VAGUS NERVE STIMULATION INTENSITY REGULATES TARGETED PLASTICITY

by

Robert Andrew Morrison

APPROVED BY SUPERVISORY COMMITTEE:

Michael P. Kilgard, Chair

Seth A. Hays

Christa K. McIntyre

Robert L. Rennaker II

I dedicate this work to my family, Jennifer Morrison, Stephen Morrison, Loretta Morrison, and Caroline Abe.

VAGUS NERVE STIMULATION INTENSITY REGULATES TARGETED PLASTICITY

by

ROBERT ANDREW MORRISON, BS, MS

DISSERTATION

Presented to the Faculty of

The University of Texas at Dallas

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY IN

COGNITION AND NEUROSCIENCE

THE UNIVERSITY OF TEXAS AT DALLAS

May 2021

ACKNOWLEDGEMENTS

I thank my mother, Jennifer Morrison, my father, Stephen Morrison, and my stepmother, Loretta Morrison. They have always been supportive, loving, and caring, and I could not have asked for a better family to be a part of. I also want to thank my wife, Caroline Abe, for always being there for me and being a constant companion. If it were not for meeting Caroline back in high school, I certainly would not have enrolled at The University of Texas at Dallas, would not have entered the field of neuroscience, and would not have written this dissertation. All of my successes have been possible only with Caroline's help, so they are not just mine, but hers as well.

I would like to thank David Pruitt for helping to train me during my undergraduate career. David took me in as a volunteer on his research team, hired me as a research technician, and eventually convinced me to enroll in the doctoral program. David played a large role in my early neuroscience career, and it has been a pleasure working for him as an undergraduate, and alongside him as a graduate student.

Thank you to all the undergraduate researchers, volunteers, and technicians on my team, past and present. This is not just my work but also theirs. Only through their hard work and determination has any of this been possible. I especially want to thank Jobin Mathew, Stephanie Abe, and Maddie Stevens. Jobin, Stephanie, and Maddie have gone above and beyond expectations, putting an exceptional amount of work into these projects. Conducting research with them is always easy because they are reliable, intelligent, and honestly just fun to work with. A special thanks also goes to Tanya Danaphongse, who has poured so much work into this research, and whose friendship I am always grateful for.

Next, I would like to thank Katy Adcock. Managing a research team with Katy all of these years has been a treat. Katy and I joined the doctoral program at the same time, and we've helped each other navigate the many ups and downs graduate students inevitably face. I will miss sitting back to back at our desks, trying to hide from everyone in our corner of the cubicles to get more work done but ultimately distracting one another. She has been an excellent research partner, and more importantly an amazing friend.

Thank you to my advisors, Dr. Michael Kilgard and Dr. Seth Hays, who have reshaped the method in which I think about just about everything. I am so grateful for the atmosphere they have cultivated at the Texas Biomedical Device Center. Graduate school will always be difficult and involve a large amount of stress, late nights, and uncertainty, but both of them always emphasized work-life balance and made the lab a rewarding place to work. Dr. Kilgard is a fantastic teacher and mentor. One thing I appreciate most about his mentorship is how much time and effort he puts into working with his students. Dr. Kilgard's office was always open, and after every meeting with him his whiteboard was always full of ideas. He always remained enthusiastic about our work, and that enthusiasm was contagious. Thank you also to Seth, who spent so much time teaching me how to write and communicate ideas effectively. I now have dozens of folders full of drafts covered top to bottom in comments and red text from Seth. I thought initially the alarming amount of red was because I was exceptionally bad at writing, but it turns out it is because he is a very thorough, thoughtful reviewer and advisor.

Thank you to Daniel Hulsey, who first helped me get settled in the lab and continued to be a great co-worker and friend throughout the rest of my time in the doctoral program. Thank you to Dr.

Van Miller, who made a great impact on my undergraduate trajectory in his neuroscience classes, and later helped me develop my educating skills as a teacher's assistant.

Pursuing my doctorate has been a transformative experience that has had a profound impact on my life. Many people have contributed both directly and indirectly to that experience. Thank you to everyone who made this work possible, both in and out of the lab.

May 2021

VAGUS NERVE STIMULATION INTENSITY REGULATES TARGETED PLASTICITY

Robert Andrew Morrison, PhD
The University of Texas at Dallas, 2021

Supervising Professor: Michael P. Kilgard

Vagus nerve stimulation (VNS) paired with motor rehabilitation enhances recovery of function after neurological injury in rats and humans. This effect is ascribed to VNS-dependent facilitation of synaptic plasticity in motor networks triggered by increases in neuromodulatory activity. Based on plasticity's role in VNS-enhancement of rehabilitation, it is possible that greater levels of synaptic reorganization lead to greater recovery. Thus, defining stimulation strategies that maximize plasticity may provide a means to optimize the efficacy of VNS therapy, improving subsequent recovery for patients. The stimulation parameters of VNS, including intensity, frequency, and duration, can influence the level of activity in relevant neuromodulatory nuclei. However, levels of neuromodulatory activity alone are not an accurate predictor of degree of plasticity. Previous studies in auditory cortex report an inverted-U relationship between VNS intensity and plasticity, such that moderate intensity VNS yields greater cortical plasticity than low or high intensity VNS.

Here, we first investigate the effects of increasing VNS intensity on motor cortex plasticity when paired with forelimb training. We demonstrate that there is an inverted-U relationship between

VNS intensity and subsequent plasticity, as VNS at moderate intensities paired with forelimb training drives expansion of associated forelimb representations in motor cortex, while low and high intensities do not. We then go on to investigate VNS-mediated plasticity in jaw motor cortex using a new behavioral paradigm emphasizing the jaw musculature, demonstrating that VNS can enhance synaptic reorganization in orofacial circuits. We validate this new behavioral paradigm by re-examining the inverted-U relationship between VNS intensity and degree of plasticity, replicating our previous findings with a higher resolution and demonstrating that there is a narrower range of effective VNS intensities than previously thought.

Although high intensity VNS fails to enhance plasticity when delivered alone, it is unclear whether the mechanisms engaged by high intensity VNS interact with and disrupt subsequent moderate intensity VNS. We tested the interaction of moderate and high intensity VNS trains to probe the mechanisms that may underlie VNS-dependent plasticity, showing that high intensity VNS engages mechanisms that disrupt VNS-dependent synaptic plasticity.

Lastly, based on our findings that VNS can enhance synaptic plasticity in orofacial circuits, we discuss the possibility of using VNS as an adjuvant to rehabilitation for post-stroke motor speech disorders. VNS paired with upper limb rehabilitation enhances upper limb function after stroke, and our findings suggests that VNS may enhance synaptic plasticity in networks related to post-stroke motor speech disorders in a similar manner. We outline the rationale for pairing VNS with rehabilitation for dyspraxia and dysphagia to enhance plasticity in orofacial circuits mediating orofacial function, which could lead to greater recovery than with just rehabilitation alone.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Acquiring and maintaining new motor skills is an ongoing process that takes place at every stage of life. During motor skill learning, movements that are initially isolated and uncoordinated can, through repetitive training, become fluid and effortless [1]. Our ability to learn new motor skills is due in large part to the nervous system's capacity to reorganize and refine neural connections through a process called synaptic plasticity.

Dr. Wilder Penfield first documented organization of the somatosensory cortices decades ago. Through a series of brain surgeries using only local anesthesia with patients remaining conscious, Penfield utilized several techniques including intracortical electrical stimulation, ablation, and patient interviews to construct functional maps of the sensory and motor cortices [2]. These maps revealed that the cortex is topographically organized into sections representing different parts of the body. For example, in the motor cortex, electrical stimulation of the area representing the hand would elicit involuntary hand movement in patients. The area of cortex these sections encompass are not proportional to the size of the body part in which they represent, but instead are proportional to their density of innervation, resulting in more cortical area being devoted to representation of the hands and face than the legs or trunk. These maps greatly contributed to our current day understanding of localization of function in the cortex.

Later, it was demonstrated that these representations in somatosensory areas were not static, but could change based on experience. Nudo, Merzenich, and colleagues observed that forelimb behavioral training expanded task-associated forelimb representations in the primary

motor cortex of adult squirrel monkeys while contracting non-associated representations [3]. Later, Alvaro Pascual-Leone and colleagues showed that after daily one-handed piano lessons, subjects had expanded digit motor representation corresponding to their trained hand, but not the untrained [4]. In recent years, advances in imaging techniques have supported the idea that neuroplasticity underpins motor skill learning through rearrangement and refinement of circuits during repetitive training [1,5,6].

During skill learning, practice and repetition gradually refine once novice circuits into expert ones. The networks that execute learned skills can become incredibly robust, with individual synapses remaining stable on the timescale of years [7]. However, when these expert circuits become damaged after neurological injury, performance of previously learned skills can be near impossible. One such injury that commonly interferes with motor networks is stroke, which affects nearly 800,000 people every year and is the largest cause of long-term disability in the United States [8]. Disability after stroke can encompass a diverse array of symptoms depending on the location and size of neuronal cell death. One of the most common impairments is upper limb dysfunction, which affects roughly 75% of stroke patients [9].

Even in an injured state, the nervous system can still adapt and reorganize. Post-stroke increases in plasticity are often observed in perilesional and contralateral areas [10,11]. This reorganization is thought to contribute to spontaneous recovery in the weeks after injury as affected circuits attempt to compensate for loss of function in damaged areas [10,11]. Similar to skill learning under healthy conditions, repetitive training after stroke can further enhance plasticity, reorganizing neural circuits and bypassing injured areas to support increases in performance [12,13]. Physical rehabilitation is the most common form of post-stroke treatment, and patients

who undergo rehabilitation can see increases in motor function. This recovery, however, is often only partial and incomplete [9]. The prevalence of stroke patients who see only partial recovery after undergoing intensive physical therapy suggests that there is a need for further intervention. Given the importance of neuroplasticity underlying post-stroke spontaneous recovery and improvements after physical rehabilitation, it is reasonable to conclude that interventions that further enhance the neuroplasticity produced by rehabilitation could lead to greater functional outcomes.

Vagus nerve stimulation (VNS) has recently emerged as a method of enhancing rehabilitation for a wide range of neurological disorders affecting motor function including stroke, traumatic brain injury, and spinal cord injury [14–22]. VNS increases the effects of rehabilitation through targeted enhancement of synaptic plasticity in central networks after injury. Electrical stimulation of the vagus nerve immediately enhances neuromodulatory function. Transient bursts of VNS rapidly activate the noradrenergic locus coeruleus (LC) and cholinergic nucleus basalis (NB), two major neuromodulatory centers in the brain [23–28]. Coincident release of these pro-plasticity neuromodulators coupled with neural activity related to rehabilitation facilitates synaptic plasticity in task-specific activated circuits [23,26,27,29,30]. This targeted enhancement of plasticity in central networks after injury is believed to underlie VNS-dependent improvements in functional recovery [19,20]. Further supporting that increases in plasticity drive VNS-enhancement of recovery, blocking neuromodulatory action of the LC and NB prevents synaptic reorganization in sensorimotor areas [24,28], as well as VNS-mediated recovery after injury [31].

Today, VNS has seen success in enhancing recovery after stroke in the clinic [21,22], as well as in a diverse set of preclinical neurological injury models [14–20]. Because of these

successes, much focus has been shifted toward understanding the neural mechanisms supporting VNS-mediated plasticity, and investigation of potential ways to refine and further enhance VNS-therapy. Because reorganization of circuits involved in rehabilitation appears to be of the utmost importance in VNS-therapy, it is possible that increasing the magnitude of VNS-mediated plasticity could further enhance recovery. One possible means of enhancing the magnitude of VNS-mediated plasticity is optimizing the electrical stimulation parameters of VNS.

The stimulation parameters of VNS, including intensity, frequency, and duration, can influence the level of activity in neuromodulatory nuclei [23]. However, it appears that level of neuromodulatory activity alone is not an accurate predictor of degree of plasticity. Chapter 2 of this dissertation will investigate the optimal range of VNS current intensities needed to enhance synaptic plasticity during motor training, and will demonstrate an inverted-U relationship between VNS intensity and cortical plasticity, such that moderate intensities increase plasticity, while low or high intensity VNS does not. This chapter will demonstrate that precise delivery of VNS parameters are critical to enhancing synaptic plasticity in motor cortex, and in turn, recovery after neurological injury.

A wide range of electrical parameters can influence VNS-mediated plasticity. As such, studies must be conducted to measure the relationship between these parametric changes, synaptic rearrangement, and any interactions that could complicate clinical implementation of VNS. Currently, studies characterizing the effects of VNS are tedious and time-intensive, hampering the speed at which they can be conducted. Chapter 3 of this dissertation will outline development and validation of a rapid way to test VNS parameters using a novel behavioral model in which VNS is paired with jaw movement during chewing, subsequently enhancing plasticity of jaw areas in

motor cortex. The speed of this new behavioral model allowed for a replication and a refinement of the findings in Chapter 2. Furthermore, Chapter 3 will discuss implications for the use of this new jaw-plasticity paradigm, and its potential future applications in widening treatment options for sufferers of neurological injury, possibly expanding the use of VNS to treat motor speech and swallowing disorders commonly experienced after stroke.

Chapter 4 will investigate the temporal dynamics of the mechanisms underlying VNS-mediated plasticity and the inverted-U relationship between VNS intensity and synaptic plasticity observed in Chapters 2 and 3. Findings in this chapter have implications for clinical translation of VNS-therapy, and give a better understanding of the neuromodulatory pathways needed to enhance synaptic plasticity. Chapter 5 will outline evidence supporting the use of VNS as a potential adjuvant to speech therapy in treating motor speech dysfunction.

References

[1] Willingham DB. A neuropsychological theory of motor skill learning. Psychol Rev 1998;105:558–84. doi:10.1037/0033-295X.105.3.558.

[2] Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 1937;60:389–443. doi:10.1093/brain/60.4.389.

[3] Nudo R, Milliken G, Jenkins W, Merzenich M. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. J Neurosci 1996;16:785–807. doi:10.1523/JNEUROSCI.16-02-00785.1996.

[4] Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol 1995;74:1037–45. doi:10.1152/jn.1995.74.3.1037.

[5] Dayan E, Cohen LG. Neuroplasticity Subserving Motor Skill Learning. Neuron 2011;72:443–54. doi:10.1016/j.neuron.2011.10.008.

[6] Kami A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. Nature 1995;377:155–8. doi:10.1038/377155a0.

[7] Yang G, Pan F, Gan W-B. Stably maintained dendritic spines are associated with lifelong memories. Nature 2009;462:920–4. doi:10.1038/nature08577.

[8] Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, et al. Heart Disease and Stroke Statistics—2020 Update: A Report From the American Heart Association. Circulation 2020;141. doi:10.1161/CIR.0000000000000757.

[9] Dobkin BH. Strategies for stroke rehabilitation. Lancet Neurol 2004;3:528–36. doi:10.1016/S1474-4422(04)00851-8.

[10] Takatsuru Y, Fukumoto D, Yoshitomo M, Nemoto T, Tsukada H, Nabekura J. Neuronal Circuit Remodeling in the Contralateral Cortical Hemisphere during Functional Recovery from Cerebral Infarction. J Neurosci 2009;29:10081–6. doi:10.1523/JNEUROSCI.1638-09.2009.

[11] Alia C, Spalletti C, Lai S, Panarese A, Lamola G, Bertolucci F, et al. Neuroplastic Changes Following Brain Ischemia and their Contribution to Stroke Recovery: Novel Approaches in Neurorehabilitation. Front Cell Neurosci 2017;11. doi:10.3389/fncel.2017.00076.

[12] Calautti C, Baron J-C. Functional Neuroimaging Studies of Motor Recovery After Stroke in Adults. Stroke 2003;34:1553–66. doi:10.1161/01.STR.0000071761.36075.A6.

[13] Zhang J, Meng L, Qin W, Liu N, Shi FD, Yu C. Structural damage and functional reorganization in Ipsilesional M1 in well-recovered patients with subcortical stroke. Stroke 2014. doi:10.1161/STROKEAHA.113.003425.

[14] Pruitt DT, Schmid AN, Kim LJ, Abe CM, Trieu JL, Choua C, et al. Vagus Nerve Stimulation Delivered with Motor Training Enhances Recovery of Function after Traumatic Brain Injury. J Neurotrauma 2016;33:871–9. doi:10.1089/neu.2015.3972.

[15] Khodaparast N, Hays SA, Sloan AM, Hulsey DR, Ruiz A, Pantoja M, et al. Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. Neurobiol Dis 2013;60:80–8. doi:10.1016/j.nbd.2013.08.002.

[16] Khodaparast N, Hays SA, Sloan AM, Fayyaz T, Hulsey DR, Rennaker RL, et al. Vagus Nerve Stimulation Delivered During Motor Rehabilitation Improves Recovery in a Rat Model of Stroke. Neurorehabil Neural Repair 2014;28:698–706. doi:10.1177/1545968314521006.

[17] Hays SA, Khodaparast N, Ruiz A, Sloan AM, Hulsey DR, Rennaker RL, et al. The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. Neuroreport 2014;25:682–8. doi:10.1097/WNR.0000000000000154.

[18] Hays SA, Ruiz A, Bethea T, Khodaparast N, Carmel JB, Rennaker RL, et al. Vagus nerve stimulation during rehabilitative training enhances recovery of forelimb function after ischemic stroke in aged rats. Neurobiol Aging 2016;43:111–8. doi:10.1016/j.neurobiolaging.2016.03.030.

[19] Meyers EC, Solorzano BR, James J, Ganzer PD, Lai ES, Rennaker RL, et al. Vagus Nerve Stimulation Enhances Stable Plasticity and Generalization of Stroke Recovery. Stroke 2018;49:710–7. doi:10.1161/STROKEAHA.117.019202.

[20] Ganzer PD, Darrow MJ, Meyers EC, Solorzano BR, Ruiz AD, Robertson NM, et al. Closed-loop neuromodulation restores network connectivity and motor control after spinal cord injury. Elife 2018;7:1–19. doi:10.7554/eLife.32058.

[21] Dawson J, Pierce D, Dixit A, Kimberley TJ, Robertson M, Tarver B, et al. Safety, Feasibility, and Efficacy of Vagus Nerve Stimulation Paired With Upper-Limb Rehabilitation After Ischemic Stroke. Stroke 2016;47:143–50. doi:10.1161/STROKEAHA.115.010477.

[22] Kimberley TJ, Pierce D, Prudente CN, Francisco GE, Yozbatiran N, Smith P, et al. Vagus nerve stimulation paired with upper limb rehabilitation after chronic stroke: A blinded randomized pilot study. Stroke 2018. doi:10.1161/STROKEAHA.118.022279.

[23] Hulsey DR, Riley JR, Loerwald KW, Rennaker RL, Kilgard MP, Hays SA. Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. Exp Neurol 2017;289:21–30. doi:10.1016/j.expneurol.2016.12.005.

[24] Hulsey DR, Hays SA, Khodaparast N, Ruiz A, Das P, Rennaker RL, et al. Reorganization of Motor Cortex by Vagus Nerve Stimulation Requires Cholinergic Innervation. Brain Stimul 2016;9:174–81. doi:10.1016/j.brs.2015.12.007.

[25] Nichols JA, Nichols AR, Smirnakis SM, Engineer ND, Kilgard MP, Atzori M. Vagus nerve stimulation modulates cortical synchrony and excitability through the activation of muscarinic receptors. Neuroscience 2011;189:207–14. doi:10.1016/j.neuroscience.2011.05.024.

[26] Dorr AE. Effect of Vagus Nerve Stimulation on Serotonergic and Noradrenergic Transmission. J Pharmacol Exp Ther 2006;318:890–8. doi:10.1124/jpet.106.104166.

[27] Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119:124–32. doi:10.1016/j.brainres.2006.08.048.

[28] Hulsey DR, Shedd CM, Sarker SF, Kilgard MP, Hays SA. Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. Exp Neurol 2019. doi:10.1016/j.expneurol.2019.112975.

[29] Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, et al. Neuromodulators Control the Polarity of Spike-Timing-Dependent Synaptic Plasticity. Neuron 2007;55:919–29. doi:10.1016/j.neuron.2007.08.013.

[30] He K, Huertas M, Hong SZ, Tie XX, Hell JW, Shouval H, et al. Distinct Eligibility Traces for LTP and LTD in Cortical Synapses. Neuron 2015. doi:10.1016/j.neuron.2015.09.037.

[31] Meyers EC, Kasliwal N, Solorzano BR, Lai E, Bendale G, Berry A, et al. Enhancing plasticity in central networks improves motor and sensory recovery after nerve damage. Nat Commun 2019;10:5782. doi:10.1038/s41467-019-13695-0.

CHAPTER 2

VAGUS NERVE STIMULATION INTENSITY INFLUENCES MOTOR CORTEX PLASTICITY

Authors: Robert A. Morrison[1,2,*], Daniel R. Hulsey[2], Katherine S. Adcock[1,2], Robert L. Rennaker II[1,2,3], Michael P. Kilgard[1,2,3], Seth A. Hays[1,2,3]

Affiliations:

[1] The University of Texas at Dallas, School of Behavioral Brain Sciences, Richardson, TX.

[2] The University of Texas at Dallas, Texas Biomedical Device Center, Richardson, TX.

[3] The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, Richardson, TX.

* Correspondence to: robert.morrison@utdallas.edu

Reprinted with permission from Elsevier: *Brain Stimulation*, Volume 12, Issue 2, 2019

> *Vagus nerve stimulation intensity influences motor cortex plasticity*, Robert A. Morrison, Daniel R. Hulsey, Katherine S. Adcock, Robert L. Rennaker, Michael P. Kilgard, Seth A. Hays, *Brain Stimulation*, ISSN 1935-861X, https://doi.org/10.1016/j.brs.2018.10.017.

Abstract

Background: Vagus nerve stimulation (VNS) paired with forelimb motor training enhances reorganization of movement representations in the motor cortex. Previous studies have shown an inverted-U relationship between VNS intensity and plasticity in other brain areas, such that moderate intensity VNS yields greater cortical plasticity than low or high intensity VNS. However, the relationship between VNS intensity and plasticity in the motor cortex is unknown.

Objective: In this study we sought to test the hypothesis that VNS intensity exhibits an inverted-U relationship with the degree of motor cortex plasticity in rats.

Methods: Rats were taught to perform a lever pressing task emphasizing use of the proximal forelimb musculature. Once proficient, rats underwent five additional days of behavioral training in which low intensity VNS (0.4 mA), moderate intensity VNS (0.8 mA), high intensity VNS (1.6 mA), or sham stimulation was paired with forelimb movement. 24 hours after the completion of behavioral training, intracortical microstimulation (ICMS) was used to document movement representations in the motor cortex.

Results: VNS delivered at 0.8 mA caused a significant increase in motor cortex proximal forelimb representation compared to training alone. VNS delivered at 0.4 mA and 1.6 mA failed to cause a significant expansion of proximal forelimb representation.

Conclusion: Moderate intensity 0.8 mA VNS optimally enhances motor cortex plasticity while low intensity 0.4 mA and high intensity 1.6 mA VNS fail to enhance plasticity. Plasticity in the motor cortex exhibits an inverted-U function of VNS intensity similar to previous findings in auditory cortex.

Introduction

Vagus nerve stimulation (VNS) has recently emerged as a method of enhancing rehabilitation for a wide range of neurological disorders affecting motor function including stroke, traumatic brain injury, and spinal cord injury [1–11]. Recovery is thought to be associated with plasticity in central networks after injury [12,13]. VNS is believed to promote recovery by inducing plasticity in networks activated during rehabilitation [12]. Thus, increasing the amount of VNS-mediated plasticity could lead to enhanced recovery.

VNS drives rapid engagement of the neuromodulators acetylcholine and norepinephrine, which act synergistically to strengthen synaptic connections in activated circuits [14–18]. Repeatedly pairing VNS with a sensory or motor event drives robust, targeted cortical plasticity [1,19–22]. For example, repeatedly pairing VNS with forelimb training increases forelimb representation in the motor cortex [22].

A number of stimulation parameters influence the magnitude of plasticity driven by VNS in the auditory cortex, but the effect of these parameters on motor cortex plasticity remains largely unexplored [23]. Increasing VNS intensity drives monotonic increases in neural activity in the locus coeruleus (LC), an area necessary for the effects of VNS on central nervous system [18,24–26]. Thus, VNS intensity and plasticity may be linearly related, where higher intensities of VNS yield greater plasticity. Alternatively, studies in auditory cortex and hippocampus reveal an inverted-U relationship between VNS intensity and plasticity, where low and high intensity VNS drive little to no plasticity, while moderate intensity VNS significantly enhances plasticity [23,27–

29]. Here we sought to test the hypothesis that motor cortex plasticity in rats exhibits an inverted-U relationship to VNS intensity.

Methods

All experimental procedures, statistical comparisons, and exclusion criteria were preregistered before beginning data collection (https://osf.io/3bxgc/).

Subjects

Forty-six female Sprague-Dawley rats weighing approximately 250 grams were used in this experiment. All rats were housed in a reversed 12:12 hour light-dark cycle. Rats were food restricted on weekdays during behavioral shaping and training with ad libitum access to food on weekends. All rats were maintained at or above 85% body weight. All handling, housing, stimulation, and surgical procedures were approved by The University of Texas at Dallas Institutional Animal Care and Use Committee.

Behavioral Training

Rats were trained to perform an automated lever pressing task [30]. The behavioral training apparatus consisted of an acrylic cage with a slot located at the front right for access to a lever positioned outside the cage (Fig. 2.1A). The slot was situated so that rats were only able to use their right forelimb to reach for the lever. A potentiometer affixed to the lever recorded the angle of the lever relative to the horizontal. The lever had a range of motion of 13°, and any lever

depression exceeding 9.5° was considered a press. A spring provided approximately 28 grams of resistance to bring the lever back to a horizontal position after it had been depressed. An audio cue signaled successful presses. All rats were trained to depress the lever twice in rapid succession. If the second press occurred within 500 ms of the first, the trial was recorded as a success and a food pellet was delivered (45 mg dustless precision pellet, BioServ, Frenchtown, NJ) (Fig. 2.1B). If the lever was not depressed a second time or the second press occurred longer than 500 ms after the first press, the trial was recorded as a failure and no food pellet was delivered.

Behavioral shaping occurred in stages. Early in shaping, the lever extended into the behavioral cage and a single press was required for a food pellet to be dispensed. The lever was moved back gradually until the tip of the lever was positioned 2.5 cm away from the cage. Finally, a second press within 500 ms of the first press was required for a food pellet to be dispensed. Rats performed the task for two 30 minute periods five days a week, with each 30-minute session being separated by at least 2 hours. Rats received a supplemental 10 grams of food pellets if they did not receive at least 100 pellets in a day. Once proficient at the task, rats were implanted with VNS cuffs and recovered for 7 days before returning to behavioral testing.

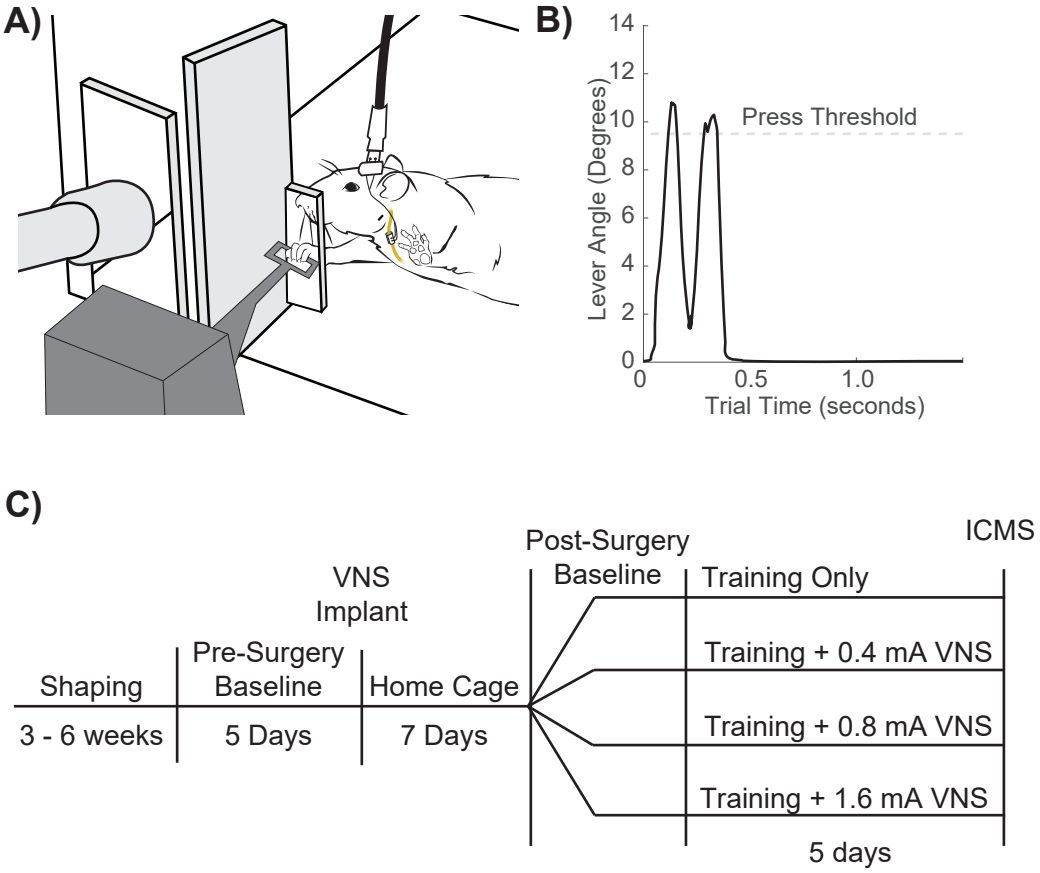


Figure 2.1: Lever pressing task and experimental design

(A) Illustration of rat performing the lever pressing task. The stimulating cable plugged into the headmounted-connector, the subcutaneous stimulation leads and nerve cuff, and the vagus nerve are shown. (B) Representative trial depicting a double press. (C) Timeline of experimental design.

Surgical Implantation

Rats were implanted with a stimulating cuff on the left cervical vagus nerve as described in previous studies [4–7]. Rats were anesthetized with ketamine hydrochloride (50 mg/kg, i.p.), xylazine (20 mg/kg, i.p.), and acepromazine (5 mg/kg, i.p.), and were placed in a stereotactic apparatus. An incision was made down the midline of the head to expose the skull. Bone screws

were inserted into the skull at points surrounding the lamboid suture and over the cerebellum. A two-channel connector was mounted to the screws using acrylic.

