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(54) **VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS**

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(21) Appl. No.: **18/295,251**

(22) Filed: **Apr. 3, 2023**

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(63) Continuation-in-part of application No. 17/599,594, filed on Sep. 29, 2021, filed as application No. PCT/US2020/027906 on Apr. 13, 2020.

(60) Provisional application No. 63/326,794, filed on Apr. 1, 2022, provisional application No. 62/833,631, filed on Apr. 12, 2019.

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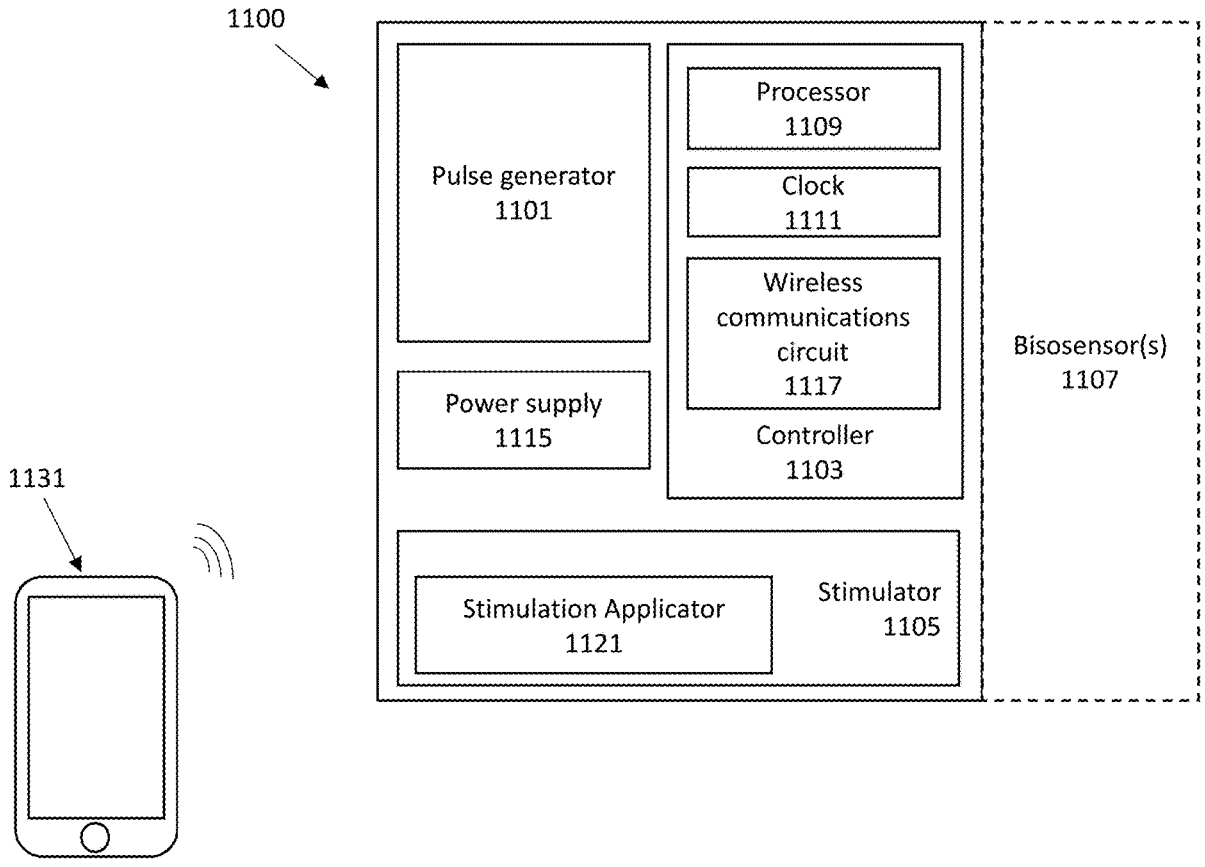
A61N 1/05 (2006.01)

(52) **U.S. Cl.**

CPC ***A61N 1/36053*** (2013.01); ***A61N 1/0556*** (2013.01); ***A61N 1/36135*** (2013.01); ***A61N 1/36171*** (2013.01)

(57) **ABSTRACT**

Systems, devices, and methods for using vagus nerve stimulation to treat demyelination disorders and/or disorder of the blood brain barrier are described. The vagus nerve stimulation therapy described herein is configured to reduce or prevent demyelination and/or promote remyelination to treat various disorders related to demyelination, such as multiple sclerosis. A low duty cycle stimulation protocol with a relatively short on-time and a relatively long off-time can be used.



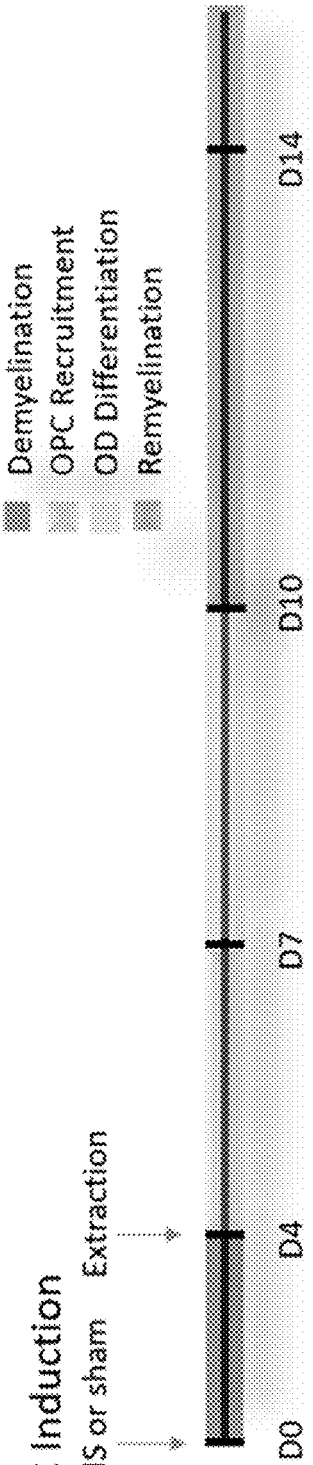

LPC Induction
+ VNS or sham
Extraction
D0
D4
D7
D10
D14
Demyelination
OPC Recruitment
OD Differentiation
Remyelination

FIG. 1

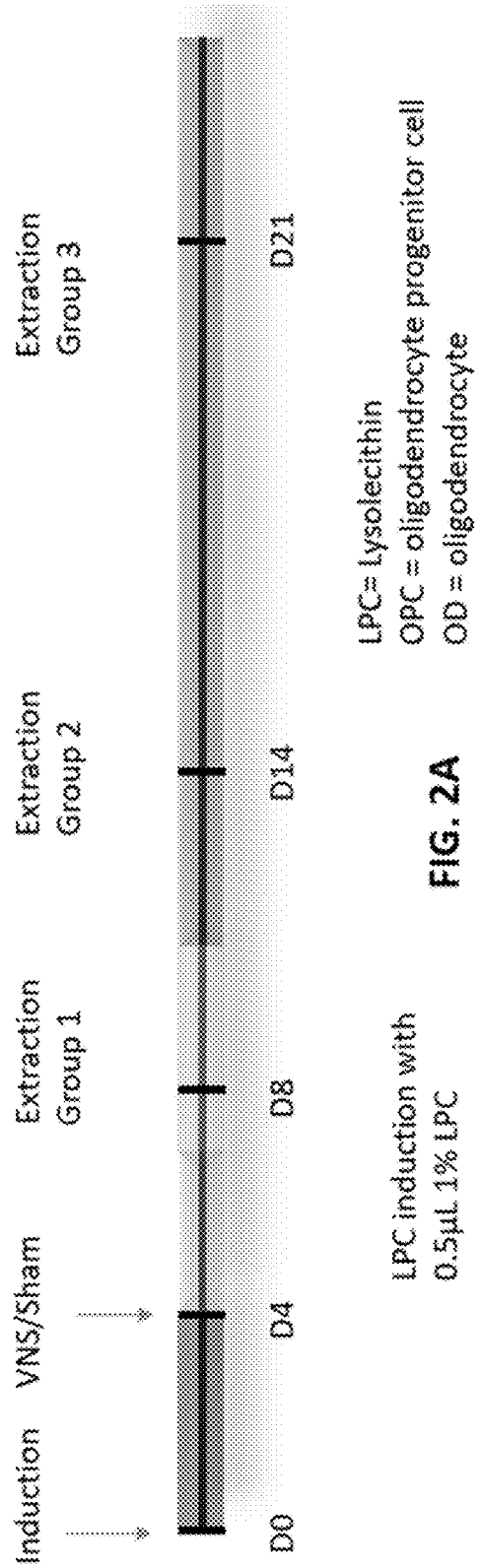

LPC Induction
VNS/Sham
Extraction Group 1
Extraction Group 2
Extraction Group 3
D0
D4
D8
D14
D21
LPC induction with 0.5µL 1% LPC
LPC= Lysolecithin
OPC = oligodendrocyte progenitor cell
OD = oligodendrocyte

FIG. 2A

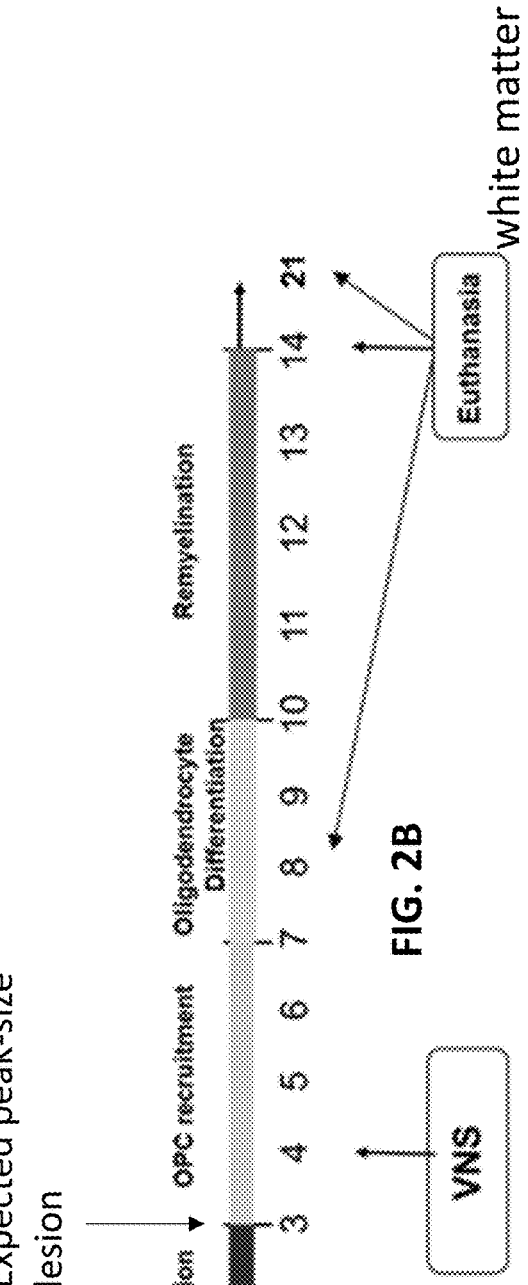


FIG. 2B

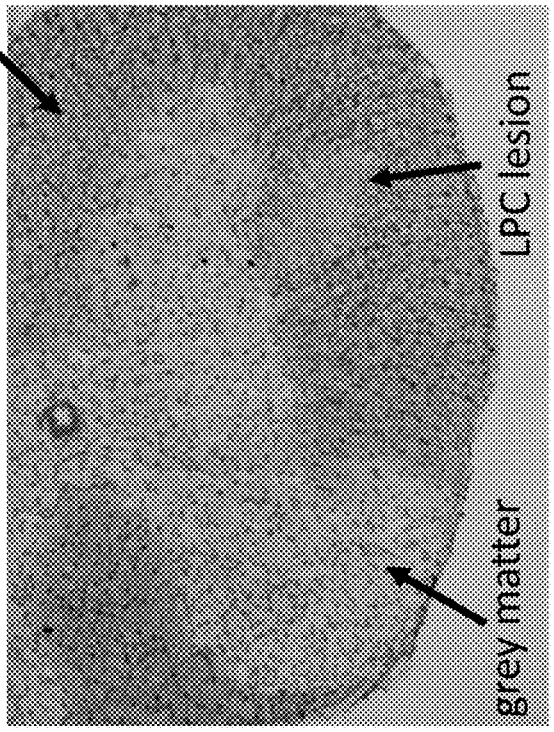


FIG. 3B

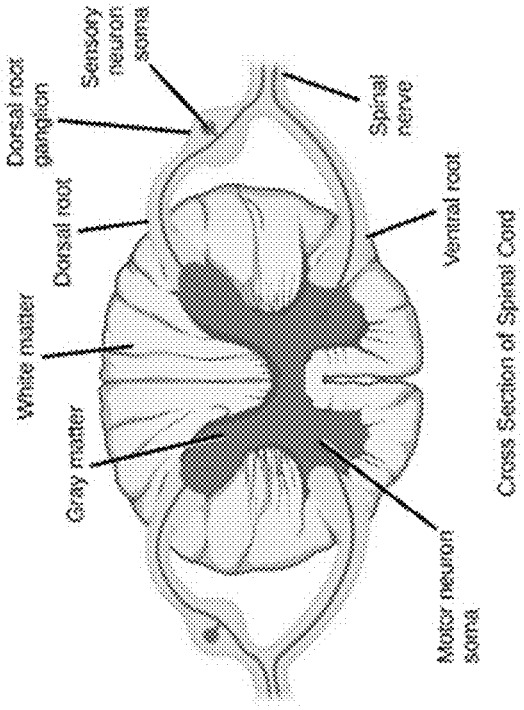


FIG. 3A

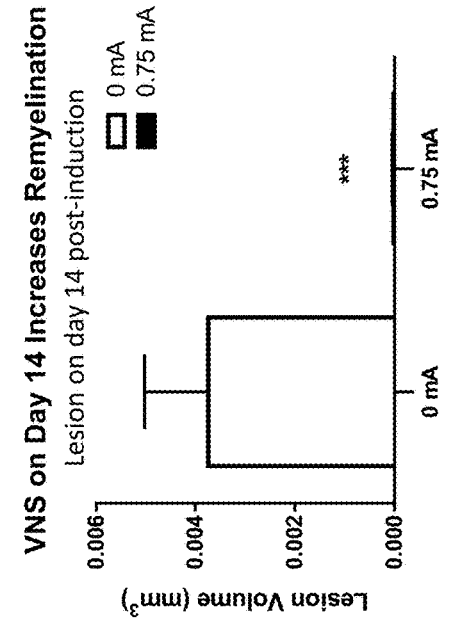


FIG. 4B

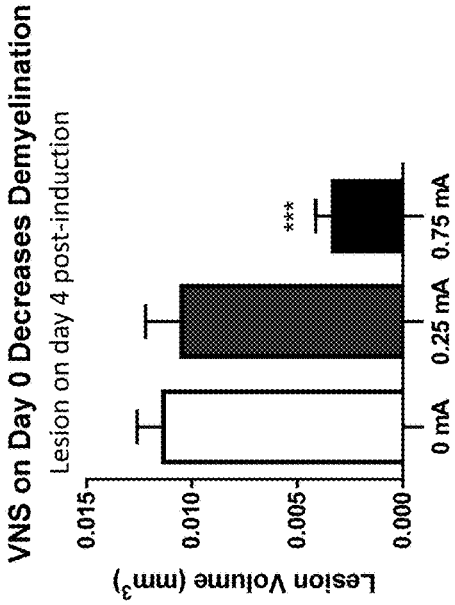


FIG. 4A

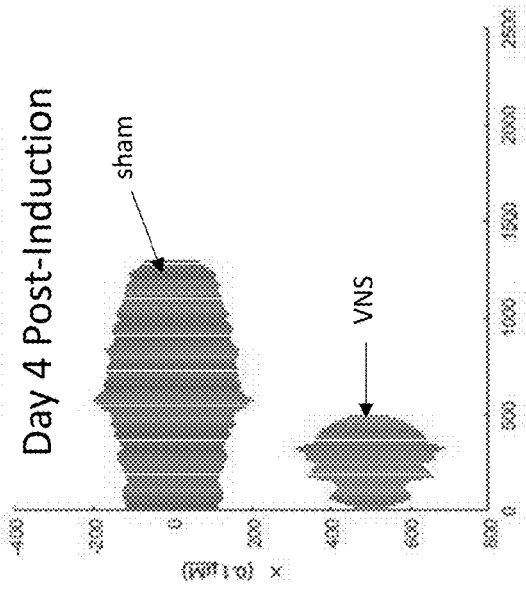


FIG. 4D

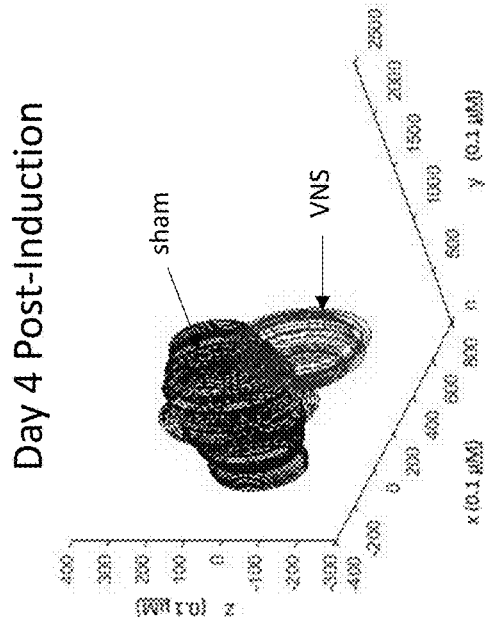


FIG. 4C

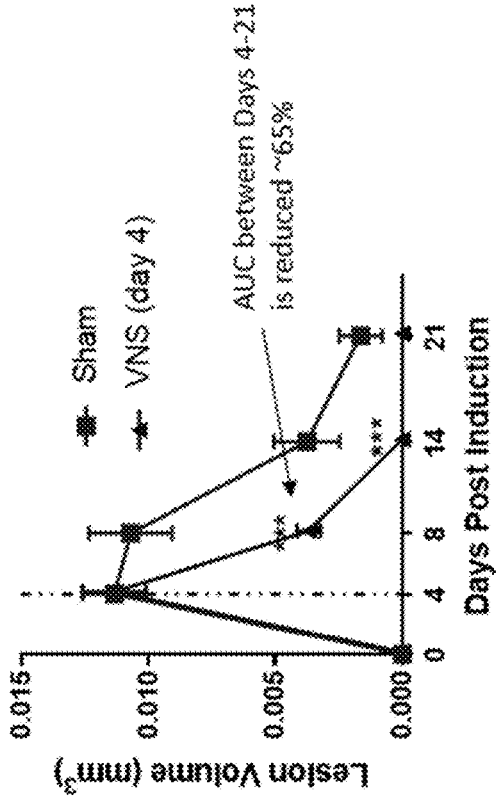


FIG. 5A

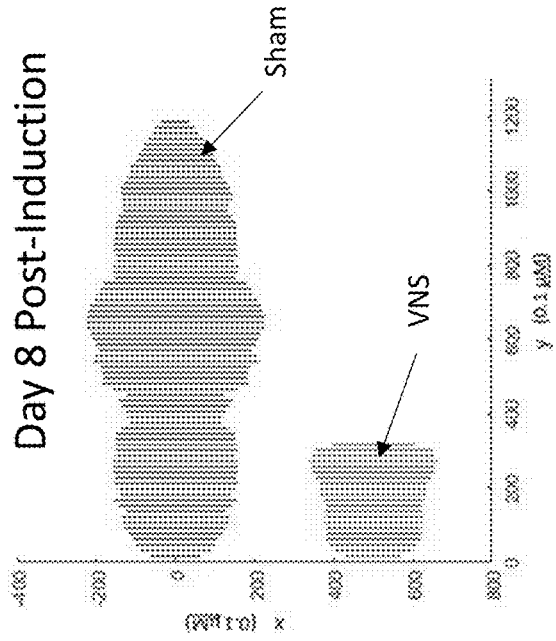


FIG. 5C

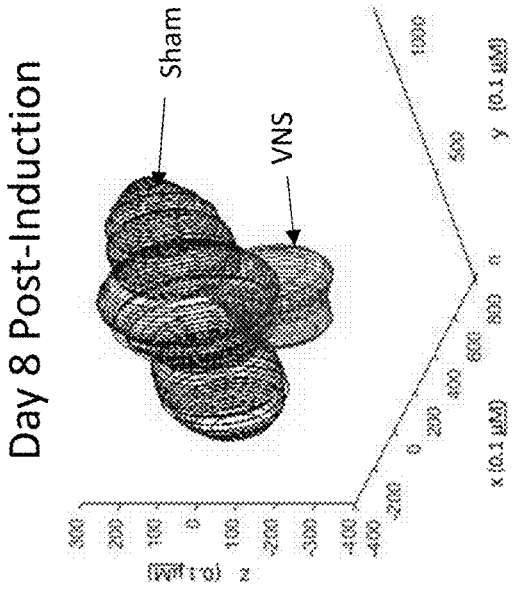


FIG. 5B

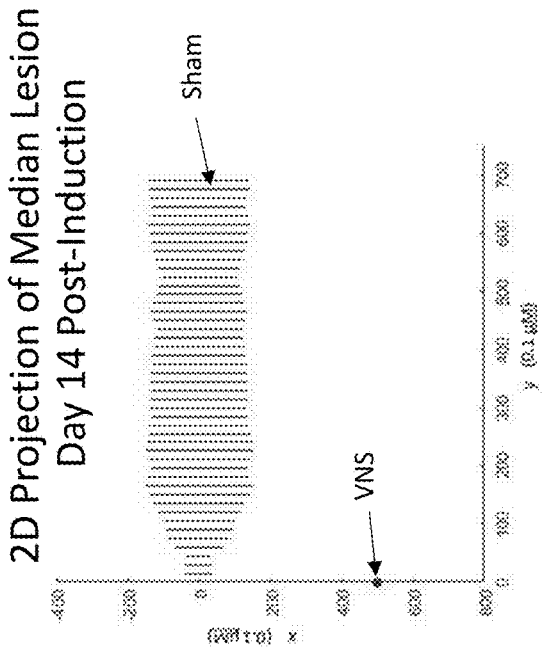


FIG. 5E

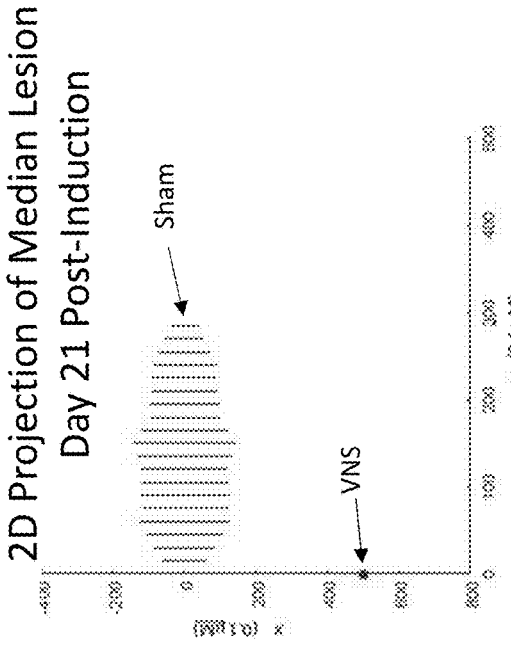


FIG. 5G

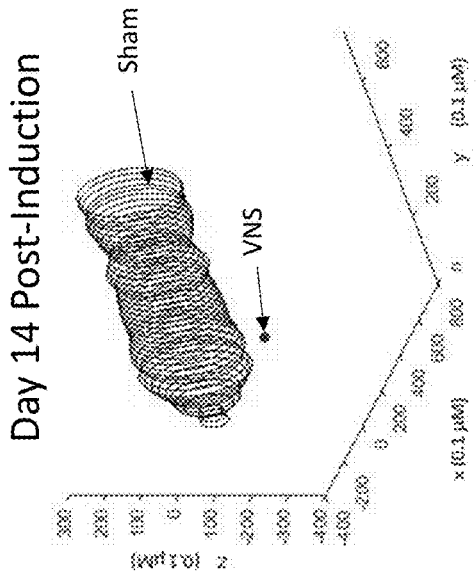


FIG. 5D

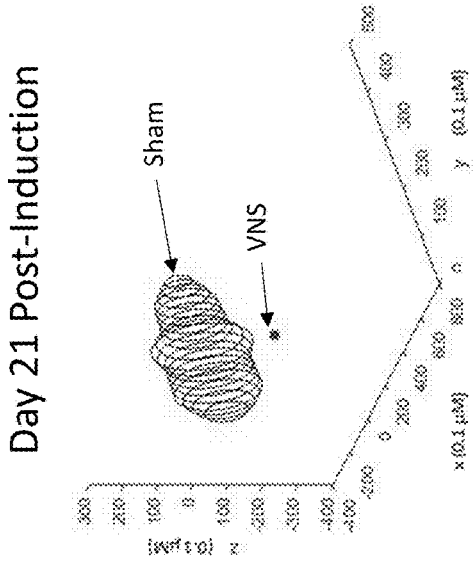


FIG. 5F

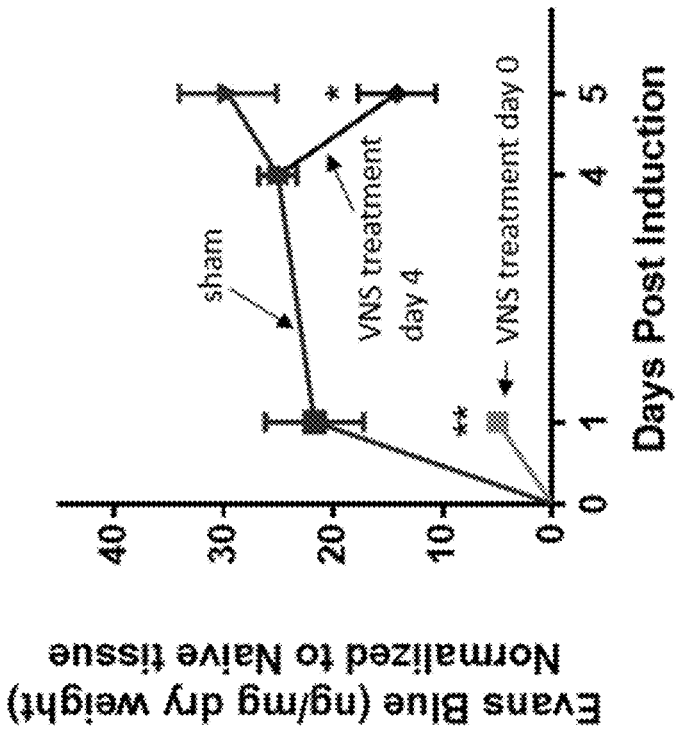

Evans Blue (ng/mg dry weight)
Normalized to Naive tissue
40
30
20
10
0
0
1
4
5
Days Post Induction
sham
VNS treatment
day 4
VNS treatment day 0
**
*
*

FIG. 6B

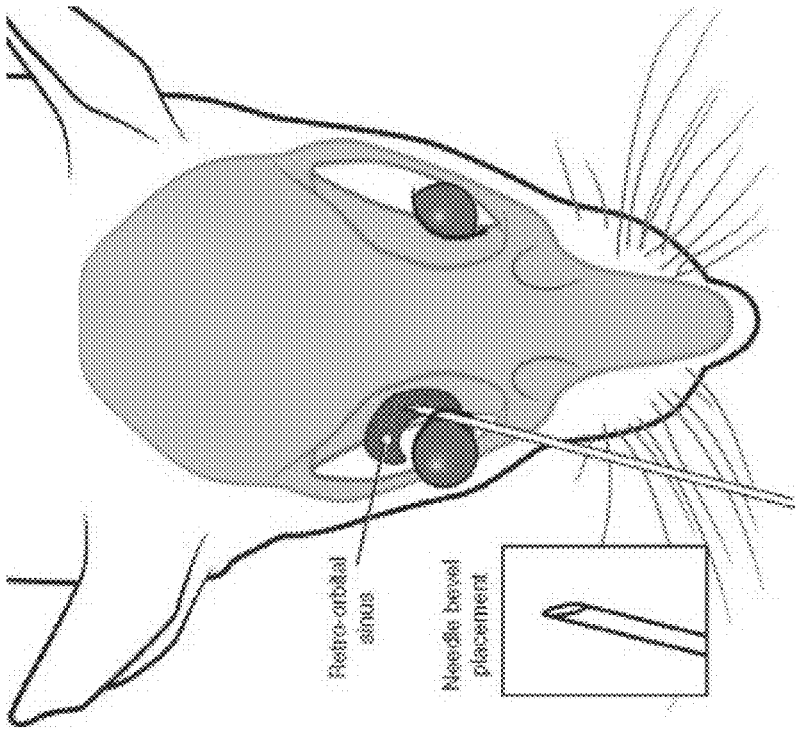

Retro-orbital sinus
Needle bevel placement

FIG. 6A

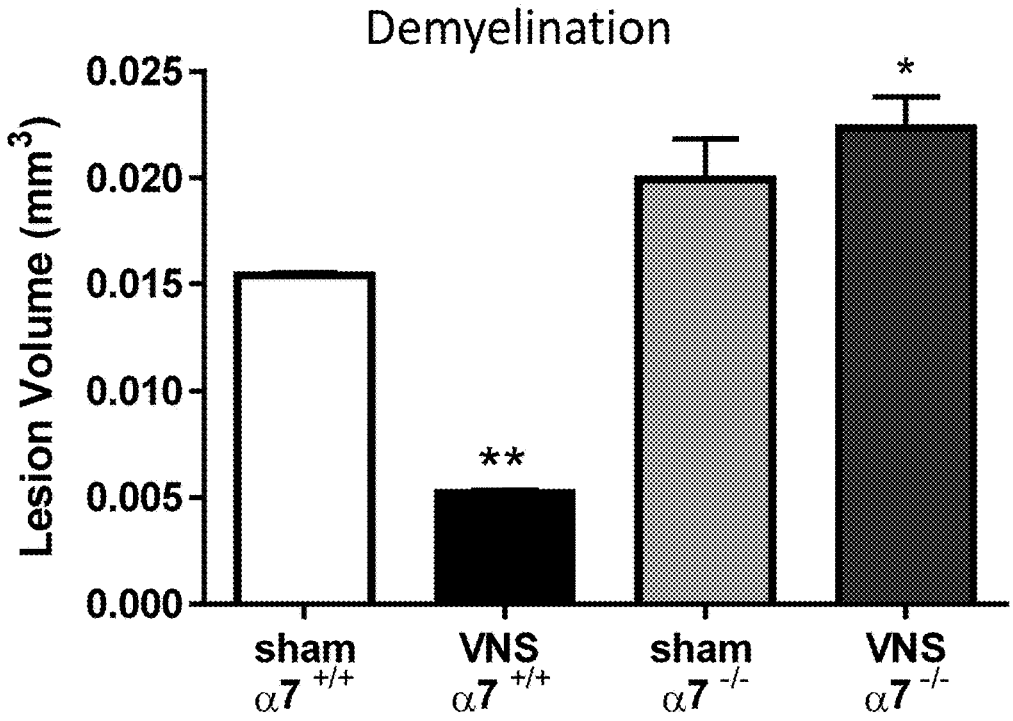

Demyelination
Lesion Volume (mm3)
0.025
0.020
0.015
0.010
0.005
0.000
**
*
sham α7 +/+
VNS α7 +/+
sham α7 -/-
VNS α7 -/-

