Supplemental Table 1

Experimental Results/Details of Chronically Cuffed Cervical Vagus Nerve on Rats

Animal Name	H&E Histology	Visual Inspection *	CNAP Detected in Stimulation?	FG Transport to Medulla RV (not cuffed)	FG Transport to Medulla LV (cuffed) **	Cytokine Analysis ***	Days Implanted
eRx62	Not observed	N/A (not cuffed)	N/A (control)	N/A (control)	N/A (control)	*Control (no device)*	N/A
eRx63	Not observed	N/A (not cuffed)	N/A (control)	N/A (control)	N/A (control)	Control (no device/LPS)	N/A
eRx104	Not observed	N/A (not cuffed)	N/A (dummy device)	Expected transport	Expected transport	*Control (dummy device)*	22
eRx105	Not observed	N/A (not cuffed)	N/A (dummy device)	Expected transport	Expected transport	*Control (dummy device)*	23
eRx106	Not observed	N/A (not cuffed)	N/A (dummy device)	Expected transport	Expected transport	*Control (dummy device)*	23
eRx110	Not observed	N/A (not cuffed)	N/A (dummy headcap)	Expected transport	Expected transport	*Control (dummy device)*	21
eRx114	Not observed	N/A (not cuffed)	N/A (dummy headcap)	Expected transport	Expected transport	*Control (dummy device)*	17
eRx115	Not observed	N/A (not cuffed)	N/A (dummy headcap)	Expected transport	Expected transport	Control (dummy, no LPS)	17
eRx127	Normal	Moderate migration	N/A (no metal in cuff)	Expected transport	Expected transport	NA (no blood collection)	32
eRx128	Normal	Severe migration	N/A (no metal in cuff)	Expected transport	Complete suppression	NA (no blood collection)	32
eRx132	Normal	Severe migration	N/A (no metal in cuff)	Expected transport	Complete suppression	*Control (no metal in cuff)*	38
eRx137	Normal	Moderate migration	N/A (no metal in cuff)	Expected transport	Complete suppression	*Control (no metal in cuff)*	30
eRx138	Normal	Severe migration	N/A (no metal in cuff)	Expected transport	Complete suppression	*Control (no metal in cuff)*	30
9909	Not observed	Moderate migration	N/A (not powered)	Expected transport	Complete suppression	Control (no stimulation)	71
9932	Not observed	Severe migration	N/A (not powered)	Expected transport	Complete suppression	Control (no stimulation)	42
eRx75	Not observed	Severe migration	N/A (not powered)	Expected transport	Heavy suppression	*Control (no stimulation)*	13
eRx66	Not observed	not observed	No detection / bad signal	Expected transport	Complete suppression	NA (no blood collection)	45
9915	Not observed	Severe migration	Possible CNAP / ambiguous	Expected transport	Complete suppression	Stimulated	57
eRx61	Not observed	not observed	No detection / bad signal	Expected transport	Complete suppression	Stimulated	62
eRx80	Not observed	not observed	No detection / bad signal	Expected transport	Complete suppression	Stimulated	37
eRx83	Not observed	not observed	No detection / bad signal	Expected transport	Complete suppression	*Stimulated*	29
eRx88	Not observed	not observed	No detection / bad signal	Expected transport	Complete suppression	*Stimulated*	21
eRx111	Not observed	Severe migration	No detection / no recording	Expected transport	Complete suppression	Stimulated	18
eRx113	Not observed	not observed	No detection / no recording	Expected transport	Complete suppression	*Stimulated*	18
eRx64	Not observed	not observed	Possible CNAP / ambiguous	Expected transport	Complete suppression	Stimulated	51
9937	Not observed	Moderate migration	Possible CNAP / ambiguous	Expected transport	Complete suppression	Stimulated	38
eRx53	Not observed	not observed	Possible CNAP / ambiguous	Expected transport	Complete suppression	Stimulated	48
eRx55	Not observed	not observed	Possible CNAP / ambiguous	Expected transport	Complete suppression	Stimulated	44
eRx68	Not observed	Moderate migration	No detection / bad signal	Expected transport	Complete suppression	*Stimulated*	21
eRx81	Not observed	Moderate migration	Possible CNAP / ambiguous	Expected transport	Complete suppression	Stimulated	37
eRx118	Not observed	Moderate migration	No detection / no recording	Expected transport	Complete suppression	Stimulated	14
eRx102	Not observed	not observed	Conclusive CNAP	Expected transport	Complete suppression	Stimulated	23
eRx103	Not observed	Mild or no migration	Conclusive CNAP	Expected transport	Light suppression	Control (no LPS)	22
eRx108	Not observed	not observed	Conclusive CNAP	Expected transport	Complete suppression	Stimulated	21
eRx109	Not observed	not observed	Conclusive CNAP	Expected transport	Complete suppression	Stimulated	21
eRx71	Not observed	Mild or no migration	No detection / bad signal	Expected transport	Complete suppression	Stimulated	44
eRx72	Not observed	Mild or no migration	Possible CNAP / ambiguous	Expected transport	Complete suppression	*Stimulated*	13
eRx116	Not observed	Mild or no migration	Possible CNAP / ambiguous	Expected transport	Heavy suppression	*Stimulated*	17
eRx119	Not observed	Mild or no migration	No detection / bad signal	Expected transport	Moderate suppression	Stimulated	14
eRx141	Normal	Mild or no migration	Possible CNAP / ambiguous	Expected transport	Complete suppression	*Stimulated*	27
eRx142	Normal	Mild or no migration	Conclusive CNAP	Expected transport	Complete suppression	*Stimulated*	26
eRx143	Normal	Mild or no migration	Possible CNAP / ambiguous	Expected transport	Complete suppression	*Stimulated*	26
eRx144	Not observed	Mild or no migration	Conclusive CNAP	Expected transport	Complete suppression	*Stimulated*	36
eRx147	Not observed	Mild or no migration	Conclusive CNAP	Expected transport	Complete suppression	Stimulated	36
eRx148	Not observed	Mild or no migration	Conclusive CNAP	Expected transport	Complete suppression	Stimulated	36
eRx150	Not observed	Mild or no migration	Possible CNAP / ambiguous	Expected transport	Complete suppression	Stimulated	34
eRx152	Not observed	Mild or no migration	Conclusive CNAP	Expected transport	Complete suppression	Control (no LPS)	34