An incision was made on the left side of the neck and the overlying musculature was blunt dissected to reveal the vagus nerve. The nerve was gently dissected away from the carotid artery. A cuff electrode was implanted surrounding the vagus nerve with 2 leads tunneled subcutaneously to connect with a 2-channel connector fixed with acrylic to the skull. Nerve activation was confirmed by observation of a ≥ 5% drop in blood oxygen saturation in response to a 10 s stimulation train of 30 Hz VNS consisting of 800 μA, 100 μs biphasic pulses, as in previous studies. The head and neck incisions were then sutured. Rats received subcutaneous injections of 4 mL 50:50 0.9% saline 5% dextrose solution and sustained release Buprenorphine (0.3 mg/kg). A seven-day recovery period followed surgery and rats were given one Baytril tablet per day (2 mg/tablet, BioServ, Frenchtown, NJ).

Vagus Nerve Stimulation

After surgery rats were randomly sorted into four groups that received lever training where successful presses were paired with 0.4 mA VNS (n = 9), 0.8 mA VNS (n = 7), 1.6 mA VNS (n = 8), or sham stimulation (n = 9) (Fig. 2.1C). All rats, regardless of experimental group, were connected via headmount-connector to a stimulation cable. In the initial sessions after implantation rats were allowed to acclimate to being attached to stimulating cables until they performed at least 100 successful trials per day. Once acclimated, rats then underwent five days of training and received VNS according to their group. VNS consisted of a 500 ms train of 100

μs biphasic pulses at a frequency of 30 Hz with an amplitude of either 0.4 mA, 0.8 mA, 1.6 mA, or 0 mA, as appropriate for each group. During daily VNS sessions, a digital oscilloscope (PicoScope 2204A, PP906, Pico Technology) was used to monitor cuff impedance to ensure nerve cuff functionality.

Intracortical Microstimulation Mapping

Within 24 hours of their last behavioral session rats underwent intracortical microstimulation (ICMS) as previously described [22,31–34]. Rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/k, i.p.). Toe-pinch response and whisking were used to determine when supplemental doses were needed in order to maintain a constant state of anesthesia for the procedure. To evaluate nerve cuff functionality, nerve activation was confirmed by observation of a ≥ 5% drop in blood oxygen saturation in response to a 10 s stimulation train of 30 Hz VNS consisting of 800 μA, 100 μs biphasic pulses. Rats that failed to demonstrate stimulation-induced depression in oxygen saturation were excluded, as defined in the pre-registration.

Rats were placed in a stereotactic apparatus and a craniotomy and duratomy were performed to expose the left motor cortex (4 mm to -3 mm AP and 0.25 mm to 5 mm ML). A cisternal drain was created to minimize cortical swelling. A tungsten electrode with an impedance of approximately 0.7 MΩ (FHC, Tungsten Microelectrode - UEWMEGSEBN3M) was lowered into the brain to a depth of 1.8 mm. Stimulation sites were then chosen at random on a grid with sites set 500 μm apart from each other. Sites were at least 1 mm away from the previous site

whenever possible. Stimulations consisted of a 40 ms pulse train of monophasic 200 μs cathodal pulses delivered at a frequency of 286 Hz. ICMS was conducted blinded with two experimenters, as previously described [22,35]. The first experimenter placed the electrode and recorded the data for each site. The second experimenter, blinded to group and electrode location, delivered stimulations and classified movements. Stimulation was increased from 20 μA until a movement was observed or until a maximum of 250 μA was reached. Movements were classified into the following categories: proximal forelimb, distal forelimb, head, and hindlimb. All raw data from ICMS is available in the appendix (Appendix A).

Subject Exclusion

All subject exclusion criteria was preregistered before data collection began (https://osf.io/3bxgc/). 33 subjects were analyzed in the final results of the study out of a total of 46 subjects. Of the 13 subjects not used in final analysis, 2 subjects were excluded due to loss of function of nerve cuffs, 6 subjects were excluded due to head-connector failure during behavioral training, and 5 subjects were excluded due to death during or immediately after VNS implantation or before ICMS surgery.

Statistics

Statistical methods and comparisons were preregistered and defined *a priori* (https://osf.io/3bxgc/). The primary outcome of this study was area of motor cortex generating

proximal forelimb movements. All other movement representations and behavioral performance data were analyzed as secondary outcome measures. A one-way ANOVA was used to compare experimental group representation sizes. Behavioral data was compared using one-way ANOVA. Bonferroni corrected unpaired two-tailed t-tests at an alpha of 0.0083 were used to identify any between group differences, as appropriate. All data are reported as mean ± SEM. "*" indicates Bonferroni-corrected significant differences in all figures.

Results

We sought to evaluate the effect of varying stimulation intensity on VNS-dependent plasticity in motor cortex. To do so, rats were shaped on an automated lever-pressing task emphasizing the proximal forelimb musculature. Once proficient, rats underwent five additional days of behavioral training in which low intensity VNS (0.4 mA), moderate intensity VNS (0.8 mA), high intensity VNS (1.6 mA), or sham stimulation was paired with forelimb movement (Fig. 2.1). Following completion of behavioral training, intracortical microstimulation (ICMS) was used to document movement representations in the motor cortex.

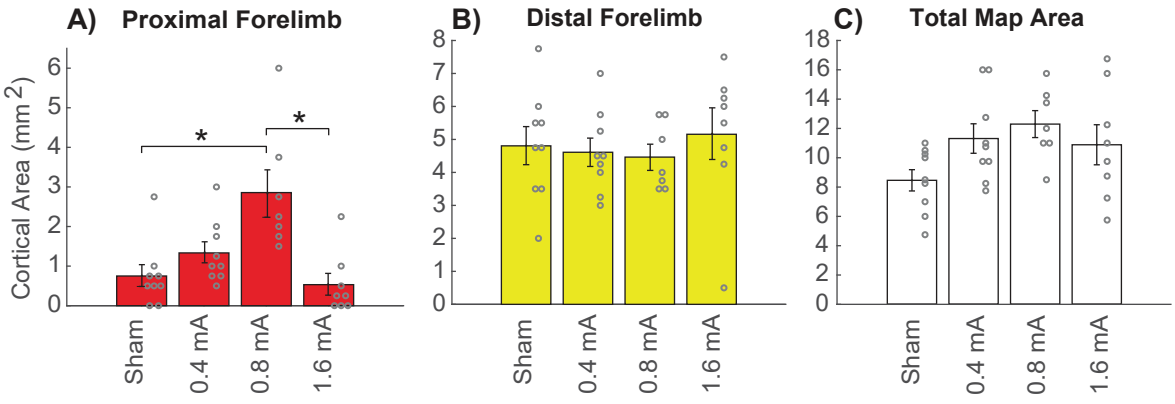


Figure 2.2: Moderate intensity VNS enhances plasticity in motor cortex

(A) Moderate intensity 0.8 mA VNS paired with forelimb motor training significantly increases the movement representation of the proximal forelimb in motor cortex compared to equivalent motor training without VNS (Sham). Low intensity 0.4 mA VNS and high intensity 1.6 mA VNS both fail to increase proximal forelimb representation compared to Sham. (B) No difference was observed in the area of distal forelimb representation across groups, indicating that VNS-dependent plasticity is specific to the trained movement. (C) No change in the total area of motor cortex was observed across groups. Circles depict individual subjects. Bars represent mean ± SEM. * denotes significant differences using Bonferroni-corrected $p < 0.0083$.

Moderate intensity 0.8 mA VNS enhances plasticity in motor cortex

Previous studies indicate that moderate intensity VNS paired with training drives substantial reorganization of cortical networks [23,27–29]. Group analysis of proximal forelimb representation area revealed a significant effect between groups (one-way ANOVA, $F[3,29] = 8.03$, $p = 4.79 \times 10^{-4}$). Moderate intensity 0.8 mA VNS paired with motor training significantly increased proximal forelimb representation compared to equivalent motor training without VNS (0.8 mA VNS: 2.86 ± 0.59 mm^2; sham: 0.75 ± 0.27 mm^2, Unpaired t-test, $t(14) = -3.48$, $p = 3.69 \times 10^{-3}$) (Fig. 2.2A, Fig. 2.3). These results are consistent with previous studies and demonstrate

that pairing moderate intensity VNS with forelimb training enhances plasticity in motor cortex [22,35].

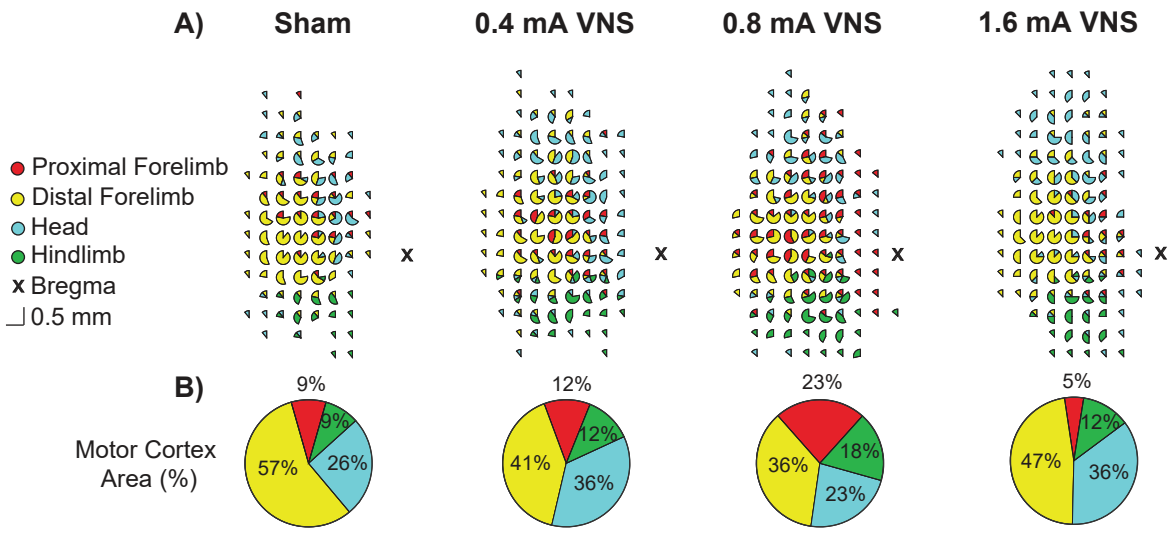


Figure 2.3: Average motor cortex movement representations

(A) Cumulative representations from all ICMS maps expressed as a percentage of representations observed at each electrode penetration for each group. (B) Average percentage of the total map devoted to each movement representation. Moderate intensity 0.8 mA VNS paired with forelimb training significantly increases the amount of motor cortex that represents the proximal forelimb compared equivalent training paired with high intensity 1.6 mA VNS.

Low intensity 0.4 mA VNS and high intensity 1.6 mA VNS fail to enhance plasticity in motor cortex

Next, we evaluated the effects of low intensity 0.4 mA VNS and high intensity 1.6 mA VNS paired with motor training. Low intensity VNS failed to increase proximal forelimb representation compared to equivalent motor training without VNS (0.4 mA VNS: 1.33 ± 0.26 mm^2, sham: 0.75 ± 0.27 mm^2, Unpaired t-test, $t(16) = -1.54$, $p = 0.14$) (Fig. 2.2A). Similarly, high

intensity VNS also failed to increase proximal forelimb representation compared to equivalent motor training without VNS (1.6 mA VNS: 0.53 ± 0.27 mm^2, sham: 0.75 ± 0.27 mm^2, Unpaired t-test, $t(15) = 0.56$, $p = 0.58$). High intensity VNS also resulted in significantly less proximal forelimb representation than moderate intensity VNS (1.6 mA VNS: 0.53 ± 0.27 mm^2, 0.8 mA VNS: 2.86 ± 0.59 mm^2, Unpaired t-test, $t(13) = 3.71$, $p = 2.60 \times 10^{-3}$). Together these results suggest that VNS intensity has an inverted-U relationship with the magnitude of plasticity, consistent with previous studies in auditory cortex [23].

VNS-mediated plasticity is specific to the trained movement

Group analysis of other movement representations showed no significant differences between groups (one-way ANOVA, distal forelimb: $F[3,29] = 0.28$, $p = 0.84$, sham: 4.81 ± 0.56 mm^2, 0.4 mA VNS: 4.61 ± 0.41 mm^2, 0.8 mA VNS: 4.46 ± 0.38 mm^2, 1.6 mA VNS: 5.16 ± 0.76 mm^2; head: $F[3,29] = 2.29$, $p = 0.10$, sham: 2.17 ± 0.44 mm^2, 0.4 mA VNS: 4.08 ± 0.66 mm^2, 0.8 mA VNS: 2.82 ± 0.41 mm^2, 1.6 mA VNS: 4.00 ± 0.74 mm^2; hindlimb: $F[3,29] = 2.14$, $p = 0.12$, sham: 0.75 ± 0.20 mm^2, 0.4 mA VNS: 1.36 ± 0.44 mm^2, 0.8 mA VNS: 2.18 ± 0.54 mm^2, 1.6 mA VNS: 1.34 ± 0.35 mm^2) (Fig. 2.2B). Additionally, no differences in total motor cortex area were observed (one-way ANOVA, $F[3,29] = 2.56$, $p = 0.07$) (Fig. 2.2C). Group analysis of average response thresholds also showed no significant differences between groups (one-way ANOVA, $F[3,29] = 2.05$, $p = 0.13$). These results confirm that VNS-dependent enlargement of cortical movement representations is restricted to the paired movement and does not broadly influence cortical representations.

VNS does not alter behavioral performance

We tested whether differences in behavioral performance could account for observed VNS-dependent increase in proximal forelimb representation. Between-group analysis during behavioral training revealed no significant differences in total successful trials (one-way ANOVA, $F[3,29] = 0.42$, $p = 0.74$) total trials (one-way ANOVA, $F[3,29] = 1.31$, $p = 0.29$) success rate (one-way ANOVA, $F[3,29] = 0.75$, $p = 0.53$) nor inter-press-interval (one-way ANOVA, $F[3,29] = 2.49$, $p = 0.08$). Group analysis also revealed no differences in the number of stimulations received between groups during behavioral training (one-way ANOVA, $F[3,29] = 0.99$, $p = 0.41$) (Fig. 2.4A) or the average time between stimulations (one-way-ANOVA, $F[3,29] = 1.19$, $p = 0.33$) (Fig. 2.4B) which had a mean of 11.06 ± 1.13 seconds. These results suggest that expansion of map representations driven by moderate intensity VNS cannot be ascribed to changes in motivation, motor performance, or the amount of stimulations administered during training.

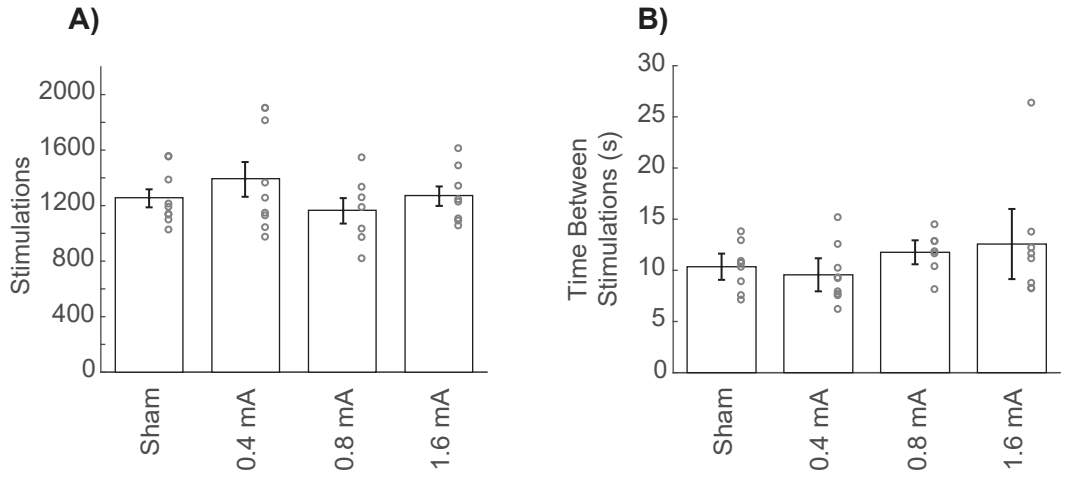


Figure 2.4: Amount of training or stimulation cannot explain moderate intensity VNS-dependent enhancement of plasticity

(A) No difference in the total number of stimulations received was observed across groups. (B) Additionally, no difference in the timing between stimulations was observed across groups. Together, these findings indicate that differences in the amount of stimulation or the timing between stimulations cannot account for the increased in proximal forelimb representation driven by moderate intensity 0.8 mA VNS. Circles depict individual subjects. Bars represent mean ± SEM.

Discussion

Repeatedly pairing VNS with motor or sensory training yields robust, specific cortical plasticity [1,19–22]. However, little is known about the stimulation parameters that most effectively drive VNS-dependent enhancement of plasticity in motor cortex. In this study, we report that the intensity of VNS paired with forelimb training exhibits an inverted-U relationship with plasticity, such that moderate intensity stimulation yields greater motor cortical reorganization than low or high intensity stimulation. VNS-dependent enhancement of plasticity is specific to the trained movement, with no changes observed in the representation of untrained

movements. Differences in behavioral performance or number of stimulations administered cannot account for the observed enhancement of plasticity. These results provide evidence to guide the selection of parameters for VNS applications that aim to enhance motor cortical plasticity and promote recovery of motor function after neurological injury [1,2,4–11].

Consistent with findings reported here, previous studies of VNS document an inverted-U relationship between stimulation intensity and memory or plasticity in other brain regions, with moderate intensity VNS being most effective [23,27,36]. In the auditory cortex, low intensity 0.4 mA VNS was comparably effective to moderate intensity 0.8 mA VNS, while here we report that 0.4 mA VNS fails to significantly enhance plasticity in motor cortex. It is possible that the difference in the effect of low intensity VNS arises from fundamental differences in auditory and motor cortices. Alternatively, this discrepancy could be explained by differences in interval between stimulations. In studies evaluating plasticity in auditory cortex, stimulations occur at a fixed interval of 30 seconds. In the present study, the interval between stimulations is variable and depends on the task performance, but averages approximately 11 seconds. The interval between stimulations is a major determinant of the degree of VNS-dependent plasticity, and reducing the amount of time between stimulations reduces VNS efficacy in auditory cortex [37]. Thus, the shorter interval in this study may account for the absence of plasticity using low intensity VNS and raises the possibility that lengthening the time between stimulations could restore VNS-dependent enhancement of motor cortex plasticity at low intensity stimulation. The results from the current study corroborate previous reports in which high intensity 1.6 mA VNS fails to enhance plasticity or memory [23,27–29].

While the vagus nerve mediates parasympathetic activity via descending projections, ascending projections are able to communicate information regarding arousing events from the periphery to the central nervous system. Ascending activation of the vagus nerve can occur in response to both beneficial stimuli, such as eating and digestion, or stimuli associated with a negative valence, such as stress, fear, and inflammation [38,39]. Vagal signals resulting from these events are able to enhance memory and promote learning, and several studies have demonstrated that the vagus nerve is required for peripherally-mediated enhancement of memory because the effect is blocked by vagal transection [29,40]. The locus coeruleus (LC), a major source of cortical norepinephrine, demonstrates rapid, phasic activation and releases norepinephrine in response to increasing intensities of VNS [14,18,25]. Similar to the effects of vagotomy on memory enhancement, lesions of the LC block central effects of VNS, suggesting norepinephrine plays a major role in VNS efficacy [26]. Thus, the vagus nerve is positioned to influence plasticity in response to a variety of peripheral stimuli and control of neuromodulatory networks by ascending vagal projections likely underlies VNS-dependent plasticity observed in response to electrical stimulation.

A variety of neuronal mechanisms could account for the inverted-U relationship observed between VNS intensity and enhancement of plasticity. VNS promotes plasticity by engaging multiple neuromodulatory networks during training [18,25]. Activation of these opposing neuromodulator actions or desensitization of receptors provide a mechanistic basis for the inverted-U. One potential explanation reflects the activation of opposing processes with different activation thresholds. In this case, VNS at moderate intensities sufficiently activates a low–threshold, pro-plasticity process and avoids activation of a high-threshold, anti-plasticity process,

resulting in robust enhancement of plasticity (Fig. 2.5). Low stimulation intensities would fail to sufficiently activate the pro-plasticity process, precluding effective plasticity. At high stimulation intensities, activation of the anti-plasticity process would dominate and similarly prevent the enhancement of plasticity. Adrenergic receptors, which are activated by VNS-dependent norepinephrine release, express features that could underlie these opposing systems. Moderate intensities of VNS may result in sufficient norepinephrine release to engage higher-affinity α_2-receptors and promote potentiation, whereas high intensity stimulation may increase norepinephrine levels further to activate lower-affinity β-receptors and oppose potentiation. Indeed, this concentration dependent control of the polarity of plasticity by adrenergic receptors has been described [41]. In addition to norepinephrine, several other neuromodulatory systems have been linked to the action of VNS in the central nervous system, including serotonin and acetylcholine [42–44]. Similar principles governing these neuromodulatory systems could also give rise to the inverted-U effect of VNS on plasticity.

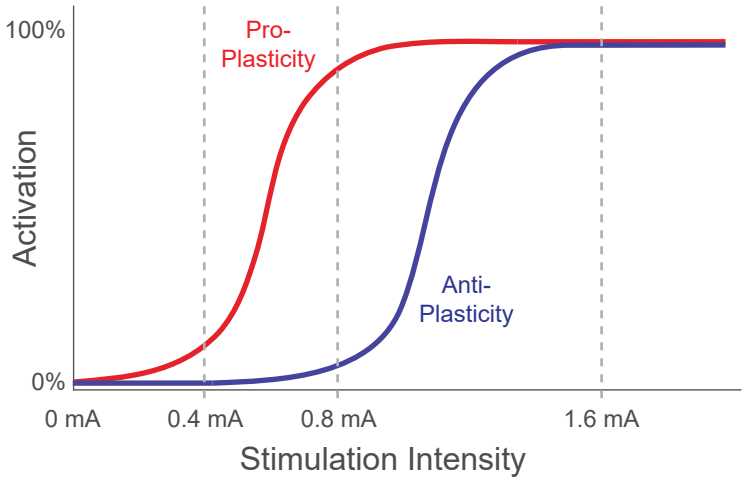


Figure 2.5: Model of the inverted-U relationship between VNS intensity and cortical plasticity

One potential explanation to account for the inverted-U relationship between VNS intensity and enhancement of plasticity relies on engagement of two opposing processes. At low stimulation intensities, VNS fails to drive sufficient activation of a low-threshold, pro-plasticity process (red) and thus fails to drive plasticity. At moderate stimulation intensities, VNS activates the low-threshold pro-plasticity process and avoids activation of a high-threshold, anti-plasticity process (blue), resulting in robust enhancement of plasticity. At high stimulation intensities, the anti-plasticity process dominates and prevents effective enhancement of plasticity.

Alternatively, a single desensitizing system could explain the inverted-U response. Moderate intensity stimulation would provide sufficient activation with minimal desensitization, while high levels of stimulation would result in overactivation and reduction of the response. G-protein coupled receptors, such as those which likely mediate the response to VNS-dependent engagement of the noradrenergic, cholinergic, and serotonergic systems, exhibit notable desensitization and may explain the inverted-U response to VNS [45]. Other effects of VNS on cortical neurons, including desynchronization and activation of hyperpolarizing currents, could potentially produce the network consequences observed in this study [44,46,47]. However, since

cortical reorganization was assessed the day after the cessation of VNS, these neuronal effects would need to engender plasticity in order to mediate the lasting changes in movement representations. While all of these models can account for the inverted-U, they are not mutually exclusive and various other systems likely contribute. Future studies directed at manipulating the activation of neuromodulatory networks could provide insight into the neuronal mechanisms that underlie VNS-dependent enhancement of plasticity. Additionally, a clear understanding of activation of these neuromodulatory systems may lead to pharmacological manipulations to potentiate the effects of VNS.

At moderate intensity stimulation, the reorganization of cortical movement representations is likely produced by temporally-precise engagement of neuromodulatory activation by VNS. During performance of the behavioral task in this study, networks in motor cortex generate activity to produce movement of the proximal forelimb muscles. Delivery of VNS concurrent with movement-related neural activity provides precisely-timed neuromodulatory feedback, which likely facilitates canonical spike-timing-dependent plasticity mechanisms to enhance plasticity within the active motor networks [17,48,49]. The degree of activation of these neuromodulatory networks is contingent upon the intensity of VNS [18], and results from the present study support the notion that moderate intensity VNS produces favorable neuromodulatory activation to facilitate plasticity. This VNS-dependent enhancement of plasticity forms the basis for the use of VNS in treatment of movement disorders.

A number of studies in preclinical models and humans demonstrate that VNS paired with rehabilitative training supports recovery in a wide range of neurological disorders including stroke, traumatic brain injury, spinal cord injury [1–11]. Recovery after these injuries is thought to be

dependent on plasticity in central networks after injury [12,13]. Indeed, VNS paired with rehabilitative training drives large-scale synaptic reorganization in motor control networks after stroke and spinal cord injury [10,12]. The VNS-dependent plasticity in corticospinal, corticorubral, and propriospinal networks likely underpins the improvements in recovery of function. Consequently, there is great interest in identifying paradigms that maximize plasticity and thereby yield greater recovery. The present study characterizes the effect of stimulation intensity across a range of parameters and establishes a framework for future studies to directly evaluate the effect of varying VNS intensity on plasticity and recovery after neurological injury. Ultimately, these findings may facilitate determination of optimal parameters for clinical application.

Acknowledgements

We would like to thank Tanya Danaphongse and David Pruitt for surgical support, and Christine Mai Shedd, Aaron Kuo, Yun-Yee Tsang, Prathima Kandukuri, Ayushi Bisaria, Stephanie Abe, Alissar Zammam, Naser Asfoor, Eric Bellinghausen, Nicole Pillai, and Joanna John for behavioral training. We would also like to thank Sadmaan Sarker, Peyton Demetrovich, Joy Mong, and Helena Zhang for support in ICMS. Further thanks goes to Kimi Rahebi, Lena Lynn Sadler, and Matt Buell for vagus nerve cuff construction.

Sources of funding

This work was supported by the National Institutes of Health R01NS085167 and R01NS094384 and by the Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO) Electrical Prescriptions (ElectRx) program under the auspices of Dr. Eric Van Gieson through the Space and Naval Warfare Systems Center, Pacific Cooperative Agreement No. N66001-15-2-4057 and the DARPA BTO Targeted Neuroplasticity Training (TNT) program under the auspices of Dr. Tristan McClure-Begley through the Space and Naval Warfare Systems Center, Pacific Grant/Contract No. N66001-17-2-4011.

Conflict of interest disclosures

MPK has a financial interesting in MicroTransponder, Inc., which is developing VNS for stroke and tinnitus. All other authors declare no conflicts of interest.

References

[1] Hays SA, Rennaker RL, Kilgard MP. Targeting Plasticity with Vagus Nerve Stimulation to Treat Neurological Disease. Prog Brain Res 2013;207:275–99. doi:10.1016/B978-0-444-63327-9.00010-2.

[2] Pruitt DT, Schmid AN, Kim LJ, Abe CM, Trieu JL, Choua C, et al. Vagus Nerve Stimulation Delivered with Motor Training Enhances Recovery of Function after Traumatic Brain Injury. J Neurotrauma 2016;33:871–9. doi:10.1089/neu.2015.3972.

[3] Kimberley TJ, Pierce D, Prudente CN, Francisco GE, Yozbatiran N, Smith P, et al. Vagus Nerve Stimulation Paired With Upper Limb Rehabilitation After Chronic Stroke. Stroke 2018;49:2789–92. doi:10.1161/STROKEAHA.118.022279.

[4] Khodaparast N, Hays SA, Sloan AM, Hulsey DR, Ruiz A, Pantoja M, et al. Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. Neurobiol Dis 2013;60:80–8. doi:10.1016/j.nbd.2013.08.002.

[5] Khodaparast N, Hays SA, Sloan AM, Fayyaz T, Hulsey DR, Rennaker RL, et al. Vagus Nerve Stimulation Delivered During Motor Rehabilitation Improves Recovery in a Rat Model of Stroke. Neurorehabil Neural Repair 2014;28:698–706. doi:10.1177/1545968314521006.

[6] Hays SA, Khodaparast N, Ruiz A, Sloan AM, Hulsey DR, Rennaker RL, et al. The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. Neuroreport 2014;25:682–8. doi:10.1097/WNR.0000000000000154.

[7] Hays SA, Khodaparast N, Hulsey DR, Ruiz A, Sloan AM, Rennaker RL, et al. Vagus Nerve Stimulation During Rehabilitative Training Improves Functional Recovery After Intracerebral Hemorrhage. Stroke 2014;45:3097–100. doi:10.1161/STROKEAHA.114.006654.

[8] Khodaparast N, Kilgard MP, Casavant R, Ruiz A, Qureshi I, Ganzer PD, et al. Vagus Nerve Stimulation During Rehabilitative Training Improves Forelimb Recovery After Chronic Ischemic Stroke in Rats. Neurorehabil Neural Repair 2016;30:676–84. doi:10.1177/1545968315616494.

[9] Hays SA, Ruiz A, Bethea T, Khodaparast N, Carmel JB, Rennaker RL, et al. Vagus nerve stimulation during rehabilitative training enhances recovery of forelimb function after ischemic stroke in aged rats. Neurobiol Aging 2016;43:111–8. doi:10.1016/j.neurobiolaging.2016.03.030.

[10] Meyers EC, Solorzano BR, James J, Ganzer PD, Lai ES, Rennaker RL, et al. Vagus Nerve Stimulation Enhances Stable Plasticity and Generalization of Stroke Recovery. Stroke 2018;49:710–7. doi:10.1161/STROKEAHA.117.019202.

[11] Dawson J, Pierce D, Dixit A, Kimberley TJ, Robertson M, Tarver B, et al. Safety, Feasibility, and Efficacy of Vagus Nerve Stimulation Paired With Upper-Limb Rehabilitation After Ischemic Stroke. Stroke 2016;47:143–50. doi:10.1161/STROKEAHA.115.010477.

[12] Ganzer PD, Darrow MJ, Meyers EC, Solorzano BR, Ruiz AD, Robertson NM, et al. Closed-loop neuromodulation restores network connectivity and motor control after spinal cord injury. Elife 2018;7:1–19. doi:10.7554/eLife.32058.