FIG. 7A

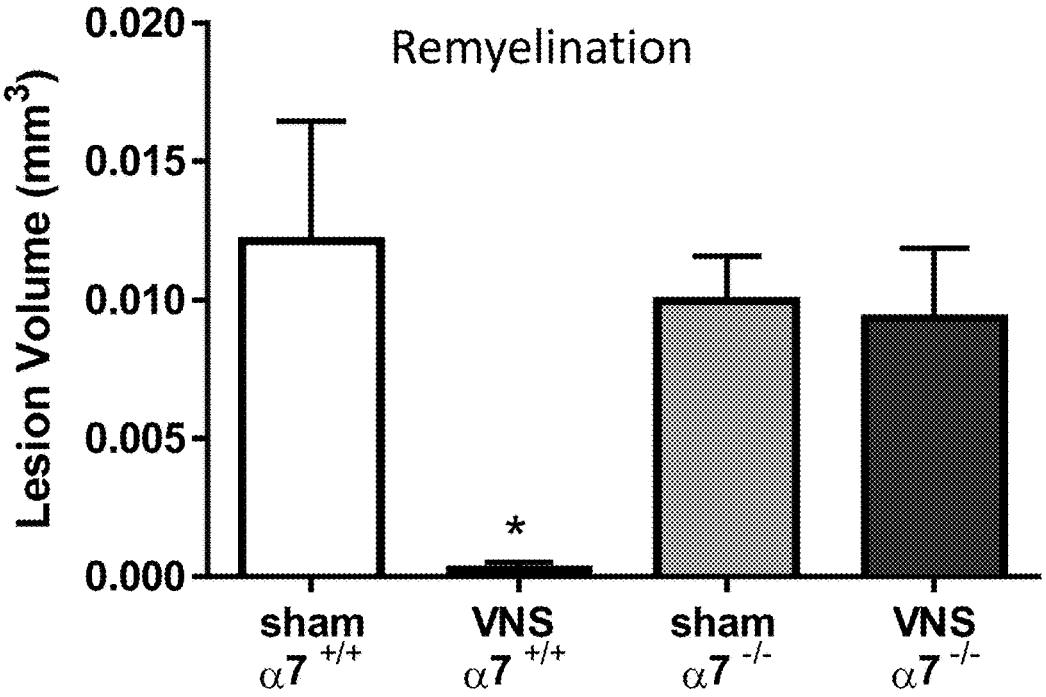

Remyelination
Lesion Volume (mm3)
0.020
0.015
0.010
0.005
0.000
*
sham α7 +/+
VNS α7 +/+
sham α7 -/-
VNS α7 -/-

FIG. 7B

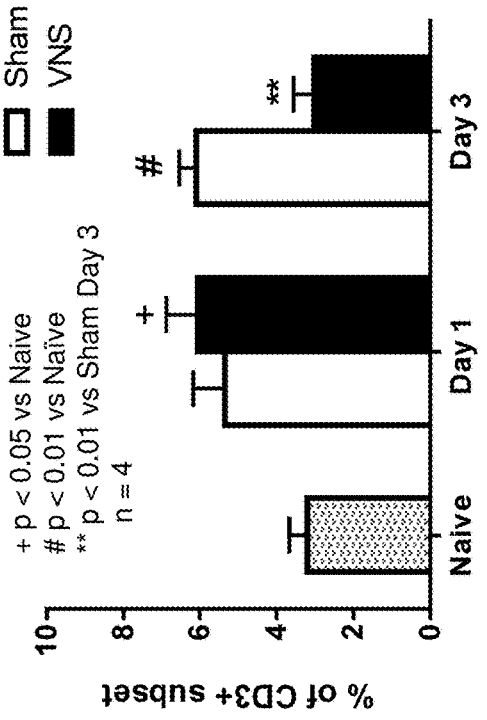

Sham
VNS
+ p < 0.05 vs Naive
# p < 0.01 vs Naïve
** p < 0.01 vs Sham Day 3
n = 4
% of CD3+ subset
0
2
4
6
8
10
Naive
Day 1
Day 3
+
#
**

FIG. 8


Sham
VNS
^ p < 0.001 vs Naive
# p < 0.01 vs Naive
** p < 0.01 vs Sham
Day 1
n = 4
% of CD11b+ CD45+ subset
0
2
4
6
8
10
12
Naive
Day 1
Day 3
^
**
#

FIG. 9

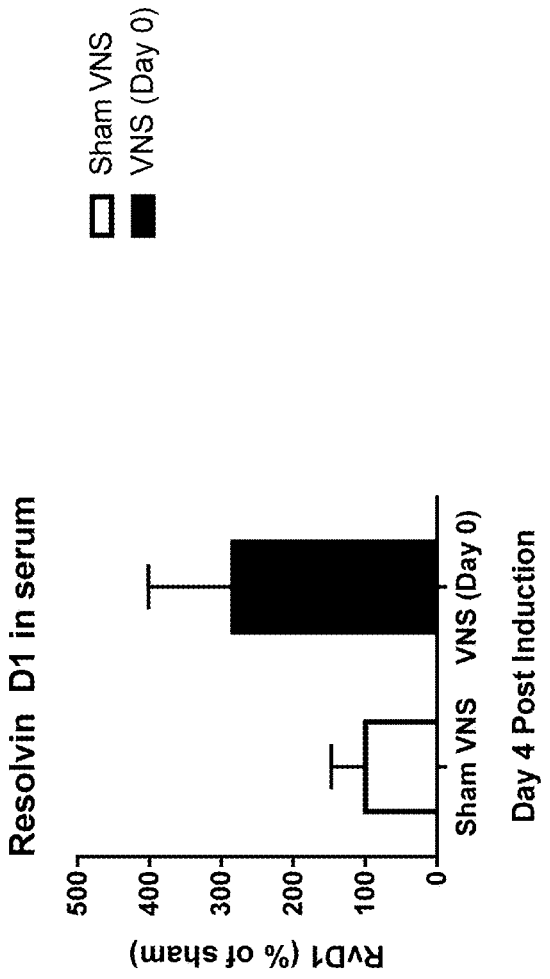


FIG. 10A

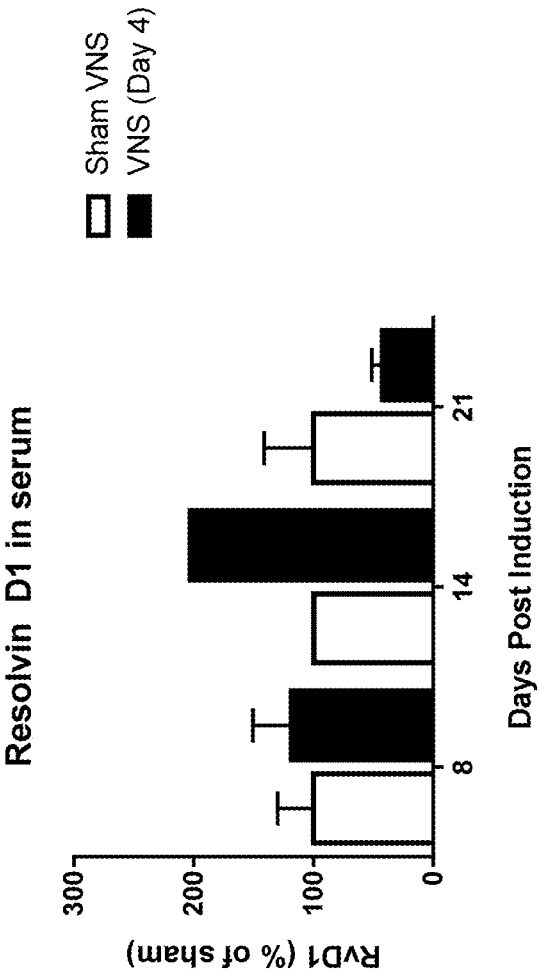


FIG. 10B

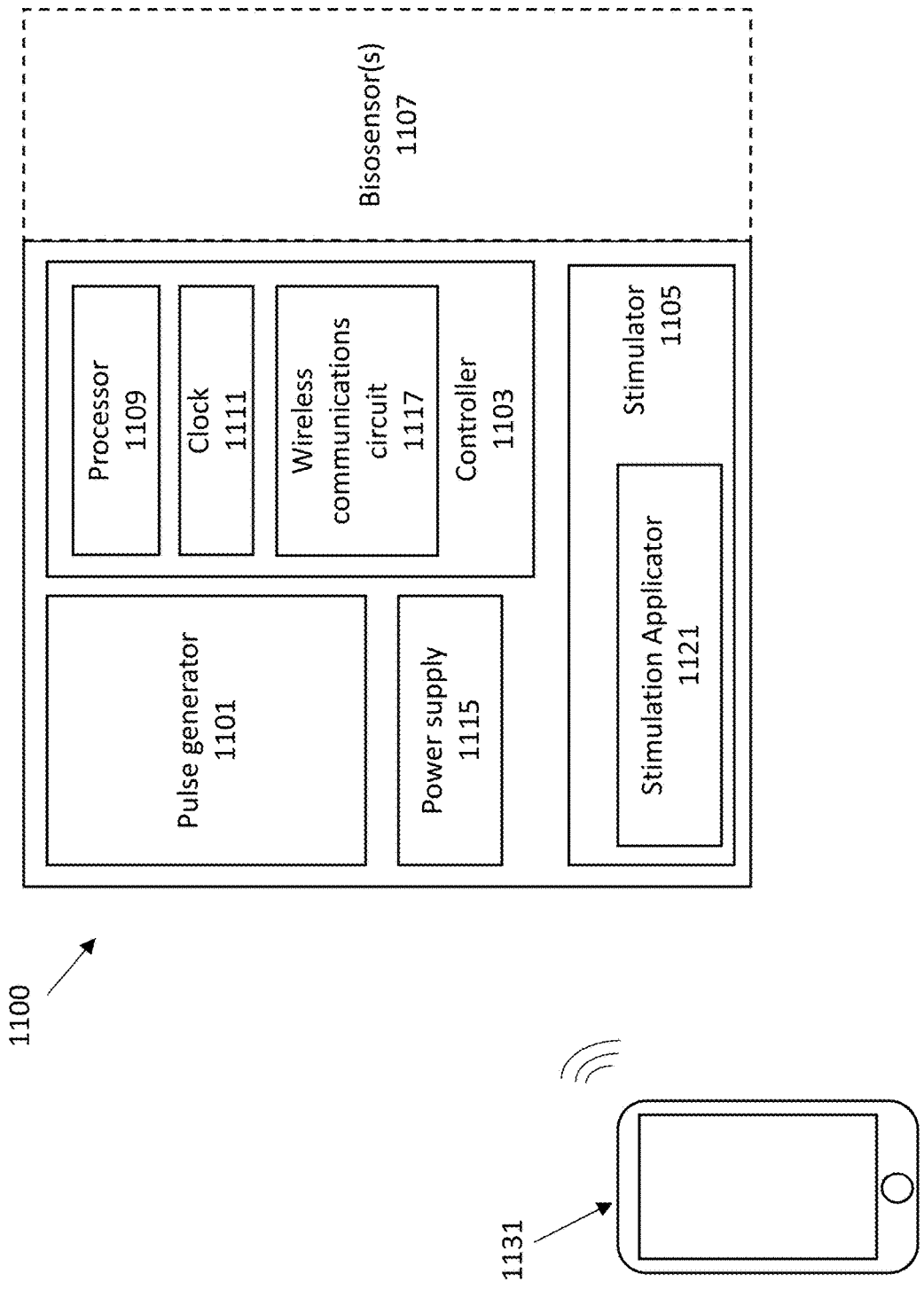

Bisosensor(s)
1107
Processor
1109
Clock
1111
Wireless communications circuit
1117
Controller
1103
Stimulator
1105
Stimulation Applicator
1121
Pulse generator
1101
Power supply
1115
1100
1131

FIG. 11

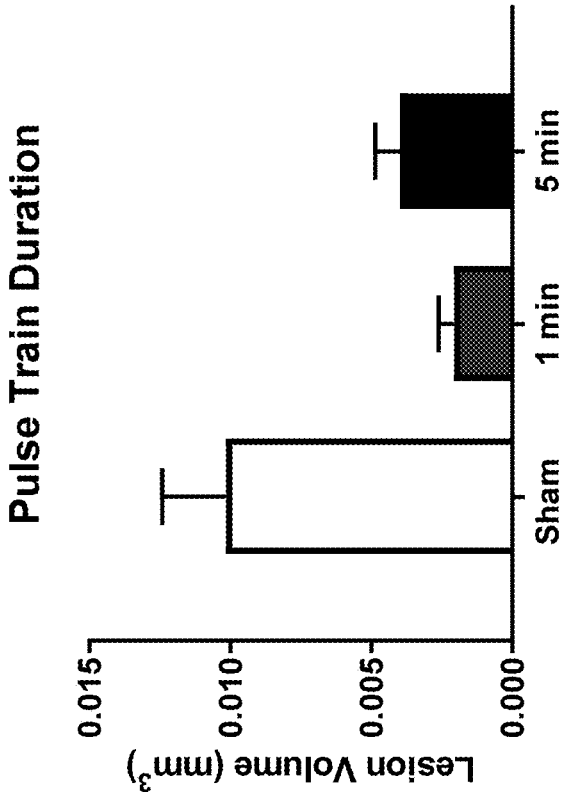

Pulse Train Duration
Lesion Volume (mm³)
0.015
0.010
0.005
0.000
Sham
1 min
5 min

FIG. 13

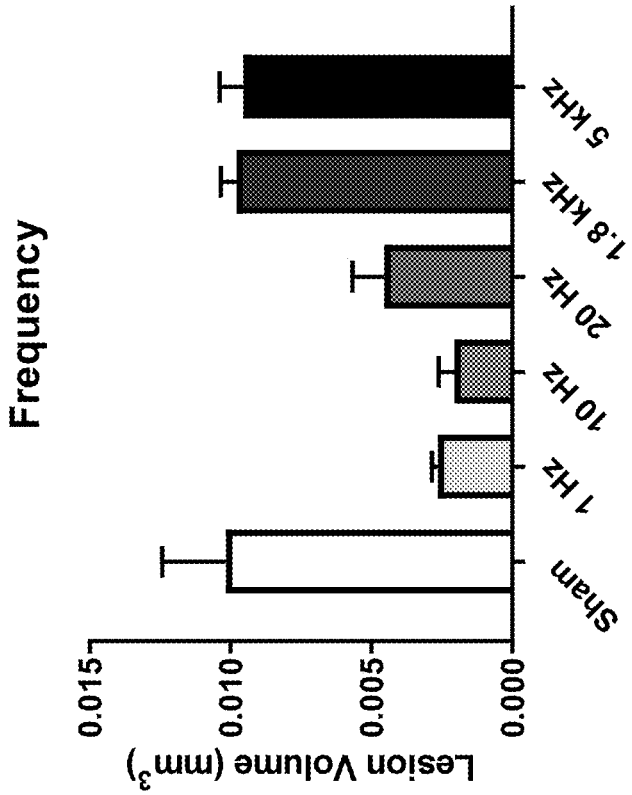

Frequency
Lesion Volume (mm³)
0.015
0.010
0.005
0.000
Sham
1 Hz
10 Hz
20 Hz
1.8 kHz
5 kHz

FIG. 12


VNS increases myelin debris uptake

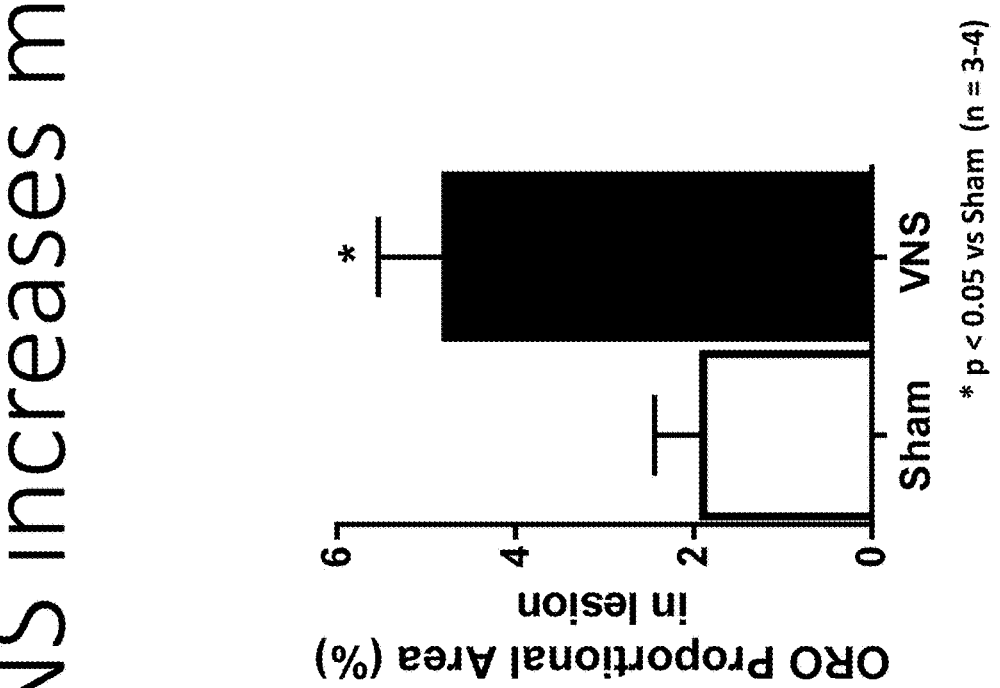

ORO Proportional Area (%)
in lesion
6
4
2
0
*
Sham
VNS
* p < 0.05 vs Sham (n = 3-4)

FIG. 14

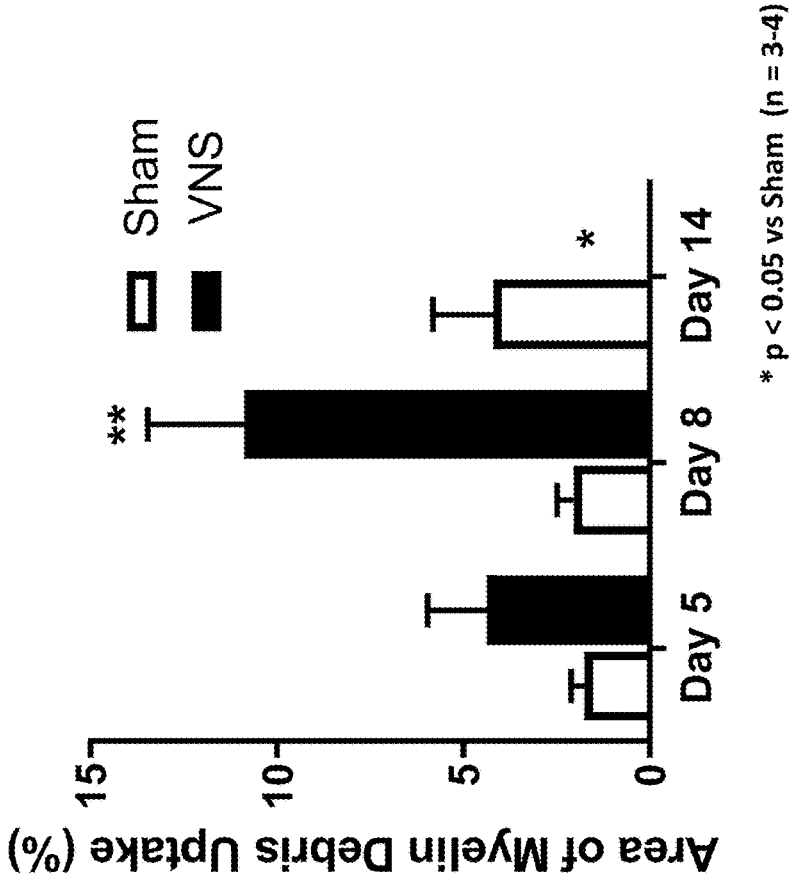

Area of Myelin Debris Uptake (%)
15
10
5
0
Sham
VNS
**
*
Day 5
Day 8
Day 14
* p < 0.05 vs Sham (n = 3-4)

FIG. 15

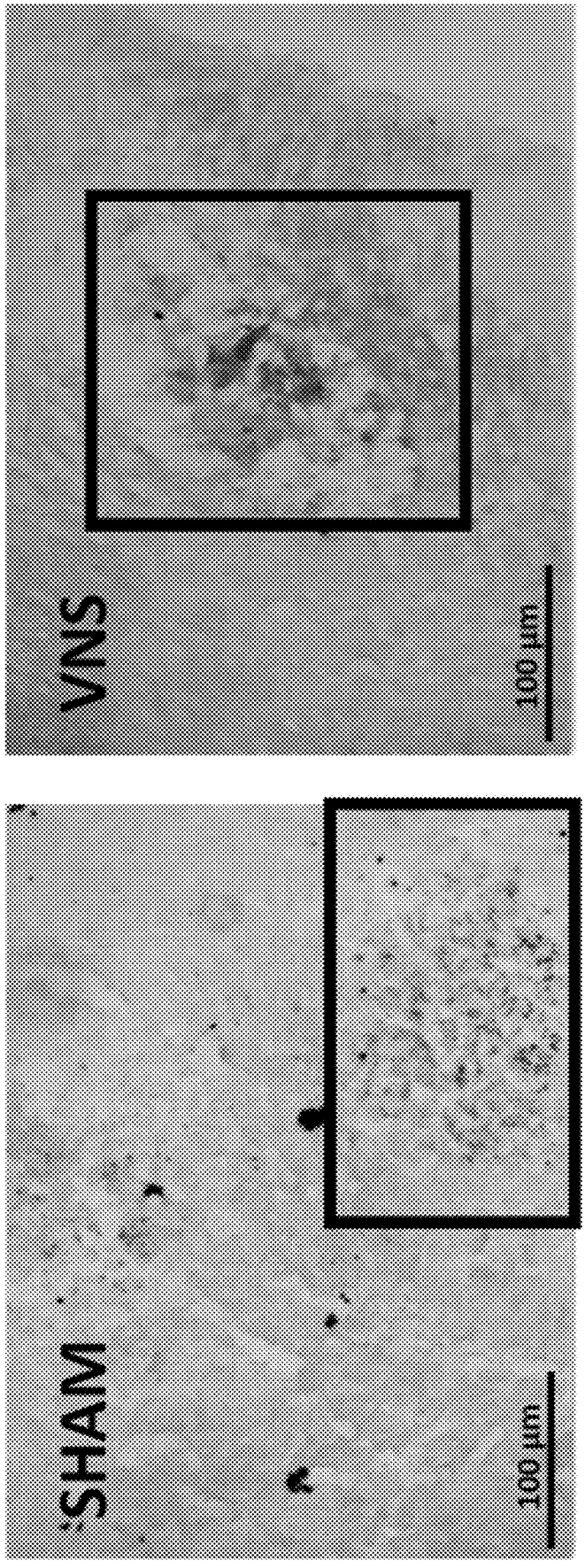

Representative Oil Red O staining
Treatment paradigm A, Day 4 post-induction
SHAM
100 µm
VNS
100 µm
* Box represents region of interest where lesion is located