Table illustrates results from chronic testing for 47 rats: Blue = a good or expected result; Red = a bad or unexpected result; Yellow = indeterminant result; White = not tested or not applicable in that animal.

* "Moderate" migration was defined as an electrode(s) that was generally in the correct location but had moved laterally ~1mm or less and/or appeared to have rotated ~30 degrees or less. The nerve still appeared to be intact and encompassed in the cuff electrode. "Severe" was be defined as an electrode(s) that had obvious migrations of >1mm laterally, rotation of >30 degrees, and/or signs of significant pulling or tearing of the encompassed vagus nerve.

** "Heavy" suppression was quantified as having no more than 15 illuminated cells in any given medulla section. "Moderate" suppression was quantified as having more than 15 illuminated cells in any given medulla section, but still had a majority of cells suppressed. "Light" suppression was quantified as having observable cell suppression, but the majority of cells illuminated.

*** Highlighted (green) and italicized items indicate animals that were included in our cytokine analyses.

Supplemental Table 2

Experimental Results/Details of Chronically Cuffed Cervical Vagus Nerve on Rats Used for Gastric Emptying Experiments

Animal Name	Control Scan	Stimulation Scan	Necropsy Nerve Inspection *	FG Transport to Medulla RV (not cuffed)	FG Transport to Medulla LV (cuffed) **	Medulla FG Brightness	Nodose FG Brightness	Days Implanted
eRx157 ***	Good Results	N/A	N/A	N/A	N/A	N/A	N/A	23
eRx159	Good Results	Good Results	Mild or no migration	Expected Transport	Heavy Suppression	Bright	Bright	34
eRx160	Good Results	Total Apnia, Stopped Stim	Mild or no migration	Expected Transport	Moderate Suppression	Bright	Dull But Present	35
eRx161	Good Results	Apnia During Stim	Mild or no migration	Expected Transport	Complete Suppression	Bright	Bright	47
eRx162	Good Results	Good Results	Mild or no migration	Expected Transport	Moderate Suppression	Bright	Bright	46
eRx163	Breathing Issues	Apnia During Stim	Moderate Migration/Pulling	Expected Transport	Complete Suppression	Dull But Present	Dull But Present	48

Table illustrates results from chronic testing for 6 rats: Blue = a good or expected result; Red = a bad or unexpected result; Yellow = indeterminant result; White = not tested or not applicable in that animal.

* “Moderate” migration was defined as an electrode(s) that was generally in the correct location but had moved laterally ~1mm or less and/or appeared to have rotated ~30 degrees or less. The nerve still appeared to be intact and encompassed in the cuff electrode.

** “Heavy” suppression was quantified as having no more than 15 illuminated cells in any given medulla section. “Moderate” suppression was quantified as having more than 15 illuminated cells in any given medulla section, but still had a majority of cells suppressed.

