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Vagus Nerve Stimulation during Rehabilitative Training Improves Functional Recovery after Intracerebral Hemorrhage

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Abstract

Background and Purpose—Vagus nerve stimulation (VNS) delivered during rehabilitative training enhances neuroplasticity and improves recovery in models of cortical ischemic stroke. However, VNS therapy has not been applied in a model of subcortical intracerebral hemorrhage (ICH). We hypothesized that VNS paired with rehabilitative training after ICH would enhance recovery of forelimb motor function beyond rehabilitative training alone.

Methods—Rats were trained to perform an automated, quantitative measure of forelimb function. Once proficient, rats received an intrastriatal injection of bacterial collagenase to induce ICH. Rats then underwent VNS paired with rehabilitative training (VNS+Rehab; N = 14) or rehabilitative training without VNS (Rehab; N = 12). Rehabilitative training began at least 9 days after ICH and continued for 6 weeks.

Results—VNS paired with rehabilitative training significantly improved recovery of forelimb function compared to rehabilitative training without VNS. The VNS+Rehab group displayed a 77% recovery of function, while the Rehab group only exhibited 29% recovery. Recovery was sustained after cessation of stimulation. Both groups performed similar amounts of trials during rehabilitative and lesion size was not different between groups.

Conclusions—VNS paired with rehabilitative training confers significantly improved forelimb recovery following ICH compared to rehabilitative training without VNS.

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Disclosures:

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

Keywords

Vagus nerve; vagal stimulation; intracerebral hemorrhage; recovery; rehabilitation

INTRODUCTION

Spontaneous intracerebral hemorrhage (ICH) is a devastating subtype of stroke and often leaves survivors with significant disability¹. There is no consistently effective post-stroke rehabilitative intervention; therefore, methods to improve recovery of motor function represent a significant clinical need.

Neuroplasticity is believed to support recovery of function after stroke, so methods that enhance plasticity may promote greater recovery after ICH. Stimulation of the vagus nerve releases neuromodulators associated with plasticity²⁻⁴. Consequently, vagus nerve stimulation (VNS) paired with forelimb training drives robust neuroplasticity⁵. Based on this enhancement of plasticity, VNS paired with rehabilitative training represents a potential method to improve recovery after stroke. Studies in models of ischemic stroke demonstrate that VNS paired with rehabilitation results in significantly greater recovery of forelimb strength and movement speed than extensive rehabilitative training without VNS⁶⁻⁸. VNS paired with rehabilitation is currently being investigated in ischemic stroke patients⁹.

Despite the efficacy of VNS paired with rehabilitation after cortical ischemic stroke, ICH bears different pathological features which may interfere with the beneficial effects of VNS. In this study, we evaluate whether VNS paired with rehabilitative training can improve recovery of motor function beyond rehabilitative training without VNS in a rat model of ICH.

METHODS**Subjects**

All procedures were approved by the University of Texas Institutional Animal Care and Use Committee. Fifty-eight female Sprague-Dawley rats (Charles River), weighing approximately 250 grams at the beginning of the experiment, were used. The rats were individually housed in a 12:12 hr reversed light cycle environment and were food deprived to no less than 85% of their normal body weight during training.

Behavioral Training

The bradykinesia assessment task (Vulintus Inc., Dallas, TX) was performed as previously described¹⁰ (Supplementary data). Once proficient at the task, rats received a lesion and VNS implant. After 7 days of recovery, rats returned for post-lesion testing and were then assigned to groups (Supplementary data). Rehabilitative training continued for the following 6 weeks.

ICH and VNS implant surgery

ICH was performed similar to previous descriptions¹¹. Rats were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Body temperature was maintained at 37°C throughout the surgery. 0.18U bacterial collagenase Type IV-S (Sigma-Aldrich Corp. St. Louis, MO, USA) in 1.0 µL saline was injected into the left hemisphere at 3.0 mm lateral, 6.0 mm ventral relative to bregma using a 26-gauge Hamilton syringe. Injections took place over a 2 min period, and the syringe remained in place for additional 3 min. A two-channel connector was then affixed to the skull and a bipolar stimulating cuff with platinum-iridium leads (5 kΩ impedance) was implanted around the left cervical vagus nerve, as previously described^{5–8,12}. Amoxicillin (5 mg) and carprofen (1 mg) tablets were provided for three days post-surgery.

