Herpesviruses are neurotropic, frequently associated with CFS, and are characterized by their tendency to lay latent in nerve tissue until reactivated by stress or illness. CFS symptoms often begin following a period of stress or illness

**Paraganglia:** Ganglia of the sensory vagus nerve that are embedded in or near most trunk organs. These immunoprivileged and glia-rich sites are potential sites for viral infection to cause glial signaling of the vagus nerve

**Proinflammatory cytokine:** A class of neuroexcitatory innate immune system proteins that includes IL-1beta, IL-6 and TNF-alpha. Proinflammatory cytokines are released locally by immune cells, including glial cells, when these cells encounter a pathogen

**Sensory vagus nerve:** The afferent division of the tenth cranial nerve. The sensory vagus nerve innervates every major trunk organ, especially tissues that are likely to contact pathogens. It is sensitive to proinflammatory cytokines, and upon contact signals the brain to begin sickness behavior

**Sickness behavior:** Involuntary behavioral changes, such as fatigue, that are triggered by innate immune system activation. Sickness behavior is brain-based and triggered by cytokine signaling of the vagus nerve. The vagus nerve infection of hypothesis states that CFS is a pathological version of normal sickness behavior (see Table 1)

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The study of phenomena – such as sickness behavior – that sit at the intersection of behavior, brain biology, and immunology, is a relatively new field of study known as *psychoneuroimmunology* [14]. Because psychoneuroimmunology spans several scientific domains, and readers may not be familiar with them all, I will give ample background for each. To understand the VNIH, one must understand each part of the connection among behavior (“psycho-”), the nervous system (“-neuro-”) and the innate immune system (“-immunology”). In this speculative article, I will begin with a discussion of neurotropic viruses as a model pathogen for CFS, and explain how an active virus can trigger a localized immune response. I will then describe how one class of molecules, proinflammatory cytokines, turns this local immune response into an organism-wide immune response, which includes involuntary behaviors such as fatigue. I will explain the vagus nerve’s vital role in this process, which is the crux of the VNIH. I will then use existing neuropathic pain literature as a template for explaining how an infection on the vagus nerve could lead to ongoing CFS symptoms. Finally, I will suggest how the VNIH of CFS might be empirically evaluated with patient studies and animal models, and I will also describe potential treatment strategies.

A caveat: Because fatigue and many other symptoms associated with CFS are part of the general innate immune response to infection, and because there are currently no definitive diagnostic tests for CFS, it is unlikely that all CFS cases have the same etiology. Thus, the VNIH is not intended to be an all-inclusive explanation for every case of

intractable fatigue. Rather, I merely intend to hypothesize a mechanism by which many – and possibly most – cases of CFS may arise.

### Neurotropic viruses

The association of many different types of infection with CFS is currently an inconsistency in the literature. These seemingly conflicting findings may instead provide evidence of a chronic neuroimmune activation (described in more detail in later sections) that can be caused by any pathogen, including viruses or bacteria. The suggestion that the location of infection matters more than the specific infection type is at the core of the VNIH of CFS. However, neurotropic viruses are the type of pathogen most commonly associated with CFS. Because the VNIH of CFS is based upon the infection of nerve tissue, this is likely not a coincidence: neurotropic viruses are characterized by their affinity for invading neural tissue, especially afferent sensory nerves [15]. As a large and widely permeating afferent sensory nerve that highly innervates the organs that are most likely to come into contact with foreign pathogens, the afferent vagus nerve and associated glial cells are prominent targets for neurotropic virus infection and the subsequent general immune response. I will briefly review some relevant information about neurotropic viruses, however it is important to point out that those viruses and bacteria which are not classically considered to be particularly neurotropic could actually be the cause of CFS if they infect the vagus nerve.

Neurotropic viruses implicated in CFS include the eight human herpesvirus types [16], especially human herpesvirus type 6 (HHV-6) [4,7,10,17], and HHV-5 (cytomegalovirus) [5]. Although it is immunotropic more often than neurotropic (it can be both, and the vagus nerve directly synapses with immune cells), HHV-4 (Epstein-Barr virus) is also commonly associated with CFS [10,18,19]. Herpesviruses are characterized by their ability to become latent, especially in the ganglia of nervous and lymphoid tissues [20]. Even though initial infection may have occurred within the first 10 years of life [15], neurotropic viruses such as herpesvirus can be reactivated even in the healthiest adults [21]. As these viruses tend to remain latent until reactivation during stress or illness, it follows that CFS patients usually report that their symptoms began during a period of stress or with a normal cold or flu [22].

While latency tends to occur within nerve tissue, upon reactivation, the viral infection spreads to the extracellular space. There, satellite glial cells envelop the viral particles [15]. These satellite glial cells proliferate and activate, releasing neuroexcitatory mediators such as immune proteins called *proinflammatory cytokines*, and other substances which are described below [23,24]. The release of proinflammatory cytokines is a general response by glia and other immune cells like interleukin-producing cells (white blood cells) to encountering *any* virus or bacteria anywhere in the body. These locally-released cytokines are detected by the nearest sensory vagus nerve chemoreceptors, causing an afferent signal to the brain. The brain then initiates fatigue and several other symptoms that overlap with CFS (see Table 1). The premise of the VNIH of CFS is that when a neurotropic virus or any other pathogen infects *the vagus nerve itself*, cytokines are released directly onto sensitive vagus nerve receptors and this normal immune response becomes pathologically intense. Here, I will provide some background and detail to the general immune response and how it relates to CFS symptoms.