*** eRx157 knocked its headcap off before the scheduled stimulation scan and was euthanized.

Supplemental Table 3

Overview of 17 Previous Studies Using Chronic Vagus Nerve Cuffing

Topic			Animals			Cuffs				Tests Done				Reference
Biological Topic	Fiber Target (Afferent / Efferent)	Nerve Cuffed	Breed (Rat)	Weight	Sample Size	Cuff Type	Cuff Inner Diameter	Duration Cuffed	Cuffing Technique	CNAP Recorded	Nerve Histology	Fluorogold Used	Inflammation Measured	Author
Seizure suppression	Afferent	Left	Sprague-Dawley	--	34	--	--	4 days	Nerve isolated (assumed), cuffed	No	No	No	No	Krahl et al. (2001) [22]
Anti-depression	Afferent	Left	Sprague-Dawley	275g+	5-6 per group	Cyberonics helical	1mm (assumed)	2 days + (1 hour to 90 days)	Cuffs wrapped around nerve & carotid; leads suture to muscle	No	No	No	No	Dorr et al. (2006) [23]
Tremor suppression	Afferent	Left	Long-Evans	300-350g	7	Cyberonics helical	1mm	4+ days	Cuffs wrapped around nerve & carotid	No	No	No	No	Handforth et al. (2001) [24]
Hippocampal neuronal plasticity	Afferent	Left	Sprague-Dawley	250-300g	6 per group	Cyberonics helical	--	2 days + 1 month	Cuffs wrapped around nerve & carotid	No	No (Brain)	No	No	Biggio et al. (2009) [25]
Neuroprotective effects in brain	Afferent	Left	Wistar	Variable	24	Cyberonics helical	--	13 weeks	Cuffs wrapped around nerve (carotid unspecified)	No	No (Brain)	No	Yes (Plasma, brain)	Chunchai et al. (2016) [26]
Brain biomarkers / anti-depression	Afferent	Left	Sprague-Dawley	250-350g	29	Cyberonics helical	--	4 weeks	References Dorr et al.	No	No	No	No	Cunningham et al. (2008) [15]
Anti-depression	Afferent	Left	Sprague-Dawley	250-300g	45	Self-sizing spiral cuff	1mm	8 weeks	Nerve isolated; wrapped with cuff	No	No (Brain)	No	No	Grimonprez et al. (2015) [27]
Body weight / fat mass	Both Possible	Left	Sprague-Dawley	250-300g	13 max per group	Cyberonics helical	--	2 days + 4 weeks	Cuffs wrapped around nerve (carotid unspecified)	No	No	No	No	Banni et al. (2012) [72]
Pain memory	Afferent	Left	Sprague-Dawley	300-350g	8 max per group	PVS sheath	1 mm	16 days	Nerve isolated; pvc around nerve; leads sutured to cleidomastoid	No	No	No	No	Zhang et al. (2013) [28]
Fear / memory conditioning	Afferent	Left	Sprague-Dawley	250-300g	40	Silicone tube	1 mm	13 days	Nerve isolated; cuffed	No	No	No	No	Pena et al. (2014) [29]
Enhanced memory	Both	Left	Long-Evans	350-450g	64	PVC sheath	1 mm	~3 days	Nerve isolated; pvc around nerve; leads sutured to cleidomastoid; bone wax inside cuff	Yes	No	No	No	Clark et al. (1998) [14]
Bowel Inflammation	Both	Left	Sprague-Dawley	--	48	Cyberonics helical	--	17 days	Cuffs wrapped around nerve & carotid	No	No (Colon)	No	Yes (Colon)	Meregnani et al. (2011) [34]
Cardiac remodeling (heart failure)	Both	Left	Sprague-Dawley	--	63	Silicone tube	0.75 mm	14 weeks	Nerve isolated; cuffed/sutured shut; strain relief cuff placed around nerve cuff and artery	No	No	No	No	Agarwal et al. (2016) [30]
Hypertension, Cardiovascular damage	Both	Right	Rat (not specified)	--	24	Wire with Kwik-Sil	wrapped wire	4-5 weeks	Nerve isolated; wire wrapped around; Kwik-Sil encapsulated	No	No	No	Yes (Serum)	Chapleau et al. (2016) [31]
Negative heart impacts	Both	Right	Sprague-Dawley	250-300g	9	Cyberonics helical	1.5 mm	10 weeks	Cuffs wrapped around nerve & carotid	No	No	No	No	Lee et al. (2016) [32]
Artery pressure / heart rate	Both	Right	Sprague-Dawley	250-350g	--	Silicone tube	0.5 mm	up to 3 months	Nerve isolated, cuffed/sutured shut	No	No	No	No	Zheng et al. (2014) [33]
Larynx action potentials	Efferent	Left	Wistar	250-350g	21 chronic	Self-sizing silicone tube	1 mm	8 weeks	Nerve isolated, cuffed	Yes	No	No	No	El Tahry et al. (2011) [35]
Inflammation / Gastric Emptying	Efferent	Left	Sprague-Dawley	190-300g	53	Silicone tube	0.75 mm	13-71 days	Nerve isolated, cuffed	Yes	Yes	Yes	Yes (Plasma)	Somann et al (2017)*

