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The timing and amount of vagus nerve stimulation during rehabilitative training affect post-stroke recovery of forelimb strength

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Abstract

Loss of upper arm strength after stroke is a leading cause of disability. Strategies that can enhance the benefits of rehabilitative training could improve motor function after stroke. Recent studies in a rat model of ischemic stroke demonstrate that vagus nerve stimulation (VNS) paired with rehabilitative training substantially improves recovery of forelimb strength compared to extensive rehabilitative training without VNS. Here we report that the timing and amount of stimulation affect the degree of forelimb strength recovery. Similar amounts of delayed VNS delivered two hours after daily rehabilitative training sessions resulted in significantly less improvement compared to VNS that is paired with identical rehabilitative training. Significantly less recovery also occurred when several-fold more VNS was delivered during rehabilitative training. Both delayed and additional VNS confer moderately improved recovery compared to extensive rehabilitative training without VNS, but fail to enhance recovery to the same degree as VNS that is timed to occur with successful movements. These findings confirm that VNS paired with rehabilitative training holds promise for restoring forelimb strength post-stroke and indicate that both the timing and amount of VNS should be optimized to maximize therapeutic benefits.

Keywords

Vagus nerve stimulation; VNS; Stroke; Recovery; Rehabilitation; Rehabilitative training; Strength; Weakness; Forelimb

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Conflict of Interest Statement:

MPK is a consultant and has a financial interest in MicroTransponder, Inc. AMS is an employee of, and RLR owns, Vulintus, LLC. Other authors declare no conflicts of interest.

Intro

As many as 795,000 Americans suffer a stroke each year. As a result, stroke is a leading cause of disability, with many patients displaying chronic impairment of the upper limbs. Loss of strength is believed to be the major contributing factor to post-stroke disability [1]. Physical rehabilitation is insufficient to restore function in the majority of patients; therefore, strategies that enhance the recovery of upper arm strength are needed.

Adjuvants to physical therapy that increase neural plasticity would be expected to improve stroke recovery [2]. Recent studies in rats have demonstrated that stimulation of the vagus nerve paired with forelimb movement causes substantial reorganization of primary motor cortex [3]. Stimulation of the vagus nerve paired with forelimb movement during rehabilitative training can substantially improve recovery of motor function after stroke. In one study, vagus nerve stimulation (VNS) paired with rehabilitative training normalized forelimb strength, while extensive rehabilitation without VNS provided only modest gains [4]. A second study demonstrated that VNS paired with rehabilitative training improves recovery of forelimb speed after stroke compared to rehabilitative training alone [5]. In addition to preclinical evidence of efficacy, VNS represents a potential stroke therapy because it is FDA approved for the treatment of epilepsy and is well-tolerated by patients [6]. Previous stimulation paradigms and those described in this study deliver much less total charge than is used for epilepsy control [4, 7], suggesting that VNS paired with physical rehabilitation for stroke recovery can be safely delivered with potentially even fewer adverse effects. Delivery of VNS timed to coincide with tones is effective in treating chronic tinnitus in animal models and patients [7, 8].

In order to further evaluate VNS combined with physical therapy for post-stroke rehabilitation, we tested the effectiveness of different stimulation paradigms to restore forelimb strength after ischemic lesion of the motor cortex in rats. If VNS acts to reinforce the effects of rehabilitative training to improve recovery of motor function, a delay between rehabilitative training and VNS delivery would be expected to reduce the beneficial effect of VNS. However, several studies have shown that activation of the vagus nerve can promote memory retention when delivered up to hours after training [9]. In this study we evaluated whether VNS delivered hours after rehabilitative training was effective at improving stroke recovery.

Our previous findings showed short durations of VNS delivered during rehabilitative training results in significant recovery of forelimb strength. It is not known whether additional stimulation enhances stroke recovery. Memory enhancement driven by VNS displays an inverted-U shaped response for stimulation intensity, suggesting that greater stimulation might not increase VNS efficacy. In this study, we also evaluated whether additional VNS delivered during rehabilitate training is more or less effective compared to the amount of VNS delivered in our earlier studies.