### Proinflammatory cytokines, the innate immune system, and sickness behavior

Over one hundred years ago, Kuniomi Ishimori, a Japanese physiologist, made an important discovery about the biological

**Table 1**

Many of the most fundamental chronic fatigue syndrome (CFS) symptoms are also proinflammatory cytokine-mediated aspects of the normally adaptive acute phase response and sickness behavior. Example citations are listed here.

Symptom	Part of acute phase response?	Proinflammatory cytokine mediated?	Common CFS symptom?
Fatigue	Yes [25]	Yes [25]	Yes [1]
Sleep architecture changes	Yes [14]	Yes [26–28]	Yes [1]
Fever	Yes [29,30]	Yes [25,26,31]	Yes [2]
Loss of appetite	Yes [32]	Yes [25,33,34]	Yes [2]
Musculoskeletal pains (myalgia)	Yes [14,35]	Yes [14,35]	Yes [1]
Hyperalgesia	Yes [14,35]	Yes [14,35,36]	Yes [37,38]
Cognitive impairments	Yes [30,39,40]	Yes [40–45]	Yes [1]
Depression/malaise	Yes [30]	Yes [46]	Yes [2]
Zinc depletion	Yes [14,47,48]	Yes [47]	Yes [49]

cause of fatigue. He extracted cerebrospinal fluid from sleep-deprived dogs and injected it into well-rested dogs, which promptly fell into a deep sleep (reviewed and translated in [50]). What Ishimori described as “a powerful sleep-inducing substance” (translated in [50], p. 519) is now known to be proinflammatory cytokines. In addition to being expressed in a circadian fashion to regulate normal sleep [51], proinflammatory cytokines are also part of the non-specific immune response to infection (for reviews see [24,42]). Proinflammatory cytokines are a class of immune signaling molecule that includes interleukins (IL) such as IL-1beta and IL-6 as well as tumor necrosis factor alpha (TNF-alpha). The word “interleukin” means “among white blood cells,” implying cytokines’ normal paracrine function: proinflammatory cytokines from the periphery can and do sometimes accumulate in blood at detectable levels and act upon the brain in an endocrine fashion, but are mostly paracrine and autocrine signalers [52,53]. This is an important point that will be revisited later: in the response to a localized infection, cytokines stay relatively localized and often do not enter the general circulation. The notoriously inconsistent cytokine studies in the CFS literature (e.g. [54–57]) often assay circulating cytokine levels in peripheral blood plasma, and may be failing to detect cytokines responding to a localized infection, for example an infection localized to a particular vagus nerve paranglia.

Vertebrate immune systems have two divisions: the *acquired* (or specific) and the *innate* (or non-specific) immune systems. The acquired immune system is the “antibody division” from which a pathogen-specific defense is mounted. For example, an antibody against HHV-6 would not recognize or combat a xenotropic murine leukemia virus-related virus (XMRV). In contrast, the innate immune system is the more evolutionarily ancient division and mounts the same general response, called the *acute phase response*, regardless of the specific invading pathogen.

When otherwise healthy individuals become sick with almost any form of illness or infection, they are likely to behave in predictable ways: they will stay in bed and, despite resting more than usual, they will still feel exhausted. They are likely to feel sore all over (referred to as *myalgia*), have a fever, and are unlikely to have the same healthy appetite or feel as mentally sharp as when they are not sick. The behavioral and motivational component of the acute phase response in humans and other complex organisms is called *sickness behavior* (for review, see [24,42]). Sickness behavior includes fatigue and is a brain-based, involuntary function of the immune response. Proinflammatory cytokine signaling of the vagus nerve is critical to the initiation of the acute phase response and sickness behavior, which subjectively feels like a less severe version of CFS but serves an important function.

Such behavioral aspects of the immune response occur because they are adaptive: they divert an organism’s energy resources away from motor activity, digestion, reproduction and cognition, and toward the immune response, in order to better cope with fighting pathogens [58,59]. However, these adaptive changes may