**This study included for comparison purposes*

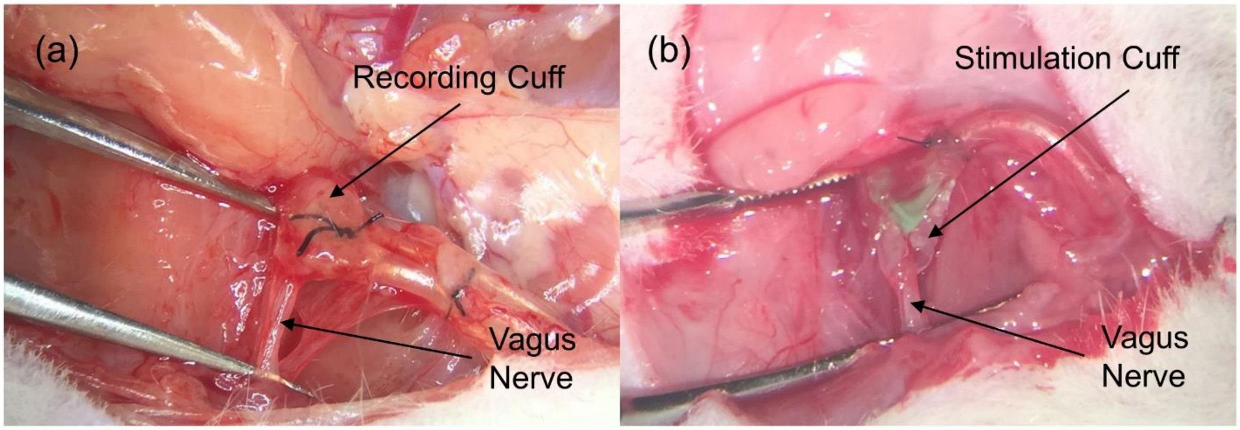
Supplemental Table 4

Stimulation Details of 17 Previous Studies Using Chronic Vagus Nerve Cuffing

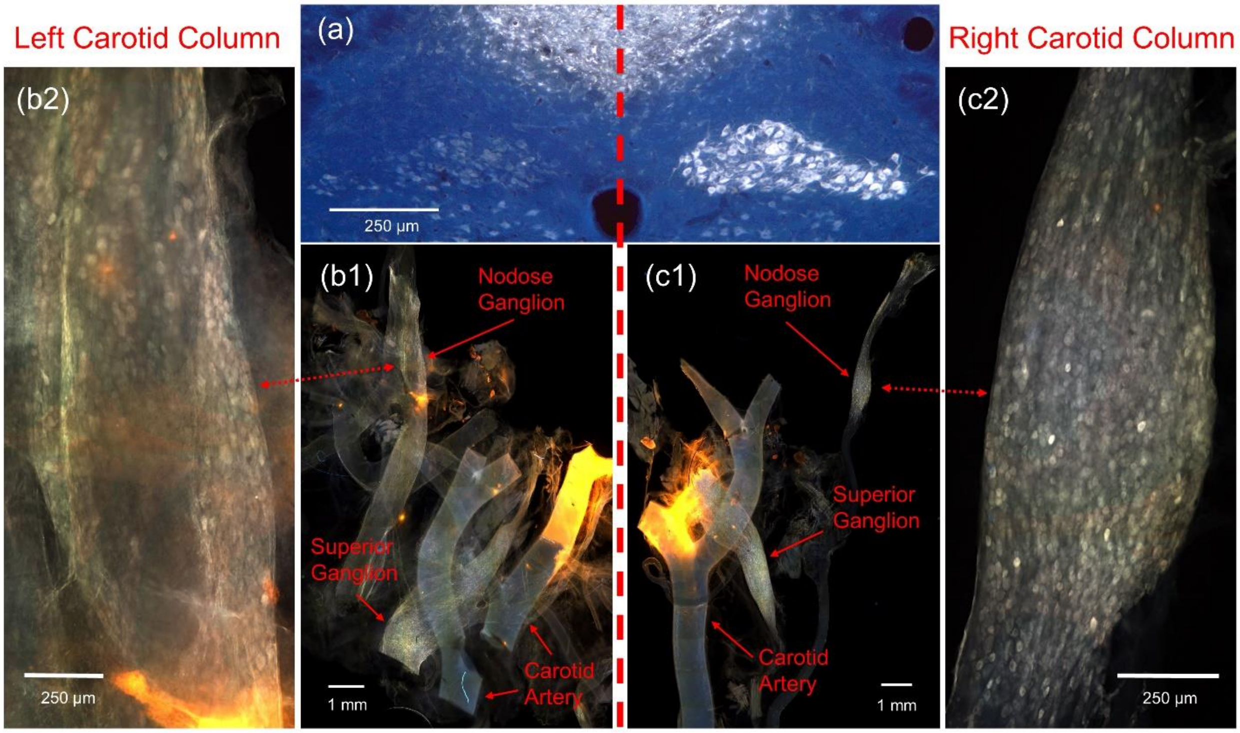
Stimulation Parameters			Stimulation Protocols			Additional Stimulation Details		Reference
Amplitude	Pulse Width (μs)	Frequency (Hz)	Pulse Details	Stimulation Duration	Stimulation Timeline	Delivery Mechanism	Stimulation Verification	Author
1 mA	500	20	Square-wave pulses	15 minute continuous pulse train	Half stimulated after 2 days, the remainder after 4 days	-	-	Krahl et al. (2001) [22]
0.25 mA	500	20	-	30 sec on, 5 min off, continuous	1 hour, 1 day, 3 day, 2-week, 3-week, & 3-month VNS groups	Implanted Cyberonics 102 Pulse Stimulator	Lead impedance check, no functional test presented	Dorr et al. (2006) [23]
0.5 mA	500	20	Balanced biphasic	5 min continuous pulse train	4+ day recovery, then 5 consecutive 20 min treatments (5 min baseline, 5 min acute VNS, 10 min post period)	A-M Systems Model 2100 stimulator, tethered to headcap	-	Handforth et al. (2001) [24]
1.5 mA	500	30	-	30 sec on, 5 min off, continuous	2 day recovery, then 3 hour or 1 month VNS	Implanted Cyberonics stimulator	-	Biggio et al. (2009) [25]
0.5 - 0.75 mA	500	20	-	14 sec on, 48 sec off, continuous	1 week recovery, then 12 week VNS	Implanted Cyberonics Demipulse stimulator	-	Chunchai et al. (2016) [26]
0.25 mA	250	20	-	30 sec per 5 min, continuous	1 week recovery, then 2 hour or 3 week VNS	Implanted Cyberonics stimulator	-	Cunningham et al. (2008) [15]
0.25, 0.5, or 1 mA	250	30	-	7 sec on, 18 sec off, continuous	1 week recovery, 5 week baseline, then 2 week VNS	Stimulator tethered to headcap	Daily impedance test with 1mA square wave	Grimonprez et al. (2015) [27]
1.5 mA	500	30	-	30 sec on, 5 min off, continuous	2 day recovery, then 3 hour or 4 week VNS	Implanted Cyberonics Demipulse Model 103 stimulator	Dedicated test pre-implant	Banni et al. (2012) [72]