[13] Pruitt DT, Danaphongse TT, Schmid AN, Morrison RA, Kilgard MP, Rennaker RL, et al. Traumatic Brain Injury Occludes Training-Dependent Cortical Reorganization in the Contralesional Hemisphere. J Neurotrauma 2017;34:2495–503. doi:10.1089/neu.2016.4796.

[14] Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119:124–32. doi:10.1016/j.brainres.2006.08.048.

[15] Dorr AE. Effect of Vagus Nerve Stimulation on Serotonergic and Noradrenergic Transmission. J Pharmacol Exp Ther 2006;318:890–8. doi:10.1124/jpet.106.104166.

[16] Follesa P, Biggio F, Gorini G, Caria S, Talani G, Dazzi L, et al. Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. Brain Res 2007;1179:28–34. doi:10.1016/j.brainres.2007.08.045.

[17] Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, et al. Neuromodulators Control the Polarity of Spike-Timing-Dependent Synaptic Plasticity. Neuron 2007;55:919–29. doi:10.1016/j.neuron.2007.08.013.

[18] Hulsey DR, Riley JR, Loerwald KW, Rennaker RL, Kilgard MP, Hays SA. Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. Exp Neurol 2017;289:21–30. doi:10.1016/j.expneurol.2016.12.005.

[19] Engineer ND, Riley JR, Seale JD, Vrana WA, Shetake JA, Sudanagunta SP, et al. Reversing pathological neural activity using targeted plasticity. Nature 2011;470:101–4. doi:10.1038/nature09656.

[20] Shetake JA, Engineer ND, Vrana WA, Wolf JT, Kilgard MP. Pairing tone trains with vagus nerve stimulation induces temporal plasticity in auditory cortex. Exp Neurol 2012;233:342–9. doi:10.1016/j.expneurol.2011.10.026.

[21] Engineer CT, Engineer ND, Riley JR, Seale JD, Kilgard MP. Pairing Speech Sounds With Vagus Nerve Stimulation Drives Stimulus-specific Cortical Plasticity. Brain Stimul 2015;8:637–44. doi:10.1016/j.brs.2015.01.408.

[22] Porter BA, Khodaparast N, Fayyaz T, Cheung RJ, Ahmed SS, Vrana WA, et al. Repeatedly Pairing Vagus Nerve Stimulation with a Movement Reorganizes Primary Motor Cortex. Cereb Cortex 2012;22:2365–74. doi:10.1093/cercor/bhr316.

[23] Borland MS, Vrana WA, Moreno NA, Fogarty EA, Buell EP, Sharma P, et al. Cortical Map Plasticity as a Function of Vagus Nerve Stimulation Intensity. Brain Stimul 2016;9:117–23. doi:10.1016/j.brs.2015.08.018.

[24] Castoro MA, Yoo PB, Hincapie JG, Hamann JJ, Ruble SB, Wolf PD, et al. Excitation properties of the right cervical vagus nerve in adult dogs. Exp Neurol 2011;227:62–8. doi:10.1016/j.expneurol.2010.09.011.

[25] Hulsey DR. Neuromodulatory pathways required for targeted plasticity therapy. The University of Texas at Dallas, 2018.

[26] Krahl SE, Clark KB, Smith DC, Browning RA. Locus Coeruleus Lesions Suppress the Seizure-Attenuating Effects of Vagus Nerve Stimulation. Epilepsia 1998;39:709–14. doi:10.1111/j.1528-1157.1998.tb01155.x.

[27] Clark KB, Krahl SE, Smith DC, Jensen RA. Post-training Unilateral Vagal Stimulation Enhances Retention Performance in the Rat. Neurobiol Learn Mem 1995;63:213–6. doi:10.1006/nlme.1995.1024.

[28] Clark KB, Smith DC, Hassert DL, Browning RA, Naritoku DK, Jensen RA. Posttraining Electrical Stimulation of Vagal Afferents with Concomitant Vagal Efferent Inactivation Enhances Memory Storage Processes in the Rat. Neurobiol Learn Mem 1998;70:364–73. doi:10.1006/nlme.1998.3863.

[29] Clark KB, Naritoku DK, Smith DC, Browning RA, Jensen RA. Enhanced recognition memory following vagus nerve stimulation in human subjects. Nat Neurosci 1999;2:94–8. doi:10.1038/4600.

[30] Hays SA, Khodaparast N, Sloan AM, Fayyaz T, Hulsey DR, Ruiz AD, et al. The bradykinesia assessment task: An automated method to measure forelimb speed in rodents. J Neurosci Methods 2013;214:52–61. doi:10.1016/j.jneumeth.2012.12.022.

[31] Kleim JA, Bruneau R, Calder K, Pocock D, VandenBerg PM, MacDonald E, et al. Functional Organization of Adult Motor Cortex Is Dependent upon Continued Protein Synthesis. Neuron 2003;40:167–76. doi:10.1016/S0896-6273(03)00592-0.

[32] Neafsey EJ, Sievert C. A second forelimb motor area exists in rat frontal cortex. Brain Res 1982;232:151–6. doi:10.1016/0006-8993(82)90617-5.

[33] Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, et al. The organization of the rat motor cortex: A microstimulation mapping study. Brain Res Rev 1986;11:77–96. doi:10.1016/0165-0173(86)90011-1.

[34] Pruitt DT, Schmid AN, Danaphongse TT, Flanagan KE, Morrison RA, Kilgard MP, et al. Forelimb training drives transient map reorganization in ipsilateral motor cortex. Behav Brain Res 2016;313:10–6. doi:10.1016/j.bbr.2016.07.005.

[35] Hulsey DR, Hays SA, Khodaparast N, Ruiz A, Das P, Rennaker RL, et al. Reorganization of Motor Cortex by Vagus Nerve Stimulation Requires Cholinergic Innervation. Brain Stimul 2016;9:174–81. doi:10.1016/j.brs.2015.12.007.

[36] Zuo Y, Smith DC, Jensen RA. Vagus nerve stimulation potentiates hippocampal LTP in freely-moving rats. Physiol Behav 2007;90:583–9. doi:10.1016/j.physbeh.2006.11.009.

[37] Borland MS, Engineer CT, Vrana WA, Moreno NA, Engineer ND, Vanneste S, et al. The Interval Between VNS-Tone Pairings Determines the Extent of Cortical Map Plasticity. Neuroscience 2018;369:76–86. doi:10.1016/j.neuroscience.2017.11.004.

[38] Schwartz MW. Central Nervous System Regulation of Food Intake. Obesity 2006;14:1S–8S. doi:10.1038/oby.2006.275.

[39] Maier SF, Goehler LE, Fleshner M, Watkins LR. The role of the vagus nerve in cytokine-to-brain communication. Ann N Y Acad Sci 1998;840:289–300. doi:10.1111/j.1749-6632.1998.tb09569.x.

[40] Williams CL, Jensen RA. Effects of vagotomy on leu-enkephalin-induced changes in memory storage processes. Physiol Behav 1993;54:659–63. doi:10.1016/0031-9384(93)90073-O.

[41] Salgado H, Köhr G, Treviño M. Noradrenergic 'Tone' Determines Dichotomous Control of Cortical Spike-Timing-Dependent Plasticity. Sci Rep 2012;2:417. doi:10.1038/srep00417.

[42] Manta S, El Mansari M, Debonnel G, Blier P. Electrophysiological and neurochemical effects of long-term vagus nerve stimulation on the rat monoaminergic systems. Int J Neuropsychopharmacol 2013;16:459–70. doi:10.1017/S1461145712000387.

[43] Manta S, Dong J, Debonnel G, Blier P. Optimization of vagus nerve stimulation parameters using the firing activity of serotonin neurons in the rat dorsal raphe. Eur Neuropsychopharmacol 2009;19:250–5. doi:10.1016/j.euroneuro.2008.12.001.

[44] Nichols JA, Nichols AR, Smirnakis SM, Engineer ND, Kilgard MP, Atzori M. Vagus nerve stimulation modulates cortical synchrony and excitability through the activation of muscarinic receptors. Neuroscience 2011;189:207–14. doi:10.1016/j.neuroscience.2011.05.024.

[45] Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 2004;27:107–44. doi:10.1146/annurev.neuro.27.070203.144206.

[46] Chase MH, Nakamura Y, Clemente CD, Sterman MB. Afferent vagal stimulation: Neurographic correlates of induced eeg synchronization and desynchronization. Brain Res 1967;5:236–49. doi:10.1016/0006-8993(67)90089-3.

[47] Zagon A, Kemeny AA. Slow Hyperpolarization in Cortical Neurons: A Possible Mechanism Behind Vagus Nerve Simulation Therapy for Refractory Epilepsy? Epilepsia 2000;41:1382–9. doi:10.1111/j.1528-1157.2000.tb00113.x.

[48] Kilgard MP. Harnessing plasticity to understand learning and treat disease. Trends Neurosci 2012;35:715–22. doi:10.1016/j.tins.2012.09.002.

[49] He K, Huertas M, Hong SZ, Tie X, Hell JW, Shouval H, et al. Distinct Eligibility Traces for LTP and LTD in Cortical Synapses. Neuron 2015;88:528–38. doi:10.1016/j.neuron.2015.09.037.

CHAPTER 3

A LIMITED RANGE OF VAGUS NERVE STIMULATION INTENSITIES PRODUCE MOTOR CORTEX REORGANIZATION WHEN DELIVERED DURING TRAINING

Authors: Robert A. Morrison[1,2,*], Tanya T. Danaphongse[2], David T. Pruitt[2], Katherine S. Adcock[1,2], Jobin K. Mathew[2], Stephanie T. Abe[2], Dina M. Abdulla[1,2], Robert L. Rennaker[1,2], Michael P. Kilgard[1,2], Seth A. Hays[2,3]

Affiliations:

[1] The University of Texas at Dallas, School of Behavioral Brain Sciences, Richardson, TX.

[2] The University of Texas at Dallas, Texas Biomedical Device Center, Richardson, TX.

[3] The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, Richardson, TX.

* Correspondence to: robert.morrison@utdallas.edu

Reprinted with permission from Elsevier*: Behavioural Brain Research*, Volume 391, 2020

> *A limited range of vagus nerve stimulation intensities produce motor cortex reorganization when delivered during training,* Robert A. Morrison, Tanya T. Danaphongse, David T. Pruitt, Katherine S. Adcock, Jobin K. Mathew, Stephanie T. Abe, Dina M. Abdulla, Robert L. Rennaker, Michael P. Kilgard, Seth A. Hays*, Behavioural Brain Research*, ISSN 0166-4328, https://doi.org/10.1016/j.bbr.2020.112705.

Abstract

Pairing vagus nerve stimulation (VNS) with rehabilitation has emerged as a potential strategy to improve recovery after neurological injury, an effect ascribed to VNS-dependent enhancement of synaptic plasticity. Previous studies demonstrate that pairing VNS with forelimb training increases forelimb movement representations in motor cortex. However, it is not known whether VNS-dependent enhancement of plasticity is restricted to forelimb training or whether VNS paired with other movements could induce plasticity of other motor representations. We tested the hypothesis that VNS paired with orofacial movements associated with chewing during an unskilled task would drive a specific increase in jaw representation in motor cortex compared to equivalent behavioral experience without VNS. Rats performed a behavioral task in which VNS at a specified intensity between 0 and 1.2 mA was paired with chewing 200 times per day for five days. Intracortical microstimulation (ICMS) was then used to document movement representations in motor cortex. VNS paired with chewing at 0.8 mA significantly increased motor cortex jaw representation compared to equivalent behavioral training without stimulation (Bonferroni-corrected unpaired t-test, $p < 0.01$). Higher and lower intensities failed to alter cortical plasticity. No changes in other movement representations or total motor cortex area were observed between groups. These results demonstrate that 0.8 mA VNS paired with training drives robust plasticity specific to the paired movement, is not restricted to forelimb representations, and occurs with training on an unskilled task. This suggests that moderate intensity VNS may be a useful adjuvant to enhance plasticity and support benefits of rehabilitative therapies targeting functions beyond upper limb movement.

Introduction

Vagus nerve stimulation (VNS) paired with rehabilitative training has emerged as a strategy to enhance recovery in the context of a wide range of neurological disorders including stroke, traumatic brain injury, and spinal cord injury [1–7]. Brief bursts of VNS rapidly activate the noradrenergic locus coeruleus (LC) and cholinergic nucleus basalis, two major neuromodulatory centers in the brain [8–13]. Coincident release of these pro-plasticity neuromodulators coupled with neural activity related to rehabilitation facilitates synaptic plasticity in task-specific activated circuits [8,12–15]. This targeted enhancement of plasticity in central networks after injury is believed to underlie VNS-dependent improvements in functional recovery [6,7].

A number of studies demonstrate that repeatedly pairing VNS with forelimb training drives specific reorganization of forelimb movement representations in motor cortex [9,10,16,17]. VNS-mediated plasticity exhibits an inverted-U relationship with stimulation intensity, such that low and high intensities fail to drive plasticity and moderate intensities significantly enhance plasticity [17,18]. This function has been coarsely defined; however, considering the importance of VNS-dependent enhancement of plasticity in its therapeutic effects, a finer delineation of the interaction between stimulation intensity and the magnitude of VNS-dependent plasticity is merited. In the present study, we sought to carefully define the relationship of plasticity at small increments of VNS intensity. Additionally, while the ability of VNS to enhance plasticity in forelimb circuits has been well-documented, VNS-mediated expansion of non-forelimb motor cortex representations has not been evaluated. Movement of the jaw muscles during chewing is a simple, innate motor behavior that represents a testbed for evaluating VNS-dependent plasticity. Here, we

sought to leverage this simple behavior and test the hypothesis that VNS paired with orofacial movements associated with chewing would drive a specific increase in jaw representation in motor cortex compared to equivalent behavioral experience without VNS. Rats were trained to perform a simple behavioral task in which either short bursts of VNS at 0.4, 0.6, 0.8, 1.0, 1.2 mA, or sham stimulation were delivered coincident with chewing over the course of five days. After the final day of training, movement representations in motor cortex were assessed with intracortical microstimulation (ICMS). The results from this study provide a framework to develop other VNS-based therapy regimens to target improved recovery beyond upper limb motor function and independently confirm the existence of an inverted-U relationship between VNS intensity and plasticity.

Methods

Experimental procedures, statistical comparisons, and exclusion criteria were preregistered before beginning data collection (https://osf.io/594za/).

Subjects

Fifty-three female Sprague Dawley rats weighing approximately 250 grams and aged approximately 6 months were used in this study (Charles River Labs). All rats were housed in a reversed 12:12 hour light-dark cycle. Rats that underwent behavioral training were food restricted on weekdays during shaping and training with ad libitum access to food on weekends. All rats were maintained at or above 85% body weight relative to their initial weight upon beginning

behavioral testing. All handling, housing, stimulation, and surgical procedures were approved by The University of Texas at Dallas Institutional Animal Care and Use Committee.

Behavioral Training

Rats were trained on a simple automated behavioral task that allowed triggering of VNS during chewing and associated orofacial movements involved in eating behavior. The behavioral training apparatus consisted of an acrylic cage with a nosepoke food dispenser at one end (Fig. 3.1A). A food pellet (45 mg dustless precision pellet, BioServ, Frenchtown, NJ) was delivered to the food dispenser. An infrared beam sensor positioned in the food dispenser was used to determine when the rat entered the nosepoke to retrieve the food pellet. Upon breaking the infrared beam, another pellet was dispensed after an 8 second delay. Each behavioral session continued until either 100 pellets had been dispensed, or until 1 hour had elapsed. Rats received a supplement of 10 grams of food pellets if they did not receive at least 100 pellets in a day to maintain weight.

Rats performed training sessions twice per day, 5 days per week, with daily sessions separated by at least 2 hours until they could reliably consume 100 pellets within 1 hour each session. Rats were then implanted with a VNS cuff and recovered for 7 days in their home cage. Seven days after surgery, rats were allocated to groups to receive 5 days of training paired with either sham stimulation or VNS at varying intensities. Rats in VNS groups received a 500 ms burst of stimulation triggered 3 seconds after nosepoke beam break, which resulted in reliable delivery of VNS during chewing and orofacial movement (Fig. 3.1B). While this timing scheme was developed to ensure VNS delivery during chewing, eating behavior requires complex coordination of sensorimotor function and many other associated orofacial movements, such as

swallowing, and licking, are co-occurring. Here, we will use the term "chewing" to describe the primary action taking place after food pellet retrieval and use the term to encompass all other sensorimotor actions taking place alongside it. At the conclusion of behavioral training, all rats underwent ICMS mapping. Untrained rats were not included as a control group in the current study, as previous reports demonstrate no significant differences in motor cortex representation between naïve and sham animals as a result of behavioral training [9,16].

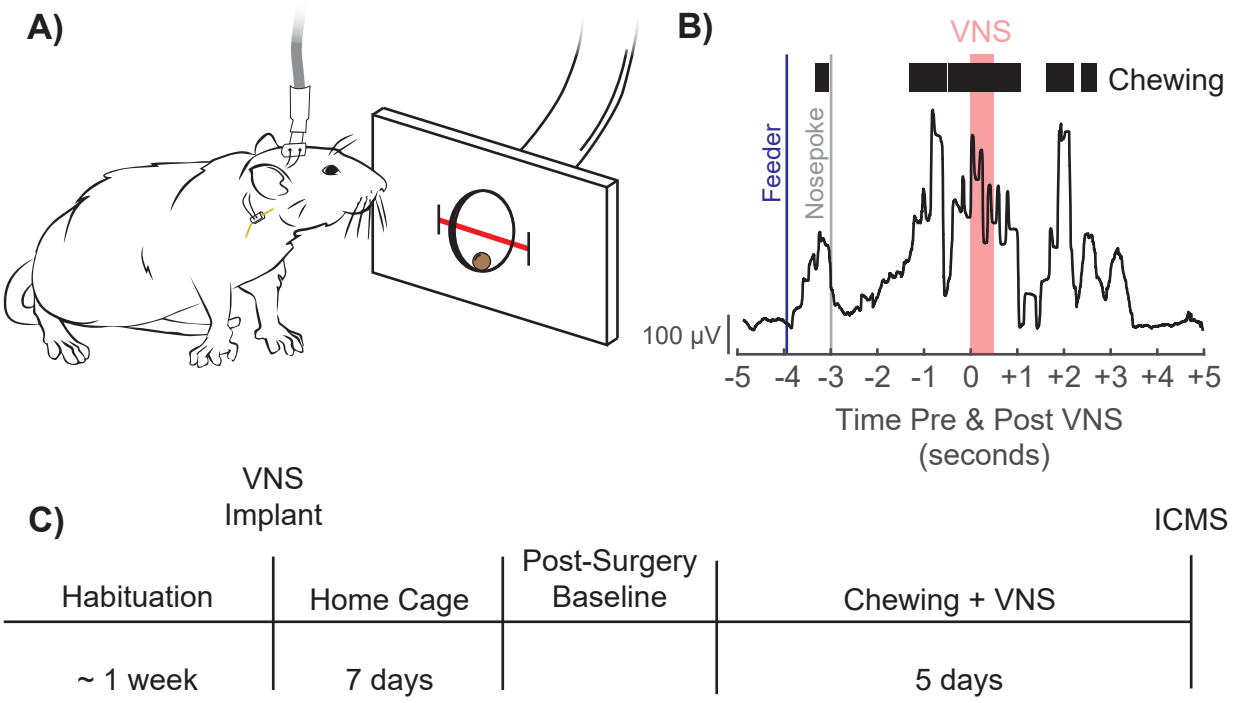


Figure 3.1: Behavioral task and experimental design

(A) Illustration of a rat performing the task. A stimulating cable stimulating cable plugged into a headmount-connector, the subcutaneous stimulation leads and nerve cuff, and the vagus nerve are shown. A feeder dispenses food pellets into a nosepoke and an infrared beam monitors movement into and out of the nosepoke. B) Representative image depicting task performance, superior

masseter EMG activity, and the relative timing of pellet dispensal and VNS. C) Experimental timeline.

Surgical Implantation

All surgeries were performed using aseptic technique under general anesthesia. Rats were implanted with a stimulating cuff on the left cervical vagus nerve as described in previous studies [2–4,17,19]. Rats were deeply anesthetized with ketamine hydrochloride (50 mg/kg, i.p.), xylazine (20 mg/kg, i.p.), and acepromazine (5 mg/kg, i.p.), and were placed in a stereotactic apparatus. Anesthesia level was maintained stably throughout surgery based on assessment of breathing rate, vibrissae whisking, toe pinch reflex, and pulse oximetry. An incision was made down the midline of the head to expose the skull. Bone screws were inserted into the skull at points surrounding the lamboid suture and over the cerebellum. A two-channel connector was mounted to the screws using acrylic. The rat was then removed from the stereotactic apparatus and placed in a supine position.

An incision was made on the left side of the neck and the overlying musculature was blunt dissected to reveal the left cervical vagus nerve. The nerve was gently dissected away from the carotid artery. A cuff electrode was implanted surrounding the vagus nerve, and the leads were tunneled subcutaneously to connect with the two-channel connector mounted on the skull. Nerve activation was confirmed by observation of a $\geq$ 5% drop in blood oxygen saturation in response to a 10 s stimulation train of VNS consisting of 0.8 mA, 100 μs biphasic pulses at 30 Hz, as in previous studies [17]. The head and neck incisions were then sutured, and rats received subcutaneous injections of 4 mL 50:50 0.9% saline 5% dextrose solution. A seven-day recovery period followed surgery.

Vagus Nerve Stimulation

After surgery, rats were randomly sorted into groups to receive either VNS at 0.4 mA (n = 3), 0.6 mA (n = 6), 0.8 mA (n = 8), 1.0 mA (n = 6), 1.2 mA (n = 4), or sham stimulation (n = 5) paired with chewing during behavioral training. During behavioral training, all rats, regardless of experimental group, were connected via headmount connector to a stimulation cable. Animals receiving sham stimulation were connected to the cable, but did not receive stimulation. In the initial sessions after implantation, rats were allowed to acclimate to being attached to stimulating cables until they performed at least 100 successful trials per day. Once acclimated, rats then underwent five days of training and received VNS or sham stimulation according to their group. Each 0.5 s stimulation train consisted of a 100 μsec biphasic pulses delivered at 30 Hz at an intensity of either 0.4, 0.6, 0.8, 1.0, or 1.2 mA, as appropriate for each experimental group. VNS was triggered 3 seconds after nosepoke beam break once a pellet had been dispensed during behavioral training, allowing stimulation to be delivered coincident with chewing of the pellet (Fig. 3.1B). A digital oscilloscope (PicoScope 2204A, PP906, Pico Technology) was used to monitor voltage across the vagus nerve during each stimulation to ensure cuff functionality.

Electromyography

Electromyography (EMG) was used to evaluate the timing of VNS administration relative to rhythmic jaw movement indicating chewing during behavioral training in a subset of rats. During EMG electrode implantation, rats were anesthetized, mounted in a stereotactic apparatus, and a four-channel headmount-connector was affixed to the skull, as described above for vagus

nerve cuff implantation. Two bone screws welded to platinum-iridium leads were inserted in the skull and served as ground and reference connections. A ball electrode on the end of a third platinum iridium lead was tunneled subcutaneously and placed over the left superior masseter. The exposed headmount was encapsulated in acrylic and the skin incision was sutured closed.

During behavioral training, left superior masseter EMG activity was monitored using an amplifier (Model 1700 Differential AC Amplifier, AM-Systems) and a digital oscilloscope (PicoScope 2204A, PP906, Pico Technology) recording at a sampling rate of 10,000 Hz. EMG signals were bandpass filtered offline (Butterworth filter, 100 to 600 Hz) and an envelope detector applied (RMS, 250 ms window). Superior masseter on/off detection was set to a threshold of 300 μV. EMG recording of superior masseter activity was used to assess the accuracy of VNS timing relative to rhythmic jaw movement during chewing after pellet retrieval. A successful VNS pairing was considered to be a stimulation that occurred within two seconds after the onset of superior masseter movement exceeding detection threshold, a window that has been shown to be effective for VNS-mediated plasticity after pairing with a motor activity [7].

Intracortical Microstimulation Mapping

Within 24 hours of their last behavioral session, rats underwent ICMS to derive functional representation maps according to standard procedures [16,17,20–23]. Rats were anesthetized by intraperitoneal injections of ketamine hydrochloride (75 mg/kg) and xylazine (5 mg/kg) and received supplemental doses of ketamine (25 mg/kg) and xylazine (1.5 mg/kg) as necessary throughout the procedure in order to maintain a consistent level of anesthesia as indicated by

breathing rate, vibrissae whisking, and toe pinch reflex. Rats were placed in a stereotactic apparatus and a craniotomy and durotomy were performed to expose the left motor cortex (4 mm to -3 mm AP and 0.25 mm to 5 mm ML). To prevent cortical swelling, a small incision was made in the cisterna magna.

A tungsten electrode with an impedance of approximately 0.7 MΩ (UEWMEGSEBN3M, FHC, Bowdoin, ME) was lowered into the brain to a depth of 1.8 mm, targeting motor outputs in layer V. Stimulation sites were then chosen at random on a grid with sites set 500 μm apart from each other. The next stimulation site was placed at least 1 mm away from the previous site whenever possible. Stimulation consisted of a 40 ms pulse train of 10 monophasic 200 μs cathodal pulses. Stimulation was increased from 10 μA until a movement was observed or until a maximum of 250 μA was reached. ICMS was conducted blinded with two experimenters, as previously described [9,16,17]. The first experimenter placed the electrode and recorded the data for each site. The second experimenter, blinded to group and electrode location, delivered stimulations and classified movements. Movements were classified into the following categories: jaw, neck, vibrissa, proximal forelimb, distal forelimb, and hindlimb. To confirm nerve cuff functionality, nerve activation was confirmed prior to ICMS by observation of a ≥ 5% drop in blood oxygen saturation in response to a 10 s stimulation train of VNS consisting of 0.8 mA, 100 μs biphasic pulses at 30 Hz, following standard procedures [17,24]. This procedure was performed prior to ICMS to avoid risk of damaging the nerve cuff leads during the ICMS surgery. Nerve cuff functionality was assessed in all subjects, regardless of group, and typically preceded ICMS by at least 90 minutes. All raw data from ICMS is available in the appendix (Appendix B).

Subject Exclusion

All exclusion criteria were preregistered before data collection began. Thirty-two subjects were analyzed in the final results of the study out of a total of 53 subjects. Of the 21 subjects not used in final analysis, seven subjects were excluded due to non-functional stimulating cuffs, four subjects were excluded to a lack of drop in blood oxygen saturation in response to VNS, seven subjects were excluded due to mortality during surgical procedures, and three subjects were excluded due to mechanical failure of the headmount during behavioral training. Non-functional stimulating cuffs were identified by digital oscilloscope readings exceeding 40 V peak-to-peak, as in previous studies [17].

Statistics

Statistical methods and planned comparisons were preregistered and defined *a priori*. The primary outcome of this study was area of motor cortex generating jaw movements. All other movement representations were analyzed as secondary outcome measures. A one-way ANOVA was used to identify differences across groups. *Post hoc* Bonferroni-corrected unpaired two-tailed t-tests were used compare between individual groups, as appropriate. Statistical tests for each comparison are noted in the text (Table 3.1). All data are reported as mean ± SEM.

Results

We sought to investigate whether pairing VNS with movement of the jaw and orofacial muscles would enhance jaw representation in motor cortex in an intensity-dependent manner. To do so, rats performed a simple behavioral task in which VNS at varying intensities or sham stimulation was timed to occur during chewing approximately 200 times per day for five days. Assessment of EMG activity in the superior masseter confirmed that VNS delivery occurred within 2 seconds of rhythmic jaw activity on 100% of trials; a time window that has been shown to be effective for VNS-mediated effects [7]. The day following the final day of behavioral training, intracortical microstimulation (ICMS) was used to document movement representations in the motor cortex.

Moderate intensity VNS paired with chewing increases jaw representation in motor cortex

Group analysis of motor cortex area eliciting jaw movement revealed significant differences between groups (One-way ANOVA, $F[5,26] = 3.1332$ $p = 0.024$). Moderate intensity 0.8 mA VNS paired with chewing significantly increased jaw representation compared to equivalent training on the task without VNS (Fig. 3.2A, Table 3.1, Unpaired t-test, $p = 0.003$). This is consistent with previous studies reporting that 0.8 mA VNS enhances training-specific plasticity in cortical networks [9,10,16,17]. No significant differences in jaw representation were observed after unpaired t-tests between sham and 0.4 mA ($p = 0.75$), 0.6 mA ($p = 0.78$), 1.0 mA ($p = 0.40$), or 1.2 mA ($p = 0.80$) VNS groups (Fig. 3.2A, Table 3.1). These results support two conclusions. First, VNS paired with unskilled behavioral tasks can drive robust plasticity in motor

cortex. Second, there is a relatively narrow range of stimulation VNS intensities that support cortical plasticity.

As expected, no differences in the cortical representation of untrained movements were observed between groups, suggesting that VNS-dependent plasticity is specific to the paired movement (Appendix B). Additionally, consistent with previous studies, total motor cortex area eliciting movements was also not affected by VNS (Fig. 3.2B; One-way ANOVA, $F[5,26] = 0.330$, $p = 0.890$). As expected, group analysis revealed no differences across groups in average stimulation threshold required to elicit movement (One-way ANOVA, $F[5,26] = 0.503$, $p = 0.801$). Together, these findings confirm that VNS-mediated plasticity is specific to the paired movement [6,7,9,16,17,25].