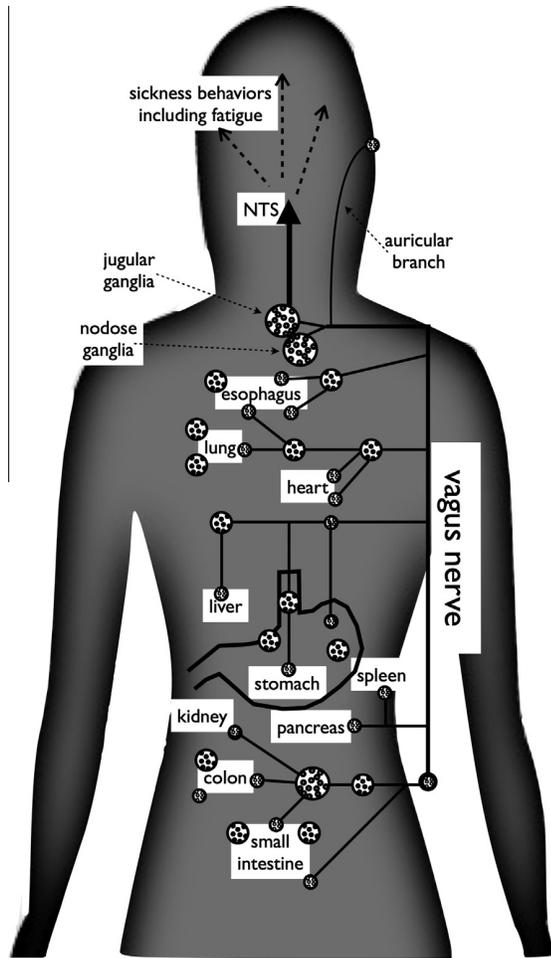
become pathological. As depicted in Table 1, there is a striking overlap between the set of behavioral changes called sickness behavior and the symptoms of CFS. The VNIH of CFS is based on the idea that CFS symptoms are an inappropriately strong and long-lasting expression of normally adaptive sickness behavior. Understanding the manner by which cytokines cause this behavior is the focus of the next section.

### The vagus nerve is a sensitive detector of proinflammatory cytokines

Given the fact that cytokines are produced locally at the site of an infection, how do they come to induce sickness behavior, which like all behavior is directed by the brain? As large, hydrophilic, polypeptide protein molecules, proinflammatory cytokines do not easily cross the blood–brain barrier to have their effect directly on the brain [24]. Instead, the immune system must act like a diffuse sensory organ that senses and then communicates the existence of peripheral infection to the brain [14]. One of the most important ways this is accomplished is when proinflammatory cytokines released at the site of a peripheral infection trigger a signal to the brain via the 10th cranial nerve, the *vagus nerve* [23,60].

Vagus nerve dysfunction has been found in CFS patients. The vagus nerve is a key means of communication for the parasympathetic nervous system. As such, the level of control that the parasympathetic nervous system exerts over the sympathetic nervous system is known as vagal tone. Vagal tone is often operationalized as the change in heart rate with respiration (referred to as respiratory sinus arrhythmia). CFS patients have abnormal vagal tone at rest [61], during head tilting [62–65], very mild exercise [66], and slightly more strenuous exercise (treadmill walking) [67]. The VNIH would contend that these findings are due to the vagus nerve’s role in cytokine signaling.

The word “vagus” means “wandering” in Latin: it is a long, highly branched nerve that travels throughout the viscera (see Fig. 1 for a highly simplified schematic of gross vagus nerve anatomy). Due to this anatomy, the vagus nerve is likely to encounter even localized proinflammatory cytokine responses. The sensory vagus nerve contains chemoreceptors that are sensitive to the presence of proinflammatory cytokines [68]. It innervates tissues that are often the initial contact points for foreign pathogens, such as the mucosa of the esophagus, gastrointestinal lining, lungs, and lymph nodes [23,68–71]. The vagus nerve also innervates most other important trunk organs such as the spleen, liver, heart, bladder, and pancreas [68,72,73]. In the vicinity of or often embedded in those target organs are vagus nerve *paranglia* [23,74], which are dense with proinflammatory cytokine chemoreceptors [75]. In fact, paranglia are found in most major branches of this highly branched nerve [76]. All of these factors maximize the chances for the vagus nerve to come into contact with a localized cytokine response. There is more anatomical evidence that the vagus nerve is



**Fig. 1.** A highly simplified schematic of vagus nerve anatomy. Circles represent ganglia and paraganglia, which contain both glial cells and sensory vagus nerve chemoreceptors. A viral or bacterial infection within any ganglia or paraganglia causes glial activation, leading to the release of proinflammatory cytokines and other neuroexcitatory mediators. The resulting afferent signal enters the brain at the nucleus tractus solitarius (NTS), and triggers sickness behaviors. When normal glial cell activation becomes pathological as it does in neuropathic pain conditions, the signal is intensified and intractable, leading to CFS.

evolved to be sensitive to small amounts of cytokine: as a key neuroimmune link, some vagal terminals form direct synapse-like connections with proinflammatory cytokine-producing lymphocytes [77]. An additional factor is the close proximity of the vagus nerve to another type of cytokine-producing cell: glial cells.

Glial cells (e.g. astrocytes and oligodendrocytes in the central nervous system or satellite glial cells, Schwann cells, and enteric glial cells in the peripheral nervous system) were once thought to be nothing more than scaffolding for neurons and nerves (*glia* is Greek for “glue”). Recent research demonstrates that this is far from the case, and that glia are a vital part of most, if not all, nervous system signaling [78,79]. It follows that glial dysfunction can be an important factor in disorders of the nervous system, and the VNIH postulates that pathogen-activated glial cells cause pathologically strong vagus nerve signaling to the brain. This pathological signaling occurs when pathogen-activated glial cells release neuroexcitatory substances such as proinflammatory cytokines, excitatory amino acids (e.g. glutamate), nitric oxide, nerve growth factor, reactive oxygen species, and prostaglandins [35,80] onto the vagus nerve’s sensory terminals. The VNIH of CFS advances the novel idea that, while normal immune cell cytokine signaling

leads to appropriate sickness behavior, inappropriate glial cell signaling can lead to CFS. Here, evidence of the vagus nerve’s involvement in sickness behavior is reviewed.