Methods

Subjects

Forty-six adult female Sprague-Dawley rats, approximately 4 months old and weighing approximately 250 grams when the experiment began, were used. The rats were housed in a 12:12 hr reversed light cycle environment and behavioral testing took place during the dark cycle in order to increase daytime activity levels. Rats were food deprived to no less than 85% of their normal body weight during training. Data from some rats in the paired VNS ($N = 6$) and rehabilitative training alone ($N = 9$) groups was published in a previous study [4]. We include this data along with additional interleaved rats in the same treatment groups to allow comparison with previous data and reduce the number of rats used for this study. Eleven rats were removed from the study due to a lack of impairment (defined as a post-lesion reduction in hit rate of less than 20%) during the post-lesion assessment. Three rats were removed from the study due to device failure within the first four weeks. If device failure occurred after more than four weeks of therapy, data from these rats was included ($n = 4$). All handling, housing, surgical procedures, and behavioral training of the rats were approved by the University of Texas at Dallas Institutional Animal Care and Use Committee.

Isometric Force Task

The isometric force task was used as previously described [4, 10]. Rats were trained to reach out through a narrow slot in the cage and pull a handle attached to a force transducer (Motor Pull Device and Motor Controller, Vulintus LLC, Sachse, TX). Force measurements were sampled at 20 or 100 Hz and measured with ± 1 gram accuracy. Custom software was used to control the task and collect data. If pull force exceeded 120 g within 2 s of initial contact with the handle, the trial was recorded as a success and the software triggered an automated pellet dispenser (Vulintus LLC, Sachse, TX) to deliver a sucrose pellet (45mg dustless precision pellet, BioServ, Frenchtown, NJ) to a receptacle located in the front left corner of the cage. If the force did not exceed 120 g with 2 s, the trial was recorded as a failure and no reward was given.

Training sessions lasted 30 minutes and were conducted twice daily, five days a week, with sessions on the same day separated by at least 2 hours. Shaping was conducted in stages as previously described [4, 10]. Once proficient, rats were held at a pre-lesion stage until they had 10 successive sessions averaging over 85% hit rate. The pre-lesion data reported in this study is the average of these 10 sessions. After this point, the rats were given an ischemic lesion followed by seven days of recovery, after which they returned for testing until they had 4 sessions with greater than 10 trials each during the post-lesion assessment. Rats then proceeded to the therapy stage where VNS was delivered as appropriate for 25 days. A subset of rats underwent 2-5 days of additional testing without VNS (Week 6) to assess persistent effects of VNS.

Unilateral motor cortex ischemic lesion

Unilateral ischemic lesions of primary motor cortex were performed as previously described [4, 5, 10, 11]. Rats were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and

xylazine (10 mg/kg, i.p.) and given supplemental doses as needed. A craniotomy exposed primary motor cortex contralateral to the trained forelimb. Injections of 1.0 μ L endothelin-1 (6.66nL/s, Bachem, Torrance, CA, 1 mg/mL in saline) were made at eight different locations within the forelimb area of motor cortex: AP 2.5, 1.5, 0.5, & -0.5 mm and ML 2.5 & 3.5 mm from bregma and 1.8 mm below the cortical surface. The craniotomy was covered with KwikCast (World Precision Instruments, Sarasota, FL), sealed acrylic, and skin was sutured.

Vagus Nerve Cuff Implantation

All subjects underwent headcap and VNS cuff implantations, as previously described [3–5, 7, 12]. Immediately following lesion surgery, a two channel connector was attached with acrylic to four skull screws. An incision and blunt dissection of the neck exposed the left cervical vagus nerve. Stimulation of the left branch of the vagus avoids cardiac complications [4, 5, 7]. The nerve was placed inside the cuff (5–6 k Ω impedance), and cuff leads were tunneled subcutaneously and attached to the two-channel connector atop the skull. Rats were provided amoxicillin (5 mg) and carprofen (1 mg) for three days following surgery.

Application of VNS

Behavioral testing sessions were identical for all rats. When appropriate, VNS was delivered as a 500 ms train of 15 biphasic 0.8 mA 100 μ s phase duration pulses at 30 Hz, the same parameters as previous studies [4, 5, 7]. The headcaps of all subjects were connected with a tether wire during post-implantation sessions. The Rehab group freely performed the task without VNS delivery (Fig. 1). The Paired VNS group received VNS during rehabilitative training sessions on successful trials for approximately 6,500 total stimulations over 25 day therapy period. At least 2 hr after the completion of daily training sessions, the Delayed VNS group received VNS delivered every 10 s for 1 hr in a dummy cage without a manipulandum each day, for 9000 stimulations over the 25 days. The delayed stimulation protocol was designed to ensure sufficient stimulation and was based on previous data in which the subject with the most paired VNS received 8024 stimulations over 25 days. The Extra VNS group received VNS during rehabilitative training sessions at 1 and 3 s pseudorandom inter-stimulus intervals (averaging stimulation every 2 s). This resulted in an approximately six-fold increase in amount of VNS compared to Paired VNS, with 45,000 total stimulations over the 25 day therapy period.