FIG. 16

FIG. 17

Treatment paradigm B, Day 8 post-induction

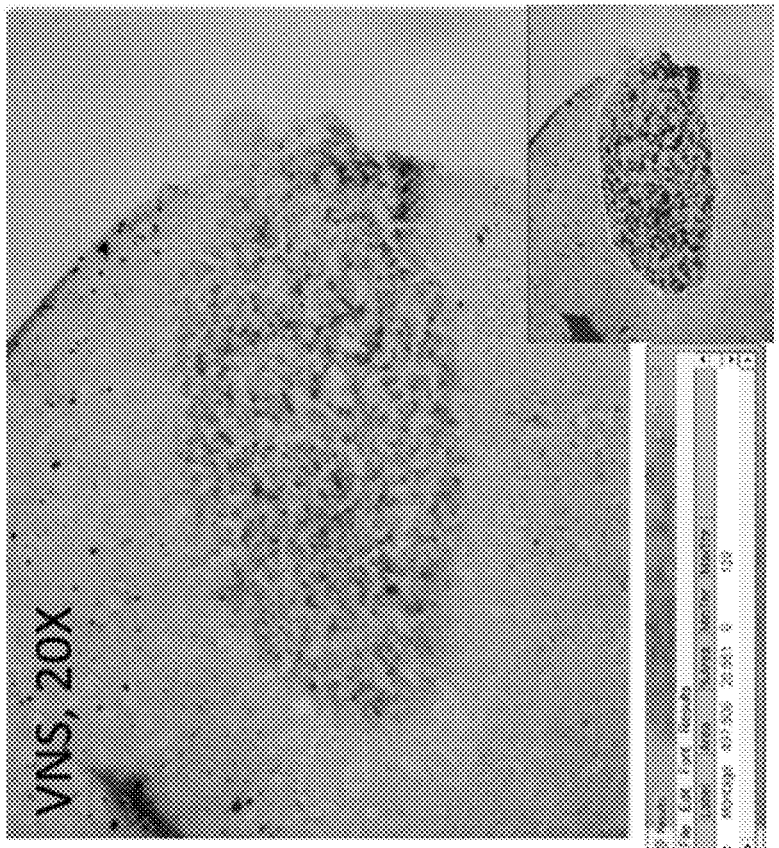


FIG. 19

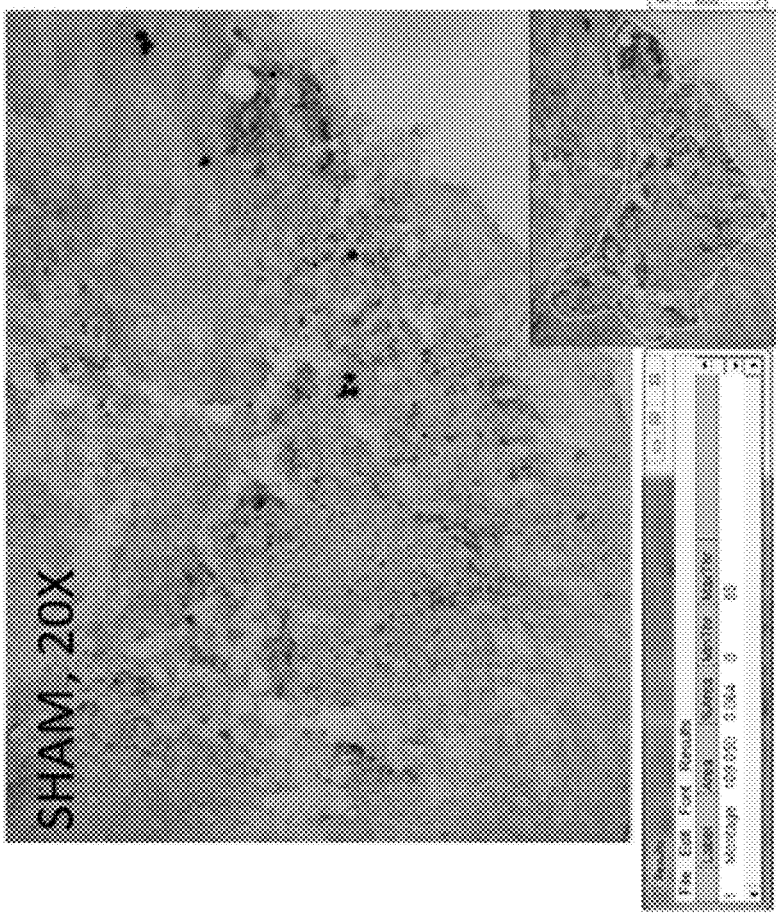


FIG. 18

REMYELINATION IS ENHANCED IN AGED (19 MONTHS) MICE

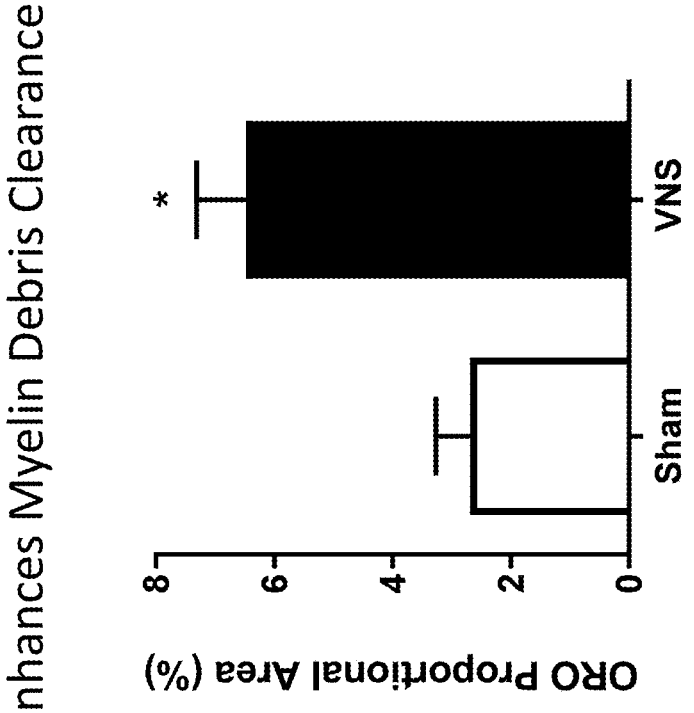


FIG. 21

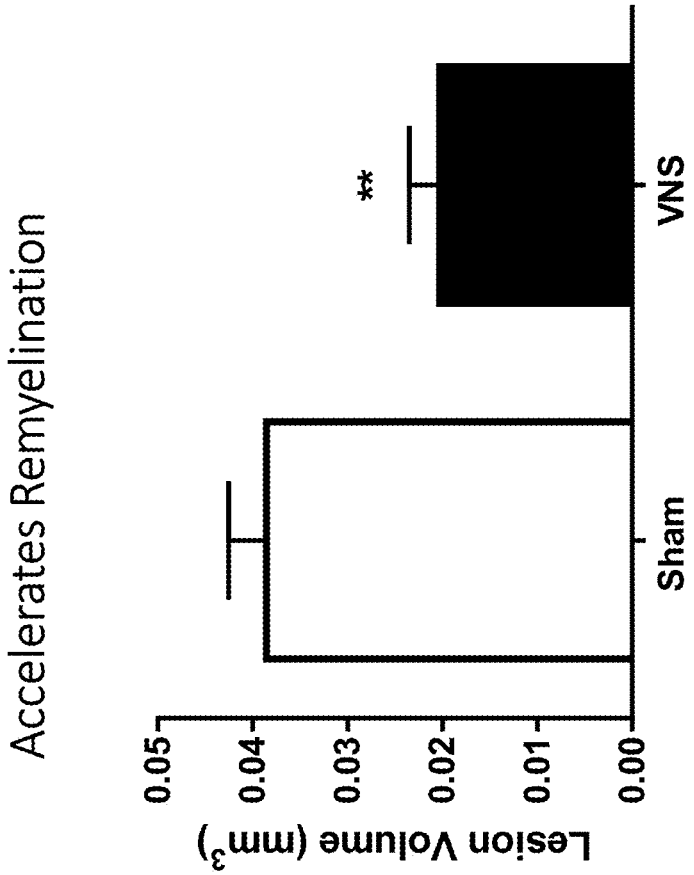


FIG. 20

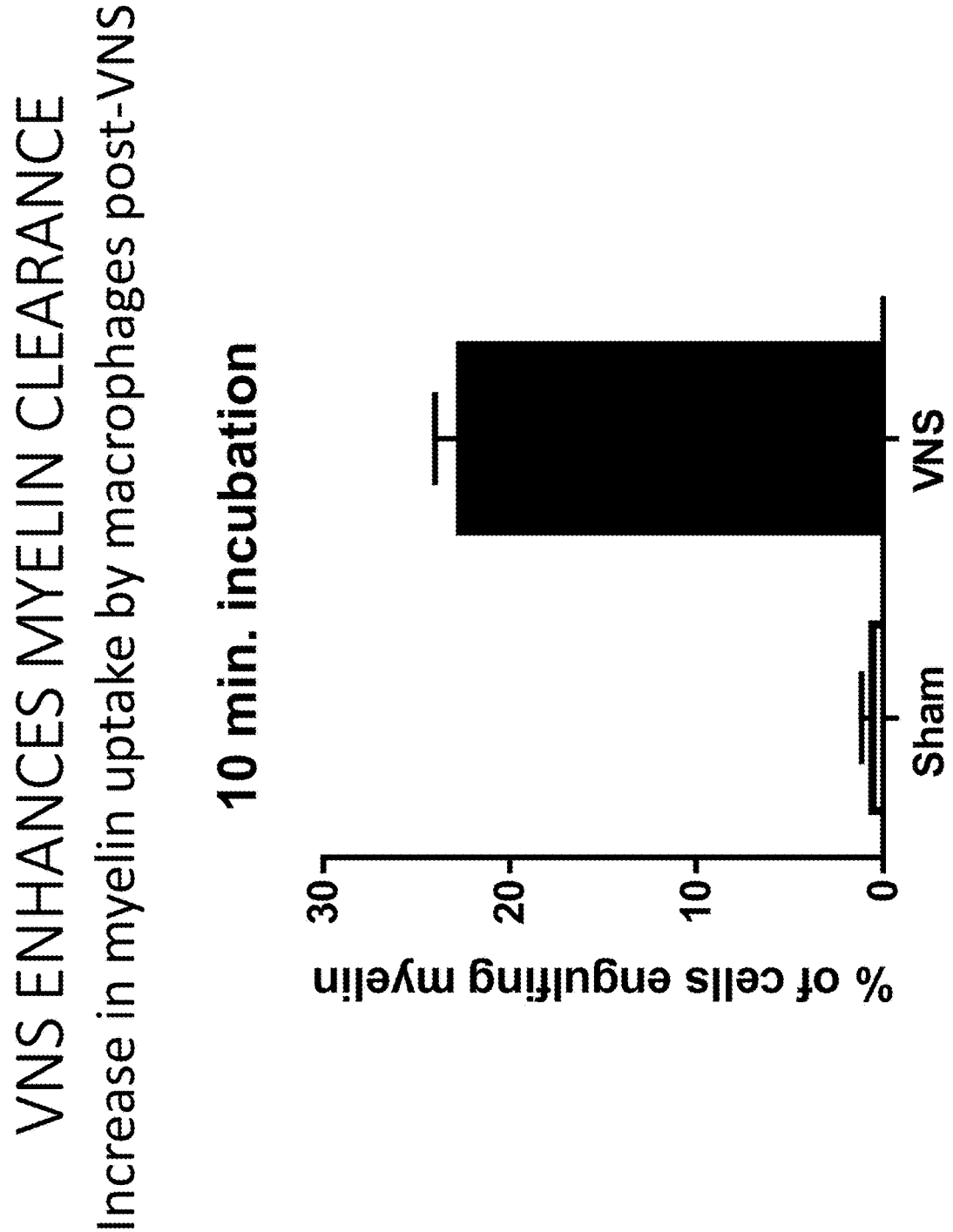

VNS ENHANCES MYELIN CLEARANCE
Increase in myelin uptake by macrophages post-VNS
10 min. incubation
% of cells engulfing myelin
30
20
10
0
Sham
VNS

FIG. 22

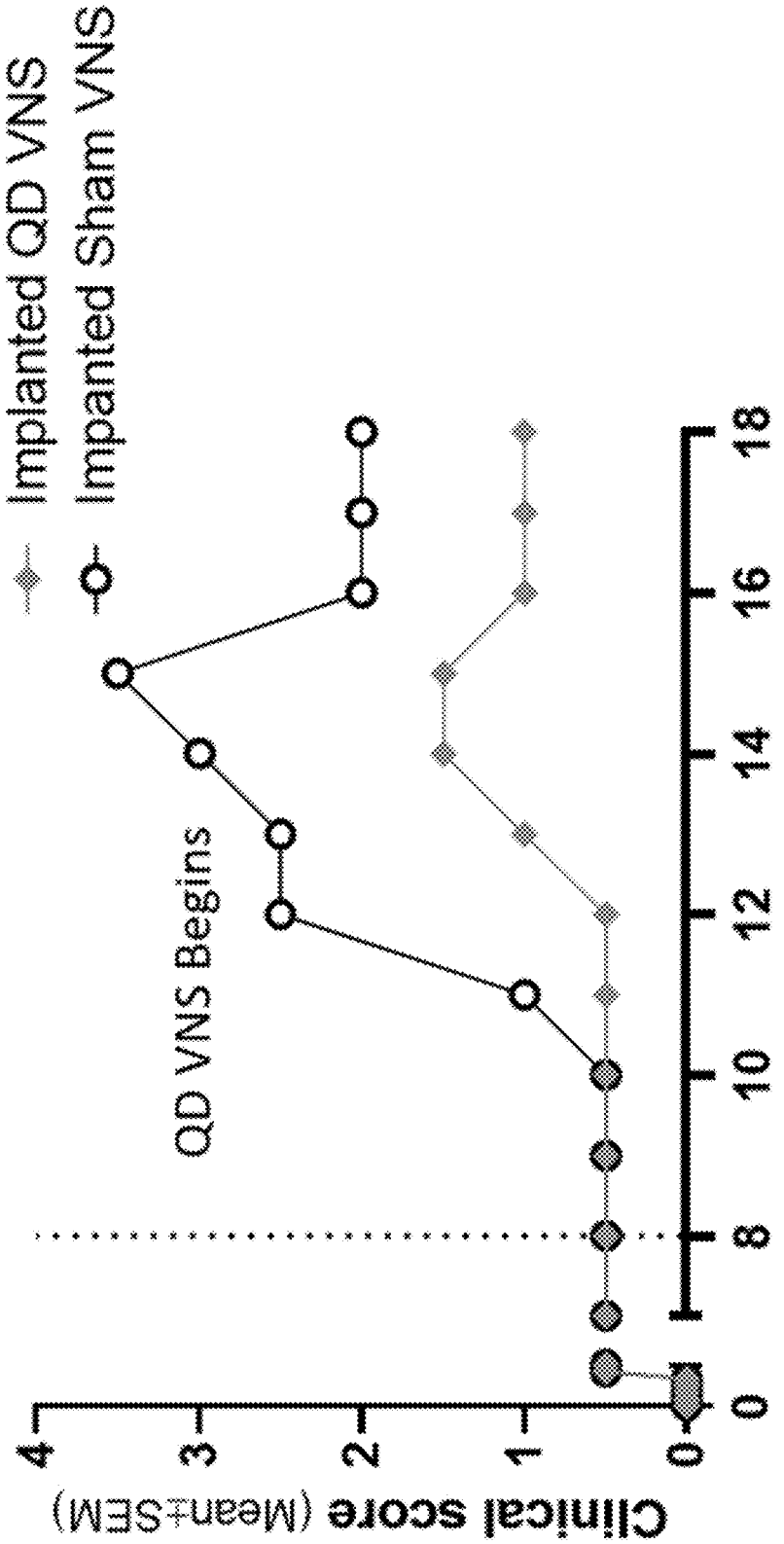

Implanted QD VNS
Impanted Sham VNS
QD VNS Begins
Clinical score (Mean±SEM)
Day after immunization
0
1
2
3
4
0
8
10
12
14
16
18

FIG. 23

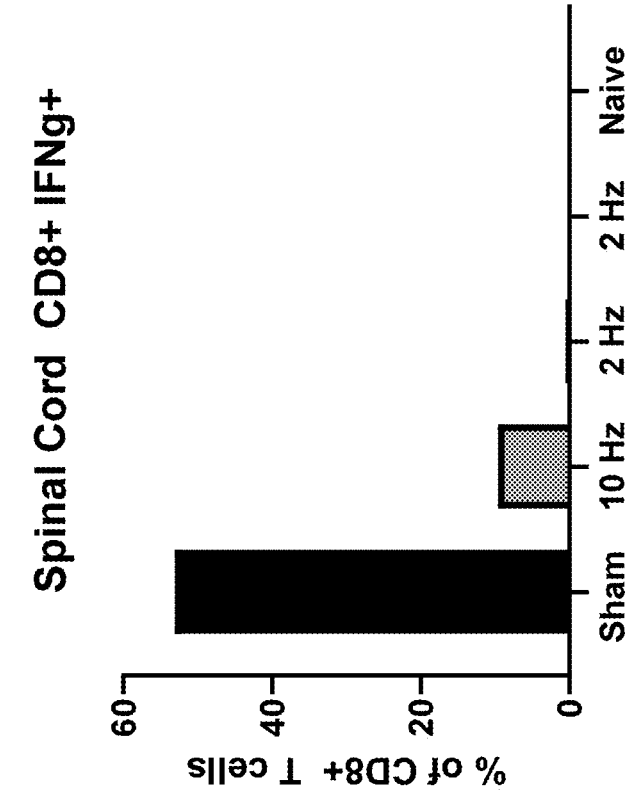

Spinal Cord CD8+ IFNg+
% of CD8+ T cells
0
20
40
60
Sham
10 Hz
2 Hz
2 Hz
Naive

FIG. 25

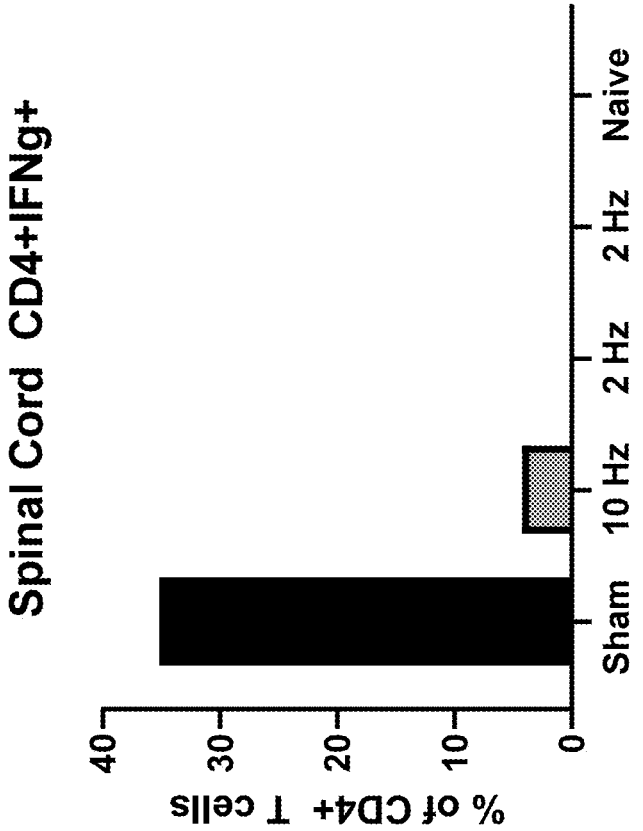

Spinal Cord CD4+IFNg+
% of CD4+ T cells
0
10
20
30
40
Sham
10 Hz
2 Hz
2 Hz
Naive

FIG. 24

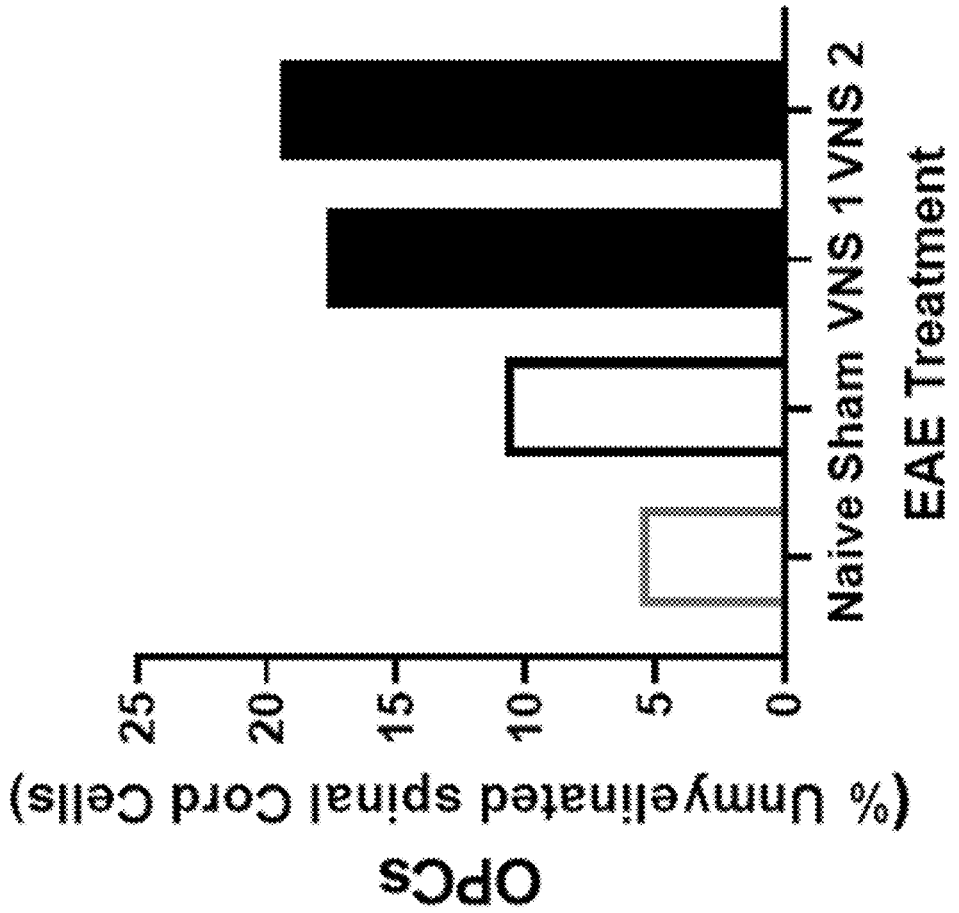

OPCs
(% Unmyelinated spinal Cord Cells)
25
20
15
10
5
0
Naive
Sham
VNS 1
VNS 2
EAE Treatment

FIG. 26

VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/326,794, titled "NERVE STIMULATION TO ACTIVATE OLIGODENDROCYTE PROGENITOR CELLS," filed on Apr. 1, 2022, herein incorporated by reference in its entirety. This application also claims priority as a continuation-in-part to U.S. patent application Ser. No. 17/599,594, titled "VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS," filed on Sep. 29, 2021, now U.S. Patent Application Publication No. 2022/0193413, which is a national phase application under 35 U.S.C. 371 of International Patent Application No. PCT/US2020/027906, filed Apr. 13, 2020, titled "VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS," now International Patent Application Publication No. WO 2020/210786, which claims priority to U.S. Provisional Patent Application No. 62/833,631, filed Apr. 12, 2019, titled "VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS," each of which is herein incorporated by reference in its entirety.

[0002] This patent application may be related to U.S. patent application Ser. No. 16/158,222 filed on Oct. 11, 2018, titled "VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS," which claims priority to U.S. Provisional Patent Application No. 62/572,374, filed on Oct. 13, 2017, titled "VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS," and U.S. Provisional Patent Application No. 62/576,547, filed Oct. 24, 2017, titled "VAGUS NERVE STIMULATION TO TREAT NEURODEGENERATIVE DISORDERS," each of which is herein incorporated by reference in its entirety.

INCORPORATION BY REFERENCE

[0003] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BACKGROUND

[0004] A variety of central nervous system (CNS) demyelinating disorders, including multiple sclerosis, acute disseminated encephalomyelitis and neuromyelitis optica spectrum disorders, are difficult to effectively treat. For example, multiple sclerosis (MS) is a neurodegenerative and neuroinflammatory disease characterized by demyelination of nerves in the central nervous system. Although the root cause of demyelination is not well understood, it generally is associated with the formation of lesions on the myelin sheaths and inflammation. Currently, there is no known cure for MS. Current treatments, with modest success, are primarily directed to treating acute attacks and reducing the frequency of attacks in the relapsing-remitting subtype of the disease or treating the symptoms. However, current therapies at best only slow the progression of the disease, and no therapy to date has demonstrated an ability to remyelinate nerves.

[0005] Therefore, it would be desirable to provide additional treatment methods and systems that can be used independently or in conjunction with other therapies to reduce the rate or amount of demyelination. Furthermore, it would be desirable to provide a therapy that remyelinates nerves and reverses the progression demyelination.

SUMMARY OF THE DISCLOSURE

[0006] The present invention relates generally to vagus nerve stimulation to treat neurodegenerative and neuroinflammatory disorders, and more specifically to vagus nerve stimulation to reduce demyelination and/or to promote remyelination to treat various neurodegenerative and neuroinflammatory disorders such as multiple sclerosis.

[0007] For example, described herein are apparatuses (e.g., devices and/or systems) for reducing demyelination and/or increase remyelination by stimulation of a vagus nerve. These apparatuses may be implants or implanted into the patient's body. Any of these apparatuses may include: a biosensor configured to detect one or more biomarkers; a stimulator configured to apply stimulation to the vagus nerve; and a controller coupled to the biosensor and the stimulator and configured to apply stimulation to the vagus nerve from the stimulator sufficient to reduce demyelination and/or increase remyelination of nerves within the patient when the biosensor detects a biomarker indicative of demyelination. In some variations, these apparatuses include an implant comprising a stimulator (e.g., a waveform and/or pulse generator, an oscillator, a power supply and/or power regulation circuit, etc.), a stimulation applicator (e.g., one or more electrodes, mechanical transducers, etc.), and a controller. The controller may be configured as a microcontroller and may be in electrical communication with the stimulator so as to control operation of the stimulator. The controller may include one or more processors, a memory and/or a timer. The stimulator and/or controller may be in electrical communication, one or more stimulation applicators. In some variations the controller may include or be in communication with wireless communications circuitry for wirelessly communicating with one or more remote processors. The remote processor may be a hand-held device (e.g., smartphone, wearable electronics, etc.). The controller may optionally be in communication with one or more biosensors that may be included with the implant or may be remote from the implant (e.g., may be wearable, single-use, etc.). In some variations the biosensors are wirelessly connected to the apparatus.

[0008] In some variations the apparatus may be used without a biosensor. For example, the apparatus may be configured to periodically and/or on demand apply VNS treatment to prevent or reduce demyelination. The apparatus may be configured to apply VNS treatment doses once multiple times per day (e.g., 1× day, 2×, day, 3×, day, 4× day, 5× day, 6× day), or every other day, or every 3 days, etc. In some variations the apparatus may be configured to both automatically apply a VNS treatment dose on a predetermined and/or adjustable scheduled, as well as provide VNS treatment doses based on input from a user (e.g., patient, physician, etc., including "on demand" doses) and/or based on detection of a biomarker indicative of an actual or potential increase in demyelination.

[0009] In any of these variations, a biosensor may be configured to detect one or more markers (e.g., biomarkers) from the patient's body, including from the patient's blood

and/or cerebrospinal fluid. Examples of biomarkers may be found herein. The biosensor may be part of the implanted apparatus, or it may be connected to the apparatus (e.g., the controller) via a wired or wireless communication. The biosensor may be configured to detect any biological marker, including chemical markers (e.g., a protein, nucleotide, e.g., RNA, DNA, microRNA, etc., lipid, carbohydrate, etc.), as well as functional markers (nerve conduction, etc.), body temperature, and the like. For example, in some variations, the biosensor is configured to detect temperature.

[0010] In general, the apparatuses described herein may be configured to be inserted or implanted into the body. For example, the apparatus may be configured to be implanted. The apparatus may include a stimulation applicator (also referred to as simply a stimulator or a VNS treatment stimulator) that may be a mechanical and/or electrical stimulator. A mechanical stimulator may be a piezoelectric driver that may vibrate and/or apply pressure to the tissue, including to the vagus nerve, in the VNS treatment parameters, such as mechanical stimulation of the vagus nerve at between 1-2 kHz for a treatment time (e.g., between 1 ms and 5 minutes, e.g., 10 ms-10 sec, etc.). Alternatively or additionally, the stimulation applicator may be an electrical stimulation applicator and may include one or more (e.g., two or more) electrodes configured to apply electrical stimulation to the vagus nerve. For example, electrical stimulation of about 0.1 mA to 10 mA (e.g., between 1 mA-5 mA), at a frequency of between about 1 Hz and about 2 kHz (e.g., between about 1-100 Hz), where the pulses applied have a pulse width of between about (50-500 usec, e.g., between about 100-300 usec). The controller may be configured to enforce an 'off-time' following a VNS treatment dose of between about 10 minute and 12 hours (e.g., between about 2 hours and 10 hours, between about 3 hours and 6 hours, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, etc.). For example, the stimulator may include an electrode configured to apply electrical energy to the vagus nerve.

[0011] In some variation the apparatus is configured to apply VNS treatment to the patient in which the VNS treatment is electrical stimulation. For example, the VNS treatment may include the application of electrical energy at between about 1-100 Hz (e.g., between about 1-50 Hz, between about 1-20 Hz, between about 5-30 Hz, between about 5-15 Hz, approximately 5 Hz, approximately 10 Hz, approximately 15 Hz, etc.). The energy may have a peak amplitude of between about 0.1 mA and about 2 mA (e.g., between about 0.2 mA and about 1.8 mA, between about 0.5 mA and about 1.5 mA, between about 0.5 mA and about 1 mA, between about 0.1 mA and about 1 mA, approximately 0.5 mA, approximately 0.75 mA, approximately 1 mA, etc.). Alternatively the applied energy may have an average amplitude of between about 0.1 mA and about 2 mA (e.g., between about 0.2 mA and about 1.8 mA, between about 0.5 mA and about 1.5 mA, between about 0.5 mA and about 1 mA, between about 0.1 mA and about 1 mA, approximately 0.5 mA, approximately 0.75 mA, approximately 1 mA, etc.). The applied energy is typically pulsed, and may be pulsed square waves, sinusoidal waves, triangular waves, etc. The applied energy may be biphasic or monophasic. For example, the applied energy may be biphasic. The applied VNS treatment may be a constant biphasic pulse train having a frequency of between 1-100 Hz (e.g., 10 Hz) and a peak amplitude of between about 0.5 mA and 2 mA (e.g., approximately 0.75 mA). Any of the methods for treatment described herein may be configured to apply this type of VNS treatment.

[0012] Any of the apparatuses (e.g., devices, systems, etc.) described herein may be configured to be implanted on the vagus nerve. Thus, any of these apparatuses may be implanted via a nerve sheath or nerve cuff configured to secure the apparatus onto the nerve and/or prevent movement of the apparatus relative to the nerve and/or insulate the apparatus from other tissues. The implanted apparatus may be implanted in any appropriate location on the nerve, including one or around the vagus nerve at the upper chest, or on or around the vagus nerve at a sub-diaphragmatic location. The implant may be a leadless implant that is connected to the vagus (see, e.g., U.S. Pat. Nos. 8,412,338, 8,612,002, 8,886,339, and 8,788,034, each of which is herein incorporated by reference in its entirety). For example, any of these apparatuses may include a nerve cuff configured to secure the stimulator to the vagus nerve. Alternatively, any of these apparatuses may include a lead connecting the micro stimulator and/or other components to the stimulation applicator on/around the vagus nerve via one or more leads.

[0013] As mentioned, any of these apparatuses may be configured to apply VNS treatment comprising a low duty-cycle electrical stimulation of between about 0.25 mA and about 5 mA to the vagus nerve for less than about 2 minutes. The apparatus may be configured to provide an off-time of at least x minutes/hours (e.g., 10 minutes, 20 minutes, 30 minutes, 40 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, etc.).

[0014] Any of the apparatuses described herein may be configured to perform a method of reducing demyelination in a patient diagnosed with or at risk of a disorder involving demyelinated nerves (e.g., including but not limited to methods of treating a disorder and/or disease associated with demyelination, such as multiple sclerosis). For example, a method of reducing demyelination (and/or a method of increasing remyelination) may be a method comprising detecting a marker for demyelination and applying stimulation to the vagus nerve to reduce demyelination of nerves within the patent.

[0015] Applying stimulation to the vagus nerve includes applying VNS treatment and may comprise, for example, applying electrical stimulation of between about 0.25 and about 5 mA to the vagus nerve for less than about 2 minutes. In some variations this may include waiting for an off-time (e.g., an off-time of at least 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, etc.).

[0016] Any of these methods may include applying non-invasive stimulation to the vagus nerve. For example, the simulation may be through a transdermal (e.g., via a surface electrode and/or mechanical stimulation, including ultrasound) route over a portion of the vagus nerve. The vagus nerve includes a number of branches or extensions that may be accessed and/or targeted from outside of the body either mechanically and/or electrically. For example, non-invasive application may include ultrasound stimulation of the vagus nerve. Any of these methods may include applying transdermal electrical stimulation (TENS), or the like.

[0017] Any of the methods described herein may include monitoring, e.g., periodically, on demand, and/or continuously, one or more markers (e.g., biomarkers) for demyelination or a risk of demyelination. As mentioned, any appro-

priate method or apparatus for monitoring demyelination or a risk of demyelination may be used. For example any of these methods may include detecting a marker for demyelination comprising monitoring the patient's temperature. A change (including an increase) in core body temperature has been linked to an increase in symptoms in demyelination disorders, including but not limited to MS.