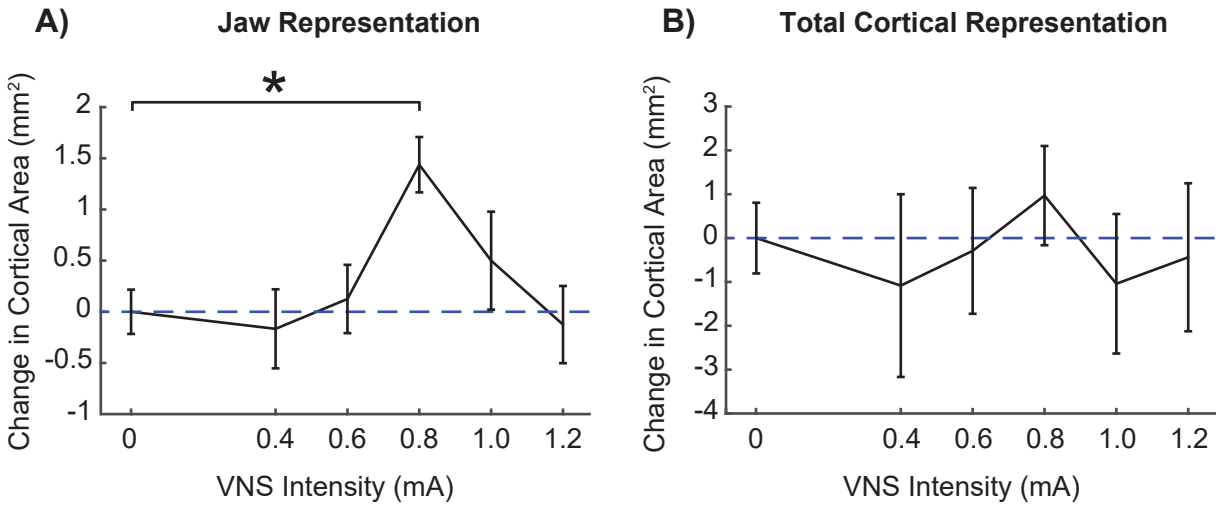


Figure 3.2: VNS paired with chewing and associated orofacial movements enhances jaw-specific plasticity in motor cortex

(A) 0.8 mA VNS paired with chewing and associated orofacial movements significantly increases jaw movement representation area in motor cortex compared to equivalent behavioral experience without VNS and VNS at 0.4, 0.6, 1.0, and 1.2 mA. (B) No change in total motor cortex area was observed between groups. Bars represent mean ± SEM. "*" indicates $p < 0.01$.

VNS does not alter behavioral performance

Group analysis of the timing between behavioral trials revealed no differences between groups (One-way ANOVA, $F[5,26] = 1.437$, $p = 0.246$). Furthermore, group analysis of the number of total stimulations given was not significantly different between groups (One-way ANOVA, $F[5,26] = 1.248$, $p = 0.316$). These results suggest that VNS does not alter behavioral performance or motivation and does not influence eating behavior.

Table 3.1: Area of movement representations from ICMS

Group analysis reveals a significant effect of group in motor cortex jaw representation only. A one-way ANOVA was used to identify differences across groups. Post hoc Bonferroni-corrected unpaired two-tailed t-tests with an alpha of 0.01 were used compare between individual groups, as appropriate. All data is displayed as mean ± SEM in mm^2. Bolded results indicate significance.

Group	Cortical area of movement representation (mm^2)					
	Jaw	Proximal Forelimb	Distal Forelimb	Vibrissae	Neck	Hindlimb
Sham	1.25 ± 0.22	1.95 ± 0.86	4.00 ± 0.88	1.65 ± 0.68	0.15 ± 0.10	1.50 ± 0.31
0.4 mA	1.08 ± 0.55	1.25 ± 0.52	4.33 ± 0.79	1.17 ± 0.73	0.08 ± 0.08	1.50 ± 0.38
0.6 mA	1.38 ± 0.33	0.17 ± 0.12	5.46 ± 0.37	1.46 ± 0.48	0.21 ± 0.16	1.54 ± 0.22
0.8 mA	**2.69 ± 0.27**	1.13 ± 0.34	4.44 ± 0.46	2.16 ± 0.74	0.25 ± 0.09	0.81 ± 0.35
1.0 mA	1.75 ± 0.48	0.54 ± 0.19	4.46 ± 0.63	1.21 ± 0.23	0.08 ± 0.05	1.42 ± 0.42
1.2 mA	1.13 ± 0.46	1.69 ± 0.58	4.94 ± 1.00	1.63 ± 0.97	0.06 ± 0.06	0.63 ± 0.30
ANOVA Results	F = 3.1332 **p = 0.024**	F = 2.204 p = 0.084	F = 0.637 p = 0.674	F = 0.340 p = 0.884	F = 0.527 p = 0.753	F = 1.279 p = 0.303

Discussion

Previous studies demonstrate that repeatedly pairing VNS with a forelimb movement drives targeted motor cortex plasticity [9,10,16,17]. In the present study, we sought to investigate whether closed-loop VNS could be applied during a distinct training paradigm to enhance plasticity of other movement representations. Here, we provide the first report that VNS is able drive robust plasticity when paired with training on an unskilled task emphasizing non-forelimb musculature. This VNS-dependent increase in jaw area occurs in the absence of changes in other representations, indicating that VNS enhances plasticity specific to the trained movement. The

findings from this study raise the possibility that VNS may be a useful adjuvant to enhance rehabilitative therapies targeting functions beyond upper limb movement.

Closed-loop VNS has emerged as a strategy to augment the effects of rehabilitation, an effect ascribed to VNS-dependent enhancement of synaptic plasticity [1–7]. Here, we show that pairing VNS with activation of the jaw muscles during chewing enhances plasticity specific to the paired movement. The observation of increased jaw representation in the present study demonstrates that the plasticity-enhancing effects of VNS paired with motor training are not restricted to forelimb movements, but can extend to other motor circuits depending on the training regimen. While forelimb dysfunction is common after stroke, roughly one third of those who undergo a stroke develop a speech aphasia and half will experience dysphagia [26–28]. Previous studies show increases in articulation and speech production in stroke patients with chronic Broca's aphasia after melodic intonation therapy, as well as increases in perilesional white matter tract plasticity [29]. Cortical plasticity has also been implicated in recovery from dysphagia after stroke [30]. While the present study clearly does not approach the complexity of motor control necessary for speech production or modelling dysphagia, the jaw-related plasticity after VNS pairing reported here raises the possibility that pairing VNS with speech therapy may represent a means to enhance plasticity in networks associated with speech production and swallowing and thereby facilitate rehabilitation.

An inverted-U relationship between VNS intensity and plasticity has been observed previously, but has not been described with the resolution reported here [17,18]. While it has been repeatedly demonstrated that VNS at 0.8 mA is able to increase cortical plasticity and enhance recovery after neurological injury [1–7,9,17], that VNS at a slightly lower 0.6 mA or slightly

higher 1.0 mA intensity fails to significantly alter motor cortex plasticity highlights a relatively narrow range of effective parameters. Stimulation intensities above 0.1 mA reliably activate afferent fibers and evoke robust spiking activity in the noradrenergic LC, a neuromodulatory center required for VNS-dependent enhancement of plasticity [8,31]. The absence of plasticity in this study at stimulation intensities well above the threshold to drive LC activity indicates that a particular level of network activation must be reached. However, the failure of higher intensity stimulation to enhance plasticity suggests that over-activation of these networks also prevents plasticity. Moreover, the absence of plasticity at higher stimulation intensities demonstrates that any direct motor or somatosensory activation in response to VNS, such as via activation of the recurrent laryngeal nerve, cannot account for plasticity observed with moderate intensity stimulation and instead is consistent with notion that VNS engages neuromodulatory networks to enhance plasticity. In practical terms, the apparently narrow range of effective VNS intensities observed here calls attention to the need for parameter optimization studies for active stimulation-based therapies.

While the inverted-U relationship between stimulation intensity and VNS-dependent enhancement of plasticity has been repeatedly observed in a number of conditions, the neuronal mechanisms that underlie this relationship are unclear. It is possible that high intensity VNS fails to enhance cortical plasticity due to overactivation and desensitization of relevant neuromodulatory systems [9,10,31]. G-protein coupled receptors for norepinephrine, acetylcholine, and serotonin, all of which are engaged by VNS and required for VNS-dependent plasticity, are known to exhibit profound desensitization [32]. Alternatively, activation of opposing classes of receptors could account for the inverted-U. Increases in VNS intensity drive

monotonic increases in LC activation which increases levels of norepinephrine in motor cortex [8]. VNS at low intensities could thus produce activation of high affinity α-adrenergic receptors to support synaptic plasticity. As VNS intensity increases LC activation and subsequently norepinephrine levels, low affinity β-adrenergic receptors would be recruited and oppose the action of the α-adrenergic receptors, thus promoting stability in cortical networks. Under this configuration, moderate intensities of VNS would produce maximal activation of pro-plasticity α-receptors and minimize activation of pro-stability β-receptors. Indeed, the opposing effects of differential activation of noradrenergic receptors on plasticity has previously been described and could account for the findings reported here [17,33,34]. Future studies to delineate the role of these neuromodulatory systems on VNS-dependent plasticity may open avenues for concomitant pharmacological manipulation of VNS effects.

Engagement of different afferent fiber populations represents another potential mechanism underlying the inverted-U relationship of VNS intensity and plasticity. However, several pieces of evidence suggest that engagement of neuromodulatory networks, rather than activation of different fiber populations, determines VNS-dependent plasticity. While the intensities utilized in the present study span much of the range of A- and B-fiber thresholds, the roughly linear relationship of spike activity in the LC and VNS intensity would not be predicted by the approximate step function activation of two fiber types [8,35]. Moreover, a number of previous studies demonstrate that parameters that influence the timing of stimulation, such as frequency and duration, which would not recruit different fiber types, strongly influence the magnitude of VNS-dependent plasticity [36,37]. These features are consistent with a mechanism whereby VNS activates neuromodulatory networks that integrate the intensity and timing of stimulation to

determine plasticity. Furthermore, activation of A- and B-fibers during VNS and their subsequent effects on respiration and cardiac function do not appear to predict effective parameters [38]. Still, a reliable biomarker may exist that could be used to predict stimulation parameters that drive plasticity most, and would prove useful to further clinical translation of VNS paired rehabilitation.

While VNS has been demonstrated to support plasticity with both jaw and forelimb-specific training regimens, the anatomy underlying jaw and forelimb movement is divergent. VNS enhances plasticity and recovery for a variety of forelimb tasks utilizing either the proximal or distal forelimb musculature, but the motor output pathways underlying these movements are predominately corticospinal in nature [1,2,4–7,9,16,17,25]. Rhythmic jaw movement can be evoked by a diverse group of brain areas that contribute to masticatory behavior. While descending projections have been traced from jaw motor cortex to the trigeminal motor neurons and pontobulbar reticular formation, several corticobulbar and medullar nuclei are implicated in both central rhythm generation of jaw movements as well as sensory integration and movement correction [39–41]. That these unique pathways appear to have a common mechanism of VNS-mediated plasticity suggests that VNS is highly adaptable at potentiating activated circuits regardless of underlying anatomy. Indeed, VNS-mediated plasticity and enhancement of recovery has been observed in distinct subcortical areas dependent on spared pathways after different levels and totalities of spinal cord injury [7]. VNS-mediated plasticity has also been observed in subcortical auditory nuclei as well as primary auditory cortex after VNS-tone pairing [42]. Together, these findings support a mechanism by which VNS-dependent engagement of neuromodulatory networks acts as a general means to facilitate synaptic plasticity, and the neural activity associated with training that coincides with delivery of VNS, such as rhythmic jaw

movement associated with chewing in the present study, dictates the networks that undergo reorganization. Thus, in addition to the reorganization of movement representations in motor cortex, VNS-dependent enhancement of plasticity is likely co-occurring in other networks involved in orofacial sensorimotor function, including the supplemental motor area, cortical masticatory area (CMA), insula, and somatosensory areas as well. Indeed, neuroplasticity in areas such as these after training on both skilled and semi-automatic orofacial tasks has been observed [43,44].

In previous preclinical studies evaluating VNS-dependent enhancement of plasticity, it was necessary to train rats for several weeks to acquire proficiency on a behavioral task before initiating VNS pairing. In this study, however, we describe a VNS-pairing paradigm in which little training is necessary. This substantial reduction in the total amount of time promotes efficient testing, which is critical to studies aimed at parametrically characterizing stimulation parameters that typically require a large number of experimental groups. The robust increase in jaw representation driven by VNS, coupled with the rapid testing afforded by the simple behavioral paradigm, lay a framework for efficient optimization of stimulation parameters, which is necessary to facilitate clinical translation of VNS therapies.

There are a number of limitations in the current study that merit consideration. First, evaluation was restricted to female rats, because VNS and parameters have been extensively validated in this sex [9,16,17,23,25,31]. The use of female rats precludes direct conclusions about sex-specific differences in nerve activation and could potentially be confounded by timing of stimulation during the estrus cycle. While there is no evidence of a sex-specific effect of VNS on the central nervous system in humans [45–47], future studies should incorporate male rats to

evaluate any differences in stimulation parameters. Secondly, the present study focused on quantification of gross jaw movement and did not further classify any sub-jaw or other orofacial movement representations. More precise methods, such as EMG, could be used to identify sub-circuit changes in response to VNS pairing. Identification of the specific facial representations that reorganize after VNS paired training on an orofacial task would undoubtedly be useful for assessing the feasibility of using VNS for treating complex disabilities after stroke, such as speech aphasia or dysphagia.

Acknowledgements

We would like to thank Daniel Hulsey for help with planning and experimental design. We also thank Harshini Rallapalli, Daniel Razick, Madison Stevens, Sarah Jacob, Jennifer Derma, Ariba Hanif, Anjana Swami, Delphi Uthirakulathu, Vikram Ezhil, Kamal Safadi, Alissar Zammam, Abarnaa Vs, Aaron Kuo, Ayushi Bisaria, Yun-Yee Tsang, and Prathima Kandukuri for help with behavioral training and animal care, and Lena Lynn Sadler and Matt Buell for vagus nerve cuff construction. We would also like to thank Katelyn Millay, Eric Meyers, Holle Carey, Andrew Sloan, Jonathan Riley, and Michael Darrow for help developing behavioral programs and testing equipment. We also thank Nikki Simmons for help with artwork.

Sources of Funding

This work was supported by the National Institutes of Health R01NS085167 and R01NS094384 and by the Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO) Electrical Prescriptions (ElectRx) program under the auspices of Dr. Eric Van Gieson through the Space and Naval Warfare Systems Center, Pacific Cooperative Agreement No. N66001-15-2-4057 and the DARPA BTO Targeted Neuroplasticity Training (TNT) program under the auspices of Dr. Tristan McClure-Begley through the Space and Naval Warfare Systems Center, Pacific Grant/Contract No. N66001-17-2-4011.

Conflict of Interest

MPK has a financial interesting in MicroTransponder, Inc., which is developing VNS for stroke. All other authors declare no conflicts of interest.

References

[1] Pruitt DT, Schmid AN, Kim LJ, Abe CM, Trieu JL, Choua C, et al. Vagus Nerve Stimulation Delivered with Motor Training Enhances Recovery of Function after Traumatic Brain Injury. J Neurotrauma 2016;33:871–9. doi:10.1089/neu.2015.3972.

[2] Khodaparast N, Hays SA, Sloan AM, Hulsey DR, Ruiz A, Pantoja M, et al. Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. Neurobiol Dis 2013;60:80–8. doi:10.1016/j.nbd.2013.08.002.

[3] Khodaparast N, Hays SA, Sloan AM, Fayyaz T, Hulsey DR, Rennaker RL, et al. Vagus Nerve Stimulation Delivered During Motor Rehabilitation Improves Recovery in a Rat Model of Stroke. Neurorehabil Neural Repair 2014;28:698–706. doi:10.1177/1545968314521006.

[4] Hays SA, Khodaparast N, Ruiz A, Sloan AM, Hulsey DR, Rennaker RL, et al. The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. Neuroreport 2014;25:682–8. doi:10.1097/WNR.0000000000000154.

[5] Hays SA, Ruiz A, Bethea T, Khodaparast N, Carmel JB, Rennaker RL, et al. Vagus nerve stimulation during rehabilitative training enhances recovery of forelimb function after ischemic stroke in aged rats. Neurobiol Aging 2016;43:111–8. doi:10.1016/j.neurobiolaging.2016.03.030.

[6] Meyers EC, Solorzano BR, James J, Ganzer PD, Lai ES, Rennaker RL, et al. Vagus Nerve Stimulation Enhances Stable Plasticity and Generalization of Stroke Recovery. Stroke 2018;49:710–7. doi:10.1161/STROKEAHA.117.019202.

[7] Ganzer PD, Darrow MJ, Meyers EC, Solorzano BR, Ruiz AD, Robertson NM, et al. Closed-loop neuromodulation restores network connectivity and motor control after spinal cord injury. Elife 2018;7:1–19. doi:10.7554/eLife.32058.

[8] Hulsey DR, Riley JR, Loerwald KW, Rennaker RL, Kilgard MP, Hays SA. Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. Exp Neurol 2017;289:21–30. doi:10.1016/j.expneurol.2016.12.005.

[9] Hulsey DR, Hays SA, Khodaparast N, Ruiz A, Das P, Rennaker RL, et al. Reorganization of Motor Cortex by Vagus Nerve Stimulation Requires Cholinergic Innervation. Brain Stimul 2016;9:174–81. doi:10.1016/j.brs.2015.12.007.

[10] Hulsey DR. Neuromodulatory pathways required for targeted plasticity therapy. 2018.

[11] Nichols JA, Nichols AR, Smirnakis SM, Engineer ND, Kilgard MP, Atzori M. Vagus nerve stimulation modulates cortical synchrony and excitability through the activation of muscarinic receptors. Neuroscience 2011;189:207–14. doi:10.1016/j.neuroscience.2011.05.024.

[12] Dorr AE. Effect of Vagus Nerve Stimulation on Serotonergic and Noradrenergic Transmission. J Pharmacol Exp Ther 2006;318:890–8. doi:10.1124/jpet.106.104166.

[13] Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119:124–32. doi:10.1016/j.brainres.2006.08.048.

[14] Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, et al. Neuromodulators Control the Polarity of Spike-Timing-Dependent Synaptic Plasticity. Neuron 2007;55:919–29. doi:10.1016/j.neuron.2007.08.013.

[15] He K, Huertas M, Hong SZ, Tie XX, Hell JW, Shouval H, et al. Distinct Eligibility Traces for LTP and LTD in Cortical Synapses. Neuron 2015. doi:10.1016/j.neuron.2015.09.037.

[16] Porter BA, Khodaparast N, Fayyaz T, Cheung RJ, Ahmed SS, Vrana WA, et al. Repeatedly Pairing Vagus Nerve Stimulation with a Movement Reorganizes Primary Motor Cortex. Cereb Cortex 2012;22:2365–74. doi:10.1093/cercor/bhr316.

[17] Morrison RA, Hulsey DR, Adcock KS, Rennaker RL, Kilgard MP, Hays SA. Vagus nerve stimulation intensity influences motor cortex plasticity. Brain Stimul 2018. doi:10.1016/j.brs.2018.10.017.

[18] Borland MS, Vrana WA, Moreno NA, Fogarty EA, Buell EP, Sharma P, et al. Cortical Map Plasticity as a Function of Vagus Nerve Stimulation Intensity. Brain Stimul 2016;9:117–23. doi:10.1016/j.brs.2015.08.018.

[19] Hays SA, Khodaparast N, Hulsey DR, Ruiz A, Sloan AM, Rennaker RL, et al. Vagus Nerve Stimulation During Rehabilitative Training Improves Functional Recovery After Intracerebral Hemorrhage. Stroke 2014;45:3097–100. doi:10.1161/STROKEAHA.114.006654.

[20] Kleim JA, Bruneau R, Calder K, Pocock D, VandenBerg PM, MacDonald E, et al. Functional Organization of Adult Motor Cortex Is Dependent upon Continued Protein Synthesis. Neuron 2003;40:167–76. doi:10.1016/S0896-6273(03)00592-0.

[21] Neafsey EJ, Sievert C. A second forelimb motor area exists in rat frontal cortex. Brain Res 1982;232:151–6. doi:10.1016/0006-8993(82)90617-5.

[22] Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, et al. The organization of the rat motor cortex: a microstimulation mapping study. Brain Res 1986;396:77–96.

[23] Pruitt DT, Schmid AN, Danaphongse TT, Flanagan KE, Morrison RA, Kilgard MP, et al. Forelimb training drives transient map reorganization in ipsilateral motor cortex. Behav Brain Res 2016;313:10–6. doi:10.1016/j.bbr.2016.07.005.

[24] Rios M, Bucksot J, Rahebi K, Engineer C, Kilgard M, Hays S. Protocol for Construction of Rat Nerve Stimulation Cuff Electrodes. Methods Protoc 2019. doi:10.3390/mps2010019.

[25] Pruitt DT, Danaphongse TT, Schmid AN, Morrison RA, Kilgard MP, Rennaker RL, et al. Traumatic Brain Injury Occludes Training-Dependent Cortical Reorganization in the Contralesional Hemisphere. J Neurotrauma 2017;34:2495–503. doi:10.1089/neu.2016.4796.

[26] Engelter ST, Gostynski M, Papa S, Frei M, Born C, Ajdacic-Gross V, et al. Epidemiology of Aphasia Attributable to First Ischemic Stroke. Stroke 2006;37:1379–84. doi:10.1161/01.STR.0000221815.64093.8c.

[27] Laska AC, Hellblom A, Murray V, Kahan T, Von Arbin M. Aphasia in acute stroke and relation to outcome. J Intern Med 2001;249:413–22. doi:10.1046/j.1365-2796.2001.00812.x.

[28] Permsirivanich W, Tipchatyotin S, Wongchai M, Leelamanit V, Setthawatcharawanich S, Sathirapanya P, et al. Comparing the effects of rehabilitation swallowing therapy vs. Neuromuscular electrical stimulation therapy among stroke patients with persistent pharyngeal dysphagia: A randomized controlled study. J Med Assoc Thail 2009.

[29] Schlaug G, Marchina S, Norton A. Evidence for Plasticity in White-Matter Tracts of Patients with Chronic Broca's Aphasia Undergoing Intense Intonation-based Speech Therapy. Ann N Y Acad Sci 2009;1169:385–94. doi:10.1111/j.1749-6632.2009.04587.x.

[30] Barritt AW, Smithard DG. Role of cerebral cortex plasticity in the recovery of swallowing function following dysphagic stroke. Dysphagia 2009. doi:10.1007/s00455-008-9162-3.

[31] Hulsey DR, Shedd CM, Sarker SF, Kilgard MP, Hays SA. Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. Exp Neurol 2019. doi:10.1016/j.expneurol.2019.112975.

[32] Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 2004;27:107–44. doi:10.1146/annurev.neuro.27.070203.144206.

[33] Salgado H, Köhr G, Treviño M. Noradrenergic 'Tone' Determines Dichotomous Control of Cortical Spike-Timing-Dependent Plasticity. Sci Rep 2012;2:417. doi:10.1038/srep00417.

[34] Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 2005;28:403–50. doi:10.1146/annurev.neuro.28.061604.135709.

[35] McAllen RM, Shafton AD, Bratton BO, Trevaks D, Furness JB. Calibration of thresholds for functional engagement of vagal A, B and C fiber groups in vivo . Bioelectron Med 2018. doi:10.2217/bem-2017-0001.

[36] Buell EP, Borland MS, Loerwald KW, Chandler C, Hays SA, Engineer CT, et al. Vagus Nerve Stimulation Rate and Duration Determine whether Sensory Pairing Produces Neural Plasticity. Neuroscience 2019. doi:10.1016/j.neuroscience.2019.03.019.

[37] Buell EP, Loerwald KW, Engineer CT, Borland MS, Buell JM, Kelly CA, et al. Cortical map plasticity as a function of vagus nerve stimulation rate. Brain Stimul 2018;11:1218–24. doi:10.1016/j.brs.2018.07.045.

[38] Bucksot JE, Morales Castelan K, Skipton SK, Hays SA. Parametric characterization of the rat Hering-Breuer reflex evoked with implanted and non-invasive vagus nerve stimulation. Exp Neurol 2020;327:113220. doi:10.1016/j.expneurol.2020.113220.

[39] Chandler SH, Goldberg LJ. Intracellular analysis of synaptic mechanisms controlling spontaneous and cortically induced rhythmical jaw movements in the guinea pig. J Neurophysiol 1982;48:126–38. doi:10.1152/jn.1982.48.1.126.

[40] Nakamura Y, Katakura N. Generation of masticatory rhythm in the brainstem. Neurosci Res 1995;23:1–19. doi:10.1016/0168-0102(95)90003-9.

[41] Lund JP. Mastication and its Control by the Brain Stem. Crit Rev Oral Biol Med 1991;2:33–64. doi:10.1177/10454411910020010401.

[42] Borland MS, Vrana WA, Moreno NA, Fogarty EA, Buell EP, Vanneste S, et al. Pairing vagus nerve stimulation with tones drives plasticity across the auditory pathway. J Neurophysiol 2019;122:659–71. doi:10.1152/jn.00832.2018.

[43] Sessle BJ, Adachi K, Avivi-Arber L, Lee J, Nishiura H, Yao D, et al. Neuroplasticity of face primary motor cortex control of orofacial movements. Arch Oral Biol 2007;52:334–7. doi:10.1016/j.archoralbio.2006.11.002.

[44] Avivi-Arber L, Lee J-C, Yao D, Adachi K, Sessle BJ. Neuroplasticity of face sensorimotor cortex and implications for control of orofacial movements. Jpn Dent Sci Rev 2010;46:132–42. doi:10.1016/j.jdsr.2009.11.007.

[45] Dawson J, Pierce D, Dixit A, Kimberley TJ, Robertson M, Tarver B, et al. Safety, Feasibility, and Efficacy of Vagus Nerve Stimulation Paired With Upper-Limb Rehabilitation After Ischemic Stroke. Stroke 2016;47:143–50. doi:10.1161/STROKEAHA.115.010477.

[46] Kimberley TJ, Pierce D, Prudente CN, Francisco GE, Yozbatiran N, Smith P, et al. Vagus nerve stimulation paired with upper limb rehabilitation after chronic stroke: A blinded randomized pilot study. Stroke 2018. doi:10.1161/STROKEAHA.118.022279.

[47] Elliott RE, Morsi A, Kalhorn SP, Marcus J, Sellin J, Kang M, et al. Vagus nerve stimulation in 436 consecutive patients with treatment-resistant epilepsy: Long-term outcomes and predictors of response. Epilepsy Behav 2011. doi:10.1016/j.yebeh.2010.10.017.

CHAPTER 4

HIGH INTENSITY VNS DISRUPTS VNS-MEDIATED PLASTICITY IN MOTOR CORTEX

Authors: Robert A. Morrison[1,2,*], Tanya T. Danaphongse[2], Stephanie T. Abe[2], Madison E. Stevens[2], Vikram Ezhil[1,2], Armin Seyedahmadi[2], Katherine S. Adcock[1,2], Robert L. Rennaker[1,2], Michael P. Kilgard[1,2], Seth A. Hays[1,2,3]

Affiliations:

[1] The University of Texas at Dallas, School of Behavioral Brain Sciences, Richardson, TX.

[2] The University of Texas at Dallas, Texas Biomedical Device Center, Richardson, TX.

[3] The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, Richardson, TX.

[*] Correspondence to: robert.morrison@utdallas.edu

Reprinted with permission from Elsevier*:* *Brain Research*, Volume 1756, 2021

> *High intensity VNS disrupts VNS-mediated plasticity in motor cortex,* Robert A. Morrison, Tanya T. Danaphongse, Stephanie T. Abe, Madison E. Stevens, Vikram Ezhil, Armin Seyedahmadi, Katherine S. Adcock, Robert L. Rennaker, Michael P. Kilgard, Seth A. Hays*, Brain Research*, ISSN 0006-8993, https://doi.org/10.1016/j.brainres.2021.147332.

Abstract

Vagus nerve stimulation (VNS) paired with motor rehabilitation enhances recovery of function after neurological injury in rats and humans. This effect is ascribed to VNS-dependent facilitation of plasticity in motor networks. Previous studies document an inverted-U relationship between VNS intensity and cortical plasticity, such that moderate intensities increase plasticity, while low or high intensity VNS does not. We tested the interaction of moderate and high intensity VNS trains to probe the mechanisms that may underlie VNS-dependent plasticity. Rats performed a behavioral task where VNS was paired with jaw movement during chewing. For five days, subjects received 100 pairings of moderate intensity VNS (Standard VNS), 100 pairings alternating between moderate and high intensity VNS (Interleaved VNS), or 50 pairings of moderate intensity VNS (Short VNS) approximately every 8 seconds. After the final behavioral session, intracortical microstimulation (ICMS) was used to evaluate movement representations in motor cortex. 100 pairings of moderate intensity VNS enhanced motor cortex plasticity. Replacing half of moderate intensity stimulation with high intensity VNS blocked this enhancement of plasticity. Removing high intensity stimulation, leaving only 50 pairings of moderate intensity VNS, reinstated plasticity. These results demonstrate that there is a period for at least 8 seconds after high intensity stimulation in which moderate intensity VNS is not able to engage mechanisms required for synaptic reorganization. More importantly, this study demonstrates that changes in stimulation parameters are a critical determinant of the magnitude of plasticity and likely the efficacy of VNS-enhanced recovery.

Introduction

Vagus nerve stimulation (VNS) paired with rehabilitation has recently emerged as a strategy to enhance recovery of motor function after a range of neurological injuries including stroke, traumatic brain injury, neuropathy, and spinal cord injury [1-11]. Greater recovery is attributed to VNS-dependent enhancement of synaptic plasticity in motor and sensory networks within the central nervous system during rehabilitation [12,13]. Thus, defining stimulation strategies that maximize plasticity may provide a means to optimize the efficacy of VNS therapy.

Activation of several neuromodulatory nuclei are required for VNS-mediated synaptic plasticity. VNS rapidly engages the nucleus basalis (NB) and locus coeruleus (LC), which in turn release the neuromodulators acetylcholine and norepinephrine, respectively, throughout the brain [14–18]. Acetylcholine and norepinephrine, as well as serotonin, bind to and activate G-protein coupled receptors (GPCRs) [19–22]. Simultaneous activation of these GPCRs paired with the neural activity associated with rehabilitation transiently increases synaptic plasticity, resulting in VNS potentiating neural circuits that contribute to recovery and enhancing the therapeutic effects of rehabilitation [23].

The stimulation parameters of VNS, including intensity, frequency, and duration, can influence the level of activity in neuromodulatory nuclei [18]. For example, VNS intensity scales linearly with LC activation, such that 1.6 mA VNS promotes twice the neuromodulator release of 0.8 mA VNS. Paradoxically, this increase in neuromodulatory activity does not translate to increases in the magnitude of synaptic plasticity. Instead, VNS-directed plasticity exhibits an inverted-U relationship with increases in stimulation intensity, such that 0.8 mA VNS significantly

increases synaptic plasticity while 0.4 and 1.6 mA VNS do not [24–26]. Highlighting the clinical importance of these findings, an equivalent inverted-U effect is observed between VNS intensity and recovery after stroke [7].