Statistics

All data are reported as the mean $\pm$ SEM. Significant effects of treatment were determined using one-way or two-way ANOVA and *post hoc* unpaired t-tests where appropriate. Paired t-tests were used to compare pre-lesion and post-therapy (Week 6) performance for the same subjects. Statistical tests for each comparison are noted in the text. Alpha level was set at 0.05 for all comparisons.

Results

Rats were trained to perform the isometric force task and reached stable proficiency in 30 ± 3 days. Before lesion, maximal pull force was 148.8 ± 1.9 g, with most trials exceeding the

120 g hit threshold. No significant difference was observed in pre-lesion maximal force between groups (Fig. 2A, PRE, One-way ANOVA, $F[3, 27] = 1.55$, $P = 0.22$). Hit rate was $86.4 \pm 0.6\%$, with no differences observed between groups (Fig. 2B, PRE, One-way ANOVA, $F[3, 27] = 0.95$, $P = 0.43$). As expected, ischemic lesion reduced multiple measures of forelimb function. Maximal pull force was significantly reduced compared to pre-lesion levels (Fig. 2A, POST: 100.8 ± 2.5 g; paired t-test, $P = 1.12 \times 10^{-15}$ compared to pre-lesion). No differences were observed between groups (One-way ANOVA, $F[3, 27] = 1.58$, $P = 0.22$). Similarly, hit rate was significantly reduced after lesion (Fig. 2B, POST: $36.9 \pm 2.6\%$; paired t-test, $P = 5.74 \times 10^{-19}$ compared to pre-lesion) with no differences observed between groups (One-way ANOVA, $F[3, 27] = 0.98$, $P = 0.42$).

VNS paired with forelimb training enhances recovery of forelimb strength after stroke

As previously reported, VNS paired with successful trials during rehabilitative training (Paired VNS, $n = 8$) significantly improved recovery of maximal force compared to rehabilitative training without VNS (Rehab, $n = 10$), indicative of a restoration of forelimb strength (Paired VNS vs. Rehab, Two-way ANOVA, $F[1, 95] = 53.86$, $P = 7.25 \times 10^{-11}$). Paired VNS also significantly improved hit rate compared to Rehab (Paired VNS vs. Rehab, Two-way ANOVA, $F[1, 95] = 39.88$, $P = 8.63 \times 10^{-9}$). VNS paired with rehabilitative training resulted in increased maximal pull force (Paired VNS vs. Rehab, unpaired t-test, all $P < 0.05$ for Weeks 2-6) and hit rate (Paired VNS vs. Rehab, unpaired t-test, all $P < 0.05$ for Weeks 2-6) beginning at the second week of therapy compared to rehabilitative training without VNS. Paired VNS resulted in a $95.0 \pm 5.1\%$ recovery of forelimb strength at the end of therapy, and forelimb strength was not significantly different from pre-lesion levels (Paired VNS, Pre-lesion vs. Week 6, paired t-test, $P = 0.13$, $n = 7$), indicative of a full recovery. Rehab restored $40.7 \pm 18.8\%$ of forelimb strength and displayed a significant deficit compared to pre-lesion levels (Rehab, Pre-lesion vs. Week 6, paired t-test, $P = 0.011$, $n = 10$), suggesting a long-lasting impairment in strength. These findings indicate the VNS paired with rehabilitative training substantially improves recovery of forelimb strength after stroke.