[0018] Any of the methods and apparatuses described herein may be used with or linked to markers for the integrity of the blood-brain barrier. The methods and apparatuses described herein generally improve the integrity of the blood-brain barrier. Thus, any marker linked to leakage or loss of integrity of the blood-brain barrier may be used to trigger VNS therapy as described herein. Examples of markers may include Serum S100β, as well as imaging modalities such as contrast-enhanced magnetic resonance imaging, CT-scan and lumbar puncture.

[0019] A detection of one or more markers (e.g., biomarkers) for demyelination may include determining a level of tumor necrosis factor in a blood or cerebrospinal fluid sample.

[0020] For example, described herein are methods (e.g., methods of treating a demyelination disorder, such as but not limited to MS, and/or methods of reducing or reversing demyelination) that include: detecting demyelination in a patient, and applying stimulation to the vagus nerve to increase the remyelination of nerves within the patent.

[0021] For example, any of these methods may include repeatedly applying a low duty-cycle electrical stimulation of between about 0.25 and about 5 mA to the patient's vagus nerve for less than about 2 minutes, followed by an off-time (e.g., of between about 10 minutes and about 48 hours) before the next stimulation.

[0022] Any of these methods and apparatuses may also include or be adapted to include the concurrent (immediately before, during or after, including systemically and/or locally) treatment with one or more pharmacological agents, particularly those that are believed to help with a demyelinating condition, such as (but not limited to) MS. For example, any of these method may include concurrently treating with a pharmacological agent such as one or more of: interferon beta-1a, interferon beta-1b, glatiramer acetate, glatiramer acetate, peginterferon beta-1a, daclizumab, teriflunomide, fingolimod, dimethyl fumarate, alemtuzumab, mitoxantrone, ocrelizumab, natalizumab.

[0023] As mentioned, any of the methods and apparatuses described herein may include continuously monitoring the patient for demyelination or a condition implicated in demyelination. For example, any of these methods and apparatuses described herein may include monitoring the patient for a marker related to a diseased selected from the group consisting of neurodegenerative diseases, neuroinflammatory diseases, and neuropathies. In some examples, the method includes detecting demyelination in a patient by detecting a marker related to MS. For example, the marker (e.g., biomarker) may be selected from the group including: neurofilament, glial fibrillary acidic protein, the monocyte macrophage marker CD163, the glial activation marker YKL-40, the B cell chemoattractant CXCL13, miRNA, mRNA, myelin reactive t cells, Kir4.1 antibodies, osteopontin, and microbiome associated lipopeptides.

[0024] In particular, described herein are methods and apparatuses for reducing or preventing demyelination and/or for increasing remyelination by stimulation of a vagus nerve. For example, an apparatus (e.g., a system, device, assembly, etc., including implants), may include: a vagus nerve stimulator configured to be implanted over or adjacent to a vagus nerve; one or more electrodes on the vagus nerve stimulator configured to apply electrical stimulation to the vagus nerve; and a controller coupled to the vagus nerve stimulator and configured to apply electrical stimulation to the vagus nerve from the one or more electrodes, wherein the controller is constrained to apply a charge per day of between 2.5 nC and 7.5 mC to reduce demyelination and/or increase remyelination within the patient. This apparatus may be a system.

[0025] The system may include an input configured to receive one or more marker level indicators, wherein the controller is configured to adjust the applied charge based on the one or more marker level indicators. For example, the system may include a biosensor configured to detect the marker from the patient's blood and/or cerebrospinal fluid and to determine a maker level indicator.

[0026] The controller may be configured to deliver the electrical stimulation during one or more dose sessions of about 5 minutes or less (e.g., 4 min or less, 3 min or less, 2 min or less, 1 min or less, etc.). The controller may be configured to apply the charge per day at a frequency of between 1 and 20 Hz. In some variations the controller is configured to apply the charge per day at a frequency of between 1 and 12 Hz.

[0027] In any of these apparatuses, the system is configured to be implanted.

[0028] Any of these systems may include a nerve cuff configured to secure the vagus nerve stimulator to the vagus nerve. The controller may be configured to apply the charge per day at two distinct frequencies between 1 and 20 Hz. The controller may be configured to apply a first dose of the electrical stimulation to reduce demyelination at a first frequency between 1 and 20 Hz, and a second dose of electrical stimulation to increase remyelination within the patient at a second frequency that is higher than the first frequency. For example, the first dose of electrical stimulation may have a frequency less than 10 Hz, and the second dose of electrical stimulation has a frequency ranging from 10 Hz and 30 Hz. In some variations the first dose of electrical stimulation has a frequency of ranging from 1 Hz and 5 Hz, and the second dose of electrical stimulation has a frequency ranging from 10 Hz and 30 Hz.

[0029] Also described herein are method of increasing clearance of myelin debris in a patient diagnosed with or at risk of a disorder involving demyelinated nerves, the method comprising applying vagus nerve stimulation to the patient of between 2.5 nC to 7.5 mC per day. The Applying may comprise applying the vagus nerve stimulation at between 0.1 and 20 Hz to the vagus nerve. In some variations, applying comprises applying the vagus nerve stimulation for less than about 5 minute each day (e.g., less than about 4 min per day, less than about 3 min per day, less than about 2 min per day, less than about 1 min per day, etc.). Applying may comprise applying stimulation to the vagus nerve from an implanted neurostimulator attached or adjacent to the vagus nerve.

[0030] Any of these methods may include adjusting the applied vagus nerve stimulation based on the level of a marker. For example, the method may include detecting a marker for demyelination in a blood, sputum, and/or cerebrospinal fluid sample.

[0031] A system for reducing demyelination and/or increasing remyelination by stimulation of a vagus nerve may include: a vagus nerve stimulator configured to be implanted over or adjacent to a vagus nerve; one or more electrodes on the vagus nerve stimulator configured to apply electrical stimulation to the vagus nerve; and a controller coupled to the vagus nerve stimulator and configured to apply electrical stimulation to the vagus nerve from the one or more electrodes, wherein the controller is constrained to apply a low duty-cycle electrical stimulation for a duration of between 1 second and 5 minutes per day, the electrical stimulation comprising a first dose of electrical stimulation to reduce demyelination at a first frequency of between 1 and 20 Hz, and a second dose of electrical stimulation to increase remyelination at a second frequency that is higher than the first frequency.

[0032] The controller may be configured to apply electrical stimulation between 1 and 24 times per day. The frequency of the first dose of electrical stimulation may be between 1 Hz to 10 Hz. The frequency of the first dose of electrical stimulation may be between 1 Hz to 5 Hz. The frequency of the second dose of electrical stimulation may be between 10 Hz to 30 Hz. The first dose of electrical stimulation may have a frequency less than 5 Hz, and the second dose of electrical stimulation may have a frequency ranging from 10 Hz and 30 Hz.

[0033] As mentioned, the controller may be configured to modulate the electrical stimulation based on feedback from a user or based on one or more biomarkers associated with demyelination. The controller may be configured to reduce the frequency of the electrical stimulation based on the feedback. For example, a controller may be configured to adjust the duration of the first dose and the second dose based on the feedback.

[0034] A method of reducing demyelination and/or increasing remyelination in a patient having a disorder involving demyelinated nerves may include: applying a low duty-cycle electrical stimulation for a total duration of between 1 second and 5 minutes per day, the electrical stimulation comprising a first dose of electrical stimulation to reduce demyelination at a first frequency of between 1 and 20 Hz, and a second dose of electrical stimulation to increase remyelination at a second frequency that is higher than the first frequency. The low duty-cycle electrical stimulation may be applied, e.g., from 1 to 24 times per day.

[0035] The first frequency may range from 1 Hz to 10 Hz. In some variations, the first frequency ranges from 1 Hz to 5 Hz. The second frequency may range from 10 Hz to 30 Hz. The first dose of electrical stimulation may have a frequency less than 5 Hz, and the second dose of electrical stimulation may have a frequency ranging from 10 Hz and 30 Hz.

[0036] Any of these method may also include modulating the electrical stimulation based on feedback from a user or based on one or more biomarkers associated with demyelination. The controller may be configured to adjust the duration of the first dose and the second dose based on the feedback.

[0037] As described herein, any of these methods may include concurrently administering one or more of an Interferon β drug, glatiramer acetate, and daclizumab in combination with applying the low duty-cycle electrical stimulation to target interferon β-1a and 1b receptors and T-cell activation to reduce central nervous system inflammation and demyelination. For example, any of these methods may include administering one or more of fingolimod, teriflunomide, and dimethyl fumarate in combination with applying the low duty-cycle electrical stimulation to target lymphocyte migration or activation to reduce central nervous system inflammation and demyelination. Any of these methods may include administering one or more of mitoxantrone, alemtuzumab, ocrelizumab, and natalizumab in combination with applying the low duty-cycle electrical stimulation to induce DNA breakage, CD52 to induce cell lysis, B-cell CD20 antigen for depletion, and/or integrin receptors to alter leukocyte migration, to reduce central nervous system inflammation and demyelination. In some variations, these methods may include administering one or more of clemastine, a Selective Estrogen Receptor Modulator (SERM), and other drugs targeting oligodentrocyte progenitor cells to enhance maturation into myelin-producing oligodendrocytes to enhance remyelination and clinical recovery from central nervous system damage.

[0038] Embodiments of the invention relate generally to apparatuses (e.g., devices, systems) and methods for stimulation of one or more nerves (e.g., vagus nerve, splenic nerve, hepatic nerve, facial nerve, and trigeminal nerve, etc.) to treat neurodegenerative and neuroinflammatory disorders, and more specifically apparatuses and methods for nerve stimulation to increase the number and/or activity of oligodendrocyte progenitor cells (OPCs) which may assist in reducing demyelination and/or may promote remyelination to treat various neurodegenerative and neuroinflammatory disorders such as multiple sclerosis.

[0039] In particular, described herein are methods and apparatuses for stimulating a nerve to increase the number of oligodendrocyte progenitor cells (OPCs), which are key cells in repairing the damage caused by oligodendrocyte death and demyelination. These cells must be recruited to the damaged site where they proliferate. Once the myelin debris is cleared by other cells (macrophages, microglia, and astrocytes) the OPCs are able to differentiate into mature oligodendrocytes and lay down new myelin.

[0040] Although we have previously described the use of nerve (e.g., vagus nerve) stimulation to treat disorders of demyelination (e.g., US20190111263A1) and/or to reduce demyelination/increase remyelination (e.g., PCTUS2020027906), however it was not previously suspected that the effect may be due in large part to an increase in oligodendrocyte progenitor cells (OPCs) in at least some of the high-responding patients. As a result, it was not suggested to modulate the timing, dose and/or co-administration with one or more therapies (e.g., drug therapies) specifically related to oligodendrocyte progenitor cell activity and life cycle. Described herein are methods and apparatuses for preventing or treating neurodegenerative disorders including, but not limited to Multiple Sclerosis (MS).

[0041] For example, the methods and apparatuses (devices, systems, etc.) described herein may include nerve (e.g., vagus nerve, etc.) stimulation to treat neurodegenerative and neuroinflammatory disorders, such as vagus nerve stimulation to reduce demyelination and/or to promote remyelination to treat various neurodegenerative and neuroinflammatory disorders such as MS. These methods and apparatuses may be configured specifically to optimize the effects of oligodendrocyte progenitor cells (OPCs) and in particular, to optimize the increase in number of OPCs when applying stimulation to the nerve. For example, the methods described herein may be configured to be periodically per-

formed at times of the day that may dramatically increase the effect on OPCs. Lymphocytes are typically maximized within the spleen or within the blood at three times during a 24 hour period, and lymphocytes may assist in development of oligodendrocytes from OPCs. Thus it may be particularly beneficial to provide stimulation during these times, or at intervals spaced by these same time periods; even if the times do not overlap perfectly, the regularity may provide a more steady-state response. For example, most humans have an increased lymphocytes in the blood or in the tissues at noon, midnight, and 7 AM. Thus, it may be surprisingly beneficial to stimulate at these times, or three times per day separated by 12 hours, 7 hours and five hours (e.g., at time 0, then 5 hours later, than 7 hours later, than 12 hours later, then repeating the cycle, 5 hours later, 7 hours later, 12 hours later, etc.). The initial stimulation may be at, e.g., +/−3 hours from midnight, but the second stimulation may be at 5 hours from that, then 7 hours from the second stimulation, then 12 hours from the third stimulation, then repeat at 5 hours again, etc.

[0042] In some cases, the patient's actual pattern for nerve (e.g., vagus nerve) stimulation may be set to occur when lymphocytes are maximized within the spleen or within the blood of the patient to be treated. The pattern may be set daily.

[0043] For example, described herein are apparatuses (e.g., devices and/or systems) for reducing demyelination and/or increase remyelination by stimulation of a vagus nerve. These apparatuses may be implants or implanted into the patient's body. Any of these apparatuses may include: a time to trigger stimulation at 3 times during the day, separated by approximately 5 hrs, 7 hours and 12 hours, before repeating this cycle daily. Alternatively or additionally, the apparatus may be configured to detect lymphocytes and to trigger stimulation as described herein at various times (e.g., three times during the day) when lymphocytes are maximized within the spleen or within the blood. The stimulator may be configured to apply stimulation to a nerve (e.g., vagus nerve, splenic nerve, hepatic nerve, facial nerve, and trigeminal nerve). These apparatuses may include a controller coupled to the biosensor (if present) for detecting biomarker levels, and a pulse generator and power supply. These apparatus may further be configured to apply stimulation to the nerve from the stimulator sufficient to increase the number of oligodendrocyte progenitor cells (OPCs). In some variations, these apparatuses include an implant comprising a stimulator (e.g., a waveform and/or pulse generator, an oscillator, a power supply and/or power regulation circuit, etc.), a stimulation applicator (e.g., one or more electrodes, mechanical transducers, etc.), and controller. The controller may be configured as a microcontroller and may be in electrical communication with the stimulator so as to control operation of the stimulator. The controller may include one or more processors, a memory and/or a timer. The stimulator and/or controller may be in electrical communication, one or more stimulation applicators. In some variations the controller may include or be in communication with wireless communications circuitry for wirelessly communicating with one or more remote processors. The remote processor may be a hand-held device (e.g., smartphone, wearable electronics, etc.). The controller may optionally be in communication with one or more biosensors that may be included with the implant or may be remote from the implant (e.g., may be wearable, single-use, etc.). In some variations the biosensors are wirelessly connected to the apparatus.

[0044] In some variations the apparatus may be used without a biosensor. For example, the apparatus may be configured to periodically and/or on demand apply treatment to increase the number of oligodendrocyte progenitor cells (OPCs). The apparatus may be configured to apply treatment doses multiple times per day (2×, day, 3×, day, 4× day, etc.), or every other day, or every 3 days, etc. However, as described herein it may be particularly beneficial to apply stimulation 3× day, and/or at times when lymphocytes are maximal in the blood. In some variations the apparatus may be configured to both automatically apply a treatment dose on a predetermined and/or adjustable scheduled, as well as provide treatment doses based on input from a user (e.g., patient, physician, etc., including "on demand" doses) and/or based on detection of a biomarker (e.g., of lymphocyte level).

[0045] In general, the apparatuses described herein may be configured to be inserted or implanted into the body. For example, the apparatus may be configured to be implanted. The apparatus may include a stimulation applicator (also referred to as simply a stimulator or a treatment stimulator) that may be a mechanical and/or electrical stimulator. A mechanical stimulator may be a piezoelectric driver that may vibrate and/or apply pressure to the tissue, including to the vagus nerve, in the treatment parameters, such as mechanical stimulation of the nerve at between 1-2 kHz for a treatment time (e.g., between 1 ms and 5 minutes, e.g., 10 ms-10 sec, etc.). Alternatively or additionally, the stimulation applicator may be an electrical stimulation applicator and may include one or more (e.g., two or more) electrodes configured to apply electrical stimulation to the nerve. For example, electrical stimulation of about 0.1 mA to 10 mA (e.g., between 1 mA-5 mA), at a frequency of between about 1 Hz and about 2 kHz (e.g., between about 1-100 Hz), where the pulses applied have a pulse width of between about (50-500 usec, e.g., between about 100-300 usec). The controller may be configured to enforce an 'off-time' following a treatment dose of between about 10 minute and 12 hours (e.g., between about 2 hours and 10 hours, between about 3 hours and 6 hours, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, etc.). For example, the stimulator may include an electrode configured to apply electrical energy to the nerve.

[0046] In some variation the apparatus is configured to apply treatment to the patient in which the treatment is electrical stimulation. For example, the treatment may include the application of electrical energy at between about 1-100 Hz (e.g., between about 1-50 Hz, between about 1-20 Hz, between about 5-30 Hz, between about 5-15 Hz, approximately 5 Hz, approximately 10 Hz, approximately 15 Hz, etc.). The energy may have a peak amplitude of between about 0.1 mA and about 2 mA (e.g., between about 0.2 mA and about 1.8 mA, between about 0.5 mA and about 1.5 mA, between about 0.5 mA and about 1 mA, between about 0.1 mA and about 1 mA, approximately 0.5 mA, approximately 0.75 mA, approximately 1 mA, etc.). Alternatively the applied energy may have an average amplitude of between about 0.1 mA and about 2 mA (e.g., between about 0.2 mA and about 1.8 mA, between about 0.5 mA and about 1.5 mA, between about 0.5 mA and about 1 mA, between about 0.1 mA and about 1 mA, approximately 0.5

mA, approximately 0.75 mA, approximately 1 mA, etc.). The applied energy is typically pulsed, and may be pulsed square waves, sinusoidal waves, triangular waves, etc. The applied energy may be biphasic or monophasic. For example, the applied energy may be biphasic. The applied treatment may be a constant biphasic pulse train having a frequency of between 1-100 Hz (e.g., 10 Hz) and a peak amplitude of between about 0.5 mA and 2 mA (e.g., approximately 0.75 mA). Any of the methods for treatment described herein may be configured to apply this type of treatment.

[0047] Any of the apparatuses (e.g., devices, systems, etc.) described herein may be configured to be implanted on the nerve. Thus, any of these apparatuses may be implanted via a nerve sheath or nerve cuff configured to secure the apparatus onto the nerve and/or prevent movement of the apparatus relative to the nerve and/or insulate the apparatus from other tissues. The implanted apparatus may be implanted in any appropriate location on the nerve, including one or around the vagus nerve at the upper chest, or on or around the vagus nerve at a sub-diaphragmatic location. The implant may be a leadless implant that is connected to the vagus (see, e.g., U.S. Pat. Nos. 8,412,338, 8,612,002, 8,886,339, and 8,788,034, each of which is herein incorporated by reference in its entirety). For example, any of these apparatuses may include a nerve cuff configured to secure the stimulator to the vagus nerve. Alternatively, any of these apparatuses may include a lead connecting the micro stimulator and/or other components to the stimulation applicator on/around the vagus nerve via one or more leads.

[0048] As mentioned, any of these apparatuses may be configured to apply treatment comprising a low duty-cycle electrical stimulation of between about 0.25 mA and about 5 mA to the vagus nerve for less than about 2 minutes. The apparatus may be configured to provide an off-time of at least x minutes/hours (e.g., 10 minutes, 20 minutes, 30 minutes, 40 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, etc.).

[0049] Any of the apparatuses described herein may be configured to perform a method of reducing demyelination in a patient diagnosed with or at risk of a disorder involving demyelinated nerves (e.g., including but not limited to methods of treating a disorder and/or disease associated with demyelination, such as multiple sclerosis).

[0050] Applying stimulation to the nerve includes applying treatment and may comprise, for example, applying electrical stimulation of between about 0.25 and about 5 mA to the nerve for less than about 2 minutes. In some variations this may include waiting for an off-time (e.g., an off-time of at least 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, etc.).

[0051] Any of these methods may include applying non-invasive stimulation to the target nerve (e.g., vagus nerve, splenic nerve, hepatic nerve, facial nerve, and trigeminal nerve) or organ (e.g. spleen). For example, the simulation may be through a transdermal (e.g., via a surface electrode and/or mechanical stimulation, including ultrasound) route over a portion of the vagus nerve. The target nerve or organ (vagus nerve, splenic nerve, hepatic nerve, facial nerve, and trigeminal nerve) may include a number of branches or extensions that may be accessed and/or targeted from outside of the body (or in some examples from within the body) either mechanically and/or electrically. For example, non-invasive application may include ultrasound stimulation of the vagus nerve or spleen. Any of these methods may include applying transdermal electrical stimulation (TENS), or the like.

[0052] Any of the methods described herein may include monitoring, e.g., periodically, on demand, and/or continuously, one or more biomarkers from blood, serum, sputum, or cerebral spinal fluid (e.g., neurofilament light chain, lymphocyte level, etc.). For example any of these methods may include detecting a marker demyelination or neurodegredation.

[0053] Any of these methods and apparatuses may also include or be adapted to include the concurrent (immediately before, during or after, including systemically and/or locally) treatment with one or more pharmacological agents, particularly those that are believed to help with a demyelinating or neurodegradating condition, such as (but not limited to) MS. For example, any of these methods may include concurrently treating with a pharmacological agent such as one or more of: interferon beta-1a, interferon beta-1b, glatiramer acetate, glatiramer acetate, peginterferon beta-1a, daclizumab, teriflunomide, fingolimod, dimethyl fumarate, alemtuzumab, mitoxantrone, ocrelizumab, natalizumab. For example, these methods and apparatuses may also include or be adapted to include the concurrent (immediately before, during or after, including systemically and/or locally) treatment with one or more injectable medications: Avonex® (interferon beta-1a), Betaseron® (interferon beta-1b), Copaxone® (glatiramer acetate), Extavia® (interferon beta-1b), Glatiramer Acetate Injection (glatiramer acetate-generic equivalent of Copaxone 20 mg and 40 mg doses), Glatopa® (glatiramer acetate-generic equivalent of Copaxone 20 mg and 40 mg doses), Kesimpta® (ofatumumab), Plegridy® (peginterferon beta-1a), and Rebif® (interferon beta-1a). These methods and apparatuses may also include or be adapted to include the concurrent (immediately before, during or after, including systemically and/or locally) treatment with one or more oral medications: Aubagio® (teriflunomide), Bafiertam™ (monomethyl fumarate), Dimethyl Fumarate (dimethyl fumarate—generic equivalent of Tecfidera), Gilenya® (fingolimod), Mavenclad® (cladribine), Mayzent® (siponimod), Ponvory™ (ponesimod), Tecfidera® (dimethyl fumarate), Vumerity® (diroximel fumarate), and Zeposia® (ozanimod). These methods and apparatuses may also include or be adapted to include the concurrent (immediately before, during or after, including systemically and/or locally) treatment with one or more infused medications: Lemtrada® (alemtuzumab), Novantrone® (mitoxantrone), Ocrevus® (ocrelizumab), and Tysabri® (natalizumab).

[0054] Any of the methods and apparatuses described herein may also include or be adapted to include the concurrent (immediately before, during or after, including systemically and/or locally) treatment with one or more acetylcholinesterase inhibitors which may act to enhance the effects of the nerve stimulation in its various therapeutic outcomes, such as (but not limited to): donepezil, rivastigmine, memantine, galantamine, and tacrine.

[0055] As mentioned, any of the methods and apparatuses described herein may include continuously monitoring the patient for demyelination or a condition implicated in demyelination. For example, any of these methods and apparatuses described herein may include also monitoring the patient for a marker related to a disease selected from the group consisting of neurodegenerative diseases, neuroin-

flammatory diseases, and neuropathies. In some examples, the method includes detecting demyelination in a patient by detecting a marker related to MS. For example, the marker (e.g., biomarker) may be selected from the group including: neurofilament, glial fibrillary acidic protein, the monocyte macrophage marker CD163, the glial activation marker YKL-40, the B cell chemoattractant CXCL13, miRNA, mRNA, myelin reactive t cells, Kir4.1 antibodies, osteopontin, and microbiome associated lipopeptides.

[0056] In particular, described herein are methods and apparatuses for reducing or preventing demyelination and/or for increasing remyelination by stimulation of a vagus nerve. For example, an apparatus (e.g., a system, device, assembly, etc., including implants), may include: a vagus nerve stimulator configured to be implanted over or adjacent to a vagus nerve; one or more electrodes on the vagus nerve stimulator configured to apply electrical stimulation to the vagus nerve; and a controller coupled to the vagus nerve stimulator and configured to apply electrical stimulation to the vagus nerve from the one or more electrodes, wherein the controller is constrained to apply a charge per day of between 2.5 nC and 7.5 mC to reduce demyelination and/or increase remyelination within the patient. This apparatus may be a system.

[0057] The controller may be configured to deliver the electrical stimulation during one or more dose sessions of about 5 minutes or less (e.g., 4 min or less, 3 min or less, 2 min or less, 1 min or less, etc.). The controller may be configured to apply the charge per day at a frequency of between 1 and 20 Hz. In some variations the controller is configured to apply the charge per day at a frequency of between 1 and 12 Hz.

[0058] In any of these apparatuses, the system is configured to be implanted.

[0059] Any of these systems may include a nerve cuff configured to secure the vagus nerve stimulator to the vagus nerve. The controller may be configured to apply the charge per day at two distinct frequencies between 1 and 20 Hz. The controller may be configured to apply a first dose of the electrical stimulation to reduce demyelination at a first frequency between 1 and 20 Hz, and a second dose of electrical stimulation to increase remyelination within the patient at a second frequency that is higher than the first frequency. For example, the first dose of electrical stimulation may have a frequency less than 10 Hz, and the second dose of electrical stimulation has a frequency ranging from 10 Hz and 30 Hz. In some variations the first dose of electrical stimulation has a frequency of ranging from 1 Hz and 5 Hz, and the second dose of electrical stimulation has a frequency ranging from 10 Hz and 30 Hz.

[0060] The first frequency may range from 1 Hz to 10 Hz. In some variations, the first frequency ranges from 1 Hz to 5 Hz. The second frequency may range from 10 Hz to 30 Hz. The first dose of electrical stimulation may have a frequency less than 5 Hz, and the second dose of electrical stimulation may have a frequency ranging from 10 Hz and 30 Hz.

[0061] For example, described herein are methods for increasing oligodendrocyte progenitor cells (OPCs) by stimulation of a nerve, the method comprising: identifying that a patient has a decreased level of oligodendrocyte progenitor cells (OPCs); applying electrical stimulation to a nerve from an implanted neurostimulator, wherein the applied electrical stimulation to the nerve is delivered as a charge per day of between 2.5 nC and 7.5 mC to increase the level of OPCs.

[0062] Any of these methods may include adjusting the electrical stimulation based on the level of OPCs. In some example, the methods may include adjusting the electrical stimulation based on the time of day.

[0063] The electrical stimulation may be delivered as three or more stimulation per day on a repeating cycle of waiting 5 hours or more between stimulations. For example, the repeating cycle comprises waiting 5 hours, then 7 hours, then 2 hours between the application of stimulation to the nerve.

[0064] In some examples applying comprises applying electrical stimulation to the nerve at between 0.1 and 20 Hz to the vagus nerve. For example, applying may include applying electrical stimulation to the nerve for less than 10 minute each day. In some examples, applying comprises applying s electrical stimulation to the nerve from an implanted neurostimulator attached or adjacent to a vagus nerve or a splenic nerve.

[0065] For example, a method for increasing oligodendrocyte progenitor cells (OPCs) by stimulation of a nerve may include: measuring or deriving a level of OPCs within a patient; adjusting an electrical stimulation based on the level OPCs within the patient; and applying the electrical stimulation to a nerve of the patient from an implanted neurostimulator, wherein the applied electrical stimulation to the nerve is delivered as a charge per day of between 2.5 nC and 7.5 mC to increase the level of OPCs.

[0066] Any of the systems described herein may be systems for increasing oligodendrocyte progenitor cells (OPCs) by stimulation of a nerve. For example, a system may include: a nerve stimulator configured to be implanted; one or more electrodes of the nerve stimulator configured to apply electrical stimulation to the nerve; and a controller coupled to the nerve stimulator and configured to apply electrical stimulation to the nerve from the one or more electrodes, wherein the controller is configured to apply a charge per day of between 2.5 nC and 7.5 mC, wherein the controller is configured to adjust the applied electrical stimulation based on a measured or derived level of OPCs within the patient. The system may include an input configured to receive the measured or derived level of OPCs within the patient.

[0067] Any of these systems may include a biosensor configured to detect a marker for the level of OPCs within the patient. The controller may be configured to deliver the electrical stimulation based on the timing of the peak levels of lymphocytes within the patient's blood. In some examples the controller is configured to deliver the electrical stimulation during a one or more dose sessions of 5 or fewer minutes. As mentioned, the controller may be configured to apply the charge per day at a frequency of between 1 and 20 Hz. The controller may be configured to apply the charge per date at a frequency of between 1 and 12 Hz. Any of these systems may be configured to be implanted. For example, the system may include a nerve cuff configured to secure the vagus nerve stimulator to the nerve (e.g., a vagus nerve, splenic nerve, etc.). The controller may be configured to apply the charge per day at two distinct frequencies between 1 and 20 Hz.

[0068] All of the methods and apparatuses described herein, in any combination, are herein contemplated and can be used to achieve the benefits as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0069] The novel features of the invention are set forth with particularity in the claims that follow. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0070] FIG. **1** illustrates a typical example of the 4 epochs that follow lysolecithin injection to the spinal cord in a model used to study multiple sclerosis.