### Cytokine to vagus nerve to brain communication induces sickness behavior

When immune cells such as glial cells or monocytes detect a pathogen, they release proinflammatory cytokines. The sensory terminals of the afferent vagus nerve that detect those cytokines send a signal to the brain, synapsing in prominent ganglia such as the jugular (superior) and nodose (inferior) ganglia, and then entering the central nervous system at the nucleus tractus solitarius (NTS) in the medulla oblongata [81]. There is good evidence from animal research that this signaling pathway from proinflammatory cytokine to vagus nerve to brain is the cause of each aspect of sickness behavior listed in Table 1 (see also [23,53]). This is important for the VNIH of CFS because an infection anywhere along this pathway could cause the exaggerated sickness behaviors seen in CFS.

Sick animals that have had their vagus nerve cut do not “act” sick: rodent studies have demonstrated that the vagus nerve is critical for the expression of sickness behavior in response to peripheral infection [60]. In rats, injection of peripheral cytokines causes vagus nerve electrical activity [82,83] and increases the activity in the nodose ganglion [84]. Furthermore, when otherwise healthy rodents are injected with proinflammatory cytokines, pathogens, or lipopolysaccharide (LPS, a molecule that activates the immune system by mimicking foreign pathogens), they show the type of sickness behaviors that are seen in CFS. However, these responses are blocked or attenuated by transectioning the abdominal vagus nerve. This includes significantly reduced social interaction and exploration [84–86] and sleep stage architecture changes [26,87] as well as other responses relevant to CFS, such as fever and hyperalgesia in rat [53,88–90] and fever in guinea pig [91].

Under the experimental conditions discussed above, proinflammatory cytokines are in relatively very high circulating concentrations, modeling a response to a severe systemic infection. However, even at the relatively low concentrations of endogenous proinflammatory cytokines seen during a normal, more localized peripheral infection, the vagus nerve sends the message to the brain to involuntarily cease non-essential energy use, engaging in sickness behavior. So what would happen if, instead of sensing proinflammatory cytokines in low concentrations in the periphery, vagus nerve receptors were directly and ceaselessly bombarded with these cytokines? The symptoms of sickness behavior would be severe and intractable, and could occur even in the absence of evidence of peripheral infection, just like in CFS. Such a state requires two conditions to be met: (1) vagus nerve proximity to cytokine-producing cells, and (2) pathological overproduction of cytokines by those cells. In the following sections, I review evidence that (1) vagus nerve chemoreceptors are uniquely exposed to glial cell cytokine signaling and that (2) there is strong evidence from the neuropathic pain literature that cytokine production from glial cells can become pathological.

### The vagus nerve is enveloped in glia

The gross vagus anatomy described above maximizes sensitive chemoreceptors’ chances for contact with cytokines released in response to peripheral infection. The cellular anatomy of vagus ganglia and paraganglia also makes the vagus nerve particularly sensitive to cytokine signaling from activated glia. The vagus nerve is densely enveloped in satellite glial cells [74], which produce proinflammatory cytokines and other neuroexcitatory mediators

when activated, and in each of the many vagal paraganglia are chemoreceptors for cytokines [75]. While vagal parasympathetic paraganglia are still not well characterized, they are thought to be fairly similar in structure to sympathetic paraganglia, featuring a very small (~20 nm) space between satellite glial cells and neurons, giving glia tight control over the paraneuronal space [74] and allowing for even minute quantities of proinflammatory cytokine released into this space to greatly increase relative concentrations available to vagal chemoreceptors. Given that these sensitive chemoreceptors can initiate sickness behavior after detecting relatively sparse proinflammatory cytokines released by circulating white blood cells, the concentrated cytokine response of activated glia within a paraganglion is quite likely to cause sickness behavior. The neuropathic pain literature offers a specific mechanism by which this normal signaling can become pathological, leading a normal sickness behavior response to become CFS.

### Neuropathic pain as a mechanistic model for dysfunctional glial cell signaling

Much progress has been made in elucidating the crucial role of glial cells' cytokine signaling in neuropathic pain states [35,80,92,93]. The VNIH simply contends that the same process that causes pathologically exaggerated pain in pain-transmitting nerves (such as virus infection in cranial nerve 5, the trigeminal nerve, leading to shingles) would cause pathologically exaggerated sickness behavior in the nerve that transmits the signal for sickness behavior (cranial nerve 10, the vagus nerve).