VNS delivered after forelimb training is less effective than VNS paired with forelimb training

We hypothesized that VNS delivered after the daily therapy session would be less effective at improving recovery than VNS paired with training. To test this, a group of rats underwent identical rehabilitative training but received VNS delivered 2 hours after daily training sessions (Fig. 1; Delayed VNS, $n = 7$). Delayed VNS is less effective at improving forelimb strength (Paired VNS vs. Delayed VNS, Two-way ANOVA, $F[1, 76] = 22.48$, $P = 9.71 \times 10^{-6}$) and hit rate (Paired VNS vs. Delayed VNS, Two-way ANOVA, $F[1, 76] = 14.24$, $P = 3.0 \times 10^{-4}$) than paired VNS. Delayed VNS results in significantly lower maximal pull force on the last two weeks of therapy compared to paired VNS (Paired VNS vs. Delayed VNS, unpaired t-test, all $p < 0.01$ for Weeks 5 and 6). Similar results are observed for hit rate (Paired VNS vs. Delayed VNS, unpaired t-test, all $p < 0.01$ for Weeks 5 and 6). These findings suggest that VNS delayed after rehabilitative training is less effective than VNS paired with forelimb movement during rehabilitative training.

Delayed VNS resulted in an improvement in recovery of forelimb strength (Rehab vs. Delayed VNS, Two-way ANOVA, $F[1, 89] = 9.28$, $P = 0.003$) and hit rate (Rehab vs. Delayed VNS, Two-way ANOVA, $F[1, 89] = 6.34$, $P = 0.014$) compared to rehabilitative training without VNS. However, at most weeks, no significant differences were observed in maximal pull force (Rehab vs. Delayed VNS, unpaired t-test, $P = 0.027$ for Week 5, all other weeks $P > 0.05$) or hit rate performance (Rehab vs. Delayed VNS, unpaired t-test, $P = 0.039$ for Week 5, all other weeks $P > 0.05$). While the group mean at each time point displays a modest increase, most time points fail to reach statistical significance, suggesting that delayed VNS is not consistently better than rehabilitative training without VNS. Delayed VNS caused a $65.7 \pm 18.6\%$ recovery of forelimb strength after therapy. Maximal pull force was still significantly impaired compared to pre-lesion levels (Delayed VNS, Pre-lesion vs. Week 6, paired t-test, $P = 0.042$, $n = 6$), demonstrating an incomplete recovery of strength. These findings establish that delayed VNS is less effective at improving forelimb strength after stroke than VNS paired with rehabilitative training.

Additional VNS is less effective than VNS paired with forelimb training

Additional VNS delivered during rehabilitative training may promote enhanced recovery or could desensitize the response and subsequently reduce post-stroke recovery. To examine the effects of additional VNS, a group of rats underwent identical rehabilitative training, but received VNS delivered on average every 2 seconds during the training session (Fig. 1; Extra VNS, $n = 6$), resulting in an approximately six-fold increase in the number of stimulations. Extra VNS did not improve recovery compared to paired VNS. Rather, extra VNS results in a trend toward a decrease in recovery of forelimb strength (Paired VNS vs. Extra VNS, Two-way ANOVA, $F[1, 69] = 3.80$, $P = 0.055$). Extra VNS results in a statistically significant reduction in hit rate compared to paired VNS (Paired VNS vs. Extra VNS, Two-way ANOVA, $F[1, 69] = 4.57$, $P = 0.036$). These findings suggest that additional VNS that is not precisely paired with movement is less effective at enhancing recovery than paired VNS.

Extra VNS results in only moderately improved recovery of maximal pull force compared to rehabilitative training without VNS (Rehab vs. Extra VNS, Two-way ANOVA, $F[1, 82] = 14.23$, $P = 3.0 \times 10^{-4}$) which reaches significance at week 2 (Rehab vs. Extra VNS, unpaired t-test, $P = 0.01$ for Week 2). No significant differences were observed at other time points, suggesting a transient improvement that is absent by the end of therapy (Rehab vs. Extra VNS, unpaired t-test, all $P > 0.05$ for Weeks 1,3-6). Similar results were obtained for hit rate performance (Rehab vs. Extra VNS, Two-way ANOVA, $F[1, 82] = 9.82$, $P = 0.002$); unpaired t-test, $P = 0.014$ for Week 2, all $P > 0.05$ for Weeks 1,3-6). Extra VNS resulted in $83.6 \pm 20.1\%$ recovery of forelimb strength after therapy, demonstrating a trend toward a deficit compared to pre-lesion levels (Extra VNS, Pre-lesion vs. Week 6, paired t-test, $P = 0.379$, $n = 4$). These findings indicate that additional VNS confers some recovery, but is less effective than VNS paired with successful trials.