While the absence of VNS-mediated plasticity at 0.4 mA can be ascribed to insufficient activation of neuromodulatory nuclei and minimal activation of GPCRs required for synaptic reorganization, the neural mechanisms underlying an absence of plasticity at higher intensities such as 1.6 mA are unclear. Overactivation of neuromodulatory systems at high stimulation intensities could lead to desensitization of mechanisms promoting VNS-mediated plasticity. If so, high intensity VNS should interfere with moderate intensity VNS and suppress plasticity-enhancing effects for the duration of this desensitization. Alternatively, if high intensity VNS does not desensitize neuromodulatory pathways critical for VNS-mediated plasticity, there should be no temporal interaction between the two, and moderate intensity VNS should still enhance plasticity.

Here, we tested the temporal interaction of moderate and high intensity VNS trains to probe the potential mechanisms that may underlie VNS-dependent plasticity. To do so, we conducted an experiment in rats pairing both 0.8 mA and 1.6 mA VNS during motor training, and using intracortical microstimulation (ICMS) we observed the magnitude of subsequent motor cortex plasticity (Fig. 4.1).

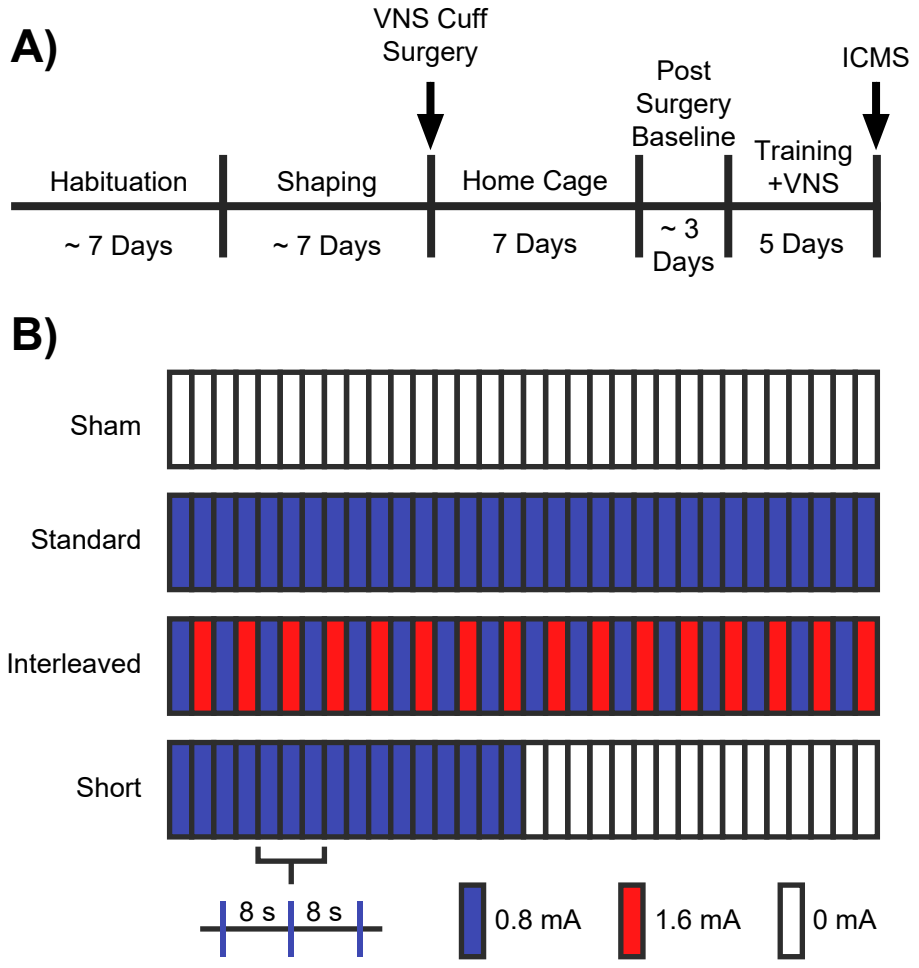


Figure 4.1: Behavioral task and experimental design

(A) Experimental timeline. (B) Overview of experimental groups. Groups received different paradigms of VNS paired with behavioral training: 100 pairings at a moderate intensity (Standard VNS), 100 pairings of interleaved moderate and high intensity stimulation (Interleaved VNS), 50 parings at a moderate intensity (Short VNS), or equivalent behavioral training with without VNS (Sham).

Results

Pairing trains of VNS with motor training drives robust, specific plasticity in motor cortex [13,24,28,33,35]. The magnitude of plasticity displays an inverted-U relationship with stimulation intensity, such that moderate intensity VNS enhances plasticity while high intensity VNS fails to enhance plasticity [24,25]. Here, we tested the temporal interaction of moderate and high intensity VNS trains to probe the potential mechanisms that may underlie VNS-dependent plasticity. To do so, rats performed a simple behavioral task for five days during which VNS at either moderate or both moderate and high intensities was paired with jaw movement during chewing (Fig. 4.1). Within 24 hours of the final behavioral training session, motor cortex movement representations were documented using intracortical microstimulation (ICMS) with area of jaw representation as the primary experimental outcome.

Moderate intensity VNS at 0.8 mA paired with motor training enhances motor cortex plasticity

We first sought to confirm that pairing standard, moderate intensity VNS with motor training could enhance motor cortex plasticity. Group analysis of motor cortex area eliciting jaw movement revealed significant differences between groups (One-way ANOVA, $F[3,19] = 8.170$, $p = 0.004$). Standard VNS paired with chewing significantly increased jaw representation compared to equivalent training on the task without VNS (Standard VNS: 2.69 ± 0.27 mm^2; Sham: 1.25 ± 0.24 mm^2, Unpaired t-test, $p = 0.004$) (Fig. 4.2A). These findings confirm that VNS at moderate intensity 0.8 mA paired with training enhances motor cortex plasticity, as previously reported [24,28,33,35].

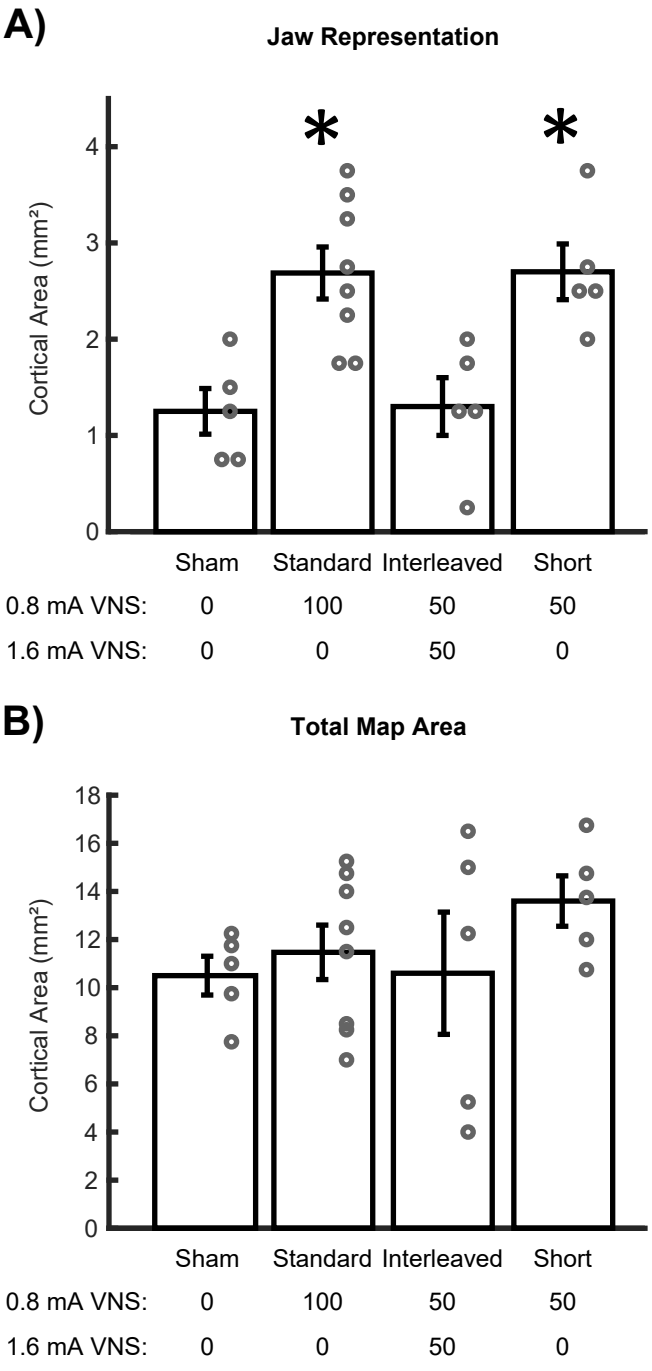


Figure 4.2: High intensity VNS disrupts cortical plasticity

(A) Standard VNS and Short VNS paired with chewing significantly increases jaw movement representation area in motor cortex compared to equivalent behavioral experience without VNS.

Interleaved VNS fails to enhance plasticity. (B) No change in total motor cortex area was observed between groups. Bars represent mean ± SEM. "*" indicates $p < 0.008$.

Adding high intensity 1.6 mA VNS interleaved with 0.8 mA VNS disrupts motor cortex plasticity

Next, we interleaved high intensity 1.6 mA VNS, which does not enhance plasticity [24–26], alongside pro-plasticity moderate intensity 0.8 mA VNS to clarify the action of high intensity VNS. Interleaved VNS resulted in significantly less jaw representation in motor cortex compared to Standard VNS (Interleaved VNS: 1.30 ± 0.30 mm^2; Standard VNS: 2.69 ± 0.27 mm^2, Unpaired t-test, $p = 0.007$). Interleaved VNS paired with chewing also failed to enhance jaw representation in motor cortex compared to Sham animals (Interleaved VNS: 1.30 ± 0.30 mm^2; Sham: 1.25 ± 0.24 mm^2, Unpaired t-test, $p = 0.899$) (Fig. 4.2A). This demonstrates that VNS at high intensities interferes with moderate 0.8 mA VNS-mediated plasticity.

0.8 mA VNS alone enhances VNS-mediated motor cortex plasticity

To confirm that high intensity stimulation was disrupting VNS-mediated plasticity, we removed the 1.6 mA stimulation, leaving only 50 moderate intensity 0.8 mA stimulations per session (Short VNS). Removal of high intensity stimulation restored motor cortex plasticity. Short VNS significantly increased jaw representation compared to Sham animals (Short VNS: 2.70 ± 0.29 mm^2; Sham: 1.25 ± 0.24 mm^2, Unpaired t-test, $p = 0.005$) and comparable to Standard VNS (Short VNS: 2.70 ± 0.29 mm^2; Standard VNS: 2.69 ± 0.27 mm^2, Unpaired t-test, $p = 0.976$) (Fig. 4.2A). These findings additionally confirm that moderate intensity stimulation enhances cortical plasticity, and high intensity stimulation prevents VNS-dependent plasticity.

VNS does not affect total motor cortex representation or movement thresholds

Consistent with previous studies [24,28,33,35], no differences in other cortical movement representations were observed between groups, indicating that VNS-dependent synaptic plasticity is specific to the paired movement (One-way ANOVA, forelimb: $F[3,19] = 0.426$, $p = 0.661$; vibrissa: $F[3,19] = 0.376$, $p = 0.693$; neck: $F[3,19] = 0.268$, $p = 0.769$; hindlimb: $F[3,19] = 2.097$, $p = 0.157$). Additionally, total motor cortex area was also not affected by VNS (One-way ANOVA, $F[3,19] = 1.796$, $p = 0.200$) (Fig. 4.2B). As expected, group analysis revealed no differences across groups in average stimulation threshold required to elicit movement (One-way ANOVA, $F[3,19] = 2.049$, $p = 0.141$).

Discussion

VNS has repeatedly proven effective at enhancing cortical plasticity using a stimulation paradigm of 0.8 mA, 100 μs pulse width, 30 Hz frequency with a pulse train of 0.5 s. Variation of these stimulation parameters can significantly influence the degree of subsequent cortical plasticity. There is an inverted-U relationship between degree of synaptic plasticity and pulse duration [36], frequency [36], and intensity of VNS [24–26,37–39]. Intensity has been the most studied of these parameters, with high intensity VNS consistently failing to enhance synaptic plasticity compared to moderate intensities. Although high intensity VNS fails to enhance plasticity when delivered alone, it is unclear whether the mechanisms engaged by high intensity VNS interact with and disrupt subsequent moderate intensity VNS.

In this study, we tested the temporal interaction of moderate and high intensity VNS to probe the potential mechanisms that may underlie VNS-dependent plasticity. To do so, we

conducted an experiment in rats pairing both 0.8 mA and 1.6 mA approximately every 8 seconds VNS during motor training, and using ICMS we observed the magnitude of subsequent motor cortex plasticity. We confirm moderate intensity 0.8 mA VNS enhances plasticity. Replacing half of moderate intensity stimulation with high intensity 1.6 mA VNS blocks this VNS-dependent enhancement of plasticity. Removing the high intensity stimulation reinstates plasticity. These results demonstrate that there is a period of time lasting at least 8 seconds after high intensity stimulation in which moderate intensity VNS is not able to enhance synaptic plasticity.

This study leveraged the inverted-U relationship between VNS intensity and was designed to differentiate between two possible outcomes (Fig. 4.3). In the first option, high intensity stimulation engages a signaling pathway with a rapid decay, and thus does not interfere with moderate intensity (Fig. 4.3B). Here, high intensity stimulation trains result in activation that exceed the effective range, indicated in green, but the signal engagement rapidly decays to baseline levels. Subsequent trains of moderate intensity stimulation then produce activation that peaks in the effective range, and thus cortical plasticity is enhanced. In the second option, high intensity stimulation engages a signaling pathway that slowly decays, and as a result, interacts with the plasticity-enhancing action of moderate intensity stimulation trains (Fig. 4.3C). In this scenario, high intensity VNS results in engagement of a signaling pathway that exceeds the effective range and slowly decays. Subsequent moderate intensity trains build on this overactivation and push the signal engagement further out of the effect range, resulting in no enhancement of plasticity. Our findings are consistent with the second option (Fig. 4.3C). This indicates that the action of trains of VNS delivered within the order of ten seconds may interact, which raises the need to consider this interaction when selecting stimulation paradigms.

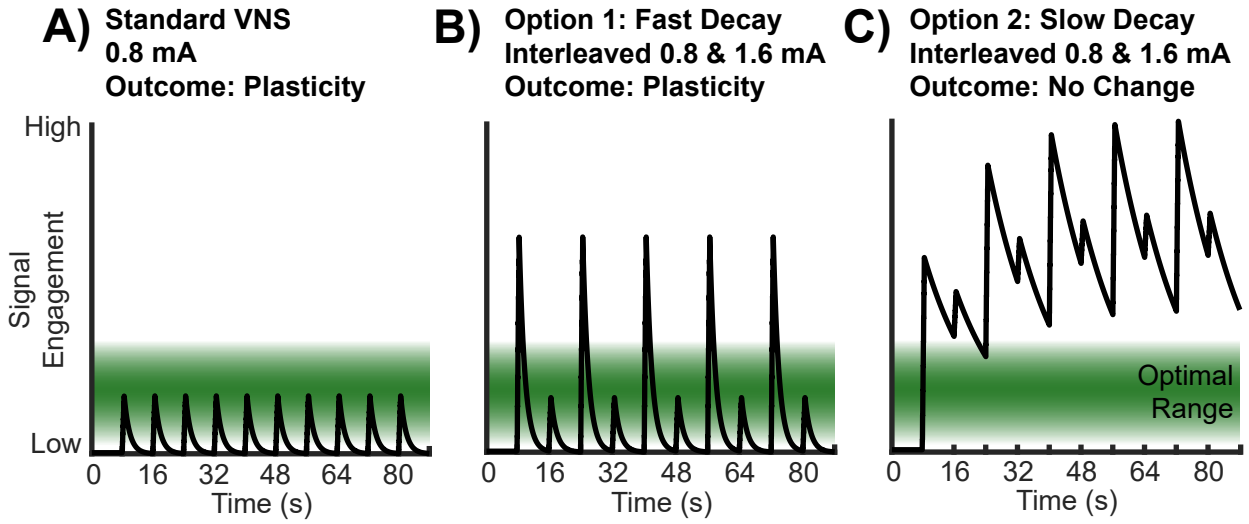


Figure 4.3: Conceptual models of the temporal engagement of plasticity enhancement pathways by VNS

(A) The green band indicates the level of engagement of signaling pathways that promotes plasticity. Moderate intensity trains of VNS produce signal engagement that peaks within the effective range, resulting in enhanced synaptic plasticity observed in the Standard VNS group. (B) In this model, signal levels decay rapidly. High intensity VNS produces activation that peaks above the effective range and thus does not facilitate plasticity. However, interleaved moderate intensity trains produce signal engagement within the effective range, which results in enhanced plasticity. In this model, the Interleaved VNS group would exhibit enhanced plasticity. Our results are inconsistent with this model. (C) In this model, signal levels decay slowly. High intensity VNS produces activation that peaks above the effective range. Interleaved moderate intensity trains build on this overactivation and also peak outside the optimal range, thus no enhancement of plasticity is observed. In this model, the Interleaved VNS group would not exhibit enhanced plasticity, which is accordant with the results reported in this study.

The mechanisms engaged by VNS may provide insight into why high intensity VNS fails to promote plasticity, and why it continues to disrupt the plasticity-enhancing effects of subsequent moderate intensity stimulation. VNS rapidly engages the NB and LC, which release the neuromodulators acetylcholine and norepinephrine. Increasing the current intensity of VNS proportionally increases the release of these neuromodulators [18]. Acetylcholine and

norepinephrine then activate GPCRs, which in turn signal for the production of new proteins and other cellular machinery needed for synaptic reorganization. High intensity VNS may disrupt this process due to an over-release of neuromodulators and overactivation and desensitization of these GPCRs, which are known to exhibit notable short-term and long-term desensitization [19,20,33,35]. This desensitization could be due to overstimulation and subsequent inactivation of relevant G-proteins via β-arrestin, which can occur on a timescale of minutes [19,20], or due to GPCR internalization or even lysosomal degradation, which may have long-lasting effects on a timescale of hours or days [20]. The timescales of these forms of desensitization could account for the ability of high intensity VNS to disrupt the effects of subsequent moderate intensity stimulation.

Alternatively, mechanisms other than GPCR desensitization may be at work. Activation of opposing families of adrenergic receptors could account for the disruption of VNS-mediated plasticity after high intensity stimulation. Increasing VNS intensity drives proportionate increases in LC activity, which in turn increases levels of norepinephrine release in motor cortex [18]. Activation of high affinity α-receptors and low affinity β-receptors differentially modulate the outcome of spike-timing-dependent plasticity depending on norepinephrine concentration, leading to either long-term potentiation or depression of synapses [40]. Under this model, moderate intensities of VNS would produce maximal activation of pro-plasticity α-receptors and minimize activation of pro-stability β-receptors, while high intensity VNS would activate β-receptors, leading to a robust, overriding stability of circuits. Indeed, similar activation of noradrenergic receptors and their effects on plasticity has been previously described at timescales that could account for the disruptive effects of high intensity VNS reported here [24,40–42].

Another possibility for high intensity stimulation's disruption of VNS-mediated plasticity is recruitment of inhibitory interneurons, resulting in increased stability of activated networks. Cholinergic modulation of sensory stimuli via GABAergic interneurons in cortico-cortical networks can influence learning and memory retrieval and may play a role in the selective potentiation of circuits paired with VNS during training [43]. Varying the concentration of acetylcholine can differentially activate feedback inhibition mechanisms via muscarinic receptors, which can lead to either suppression or amplification of sensory information in cortical networks. Under this model, high intensity VNS raises cholinergic levels in motor cortex to the point that inhibitory mechanisms dominate, causing the nervous system to promote circuit stability and overriding any signal from moderate intensity VNS for synapses to reorganize.

VNS can selectively enhance synaptic reorganization when paired with an array of training and rehabilitative paradigms [2,12,24,26,28,33,35]. Furthermore, VNS-paired rehabilitation enhances recovery compared to rehabilitation alone in a number of injuries including stroke, traumatic brain injury, neuropathy, and spinal cord injury [1–5,8,10,12]. VNS parameters that enhance plasticity yield recovery, while those that fail to enhance plasticity do not provide any functional benefits [7]. This suggests that synaptic reorganization is the driving force behind VNS-mediated recovery after injury.

The range of VNS intensities that enhance synaptic plasticity is narrow [26], and therefore it is of the upmost importance to accurately deliver stimulation, as small deviations from optimal stimulation parameters could result in a lack of treatment efficacy. Variations in nerve cuff implantation, scarring, and natural variations between patients could alter vagal fiber activation [44], which could conceivably alter neuromodulatory response and the resulting magnitude of

synaptic reorganization. In order to counteract these variations it would seem reasonable to employ a VNS-rehabilitation paradigm in which the intensities of VNS ramp from slightly below to slightly above 0.8 mA throughout treatment to ensure patients receive optimal vagal fiber activation. However, we demonstrate that high intensity stimulation can disrupt VNS-mediated plasticity, meaning such a paradigm could be counterproductive to patient recovery. A better understanding of the neural basis for VNS-mediated synaptic plasticity at moderate intensities, and disruption of this synaptic plasticity at higher intensities, could open the door for greater optimization and increased treatment efficacy in the clinic.

Methods

Subjects

Thirty-six female Sprague Dawley rats weighing approximately 250 grams were used in this study (Charles River Labs). All rats were housed in a reversed 12:12 hour light-dark cycle. Rats that underwent behavioral training were food restricted on weekdays during shaping and training with ad libitum access to food on weekends. All rats were maintained at or above 85% body weight relative to the beginning of shaping. All handling, housing, stimulation, and surgical procedures were approved by The University of Texas at Dallas Institutional Animal Care and Use Committee. Data from a subset of subjects used in this study has been previously published [26]. All subjects were run concurrently.

Behavioral training

Rats were trained on a simple automated behavioral task that allowed triggering of VNS during chewing [26]. The behavioral training apparatus consisted of an acrylic cage with a nosepoke food dispenser at one end. A food pellet (45 mg dustless precision pellet, BioServ, Frenchtown, NJ) was delivered to the food dispenser. An infrared beam sensor positioned in the food dispenser was used to determine when the rat entered the nosepoke to retrieve the food pellet. Upon breaking the infrared beam, another pellet was dispensed after an eight second delay. Additionally, in the appropriate groups, VNS was triggered 3 seconds after beam break. This stimulation timing results in reliable delivery of VNS during chewing [26]. Each behavioral session continued until either 100 pellets had been dispensed, or until 1 hour had elapsed. Rats received a supplement of approximately 100 food pellets if they did not receive at least 100 pellets in a day to maintain weight.

Rats performed the task twice per day, 5 days per week, with daily sessions separated by at least 2 hours. Rats were shaped on the task until they reliably consumed 100 pellets within 1 hour each session (Fig. 4.1A). Rats were then implanted with a VNS cuff and recovered for 7 days in their home cage with ad libitum access to food and water. Seven days after surgery, rats were allocated to one of four groups to receive 10 additional training sessions over 5 days. Groups received either VNS at 0.8 mA paired with 100 trials per session (Standard VNS), VNS at 0.8 mA paired with 50 trials per session (Short VNS), VNS alternating between 0.8 and 1.6 mA on each successive trial for 100 trials per session (Interleaved VNS), or sham stimulation (Sham) (Fig. 4.1B). Each train of VNS consisted of a 500 ms burst triggered 3 seconds after nosepoke beam

break, which reliably results in delivery of VNS during chewing [26]. Twenty-four hours after the conclusion of behavioral training, all rats underwent ICMS motor cortex mapping.

Surgical implantation

All surgeries were performed using aseptic technique under general anesthesia. Rats were implanted with a stimulating cuff on the left cervical vagus nerve as described in previous studies [3–5,24,27]. Rats were anesthetized with ketamine hydrochloride (50 mg/kg, i.p.), xylazine (20 mg/kg, i.p.), and acepromazine (5 mg/kg, i.p.), and were placed in a stereotactic apparatus. An incision was made down the midline of the head to expose the skull. Bone screws were inserted into the skull at points surrounding the lamboid suture and over the cerebellum. A two-channel connector was mounted to the screws using acrylic. The rat was then removed from the stereotactic apparatus and placed in a supine position.

An incision was made on the left side of the neck and the overlying musculature was blunt dissected to reveal the left cervical vagus nerve. The nerve was gently dissected away from the carotid artery. A cuff electrode was implanted surrounding the vagus nerve, and the leads were tunneled subcutaneously to connect with the two-channel connector mounted on the skull. Nerve activation was confirmed by observation of a $\geq 5\%$ drop in blood oxygen saturation in response to a 10 s stimulation train of VNS, as in previous studies [24]. The head and neck incisions were then sutured, and rats received subcutaneous injections of 4 mL 50:50 0.9% saline 5% dextrose solution. A seven day recovery period followed surgery during which animals did not perform behavioral training. All rats underwent implantation procedures.

Vagus nerve stimulation

Upon return to behavioral testing after surgery, rats were randomly assigned to groups to receive either 100 stimulations of VNS at 0.8 mA per session (Standard VNS, n = 8), 50 stimulations of 0.8 mA VNS per session (Short VNS, n = 5), 100 stimulations of VNS alternating between 0.8 and 1.6 mA per session (Interleaved VNS, n = 5), or equivalent behavioral training without stimulation (Sham, n = 5) (Fig. 4.1B). In the initial sessions after implantation, no stimulation was delivered in any group while rats were allowed to acclimate to being attached to stimulating cables until they reliably consumed 100 pellets in a one hour session. Once acclimated, rats then underwent five days of training and received VNS stimulation according to their group. VNS was triggered 3 seconds after nosepoke beam break once a pellet had been dispensed during behavioral training, resulting in stimulation that was consistently delivered during chewing of the pellet [26]. Each 0.5 s stimulation train consisted of 100 μsec biphasic pulses delivered at 30 Hz at an intensity of either 0.8 mA or 1.6 mA, as appropriate for each experimental group. A digital oscilloscope (PicoScope 2204A, PP906, Pico Technology) was used to monitor voltage across the electrodes during each stimulation to ensure cuff functionality.

Intracortical microstimulation mapping

Approximately 24 hours after their last behavioral session, rats underwent ICMS to derive cortical movement representation maps according to standard procedures [24,28–32]. Rats were anesthetized with intraperitoneal injections of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg). Rats received supplemental doses of ketamine as necessary throughout the procedure in order to maintain a consistent level of anesthesia as indicated by breathing rate, vibrissae

whisking, and toe pinch reflex. Rats were placed in a stereotactic apparatus and a craniotomy and durotomy were performed to expose the left motor cortex (4 mm to -3 mm AP and 0.25 mm to 5 mm ML). To prevent cortical swelling, a small incision was made in the cisterna magna.

Connected to a pulse stimulator (Model 2100, A-M Systems, Sequim, WA, ±100 V), a tungsten electrode (0.65 ± 0.8 MΩ) (UEWMEGSEBN3M, FHC, Bowdoin, ME) was lowered into the brain to a depth of 1.8 mm. Stimulation sites were chosen at random on a grid with sites set 500 μm apart from each other. The next stimulation site was placed at least 1 mm away from the previous site whenever possible. Stimulation consisted of a 40 ms pulse train of 10 monophasic 200 μs cathodal pulses. Stimulation was increased from 10 μA until a movement was observed or until a maximum of 250 μA was reached. ICMS was conducted blinded with two experimenters, as previously described [24,28,33]. The first experimenter placed the electrode and recorded the data for each site. The second experimenter, blinded to group and electrode location, delivered stimulations and classified movements. Movements were classified into the following categories: jaw, neck, vibrissa, forelimb, and hindlimb. After the completion of ICMS, VNS cuff functionality was confirmed by a stimulation-evoked decrease in blood oxygen saturation in response to a 10 s VNS train, as previously described [24,34]. All maps from ICMS are included in the appendix (Appendix C).

Subject exclusion

Twenty-three subjects were analyzed in the final results of the study out of a total of 36 subjects. Of the 13 subjects not used in final analysis, 5 subjects were excluded due to non-

functional stimulating cuffs (indicated by digital oscilloscope readings exceeding 40 V peak-to-peak), 3 subjects were excluded due to headcap failure, 3 subjects were excluded due to a lack of drop in blood oxygen saturation in response to VNS during ICMS, 1 subject died during VNS surgery, and 1 subject died during ICMS surgery. All exclusion criteria were predefined before beginning data collection and are consistent with previous studies [24,26,28,33,35].

Statistics

The primary outcome of this study was area of motor cortex generating jaw movements. All other movement representations were analyzed as secondary outcome measures. A one-way ANOVA was used to identify differences across groups. *Post hoc* unpaired two-tailed t-tests using a Bonferroni-corrected alpha of 0.008 were used to determine statistically significant differences between individual groups, as appropriate. Statistical tests for each comparison are noted in the text. All data are reported as mean ± SEM.

Acknowledgements

We would like to thank Sarah Jacob, Anjana Swami, Kamal Safadi, Anusheh Kashif, Armaan Somaney, Amina Aslam, Yabsera Mesfin, Vishal Kumar, and Zainab Haider for help with behavioral training. We would also like to thank David Pruitt and Jesse Bucksot for help with programming and troubleshooting.

Sources of funding

This work was supported by the National Institutes of Health R01NS094384 and R01NS103803 and by the DARPA BTO Targeted Neuroplasticity Training (TNT) program under the auspices of Dr. Tristan McClure-Begley through the Space and Naval Warfare Systems Center, Pacific Grant/Contract No. N66001-17-2-4011.

Conflict of interest

MPK has a financial interest in MicroTransponder, Inc., which is developing VNS for stroke. RLR has a financial interest in Teliatry, Inc., and X-Nerve, Inc., which may commercialize targeted plasticity therapy. All other authors declare no conflicts of interest.

References

[1] Hays SA, Ruiz A, Bethea T, Khodaparast N, Carmel JB, Rennaker RL, et al. Vagus nerve stimulation during rehabilitative training enhances recovery of forelimb function after ischemic stroke in aged rats. Neurobiol Aging 2016;43:111–8. doi:10.1016/j.neurobiolaging.2016.03.030.

[2] Meyers EC, Solorzano BR, James J, Ganzer PD, Lai ES, Rennaker RL, et al. Vagus Nerve Stimulation Enhances Stable Plasticity and Generalization of Stroke Recovery. Stroke 2018;49:710–7. doi:10.1161/STROKEAHA.117.019202.

[3] Khodaparast N, Hays SA, Sloan AM, Hulsey DR, Ruiz A, Pantoja M, et al. Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. Neurobiol Dis 2013;60:80–8. doi:10.1016/j.nbd.2013.08.002.