Group assignment and exclusion criteria

The Rehab group (N = 12) underwent rehabilitative training for 6 weeks, which consisted of freely performing the task during training sessions. The VNS+Rehab group (N = 14) underwent identical rehabilitative training, but received VNS during training based on one of two paradigms (see Supplementary data). One group (N = 8) received VNS on successful trials, similar to previous studies^{6–8}. Another group (N = 6) received VNS on all trials. VNS was delivered using identical parameters to previous studies: 500 ms train, 15 biphasic 0.8 mA pulses, 100 µs each, 30 Hz^{5–8}. No stimulation was delivered on the sixth week in any group to allow assessment of effects persisting after VNS cessation. Estrous phase was not monitored during the study because behavioral testing and stimulation occur over multiple cycles. Experimenters were blind to treatment group during testing, and automated data analysis eliminated any bias¹⁰. Thirty-two rats were excluded from the main text due to: 1) death, 2) failure to demonstrate a post-lesion impairment, 3) impairment too severe to perform task, or 4) stimulation device failure (see Supplementary data). Data for all subjects is included in the supplementary information. Exclusion had minimal effects on statistical comparisons.

Histological Processing

Following behavioral testing, subjects were perfused with 4% paraformaldehyde. Cresyl violet staining and analysis were performed as previously described^{6,7}. Histology could not be performed on 3 of the 26 included subjects due to technical difficulties.

Statistics

All data are expressed as mean ± SEM. Significant differences between groups were determined using two-way ANOVA or two-tailed t-tests where appropriate. Alpha level was set at 0.05 for all comparisons.

RESULTS

Prior to ICH, all rats were highly proficient at the task (Supplementary Video 1). No significant difference in hit rate, second press latency, or number of trials was observed between groups (PRE, Fig. 1; Rehab v. VNS+Rehab, unpaired t-test, all $P > 0.05$). ICH significantly worsened multiple measures of forelimb performance in both groups

(Supplementary Video 2). No differences were observed in post-lesion performance metrics between groups (POST, Fig. 1, unpaired t-test, all $P > 0.05$).

VNS paired with rehabilitative training (VNS+Rehab, Supplementary Video 3) significantly enhances recovery compared to rehabilitative training without VNS (Rehab, Supplementary Video 4). ANOVA comparing hit rate for Rehab and VNS+Rehab groups over the course of therapy (weeks 1–6) revealed a significant effect of treatment (Fig. 1A, Two-way ANOVA, $F[1,144] = 39.59$, $P = 3.54 \times 10^{-9}$). Enhanced recovery is maintained on week 6 after the cessation of stimulation. At the conclusion of therapy, VNS+Rehab group demonstrated significantly greater recovery of initial impairment compared to Rehab (Rehab: $29.4 \pm 12.0\%$ recovery, VNS+Rehab: $76.8 \pm 11.3\%$ recovery; unpaired t-test, $P = 0.0062$). ANOVA on second press latency over the course of therapy reveals a significant effect of treatment, indicating that recovery of forelimb movement speed is enhanced by VNS+Rehab (Fig. 1B, Two-way ANOVA, $F[1,144] = 57.67$, $P = 3.58 \times 10^{-12}$).

Total number of trials per day during therapy failed to demonstrate a significant effect of treatment (Fig. 1C, Two-way ANOVA, $F[1,144] = 0.23$, $P = 0.633$), indicating that VNS does not affect training intensity. No differences in tissue loss were observed across groups (Fig. 2, Rehab: $13.55 \pm 2.56 \text{ mm}^3$, VNS+Rehab: $11.09 \pm 1.58 \text{ mm}^3$, unpaired t-test, $P = 0.38$). No lesion metrics were correlated with impairment in individual subjects (see Supplementary data).

DISCUSSION

Previous studies show that VNS paired with rehabilitative training improves recovery of forelimb speed and strength after cortical ischemic lesion^{6–8}. The results from the present study extend the efficacy of VNS to a model of ICH that includes subcortical damage to both white and gray matter¹³. VNS therapy therefore may be useful in stroke patients bearing similar pathology and could potentially generalize to other mechanisms of brain injury.