[0071] FIGS. **2**A and **2**B illustrate the experimental protocols used to study demyelination and remyelination.

[0072] FIG. **3**A illustrates a cross-section of a healthy spinal cord.

[0073] FIG. **3**B illustrates a stained cross-section of a spinal cord with a lesion (which may be considered a demyelination) induced by lysolecithin injection.

[0074] FIGS. **4**A-**4**D are graphs that show that vagus nerve stimulation reduced the amount of demyelination that resulted from lysolecithin injection. FIG. **4**A is a graph showing the effect of vagal nerve stimulation (VNS) on demyelination with various levels of stimulation (0 mA, 0.25 mA, and 0.75 mA) four days following inducing of a demyelinating lesion. FIG. B shows the increase in remyelination by two weeks after inducing the demyelinating lesion without VNS treatment (0 mA) and with VNS treatment (0.75 mA), showing a rapid remyelination when VNS is applied. FIG. **4**C is a 3D graph of the lesion size variation by depth at four days post induction of the demyelinating lesion with and without VNS treatment. FIG. **4**D is a 2D projection graph of the median lesion four days post-induction of the demyelinating lesion comparing sham (no VNS treatment) and VNS treatment.

[0075] FIGS. **5**A-**5**G are graphs that show that vagus nerve stimulation increased the rate and/or amount of remyelination. FIG. **5**A is a graph showing the change in demyelination (determined by the change in induced lesion volume) following induction of demyelination with and without VNS treatment, showing an approximately 65% reduction in the area under the lesion volume (mm^3)/days post induction. FIG. **5**B is a 3D representation of the demyelination (lesion) size variation with depth for no VNS treatment (sham) and VNS treatment. FIG. **5**C is a 2D projection of median lesion volume eight days post-induction of demyelination (e.g., lesion) with VNS treatment and without VNS treatment (sham). FIG. **5**D is a 3D representation of demyelination (lesion size) variation with depth at day 14 following inducing of demyelination (day 14 post induction) with VNS treatment (VNS) and without VNS treatment (sham). FIG. **5**E is a 2D projection of median demyelination (lesion) at two weeks post-induction of demyelination with VNS treatment and without VNS treatment ("sham"). FIG. **5**F is a 3D representation of demyelination (lesion size) variation with depth at day 21 following inducing of demyelination (day 14 post induction) with VNS treatment (VNS) and without VNS treatment (sham). FIG. **5**G is a 2D projection of median demyelination (lesion) at three weeks post-induction of demyelination with VNS treatment and without VNS treatment ("sham").

[0076] FIG. **6**A shows the experimental protocol used to show the effect of VNS treatment as described herein on vessel leakiness following post-induction of demyelination.

[0077] FIG. **6**B illustrates the use of VNS treatment as described herein to reduce the leakiness of the blood-brain barrier following induced demyelination. VNS treatment before induced demyelination prevented the passage of dye (Evans blue) through the rat model of the blood brain barrier. VNS treatment after induced demyelination reduced and reversed the leakiness. VNS treatment on Day 0 (following LPC induction) significantly decreased leukocyte infiltration 24 hours post-stimulation, while VNS treatment on Day 4 post-LPC induction significantly decreases leukocyte infiltration 24 hours post-stimulation.

[0078] FIG. **7**A illustrates the effect of the alpha-7 nicotinic acetylcholine receptors (a7 nAChR) in preventing demyelination remyelination from VNS treatment (compared to sham without VNS treatment).

[0079] FIG. **7**B illustrates the effect of the alpha-7 nicotinic acetylcholine receptors in increasing remyelination from VNS treatment (compared to sham without VNS treatment).

[0080] FIG. **8** shows the effect of VNS treatment as described herein to prevent or reverse leakiness of the blood-brain barrier compared to sham (no VNS treatment). In FIG. **9**, CD3+ T cell infiltration was significantly decreased in the VNS group on Day 3 post-LPC induction compared to Sham group by 50%.

[0081] FIG. **9** illustrates macrophage infiltration through a model of the blood-brain barrier is significantly decreased 24 hours post-demyelination induction (e.g., via LPC) with VNS treatment compared to sham (no VNS treatment) by 55%.

[0082] FIGS. **10**A and **10**B illustrate the effect of pro-resolution lipid Resolvin D1 following induction of demyelination with VNS treatment (VNS) and without VNS treatment (sham), showing Resolvin D1 (RvD1) is increased in VNS animals 4 days post-LPC induction compared to Sham animals and remains elevated 14 days post-LPC induction. Levels were decreased below that of the Sham 21 days post-LPC induction, by which time, no visible lesion is detected in VNS animals.

[0083] FIG. **11** schematically illustrates one example of an apparatus for reducing demyelination (e.g., increasing remyelination and/or reducing leakage through the blood-brain barrier), as described herein.

[0084] FIG. **12** illustrates VNS at a frequency of less than about 100 Hz (e.g., 75 Hz or less, 50 Hz or less, 40 Hz or less, 30 Hz or less, 25 Hz or less, etc.) more effective than higher frequencies, e.g., 1.8 kHz and above.

[0085] FIG. **13** illustrates significantly more lesion area remyelinated after stimulation at 1 or 5 minutes at 10 Hz, while in general, stimulation for less than 20 minutes (e.g., less than 15 minutes, less than 10 minutes, less than 9 minutes, less than 8 minutes, less than 7 minutes, less than 6 minutes, less than 5 minutes, etc.) was more effective than longer times.

[0086] FIGS. **14** and **15** illustrate vagus nerve stimulation (VNS) significantly increased myelin debris uptake when the stimulation was within the prescribed treatment ranges.

[0087] FIG. **16** shows stained sections through tissue from sham animals.

[0088] FIG. **17** shows an image from animals treated with VNS on day 4 of treatment paradigm A, post-VNS.

[0089] FIGS. **18** and **19** show analysis of the staining to quantify the intensity and extent.

[0090] FIG. **20** illustrates a 34% reduction in lesion volume in the VNS group relative to the sham group on day 10 post-induction in aged mice.

[0091] FIG. **21** illustrates a 2.4× fold increase in oil red 0 staining as a % of lesion for the VNS group compared to the sham group ($p<0.05$) on day 10 in aged mice.

[0092] FIG. **22** is a graph showing that VNS enhances myelin clearance, as evidenced by the increase in myelin uptake by macrophages following VNS (e.g., showing an increase in the percentage of cells engulfing myelin following VNS).

[0093] FIG. **23** is a graph comparing clinical scores of rats treated chronic low duty cycle VNS versus sham VNS, showing a reduction in clinical scores from implanted VNS vs. implanted sham treatment in an MS model.

[0094] FIG. **24** shows the percent of CD4+ T cells in samples from rat spinal cord treated with VNS as compared to sham, showing the VNS reduces the pathogenic CD3+/CD4+/IFNg+ TH1 cells in the spinal cord one to two days following VNS.

[0095] FIG. **25** shows the percent of CD8+ T cells in samples from rat spinal cord treated with VNS as compared to sham, showing the VNS reduces the pathogenic CD3+/CD8+/IFNg+ TH1 cells in the spinal cord one to two days following VNS.

[0096] FIG. **26** is a graph showing the effect of stimulation on a nerve of the inflammatory reflex (e.g., the vagus nerve) on oligodendrocyte progenitor cells (OPCs) in rats.

DETAILED DESCRIPTION

[0097] In general, described herein are methods and apparatuses for increasing the number of oligodendrocyte progenitor cells (OPCs) by simulation of a nerve of the inflammatory reflex, including the vagus nerve and/or splanchnic, splenic, phrenic, mesenteric ganglion, Prevertebral ganglia, Paravertebral ganglia, trigeminal, glossopharyngeal, auricular nerve, auricular vagus nerve.

[0098] Electrical and/or mechanical stimulation of the cholinergic anti-inflammatory pathway (NCAP) by stimulation of the carotid vagus nerve has been well described. For example, see U.S. Pat. Nos. 6,838,471, 8,914,114, 9,211,409, 6,610,713, 8,412,338, 8,996,116, 8,612,002, 9,162,064, 8,855,767, 8,886,339, 9,174,041, 8,788,034 and 9,211,410, each of which is herein incorporated by reference in its entirety. It has not previously been suggested that vagus nerve stimulation may be used to prevent or reduce demyelination and/or improve remyelination, and in particular, to increase the number of OPCs. Vagus nerve stimulation, through activation of both efferent and afferent pathways (or primarily through one of the efferent or afferent pathway), may be able to reduce the inflammation associated with inflammatory diseases and disorders, thereby reducing the severity of the symptoms and/or slowing, stopping, or reversing the progression of the disease. Applicants have surprisingly found that the apparatuses (e.g., systems, devices, etc.) and methods described herein may be used to stimulate the vagus nerve to reduce demyelination and/or to increase or promote remyelination. Furthermore, although the use of VNS treatment to modulate inflammation has been thought to involve afferent pathways, remyelination and demyelination may involve the efferent pathway or both the afferent and efferent pathways.

[0099] Diseases (e.g., diseases and disorder of myelination) which may benefit from VNS as described herein (e.g., the methods and apparatuses described herein) include, but are not limited to, multiple sclerosis (MS), Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis (ALS), chronic inflammatory demyelinating polyneuropathy (CIDP), and Batten disease. Other neuroinflammatory disorders may include: acute disseminated encephalomyelitis (ADEM), acute optic neuritis (AON), transverse myelitis, and Neuromyelitis optica (NMO). Neuropathies that may benefit from VNS include peripheral neuropathies, cranial neuropathies, and autonomic neuropathies. Thus any of the methods and apparatuses described herein may be used (and adapted for) treatment with any of these diseases and neuropathies.

Vagus Nerve Stimulation Systems and Devices

[0100] In some variations the devices described herein are electrical stimulation devices that may be implanted, and may be activated to apply current for a proscribed duration, followed by a period without stimulation. As described in the examples that follow, the stimulation protocol may comprise a very limited period of stimulation (e.g., an on-time of less than 5 minutes, 2 minutes, 1 minute, etc.) followed by an off-time (during which stimulation is not applied, and may be prevented from being applied) of extensive duration (e.g., greater than 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 4 hours, 12 hours, greater than 20 hours, greater than 24 hours, greater than 36 hours, greater than 48 hours, etc.). The applied energy may be electrical energy that is a fixed current having a frequency that is within the range of about 0.5 mA to 5 mA (e.g., approximately 2 mA), at a frequency of between about 1 Hz and about 1000 Hz (e.g., between 1 Hz and 100 Hz, between 1 Hz and 30 Hz, between 10 Hz and 200 Hz, etc.), where the pulses applied have a pulse width of approximately (50-500 usec, e.g., a 200 usec pulse). Thus, the duty-cycle of the applied current may be extremely low, where duty cycle may refer to the ratio of on-time/(on-time plus off-time). The stimulation is applied at an extremely low duty cycle, where duty cycle may refer to the percent of on-time to the total on-time and off-time for the ongoing treatment. For example, low duty cycle may be less than about 10, 5, 4, 3, 2, 1, or 0.5 percent of on-time to the total on time and off-time. The effect may be seen relatively quickly, and may persist over the entire off-time.

[0101] In particular, the methods and apparatuses described herein may be applied as needed, e.g., when the patient expresses or is likely to express an increased risk for demyelination and/or is experiencing (or has experienced) demyelination. Alternatively or additionally, the methods an apparatuses may be applied as needed when the patient expresses or is likely to express, and/or is experiencing (or has experienced) a leakage through the blood-brain barrier.

[0102] For example, we show herein that a low level, low duty cycle stimulation protocol (as described herein) reduces demyelination and/or increases remyelination, and prevents and/or reduces leakage through the blood-brain barrier. The effectiveness of low level, low duty cycle vagus nerve stimulation (VNS therapy) administered on even a single day results in a reduction in demyelination and an increase in remyelination seen over the course of two to three weeks. This type of stimulation contrasts with the use of a high duty cycle stimulation used by others to modulate vagus-nerve

mediated functions (such as heart rate, etc.), or treat disorders such as epilepsy and depression. An important finding here is that demyelination can be reduced and even more surprisingly, remyelination can be increased. This effect is corroborated at these low duty cycle parameters by examining the histology of the spinal cord as described later below. Although low duty cycle vagus nerve stimulation is effective and highly efficient at reducing inflammation, in some embodiments, a higher duty cycle stimulation can be used, such as a duty cycle that is greater than about 1, 2, 3, 4, 5, 10, 20, 30, 40, or 50 percent of on-time to the total on-time and off-time.

[0103] MS patients may experience circadian pattern disruptions to symptoms that may be associated with or caused in part by the circadian patterns of IL-6 levels. Optionally, drugs, such as steroids, can be used along with VNS to suppress nighttime spiking of IL-6. Similarly, VNS can be modulated, by altering the timing of the stimulations for example, to suppress nighttime spiking of IL-6 more effectively. However, one advantage of VNS is the relatively long duration of the effect after a single stimulation, which may allow suppression of IL-6 levels during both night and day, which may render unnecessary the need for supplementary drug treatment or alternative timings. In some embodiments, VNS can be given in the evening before sleep, such as 15, 30, 45, 60, 90, 120, 150, or 180 minutes before sleep, and may also be given at night during sleep, to ensure nighttime suppression of IL-6 levels. In some embodiments, the amplitude of stimulation during sleep can be lowered (e.g., less than 2, 1.5, or 1 mA) to avoid waking the patient. In some embodiments, IL-6 levels can be measured and/or monitored, and VNS can be modulated based on the measured and/or monitored IL-6 levels. Other cytokines may also be measured and/or monitored, such as IL-1, TNF, IFN-gamma, IL-12, IL-18, and GM-CSF. These other cytokines may be used instead of or in addition to IL-6, either in combination or singly.

[0104] The methods, devices and systems herein may be applied specifically to treat any disorder for which a reduction of demyelination and/or an increase in remyelination would be beneficial. For example, described herein are electrodes (e.g., cuff electrodes, microstimulators) that may be placed around the vagus nerve and may communicate with one or more stimulators configured to apply appropriate stimulation of the vagus nerve to modulate demyelination and/or remyelination. The stimulator may be implanted. In some variations the stimulator is integral to the electrodes, and may be charged externally. The extremely low duty-cycle of the technique described herein may allow the device to be miniaturized to a greater degree than previously suspected for the treatment of chronic disorders via an implantable device.

[0105] In general, a device or system for modulating demyelination and/or remyelination may include a stimulator element (e.g., an electrode, actuator, etc.) and a controller for controlling the application of stimulation by the stimulator element. A stimulator element may be configured for electrical stimulation (e.g., an electrode such as a cuff electrode, needle electrode, paddle electrode, non-contact electrode, array or plurality of electrodes, etc.), mechanical stimulation (e.g., a mechanical actuator, such as a piezoelectric actuator or the like), ultrasonic actuator, thermal actuator, or the like. In some variations the systems and/or devices are implantable. In some variations the systems and/or device are non-invasive. In general, the controller may include control logic (hardware, software, firmware, or the like) to control the activation and/or intensity of the stimulator element. The controller may control the timing (e.g., on-time, off-time, stimulation duration, stimulation frequency, etc.). In variations in which the applied energy is electrical, the controller may control the applied waveform (amplitude, frequency, burst duration/inter-burst duration, etc.). Other components may also be included as part of any of these device or system, such as a power supply (e.g., battery, inductive, capacitor, etc.), transmit/receive elements (e.g., antenna, encoder/decoder, etc.), signal generator (e.g., for conditioning or forming the applied signal waveform), and the like. In some embodiments, a rechargeable battery that may be inductively charged allows the stimulator to deliver numerous electrical stimulations before needing to be recharged. In other embodiments, one or more capacitors that can also be inductively charged can be used to store a limited amount of energy that may be sufficient to deliver a single stimulation or a daily amount of stimulations. This dramatically reduces the size and cost of the stimulator, but requires that the user charge the stimulator daily or before each use.

[0106] In one example, an implantable device for modulating demyelination and/or remyelination (and/or reducing or preventing leaking of the blood-brain barrier) includes an electrode for electrically stimulating the vagus nerve. The electrode may be, for example, a cuff electrode. The electrode may be connected (directly or via a connector) to a controller and signal generator. The signal generator may be configured to provide an electrical signal to the electrode(s). For example, the electrical signal may be an electrical waveform having a frequency of between about 0.1 Hz and about 1 KHz (e.g., 10 Hz), where the pulses applied have a pulse width of approximately (50-500 usec, e.g., a 200 usec pulse). The signal generator may be battery (and/or inductively) powered, and the electrical signal may be amplitude and/or voltage controlled. For example in some variations the device or system may be configured to apply a current that is between about 0.05 mA to 25 mA (e.g., approximately 0.5 mA, 1 mA, 2 mA, 3 mA, etc.). The electrical signal may be sinusoidal, square, random, or the like, and may be charge balanced. In general, the controller (which may be embodied in a microcontroller such as a programed ASIC), may regulate turning on and off the stimulation. For example, stimulation may be applied for an on-time of between about 0.1 sec and 10 minutes (e.g., between 1 sec and 5 minutes, between 1 sec and 2 minutes, approximately 1 minute, etc.); the stimulation may be configured to repeat automatically once every x hours or days, e.g., every other day (off time of approximately 48 hours), once a day (e.g., with an off-time of approximately 24 hours), twice a day (off-time of approximately 12 hours), three times a day (off time of approximately 8 hours), four times a day (off time of approximately 6 hours), or the like. In some variations the implant may be configured to receive control information from a communications device. The communications device may allow modification of the stimulation parameters (including off-time, on-time, waveform characteristics, etc.). The communications device may be worn, such as a collar around the neck, or handheld.

[0107] In use, an implant may be configured to be implanted so that the electrodes contact or approximate the vagus nerve or a portion of the vagus nerve. In one variation

the implant includes a cuff that at least partially surrounds the vagus (e.g., near the carotid region). The controller and/or signal generator (including any power source) may be formed as part of the cuff or may be connected to by a connector (e.g., wire).

[0108] In some variations the device may be non-invasive. For example, the device may be worn outside the body and may trigger stimulation of the vagus nerve from a site external to the body (e.g., the ear, neck, torso, etc.). A non-invasive device may include a mechanical device (e.g., configured to apply vibratory energy). In some variations the device is configured to apply ultrasound that may specifically target the vagus nerve and apply energy to activate the vagus nerve. In some variations, transcutaneous magnetic stimulation of the vagus nerve may be used.

[0109] In any of the variations described herein, the devices, system and methods may be configured to prevent desensitization of the signal in a way that would reduce or inhibit the modulation of demyelination and/or remyelination. For example in some variations, "over stimulation" of the vagus nerve, e.g., simulation at intensities that are too great or applied for too long, or outside of the frequency ranges described herein, may result in desensitization of the effect, thus further modulation may be limited or inhibited. Therefore, in some embodiments, the amplitude of stimulation may be restricted from exceeding (i.e., be less than) about 3 mA, 4 mA, or 5 mA, and/or the duty cycle may be restricted from exceeding about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25%. In some embodiments, the amplitude is also at least 0.25 mA, 0.5 mA, 0.75 mA, or 1.0 mA.

[0110] The examples illustrated above may provide insight into the devices, systems and methods of use for stimulation of the vagus nerve to modulate demyelination and/or remyelination. These methods and devices may be used to treat any indication for which modulation of demyelination and/or remyelination would be beneficial. Non-limiting examples of indications include neurodegenerative and neuroinflammatory diseases such as multiple sclerosis (MS), Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis (ALS), and Batten disease. Other examples include peripheral neuropathies, cranial neuropathies, and autonomic neuropathies. In general, these devices may offer alternative and in some ways superior treatment as compared to pharmacological interventions aimed at modulating demyelination and/or remyelination, and therefore may be used for any indication for which such pharmacological treatments are suggested or indicated. In some embodiments, the VNS treatments described herein can be used in conjunction with pharmacological treatments, particularly when the pharmacological treatment has a different mechanism of action than the VNS, which may lead to synergistic results.

[0111] Thus, the methods of modulating demyelination and/or remyelination as described herein may be used in conjunction with one or more pharmacological interventions, and particularly interventions that treat diseases associated with demyelination, neurodegeneration or neuroinflammation. For example, it may be beneficial to treat a subject receiving stimulation of the vagus nerve to modulate demyelination and/or remyelination by also providing agent such as intravenous corticosteroids (e.g., methylprednisolone), oral corticosteroids, interferons beta-1a and beta-1b, monoclonal antibodies (e.g., natalizumab, alemtuzumab, daclizumab and ocrelizumab), and immunomodulators (e.g., glatiramer acetate, mitoxantrone, fingolimod, teriflunomide, and dimethyl fumarate).

[0112] Thus, described herein are devices (VNS devices) for the treatment of neurodegenerative and/or neuroinflammatory disorders. Such devices are generally configured to apply low duty-cycle stimulation to the vagus nerve of a subject, as described in any of the variations (or sub-combinations) of these variations. In some embodiments, the patient is first diagnosed or identified with a neurodegenerative and/or neuroinflammatory disorder, particular a disorder characterized by demyelination or need for remyelination, before being implanted and treated with the VNS device.

[0113] In use, any of the methods described herein may include a step of monitoring for demyelination or demyelination-associated disorders, which may be determined through detection of a biomarker from blood and/or cerebrospinal fluid, and/or through medical imaging techniques such as MRI or CT scans. For example, as assay for an inflammatory cytokine (e.g., tumor necrosis factor) may be used to detect acute inflammatory episodes. Monitoring may be continuous or discrete (e.g., at one or more times, or time intervals). In addition or alternatively, biomarkers associated with multiple sclerosis or other neurodegenerative and/or neuroinflammatory diseases or neuropathies can be used for monitoring, depending on the disease being treated. See Housley, W. J., D. Pitt and D. A. Hafler (2015). "Biomarkers in multiple sclerosis." Clin Immunol 161(1): 51-58; and Katsavos, S. and M. Anagnostouli (2013). "Biomarkers in Multiple Sclerosis: An Up-to-Date Overview." *Mult Scler Int* 2013: 340508. For example, biomarkers found in MS serum and cerebrospinal fluid include markers of neurodegeneration including neurofilament and GFAP, the monocyte macrophage marker CD163, the glial activation marker YKL-40, the B cell chemoattractant CXCL13, miRNA and mRNA, myelin reactive t cells, Kir4.1 antibodies, osteopontin, and microbiome associated lipopeptides. Any of these biomarkers can be monitored and/or measured alone or in combination, and can be used as feedback to modulate VNS. Other biomarkers for treating MS patients in particular are listed in Table 1.

(A) Diagnostic biomarkers (criteria i, iv, v, and vi)

(1) Genetic-immunogenetic		
HLA-DRB1*1501	+++ Risk for MS	See also B, E
DR3 and DR4 haplotypes	++ Risk for MS	
HLA-DRB1*04	++ Risk for MS	
HLA-DRB1*0401	+ Risk for high familial autoimmunity in MS patients	See also F
HLA-DQ1*0102	+ Risk for MS, in coexistence with HLA-DRB1*1501	
HLA-DPB1*0501	+ Risk for opticospinal MS	
HLA-DPB1*0301	+ Risk for opticospinal MS	
IL2RA and IL7RA polymorphisms	+ Risk for MS	
EVI5, CD58, KIAA0350, and RPL5 polymorphisms	+/– Risk for MS	

-continued

(2) Laboratorial		
OCB IgG	+++ But with low specificity	See also E
KFLC	+++ But with low specificity	See also E
MRZ reaction	+++ Higher specificity than OCB IgG	See also E, F
Anti BRRF2, anti EBNA-1	++	See also B, C
Anti MBP 48-70 and 85-170	+	See also B, E
Anti MBP 43-68 and 146-170	+ Differential diagnosis with OND's	See also B, E
MBP/MOG conformational epitopes antibodies	+ But low specificity	See also B, E, F
VEGF-A	+ Lower CSF levels in all disease forms, but low specificity	See also D, E
Vitamin D	+++ Lower levels, higher risk for MS	See also C, F
TRECs	+ Lower serum levels in all disease forms, but low specificity	See also B
CSF levels of lipocalin 2	+ Higher CSF levels in MS, but low specificity	See also F
AR	+++ Differential diagnosis of MS and NMO	See also C, E
NO and NO metabolites	+ Higher CSF and serum levels in MS, but low specificity	See also C, E
NF-L	++ Higher CSF levels in MS patients	See also C, F
NAA	+++ Differential diagnosis of RRMS and NMO	See also D, E
GFAP	+++ Differential diagnosis of MS and NMO	See also C, E
	+ Differential diagnosis of MS and NMO	See also C, E
Nogo-A	++ For MS forms with prominent neurodegenerative element	See also D
(3) Imaging		
Contrast-enhanced T1 lesions	+++	See also C
Hyperintense T2-weighted lesions	+++	See also C, D, E
Corpus callosum DTI abnormalities	++ Early diagnostic biomarker	See also E
MRS findings (glutamate/ choline)	+++	See also C, D, E
PET	++ But still experimental	
EPs	+++	See also
Motor EPs	+++ Spinal cord syndrome at presentation	C,
VEMPs	+++ Brainstem dysfunction	D, E
SSR	++ Autonomic dysfunction assessment in MS patients	See also E

(B) Biomarkers of phenotypical expression (criteria ii, iv, v, and vi)

(1) Genetic-immunogenetic		
HLA-DRB1*1501	+++ Early disease onset	See also A, E
HLA-DRB1*1501	+ Risk for cognitive decline	
HLA-DRB1*01	++ Protection against malignant disease form	
ApoE ε4	++ Greater risk for mental disorders	
(2) Laboratorial		
OCB IgM against myelin lipids	+/− Aggressive disease course	See also E
EBV antibodies	+ Early disease onset	See also A, C

-continued

Anti-MBP	+++ ADEM-like onset in childhood MS	See also A, E
Anti-MOG	+++ Childhood MS, ADEM, isolated optic neuritis, anti-AQP4 (−) NMO	See also A, E, F
rMOG index	+++ Progressive disease forms	
IL-6 serum levels	+++ Age at onset	See also C
TRECs	++ Lower levels PPMS	See also A
Amyloid-(1-42)	++ Lower levels, higher risk for mental disorders	
(3) Imaging		
UCCA atrophy	+++ Progressive disease forms	See also E
NAGM DTI abnormalities	+++ Progressive disease forms	

(C) Biomarkers of demyelination-neuroinflammation-relapse (criteria i, ii, iii, iv, v, and vi)

(1) Genetic-immunogenetic		
TOB1	+++ Underexpression, higher Th1 and Th17 percentage	See also E
(2) Laboratorial		
EBV antibodies	+ Higher inflammatory activity	See also A, B
CXCL13	++ Mobilizes B-cells, T-helper cells	
CXCL12	+/− Neuroprotection against inflammation in EAE/experimental	
IFN-/TNF-a	+++ Th1 immune response	
IL-1 levels imbalance	+ Triggering factor for neuroinflammation	
IL-6	+++ B-cell and T-cell immunity link, Th17 immune response triggering factor	See also B
	++ Correlation with relapse frequency in female MS patients	
IL-10-592 position polymorphisms	++ Regulation of CNS autoimmunity	
IL-15	++ BBB disruption, enhanced CD8(+) T cytotoxicity	
IL-33	+ Increase in IFN-γ and IL-17 in mice EAE	
sICAM-1	++ Higher levels, higher inflammatory activity	See also F
	+++ Higher levels in NMO than MS-marker of BBB disruption	
sVCAM-1	+++ Higher levels in NMO than MS-marker of BBB disruption	See also F
Laminin 411	++ TH-17 enhancement	
4 Integrin	++ Correlation with gadolinium-enhanced lesions during CIS	See also E, F
Osteopontin	++ Serum and CSF elevation during relapse	
Fetuin-A	+++ Overexpression in active demyelinating lesions	See also F
Vitamin D	+++ High levels, anti-inflammatory role-lower radiological disease activity	See also A, F
CSF mature B-cells/ plasma-blasts	++ Bigger accumulation, higher inflammatory activity	
CXCR3	++ Helps T-cells to enter the brain	
CX(3)CR1	++ CD4(+)CD28(−) cytotoxic cells biomarker	
CSF CCR2(+) CCR5(+) T cells	+++ Increase during MS relapse-osteopontin enhancement	
CD56 Bright NK	++ Remission phase	
AR	+++ Biomarker of BBB disruption	See also A, E
MMP-9	++ Higher CSF levels during relapse	
Ninjurin-1	++ Upregulation in active demyelinating lesions	

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MBP and fragments	+++ Higher CSF levels during relapse	See also F
B-Crystalline	+++ Over-expression in active demyelinating lesions	
NO and metabolites	++	See also A, E
7-Ketocholesterol	++	
Glutamate	+++ Higher levels in active demyelinating lesions	
Cystine/glutamate antiporter	+ Over-expression in active demyelinating lesions	
NF-L	+++ Higher CSF levels, especially the 3rd week after relapse onset	See also A, F
GFAP	++ Higher levels during relapse	See also A, E
S100B	+/− Higher CSF levels during MS/NMO relapse	See also A, E
N-CAM	+ CSF elevation at remission onset	
BDNF	++ Lower levels inhibit demyelination and axonal loss	See also D, E, F
(3) Imaging		
Contrast-enhanced T1 lesions	+++ Active lesions	See also A
Hyperintense T2-weighted lesions	++ Combination of different mechanisms	See also A, D, E
MTR decrease	+ Demyelination and axonal loss combined	See also D
DTI abnormalities	++ Combination of different mechanisms	See also D, E
MRS findings (especially changes in glutamate and choline)	+++ Active lesions	See also A, D, E
DTS	++ Promising but still experimental	See also D
EP's delayed conduction	++ Demyelination biomarker	See also A, D, E