Types of neuropathic pain include hyperalgesia (exaggerated pain) or allodynia (interpreting non-painful stimuli as painful), which are normally adaptive mechanisms to protect a site of infection or injury. Infection can activate glial cells encapsulating synapses in the dorsal horn of the spinal cord, increasing postsynaptic sensitivity to incoming nociceptive information from the periphery. In neuropathic pain states, activated glial release of neuroexcitatory substances such as proinflammatory cytokines, glutamate, nitric oxide, nerve growth factor, reactive oxygen species, and prostaglandins leads to an amplified pain response and subjective hyperalgesia or allodynia of the individual [35,80]. It follows that release of these substances directly onto the afferent vagus nerve could lead to amplified sickness behaviors. In pain-transmitting nerves, there is a point at which the protective and adaptive pain function becomes pathological: intractable hyperalgesia or allodynia results when proinflammatory cytokine release operates as a feed-forward loop. For example, the release of IL-1 stimulates more IL-1, and activated glial cells tend to activate other glial cells [35,94]. This is a general property of glia and there is no reason to suspect that vagus nerve-associated glia would function differently than pain nerve-associated glia. Indeed, the neuropathic pain state of fibromyalgia and CFS are frequently confused or comorbid, and comorbidity may reflect a general predisposition to dysfunctional glial signaling. Thus, in hyperalgesia and allodynia in neuropathic pain as with sickness behavior in CFS, glial activation causes a normally adaptive and protective response to become a persistent and debilitating state. A normal signal in a pain-transmitting nerve leads to subjective pain. When that signal is enhanced by activated glia, it may lead to neuropathic pain. The VNIH then states that a normal signal in sensory vagus nerve leads to sickness behavior and when that signal is enhanced by activated glia it may lead to CFS.

In an elegant series of experiments characterizing the mechanisms by which central nervous system viral infection can lead to neuropathic pain, the Milligan, Maier, and Watkins group reported several findings that can be directly applied to the VNIH of CFS, and help us to account for several apparent inconsistencies

in the CFS literature (see Table 2 for a list of inconsistencies resolved by the VNIH). In a rat model, recombinant gp120, the glycoprotein of the human immunodeficiency virus-1 (HIV-1) viral envelope, was delivered by intrathecal injection at the level of lumbosacral spinal cord [95,96]. Gp120 is the component of HIV-1 that activates glial cells. From these studies, there are three major lessons relevant to the VNIH of CFS:

- 1. Not all cytokine responses that affect the central nervous system are measurable in blood.** Central nervous system viral infection leads to a proinflammatory cytokine response, caused by glial activation, which is measurable in the infected tissue and in cerebrospinal fluid sampled from near the site of infection. However, the proinflammatory cytokine response is *not* detectable in cerebrospinal fluid sampled at a distance from the site of infection or in peripheral blood. This general property is found elsewhere in the cytokine literature as well: for example, virus infection induced in mouse lung led to acute phase responses, and proinflammatory cytokine increases were found in lung lavage fluid but not peripheral blood [97]. This principle is essential to understanding why there are inconsistencies in cytokine studies of CFS patients (e.g. [57,98]): cytokines responding to local infection stay local. The cytokine profile of a given CFS patient would depend upon where along the vagal pathway the infection is, and whether blood or cerebrospinal fluid was analyzed. For example, if CFS were caused by a viral infection in one of the many abdominal vagal paraganglia that are near or embedded in their target organ or by an infection in the jugular (superior) or nodose (inferior) ganglia in the cervical carotid sheath, the cytokine response would likely not be detectable in cerebrospinal fluid and may or may not be detectable in peripheral blood. If CFS were caused by a viral infection within the NTS where the vagus nerve enters the brainstem, proinflammatory cytokines may or may not be detectable in cerebrospinal fluid, but would likely not be detectable in peripheral blood.
- 2. Cytokine profiles are dynamic.** Milligan et al. demonstrate why it may not be fruitful to focus on one particular cytokine or to attempt to find a "cytokine profile" for CFS diagnosis. The initial glia-mediated proinflammatory cytokine response to viral infection occurs in an interacting and dynamically timed cascade that changes hourly (cytokine-cytokine interactions are reviewed by Turrin and Plata-Salamán [52]). Furthermore, other studies have shown that even this complicated cascade of hour-to-hour changes has fluctuating rhythms. For example, in fibromyalgia patients as well as in healthy controls, cytokine profiles are characterized by ultradian bursts [99]. Add to that the fact that even in healthy individuals, cytokines have a circadian rhythm [100] and it becomes apparent that cytokine studies of single timepoint peripheral blood samples are likely to provide inadequate information. Many CFS patient studies have ignored these first two basic properties of cytokines: they are released locally, and their levels change in ultradian bursts within circadian rhythms.
- 3. Inhibiting glial cells can improve symptoms.** In the Milligan et al. model of perispinal infection, intrathecal injection of glial inhibitors attenuated virally-induced glial activation, proinflammatory cytokine response, and subsequent allodynia [95,96]. This is key to one potential treatment option for CFS patients, to be discussed in the treatment strategies section below.

### Implications of the hypothesis: research

The VNIH of CFS lends itself to modeling, testable hypotheses, and treatment strategies. Three main goals of related research

**Table 2**

A list of conflicting observations in the current chronic fatigue syndrome (CFS) literature and their resolution by the Vagus Nerve Infection Hypothesis (VNIH) of CFS.