0.04 or 0.4 mA	500	1	-	30 seconds acute VNS pre or post conditioning	5 day recovery, pre-condition day, then 3 consecutive conditioning days	A-M Systems Model 2100 stimulator, tethered through neck incision	Respiration and behavioral response to VNS	Zhang et al. (2013) [28]
0.4 mA	500	30	-	30 seconds	1 week recovery, then 5 conditioning/ treatment days (acute VNS on day 4)	Tucker Davis Tech. MS4 stimulator, tethered to headcap	Respiration response to 0.2 mA, 60 Hz, 10 sec VNS	Pena et al. (2014) [29]
0.2, 0.4, or 0.8 mA	500	20	Biphasic pulses	30 seconds	2 day recovery, then condition testing with acute VNS	Undefined stimulator, tethered through neck incision	Action potentials recorded	Clark et al. (1998) [14]
1 mA	500	5	-	10 sec on, 90 sec off, continuous	12 day recovery, then 3 hour VNS per day (5 days)	Grass Technologies stimulator chain, tethered through head connector	-	Meregnani et al. (2011) [34]
0.05 - 0.25 mA	200	20	-	10 sec on, 50 sec off, continuous	7 day recovery, then 6 week VNS	Undefined pulse generator and connection.	Respiration and heart rate response	Agarwal et al. (2016) [30]
3 V	1000	5	Charge balanced	1 hour on, 1 hour off, continuous	3-4 day recovery, then 4 week VNS	Implanted Harald Stauss Scientific Model RNS stimulator	Heart rate and blood pressure response	Chapleau et al. (2016) [31]
1 mA	500	20	-	7 sec on, 66 sec off, continuous	10 week VNS	Implanted Cyberonics Demipulse Model 103 stimulator	-	Lee et al. (2016) [32]
0.1 - 1 mA	200	20	Rectangular pulses	Various, intermittent	6 weeks intermittent VNS	Implanted Unimec ISE1000SA pulse generator	Heart rate and artery pressure	Zheng et al. (2014) [33]
0.04 - 0.8 mA	500	-	Block-pulses	Various to determine dose response curves	5 days / week, for 8 weeks	Undefined stimulator tethered to headcap	Action potentials recorded	El Tahry et al. (2011) [35]
Various (1 mA typical)	Various (1 ms typical)	5	Balanced biphasic square pulses	5 - 10 min pre-calibration, 5 min treatment	13 – 71 day recovery, then pre-calibration VNS 30 min before, acute VNS 30 min after endotoxin	Bionode, fully implanted or tethered to headcap	Direct recording, separate recording cuff	Somann et al (2017)*