Intensity of training was not different between groups

The intensity of rehabilitative training can affect the degree of functional recovery [13]. Therefore, we evaluated whether VNS could improve recovery by increasing the intensity of

training. Consistent with previous studies [4, 5], no differences were observed in the intensity of training for any delivery paradigms of VNS, as evidenced by a similar number of pulls performed over the course of the five week therapy period (Rehab: 34787 ± 3356 pulls, Paired VNS: 25366 ± 1871 pulls, Delayed VNS: 40972 ± 6313 pulls, Extra VNS: 34214 ± 2674 pulls; One-way ANOVA, $F[3, 27] = 2.73$, $P = 0.063$). This suggests that the differences in recovery observed between groups cannot be accounted for by differences in the intensity of training.

Discussion

This study evaluated different VNS delivery paradigms to improve recovery of forelimb strength after ischemic lesion of the motor cortex. First, we evaluated the importance of temporally precise delivery of VNS during rehabilitative training. We find that delayed VNS delivered at least 2 hours after training is less effective at improving recovery compared to VNS paired with rehabilitative training. Second, we evaluated whether additional VNS distributed in time during rehabilitative training would improve recovery. We find that rather than improving recovery, additional VNS is less effective at restoring forelimb strength than VNS paired with successful forelimb movements. The findings from these experiments suggest that temporally precise delivery of VNS paired with rehabilitative training drives the most recovery of function after stroke.

Reorganization of motor representations in the motor cortex is associated with recovery after stroke [14]. In unlesioned rats, VNS paired with forelimb training drives training-specific map reorganization in the motor cortex [3]. This robust, specific enhancement of plasticity driven by VNS is believed to underlie the improvement in functional recovery observed when VNS is paired with rehabilitative training [4, 5]. The majority of vagus nerve fibers are ascending and project to the nucleus tractus solitarius [15]. Stimulation of the vagus nerve activates neurons in the noradrenergic locus coeruleus and the cholinergic basal forebrain, resulting in release of neuromodulators throughout the central nervous system [16]. Release of neuromodulators during external events enhances event-specific cortical plasticity [17–19]. Therefore, VNS that is delivered outside of the time of motor training would not be predicted to enhance plasticity or improve recovery of function. However, previous studies have documented enhancements in memory retention when VNS is delivered after training, likely by enhancing consolidation [20]. As such, VNS may still confer beneficial effects when delayed after training. Our results indicate that delayed VNS is significantly less effective at improving recovery of forelimb function after stroke than VNS paired with rehabilitative training and is only modestly more effective than rehabilitative training without VNS. This corroborates preliminary findings from a previous study indicating that VNS must be paired with training to improve post-stroke recovery [5]. The importance of temporal precision between VNS and rehabilitative training supports a plasticity-dependent mechanism of recovery rather than alternative mechanisms that would not be predicted to require temporal precision, such as neuroprotection or modulation of the immune system. Consolidation and post-stroke recovery share common molecular mechanisms [21]; therefore, the modest improvement in recovery caused by delayed VNS compared to rehabilitative training without VNS may be due to enhancement of consolidation. Our findings are consistent with previous reports that precisely timed

stimulation methods drive more effective recovery after brain injury than techniques that do not allow precise timing [22] and suggest that clinical implementations of VNS and rehabilitative training use precisely timed delivery of stimulation to maximize benefits.

Additional VNS delivered during rehabilitative training may further increase recovery or desensitize the effectiveness of VNS and occlude recovery. Our findings indicate that delivery of six-fold more stimulation results in reduced recovery compared to less VNS paired with rehabilitative training and only moderately improved recovery compared to rehabilitative training without VNS. One limitation of the current study design is that it is not possible to precisely pair the timing of VNS during forelimb movement and also deliver several-fold more stimulation. Rats in the Paired VNS group already received stimulation on more than half of the trials during the first week of therapy, and therefore stimulation was delivered repeatedly at random intervals during training rather than precisely paired with forelimb movement in order to achieve substantially more stimulation.