(D) Biomarkers of axonal loss-neurodegeneration (criteria i, iv, v, and vi)

(1) Laboratorial		
VEGF-A	++ Lower levels, higher risk for neurodegeneration	See also A, E
14-3-3	+/- Axonal loss	
NAA	+++ Axonal loss	See also A, E
BDNF	++ Lower levels inhibit demyelination and axonal loss	See also C, E, F
Nogo-A	+++ Higher CSF levels, failure in axonal repair	See also A
(2) Imaging		
RNFL thinning	+++ Axonal loss in the optic nerve	See also E, F
Hyperintense T2-weighted lesions	++ Combination of different mechanisms	See also A, C, E
Black holes	+++ Axonal loss	See also E
MTR decrease	++ Demyelination and axonal loss combined	See also C
DTI abnormalities	++ Combination of different mechanisms	See also C, E
MRS findings (especially NAA)	++	See also A, C, E
DTS	+++ Promising but still not widely accessible	See also C
Visual and motor EPs	++	See also A, C, D

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(E) Prognostic biomarkers-biomarkers of disability progression (criteria ii, iv, v, vi, and viii)

(1) Genetic-immunogenetic		
HLA-DRB1*1501	+/− Early progression from RRMS to SPMS	See also A, B
HLA-DRB1*1501	+ Worst brain atrophy measures	
HLA-DQB1*0301	+ Worst brain atrophy measures	
HLA-DQB1*0602	+ Worst whole and gray matter atrophy measures	
TOB1	+++ Early conversion from CIS to CDMS	See also C
(2) Laboratorial		
OCB IgG	+++ Conversion from CIS to CDMS	See also A
KFLC	+++ Conversion from CIS to CDMS	See also A
OCB IgM	+/− Bad prognostic biomarker	See also B
MRZ reaction	+++ Conversion from CIS to CDMS	See also A, F
Anti-MBP	+/− Conversion from CIS to CDMS	See also A, B
Anti-MOG	+/− Conversion from CIS to CDMS	See also A, B,F
AR	++ Marker of clinical severity in NMO	See also A, C
VEGF-A	++ Lower levels, progression from RRMS to SPMS	See also A, D
NO and NO metabolites	++ Higher CSF levels, longer relapses/higher disability progression rates	See also A, C
NF-H	+++ Higher CSF levels, progressive forms/bad prognostic biomarker	
NF-H and tan	+++ Combined high CSF levels, conversion from CIS to CDMS	
Tubulin/actin	++ Higher CSF levels, progressive forms/worst disability scores	
NAA	+++ Lower CSF levels, progressive forms/worst disability scores	See also A, D
GFAP	++ Higher CSF levels, progressive MS forms/worst disability scores +++ Disability progression in NMO	See also A, C
S100B	+ Disability progression in NMO	See also A, C
BDNF	++ Lower CSF levels in SPMS patients	See also C, D, F
Unblocked α4 integrin	+ Prognostic factor of risk for PML	See also C, F
(3) Imaging		
RNFL thinning	+ Correlation with brain atrophy measures and disease progression	See also D, F
Hyperintense T2-weighted lesions	+/−	See also A, C, D
Black holes	+/−	See also D
Whole brain atrophy measures	++ Worsening rates at MS onset, prognostic biomarker of disability after 8 years	
Gray matter atrophy measures	+++ Higher worsening rates, progressive forms/early CIS conversion to RRMS	
UCCA atrophy	++ Progressive forms, good correlation with EDSS, bad prognostic in RRMS	See also B
DTI abnormalities	+++ Early prognostic biomarker of relapse	See also C, D
Corpus callosum DTI abnormalities	+++ Bad prognostic biomarker	See also A
Spinal cord DTI abnormalities	+++ Good correlation with EDSS scores	
Early MRS abnormalities	++ Bad prognostic biomarker	See also A, C, D

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Combined EPs	+++ Good prognostic biomarker, especially for benign disease forms	See also A, C, D
SSR	++ Correlation with higher EDSS scores	See also A
(F) Biomarkers of therapeutical response (criteria i, iv, v, vi, and vii)		
(1) Genetic-immunogenetic		
HLA-DRB1* 0401, 0408, 1601	+++ Higher risk for developing neutralizing antibodies against IFN-B	See also A
(2) Laboratorial		
MRZ reaction	++ B-cell immunity targeted therapy	See also A, E
Anti-MOG	++ B-cell immunity targeted therapy	See also A, B, E
Fetuin-A	+++ Decreased CSF levels in Natalizumab responders	See also C
MBP	+++ Decrease in CSF levels in methylprednizolone responders	See also C
CSF lipocalin 2	++ Decreased CSF levels in Natalizumab responders	See also A
Unblocked α4 integrin	+++ Therapeutical response to Natalizumab	See also C, E
NF-L	+++ Normalized CSF levels in Natalizumab responders	See also A, C
BDNF	+++ CSF elevation in Glatiramer Acetate responders	See also C, D, E
TRAIL	++ Serum levels good predictors of response in IFN-B	
MxA	++ Serum levels good predictors of response in IFN-B	
sVCAM	++ CSF alterations in IFN-B responders	See also C
Th17 immune profil	+/− Immune response exacerbation by IFN-B	
Vitamin D	+++ Increased levels in IFN-B responders	See also A, C
sICAM-1	+ Lower levels in Cladribine responders	See also C
sE-Selectin	+ Lower levels in Cladribine responders	
(3) Imaging		
RNFL	+++ Biomarker of therapeutical efficacy for several agents	See also D, E

Classification of biomarkers. +++ very strong correlation, ++ strong correlation, + modest correlation, and +/− controversial correlation.
Criteria used for classification., (i) Biological rationale; (ii) clinical rationale; (iii) predictability of disease initiation, reactivation or progression, or of disease differentiation; (iv) sensitivity and specificity; (v) reproducibility; (vi) practicality; (vii) correlation with therapeutical outcome; (viii) correlation with prognosis and disability. Biomarkers of more than one category are indicated in the third column.

[0114] The information described herein for the first time shows that stimulation of the vagus nerve modulates demyelination and/or remyelination and/or leaking through the blood-brain barrier. The examples provided herein are not intended to be comprehensive, but merely illustrate and embody certain variations of the invention. It is within the abilities of one of ordinary skill in the art to understand and apply, without undue experimentation, the invention as described herein.

Example 1

[0115] To study the effect of VNS on neurodegeneration and neuroinflammation, a lysolecithin (LPC)-induced MS model can be used. Lysolecithin is a bioactive pro-inflammatory lipid that is a detergent-like membrane solubilizing agent. A 1% solution of LPC can induce local demyelinating lesions when injected into the white matter of the spinal cord. Four distinct epochs occur over 14 days post-injection: (1) demyelination; (2) oligodendrocyte progenitor cell (OPC) recruitment; (3) differentiation; and (4) remyelination. FIG. **1** illustrates a typical example of the 4 epochs, where demyelination occurs from about days 0-3, OPC recruitment occurs from about days 3-7, OPC differentiation occurs from about days 7-10, and remyelination occurs from about days 10-14.

[0116] To induce a self-limited demyelinating lesion, spinal cords of female BALB/c mice were injected between T3-T5 with 1% LPC (0.5 μL at 0.25 μL/min). The procedure to inject the mice with LPC was as follows. The mouse was anesthetized and stabilized into a stereotaxic frame. A midline incision was made between the scapulae. The underlying fat pads were bluntly separated and the spinous process of the T2 vertebra was identified and a laminectomy was performed. A syringe was advanced to 0.3 mm into the spinal cord and 0.5 uL of LPC was injected at a rate of 0.250 uL/min for 2 min. The muscle and adipose tissue were sutured, and the skin was closed with surgical staples.

[0117] VNS was performed as previously described (Olofsson, Levine, et al. 2015. Bioelectronic Medicine: 37-42) on Day 0 or Day 4 post-induction with LPC. More specifically, to study the effect of VNS on demyelination, VNS (0.75-1 mA, 250 μS pulse, 10 Hz) or sham VNS (0 mA) was performed immediately following LPC administration, and the mice were euthanized on the day of expected peak lesion volume (day 4 post-induction; J Neurocytol 24(10): 775-81). The demyelination experimental protocol is summarized in FIG. **2**A.

[0118] Spinal cord lesion volumes/areas were quantified by myelin loss as assessed from luxol blue-stained, 15 μm serial sections. FIG. **3**A shows an illustration of a typical cross-section of the spinal cord, and FIG. **3**B shows a luxol blue stained cross-section of the spinal cord with an LPC induced lesion in the anterior funiculus of the white matter 5 days post-LPC injection. To study the effect of VNS on remyelination, VNS or sham VNS treatments was performed 4 days post-induction, mice were euthanized on days 8, 14, or 21 post-induction, and nerves were processed as above. The remyelination experimental protocol is summarized in FIG. **2**B. Mean lesion volumes between groups were compared by t-test.

[0119] Results: The demyelination protocol illustrated in FIG. **2**A showed that VNS inhibited demyelinated lesion progression compared to sham. On day 4 post-induction, the mice were euthanized and the spinal cord around the LPC injection site was sectioned and stained with luxol blue. As shown in FIGS. **4**A-**4**D, the mean lesion volume in the VNS group (0.75 mA) was significantly lower than in the sham group ($p=0.0023$ by t-test). VNS at 0.25 mA resulted in a mean lesion volume similar to sham VNS.

[0120] The remyelination protocol illustrated in FIG. **2**B showed that remyelination occurred at a significantly accelerated rate in the VNS group. As shown in FIGS. **5**A-**5**G, on day 8 post-induction, mean lesion volume in the VNS group was reduced. On day 14 post-induction, mean lesion volume in the VNS group was significantly lower than in the sham group. On day 14, 11 out of 12 VNS animals had no detectable lesion. By Day 21, the mean lesion volume in the sham group was almost back to baseline. FIG. **5**A shows that the area under the curve (AUC) between days 4 and 21 is reduced by about 65 percent with vagus nerve stimulation.

[0121] Conclusions: VNS reduced demyelination and accelerated remyelination, demonstrating a robust effect

after a single dose in this model. Repeated stimulation of the vagus nerve with an implanted nerve stimulator may further reduce the rate of demyelination and/or further accelerate remyelination. This will be tested in an experimental autoimmune encephalomyelitis model to further assess the potential of VNS to treat MS.

Example 2

[0122] Another study was performed to determine the effect of VNS on vessel leakiness 24 hours post-induction and stimulation. A lesion was induced as described above using LPC injection and VNS was performed immediately following induction. At 24 h, 0.15 mL of 1% Evans blue dye was injected intravenously through retro-orbital injection under anesthesia for 1 hr., as shown in FIG. **6**A. One hour later, the animals were euthanized via cervical dislocation. Measurement of extravasation in the spinal cord (SC) was determined by extracting the SC and weighing the SC wet. The SC was then dried for 24 h at 56° C. and weighed dry. The Evans blue dye was extracted with a formamide solvent for 48 h at 56° C. incubation. The supernatant was measured spectroscopically at 620 nm and the quantity of Evans blue dye was determined by interpolation from a reference curve. The quantity of Evans blue dye was normalized to the dry weight of the SC. As shown in FIG. **6**B, less Evans blue dye was extracted from the spinal cord from the mice that received VNS, which provides evidence that VNS reduces vessel leakiness 24 hours post-induction and stimulation. In addition, the amount of Evans blue dye extracted from the mice that received VNS was similar to the amount of Evans blue dye extracted from naïve mice (no LPC induced lesion).

[0123] Leakiness in the blood brain barrier may allow immune cells and inflammatory cytokines and chemokines to pass through and contribute to continued inflammation in the brain and/or spinal cord. Therefore, VNS may reduce vessel leakiness around the central nervous system (CNS), thereby reducing the recruitment of proinflammatory cells such as lymphocytes (e.g., T-cells) and macrophages to the brain and spinal cord, thereby reducing the inflammation in the CNS and reducing the amount demyelination that results from an inflammatory attack by the immune system.

Example 3

[0124] In general, the apparatuses and methods described for VNS therapy may also be used to prevent or treat increased leakiness of the blood-brain barrier, as illustrate in FIG. **6**B.

[0125] Methods: 1% LPC was injected into the spinal cord white matter of BALB/c mice. For the first intervention time point, VNS therapy or sham VNS was performed immediately after injection. 24 hours later, mice (VNS, sham VNS, and naïve (no-LPC)) are injected with 1% Evans blue dye which binds to the albumin in blood and is left to circulate for 1 hour. Spinal cords are then harvested, dried for 24 hours in pre-weighed tubes at 60° C. Dried tissues are then incubated in formamide for 48 hours. Supernatant is then extracted from the tubes and read spectroscopically at 620 nm. For the second intervention time point, VNS therapy or sham VNS therapy occurs on day 4 post-LPC induction. On day 5 post-LPC induction, Evans blue extravasation is performed the same way as described for demyelination experiment. Evans blue concentration is compared (ng/mg of tissue) and normalized to naïve animals.

[0126] Results: LPC increased blood-spinal cord leakiness. VNS therapy significantly reduced Evans blue extravasation into the spinal cord compared to sham (81% decrease) 24 hours post-LPC induction (FIG. **6**B). In addition, VNS therapy on day 4 post-LPC significantly reduced Evans blue extravasation on day 5 compared to sham (52% decrease).

[0127] Conclusion: VNS therapy increases the integrity of the blood-spinal cord barrier and subsequently reduces the extravasation of protein/Evans blue and other circulating species, including antibodies, DAMPS/PAMPS, and immunocytes into the central nervous system.

Example 4

[0128] Another experiment was performed to determine whether the effect of VNS on demyelination was a7 nicotinic acetylcholine receptor (nAChR) dependent. Two mice strains were used in the study. One mice strain is the C57 Black subtype 6 (C57BL/6), which is a common wild type strain that expresses α7 receptors and are denoted as α7+/+. The second mice strain is an α7 knockout strain of the C57BL/6 strain, which lacks the α7 receptor and are denoted as α7−/−. Each of the mice strains were given LPC injections in sham (no VNS) and VNS groups. Tissue extraction was performed 4 days post-injection. The procedure was essentially identical to the Balb/c mice demyelination experiments described above in Example 1.

[0129] As shown in FIG. **7**A, the protective effects of VNS on demyelination is α7 nAChR-dependent. VNS treatment on mice with the α7 nAChR showed a reduced lesion volume when compared with sham, while VNS treatment on mice without the α7 nAChR showed no reduction in lesion volume when compared with sham. Similarly, the remyelination effect of VNS treatment may be α7 nAChR dependent, as shown in FIG. **7**B. In this example, the effect of VNS treatment on remyelination in the presence (+/+) and absence (−/−) of the α7 nAChR due to either sham (no VNS treatment) or VNS treatment were examined, showing a substantial decrease in lesion volume, the maker for remyelination following induction of a demyelination event (e.g., application of LPC.

[0130] In FIGS. **7**A-**7**B, 1% LPC was injected into the spinal cord white matter of α7 nAChR knockout mice and C57BL/6 (wildtype) mice. For demyelination experiment, VNS treatment or sham VNS treatment, tissue collection, processing, and analysis are all the same as mentioned above for FIG. **1**A. For remyelination, VNS and sham VNS intervention occurs the same as experiment described for FIG. **4**B. Spinal cords are harvested only on day 8 post-LPC induction. Processing and analysis performed are the same as described for FIGS. **4**A-**4**B.

[0131] Result: VNS therapy decreased demyelination in wildtype C57BL/6 mice. VNS therapy did not decrease demyelination in α7 KO animals (FIG. **7**A). VNS therapy increased remyelination in wildtype animals, but did not increase remyelination in the knockouts (FIG. **7**B). Thus, the effects of VNS on demyelination and remyelination are α7-dependent.

Example 5

[0132] In general, the apparatuses and methods described for VNS therapy may also be used to prevent or treat increased immunocyte homing to the central nervous system, as illustrate in FIGS. **8** and **9**.

[0133] In FIGS. **8** and **9**, CD3+ T cell infiltration through a model for the blood-brain barrier is significantly decreased in the VNS treatment group. As shown in FIG. **8**, the CD3+ T cell infiltration through the model of the blood-brain barrier on Day 3 post-LPC induction compared to Sham group is reduced by 50%. In. FIG. **9**, the macrophage infiltration is significantly decreased 24 hours post-LPC induction in the VNS treatment group compared to the Sham (no VNS treatment) group by 55%.

[0134] Methods: Surgical procedures and VNS/sham VNS treatments remain the same from FIG. **4**A. Spinal cords from VNS therapy, sham, and naïve mice are harvested on days 1 or 3 post-LPC induction. Tissue is then digested in enzymatic cocktail for 20 minutes at 37° C. followed by trituration and filtering through a 100 μM mesh screen. Single cell suspension is then put through a density gradient to remove myelin debris from glia cells and immune cells. Once isolated, cells are blocked in FACS buffer and CD32/CD19 for a half hour to prevent unspecific antibody staining. Cells are counted and checked for viability via hemocytometer. Cells are then placed in tubes, stained for either T cells (CD3+) or macrophages (CD11b+, CD45hi) and then analyzed via flow cytometer. Populations of cells are quantified using FlowJo program.

[0135] Result: LPC increased CD3+ T cell and macrophage infiltration in the spinal cord compared to naïve tissue (FIGS. **8** and **9**). There was a significant reduction in CD3+ T cell infiltration on day 3 post-LPC induction in VNS therapy treated animals compared to sham (50% reduction) (FIG. **9**). In addition, VNS therapy resulted in a significant decrease in macrophage infiltration compared to sham 1 day post-LPC induction (55% reduction) (FIG. **9**). Thus VNS significantly reduces the infiltration of peripheral immunocytes into the CNS in this lysolecithin-induced MS model.

[0136] As shown in FIGS. **10**A-**10**B, VNS therapy also increased remyelination following a decrease in myelination. During spinal cord extractions for all prior experiments performed (see examples 1-4, above), blood was collected via cardiac puncture as well. Blood was centrifuged at 8,000×g for 5 minutes, the serum was collected and stored at −80° C. Using a Resolvin D1 ELISA kit, levels of RvD1 were measured spectroscopically from the serum of VNS and sham VNS mice for the demyelination (D4 harvest) and remyelination (D8, D14, and D21 harvests) experiments. Levels of RvD1 are analyzed (pg/mL) and represented as a percent of sham by day.

[0137] Result: as showing FIG. **10**A, VNS therapy on day 0 (LPC-induction) increased serum levels of RvD1 on day 4. As shown in FIG. **10**B, VNS on day 4 post-LPC induction also increased RvD1 in the serum levels of RvD1 with the highest concentration occurring on day 14 post-LPC induction. RvD1 levels in VNS serum were decreased as compared to sham at 21 days post-LPC induction, likely due to earlier resolution in the VNS group.

[0138] Thus, VNS therapy increases the pro-resolving lipid mediator RvD1 in serum which may contribute to the increased speed in resolution time of LPC-induced lesions compared to sham.

Example: System

[0139] FIG. **11** schematically illustrates one example of a system **1100** for treating demyelination (e.g., for treating MS, or any other demyelinating disorders). In some variations the system for reducing demyelination and/or increase remyelination by stimulation of a vagus nerve includes a controller **1103**, a stimulator **1105**, and a pulse generator **1101**. The pulse generator and stimulator may be connected to and controlled by the controller. In some variations all or some of the system may be implanted into the patient's body. All or some of the components of the apparatus may be enclosed by a housing (e.g., an implant housing). In general, the systems may also include one or more biosensor **1107** configured to detect one or more biomarkers. The biosensor may be coupled with the rest of the system (e.g., implant) or it may be separate and may communicate via a wired or wireless connection. For example the biosensor may be implanted into the body so as to sample blood, spinal fluid, or the like; in some variations the biosensor is external to the body and may be single use or configured for limited-reuse. In some variations the biosensor may include a sensor for determining a patient's physical condition (e.g., temperature, nerve conduction, etc.). In some variations the biosensor may be an immunochemical sensor configured to detect binding of one or more analytes and/or to provide a concentration.

[0140] The stimulator may be configured to apply stimulation to the vagus nerve. A stimulator may be configured for electrical stimulation, mechanical stimulation, or both. For example, the stimulator may include or be coupled with the pulse generator **1101** (e.g., waveform and/or pulse generator, oscillator, etc.). The stimulator may include one or more stimulation applicators **1121** (e.g., one or more electrodes, mechanical transducers, etc.) for contact with the tissue, including the vagus nerve.

[0141] Any of the apparatuses may also include one or more power supplies **1115**, and/or power regulation circuit, etc.

[0142] The controller is typically functionally coupled to the one or more biosensor (e.g., receiving data from the biosensor(s)) and controls the stimulator and may be configured to apply stimulation to the vagus nerve from the stimulator sufficient to reduce demyelination and/or increase remyelination of nerves within the patient when the biosensor detects a biomarker indicative of demyelination (including detecting active demyelination or a marker that is indicative of imminent active demyelination).

[0143] For example, a system may include an implant comprising a stimulator (e.g., a waveform and/or pulse generator, an oscillator, a power supply and/or power regulation circuit, etc.), a stimulation applicator (e.g., one or more electrodes, mechanical transducers, etc.), and a controller. The controller may be configured as a microcontroller and may be in electrical communication with the stimulator so as to control operation of the stimulator. The controller may include one or more processors, a memory and/or a timer. The stimulator and/or controller may be in electrical communication, one or more stimulation applicators. In some variations the controller may include or be in communication with wireless communications circuitry **1117** for wirelessly communicating with one or more remote processors **1131**. The remote processor may be a hand-held device (e.g., smartphone, wearable electronics, etc.). The controller may optionally be in communication with one or more bio sensors that may be included with the implant or may be remote from the implant (e.g., may be wearable, single-use, etc.). In some variations the biosensors are wirelessly connected to the apparatus.

[0144] The electronic device (e.g., smartphone, wearable electronics, etc.), which is in communication with controller, can be configured to accept input from the user and/or to sense one or more biomarkers for triggering a change in the electrical stimulation parameters. For instance, the user may experience a flare-up or relapse, where the user experiences the onset or worsening of symptoms (e.g., pain, muscular cramping or stiffness and/or fatigue). The electronic device can be configured to accept input from the user to indicate that they are experiencing a flare-up, which can cause the controller to adjust the stimulation parameters accordingly (e.g., increase or decrease frequency and/or current). Likewise, the electronic device may be configured to accept input from the user indicating recovery from a flare-up in which the user experiences reduction or extinction of symptoms, which can cause the controller to adjust stimulations accordingly (e.g., increase or decrease frequency and/or current). In some cases, the electronic device is configured to accept a score or rating indicating the severity of a flare-up, and the controller can adjust the stimulation parameters based on the score or rating. Alternatively or additionally, the electronic device can be configured to sense one or more biomarkers that indicate the onset of a flare-up. In some embodiments, the electronic device is configured to non-invasively detect the biomarker(s), for example, using renal biomarker(s) and/or optic nerve biomarker(s). In some embodiments, the electronic device is additionally or alternatively configured to detect the biomarker(s) from a fluid sample. The electronic device may be configured to detect a severity of the flare-up based on, for example, a measurement of the sensed biomarker(s). The controller can be configured to adjust the stimulation parameters (e.g., automatically) based on the detected biomarker(s) indicating the onset and/or diminishment of a flare-up and/or based on the severity of the flare-up.

Increased Clearance

[0145] The methods and apparatuses described above describe the treatment of multiple sclerosis (MS). More specifically, the methods and apparatuses described herein may be refined to increase the clearance of cellular debris that may be associated with MS and other neurodegenerative and/or neuroinflammatory disorders as well as acute neuronal injury. Thus, in some variations the methods and apparatuses described herein may increase clearance of neuronal cellular debris by the targeted application of charge to the vagus nerve. The targeted application of charge to the vagus nerve may enhance clearance of cellular debris, particularly in multiple sclerosis patients by modulating the activity of one or more of microglia and/or other macrophages.

[0146] Endothelial cells lining the inside of small blood vessels may promote clearance of myelin debris, a common detrimental outcome of demyelinating diseases such as multiple sclerosis (MS). Activity may also decrease due to age or other disease states. In healthy subjects, macrophages/microglia may engulf myelin debris and may otherwise perform homoeostatic activity in the normal CNS, a function associated with high motility of their ramified processes and their constant phagocytic clearance of cell debris. In some conditions, including MS, there may be a reduction of recruitment or activation of microglia to clear myelin debris. Insufficient clearance by microglia, prevalent in several neurodegenerative and/or neuroinflammatory diseases and declining with ageing, is associated with an inadequate regenerative response. The methods and apparatuses described herein may enhance the activity microvascular endothelial cells, and/or macrophages and/or microglia. Specifically, the application of vagus nerve stimulation (VNS) within a defined range of values may result in increased endothelial-mediated clearance of tissue debris and/or macrophage/microglial-mediated clearance of tissue debris.

[0147] For example, described herein are methods of treating a patient for a neurodegenerative and/or neuroinflammatory disorder and/or acute CNS injury by increasing the activity of microglia and/or other macrophages for repeating period of time and/or for a sustained period. This may be achieved by the application of vagus nerve stimulation within a range of charge (e.g., in nanocolumb to microcolumb range) of between about 2.5 nC/day (e.g., about 0.1 mA and 0.1 msec pulse-with VNS) to about 7.5 mC/day. Pulses may be applied between 0.1 and 50 Hz (e.g., between 1 and 20 Hz, etc.). The charge may be delivered either directly, e.g., by an implantable device, or indirectly, as by a transcutaneous delivery device. Outside of these ranges (e.g., the application of less than 2.5 nC/day) typically has little or no effect on the majority of patients. Similarly, the application of greater than about 7.5 mC/day may have no additional effect and in some cases may result in an inhibition of the effect. Thus it may be beneficial to limit the daily application of charge to be between about 2.5 nC and 7.5 mC per day (e.g., between about 5 nC and 7 mC, between about 10 nC and about 6.5 mC, between about 50 nC and about 6 mC, between about 100 nC and about 6 mC, etc.).

[0148] Applicants have found that the application of VNS within the recited charge range increases the clearance of debris. For example, images of neuronal tissue from animals treated within the effective range of applied charge (e.g., adjusted for animal size and stimulation location) stained for myelin debris shows an increase in intracellular breakdown products, suggested that myelin debris uptake is increased after stimulation. This increase may be sustained for a period following stimulation (e.g., over hours and days). Surprisingly, this effect was seen even with older animals (e.g., otherwise normal mice) showing an enhancement compared to controls otherwise showing normal aging effect of decrease myelin clearance.

[0149] Any of the methods described herein may target endothelial cell and/or microglia and/or macrophage activity. For example, in some variations the method and/or apparatus may include examining one or more markers for endothelial cell and/or macrophage and/or microglial activity. These markers may be particularly useful in more precisely targeting the treatment, either with just the VNS treatment alone or in combination with one or more compositions for treating a disorder involving demyelinated neurons such as (but not limited to) MS.

[0150] FIGS. **14** and **15** illustrate the increase in myelin debris update following VNS within the effective dosing range described herein. In general, tissue resolution of damage may require clearance of damaged cells and debris. In particular, in MS, the process of remyelination may be heavily dependent on endothelial, microglial and macrophage clearance of damaged cells and myelin debris (e.g., efferocytosis and phagocytosis) before oligodendrocytes can lay down new myelin on damaged axons. In MS, the cells

responsible for clearing cellular and extracellular debris may have become senescent and inefficient at general endocytosis; a similar effect may be seen in older people and in older animals, causing delayed healing and rapid progression of diseases, including, but not limited to, MS.

[0151] Stimulating the vagus nerve electrically (e.g., VNS) can increase the rate of phagocytosis and efferocytosis to increase the rate of disease resolution, demonstrated with remyelination in a rodent model of MS, as discussed above and down in FIGS. **14** and **15**. Similar processes enable repair in many other disease contexts. For example, stimulating the vagus nerve electrically can induce more efficient general endocytosis in aged rodents. This finding may be extended to humans, where VNS can reverse senescence in cells, including the cells of older individuals, to increase the rate of repair and the resolution of disease and damage, and to potentially extend life.

[0152] In FIG. **14**, the data was generated from an animal model (BALB/c mice), in which 1% lysolecithin was injected into the spinal cord white matter (e.g., 0.5 uL at 0.25 uL/min), and VNS (in this example, 0.75 mA, at 10 Hz) or sham VNS was performed immediately after injection ("paradigm A"). Animals were euthanized on day 4 post-induction and intact spinal cords were harvested. In a second treatment paradigm ("paradigm B"), similar mice were treated with VNS (0.75 mA, 10 Hz) or sham VNS was performed on day 4 post-induction. Animals were euthanized on days 5, 8, 10, or 14 post-induction and intact spinal cords were harvested.

[0153] Spinal cords were cut into 20 μM sections, and stained with oil red O, which shows the presence of fat or lipids in fresh, frozen tissue sections and is used mainly to measure lipid accumulation. Oil red O is a fat-soluble diazo dye that functions as an oil-soluble colorant (at droplets stain an intense red-orange color). Oil red O stains myelin degradation products and is used as a marker of phagocytosis. Stained area within the lesion was quantified as a percentage of the lesion.