Conflicting observation in CFS literature	VNIH resolution
Many different viruses and even bacteria have been associated with CFS [4–11] Cytokine studies are inconsistent [54–57]	Type of infection is less important than location because the same innate immune system response occurs regardless of infection type Location and severity of infection leads to different cytokine profiles in the cerebrospinal fluid and peripheral blood of individual patients [95–97] (see text for details)
The anti-HHV-6 and HHV-4 (Epstein-Barr) drug valganciclovir only helps some patients with elevated HHV-6 and HHV-4 antibody titers [101]	Antiviral drugs are not effective on infections within immunoprivileged sites such as vagal paraganglia [15]. These paraganglia are hypothesized to be the most likely location for CFS-causing infection
In general, antiviral drugs help some, but not all, patients [102]	In addition to being ineffective for intra-ganglia infections, antivirals are made for a specific type of virus. CFS can be caused by different virus types, or even by bacteria
Not all patients have the same symptoms [2]	Location of infection along the afferent vagus nerve pathway leads to different symptoms and levels of severity
Women are much more likely than men to have CFS, with reported female:male ratios of 2:1–6:1, depending on the clinical definition used [13,103–106]	The vagus nerve is a structurally and functionally sexually dimorphic organ [107–110]. The modulation by vagal afferents of sickness behavior-related hyperalgesia is sexually dimorphic [111,112]

should be experimental support for the VNIH, the development of diagnostic tools, and the development of treatments. Basic research in support of these goals should involve animal models as well as investigative patient studies. Researchers using animal models have the advantage of controlling the type, location, and severity of experimental vagus nerve infection. For example, Blessing et al. demonstrated that it is possible to conduct rat survival surgeries in which vagal ganglia are deliberately virally infected in a targeted fashion [113]. In that study, behavioral measures were not taken because the infections were very severe, causing significant swelling in the medulla and mortality within 3 days (personal communication with W.W. Blessing, October 10, 2011). Future studies should use a less debilitating viral load and should include behavioral measures of the sickness response. For example, initial studies could target prominent afferent vagus paraganglia and ganglia for experimental infection with active virus. After recovery, a forced-swim paradigm followed by measures of voluntary wheel running could serve as a model of post-exertional malaise. Rodents experiencing post-exertional malaise following forced swim would be expected to engage in significantly less voluntary wheel running. If this model works, it could be used to answer specific questions about CFS sequelae. For example, the VNIH would explain exaggerated post-exertional malaise as being the result of a normal post-exercise increase in proinflammatory cytokines [114,115] leading to an enhanced feed-forward loop of vagus nerve cytokine signaling. Therefore, one testable hypothesis would be increased vagus nerve electrical activity or increased NTS activity in vagal ganglia-infected rats after forced swim.

For reasons reviewed above, systemic measures in human CFS patients such as peripheral blood cytokine levels may not be particularly diagnostic or informative. With no blood test for CFS forthcoming, live human studies are difficult. The current gold standard of direct evidence to support the VNIH may be CFS patient cadaver studies consisting of immunohistochemical staining for activated glia, inflammation, and active virus infection within vagus nerve, its paraganglia and ganglia, or NTS. However, the most common marker for glial activation, glial fibrillary acidic protein (GFAP), may not be present in paraganglionic satellite glial cells [74]. Furthermore, given the likely difficulty finding suitable cadavers, the fact that CFS infection could be caused by any number of neurotropic viruses (some of which the majority of humans already harbor), and the difficulty of dissecting out all possible infection locations in the long and highly branched vagus nerve, other models and approaches should also be considered.

In patients, magnetic resonance imaging (MRI) after injection of gadolinium can be used to detect viral lesions in tissue within the central nervous system [116]. This can only be accomplished within the central nervous system because gadolinium contrasting

delineates a disruption of the blood–brain barrier and not a viral lesion per se. Live imaging of an infection in peripheral vagal paraganglia would be more difficult. In vivo electrophysiological recordings of vagus nerve are possible [117] but invasive. A new line of research should seek to develop novel protocols for resting-state and functional imaging of vagus nerve and brainstem NTS in CFS patients. In addition, the use of translocator protein radioligands in positron emission tomography (PET) imaging has shown promise as a method of imaging microglial activation in neurodegenerative disorder-induced neuroinflammation [118], and may prove valuable in CFS research. Such methods could provide both support for the general hypothesis and important information that could inform individual treatment strategies.

One significant ongoing barrier to human CFS research is the difficulty recruiting the most severely symptomatic patients, who are often unable to get out of bed on their own and who recognize that even the minor physical activity associated with traveling to a research facility would likely lead to a severe and sustained post-exertional malaise. Given normal individual differences in measures of vagal tone and immune physiology, studies attempting to contrast vagus function in mildly symptomatic patients versus controls may become underpowered. It is important for any CFS study to include patients with the most severe symptoms, and as such budgeting and IRB approval for home visits should be included in grant proposals when feasible.

### Implications of the hypothesis: treatment strategies

#### *Pharmacological, neurotherapeutic & surgical treatment strategies*

According to the VNIH of CFS, possible treatment strategies include glial inhibitors, specific antivirals, vagus nerve stimulation (VNS), and vagotomy. If infection-induced glial activation within vagus nerve is the central underlying cause of most CFS symptoms, then glial inhibitors could be a particularly effective treatment strategy. Glial inhibitors have shown promise as an adjunct medication for treating neuropathic pain states [119] and, given that some types have relatively minor side effects, use of glial inhibitors could become the standard treatment for CFS caused by CNS vagus nerve infection.