Two mechanisms could explain why more VNS results in less stroke recovery. Additional VNS may cause desensitization that occludes the reinforcing effects of VNS paired with forelimb movement. Previous studies using VNS to enhance neural plasticity and memory observed an inverted-U response. Moderate VNS intensity enhanced plasticity and memory, but greater and lesser stimulation intensities did not [20]. Desensitization of the G-protein coupled receptors that respond to the acetylcholine and norepinephrine released by VNS could account for these results, though many other cellular mechanisms are possible [16, 23]. A second possible explanation is that the additional VNS reinforces non-task specific movements. Paired VNS and Extra VNS rats received the same number of stimulations within 290 msec of forelimb movement (approximately 135 per session). The 765 additional stimulations delivered to the Extra VNS group occur during non-task specific movements, including movement of the unimpaired forelimb, jaw, vibrissae, and hindlimb, as well as during periods of inactivity. Stimulation during movements other than the impaired forelimb may competitively interfere with the reinforcement of impaired forelimb movements, thus leading to reduced recovery [3, 24, 25]. Our findings indicate that the amount and timing of stimulation can impact the benefits of VNS during rehabilitative training.

Insufficient intensity of rehabilitative training after a stroke can limit functional recovery [13]. Previous studies have indicated that VNS does not affect the intensity of rehabilitative training [4, 5]. The findings from the present study confirm this, as no differences in intensity of training are observed between groups. Therefore, training intensity cannot account for the differences in recovery observed between groups. With stimulation beginning at least nine days after lesion, VNS would not be predicted to confer any neuroprotective effects. Using a similar stimulation schedule, previous studies do not report a reduction in lesion size [4, 5]. Therefore, it is unlikely that reduced lesion size accounts for the differences in recovery observed between groups in this study. The lack of changes in training intensity and lesion size suggest that VNS acts through an alternative mechanism, likely by enhancing neural plasticity, to promote recovery.

Here we report that VNS paired with rehabilitative training improves recovery of forelimb function in a rat model of ischemic stroke. Delaying VNS for two hours after daily

rehabilitative training results in less recovery of forelimb function. Additional stimulation of the vagus nerve also results in less recovery. These findings indicate that VNS paired with rehabilitative training holds potential as a post-stroke therapy to improve recovery of motor function, and suggest that future implementations of the therapy should accordingly optimize the timing and amount of stimulation to maximize beneficial effects.

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References

1. Canning CG, Ada L, Adams R, O'Dwyer NJ. Loss of strength contributes more to physical disability after stroke than loss of dexterity. *Clin Rehabil.* 2004; 18:300–308. [PubMed: 15137561]
2. Nudo RJ. Plasticity. *NeuroRx.* 2006; 3:420–427. [PubMed: 17012055]
3. Porter BA, Khodaparast N, Fayyaz T, Cheung RJ, Ahmed SS, Vrana WA, Rennaker RL, Kilgard MP. Repeatedly Pairing Vagus Nerve Stimulation with a Movement Reorganizes Primary Motor Cortex. *Cerebral Cortex.* 2011; 22:2365–2374. [PubMed: 22079923]
4. Khodaparast N, Hays SA, Sloan AM, Fayyaz T, Hulse DR, Ruiz A, Pantoja M, Rennaker RL, Kilgard MP. Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. *Neurobiol Dis.* 2013; 60:80–88. [PubMed: 23954448]
5. Khodaparast N, Hays SA, Sloan AM, Fayyaz T, Hulse DR, Rennaker RL II, Kilgard MP. Vagus nerve stimulation delivered during motor rehabilitation improves recovery in a rat model of stroke. *Neurorehabil Neural Repair.* 2013; 60:80–88.
6. Schachter SC, Saper CB. Vagus nerve stimulation. *Epilepsia.* 1998; 39:677–686. [PubMed: 9670894]
7. Engineer ND, Riley JR, Seale JD, Vrana WA, Shetake JA, Sudanagunta SP, Borland MS, Kilgard MP. Reversing pathological neural activity using targeted plasticity. *Nature.* 2011; 470:101–104. [PubMed: 21228773]
8. 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: Technology at the Neural Interface.* 2013
9. McGaugh JL, Roozendaal B. Drug enhancement of memory consolidation: historical perspective and neurobiological implications. *Psychopharmacology (Berl).* 2009; 202:3–14. [PubMed: 18704369]
10. Hays SA, Khodaparast N, Sloan AM, Hulse DR, Pantoja M, Ruiz AD, Kilgard MP, Rennaker RL. The isometric pull task: a novel automated method for quantifying forelimb force generation in rats. *J Neurosci Methods.* 2012; 212:329–337. [PubMed: 23183016]
11. Hays SA, Khodaparast N, Sloan AM, Fayyaz T, Hulse DR, Ruiz AD, Kilgard MP, Rennaker RL. The bradykinesia assessment task: an automated method to measure forelimb speed in rodents. *Journal of Neuroscience Methods.* 2013; 214:52–61. [PubMed: 23353133]
12. Peña DF, Engineer ND, McIntyre CK. Rapid Remission of Conditioned Fear Expression with Extinction Training Paired with Vagus Nerve Stimulation. *Biological psychiatry.* 2012; 73:1071–1077. [PubMed: 23245749]
13. Sivenius J, Pyörälä K, Heinonen O, Salonen J, Riekkinen P. The significance of intensity of rehabilitation of stroke--a controlled trial. *Stroke.* 1985; 16:928–931. [PubMed: 3911506]