**This study included for comparison purpose*

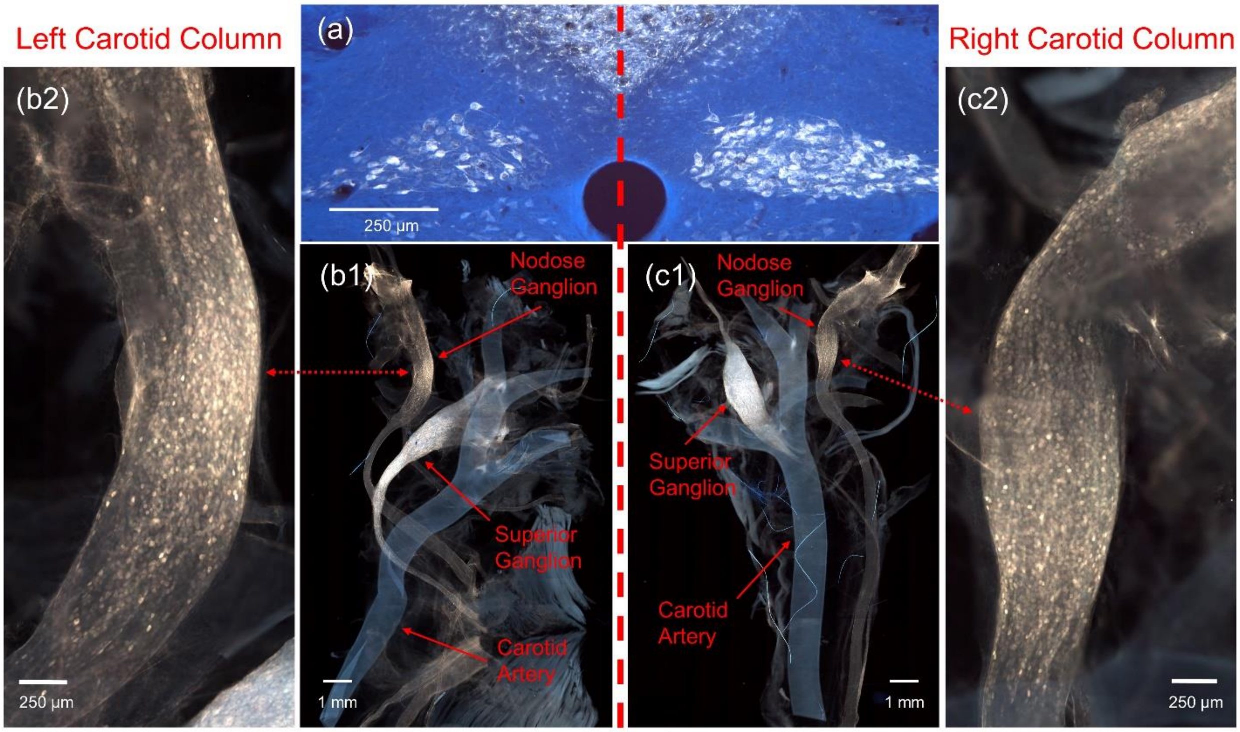
Supplemental Figure 1. Photograph showing a standard stimulation cuff used in our studies. Threaded wire electrode was used as a grounding reference for stimulation and oriented cranially.