For example, ibudilast (also known as AV411 or MN166) inhibits glial production of proinflammatory cytokines via inhibition of a proinflammatory cytokine called macrophage-migration inhibitory factor (MIF) [120]. In a series of experiments, Alexander et al. demonstrated the crucial role of MIF in the establishment, severity, and duration of neuropathic signaling in pain transmitting nerves [121]. Given the mechanistic overlap between neuropathic pain

and the VNIH of CFS, their data demonstrate that an MIF inhibitor such as ibudilast could be an effective method for reducing pathological vagus nerve signaling. They found that MIF increased transcription of proinflammatory cytokines such as IL-1beta, IL-6, and TNF-alpha within rat microglia, and treatment with MIF inhibitor led to reduction of proinflammatory cytokine transcription within rat microglia. Furthermore, MIF led to localized structural plasticity and neuroexcitability in afferent pain transmitting spinal ganglia, and increased production of the neuroexcitatory gas, nitric oxide. In addition to acting as an MIF inhibitor, ibudilast also acts as a phosphodiesterase inhibitor to inhibit production of the proinflammatory cytokine TNF-alpha by glial cells [122]. TNF-alpha is a key proinflammatory cytokine in the initial cytokine cascade and acts synergistically with other proinflammatory cytokines [52], meaning that its inhibition will also inhibit the production and efficacy of other proinflammatory cytokines. Furthermore, blocking glial TNF-alpha increases uptake and metabolism of glutamate by glial cells [123], which would attenuate a direct mechanism of vagus nerve excitation, as the terminals and ganglia of vagal afferents contain glutamate receptors [124]. Ibudilast can also prevent viral activation of microglia [125], and is safe for human use. Ibudilast is already frequently prescribed in Japan as an anti-asthmatic [126] and in Australia it is undergoing clinical trials for use in neuropathic pain states. There are also several other general glial inhibitor drugs, such as minocycline, pentoxifylline and propentofylline, all with slightly different mechanisms but often with undesirable side effects. It is likely that, just as glial inhibitors are being combined with traditional opioids for treatment of neuropathic pain states, glial inhibitors may need to be combined with appropriate antivirals for effective treatment of CFS.

Even on their own, antivirals have shown some promise in treating select groups of CFS patients. For example, in patients with elevated HHV-6 and HHV-4 (Epstein-Barr) antibody titers, valganciclovir significantly improved fatigue symptoms in a majority of patients [101]. The lack of efficacy in some patients could reflect the fact that, after neurotropic viruses have been taken up into sensory ganglia, they are protected from antiviral drugs and antibodies [15]. It could also reflect the fact that the vagus nerve was not infected by the types of virus best treated by valganciclovir, but rather by a different pathogen. According to the VNIH of CFS, many different pathogens could cause CFS, making individualized medicine crucial for proper patient care. Identifying the specific infectious agent in each patient will be critical: giving antiretroviral drugs to someone whose symptoms are caused by a non-retrovirus such as HHV-6 will do more harm than good. If the VNIH of CFS proves to be accurate, individualized treatment should include tests for each patient to identify the particular virus(es) infecting them. This of course may prove challenging because most humans are infected with certain viral strains [127], so blood tests for these viral antibodies are likely to be positive. However, the specific location of infection rather than the mere presence of infection may be the causal factor for CFS. Future CFS research could borrow from tumor imaging research and use radiolabeled antibodies to localize clusters of specific virus types *in vivo*.

If more basic research supports the vagus infection hypothesis of CFS, VNS is another potential CFS treatment that may merit investigation. Traditional VNS is invasive and involves stimulation of the cervical branch of the vagus nerve within the carotid sheath. VNS has shown promise in conditions that overlap with CFS, such as depression [128] and chronic headache [129]. There is also some indirect evidence that VNS could treat symptoms related to an ongoing acute phase response. Borovikova et al. reported that VNS with acetylcholine reduced the systemic inflammatory response to LPS in rats, including reductions in circulating proinflammatory cytokines [130]. In that same study, direct electrical stimulation of peripheral vagus during exotoxemia inhibited

TNF-alpha synthesis and peak plasma levels. However, the mechanism of action for the effect of VNS is not entirely understood and if afferent vagus excitation is the cause of CFS, VNS could make symptoms worse. In rat pain models, hyperalgesia severity changes with proinflammatory cytokine levels and, depending on the strength of stimulation, VNS can either increase or decrease baseline nociceptive thresholds [131–133]. Careful calibration of vagus nerve stimulation may be an important factor and it is likely that individual differences would play a substantial role in the effects of a given level of VNS on CFS symptoms. A newer and less invasive form of VNS involves transcutaneous stimulation of the afferent auricular branch (see Fig. 1) of the vagus nerve [134]. While this method is not as well studied as traditional cervical VNS, its effects seem to be similar and as such may be an attractive, less invasive treatment option.

Most radically, vagotomy has been used in animal models to experimentally block several aspects of sickness behavior after peripheral infection (reviewed above), and may be an option for the most severe cases of CFS. However, in rats, bilateral cervical vagotomy is fatal [60], pointing to the necessity of a targeted vagotomy. Such targeting depends on the detection of an isolated acute lesion within the afferent vagus nerve system, and this is currently not feasible. Again, this is potentially a very important problem for basic biomedical research to solve.