14. Nudo R, Friel K. Cortical plasticity after stroke: implications for rehabilitation. *Rev Neurol*. 1999; 155:713. [PubMed: 10528355]
15. Henry TR. Therapeutic mechanisms of vagus nerve stimulation. *Neurology*. 2002; 59:S3–S14. [PubMed: 12270962]
16. Hays SA, Rennaker RL II, Kilgard MP. Targeting plasticity with vagus nerve stimulation to treat neurological disease. *Prog Brain Res*. 2013; 207:275–299. [PubMed: 24309259]
17. Kilgard MP, Pandya PK, Vazquez J, Gehi A, Schreiner CE, Merzenich MM. Sensory input directs spatial and temporal plasticity in primary auditory cortex. *J Neurophysiol*. 2001; 86:326–338. [PubMed: 11431514]
18. Kasamatsu T, Watabe K, Heggelund P, Schöller E. Plasticity in cat visual cortex restored by electrical stimulation of the locus coeruleus. *Neurosci Res*. 1985; 2:365–386. [PubMed: 3929191]
19. Kilgard MP. Harnessing plasticity to understand learning and treat disease. *Trends Neurosci*. 2012; 35:715–722. [PubMed: 23021980]
20. Clark K, Krah S, Smith D, Jensen R. Post-training unilateral vagal stimulation enhances retention performance in the rat. *Neurobiol Learn Mem*. 1995; 63:213–216. [PubMed: 7670833]
21. Ploughman M, Windle V, MacLellan CL, White N, Doré JJ, Corbett D. Brain-Derived Neurotrophic Factor Contributes to Recovery of Skilled Reaching After Focal Ischemia in Rats. *Stroke*. 2009; 40:1490–1495. [PubMed: 19164786]
22. Edwardson M, Lucas T, Carey J, Fetz E. New modalities of brain stimulation for stroke rehabilitation. *Experimental Brain Research*. 2013; 224:335–358. [PubMed: 23192336]
23. Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci*. 2004; 27:107–144. [PubMed: 15217328]
24. Kleim JA, Jones TA. Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. *Journal of Speech, Language and Hearing Research*. 2008; 51:S225.
25. Allred RP, Cappellini CH, Jones TA. The “good” limb makes the “bad” limb worse: Experience-dependent interhemispheric disruption of functional outcome after cortical infarcts in rats. *Behav Neurosci*. 2010; 124:124–132. [PubMed: 20141287]

Fig. 1.

Timeline of experiment and VNS delivery. (i) Timeline of daily experimental procedures. (ii) Representative data collected for 30 sec using the isometric force task. (iii) VNS delivery matched to the data in (ii). Rats in the Rehab group do not receive VNS. The Paired VNS group received VNS with successful trials during behavioral testing sessions. The Delayed VNS group received similar amounts of VNS in a dummy cage 2 hr after the last daily training session. The Extra VNS group received 6-fold more stimulations during the behavioral testing sessions compared to Paired VNS. The gray VNS markers denote the extra stimulations which were not paired with successful trials.

Fig. 2.

The timing and amount of VNS affect the recovery of multiple parameters of forelimb function after stroke. (A) Maximal pull force per trial over the course of the experiment. Paired VNS demonstrates the greatest recovery of maximal pull force during the therapy phase (Weeks 1 – 6). (B) Hit rate over the course of the experiment. Paired VNS results in the greatest recovery of hit rate. Error bars represent SEM. * indicates a significant difference (unpaired t-test, $P < 0.05$) for each group compared to Rehab on weeks 1 – 6. The color of the * marker indicates the group for the comparison.