The collagenase injection model of ICH results in protracted neuronal death, with lesion size evolving as long as four weeks after the initial injection¹³. In the present study, VNS did not begin until at least nine days after collagenase injection, after which the lesion is predicted to have reached more than 75% of its final size¹³. As expected, we did not observe a difference in tissue loss between groups; therefore, the improved functional outcomes resulting from VNS cannot be attributed to reduced lesion size. The absence of neuroprotective effects when VNS is delivered on this timescale after lesion onset is consistent with previous studies^{6,7}. The lack of a difference in lesion size between groups suggests that VNS is not enhancing forelimb recovery through neuroprotection but rather acting through a different mechanism, such as enhancing neuroplasticity. The degree of forelimb impairment after ICH was not correlated with any of the anatomical measures in this study. This suggests that a feature not observed with gross anatomy, such as partial damage to projections or pathological plasticity, may underlie at least part of the functional impairment after ICH. VNS has been successfully employed to reverse pathological plasticity and confer benefits in chronic tinnitus patients¹⁴. Similarly, VNS paired with

rehabilitative training may promote beneficial plasticity to drive functional recovery after ICH.

Neuroplasticity is believed to be a substrate for recovery after brain damage. Similar to ischemic stroke and traumatic brain injury, rehabilitative training after ICH likely supports recovery by promoting reorganization within motor circuitry. Previous studies correlate increased dendritic complexity, a morphological feature associated with plasticity, with improved motor recovery in subjects that receive rehabilitative training after ICH¹⁵. Brain-derived neurotrophic factor (BDNF) is known to promote increased dendritic complexity, and VNS provides a potential direct link to plasticity by driving increased expression of BDNF and activation of TrkB signaling^{2,3}. Despite links to plasticity⁸, the mechanism by which VNS improves recovery after ICH remains unclear and should be addressed in future studies.

Unlike studies in models of ischemic stroke^{6,7}, VNS paired with rehabilitative training results in an incomplete recovery of forelimb function after ICH, which is likely accounted for by the differences in the lesion characteristics described above. To attempt to improve recovery, two different stimulation paradigms were employed (see Supplementary data). One group received stimulation on successful trials similar to the design used in previous studies, and the second group received stimulation on all trials, resulting in approximately 40% more stimulations over the course of the therapy. Consistent with previous reports, additional VNS does not result in greater recovery⁸. No difference in recovery was observed between either VNS paradigm. Parameters such as current intensity and timing of stimulation modulate the effects of VNS⁸. Therefore, optimizing these parameters is of key importance for clinical implementation.

VNS paired with physical rehabilitation represents a potentially attractive method to improve recovery after stroke and is currently under evaluation in ischemic stroke patients⁹. VNS is FDA-approved to treat epilepsy and depression, and more than 60,000 patients are implanted with VNS devices. VNS is safe and well-tolerated. The implementation of VNS in the present study uses 100-fold less daily stimulation than is approved for epilepsy, which may further reduce any occurrence of adverse effects. Along with the evidence of safety and preclinical efficacy of VNS paired with rehabilitation in models of ischemic and hemorrhagic stroke, this report strengthens the viability of VNS as a post-stroke therapy.

Conclusions and future directions

This study demonstrates that VNS paired with rehabilitative training improves recovery of forelimb function after ICH compared to rehabilitative training without VNS. This extends the efficacy of VNS to models that include subcortical and white matter damage. Furthermore, the beneficial effects last after the cessation of VNS, suggesting that functional improvements may be lasting. Clinical investigation in patients may be warranted. Further preclinical studies should evaluate the cellular and molecular mechanisms underlying VNS-dependent enhancement of recovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

VNS paired with rehabilitative training improves forelimb recovery after ICH. (A) VNS paired with rehabilitative training (N = 14) improves hit rate by the second week of therapy compared to rehabilitative training without VNS (N = 12). Recovery is maintained after the cessation of VNS on week six. (B) VNS paired with rehabilitative training improves second press latency compared to rehabilitative training without VNS. (C) The number of trials performed by the Rehab and VNS+Rehab groups is not different at any time point during testing. * denotes $P < 0.05$ between Rehab and VNS+Rehab group at each time point.

Fig. 2.

Lesion size is not affected by VNS. Representative images showing ICH lesions from a subject in the Rehab group (A) and the VNS+Rehab group (B). (C) No difference was observed in tissue loss between groups.