#### *Psychological and behavioral treatment strategies and the false dichotomy*

The debate over the etiology of CFS has been rife with a questionable dichotomy between mind and body. It has been argued that CFS is a psychological disorder caused by psychological mechanisms such as classical conditioning or learned helplessness (e.g. [135,136]). Strong evidence for the vagus nerve hypothesis of CFS would contradict this assumption of a purely psychological etiology to CFS. On the other side of the dichotomy lies the idea that CFS is like a broken arm, caused by a purely physical event, and in need of a purely physical cure. In some patient advocacy circles, psychological theories of CFS are so offensive that the clinical recommendation of any non-pharmacological intervention for CFS is seen to imply that CFS is a purely psychological disorder, or worse, a weakness of mind or character. Patients should be helped to understand that this is not the case and that resistance to psychological and behavioral intervention is misguided. Both cognitive behavioral therapy and graded exercise therapy have been shown in a randomized trial to be helpful for approximately 30% of individuals with CFS [137]. While these effects were moderate, the fact that 30% of patients significantly improved from psychological and behavioral interventions – without any drugs or surgery – should not be ignored.

There are two reasons that both psychological and behavioral interventions should be strongly recommended, along with the treatment options discussed above, to individuals with CFS.

1. While the VNIH of CFS posits a clearly non-psychological etiology, patients with other clearly non-psychological conditions also see physical benefits from psychological and behavioral interventions. For example, Fekete et al. reviewed evidence that such interventions could improve biomarkers for the non-psychological disorders type 2 diabetes, AIDS, and cancer [138]. Meditation improved both blood pressure and insulin sensitivity in individuals with type 2 diabetes. In individuals with HIV, cognitive behavioral stress management training, even when controlling for the effect of medication adherence, resulted in both lower viral load and greater naive T-cell count. Individuals undergoing adjuvant therapy for breast cancer who were also undergoing cognitive behavioral therapy showed improve-

ments in an indicator of immune function (lymphocyte proliferative response to challenge) relative to those who were not undergoing cognitive behavioral therapy. No one would argue that breast cancer reflects a weakness of character and yet psychological interventions help physical symptoms.

- Both cognitive behavioral therapy and graded exercise therapy can convey to understandably despondent individuals suffering from CFS that recovery is possible. Furthermore, graded exercise therapy can help overcome the atrophy of long-term muscle deconditioning, provided that post-exertional malaise does not worsen symptoms long-term. These two benefits are not directly related to vagus nerve infection, but both are crucial for recovery. The adamant refusal of some patients to engage in psychological or behavioral treatment strategies should be challenged – with empathy, logic, and information – as medically inadvisable.

Thus, previous research indicates that the best approach for combating CFS symptoms caused by vagus nerve infection may be some combination of the above strategies, for example a cocktail of glial inhibitors with an appropriate specific anti-viral agent along with cognitive behavioral therapy and graded exercise therapy. Careful clinical research should be undertaken before such a regimen is attempted.

## Conclusion

The VNIH offers CFS researchers and patients a specific mechanism for explaining symptoms, and it offers testable hypotheses and treatment strategies. According to this hypothesis, the major symptoms experienced by CFS patients represent pathologically exaggerated sickness behavior caused by infection-activated glial signaling somewhere along the afferent vagus nerve system. Several researchers have advanced theories that align with the VNIH of CFS. Many groups have pointed out that CFS symptoms are consistent with viral infection and ongoing immune activation. More specifically, Shapiro theorized that CFS could be caused by the common neurotropic herpesvirus varicella-zoster infecting the peripheral nervous system [139]. Maes has pointed out the overlap between inflammation, depression, and CFS [140]. The vagus nerve hypothesis provides an exact mechanism to these hypotheses, as well as an explanation for many of the inconsistencies in the literature (see Table 2).

According to the VNIH, both qualitative and quantitative variance in CFS symptoms between patients could be explained by the following related and interacting factors:

- Location of infection along the vagus nerve pathway
- Severity and duration of the body's sickness behavior response
- Severity and duration of inactivity
- Infection type, location of any infection outside of vagus nerve, and severity of infection

Elucidation of these four factors is likely to be critical for understanding individual patients' symptoms and determining individualized treatment strategies.

Research into the VNIH of CFS should involve several avenues. These include animal models utilizing deliberate vagus nerve infection, and human cadaver studies staining for viral infection and activated glia in vagal ganglia and paraganglia. Utilizing basic biomedical imaging research to discover a successful method for localizing active viral infection along the vagal path from peripheral to central nervous system would be of great import to both testing the hypothesis and determining effective clinical treatment. Functional studies of the vagus nerve should compare highly

symptomatic patients to healthy controls. In patients, the effectiveness of glial inhibitors can be tested, but these may not be effective in the absence of concurrent antiviral treatment. Antivirals should only be given if the specific type of virus causing the infection has been determined. VNS and vagotomy are theoretical treatment options that may benefit from validation in animal models before human studies are attempted.

## Conflict of interest

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