[4] Khodaparast N, Hays SA, Sloan AM, Fayyaz T, Hulsey DR, Rennaker RL, et al. Vagus Nerve Stimulation Delivered During Motor Rehabilitation Improves Recovery in a Rat Model of Stroke. Neurorehabil Neural Repair 2014;28:698–706. doi:10.1177/1545968314521006.

[5] Hays SA, Khodaparast N, Ruiz A, Sloan AM, Hulsey DR, Rennaker RL, et al. The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. Neuroreport 2014;25:682–8. doi:10.1097/WNR.0000000000000154.

[6] Darrow MJ, Torres M, Sosa MJ, Danaphongse TT, Haider Z, Rennaker RL, et al. Vagus Nerve Stimulation Paired With Rehabilitative Training Enhances Motor Recovery After Bilateral Spinal Cord Injury to Cervical Forelimb Motor Pools. Neurorehabil Neural Repair 2020;34:200–9. doi:10.1177/1545968319895480.

[7] Pruitt DT, Danaphongse TT, Lutchman M, Patel N, Reddy P, Wang V, et al. Optimizing Dosing of Vagus Nerve Stimulation for Stroke Recovery. Transl Stroke Res 2020. doi:10.1007/s12975-020-00829-6.

[8] Pruitt DT, Schmid AN, Kim LJ, Abe CM, Trieu JL, Choua C, et al. Vagus Nerve Stimulation Delivered with Motor Training Enhances Recovery of Function after Traumatic Brain Injury. J Neurotrauma 2016;33:871–9. doi:10.1089/neu.2015.3972.

[9] Kimberley TJ, Pierce D, Prudente CN, Francisco GE, Yozbatiran N, Smith P, et al. Vagus nerve stimulation paired with upper limb rehabilitation after chronic stroke: A blinded randomized pilot study. Stroke 2018. doi:10.1161/STROKEAHA.118.022279.

[10] Dawson J, Pierce D, Dixit A, Kimberley TJ, Robertson M, Tarver B, et al. Safety, Feasibility, and Efficacy of Vagus Nerve Stimulation Paired With Upper-Limb Rehabilitation After Ischemic Stroke. Stroke 2016;47:143–50. doi:10.1161/STROKEAHA.115.010477.

[11] Darrow MJ, Mian TM, Torres M, Haider Z, Danaphongse T, Rennaker RL, et al. Restoration of Somatosensory Function by Pairing Vagus Nerve Stimulation with Tactile Rehabilitation. Ann Neurol 2020;87:194–205. doi:10.1002/ana.25664.

[12] Ganzer PD, Darrow MJ, Meyers EC, Solorzano BR, Ruiz AD, Robertson NM, et al. Closed-loop neuromodulation restores network connectivity and motor control after spinal cord injury. Elife 2018;7:1–19. doi:10.7554/eLife.32058.

[13] Meyers EC, Kasliwal N, Solorzano BR, Lai E, Bendale G, Berry A, et al. Enhancing plasticity in central networks improves motor and sensory recovery after nerve damage. Nat Commun 2019;10:5782. doi:10.1038/s41467-019-13695-0.

[14] Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119:124–32. doi:10.1016/j.brainres.2006.08.048.

[15] Dorr AE. Effect of Vagus Nerve Stimulation on Serotonergic and Noradrenergic Transmission. J Pharmacol Exp Ther 2006;318:890–8. doi:10.1124/jpet.106.104166.

[16] Follesa P, Biggio F, Gorini G, Caria S, Talani G, Dazzi L, et al. Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. Brain Res 2007;1179:28–34. doi:10.1016/j.brainres.2007.08.045.

[17] Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, et al. Neuromodulators Control the Polarity of Spike-Timing-Dependent Synaptic Plasticity. Neuron 2007;55:919–29. doi:10.1016/j.neuron.2007.08.013.

[18] Hulsey DR, Riley JR, Loerwald KW, Rennaker RL, Kilgard MP, Hays SA. Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. Exp Neurol 2017;289:21–30. doi:10.1016/j.expneurol.2016.12.005.

[19] Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 2004;27:107–44. doi:10.1146/annurev.neuro.27.070203.144206.

[20] Rajagopal S, Shenoy SK. GPCR desensitization: Acute and prolonged phases. Cell Signal 2018. doi:10.1016/j.cellsig.2017.01.024.

[21] Lovinger DM. Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum. Neuropharmacology 2010;58:951–61. doi:10.1016/j.neuropharm.2010.01.008.

[22] Jong Y-JI, Harmon SK, O'Malley KL. Intracellular GPCRs Play Key Roles in Synaptic Plasticity. ACS Chem Neurosci 2018;9:2162–72. doi:10.1021/acschemneuro.7b00516.

[23] Engineer ND, Kimberley TJ, Prudente CN, Dawson J, Tarver WB, Hays SA. Targeted Vagus Nerve Stimulation for Rehabilitation After Stroke. Front Neurosci 2019;13. doi:10.3389/fnins.2019.00280.

[24] Morrison RA, Hulsey DR, Adcock KS, Rennaker RL, Kilgard MP, Hays SA. Vagus nerve stimulation intensity influences motor cortex plasticity. Brain Stimul 2019;12:256–62. doi:10.1016/j.brs.2018.10.017.

[25] Borland MS, Vrana WA, Moreno NA, Fogarty EA, Buell EP, Sharma P, et al. Cortical Map Plasticity as a Function of Vagus Nerve Stimulation Intensity. Brain Stimul 2016;9:117–23. doi:10.1016/j.brs.2015.08.018.

[26] Morrison RA, Danaphongse TT, Pruitt DT, Adcock KS, Mathew JK, Abe ST, et al. A limited range of vagus nerve stimulation intensities produce motor cortex reorganization when delivered during training. Behav Brain Res 2020;391:112705. doi:10.1016/j.bbr.2020.112705.

[27] Hays SA, Khodaparast N, Hulsey DR, Ruiz A, Sloan AM, Rennaker RL, et al. Vagus Nerve Stimulation During Rehabilitative Training Improves Functional Recovery After Intracerebral Hemorrhage. Stroke 2014;45:3097–100. doi:10.1161/STROKEAHA.114.006654.

[28] Porter BA, Khodaparast N, Fayyaz T, Cheung RJ, Ahmed SS, Vrana WA, et al. Repeatedly Pairing Vagus Nerve Stimulation with a Movement Reorganizes Primary Motor Cortex. Cereb Cortex 2012;22:2365–74. doi:10.1093/cercor/bhr316.

[29] Kleim JA, Bruneau R, Calder K, Pocock D, VandenBerg PM, MacDonald E, et al. Functional Organization of Adult Motor Cortex Is Dependent upon Continued Protein Synthesis. Neuron 2003;40:167–76. doi:10.1016/S0896-6273(03)00592-0.

[30] Neafsey EJ, Sievert C. A second forelimb motor area exists in rat frontal cortex. Brain Res 1982;232:151–6. doi:10.1016/0006-8993(82)90617-5.

[31] Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, et al. The organization of the rat motor cortex: a microstimulation mapping study. Brain Res 1986;396:77–96.

[32] Pruitt DT, Schmid AN, Danaphongse TT, Flanagan KE, Morrison RA, Kilgard MP, et al. Forelimb training drives transient map reorganization in ipsilateral motor cortex. Behav Brain Res 2016;313:10–6. doi:10.1016/j.bbr.2016.07.005.

[33] Hulsey DR, Hays SA, Khodaparast N, Ruiz A, Das P, Rennaker RL, et al. Reorganization of Motor Cortex by Vagus Nerve Stimulation Requires Cholinergic Innervation. Brain Stimul 2016;9:174–81. doi:10.1016/j.brs.2015.12.007.

[34] Rios M, Bucksot J, Rahebi K, Engineer C, Kilgard M, Hays S. Protocol for Construction of Rat Nerve Stimulation Cuff Electrodes. Methods Protoc 2019. doi:10.3390/mps2010019.

[35] Hulsey DR, Shedd CM, Sarker SF, Kilgard MP, Hays SA. Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. Exp Neurol 2019. doi:10.1016/j.expneurol.2019.112975.

[36] Buell EP, Borland MS, Loerwald KW, Chandler C, Hays SA, Engineer CT, et al. Vagus Nerve Stimulation Rate and Duration Determine whether Sensory Pairing Produces Neural Plasticity. Neuroscience 2019. doi:10.1016/j.neuroscience.2019.03.019.

[37] Clark KB, Krahl SE, Smith DC, Jensen RA. Post-training Unilateral Vagal Stimulation Enhances Retention Performance in the Rat. Neurobiol Learn Mem 1995;63:213–6. doi:10.1006/nlme.1995.1024.

[38] Clark KB, Smith DC, Hassert DL, Browning RA, Naritoku DK, Jensen RA. Posttraining Electrical Stimulation of Vagal Afferents with Concomitant Vagal Efferent Inactivation Enhances Memory Storage Processes in the Rat. Neurobiol Learn Mem 1998;70:364–73. doi:10.1006/nlme.1998.3863.

[39] Clark KB, Naritoku DK, Smith DC, Browning RA, Jensen RA. Enhanced recognition memory following vagus nerve stimulation in human subjects. Nat Neurosci 1999;2:94–8. doi:10.1038/4600.

[40] Salgado H, Köhr G, Treviño M. Noradrenergic 'Tone' Determines Dichotomous Control of Cortical Spike-Timing-Dependent Plasticity. Sci Rep 2012;2:417. doi:10.1038/srep00417.

[41] Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 2005;28:403–50. doi:10.1146/annurev.neuro.28.061604.135709.

[42] Hansen N, Manahan-Vaughan D. Locus Coeruleus Stimulation Facilitates Long-Term Depression in the Dentate Gyrus That Requires Activation of β-Adrenergic Receptors. Cereb Cortex 2015. doi:10.1093/cercor/bht429.

[43] Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol 2006;16:710–5. doi:10.1016/j.conb.2006.09.002.

[44] Bucksot JE, Wells AJ, Rahebi KC, Sivaji V, Romero-Ortega M, Kilgard MP, et al. Flat electrode contacts for vagus nerve stimulation. PLoS One 2019;14:e0215191. doi:10.1371/journal.pone.0215191.

CHAPTER 5

VAGUS NERVE STIMULATION AS A POTENTIAL ADJUVANT TO REHABILITATION FOR POST-STROKE MOTOR SPEECH DISORDERS

Authors: Robert A. Morrison[1,2,*], Seth A. Hays[1,2,3], Michael P. Kilgard[1,2]

Affiliations:

[1] The University of Texas at Dallas, School of Behavioral Brain Sciences, Richardson, TX.

[2] The University of Texas at Dallas, Texas Biomedical Device Center, Richardson, TX.

[3] The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, Richardson, TX.

[*] Correspondence to: robert.morrison@utdallas.edu

Traditional therapy for post-stroke motor speech disorders

Impairments affecting orofacial function are some of the most common lasting deficits after ischemic stroke, second only to hemiparesis. Roughly one third of those who undergo a stroke develop a speech impairment and nearly half will experience dysphagia [1–5]. Acquired apraxia of speech, the inability to plan movements needed for normal speech production, and dysarthria, reduced muscular coordination of speech, can have devastating effects on quality of life. Stroke patients with speech impairments are twice as likely to require admittance to long-term care facilities [6]. Thus, the development of interventions to improve speech and reduce disability after stroke are of clear clinical importance.

A diverse array of speech-language therapies are used to treat motor speech disorders. Therapy may employ one or several rehabilitative strategies targeting rate and intensity of speech, prosody, and qualities affected by improper muscle control such as phonation and resonance. Course of treatment is commonly assessed based on the patient's individual needs, and depending on severity of injury the clinician may choose to emphasize weak abilities to build strength or focus on coping strategies to circumvent particular deficits. While speech therapy is able to enhance function after stroke, many patients see only modest improvements after treatment [7,8], suggesting that there is a need for further intervention. Rehabilitative strategies that augment the effects of traditional speech therapy hold promise in reducing the disability associated with motor speech disorders, possibly enhancing recovery further.

Plasticity underlies functional improvements of motor speech control recovery

Neuroplasticity allows the brain to reorganize speech circuits disrupted by stroke and is a driving force behind recovery from motor speech disorders. Though speech production is heavily lateralized to the left hemisphere, increases in neuroplasticity during speech therapy can shift speech processing towards the right hemisphere [9]. Moreover, areas directly adjacent to the site of injury can undergo reorganization after therapy [10,11]. Plasticity in orofacial motor areas have been implicated in increases in function after speech therapy as well [12,13], suggesting that plasticity of circuits directly involved in speech production plays a significant role in mediating recovery. These increases in neuroplasticity are thought to aid in the bypassing of injured circuits contributing to motor speech impairment, allowing the nervous system to compensate for loss of function [14–16]. Given the importance of neuroplasticity underlying speech therapy and the incomplete recovery many patients experience after undergoing treatment, it is reasonable to conclude that interventions that further enhance the neuroplasticity produced by speech therapy could lead to greater functional outcomes.

Vagus nerve stimulation

Vagus nerve stimulation (VNS) has emerged as a method of enhancing rehabilitative outcomes for a wide range of neurological injuries, including stroke [17–26] (Table 5.1). VNS increases the effects of rehabilitation through targeted enhancement of synaptic plasticity in central networks after injury. Electrical stimulation of the vagus nerve immediately enhances neuromodulatory function. Bursts of VNS rapidly activate the noradrenergic locus coeruleus (LC)

and cholinergic nucleus basalis (NB), two major neuromodulatory centers in the brain [27–32]. Coincident release of these pro-plasticity neuromodulators coupled with neural activity related to rehabilitation facilitates synaptic plasticity in task-specific activated circuits [27,31–34].

VNS enhances plasticity and recovery in motor dysfunction

VNS enhances cortical representations related to a variety of motor activities. Stimulation of the vagus nerve paired with movement during motor training increases synaptic plasticity in activated circuits, selectively expanding cortical representations of the muscles active at the time of stimulation [22,28,29,35–37]. VNS-mediated synaptic plasticity also takes place in sub-cortical structures throughout task-related circuits [23,38]. The timing of VNS is of particular importance, as it is able to potentiate circuits activated within a roughly 2 second window after stimulation occurs [23]. This targeted-enhancement of plasticity has proven useful in augmenting the effects of motor rehabilitation (Table 5.1). VNS-paired stroke upper limb rehabilitation significantly enhances motor recovery compared to traditional rehabilitation alone in rats [18,20,21,39,40], and two clinical trials have now demonstrated that VNS-paired stroke rehabilitation significantly enhances functional recovery in humans [24,25].

Table 5.1: VNS enhances a wide range of rehabilitative therapies

Impairment	Cause	Intervention	Animal Evidence	Clinical Evidence
Hemiparesis	Stroke	VNS + Motor Rehabilitation	[18–23,26]	[24,25]
	Spinal Cord Injury	VNS + Motor Rehabilitation	[23,58]	[59]
	Traumatic Brain Injury	VNS + Motor Rehabilitation	[17]	
	Neuropathy	VNS + Motor Rehabilitation	[60]	
Auditory	Tinnitus	VNS + Auditory Training	[61,62]	[63–66]
Somatosensory	Stroke	VNS + Tactile Rehabilitation		[67]
	Neuropathy	VNS + Sensory Rehabilitation	[60,68,69]	
Anxiety	Post-Traumatic Stress Disorder	VNS + Prolonged Exposure Therapy	[70–72]	[73,74]
Speech Production	Stroke	VNS + Speech Therapy	N/A	

VNS as a potential adjuvant to therapy for motor speech disorders

VNS paired with upper limb rehabilitation enhances upper limb function after stroke [24,25], and recent work suggests that VNS can enhance synaptic plasticity in networks related to post-stroke motor speech disorders in a similar manner. VNS can significantly enhance synaptic plasticity in orofacial circuits [41,42]. Repeatedly pairing VNS with jaw movement increases the area of motor cortex that evokes jaw movements [41,42]. Because VNS enhances recovery from upper limb dysfunction by increasing synaptic plasticity in upper limb circuits, enhancement of speech therapy could similarly be realized via VNS-mediated plasticity in orofacial circuits. This possibility is further supported by the fact that plasticity in orofacial areas is already implicated in recovery from motor speech dysfunction [12,13], suggesting VNS could prove a useful adjuvant to enhance the effects of various traditional speech therapy interventions after stroke.

Using a paradigm similar to that of existing VNS-paired upper limb stroke rehabilitation [24,25] could allow for pairing of VNS with multiple speech therapy techniques (Table 5.2). VNS paired with specific exercises could allow for targeted enhancement of orofacial circuits involved in specific deficits, enhancing recovery. Under this rehabilitation paradigm, the therapist leading the speech therapy session would activate the patient's VNS implant via a wireless remote. The therapist would conduct speech therapy normally, triggering VNS when the patient is performing speech therapy exercises, emphasizing moments they view as conducive to recovery. This timed, performance-dependent application of VNS strives to reorganize and strengthen the circuits activated during speech therapy that mediate recovery, enhancing the effects of rehabilitation.

Table 5.2: VNS could be delivered during different therapies to treat a variety of post-stroke motor speech impairments

Impairment	Intervention to be Paired with VNS	Clinical Evidence
Non-Fluent Aphasia	Articulatory Placement	[75]
Dysarthria	Oromotor Exercises	[76]
Apraxia of speech	Articulatory Feedback	[77,78]
	Constraint-Induced Aphasia Therapy	[79,80]
	McNeill Dysphagia Therapy	[81]
Anomia	Concurrent Phonemic Cueing	[82]
Agrammatic Aphasia	Conversation Therapy	[83]

VNS as an alternative to previously studied neuromodulatory strategies in speech therapy

Increasing neuroplasticity during speech therapy to enhance the effects of treatment is not a new idea. Pharmacological augmentation of speech therapy has long been proposed as a means of increasing the magnitude of neuroplasticity during treatment to gain better recovery outcomes

[43]. However, clinical investigation of speech therapy paired with a wide range of drugs affecting neuromodulatory systems has generally shown mixed effects on recovery [43–45]. Further complicating the use of pharmacologically augmented speech therapy, while a number of these drugs of interest may enhance neuroplasticity, many have contraindications, particularly in those with underlying cardiovascular issues that can contribute to stroke. Many of these drugs activate neuromodulatory systems similar to those activated by VNS, but one critical difference is the timing of neuromodulation. The systemic nature of drugs do not lend to potentiation of circuits contributing to recovery from speech disorders, but instead, lead to a global, sustained activation of neuromodulatory systems. Alternatively, VNS-paired rehabilitation accounts for this lack of temporal specificity by only increasing neuromodulator levels transiently, allowing for the targeting of specific neural circuits mediating recovery.

Another approach to the problem of temporal specificity in neuromodulatory activity, transcranial direct current stimulation (tDCS) [46,47] and transcranial magnetic stimulation (TMS) [48] have been used in conjunction with speech therapy with more promising, yet still mixed outcomes. While timed bursts of tDCS and TMS may solve for the lack of temporal specificity seen in pharmacological augmentation of speech therapy, these treatments locally activate glutamatergic and GABAergic neurons [49,50], which may actively interfere with circuits mediating motor function. VNS, however, only increases neuromodulatory function and does not interfere with ongoing neural spiking [27–29,36,41]. The temporal specificity of VNS positions it as a promising alternative to drugs, tDCS, and TMS in treating motor speech dysfunction after stroke.

Applications for VNS-paired treatment of dysphagia

Post-stroke dysphagia is another commonly experienced disability, affecting approximately 50% to 75% of patients [5,11,51–53]. While post-stroke dysphagia is often acute, resolving within a month after injury, up to 40% of patients can still display disrupted swallowing a year after onset [54]. Chronic dysphagia increases risk for aspiration pneumonia, admittance to long-term care facilities, and death [53,55]. While behavioral mitigation strategies and diets limiting food consistency are common treatment prescriptions, these are often ineffective at improving long-term outcomes [56]. Similar to recovery from apraxia of speech, plasticity in orofacial motor areas in cortex appears to be a determinant of recovery of function in post-stroke dysphagia. After stroke, increases in oropharyngeal representation in the contralesional hemisphere accompany recovery from dysphagia [11,57]. Given the high comorbidity of post-stroke speech apraxia and dysphagia, their similarities in underlying pathologies, and their overlap in therapeutic strategies, VNS may prove an effective adjuvant to dysphagia treatment, such as oromotor exercises as well.

Conclusions

VNS has emerged as a method of enhancing rehabilitative outcomes for a wide range of neurological disorders. Here, we suggest pairing VNS with traditional speech therapy to enhance recovery from post-stroke speech motor dysfunction. We outline clinical success of VNS-paired physical rehabilitation after stroke, which demonstrates that VNS can induce plasticity in task-activated motor systems, enhancing patient recovery outcomes. Furthermore, we summarize the

observations that VNS can enhance plasticity in orofacial networks when paired with jaw movement, which supports its use as a potential adjuvant to speech therapy in treating motor speech dysfunction. Based on this evidence, we believe VNS-paired speech therapy shows promise as a means of enhancing recovery after post-stroke motor speech disorders, and future study of this new treatment has potential to increase function, and subsequently quality of life for the many suffers of these common conditions.

Sources of funding

This work was supported by the National Institutes of Health R01NS094384 and R01NS103803 and by the DARPA BTO Targeted Neuroplasticity Training (TNT) program under the auspices of Dr. Tristan McClure-Begley through the Space and Naval Warfare Systems Center, Pacific Grant/Contract No. N66001-17-2-4011.

Conflict of interest

MPK has a financial interest in MicroTransponder, Inc., which is developing VNS for stroke. All other authors declare no conflicts of interest.

References

[1] Engelter ST, Gostynski M, Papa S, Frei M, Born C, Ajdacic-Gross V, et al. Epidemiology of Aphasia Attributable to First Ischemic Stroke. Stroke 2006;37:1379–84. doi:10.1161/01.STR.0000221815.64093.8c.

[2] Laska AC, Hellblom A, Murray V, Kahan T, Von Arbin M. Aphasia in acute stroke and relation to outcome. J Intern Med 2001;249:413–22. doi:10.1046/j.1365-2796.2001.00812.x.

[3] Permsirivanich W, Tipchatyotin S, Wongchai M, Leelamanit V, Setthawatcharawanich S, Sathirapanya P, et al. Comparing the effects of rehabilitation swallowing therapy vs. neuromuscular electrical stimulation therapy among stroke patients with persistent pharyngeal dysphagia: a randomized controlled study. J Med Assoc Thai 2009;92:259–65.

[4] Flowers HL, Skoretz SA, Silver FL, Rochon E, Fang J, Flamand-Roze C, et al. Poststroke Aphasia Frequency, Recovery, and Outcomes: A Systematic Review and Meta-Analysis. Arch Phys Med Rehabil 2016;97:2188-2201.e8. doi:10.1016/j.apmr.2016.03.006.

[5] Stipancic KL, Borders JC, Brates D, Thibeault SL. Prospective Investigation of Incidence and Co-Occurrence of Dysphagia, Dysarthria, and Aphasia Following Ischemic Stroke. Am J Speech-Language Pathol 2019;28:188–94. doi:10.1044/2018_AJSLP-18-0136.

[6] Mitchell C, Gittins M, Tyson S, Vail A, Conroy P, Paley L, et al. Prevalence of aphasia and dysarthria among inpatient stroke survivors: describing the population, therapy provision and outcomes on discharge. Aphasiology 2020:1–11. doi:10.1080/02687038.2020.1759772.

[7] Mitchell C, Bowen A, Tyson S, Butterfint Z, Conroy P. Interventions for dysarthria due to stroke and other adult-acquired, non-progressive brain injury. Cochrane Database Syst Rev 2017. doi:10.1002/14651858.CD002088.pub3.

[8] Langhorne P, Bernhardt J, Kwakkel G. Stroke rehabilitation. Lancet 2011;377:1693–702. doi:10.1016/S0140-6736(11)60325-5.

[9] Wan CY, Zheng X, Marchina S, Norton A, Schlaug G. Intensive therapy induces contralateral white matter changes in chronic stroke patients with Broca's aphasia. Brain Lang 2014;136:1–7. doi:10.1016/j.bandl.2014.03.011.

[10] Schlaug G, Marchina S, Norton A. Evidence for Plasticity in White-Matter Tracts of Patients with Chronic Broca's Aphasia Undergoing Intense Intonation-based Speech Therapy. Ann N Y Acad Sci 2009;1169:385–94. doi:10.1111/j.1749-6632.2009.04587.x.

[11] Barritt AW, Smithard DG. Role of cerebral cortex plasticity in the recovery of swallowing function following dysphagic stroke. Dysphagia 2009. doi:10.1007/s00455-008-9162-3.

[12] Avivi-Arber L, Lee J-C, Yao D, Adachi K, Sessle BJ. Neuroplasticity of face sensorimotor cortex and implications for control of orofacial movements. Jpn Dent Sci Rev 2010;46:132–42. doi:10.1016/j.jdsr.2009.11.007.

[13] Ludlow CL, Hoit J, Kent R, Ramig LO, Shrivastav R, Strand E, et al. Translating Principles of Neural Plasticity Into Research on Speech Motor Control Recovery and Rehabilitation. J Speech, Lang Hear Res 2008;51. doi:10.1044/1092-4388(2008/019).

[14] Khedr EM, Abo-Elfetoh N. Therapeutic role of rTMS on recovery of dysphagia in patients with lateral medullary syndrome and brainstem infarction. J Neurol Neurosurg Psychiatry 2010;81:495–9. doi:10.1136/jnnp.2009.188482.

[15] Kumar S, Wagner CW, Frayne C, Zhu L, Selim M, Feng W, et al. Noninvasive Brain Stimulation May Improve Stroke-Related Dysphagia. Stroke 2011;42:1035–40. doi:10.1161/STROKEAHA.110.602128.

[16] Thiel A, Hartmann A, Rubi-Fessen I, Anglade C, Kracht L, Weiduschat N, et al. Effects of Noninvasive Brain Stimulation on Language Networks and Recovery in Early Poststroke Aphasia. Stroke 2013;44:2240–6. doi:10.1161/STROKEAHA.111.000574.

[17] Pruitt DT, Schmid AN, Kim LJ, Abe CM, Trieu JL, Choua C, et al. Vagus Nerve Stimulation Delivered with Motor Training Enhances Recovery of Function after Traumatic Brain Injury. J Neurotrauma 2016;33:871–9. doi:10.1089/neu.2015.3972.

[18] Khodaparast N, Hays SA, Sloan AM, Hulsey DR, Ruiz A, Pantoja M, et al. Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. Neurobiol Dis 2013;60:80–8. doi:10.1016/j.nbd.2013.08.002.

[19] Khodaparast N, Hays SA, Sloan AM, Fayyaz T, Hulsey DR, Rennaker RL, et al. Vagus Nerve Stimulation Delivered During Motor Rehabilitation Improves Recovery in a Rat Model of Stroke. Neurorehabil Neural Repair 2014;28:698–706. doi:10.1177/1545968314521006.

[20] Hays SA, Khodaparast N, Ruiz A, Sloan AM, Hulsey DR, Rennaker RL, et al. The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. Neuroreport 2014;25:682–8. doi:10.1097/WNR.0000000000000154.

[21] Hays SA, Ruiz A, Bethea T, Khodaparast N, Carmel JB, Rennaker RL, et al. Vagus nerve stimulation during rehabilitative training enhances recovery of forelimb function after ischemic stroke in aged rats. Neurobiol Aging 2016;43:111–8. doi:10.1016/j.neurobiolaging.2016.03.030.

[22] Meyers EC, Solorzano BR, James J, Ganzer PD, Lai ES, Rennaker RL, et al. Vagus Nerve Stimulation Enhances Stable Plasticity and Generalization of Stroke Recovery. Stroke 2018;49:710–7. doi:10.1161/STROKEAHA.117.019202.

[23] Ganzer PD, Darrow MJ, Meyers EC, Solorzano BR, Ruiz AD, Robertson NM, et al. Closed-loop neuromodulation restores network connectivity and motor control after spinal cord injury. Elife 2018;7:1–19. doi:10.7554/eLife.32058.

[24] Dawson J, Pierce D, Dixit A, Kimberley TJ, Robertson M, Tarver B, et al. Safety, Feasibility, and Efficacy of Vagus Nerve Stimulation Paired With Upper-Limb Rehabilitation After Ischemic Stroke. Stroke 2016;47:143–50. doi:10.1161/STROKEAHA.115.010477.

[25] Kimberley TJ, Pierce D, Prudente CN, Francisco GE, Yozbatiran N, Smith P, et al. Vagus nerve stimulation paired with upper limb rehabilitation after chronic stroke: A blinded randomized pilot study. Stroke 2018;49:2789–92. doi:10.1161/STROKEAHA.118.022279.

[26] Pruitt DT, Danaphongse TT, Lutchman M, Patel N, Reddy P, Wang V, et al. Optimizing Dosing of Vagus Nerve Stimulation for Stroke Recovery. Transl Stroke Res 2021;12:65–71. doi:10.1007/s12975-020-00829-6.

[27] Hulsey DR, Riley JR, Loerwald KW, Rennaker RL, Kilgard MP, Hays SA. Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. Exp Neurol 2017;289:21–30. doi:10.1016/j.expneurol.2016.12.005.

[28] Hulsey DR, Hays SA, Khodaparast N, Ruiz A, Das P, Rennaker RL, et al. Reorganization of Motor Cortex by Vagus Nerve Stimulation Requires Cholinergic Innervation. Brain Stimul 2016;9:174–81. doi:10.1016/j.brs.2015.12.007.

[29] Hulsey DR, Shedd CM, Sarker SF, Kilgard MP, Hays SA. Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. Exp Neurol 2019. doi:10.1016/j.expneurol.2019.112975.

[30] Nichols JA, Nichols AR, Smirnakis SM, Engineer ND, Kilgard MP, Atzori M. Vagus nerve stimulation modulates cortical synchrony and excitability through the activation of muscarinic receptors. Neuroscience 2011;189:207–14. doi:10.1016/j.neuroscience.2011.05.024.

[31] Dorr AE. Effect of Vagus Nerve Stimulation on Serotonergic and Noradrenergic Transmission. J Pharmacol Exp Ther 2006;318:890–8. doi:10.1124/jpet.106.104166.

[32] Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119:124–32. doi:10.1016/j.brainres.2006.08.048.

[33] Seol GH, Ziburkus J, Huang S, Song L, Kim IT, Takamiya K, et al. Neuromodulators Control the Polarity of Spike-Timing-Dependent Synaptic Plasticity. Neuron 2007;55:919–29. doi:10.1016/j.neuron.2007.08.013.