[0154] As shown in FIGS. **14** and **15**, vagus nerve stimulation (VNS) significantly increased myelin debris uptake when the stimulation was within the prescribed treatment ranges described herein. In FIG. **14**, there was an increase in positive staining area compared to sham control, indicating a more than double increase in phagocytosis in this region of the neural tissue. Specifically, when looking at animals treated as per treatment paradigm A, there was an approximately 2.5× fold increase in oil red O staining as a % of lesion for the VNS group compared to the sham group ($p<0.05$) on day 4. FIG. **15** shows a time course for treatment when using treatment paradigm B. in this example, there was an approximately 2.5× and 5.5× fold increase in oil red O staining as a percent of lesion for the VNS group compared to the sham group ($p<0.05$) on day 5 and day 8 respectively. On day 14 there was an increase in oil red o staining in the sham group as compared to sham day 4 and 8. There was no detectable myelin uptake in the VNS group as there were no longer any detectable lesions.

[0155] Thus, as evidenced by FIGS. **14** and **15**, VNS greatly increases myelin debris uptake by macrophages and microglia in this model as assessed by oil red O staining. An acceleration in myelin debris uptake vs sham was evident. This may also be seen from the raw data shown in FIGS. **16-19**, showing representative O Red O staining patterns from the tissue. FIGS. **16** and **18** show stained sections through tissue from sham animals. FIGS. **17** and **19** show images from animals treated with VNS on day 4 of treatment paradigm A, post-VNS (FIG. **17**) or day 8 post-induction for paradigm B (FIG. **19**). The boxed regions in FIGS. **16** and **17** show lesions. There was significantly more staining in VNS treated regions. The inset regions in the bottoms of FIGS. **18** and **19** show analysis of the staining to quantify the intensity and extent.

[0156] Similar results were seen in older mice, in which remyelination normally declines. For example, 1% lysolecithin was injected into the spinal cord white matter of in aged (19 months old) C57Black6 mice and treatment paradigm B was utilized. For example, VNS (0.75 mA, 10 Hz) or sham VNS was performed on day 4 post-induction, and animals were euthanized on day 10 post-induction, and intact spinal cords were harvested. Spinal cords were cut into 20 μM sections and stained with Luxol-fast blue or oil red O, and stained area within the lesion was quantified. As seen in FIGS. **20** and **21**, VNS both accelerated remyelination (FIG. **20**) and enhanced myelin debris clearance (FIG. **21**). As shown in FIGS. **20**, there was a 34% reduction in lesion volume in the VNS group relative to the sham group on day 10 post-induction. As shown in FIG. **21**, there was a 2.4× fold increase in oil red O staining as a % of lesion for the VNS group compared to the sham group ($p<0.05$) on day 10. Thus, in aged mice, VNS increases myelin debris uptake by macrophages and microglia as assessed by oil red O staining, and an acceleration in remyelination of the induced lesion was evident in the VNS group.

Example 6: Evidence of Enhanced Phagocytosis

[0157] As described herein, tissue resolution of damage due to an inflammatory event generally requires clearance of damaged cells and debris. In multiple sclerosis (MS), the process of remyelination is believed to be heavily dependent on endothelial, microglial and macrophage clearance of damaged cells and myelin debris (efferocytosis and phagocytosis) before oligodendrocytes can lay down new myelin on damaged axons. The cells responsible for clearing cellular and extracellular debris become senescent and inefficient at general endocytosis in older people and in older animals, causing delayed healing and rapid progression of diseases, including, but not limited to, MS.

[0158] Surprisingly, as shown herein, stimulating the vagus nerve electrically can increase the rate of phagocytosis, which in turn increases the rate of disease resolution. FIG. **22** illustrates results of an ex-vivo uptake study demonstrating evidence of enhanced myelin-uptake by macrophages following VNS in mice. The mice were treated with either a VNS dosage or a sham VNS. The macrophages were harvested from the treated mice, and the myelin uptake was quantified. Primary macrophages isolated from the mice that had VNS applied were shown to be more efficient at taking up myelin compared to the sham VNS. As shown in FIG. **22**, macrophages from the mice treated with VNS dosage mice had accelerated myelin debris uptake compared to macrophages from the sham stimulated mice. These results indicate that stimulating the vagus nerve electrically can induce more efficient general endocytosis in rodents. These result suggest VNS intervention in humans may reverse senescence in cells, to increase the rate of repair and the resolution of disease and damage, and to extend life. Similar processes may be vital to enable repair in many other disease contexts.

[0159] The results of FIG. **22** were obtained based on an ex-vivo uptake study using the following methods: BALB/c mice (from Charles River laboratories) were acclimated for 7 days, anesthetized, and treated with either a VNS dosage (60 second pulse train, 10 Hz frequency, 250 uS pulse width, 0.75 mA) or a sham VNS. After 4 hours, the mice were euthanized by CO2 asphyxiation and peritoneal macrophages extracted. The cell suspension of peritoneal macrophages was cultured on 24 well plates containing glass cover slips and incubated over night at 37° C. with 5% CO2. The cells were washed with phosphate-buffered saline (PBS) and incubated with CFSE-labeled myelin (100 μg) in complete RPMI medium for 10 minutes at 37° C. Cells were washed with PBS and fixed with 4% PFA for 10 minutes. Fixed cells were washed with PBS and stained with DAPI for 30 minutes. The stained cells were washed with PBS and the coverslips were mounted with ProLong anti-fade mounting medium and imaged using Zeiss ApoTome fluorescent microscope. Total macrophage numbers in the field of view were counted via DAPI staining. Myelin uptake was also quantified via CFSE-positive cells. The percentage of phagocytosing cells in the overall population was then calculated.

Example 7: EAE Model

[0160] FIGS. **23-25** show results from experimental autoimmune encephalomyelitis (EAE) model studies comparing low duty cycle VNS treatment versus sham VNS. FIG. **23** compares clinical symptom scores of rats treated chronic low duty cycle VNS versus sham VNS. These results show that low duty cycle VNS can delay onset of significant disease symptoms and abrogate disease severity. FIGS. **24** and **25** compare % of CD4+ T cells or CD8+ T cells in samples from rats treated with acute VNS. The results show that a single dose of VNS at 2 Hz or at 10 Hz prevents the ingress of pathogenic CD4+ and CD8+ IFNg+ Th1 cells. In MS, the progression of disease is causally linked to infiltration of immunocytes, including T helper type 1 (Th1) cells. Therapies targeting infiltration of these cells have been found to be effective and clinically approved to treat MS (e.g. natalizumab). As demonstrated within this data, low duty cycle stimulation of the vagus nerve substantially blocks the infiltration of Th1 cells, both CD4+ and CD8+ IFNg-producing cells.

[0161] The results of FIG. **23** were obtained using the following methods: female Lewis rats (from Charles River laboratories) were acclimated for 7 days. Pulse generators (Rodent-MR, SetPoint Medical) were implanted into rats, with the cuff electrode positioned around the left cervical vagus nerve. EAE was induced by injecting 0.1 mL of gpMBP (69-88) antigen with CFA emulsion mix (Cat #EK-3110, Hook Laboratories, MA) subcutaneously on both sides of lower back. The rats were weighed and monitored for clinical score as per the Hooke laboratories EAE scoring guide. Daily 60 second VNS (0.3 mA, 10 Hz QD) or sham VNS began on Day 8 day post induction.

[0162] The results of FIGS. **24** and **25** were obtained using the following methods: Female Lewis rats (from Charles River laboratories) were acclimated for 7 days and EAE was induced by injecting 0.1 mL of gpMBP (69-88) antigen with CFA emulsion mix (Cat #EK-3110, Hook Laboratories, MA) subcutaneously on both sides of lower back. One rat was maintained naïve to induction and treatment. The rats were weighed and monitored daily for clinical score as per the Hooke laboratories EAE scoring guide. On day 9 post-induction, VNS (30 seconds, 0.3 mA, 2 Hz or 10 Hz) or Sham VNS was performed under anesthesia. After 24-48 hours, the rats were euthanized by CO2 asphyxiation and the blood was collected directly by cardiac puncture. Spinal cord tissues were collected in ice cold hibernate medium. The tissues were rinsed with PBS (1×) and then digested with 1 μg/ml collagenase/dispase (Roche Life Science, Germany) in neurobasal medium (Gibco, Thermo Fisher Scientific, Waltham, Mass., USA) for 1 hour at 37° C. on a rotator-shaker. Following digestion, spinal cord tissues were washed with HBSS (Gibco™ HBSS), triturated using fire-polished glass Pasteur pipettes (Fisher Scientific, Waltham, Mass.), and filtered through a 70-μm strainer. The cell suspension was layered onto 15% BSA in HBSS and centrifuged at ×129 g for 20 min without brake. The cell pellet containing the infiltrated cells were suspended in FACS buffer (1×PBS, 1% FBS in 0.5 mM EDTA) with CD32 Mouse anti-Rat (Fc block, 1:100) blocking antibody for 30 minutes on ice. The cells were stained for external surface staining with eFluor 455UV Fix viability dye; eBioscience (1:2000), CD45-PB; Biolegend (1:100), CD11b PE-Cy7 (1:100), CD3 APC-Cy7; Novus Biologicals (1:100), CD4 Alexa Fluor 488; Bio-Rad (1:100), CD8 BUV805; BD Biosciences (1:100), in FACS blocking buffer for 30 minutes on ice. The cells were washed with PBS and fixed using CYTO-PERM fixation buffer for 10 minutes on ice for intra cellular staining with IFN-gamma eFluor 660; Thermo Fisher Scientific (1:100), 30 minutes on ice. The cells were washed and rinsed with PBS (1×) and suspended in FACS buffer and transferred to FACS tube. The stained cells were analyzed using BD FACSymphony. Thus, stimulating the vagus nerve prevents infiltration of IFNg+ T cells into the central nervous system, delays disease symptoms, and abrogates disease severity in the EAE rodent model of MS.

Stimulation Protocol—Dosing

[0163] Returning to FIGS. **12** and **13**, these figures show animal (mouse) model data illustrating the range of dosing parameters applied. In FIGS. **12** and **13** mice were treated with LPC at precise vertebral regions and VNS was applied from an implanted electrode after de-myelinating lesions were permitted to develop. For example, mice were anesthetized and stabilized into a stereotaxic frame, and a midline incision was made between the scapulae to access the spinous process of the T2-T5 vertebra; 0.5 uL of LPC was injected into the spinal cord at a rate of 0.250 uL/min for 2 min, and the incision site sutured closed. VNS was delivered on Day 4 post-induction with LPC (when peak lesion size is expected), using charge-balanced, biphasic, square pulses having a 200-250 μs pulse width at 10 Hz for 60 seconds, unless indicated otherwise. Lesions were quantified by area of myelin loss, as assessed on luxol blue stained serial sections with nuclear fast red counterstain.

[0164] As shown in FIG. **12**, applying VNS at a frequency of less than about 100 Hz (e.g., 75 Hz or less, 50 Hz or less, 40 Hz or less, 30 Hz or less, 25 Hz or less, etc.) was more effective than higher frequencies, e.g., 1.8 kHz and above. In this exemplary graph, no current was delivered to the Sham animals. All other mice were stimulated at a current of 0.75 mA, and pulse train duration of 60 s. The 5 kHz group was stimulated with 5 kHz sinusoidal pulse; all other mice were stimulated at a 0.25 ms biphasic-charge balanced pulse. As shown in FIG. **12**, significantly more lesion area was remy-

elinated after stimulation at 1, 10, or 20 Hz, and high frequency stimulation did not modulation remyelination. In general, the frequency range of between 1-10 Hz stimulation was more effective at enhancing remyelination than 20 Hz and higher.

[0165] As shown in FIG. **13**, significantly more lesion area was remyelinated after stimulation at 1 or 5 minutes at 10 Hz, while in general, stimulation for less than 20 minutes (e.g., less than 15 minutes, less than 10 minutes, less than 9 minutes, less than 8 minutes, less than 7 minutes, less than 6 minutes, less than 5 minutes, etc.) was more effective than longer times. Curiously, 1 minute of stimulation was more effective than 5 minutes of stimulation. In this example, no current was delivered to the sham animals; all other mice were stimulated with a current of 0.75 mA, 10 Hz, 0.25 ms biphasic-charge balanced pulse. The pulse train duration was varied as shown.

[0166] Based on preliminary work, similar trends are expected to be seen in human patients. For example, it is likely that relatively low-frequency stimulation (e.g., between 0.1 and 20 Hz) and lower pulse train duration (e.g., less than 5 minutes) are more effective than higher frequency stimulation at longer pulse train duration. This result is surprising and in conjunction with the findings regarding total charge per day, suggests that the total charge applied to the vagus nerve per day may be optimally between about 2.5 nC and 7.5 mC per day to modulate endothelial cells, microglia, and/or macrophage to increase clearance of cellular debris (such as myelin debris) and therefore reduce lesion volume in diseases and disorders of myelination, including MS.

[0167] The distribution of dose of VNS to be delivered may be all at once (e.g., once per day, once per every other day, once per every third day, once per ever fourth day, once per every fifth day, once per every sixth day, once per week, etc.) or distributed over each day or days (e.g., 2× per day, 3× per day, 4× per day, 5× per day, etc.). The total charge delivered per day may be less than about 7.5 mC (e.g., about 7 mC/day, about 6.5 mC/day, about 6 mC/day, about 5.5 mC/day, about 5 mC/day, etc., in some variations between about 2.5 nC/day and about 7.5 mC/day, between about 2.5 nC/day and about 7 mC/day, between about 5 nC/day and about 6.5 mC/day per day, etc.).

[0168] The dosing may be varied or adjusted. In particular, the dose may be adjusted based on the time since starting treatment. In variations in which the VNS is applied through an implanted device, the dose may be tapered over time. For example, the apparatus may reduce the number and/or intensity of VNS applied over time, e.g., from once per day to once every two or three days, to once every week, etc. In some variations the dose may be adjusted based on a concurrently delivered drug or therapy. The dose of VNS may be decreased when a drug to treat a disease or disorder of myelination is being concurrently taken.

[0169] As mentioned above, in some variations a dose may be frequency optimized around about 10 Hz (e.g., between 0.1 Hz and 20 Hz, between about 1 Hz and 15 Hz, between about 1 Hz and 12 Hz, etc.). In some cases, frequencies above 30 Hz are found to be substantially ineffectual. Preliminary data also suggests that a stimulation of 1 min or less may be sufficient to achieve a full remyelination effect per dose. Thus, in some variations the dose of VNS for treating diseases or disorders effecting myelination may be limited to between about 0.1 second and 120 seconds, e.g., less than about 120 seconds, less than 100 seconds, less than 90 seconds, less than 80 seconds, less than 70 seconds, less than 60 seconds, less than 50 seconds, less than 45 seconds, less than 40 seconds, less than 35 seconds, less than 30 seconds, etc. Any of the apparatuses described herein may be configured to limit stimulation within these effective ranges, which may prevent overshooting the effective range.

[0170] Animal data suggests that there may be a lower-bounding intensity of about 0.25 mA (in mice), below which VNS is ineffective. In humans this level may be lower or equivalent (e.g., 0.05 mA, 0.1 mA, 0.15 mA, 0.2 mA, 0.25 mA). In general, the effective range of VNS in order to achieve remyelination as described herein appears to be different from the range of VNS applied parameters to achieve other effects, including previously described modulation of inflammation. For example, the application of VNS to modulate myelination (remyelination/demyelination) appears to be more tightly bounded than anti-inflammatory effects. For example, dosing with applied energy outside of this range (e.g., less than the minimal levels, greater than the maximum levels) which may have a robust anti-inflammatory effect will have little or no effect on myelination (e.g., outside of between 2.5 nC and 7.5 mC per day).

[0171] Preliminary work in an animal model (e.g., dog) has shown that tapering the applied dose (e.g., from daily, gradually tapering to weekly) may be effective, as (surprisingly) the effects of stimulation within the effective parameter ranges may be longer lasting over time.

Multi-Modal Stimulation

[0172] As described herein, the VNS treatments described herein have been found to reduce demyelination and promote remyelination effective for treating various neurodegenerative and/or neuroinflammatory disorders such as multiple sclerosis. Although treatments for reducing demyelination may also promote remyelination and vice versa, the underlying mechanisms of demyelination and remyelination may differ. Furthermore, the optimal frequencies for VNS may be different, and may span different (and non-overlapping) ranges. Thus, the methods and apparatuses described herein may include applying different "doses" of VNS at different frequencies (e.g., different frequency ranges), to enhance either or both demyelination (e.g., clearance) and remyelination. In some variations the methods and apparatuses may adjust the duration of one frequency component over the other in order to enhance either demyelination or remyelination at different times in a patient's treatment. For example, in some variations, the method or apparatus may be configured to apply stimulation at a first frequency of between 1 and 20 Hz (e.g., between 1 and 10 Hz, between 1 and 7 Hz, between 1 and 5 Hz, etc.) to reduce or prevent demyelination, and a second dose of electrical stimulation to increase remyelination at a second frequency that is higher than the first frequency (e.g., between 10 and 30 Hz, between 15 and 30 Hz, etc.). In some variations the method or apparatus may be configured to apply the lower dose (lower frequency range to prevent or reduce demyelination) but may switch to applying either both the lower frequency range (the first frequency) and the higher frequency range (e.g., the second frequency) when a trigger event is detected. The trigger event may be triggered by the user (e.g., the patient and/or physician or other health care practitioner), e.g., in response to a demyelination event.

In some variations the trigger event may be triggered by detecting one or more biomarkers for demyelination, as described herein.

[0173] With respect to MS in particular, the methods and apparatuses described herein may be used to specifically treat relapsing-remitting MS (RRMS). As described above, these methods and apparatuses may be used to prevent or reduce the effects of periods of active inflammation ("relapses") and/or during intervals between active inflammation. Alternatively, in some variations the apparatuses and methods described herein may be used in particular to treat primary-progressive MS (PPMS). The parameters (e.g., controller, feedback, etc., including in particular the frequencies used for treatment and/or the dosing schedule) may be adjusted based on the type of MS in addition or instead of patient-specific markers (e.g., biomarkers). These methods and apparatuses may also or alternatively be adapted for treatment of second-progressive MS (SPMS) and/or progressive-relapsing MS (PPMS).

[0174] Demyelination is generally associated with an immune response and proinflammatory mechanisms. Remyelination is a regenerative process associated with immune resolution. As described herein some types of VNS stimulation (e.g., lower-frequency, such as between about 1-20 Hz, e.g., between about 1-10 Hz, between about 1-7 Hz, between about 1-5 Hz, etc.) are more preventative and may reduce the severity of demyelination, e.g., by preventing IFNg+ T-cell infiltration (e.g., by immunocytes, including Th1 cells) and myelin breakdown. Other types of VNS stimulation (e.g., higher frequency stimulation, e.g., between 10-30 Hz, e.g., between 12-30 Hz, between 15-30 Hz, between 20-30 Hz, etc.) may enhance remyelination. Thus, as mentioned above, a VNS treatment regimen can be optimized to include different stimulation modes based on whether the stimulation parameters are more effective/efficient at reducing demyelination or promoting remyelination. For example, a first stimulation mode can be optimized to target reduction of demyelination and a second stimulation mode can be optimized to target increase of remyelination. In some implementations, a VNS treatment regimen includes a combination of first mode and second mode stimulations. In other implementations, a VNS treatment regimen includes only first mode stimulation(s) or only second mode stimulation(s). Any of these systems and methods may be configured to toggle between the first mode and the second mode based on feedback (user and/or biomarker feedback), either manually, semi-automatically or automatically. In some variations the amount of first mode stimulation and second mode stimulation may be adjusted, e.g., by increasing the percentage of the total simulation, which may be limited, as described herein to a total daily amount of charge transferred (e.g., between 2.5 nC and 7.5 mC), and/or a total daily stimulation time (e.g., between 1 minute and 5 minutes per day, etc.). The percent of stimulation in the first mode and the second mode may be approximately equal (e.g., about half of the stimulation at the first mode frequency and about half of the stimulation at the second mode frequency, i.e., 50%/50%, or about 40%/60%, about 30%/70%, about 20%/80%, about 10%/90%, about 60%/40%, about 70%/30%, about 80%/20%, about 80%/10%, etc.).

[0175] In some cases, a treatment regimen includes a series of separately applied stimulations that include both first mode stimulations (targeting reduction in demyelination) and second mode stimulations (targeting increasing remyelination). For example, each first mode stimulation may be directly followed by a second mode stimulation. Alternatively, as set of multiple first mode stimulations (e.g., 2, 3, 4, 5, 6, 10, or 20) may be applied, followed by a set of multiple second mode stimulations (e.g., 2, 3, 4, 5, 6, 10, or 20). In some cases, a first set of multiple first mode stimulations (e.g., 2, 3, 4, 5, 6, 10, or 20) may be applied, followed by a single second mode stimulation, followed by a second set of multiple first mode stimulations (e.g., 2, 3, 4, 5, 6, 10, or 20). In some cases, a first set of multiple second mode stimulations (e.g., 2, 3, 4, 5, 6, 10, or 20) may be applied, followed by a single first mode stimulation, followed by a second set of multiple second mode stimulations (e.g., 2, 3, 4, 5, 6, 10, or 20). The number of stimulations and/or the total stimulation time using a particular mode can be chosen based on whether a patient's condition warrants an emphasis on demyelination reduction or remyelination promotion.

[0176] In some cases, the treatment regimen is chosen based on symptoms of the patient. For example, in some variations, one or more first mode simulations (targeting reduction in demyelination) may be administered if the patient is experiencing a flare-up in symptoms to counteract inflammation processes. Once inflammation has subsided to a sufficient extent, one or more second mode simulations (targeting increasing remyelination) may be administered to promote restorative cellular processes an immune resolution.

[0177] In some cases, the VNS treatment regimen is based on a particular neurodegenerative and/or neuroinflammatory condition or disease. For instance, a treatment regimen that includes a greater degree of first mode simulations targeting reduction in demyelination may be administered to patients whose conditions are associated with higher levels of inflammatory or pro-inflammatory cellular processes. A treatment regimen that includes a greater degree of second mode simulations targeting increasing remyelination may be administered to patients whose conditions are associated with lower levels of inflammation in order to promote healing.

[0178] In some variations, the treatment regimen is modulated based on biomarker feedback. For example, the stimulation regimen may be modified to include a greater degree of (or solely) first mode simulations targeting reduction in demyelination in response to the presence of biomarker(s) indicating demyelination. Likewise, the stimulation regimen may be modified to include a greater degree of (or solely) second mode simulations targeting remyelination in response to the presence of biomarker(s) indicating lower levels of demyelination. In some cases, the degree of the first and second mode stimulations is based on threshold levels of one or more biomarkers.

[0179] In some implementations, the stimulation parameters for reducing demyelination (first mode stimulation) includes lower frequencies compared to stimulation parameters for promoting remyelination (second mode stimulation). In some embodiments, the demyelination-reducing (first mode) frequencies are less about 10 Hz (e.g., <10 Hz, <8 Hz, <6 Hz, <4 Hz, <3 Hz, or <2 Hz). In some embodiments, the demyelination-reducing (first mode) frequencies range from about 1 Hz to about 9 Hz (e.g., 1 Hz-9 Hz, 1 Hz-8 Hz, 1 Hz-6 Hz, 1 Hz-5 Hz, 1 Hz-3 Hz, 2 Hz-9 Hz, 2 Hz-6 Hz, 2 Hz-4 Hz, or 2 Hz-3 Hz). In some embodiments, the remyelination-promoting (second mode) frequencies are

about 10 Hz or greater (e.g., 10 Hz or greater, 11 Hz or greater, 15 Hz or greater, 20 Hz or greater, or 25 Hz or greater). In some embodiments, the remyelination-promoting (second mode) frequencies range from about 10 Hz to about 30 Hz (e.g., 10 Hz-30 Hz, 10 Hz-25 Hz, 11 Hz-20 Hz, 10 Hz-15 Hz, 15 Hz-30 Hz, 15 Hz-2 Hz, or 20 Hz-30 Hz).

[0180] Any of the demyelination-reducing (first mode) and remyelination-promoting (second mode) stimulations may be characterized as having low current and/or low duty-cycle stimulation characteristics described herein. In some implementations, the demyelination-reducing (first mode) and remyelination-promoting (second mode) stimulations are characterized as having a current ranging from about 0.1 mA to about 5 mA (e.g., 0.1-4 mA, 0.1-3 mA, 0.1-2 mA, 0.1-1 mA, 0.25-1 mA, 0.1-0.75 mA, 0.25-0.75 mA, etc.), a stimulation duration ranging from about 1 second to about 5 minutes (e.g., 1 sec-5 min, 1 sec-3 min, 1 sec-2 min, 1 sec-1 min, 30 sec-1 min, or 30 sec-2 min, 30 sec-5 min, 30 sec-4 min, 30 sec-3 min, etc.), and/or have an off time between simulation ranging from about 10 minutes to about 24 hours (e.g., 10 min-24 hrs, 10 min-6 hrs, 30 min-6 hrs, 6 hrs-24 hrs, or 30 min-24 hrs).

Combination with Drugs

[0181] The methods and apparatuses described herein may be combined or used concurrently with (e.g., in conjunction with) one or more drugs, including drugs to treat a disease or disorder of myelination.

[0182] It is well known that it may be difficult to combine or use multiple drugs for treating diseases or disorders of myelination such as multiple sclerosis (MS), because such drugs may interact in undesirable and potentially dangerous ways. The methods and apparatuses described herein may be used in conjunction with one or more drugs without negative interactions between the VNS methods and apparatuses described herein and other, pharmaceutically-based therapies. The VNS methods and apparatuses described herein to treat a disease or disorder of myelination may be used in conjunction with one or more of: Avonex (interferon beta-1a), Betaseron (interferon beta-1b), Copaxone (glatiramer acetate), Extavia (interferon beta-1b), Glatiramer Acetate Injection (glatiramer acetate-generic equivalent of Copaxone 20 mg and 40 mg doses), Glatopa (glatiramer acetate-generic equivalent of Copaxone 20 mg and 40 mg doses), Plegridy (peginterferon beta-1a), Rebif (interferon beta-1a), Aubagio (teriflunomide), Gilenya (fingolimod), Tecfidera (dimethyl fumarate), Mayzent (siponimod), Mavenclad (cladribine), Lemtrada (alemtuzumab), Novantrone (mitoxantrone), Ocrevus (ocrelizumab), Tysabri (natalizumab), Solu-Medrol (methylprednisolone), Deltasone (prednisone), H.P. Acthar Gel (ACTH), and Ampyra (dalfampridine).

[0183] The methods and apparatuses described herein for applying VNS may therefore be combined with a pharmacological treatment with low risk of additional side effects, possibly because the VNS results in an enhancement of native response, such as the increase in microglia and/or macrophage activity, often a different pathway than the pharmacological agent.

[0184] In particular, the VNS methods and apparatuses described herein may be used with one or more drugs that enhance remyelination. For example, the methods and apparatuses described herein may be used with a receptor muscarinic type 3 (M3R) modulating drug. The VNS methods and apparatuses described herein may alternatively or additionally be used with a B-cell targeting drug (e.g., drugs that target cell-surface markers on B-cells and T-cells, such as cladribineand Alemtuzumab).

[0185] The immune cell modulating effects of VNS can be enhanced by, or can be used to enhance the effect of, additional drugs to treat patients with neuroinflammatory disorders such as multiple sclerosis (MS).

[0186] In some cases, the VNS treatments described herein can be used in combination with Interferon β drugs and generics, glatiramer acetate and generics, and daclizumab. Modes of action targeting interferon β-1a and 1b receptors and T-cell activation may be complimentary and additive to VNS. These combinations may reduce central nervous system inflammation and demyelination.

[0187] In some cases, the VNS treatments described herein can be used in combination with fingolimod, teriflunomide, and dimethyl fumarate. Modes of action targeting lymphocyte migration or activation can be additive to VNS. These combinations may reduce central nervous system inflammation and demyelination.

[0188] In some cases, the VNS treatments described herein can be used in combination with mitoxantrone, alemtuzumab, ocrelizumab, and natalizumab. Modes of action targeting induce DNA breakage, CD52 to induce cell lysis, B-cell CD20 antigen for depletion, and/or integrin receptors to alter leukocyte migration can be complimentary and can be additive to VNS. These combinations should reduce central nervous system inflammation and demyelination.

[0189] In some cases, the VNS treatments described herein can be used in combination with clemastine, a Selective Estrogen Receptor Modulator (SERM) such as bazedoxifene, and other drugs targeting oligodentrocyte progenitor cells to enhance maturation into myelin-producing oligodendrocytes. Mode of action targeting enhanced maturation of myelin-producing oligodendrocytes can be complementary to VNS effects on phagocytosis and can be additive. These combinations should enhance remyelination and clinical recovery from central nervous system damage.

[0190] In some variations, the therapy may include implanting an internal VNS stimulator, and after the patient is stable and healed, giving the patient a drug, and maintaining the VNS indefinitely. The use of VNS alone or in combination with other, e.g., drug therapies may be used to reduce demyelination and/or increase remyelination. In some variation the use of VNS alone or in combination with other, e.g., drug, therapies may delay the onset of MS, prevent the decline and in some cases reverse the decline in MS patients. In some cases, patients that do not respond to traditional therapies may respond to VNS therapy to treat a disease or disorder of myelination.

[0191] When the VNS as described herein is used in conjunction with a pharmaceutical (e.g., drug) therapy, the drug used may be adjusted, e.g., to reduce the dose of the pharmaceutical, thus the dosing of the additional MS drug may be reduced substantially compared to the dose without VNS. In some variations a method of treatment may include titrating the dose of the drug therapy when used in conjunction with the VNS as described herein.