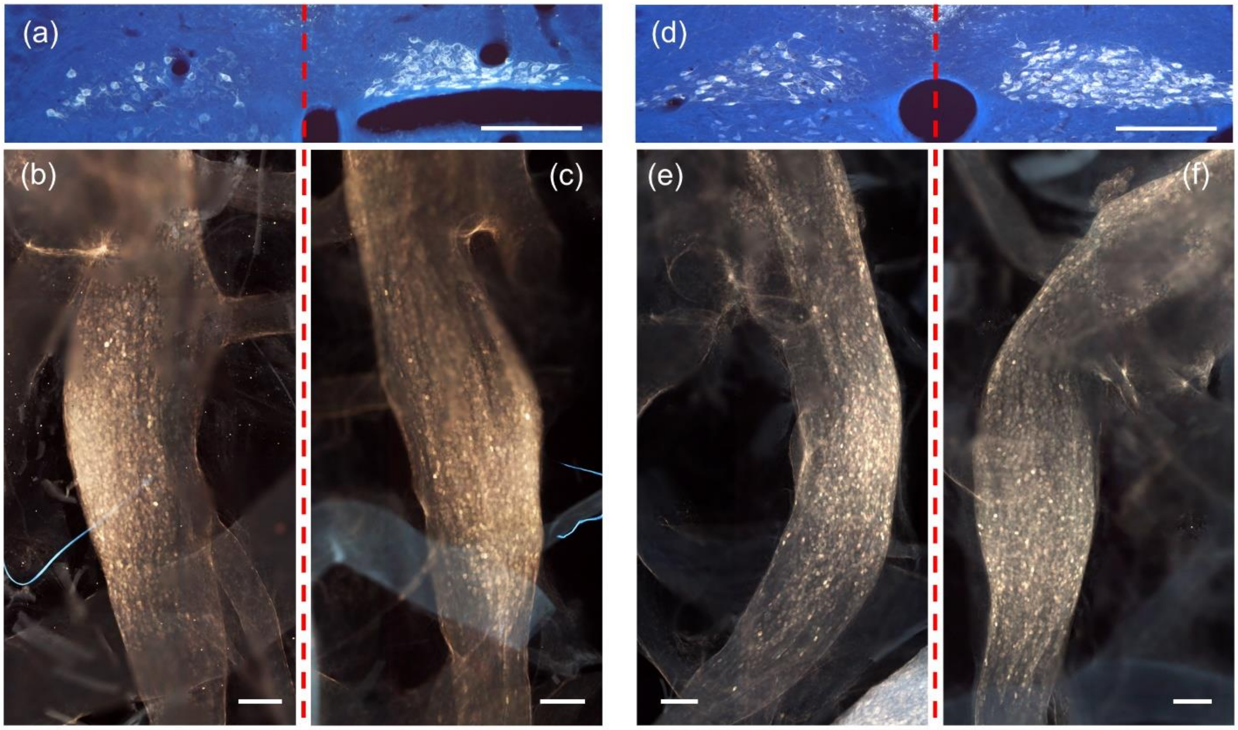
Supplemental Figure 2. Pre-perfusion visual inspection of two animals, both showing cuffs in correct location with vagus nerve intact. (**a**) From a rat that demonstrated complete suppression of Fluorogold transport (eRx71), while (**b**) demonstrated a limited but present transport (eRx103).



Supplemental Figure 3. Representative fluorescence images and image mosaics of a single rat (eRx144). (**a**) medulla image showing clear efferent fiber FG transport on the right side of the dorsal motor nucleus, and complete suppression of transport on the left side. (**b1**) tissue dissected cranial to stimulation cuff on left vagus nerve, with enhanced image of nodose ganglia (**b2**) showing FG illumination. (**c1**) cervical column of right vagus nerve and surrounding tissue, with enhanced image of nodose ganglia (**c2**) showing FG illumination. Vagus nodose ganglia from each side show similar size, shape, location, and textural illumination, indicating active afferent fiber transport in both despite a complete suppression of efferent fiber transport in the cuffed left vagus. Note that the bright yellow and orange illumination on the upper edge of the carotid artery is from ink residue used to mark the samples during explant and has no bearing on FG illumination.