[34] He K, Huertas M, Hong SZ, Tie XX, Hell JW, Shouval H, et al. Distinct Eligibility Traces for LTP and LTD in Cortical Synapses. Neuron 2015. doi:10.1016/j.neuron.2015.09.037.

[35] Porter BA, Khodaparast N, Fayyaz T, Cheung RJ, Ahmed SS, Vrana WA, et al. Repeatedly Pairing Vagus Nerve Stimulation with a Movement Reorganizes Primary Motor Cortex. Cereb Cortex 2012;22:2365–74. doi:10.1093/cercor/bhr316.

[36] Morrison RA, Hulsey DR, Adcock KS, Rennaker RL, Kilgard MP, Hays SA. Vagus nerve stimulation intensity influences motor cortex plasticity. Brain Stimul 2019;12:256–62. doi:10.1016/j.brs.2018.10.017.

[37] Tseng C-T, Brougher J, Gaulding SJ, Hassan BS, Thorn CA. Vagus nerve stimulation promotes cortical reorganization and reduces task-dependent calorie intake in male and female rats. Brain Res 2020;1748:147099. doi:10.1016/j.brainres.2020.147099.

[38] Borland MS, Vrana WA, Moreno NA, Fogarty EA, Buell EP, Vanneste S, et al. Pairing vagus nerve stimulation with tones drives plasticity across the auditory pathway. J Neurophysiol 2019;122:659–71. doi:10.1152/jn.00832.2018.

[39] Hays SA, Khodaparast N, Hulsey DR, Ruiz A, Sloan AM, Rennaker RL, et al. Vagus Nerve Stimulation During Rehabilitative Training Improves Functional Recovery After Intracerebral Hemorrhage. Stroke 2014;45:3097–100. doi:10.1161/STROKEAHA.114.006654.

[40] Khodaparast N, Kilgard MP, Casavant R, Ruiz A, Qureshi I, Ganzer PD, et al. Vagus Nerve Stimulation During Rehabilitative Training Improves Forelimb Recovery After Chronic Ischemic Stroke in Rats. Neurorehabil Neural Repair 2016;30:676–84. doi:10.1177/1545968315616494.

[41] Morrison RA, Danaphongse TT, Pruitt DT, Adcock KS, Mathew JK, Abe ST, et al. A limited range of vagus nerve stimulation intensities produce motor cortex reorganization when delivered during training. Behav Brain Res 2020;391:112705. doi:10.1016/j.bbr.2020.112705.

[42] Morrison RA, Danaphongse TT, Abe ST, Stevens ME, Ezhil V, Seyedahmadi A, et al. High intensity VNS disrupts VNS-mediated plasticity in motor cortex. Brain Res 2021;1756:147332. doi:10.1016/j.brainres.2021.147332.

[43] Berthier ML. Poststroke Aphasia. Drugs Aging 2005;22:163–82. doi:10.2165/00002512-200522020-00006.

[44] Noble S, Benfield P. Piracetam. CNS Drugs 1998;9:497–511. doi:10.2165/00023210-199809060-00006.

[45] McNeil MR, Doyle PJ, Spencer KA, Goda AJ, Flores D, Small SL. A double-blind, placebo-controlled study of pharmacological and behavioural treatment of lexical-semantic deficits in aphasia. Aphasiology 1997;11:385–400. doi:10.1080/02687039708248479.

[46] Elsner B, Kugler J, Pohl M, Mehrholz J. Transcranial direct current stimulation (tDCS) for improving aphasia in patients with aphasia after stroke. Cochrane Database Syst Rev 2015. doi:10.1002/14651858.CD009760.pub3.

[47] Doeltgen SH, Bradnam L V., Young JA, Fong E. Transcranial non-invasive brain stimulation in swallowing rehabilitation following stroke — A review of the literature. Physiol Behav 2015;143:1–9. doi:10.1016/j.physbeh.2015.02.025.

[48] Naeser MA, Martin PI, Ho M, Treglia E, Kaplan E, Bashir S, et al. Transcranial Magnetic Stimulation and Aphasia Rehabilitation. Arch Phys Med Rehabil 2012;93:S26–34. doi:10.1016/j.apmr.2011.04.026.

[49] Medeiros LF, de Souza ICC, Vidor LP, de Souza A, Deitos A, Volz MS, et al. Neurobiological Effects of Transcranial Direct Current Stimulation: A Review. Front Psychiatry 2012;3. doi:10.3389/fpsyt.2012.00110.

[50] Cirillo G, Di Pino G, Capone F, Ranieri F, Florio L, Todisco V, et al. Neurobiological after-effects of non-invasive brain stimulation. Brain Stimul 2017;10:1–18. doi:10.1016/j.brs.2016.11.009.

[51] Mann G, Hankey GJ, Cameron D. Swallowing Function After Stroke. Stroke 1999;30:744–8. doi:10.1161/01.STR.30.4.744.

[52] Singh S. Dysphagia in stroke patients. Postgrad Med J 2006;82:383–91. doi:10.1136/pgmj.2005.043281.

[53] Martino R, Foley N, Bhogal S, Diamant N, Speechley M, Teasell R. Dysphagia After Stroke. Stroke 2005;36:2756–63. doi:10.1161/01.STR.0000190056.76543.eb.

[54] Terré R, Mearin F. Resolution of Tracheal Aspiration After the Acute Phase of Stroke-Related Oropharyngeal Dysphagia. Am J Gastroenterol 2009;104:923–32. doi:10.1038/ajg.2008.160.

[55] Smithard DG, Smeeton NC, Wolfe CDA. Long-term outcome after stroke: does dysphagia matter? Age Ageing 2006;36:90–4. doi:10.1093/ageing/afl149.

[56] Carnaby G, Hankey GJ, Pizzi J. Behavioural intervention for dysphagia in acute stroke: a randomised controlled trial. Lancet Neurol 2006;5:31–7. doi:10.1016/S1474-4422(05)70252-0.

[57] Hamdy S, Aziz Q, Rothwell JC, Power M, Singh KD, Nicholson DA, et al. Recovery of swallowing after dysphagic stroke relates to functional reorganization in the intact motor cortex. Gastroenterology 1998;115:1104–12. doi:10.1016/s0016-5085(98)70081-2.

[58] Darrow MJ, Torres M, Sosa MJ, Danaphongse TT, Haider Z, Rennaker RL, et al. Vagus Nerve Stimulation Paired With Rehabilitative Training Enhances Motor Recovery After Bilateral Spinal Cord Injury to Cervical Forelimb Motor Pools. Neurorehabil Neural Repair 2020;34:200–9. doi:10.1177/1545968319895480.

[59] Kilgard MP, Rennaker RL, Hays SA, Wigginton JG, Hamilton R, Foreman M, et al. Targeted Plasticity Therapy for Upper Limb Rehabilitation in Spinal Cord Injuries. ClinicaltrialsGov 2021:NCT04288245. https://clinicaltrials.gov/ct2/show/NCT04288245.

[60] Meyers EC, Kasliwal N, Solorzano BR, Lai E, Bendale G, Berry A, et al. Enhancing plasticity in central networks improves motor and sensory recovery after nerve damage. Nat Commun 2019;10:5782. doi:10.1038/s41467-019-13695-0.

[61] Engineer CT, Engineer ND, Riley JR, Seale JD, Kilgard MP. Pairing Speech Sounds With Vagus Nerve Stimulation Drives Stimulus-specific Cortical Plasticity. Brain Stimul 2015;8:637–44. doi:10.1016/j.brs.2015.01.408.

[62] Engineer ND, Riley JR, Seale JD, Vrana WA, Shetake JA, Sudanagunta SP, et al. Reversing pathological neural activity using targeted plasticity. Nature 2011;470:101–4. doi:10.1038/nature09656.

[63] De Ridder D, Vanneste S, Engineer ND, Kilgard MP. Safety and Efficacy of Vagus Nerve Stimulation Paired With Tones for the Treatment of Tinnitus: A Case Series. Neuromodulation Technol Neural Interface 2014;17:170–9. doi:10.1111/ner.12127.

[64] Vanneste S, Martin J, Rennaker RL, Kilgard MP. Pairing sound with vagus nerve stimulation modulates cortical synchrony and phase coherence in tinnitus: An exploratory retrospective study. Sci Rep 2017;7:17345. doi:10.1038/s41598-017-17750-y.

[65] Tyler R, Cacace A, Stocking C, Tarver B, Engineer N, Martin J, et al. Vagus Nerve Stimulation Paired with Tones for the Treatment of Tinnitus: A Prospective Randomized Double-blind Controlled Pilot Study in Humans. Sci Rep 2017;7:11960. doi:10.1038/s41598-017-12178-w.

[66] De Ridder D, Kilgard M, Engineer N, Vanneste S. Placebo-controlled vagus nerve stimulation paired with tones in a patient with refractory tinnitus: A case report. Otol Neurotol 2015. doi:10.1097/MAO.0000000000000704.

[67] Kilgard MP, Rennaker RL, Alexander J, Dawson J. Vagus nerve stimulation paired with tactile training improved sensory function in a chronic stroke patient. NeuroRehabilitation 2018;42:159–65. doi:10.3233/NRE-172273.

[68] Darrow MJ, Mian TM, Torres M, Haider Z, Danaphongse T, Seyedahmadi A, et al. The tactile experience paired with vagus nerve stimulation determines the degree of sensory recovery after chronic nerve damage. Behav Brain Res 2021;396:112910. doi:10.1016/j.bbr.2020.112910.

[69] Darrow MJ, Mian TM, Torres M, Haider Z, Danaphongse T, Rennaker RL, et al. Restoration of Somatosensory Function by Pairing Vagus Nerve Stimulation with Tactile Rehabilitation. Ann Neurol 2020;87:194–205. doi:10.1002/ana.25664.

[70] Noble LJ, Gonzalez IJ, Meruva VB, Callahan KA, Belfort BD, Ramanathan KR, et al. Effects of vagus nerve stimulation on extinction of conditioned fear and post-traumatic stress disorder symptoms in rats. Transl Psychiatry 2017;7:e1217–e1217. doi:10.1038/tp.2017.191.

[71] Pena DF, Childs JE, Willett S, Vital A, McIntyre CK, Kroener S. Vagus nerve stimulation enhances extinction of conditioned fear and modulates plasticity in the pathway from the ventromedial prefrontal cortex to the amygdala. Front Behav Neurosci 2014;8. doi:10.3389/fnbeh.2014.00327.

[72] Souza RR, Robertson NM, Mathew E, Tabet MN, Bucksot JE, Pruitt DT, et al. Efficient parameters of vagus nerve stimulation to enhance extinction learning in an extinction-resistant rat model of PTSD. Prog Neuro-Psychopharmacology Biol Psychiatry 2020;99:109848. doi:10.1016/j.pnpbp.2019.109848.

[73] George MS, Ward HE, Ninan PT, Pollack M, Nahas Z, Anderson B, et al. A pilot study of vagus nerve stimulation (VNS) for treatment-resistant anxiety disorders. Brain Stimul 2008;1:112–21. doi:10.1016/j.brs.2008.02.001.

[74] Kilgard MP, Hays SA, Rennaker RL, Wigginton JG, Foreman M, Powers M, et al. Targeted Plasticity Therapy for Posttraumatic Stress Disorder. ClinicaltrialsGov 2020:NCT04064762. https://clinicaltrials.gov/ct2/show/NCT04064762.

[75] Henry ML, Hubbard HI, Grasso SM, Mandelli ML, Wilson SM, Sathishkumar MT, et al. Retraining speech production and fluency in non-fluent/agrammatic primary progressive aphasia. Brain 2018;141:1799–814. doi:10.1093/brain/awy101.

[76] Mackenzie C, Muir M, Allen C, Jensen A. Non-speech oro-motor exercises in post-stroke dysarthria intervention: a randomized feasibility trial. Int J Lang Commun Disord 2014;49:602–17. doi:10.1111/1460-6984.12096.

[77] Katz WF, McNeil MR. Studies of Articulatory Feedback Treatment for Apraxia of Speech Based on Electromagnetic Articulography. Perspect Neurophysiol Neurogenic Speech Lang Disord 2010;20:73–9. doi:10.1044/nnsld20.3.73.

[78] Ballard KJ, Wambaugh JL, Duffy JR, Layfield C, Maas E, Mauszycki S, et al. Treatment for Acquired Apraxia of Speech: A Systematic Review of Intervention Research Between 2004 and 2012. Am J Speech-Language Pathol 2015;24:316–37. doi:10.1044/2015_AJSLP-14-0118.

[79] Sickert A, Anders L-C, Munte TF, Sailer M. Constraint-induced aphasia therapy following sub-acute stroke: a single-blind, randomised clinical trial of a modified therapy schedule. J Neurol Neurosurg Psychiatry 2014;85:51–5. doi:10.1136/jnnp-2012-304297.

[80] Zhang J, Yu J, Bao Y, Xie Q, Xu Y, Zhang J, et al. Constraint-induced aphasia therapy in post-stroke aphasia rehabilitation: A systematic review and meta-analysis of randomized controlled trials. PLoS One 2017;12:e0183349. doi:10.1371/journal.pone.0183349.

[81] Carnaby GD, LaGorio L, Silliman S, Crary M. Exercise-based swallowing intervention (McNeill Dysphagia Therapy) with adjunctive NMES to treat dysphagia post-stroke: A double-blind placebo-controlled trial. J Oral Rehabil 2020;47:501–10. doi:10.1111/joor.12928.

[82] Nardo D, Holland R, Leff AP, Price CJ, Crinion JT. Less is more: Neural mechanisms underlying anomia treatment in chronic aphasic patients. Brain 2017. doi:10.1093/brain/awx234.

[83] Basso A. "Natural" conversation: A treatment for severe aphasia. Aphasiology 2010;24:466–79. doi:10.1080/02687030802714165.

APPENDIX A

SUPPLEMENTAL FIGURES FOR CHAPTER 2

Authors: Robert A. Morrison[1,2,*], Daniel R. Hulsey[2], Katherine S. Adcock[1,2], Robert L. Rennaker II[1,2,3], Michael P. Kilgard[1,2,3], Seth A. Hays[1,2,3]

Affiliations:

[1] The University of Texas at Dallas, School of Behavioral Brain Sciences, Richardson, TX.

[2] The University of Texas at Dallas, Texas Biomedical Device Center, Richardson, TX.

[3] The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, Richardson, TX.

* Correspondence to: robert.morrison@utdallas.edu

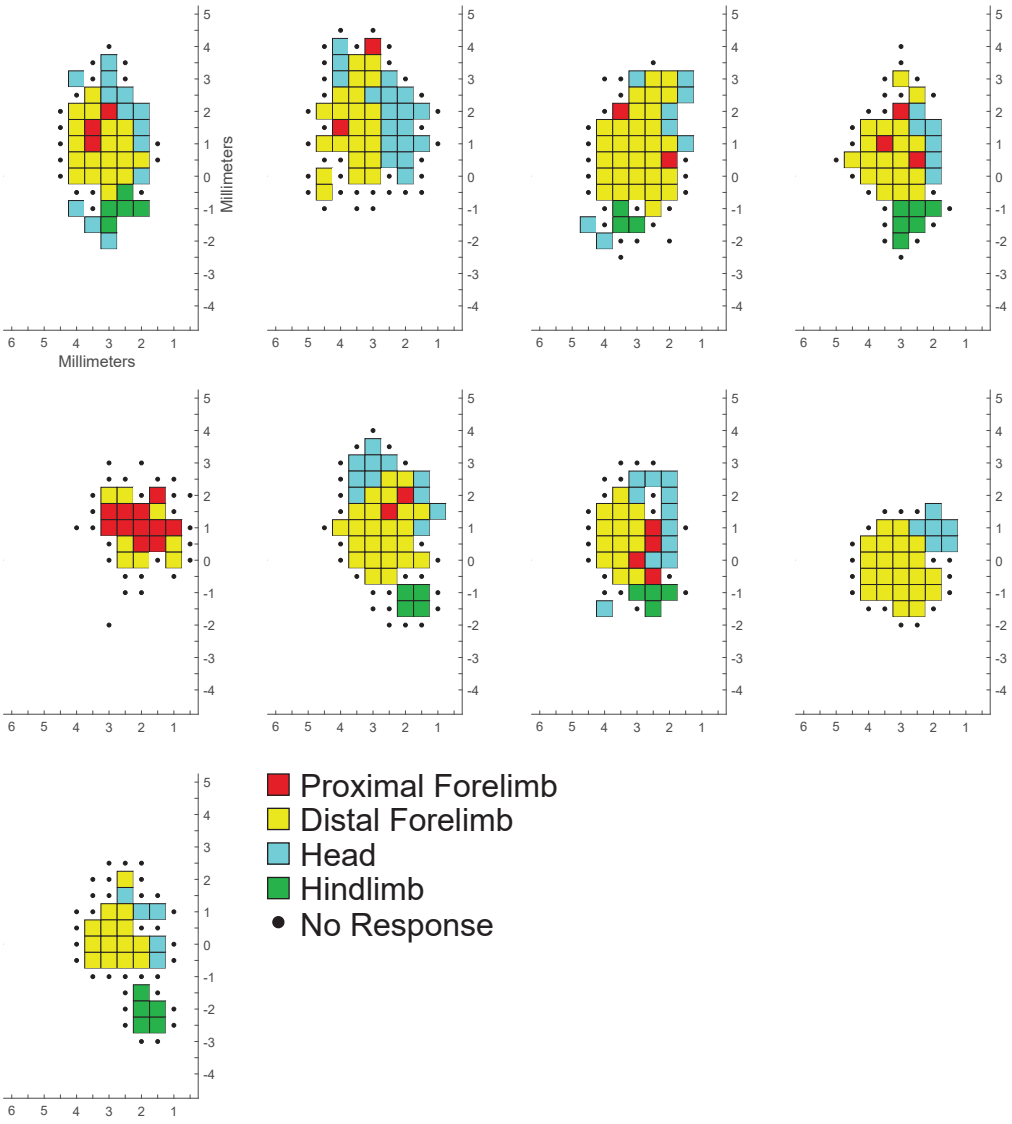
Reprinted with permission from Elsevier: *Brain Stimulation*, Volume 12, Issue 2, 2019

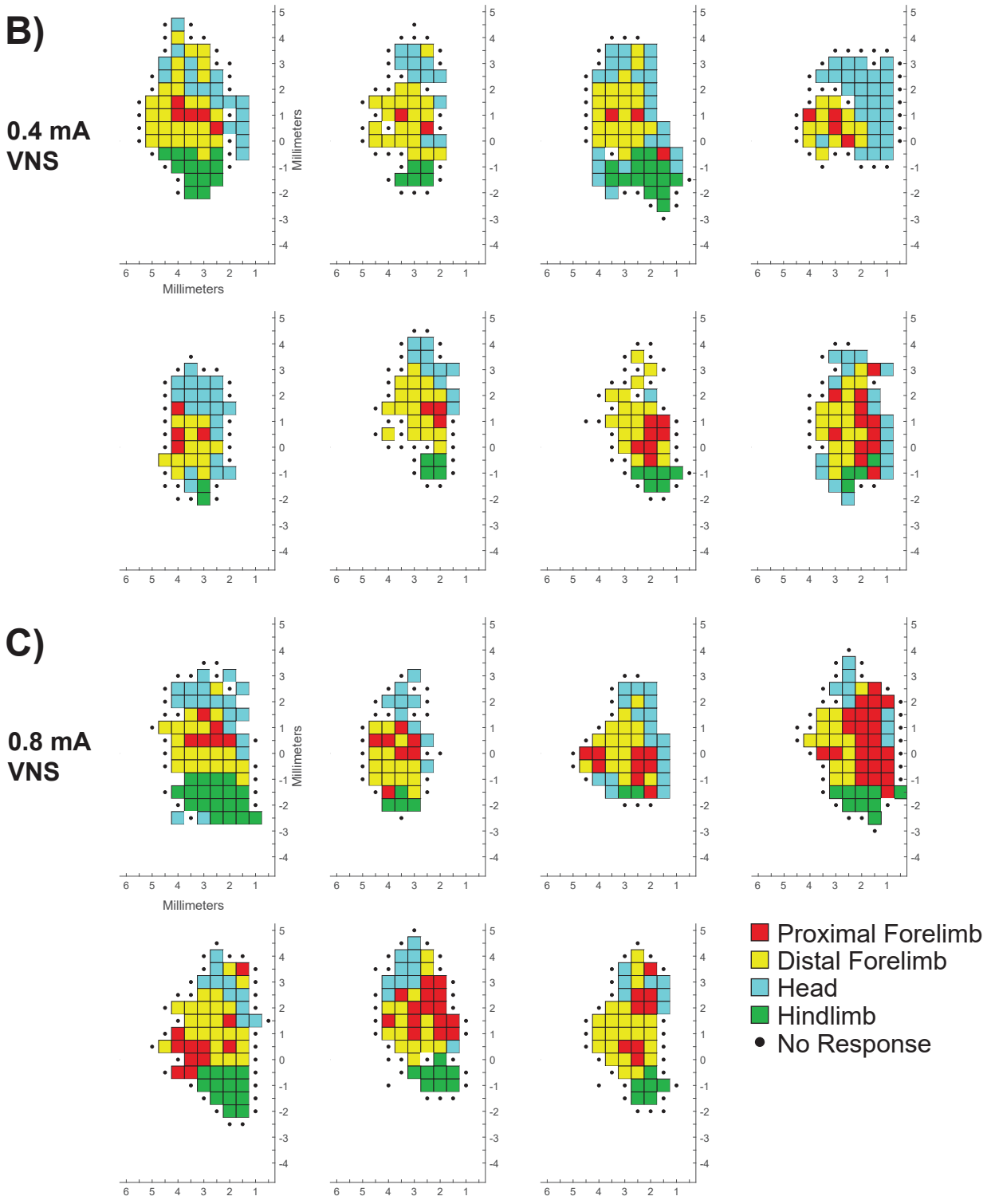
> *Vagus nerve stimulation intensity influences motor cortex plasticity*, Robert A. Morrison, Daniel R. Hulsey, Katherine S. Adcock, Robert L. Rennaker, Michael P. Kilgard, Seth A. Hays, *Brain Stimulation*, ISSN 1935-861X, https://doi.org/10.1016/j.brs.2018.10.017.

Contents:

A)

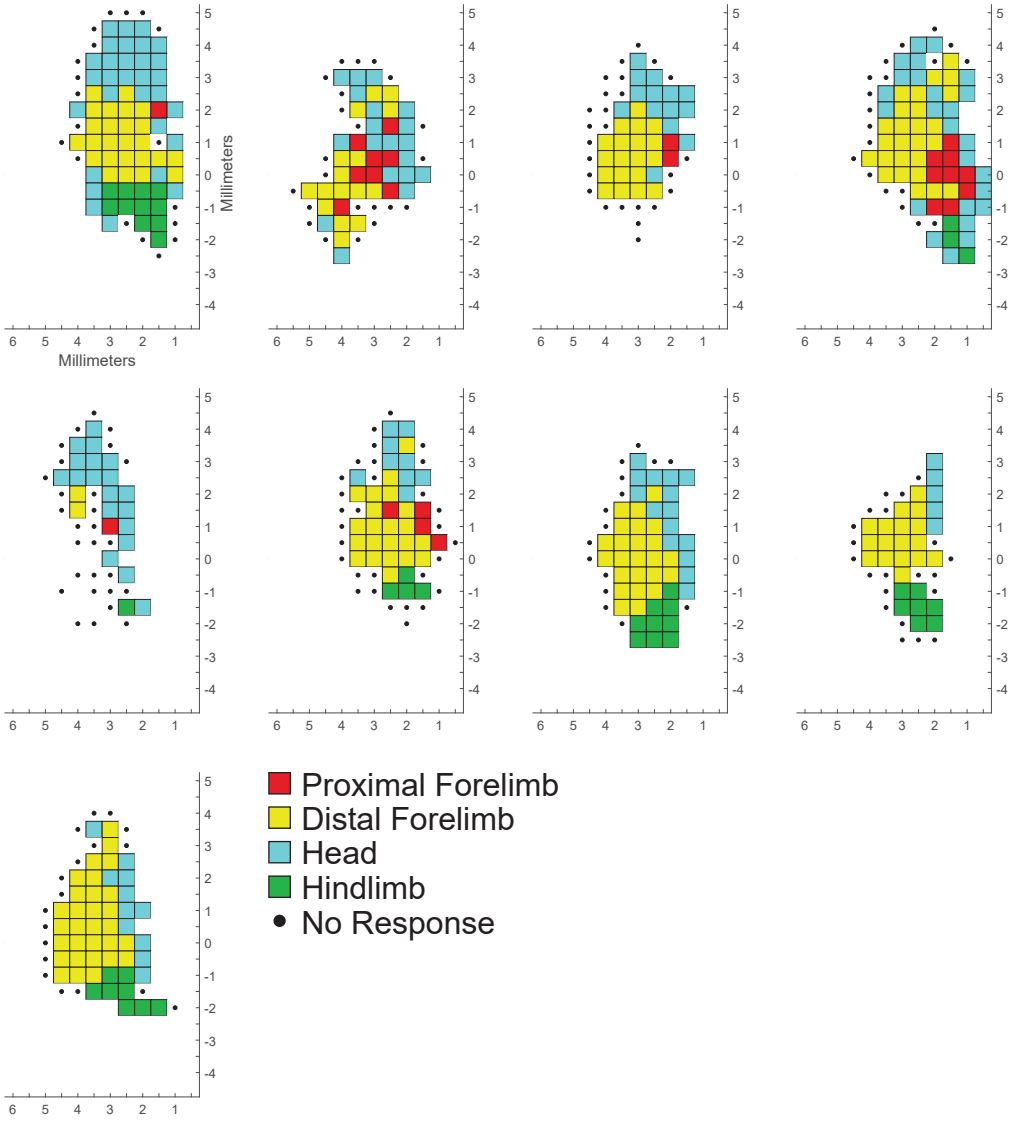
Sham


Millimeters
Millimeters
Proximal Forelimb
Distal Forelimb
Head
Hindlimb
No Response


B)
0.4 mA
VNS
C)
0.8 mA
VNS
Millimeters
Millimeters
Proximal Forelimb
Distal Forelimb
Head
Hindlimb
No Response

D)

1.6 mA VNS


Millimeters
Millimeters
Proximal Forelimb
Distal Forelimb
Head
Hindlimb
No Response

APPENDIX B

SUPPLEMENTAL FIGURES FOR CHAPTER 3

Authors: Robert A. Morrison[1,2,*], Tanya T. Danaphongse[2], David T. Pruitt[2], Katherine S. Adcock[1,2], Jobin K. Mathew[2], Stephanie T. Abe[2], Dina M. Abdulla[1,2], Robert L. Rennaker[1,2], Michael P. Kilgard[1,2], Seth A. Hays[2,3]

Affiliations:

[1] The University of Texas at Dallas, School of Behavioral Brain Sciences, Richardson, TX.

[2] The University of Texas at Dallas, Texas Biomedical Device Center, Richardson, TX.

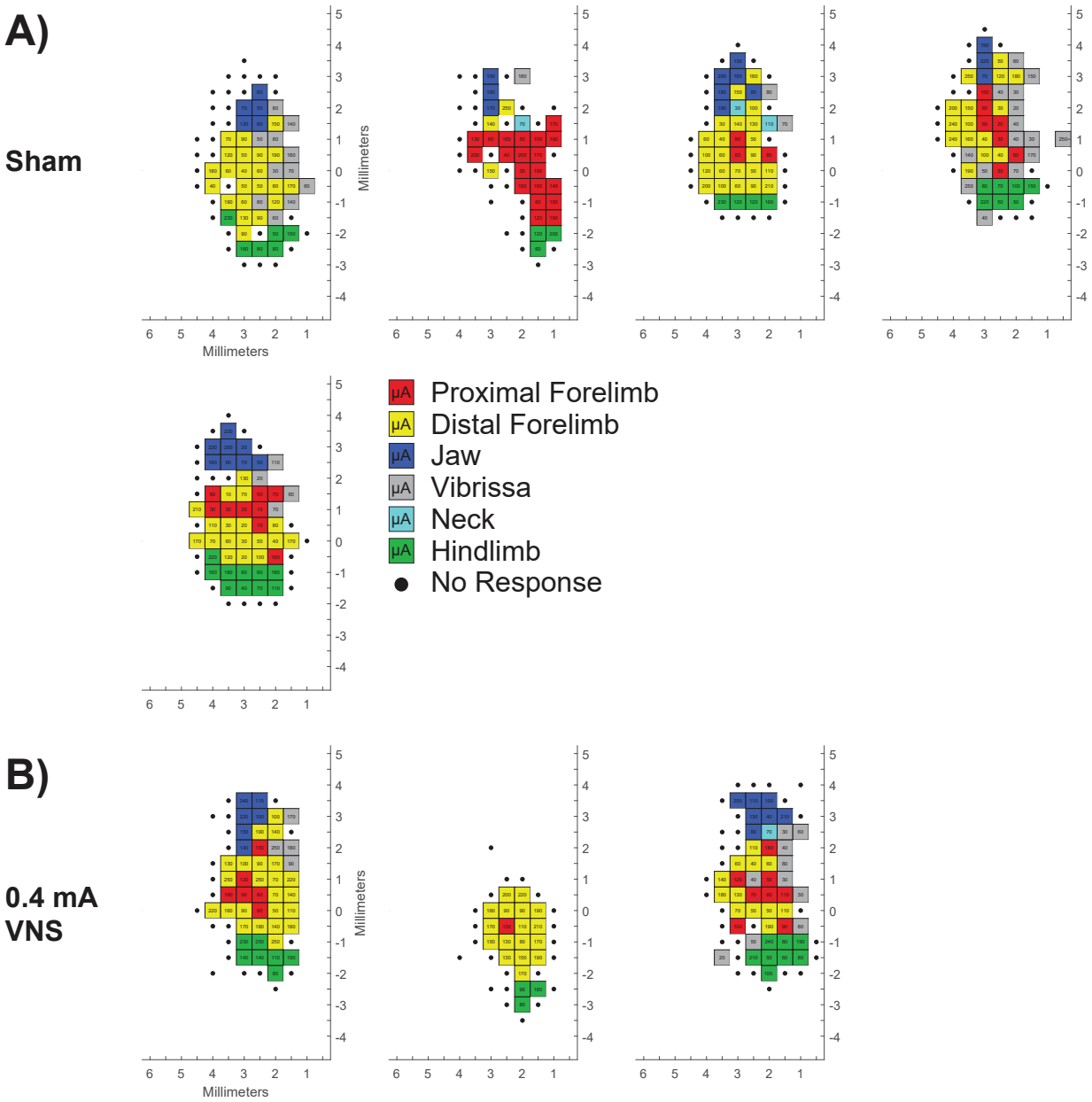
[3] The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, Richardson, TX.