VNS Implant Location

[0192] When the VNS therapy described herein include an implanted (e.g., surgically implanted) microstimulator device, in some variations the implanted device may be

attached or implanted on or adjacent to the vagus nerve at a region of the neck (cervical placement). Alternatively, the microstimulator device may be implanted in a subdiaphragmatic location, on the subdiaphragmatic vagus nerve, the splenic nerve, etc.

Transcutaneous Stimulation

[0193] Alternatively, in some variations the VNS is applied externally by one or more transcutaneous electrical stimulators. Energy may be applied externally on one or more locations such as the neck (including the sides of the neck, beneath the ears), chest (e.g., midline of chest), abdomen, ear, etc. Non-invasive (e.g., external) stimulation may be configured so that the stimulation received by the vagus nerve is within the parameters described for implantable systems as described herein. For example, a handheld or worn application may be applied to the patient's neck or other region on the skin overlying a vagus nerve region and electrical energy applied within the 0.1-30 Hz (e.g., 1-10 Hz) range, at power settings configured so that the total charge received by the vagus nerve is between 2.5 nC and 7.5 mC per day.

Feedback from MS Biomarkers

[0194] Any of the apparatuses and methods described herein may also be modulated by the use of one or more markers, and specifically, markers for myelination (including myelination fragments, myelination clearance, etc.) and/or markers for microglial and/or other macrophage activity. For example, markers may include microglia markers such as antibody markers (e.g., antibodies to fractalkine receptor (Cx3cr1), TMEM119, CD11b and CD45, ionized calcium binding adaptor molecule 1 (Iba1), CX3CR1 is the fractalkine receptor, F4/80, CD68, CD40, etc. In addition, markers may look specifically for myelin, including antibodies direct to myelin.

[0195] For example, makers may include qualitative and quantitative measurement of elevated immunoglobulins (IgG) in the CSF and/or blood. Isoelectric focusing (IEF) is one qualitative method for detection of oligoclonal bands (OCBs) used to track MS, and has been shown to have a sensitivity higher than 95% in MS15 and a specificity generally considered to be more than 86%. Any of the methods and apparatuses described herein may track the OCBs within an individual patient and adjust the therapy (e.g., applied VNS and/or VNS plus drug) based on changes in this marker. Other similar markers may include the detection of oligoclonal IgM bands, which may be predictive for a more severe disease course with a shorter time period to the next relapse.

[0196] Alternatively or additionally, antibodies directed against myelin-oligodendrocyte-glycoprotein (MOG), which is typically localized on the surface of myelin sheaths and oligodendrocytes, and/or myelin basic protein (MBP), which constitutes 30% of total central myelin protein, may be measured/or tracked to adjust the VNS therapy as described herein.

[0197] Any of the markers described herein may be specific to the disorder or disease of myelination to be treated. For example, Neuromyelitis optica (NMO) is an inflammatory demyelinating disorder that selectively affects the spinal cord and optic nerves. The presence of NMO-specific autoantibodies, NMO IgG may be used as a marker. Further, Aquaporin-4, a water channel located in astrocyte foot processes at the blood-brain barrier is a target antigen.

[0198] Other markers may include Interferon-β (IFN-β). Interferon-β, including neutralizing antibodies (NAb) to IFN-β, levels may track the progression or state of a disorder of myelination and may be used to modulate the VNS therapy. Increases (or high normalized levels) in NAb titres may indicate an increase in the dose of VNS.

[0199] Natalizumab is a humanized monoclonal antibody that binds to very late activation antigen 4 (VLA-4), an α4β1 integrin, and thereby prevents the migration of leukocytes through the blood-brain barrier. Thus, natalizumab (and/or NAb to natalizumab) may be used as a marker for modulating VNS treatment in patients.

[0200] Markers may be tested by sampling a fluid (e.g., blood, spinal fluid, etc.) either acutely periodically (during medical visits) and/or directly by an implant using one or more sensors within the device. In some variations the markers may be sampled noninvasively, by looking at, for example, retinal markers, optic nerve markers, etc.

[0201] As mentioned above any of the systems described herein can be configured to modulate stimulation based on the onset of a flare-up, in which the patient experiences the onset or worsening of symptoms (e.g., pain, muscular cramping or stiffness and/or fatigue). In some variations, the patient provides input indicating the onset of a flare-up, for example, via a portable (e.g., wearable) electronic device. In some variations, the measurement of one or more of the biomarkers can indicate whether the patient is experiencing, or about to experience, a flare-up. Treatment can then be prescribed based on whether a flare-up is occurring, about to occur, and in some cases, based on a severity of the flare-up. In some implementations, an increase in one or more biomarkers associated with demyelination and/or inflammation may indicate the onset of a flare-up, and the stimulation parameters may be modulated specifically to counteract the demyelination and/or inflammation. For example, the stimulation parameters may be modulated to those found to reduce demyelination (e.g., frequency less than 10 Hz, less than 8 Hz, less than 5 Hz, or less than 3 Hz).

[0202] These methods may be methods to treat a demyelinating disorder and/or to prevent or reduce demyelination and/or to increase remyelination. Surprisingly, stimulating a nerve of the inflammatory reflect (e.g., the vagus nerve) may increase the number of oligodendrocyte progenitor cells (OPCs), which are key cells in repairing the damage caused by oligodendrocyte death and demyelination. These cells must be recruited to the damaged site, and they proliferate. Once the myelin debris is cleared by other cells (macrophages, microglia, and astrocytes) the OPCs are able to differentiate into mature oligodendrocytes and lay down new myelin.

[0203] In diseased patients, the application of stimulation as described herein may increase the level of oligodendrocyte progenitor cells and therefore may reduce the amount of demyelination and/or increase remyelination. Surprisingly, in a standard rodent for MS model (experimental autoimmune encephalomyelitis; EAE) in rats with implanted vagus nerve stimulators that were turned on to automatically stimulate (e.g., 1 mA, 60 s, 10 Hz, three times a day), at a timepoint prior to clinical symptomatology but during which time histological changes may already occurring, 24 hours following of treatment initiation a significant increase in OPCs was observed. See, e.g., FIG. **26**.

[0204] In FIG. **26** a significant increase in OPCs was seen when MS model rats were implanted with a vagus nerve

stimulator and stimulated as indicated above, before the development of appreciable symptoms. After 24 hours (with 3 stimulation period, distributed over the day, the animals were sacrificed and their spinal cords harvested, extracted the cells, stained with and fluorescent antibody targeted to an OPC-specific protein (A2B5) and quantified the number of cells using flow cytometry. Only the non-neuronal (e.g., unmyelinated spinal cord) cells were examined by cell counting/sorting (FACS). As seen in FIG. **1**, an expected 5% of OPCs in the naïve rat, an increased amount in the sham animal, but a much higher number of OPCs in the two stimulated rats. This is a very unexpected finding as there had previously not been expected to show OPC proliferation and migration.

[0205] In this example, the stimulation parameters were: 0.2 nanocoulombs to 5 kilocoulomb/day. This may be an effective therapy for treating neuroinflammatory diseases including full spectrum of MS (clinically isolated syndrome, relapsing-remitting, secondary progressive, primary progressive) and neurodegenerative diseases in which it would be beneficial to increase the number of OPCs.

[0206] As described above, any appropriate device may be used. For example, stimulation may be by: IPG with leaded cuff, IPG with leadless cuff, transcutaneously powered (RF or ultrasound) and internally transduced to current, or stimulate (RF or US) transcutaneously to directly stimulate nerves or organs such as the liver and spleen.

[0207] The stimulation times (midnight, noon and 7 PM) were chosen to mimic the times around when lymphocytes are generally maximized within the spleen or within the blood. For humans it may translate into noon, midnight, and 7 AM, or within +/−3 hours of these times.

[0208] In general, electrical and/or mechanical stimulation of the cholinergic anti-inflammatory pathway (NCAP) by stimulation of the carotid vagus nerve has been well described. For example, see U.S. Pat. Nos. 6,838,471, 8,914,114, 9,211,409, 6,610,713, 8,412,338, 8,996,116, 8,612,002, 9,162,064, 8,855,767, 8,886,339, 9,174,041, 8,788,034 and 9,211,410, each of which is herein incorporated by reference in its entirety. It has not previously been suggested that vagus nerve stimulation may be used to prevent or reduce demyelination and/or improve remyelination. Vagus nerve stimulation, through activation of both efferent and afferent pathways (or primarily through one of the efferent or afferent pathway), may be able to reduce the inflammation associated with inflammatory diseases and disorders, thereby reducing the severity of the symptoms and/or slowing, stopping, or reversing the progression of the disease. Applicants have surprisingly found that the apparatuses (e.g., systems, devices, etc.) and methods described herein may be used to stimulate the vagus nerve to reduce demyelination and/or to increase or promote remyelination. Furthermore, although the use of VNS treatment to modulate inflammation has been thought to involve afferent pathways, remyelination and demyelination may involve the efferent pathway or both the afferent and efferent pathways.

[0209] Diseases (e.g., diseases and disorder of myelination) which may benefit from treatment as described herein (e.g., the methods and apparatuses described herein) include, but are not limited to, multiple sclerosis (MS), Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis (ALS), chronic inflammatory demyelinating polyneuropathy (CIDP), and Batten disease. Other neuroinflammatory disorders may include: acute disseminated encephalomyelitis (ADEM), acute optic neuritis (AON), transverse myelitis, and Neuromyelitis optica (NMO). Neuropathies that may benefit from VNS include peripheral neuropathies, cranial neuropathies, and autonomic neuropathies. Thus any of the methods and apparatuses described herein may be used (and adapted for) treatment with any of these diseases and neuropathies, particularly those that would benefit from an increase in OPCs.

Nerve Stimulation Systems and Devices

[0210] In some variations the devices described herein are electrical stimulation devices that may be implanted, and may be activated to apply current for a proscribed duration, followed by a period without stimulation. As described in the examples that follow, the stimulation protocol may comprise a very limited period of stimulation (e.g., an on-time of less than 5 minutes, 2 minutes, 1 minute, etc.) followed by an off-time (during which stimulation is not applied, and may be prevented from being applied) of extensive duration (e.g., greater than 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 1.5 hours, 2 hours, 4 hours, 12 hours, greater than 20 hours, greater than 24 hours, greater than 36 hours, greater than 48 hours, etc.). The applied energy may be electrical energy that is a fixed current having a frequency that is within the range of about 0.5 mA to 5 mA (e.g., approximately 2 mA), at a frequency of between about 1 Hz and about 1000 Hz (e.g., between 1 Hz and 100 Hz, between 1 Hz and 30 Hz, between 10 Hz and 200 Hz, etc.), where the pulses applied have a pulse width of approximately (50-500 usec, e.g., a 200 usec pulse). Thus, the duty-cycle of the applied current may be extremely low, where duty cycle may refer to the ratio of on-time/(on-time plus off-time). The stimulation is applied at an extremely low duty cycle, where duty cycle may refer to the percent of on-time to the total on-time and off-time for the ongoing treatment. For example, low duty cycle may be less than about 10, 5, 4, 3, 2, 1, or 0.5 percent of on-time to the total on time and off-time. The effect may be seen relatively quickly, and may persist over the entire off-time.

[0211] In particular, the methods and apparatuses described herein may be applied as needed, e.g., when the patient expresses or is likely to express an increased risk for demyelination and/or is experiencing (or has experienced) demyelination. Alternatively or additionally, the methods an apparatuses may be applied as needed when the patient expresses or is likely to express, and/or is experiencing (or has experienced) a leakage through the blood-brain barrier.

[0212] For example, we show herein that a low level, low duty cycle stimulation protocol (as described herein) reduces demyelination and/or increases remyelination, and prevents and/or reduces leakage through the blood-brain barrier. The effectiveness of low level, low duty cycle vagus nerve stimulation (VNS therapy) administered on even a single day results in a reduction in demyelination and an increase in remyelination seen over the course of two to three weeks. Note that although reference may be made to VNS, the simulation may be adapted for application to other nerves or organs as described above.

[0213] This type of stimulation contrasts with the use of a high duty cycle stimulation used by others to modulate vagus-nerve mediated functions (such as heart rate, etc.), or treat disorders such as epilepsy and depression. An important finding here is that demyelination can be reduced and

even more surprisingly, remyelination can be increased. This effect is corroborated at these low duty cycle parameters by examining the histology of the spinal cord as described later below. Although low duty cycle vagus nerve stimulation is effective and highly efficient at reducing inflammation, in some embodiments, a higher duty cycle stimulation can be used, such as a duty cycle that is greater than about 1, 2, 3, 4, 5, 10, 20, 30, 40, or 50 percent of on-time to the total on-time and off-time.

[0214] MS patients may experience circadian pattern disruptions to symptoms that may be associated with or caused in part by the circadian patterns of IL-6 levels. Optionally, drugs, such as steroids, can be used along with VNS to suppress nighttime spiking of IL-6. Similarly, VNS can be modulated, by altering the timing of the stimulations for example, to suppress nighttime spiking of IL-6 more effectively. However, one advantage of VNS is the relatively long duration of the effect after a single stimulation, which may allow suppression of IL-6 levels during both night and day, which may render unnecessary the need for supplementary drug treatment or alternative timings. In some embodiments, VNS can be given in the evening before sleep, such as 15, 30, 45, 60, 90, 120, 150, or 180 minutes before sleep, and may also be given at night during sleep, to ensure nighttime suppression of IL-6 levels. In some embodiments, the amplitude of stimulation during sleep can be lowered (e.g., less than 2, 1.5, or 1 mA) to avoid waking the patient. In some embodiments, IL-6 levels can be measured and/or monitored, and VNS can be modulated based on the measured and/or monitored IL-6 levels. Other cytokines may also be measured and/or monitored, such as IL-1, TNF, IFN-gamma, IL-12, IL-18, and GM-CSF. These other cytokines may be used instead of or in addition to IL-6, either in combination or singly.

[0215] The methods, devices and systems herein may be applied specifically to treat any disorder for which a reduction of demyelination and/or an increase in remyelination would be beneficial. For example, described herein are electrodes (e.g., cuff electrodes, microstimulators) that may be placed around the vagus nerve and may communicate with one or more stimulators configured to apply appropriate stimulation of the vagus nerve to modulate demyelination and/or remyelination. The stimulator may be implanted. In some variations the stimulator is integral to the electrodes, and may be charged externally. The extremely low duty-cycle of the technique described herein may allow the device to be miniaturized to a greater degree than previously suspected for the treatment of chronic disorders via an implantable device.

[0216] In general, a device or system for modulating demyelination and/or remyelination may include a stimulator element (e.g., an electrode, actuator, etc.) and a controller for controlling the application of stimulation by the stimulator element. A stimulator element may be configured for electrical stimulation (e.g., an electrode such as a cuff electrode, needle electrode, paddle electrode, non-contact electrode, array or plurality of electrodes, etc.), mechanical stimulation (e.g., a mechanical actuator, such as a piezoelectric actuator or the like), ultrasonic actuator, thermal actuator, or the like. In some variations the systems and/or devices are implantable. In some variations the systems and/or device are non-invasive. In general, the controller may include control logic (hardware, software, firmware, or the like) to control the activation and/or intensity of the stimulator element. The controller may control the timing (e.g., on-time, off-time, stimulation duration, stimulation frequency, etc.). In variations in which the applied energy is electrical, the controller may control the applied waveform (amplitude, frequency, burst duration/inter-burst duration, etc.). Other components may also be included as part of any of these device or system, such as a power supply (e.g., battery, inductive, capacitor, etc.), transmit/receive elements (e.g., antenna, encoder/decoder, etc.), signal generator (e.g., for conditioning or forming the applied signal waveform), and the like. In some embodiments, a rechargeable battery that may be inductively charged allows the stimulator to deliver numerous electrical stimulations before needing to be recharged. In other embodiments, one or more capacitors that can also be inductively charged can be used to store a limited amount of energy that may be sufficient to deliver a single stimulation or a daily amount of stimulations. This dramatically reduces the size and cost of the stimulator, but requires that the user charge the stimulator daily or before each use.

[0217] In one example, an implantable device for modulating demyelination and/or remyelination (and/or reducing or preventing leaking of the blood-brain barrier) includes an electrode for electrically stimulating the vagus nerve. The electrode may be, for example, a cuff electrode. The electrode may be connected (directly or via a connector) to a controller and signal generator. The signal generator may be configured to provide an electrical signal to the electrode(s). For example, the electrical signal may be an electrical waveform having a frequency of between about 0.1 Hz and about 1 KHz (e.g., 10 Hz), where the pulses applied have a pulse width of approximately (50-500 usec, e.g., a 200 usec pulse). The signal generator may be battery (and/or inductively) powered, and the electrical signal may be amplitude and/or voltage controlled. For example in some variations the device or system may be configured to apply a current that is between about 0.05 mA to 25 mA (e.g., approximately 0.5 mA, 1 mA, 2 mA, 3 mA, etc.). The electrical signal may be sinusoidal, square, random, or the like, and may be charge balanced. In general, the controller (which may be embodied in a microcontroller such as a programed ASIC), may regulate turning on and off the stimulation. For example, stimulation may be applied for an on-time of between about 0.1 sec and 10 minutes (e.g., between 1 sec and 5 minutes, between 1 sec and 2 minutes, approximately 1 minute, etc.); the stimulation may be configured to repeat automatically once every x hours or days, e.g., every other day (off time of approximately 48 hours), once a day (e.g., with an off-time of approximately 24 hours), twice a day (off-time of approximately 12 hours), three times a day (off time of approximately 8 hours), four times a day (off time of approximately 6 hours), or the like. In some variations the implant may be configured to receive control information from a communications device. The communications device may allow modification of the stimulation parameters (including off-time, on-time, waveform characteristics, etc.). The communications device may be worn, such as a collar around the neck, or handheld.

[0218] In use, an implant may be configured to be implanted so that the electrodes contact or approximate the vagus nerve or a portion of the vagus nerve. In one variation the implant includes a cuff that at least partially surrounds the vagus (e.g., near the carotid region). The controller

and/or signal generator (including any power source) may be formed as part of the cuff or may be connected to by a connector (e.g., wire).

[0219] In some variations the device may be non-invasive. For example, the device may be worn outside the body and may trigger stimulation of the vagus nerve from a site external to the body (e.g., the ear, neck, torso, etc.). A non-invasive device may include a mechanical device (e.g., configured to apply vibratory energy). In some variations the device is configured to apply ultrasound that may specifically target the vagus nerve or spleen and apply energy to the vagus nerve or spleen. In some variations, transcutaneous magnetic stimulation of the vagus nerve may be used.

[0220] In any of the variations described herein, the devices, system and methods may be configured to prevent desensitization of the signal in a way that would reduce or inhibit the modulation of demyelination and/or remyelination. For example in some variations, "over stimulation" of the vagus nerve, e.g., simulation at intensities that are too great or applied for too long, or outside of the frequency ranges described herein, may result in desensitization of the effect, thus further modulation may be limited or inhibited. Therefore, in some embodiments, the amplitude of stimulation may be restricted from exceeding (i.e., be less than) about 3 mA, 4 mA, or 5 mA, and/or the duty cycle may be restricted from exceeding about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25%. In some embodiments, the amplitude is also at least 0.25 mA, 0.5 mA, 0.75 mA, or 1.0 mA.

[0221] The examples illustrated above may provide insight into the devices, systems and methods of use for stimulation of the vagus nerve to modulate demyelination and/or remyelination. These methods and devices may be used to treat any indication for which modulation of demyelination and/or remyelination would be beneficial. Non-limiting examples of indications include neurodegenerative and neuroinflammatory diseases such as multiple sclerosis (MS), Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis (ALS), and Batten disease. Other examples include peripheral neuropathies, cranial neuropathies, and autonomic neuropathies. In general, these devices may offer alternative and in some ways superior treatment as compared to pharmacological interventions aimed at modulating demyelination and/or remyelination, and therefore may be used for any indication for which such pharmacological treatments are suggested or indicated. In some embodiments, the VNS treatments described herein can be used in conjunction with pharmacological treatments, particularly when the pharmacological treatment has a different mechanism of action than the VNS, which may lead to synergistic results.

[0222] Thus, the methods of modulating demyelination and/or remyelination as described herein may be used in conjunction with one or more pharmacological interventions, and particularly interventions that treat diseases associated with demyelination, neurodegeneration or neuroinflammation. For example, it may be beneficial to treat a subject receiving stimulation of the vagus nerve to modulate demyelination and/or remyelination by also providing agent such as intravenous corticosteroids (e.g., methylprednisolone), oral corticosteroids, interferons beta-1a and beta-1b, monoclonal antibodies (e.g., natalizumab, alemtuzumab, daclizumab and ocrelizumab), and immunomodulators (e.g., glatiramer acetate, mitoxantrone, fingolimod, teriflunomide, and dimethyl fumarate).

[0223] Thus, described herein are devices (e.g. VNS devices) for the treatment of neurodegenerative and/or neuroinflammatory disorders. Such devices are generally configured to apply low duty-cycle stimulation to the vagus nerve of a subject, as described in any of the variations (or sub-combinations) of these variations. In some embodiments, the patient is first diagnosed or identified with a neurodegenerative and/or neuroinflammatory disorder, particular a disorder characterized by demyelination or need for remyelination, before being implanted and treated with the VNS device.

[0224] It should be appreciated that all combinations of the foregoing concepts and additional concepts discussed in greater detail below (provided such concepts are not mutually inconsistent) are contemplated as being part of the inventive subject matter disclosed herein and may be used to achieve the benefits described herein.

[0225] The process parameters and sequence of steps described and/or illustrated herein are given by way of example only and can be varied as desired. For example, while the steps illustrated and/or described herein may be shown or discussed in a particular order, these steps do not necessarily need to be performed in the order illustrated or discussed. The various example methods described and/or illustrated herein may also omit one or more of the steps described or illustrated herein or include additional steps in addition to those disclosed.

[0226] When a feature or element is herein referred to as being "on" another feature or element, it can be directly on the other feature or element or intervening features and/or elements may also be present. In contrast, when a feature or element is referred to as being "directly on" another feature or element, there are no intervening features or elements present. It will also be understood that, when a feature or element is referred to as being "connected", "attached" or "coupled" to another feature or element, it can be directly connected, attached or coupled to the other feature or element or intervening features or elements may be present. In contrast, when a feature or element is referred to as being "directly connected", "directly attached" or "directly coupled" to another feature or element, there are no intervening features or elements present. Although described or shown with respect to one embodiment, the features and elements so described or shown can apply to other embodiments. It will also be appreciated by those of skill in the art that references to a structure or feature that is disposed "adjacent" another feature may have portions that overlap or underlie the adjacent feature.

[0227] Terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. For example, as used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" and/or "comprising," when used in this specification, specify the presence of stated features, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, steps, operations, elements, components, and/or groups thereof. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items and may be abbreviated as "/".

[0228] Spatially relative terms, such as "under", "below", "lower", "over", "upper" and the like, may be used herein

for ease of description to describe one element or feature's relationship to another element(s) or feature(s) as illustrated in the figures. It will be understood that the spatially relative terms are intended to encompass different orientations of the device in use or operation in addition to the orientation depicted in the figures. For example, if a device in the figures is inverted, elements described as "under" or "beneath" other elements or features would then be oriented "over" the other elements or features. Thus, the exemplary term "under" can encompass both an orientation of over and under. The device may be otherwise oriented (rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein interpreted accordingly. Similarly, the terms "upwardly", "downwardly", "vertical", "horizontal" and the like are used herein for the purpose of explanation only unless specifically indicated otherwise.

[0229] Although the terms "first" and "second" may be used herein to describe various features/elements (including steps), these features/elements should not be limited by these terms, unless the context indicates otherwise. These terms may be used to distinguish one feature/element from another feature/element. Thus, a first feature/element discussed below could be termed a second feature/element, and similarly, a second feature/element discussed below could be termed a first feature/element without departing from the teachings of the present invention.

[0230] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising" means various components can be co-jointly employed in the methods and articles (e.g., compositions and apparatuses including device and methods). For example, the term "comprising" will be understood to imply the inclusion of any stated elements or steps but not the exclusion of any other elements or steps.

[0231] As used herein in the specification and claims, including as used in the examples and unless otherwise expressly specified, all numbers may be read as if prefaced by the word "about" or "approximately," even if the term does not expressly appear. The phrase "about" or "approximately" may be used when describing magnitude and/or position to indicate that the value and/or position described is within a reasonable expected range of values and/or positions. For example, a numeric value may have a value that is +/−0.1% of the stated value (or range of values), +/−1% of the stated value (or range of values), +/−2% of the stated value (or range of values), +/−5% of the stated value (or range of values), +/−10% of the stated value (or range of values), etc. Any numerical values given herein should also be understood to include about or approximately that value, unless the context indicates otherwise. For example, if the value "10" is disclosed, then "about 10" is also disclosed. Any numerical range recited herein is intended to include all sub-ranges subsumed therein. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "X" is disclosed the "less than or equal to X" as well as "greater than or equal to X" (e.g., where X is a numerical value) is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point "15" are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0232] Although various illustrative embodiments are described above, any of a number of changes may be made to various embodiments without departing from the scope of the invention as described by the claims. For example, the order in which various described method steps are performed may often be changed in alternative embodiments, and in other alternative embodiments one or more method steps may be skipped altogether. Optional features of various device and system embodiments may be included in some embodiments and not in others. Therefore, the foregoing description is provided primarily for exemplary purposes and should not be interpreted to limit the scope of the invention as it is set forth in the claims.

[0233] The examples and illustrations included herein show, by way of illustration and not of limitation, specific embodiments in which the subject matter may be practiced. As mentioned, other embodiments may be utilized and derived there from, such that structural and logical substitutions and changes may be made without departing from the scope of this disclosure. Such embodiments of the inventive subject matter may be referred to herein individually or collectively by the term "invention" merely for convenience and without intending to voluntarily limit the scope of this application to any single invention or inventive concept, if more than one is, in fact, disclosed. Thus, although specific embodiments have been illustrated and described herein, any arrangement calculated to achieve the same purpose may be substituted for the specific embodiments shown. This disclosure is intended to cover any and all adaptations or variations of various embodiments. Combinations of the above embodiments, and other embodiments not specifically described herein, will be apparent to those of skill in the art upon reviewing the above description.

What is claimed is:

1. A method for increasing oligodendrocyte progenitor cells (OPCs) by stimulation of a nerve, the method comprising:

identifying that a patient has a decreased level of oligodendrocyte progenitor cells (OPCs);

applying electrical stimulation to a nerve from an implanted neurostimulator, wherein the applied electrical stimulation to the nerve is delivered as a charge per day of between 2.5 nC and 7.5 mC to increase the level of OPCs.

2. The method of claim **1**, further comprising adjusting the electrical stimulation based on the level of OPCs.

3. The method of claim **1**, further comprising adjusting the electrical stimulation based on the time of day.

4. The method of claim **1**, wherein the electrical stimulation is delivered as three or more stimulation per day on a repeating cycle of waiting 5 hours or more between stimulations.

5. The method of claim **1**, wherein the repeating cycle comprises waiting 5 hours, then 7 hours, then 2 hours between the application of stimulation to the nerve.

6. The method of claim **1**, wherein applying comprises applying electrical stimulation to the nerve at between 0.1 and 20 Hz to the vagus nerve.

7. The method of claim **1**, wherein applying comprises applying electrical stimulation to the nerve for less than 10 minute each day.

8. The method of claim **1**, wherein applying comprises applying s electrical stimulation to the nerve from an implanted neurostimulator attached or adjacent to a vagus nerve or a splenic nerve.

9. A method for increasing oligodendrocyte progenitor cells (OPCs) by stimulation of a nerve, the method comprising:

measuring or deriving a level of OPCs within a patient;

adjusting an electrical stimulation based on the level OPCs within the patient; and

applying the electrical stimulation to a nerve of the patient from an implanted neurostimulator, wherein the applied electrical stimulation to the nerve is delivered as a charge per day of between 2.5 nC and 7.5 mC to increase the level of OPCs.

10. A system for increasing oligodendrocyte progenitor cells (OPCs) by stimulation of a nerve, the system comprising:

a nerve stimulator configured to be implanted;

one or more electrodes of the nerve stimulator configured to apply electrical stimulation to the nerve; and

a controller coupled to the nerve stimulator and configured to apply electrical stimulation to the nerve from the one or more electrodes, wherein the controller is configured to apply a charge per day of between 2.5 nC and 7.5 mC, wherein the controller is configured to adjust the applied electrical stimulation based on a measured or derived level of OPCs within the patient.

11. The system of claim **10**, further comprising an input configured to receive the measured or derived level of OPCs within the patient.

12. The system of claim **10**, further comprising a biosensor is configured to detect a marker for the level of OPCs within the patient.

13. The system of claim **10**, wherein the controller is configured to deliver the electrical stimulation based on a timing of the peak levels of lymphocytes within the patient's blood.

14. The system of claim **10**, wherein the controller is configured to deliver the electrical stimulation during a one or more dose sessions of 5 or fewer minutes.

15. The system of claim **10**, wherein the controller is configured to apply the charge per day at a frequency of between 1 and 20 Hz.

16. The system of claim **10**, wherein the controller is configured to apply the charge per date at a frequency of between 1 and 12 Hz.

17. The system of claim **10**, wherein the system is configured to be implanted.

18. The system of claim **10**, further comprising a nerve cuff configured to secure the vagus nerve stimulator to the vagus nerve.

19. The system of claim **10**, wherein the controller is configured to apply the charge per day at two distinct frequencies between 1 and 20 Hz.

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