Supplemental Figure 4. Representative fluorescence images and image mosaics of a single rat (eRx162). (**a**) medulla image showing clear efferent fiber FG transport on the right side of the dorsal motor nucleus, and significant suppression of transport on the left side. (**b1**) cervical column dissected cranial to stimulation cuff on left vagus nerve, with enhanced image of nodose ganglia (**b2**) showing FG illumination. (**c1**) cervical column of right vagus nerve and surrounding tissue, with enhanced image of nodose ganglia (**c2**) showing FG illumination. Vagus nodose ganglia from each side show similar size, shape, location, and textural illumination, indicating similar active afferent fiber transport in both despite a suppression of efferent fiber transport in the cuffed left vagus.



Supplemental Figure 5. Comparative fluorescence images of two separate rats demonstrating healthy FG transport through efferent fibers to the DMN and afferent fibers to the nodose in uncuffed right vagus nerves, but only through afferent fibers in cuffed left vagus nerves. (a) medulla image (from eRx160) showing clear efferent fiber FG illumination on the right side of the dorsal motor nucleus, and severe suppression of FG on the left side. (b & c) corresponding fluorescence nodose images of the cuffed left and uncuffed right (respectively) vagus nerves (from eRx160). (d) medulla image (from eRx162) also showing strong efferent fiber FG illumination on the right side of the dorsal motor nucleus, and significant suppression of FG on the left side. (e & f) corresponding fluorescence nodose images of the cuffed left and uncuffed right (respectively) vagus nerves (from eRx162). Red dashed lines separate the left vagal effects from right vagal effects. Scale bars = 250 microns.

Supplemental methods for hematoxylin and eosin histology

For paraffin processing, nerve tissue was processed in a Sakura VIP6 Tissue Processor. The protocol timing used is shown in Supplemental Table 4. All solutions were used under vacuum pressure and at room temperature, except the paraffin which was infiltrated at 61º C. The tissue was then embedded in Leica Paraplast Plus paraffin and cut on a Leica FinesseME microtome at a thickness of 4 micrometers. Slides were dried in a 60º C oven for an hour and stained in a Shandon Varistain autostainer with Gill's Hematoxylin (3 min.) and Eosin Y/Phloxine B (30 sec.). Stained sections were dehydrated through graded alcohols, cleared in xylene, and coverslipped.

For plastic processing, cuffed nerve tissue was processed in a Leica TP1020 processor using the protocol timing in Supplemental Table 4. All stations, except the Acetone, were used under vacuum. The last two changes of PMMA were done by hand in a glass desiccator under vacuum. All Methyl-MethAcrylate (MMA) changes were a solution of 95% MMA (Fisher Scientific) and 5% Dibutyl Phthalate (Fisher Scientific). The cuffed nerve samples were embedded in prepolymerized molds using a solution of 95% MMA and 5% dibutyl phthalate. 0.25% Perkadox16 was used as an initiator for polymerization. The molds were then left at room temperature for a week to polymerize. After the blocks were fully polymerized, they were put in a 60º C oven for an hour to complete polymerization and evaporate any remaining MMA. The PMMA blocks were then trimmed with a Mar-med band saw and mounted on plastic blocks for sectioning in a Reichert-Jung Polycut S. Sections were taken from the PMMA blocks at a thickness of 4-5 micrometers and mounted on APES (Sigma Aldrich) coated slides. The slides were compressed in a metal press and left in a 60º C oven overnight. The slides were then de-plasticized in three changes of acetone for 10 minutes each, hydrated and stained in a Shandon Varistain autostainer with Gill's Hematoxylin (5 min) and Eosin Y/Phloxine B (2 min.). Stained sections were dehydrated and coverslipped as the paraffin sections.

Supplemental Table 4

Protocol timing used in paraffin tissue and cuff, and PMMA and cuff sample processing.

Paraffin Processing		
Station	**Solution**	**Time (min)**
1	10% NBF	0:00
2	70%ETOH	0:05
3	80%ETOH	0:05
4	95%ETOH	0:05
5	95%ETOH	0:05
6	100%ETOH	0:05
7	100%ETOH	0:05
8	100%ETOH	0:10
9	XYLENE	0:05
10	XYLENE	0:10
11	PARAFFIN	0:05
12	PARAFFIN	0:05
13	PARAFFIN	0:05
14	PARAFFIN	0:05
	Process Time	1:15
	Total Time	2:15

PMMA Processing		
Station	**Solution**	**Time (hrs)**
1	70%ETOH	1
2	95%ETOH	1
3	95%ETOH	1
4	100%ETOH	2
5	100%ETOH	2
6	100%ETOH	3
7	Acetone	2
8	Acetone	2
9	MMA 1	12
10	MMA 2	12
11	MMA 3	12
12	MMA 4	24
	Total Time (days)	74