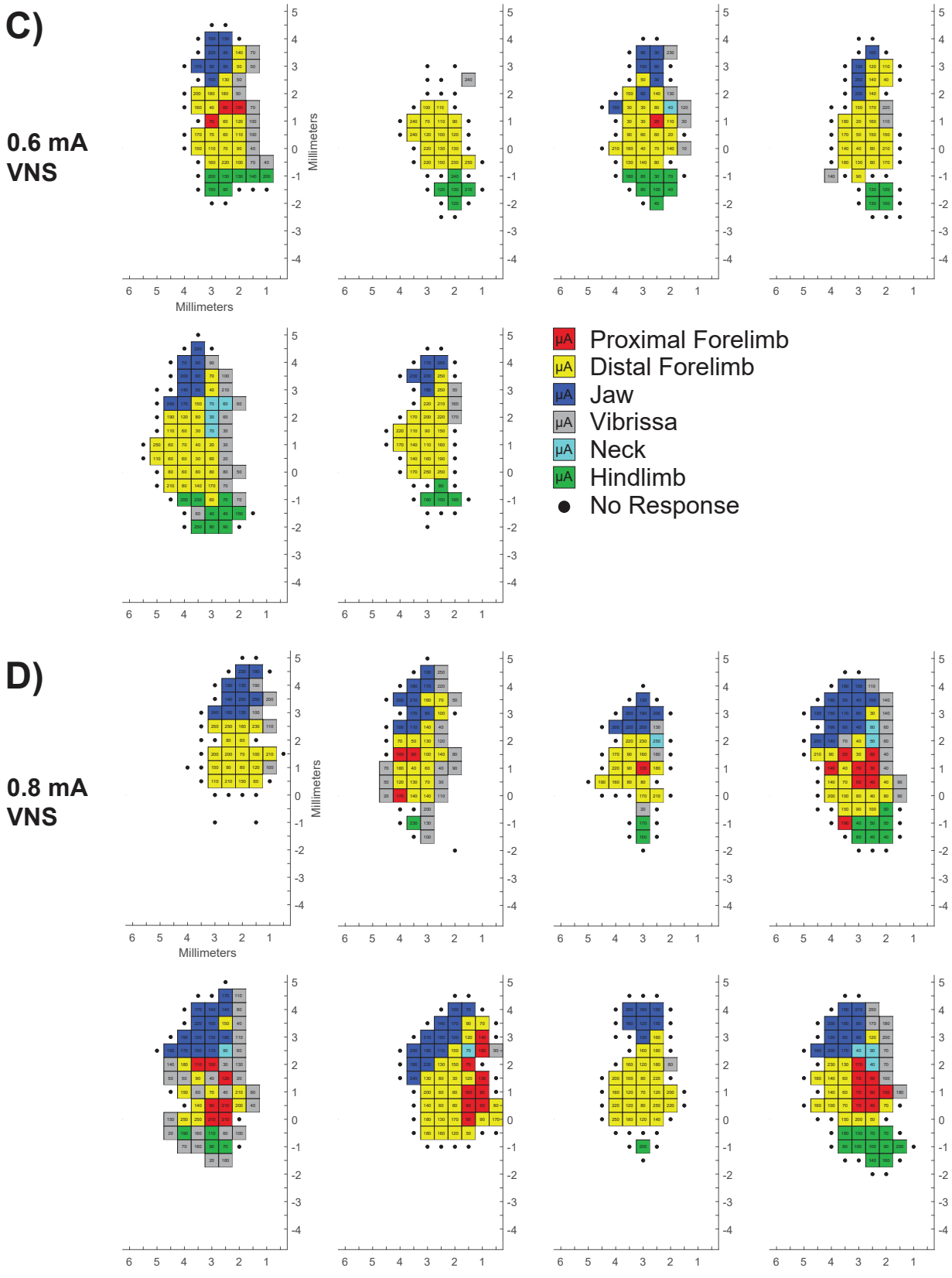
* Correspondence to: robert.morrison@utdallas.edu

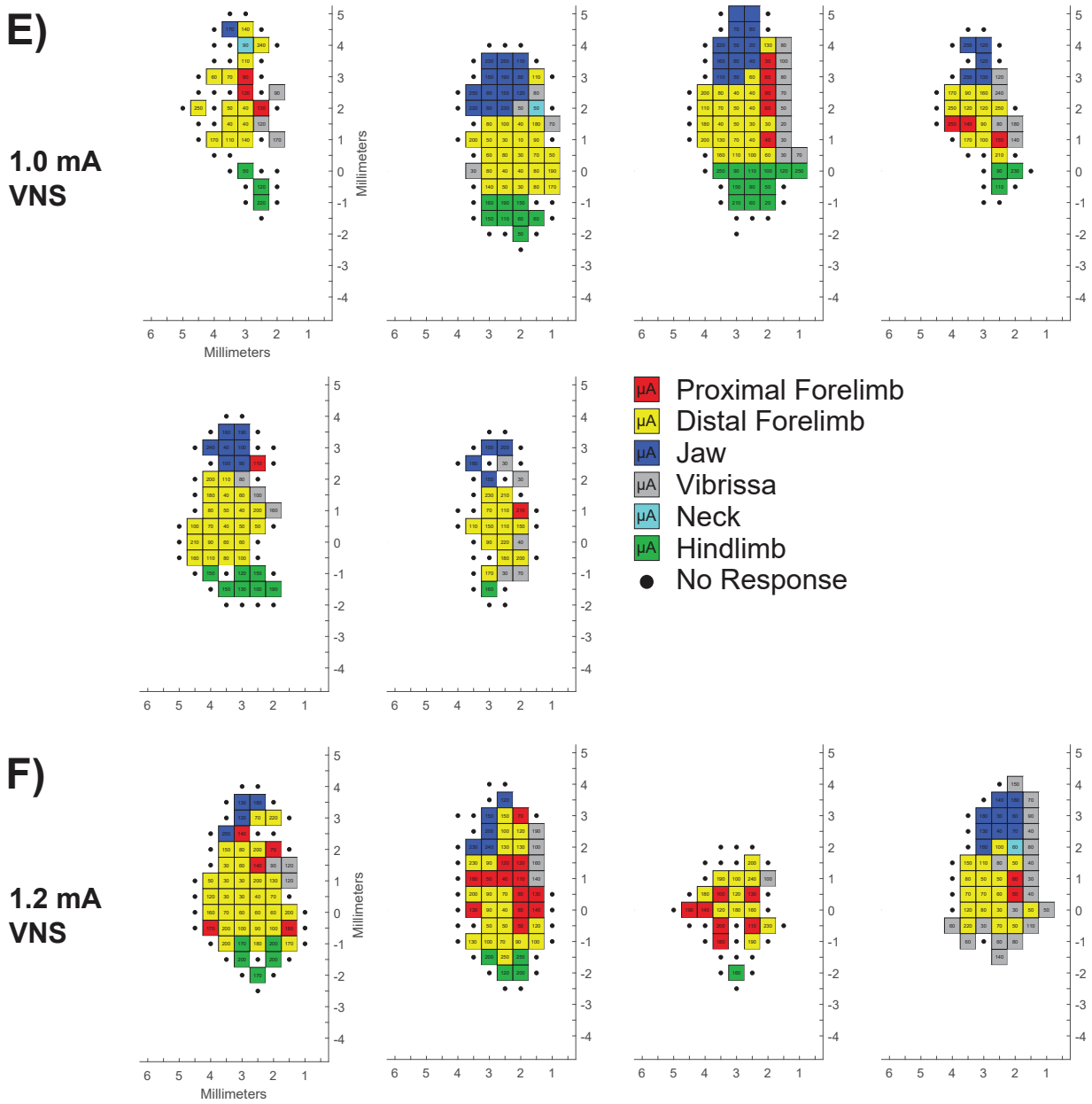
Reprinted with permission from Elsevier*: Behavioural Brain Research*, Volume 391, 2020

> *A limited range of vagus nerve stimulation intensities produce motor cortex reorganization when delivered during training,* Robert A. Morrison, Tanya T. Danaphongse, David T. Pruitt, Katherine S. Adcock, Jobin K. Mathew, Stephanie T. Abe, Dina M. Abdulla, Robert L. Rennaker, Michael P. Kilgard, Seth A. Hays*, Behavioural Brain Research*, ISSN 0166-4328, https://doi.org/10.1016/j.bbr.2020.112705.

Contents:


A)
Sham
Millimeters
Millimeters
μA Proximal Forelimb
μA Distal Forelimb
μA Jaw
μA Vibrissa
μA Neck
μA Hindlimb
No Response
B)
0.4 mA
VNS
Millimeters


C)
0.6 mA
VNS
D)
0.8 mA
VNS
Millimeters
Millimeters
Proximal Forelimb
Distal Forelimb
Jaw
Vibrissa
Neck
Hindlimb
No Response


E)
1.0 mA
VNS
F)
1.2 mA
VNS
Millimeters
Millimeters
μA Proximal Forelimb
μA Distal Forelimb
μA Jaw
μA Vibrissa
μA Neck
μA Hindlimb
No Response

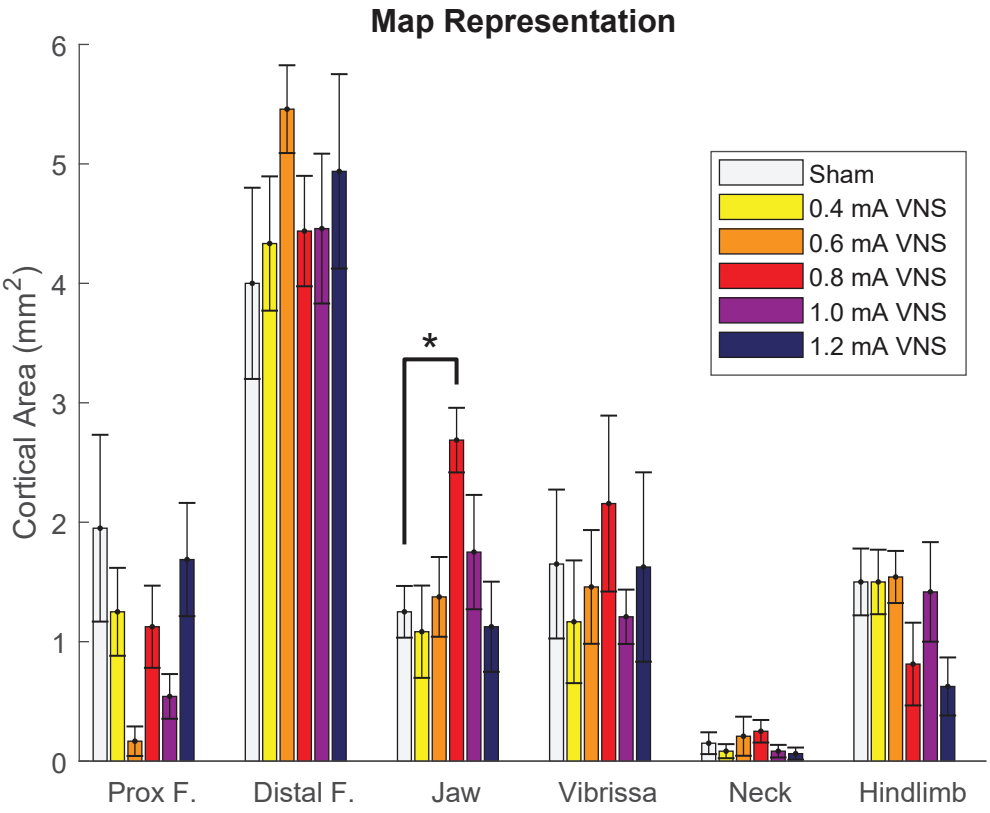


Figure B.2: All movement representations from ICMS for Chapter 3

All movement representations from ICMS. Bars represent mean ± SEM. "*" indicates $p < 0.01$

APPENDIX C

SUPPLEMENTAL FIGURES FOR CHAPTER 4

Authors: Robert A. Morrison[1,2,*], Tanya T. Danaphongse[2], Stephanie T. Abe[2], Madison E. Stevens[2], Vikram Ezhil[1,2], Armin Seyedahmadi[2], Katherine S. Adcock[1,2], Robert L. Rennaker[1,2], Michael P. Kilgard[1,2], Seth A. Hays[1,2,3]

Affiliations:

[1] The University of Texas at Dallas, School of Behavioral Brain Sciences, Richardson, TX.

[2] The University of Texas at Dallas, Texas Biomedical Device Center, Richardson, TX.

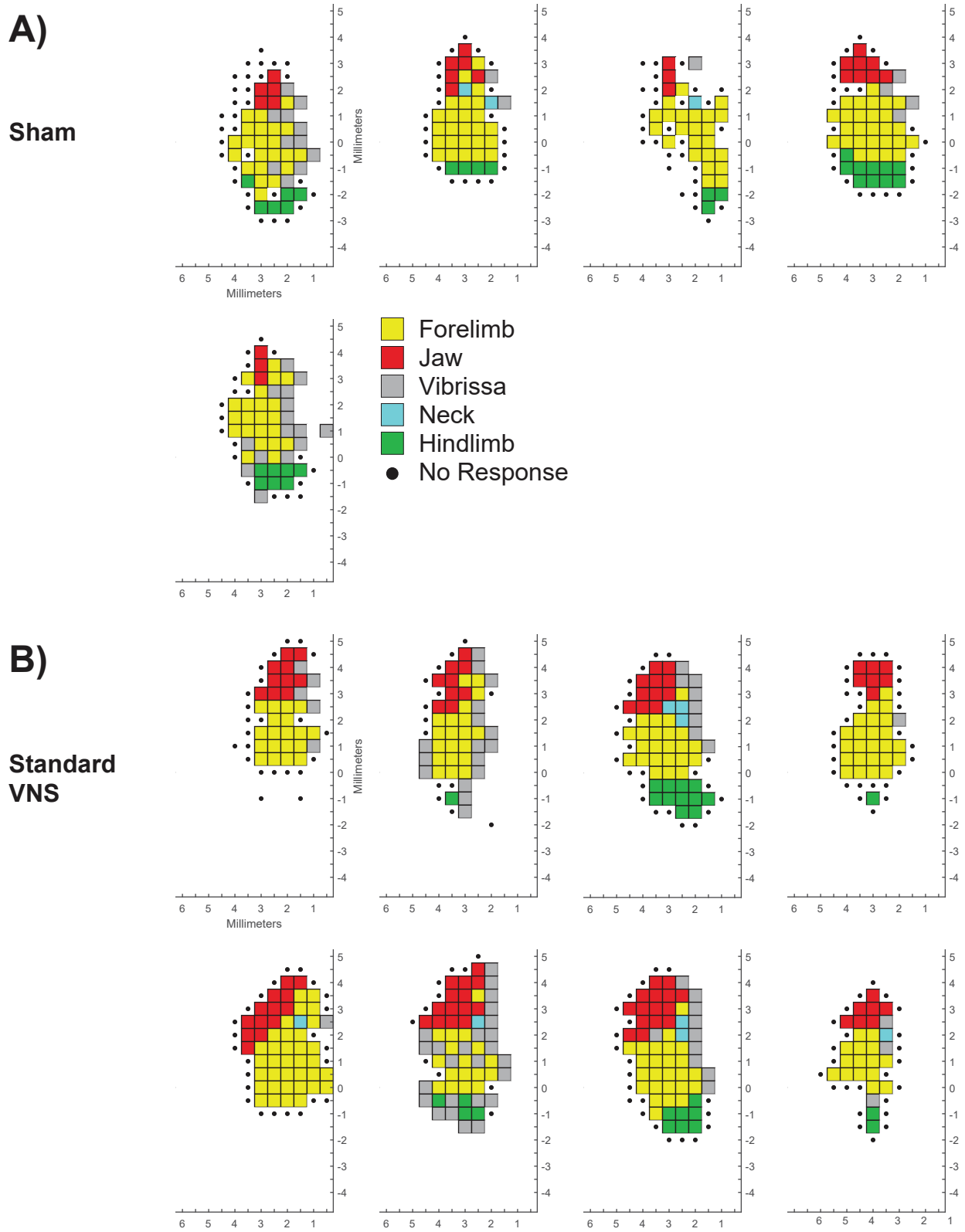
[3] The University of Texas at Dallas, Erik Jonsson School of Engineering and Computer Science, Richardson, TX.

[*] Correspondence to: robert.morrison@utdallas.edu

Reprinted with permission from Elsevier*:* *Brain Research*, Volume 1756, 2021

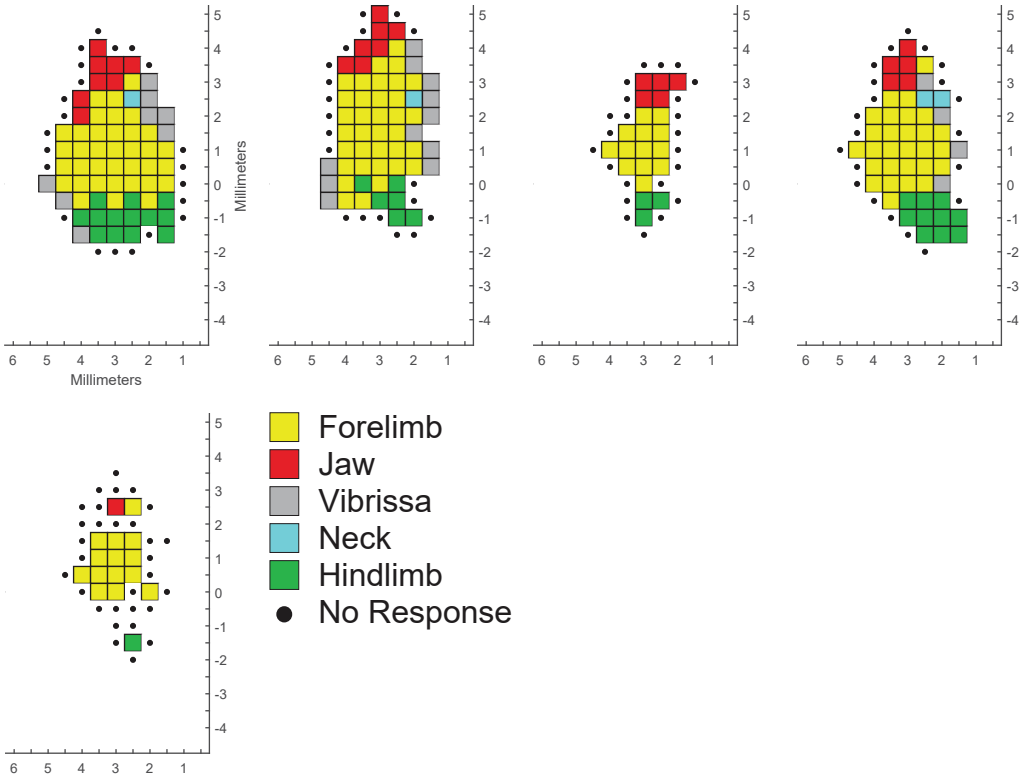
> *High intensity VNS disrupts VNS-mediated plasticity in motor cortex,* Robert A. Morrison, Tanya T. Danaphongse, Stephanie T. Abe, Madison E. Stevens, Vikram Ezhil, Armin Seyedahmadi, Katherine S. Adcock, Robert L. Rennaker, Michael P. Kilgard, Seth A. Hays*, Brain Research*, ISSN 0006-8993, https://doi.org/10.1016/j.brainres.2021.147332.

Contents:


A)
Sham
B)
Standard
VNS
Millimeters
Millimeters
Forelimb
Jaw
Vibrissa
Neck
Hindlimb
No Response

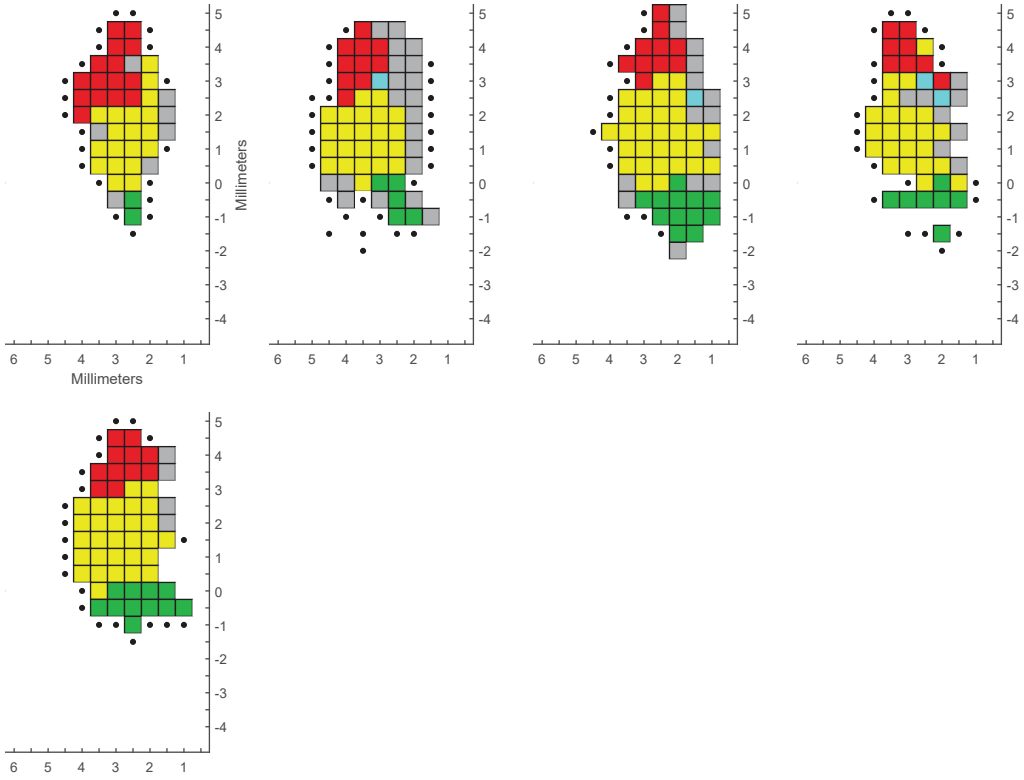
C)

Interleaved VNS



D)

Short VNS



BIOGRAPHICAL SKETCH

Robert Morrison grew up in Georgetown, TX, where he graduated high school in 2011. He then earned his Bachelors of Science in 2015 and his Masters of Science in 2018 from The University of Texas at Dallas. Robert conducted his doctoral work under the instruction of Dr. Michael Kilgard and Dr. Seth Hays, researching the use of neural implants to enhance synaptic plasticity supporting recovery after stroke and other neurological injuries. His training took place in a diverse, multidisciplinary environment at The Texas Biomedical Device Center, where he worked closely alongside engineers and clinical scientists. This allowed him to follow the impact of his studies from the lab to the clinic, giving Robert a wide, translational perspective of his work.

CURRICULUM VITAE

Robert Andrew Morrison, PhD

University of Texas at Dallas, Texas Biomedical Device Center
Robert.Morrison@utdallas.edu, Robert.Andrew.Morrison@gmail.com

EDUCATION

PhD, Cognition and Neuroscience **2016 - 2021**
University of Texas at Dallas
Advisors: Michael Kilgard, PhD; Seth Hays, PhD

MS, Applied Cognition and Neuroscience **2018**
University of Texas at Dallas
Focus in Systems Neuroscience

BS, Neuroscience **2015**
University of Texas at Dallas

RESEARCH EXPERIENCE

Graduate Research Assistant **2016 - 2021**
Texas Biomedical Device Center, University of Texas at Dallas
Advisors: Michael Kilgard, PhD; Seth Hays, PhD

- Researched the relationship between plasticity and recovery after neurological injury following rehabilitation paired with targeted plasticity therapy (TPT).
- Developed a novel rat behavioral paradigm that allowed for faster identification of effective stimulation parameters in TPT.
- Managed a team of 10-20 undergraduate researchers.
- Organized and led a bi-monthly journal club for graduate students starting in 2018.
- Conducted vagal nerve cuff implants and intracortical microstimulation surgeries.

Research Technician **2015 - 2016**
Rennaker Neural Engineering Lab, University of Texas at Dallas
Advisors: David Pruitt, PhD; Robert Rennaker, PhD

- Conducted behavioral experiments investigating vagus nerve stimulation's effect on motor recovery after traumatic brain injury and stroke in rats, and investigated the pathological effects of traumatic brain injury on cortical plasticity.
- Assisted in motor cortex intracortical microstimulation experiments.
- Performed paraformaldehyde perfusions and harvested brain tissue for histology.

Undergraduate Researcher **2014 - 2015**
Rennaker Neural Engineering Lab, University of Texas at Dallas
Advisors: David Pruitt, PhD

- Conducted rat behavioral experiments using vagus nerve stimulation paired with motor rehabilitation to treat models of traumatic brain injury and Parkinson's disease.

ACADEMIC EXPERIENCE

Graduate Teaching Assistant
School of Behavioral and Brain Sciences, University of Texas at Dallas

NSC 4356.001 – Neurophysiology, Professor: Dr. Rukhsana Sultana **Spring 2021**

- Single graduate TA for class of ~150 students. Held online office hours, managed a team of undergraduate TAs and the creation of review sessions, and graded coursework.

NSC 3361.002 – Introduction to Neuroscience, Professor: Dr. Rukhsana Sultana **Fall 2020**

- Graduate TA for class of ~100 students. Held online office hours and held weekly review sessions.

NSC 3361.001 – Introduction to Neuroscience, Professor: Dr. Van Miller **Fall 2019**

- Single graduate TA for class of 300 students. Held office hours, managed a team of undergraduate TAs and the creation of review sessions, graded coursework, and taught two lectures.

NSC 3361.003 – Introduction to Neuroscience, Professor: Dr. Xiaosi Gu **Fall 2016**

- Single TA for class of 98 students. Organized and conducted review sessions, held office hours, graded course materials, and taught five full lectures.

Neuroscience Tutor & Team Manager **2013 - 2015**
Student Success Center, University of Texas at Dallas

- Held office hours and offered one on one and small group tutoring and review for neuroscience courses.
- Managed a team of ~12 undergraduate tutors in various subjects, conducted performance evaluations, and held training sessions in tutoring and student crisis management.

AWARDS & HONORS

Dissertation Research Award: **$1500**, UT Dallas Office of Graduate Education	**2020**
Research Competition Finalist: **$50**, School of Behavioral and Brain Sciences	**2020**
PhD Research Small Grant: **$900**, UT Dallas Office of Graduate Education	**2020**
Matthew S. Perry Fellowship: **$1250**, School of Behavioral and Brain Sciences	**2019**
Preregistration Challenge Winner: **$1000**, Center for Open Science	**2018**
Travel Award: **$3000 total**, School of Behavioral and Brain Sciences	**2017 - 2019**

PEER REVIEWED PUBLICATIONS

Total citations as of 3/19/2021: **52**

6. **Morrison, R.A.**, Danaphongse, T.T., Abe, S.T., Stevens, M.A., Ezhil, V., Seyedahmadi, *et al.* (2021**)**. High intensity VNS disrupts VNS-mediated plasticity in motor cortex. *Brain Research*. https://doi.org/10.1016/j.brainres.2021.147332.

5. Adcock, K. S., Blout, A. E., **Morrison, R. A.**, Alverez-Dieppa, A., Kilgard, M. P., *et al.* (2020). Deficits in skilled motor and auditory learning in a rat model of rett syndrome. *Journal of Neurodevelopmental Disorders.* https://doi.org/10.1186/s11689-020-09330-5

4. **Morrison, R. A.**, Danaphongse, T.T., Adcock, K. S., Mathew, J.K., Abe, S.T., Abdulla, D.M., *et al*. (2020). A limited range of vagus nerve stimulation intensities produce motor cortex reorganization when delivered during training. *Behavioural Brain Research.* https://doi.org/10.1016/j.bbr.2020.112705

3. **Morrison, R. A.**, Hulsey, D. R., Adcock, K. S., Rennaker, R. L., Kilgard, M. P., & Hays, S. A. (2019). Vagus nerve stimulation intensity influences motor cortex plasticity. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2018.10.017

2. Pruitt, D. T., Danaphongse, T. T., Schmid, A. N., **Morrison, R. A.**, Kilgard, M. P., *et al.* (2017). Traumatic brain injury occludes training-dependent cortical reorganization in the contralesional hemisphere. *Journal of Neurotrauma*. https://doi.org/10.1089/neu.2016.4796

1. Pruitt, D. T., Schmid, A. N., Danaphongse, T. T., Flanagan, K. E., **Morrison, R. A.**, *et al.* (2016). Forelimb training drives transient map reorganization in ipsilateral motor cortex. *Behavioural Brain Research.* https://doi.org/10.1016/j.bbr.2016.07.005

PUBLIC PRESENTATIONS

2020, BBS Research Competition, University of Texas at Dallas; Exploring vagus nerve stimulation mediated plasticity and implications for recovery after neurological injury

2019, DCS Series, University of Texas at Dallas; Enhancing recovery after neurological injury using targeted plasticity therapy and vagus nerve stimulation

2019, Dissertation Proposal, University of Texas at Dallas; The relationship between VNS-mediated plasticity and recovery after injury

2018, Systems Neuroscience Brown Bag Series, University of Texas at Dallas; Vagus nerve stimulation intensity influences motor cortex plasticity

2017, Systems Neuroscience Brown Bag Series, University of Texas at Dallas; Cortical map plasticity as a function of vagus nerve stimulation intensity paired with motor training

CONFERENCE POSTERS & ABSTRACTS

2019, Society for Neuroscience Annual Meeting, First Author; *Investigating the interaction of vagus nerve stimulation intensity and interval on motor cortex plasticity*

2019, Society for Neuroscience Annual Meeting, Fifth Author; *Evaluating vagus nerve stimulation paired with rehabilitation for sensory and motor dysfunction after radial nerve injury*

2018, Society for Neuroscience Annual Meeting, First Author; *Timing and intensity of vagus nerve stimulation influences motor cortex plasticity*

2017, Society for Neuroscience Annual Meeting, First Author; *Cortical map plasticity as a function of vagus nerve stimulation intensity paired with motor training*

2015, Society for Neuroscience Annual Meeting, Sixth Author; *Effects of vagus nerve stimulation paired with motor training on contralesional cortical plasticity after brain injury*

PROFESIONAL AFFILIATIONS

Society for Neuroscience **2017 - Present**