Abstract

Ischemic stroke and spinal cord injury are neurological injuries that produce persisting motor deficits. Additionally, the majority of those suffering from impairments due to ischemic stroke or spinal cord injury rarely fully recover complete function with current treatment options. Here, we investigate the use of vagus nerve stimulation (VNS), which facilitates specific and long-lasting plasticity after injury. Sprague-Dawley rats affected by a spinal cord contusion at C5 and administered VNS paired with rehabilitation exhibited significant recovery of forelimb strength on an isometric pull task. We also propose similar recovery of supination function in Sprague-Dawley rats affected by endothelin-1-induced focal motor cortex ischemic stroke. Thus, VNS paired with rehabilitation may be an effective therapeutic tool in the event of neurological injury such as ischemic stroke or spinal cord injury.

Introduction

Motor function can be compromised in a number of different neurological injuries. The resulting loss of motor ability can seriously affect well-being and independence of patients. Furthermore, motor impairments are often long-lasting and difficult to treat. For instance, many skilled motor movements are lost after a motor cortex stroke (Farr and Whishaw, 2002). Additionally in spinal cord injury (SCI) which annually affects more than 10,000 people in the United States, impaired hand and arm function frequently disrupt quality of life (Beekhuizen and Field-Fote, 2005). As of yet, recovery of motor function after either motor cortex stroke or spinal cord injury is limited. Of
those who experience reduced function of the upper limb after stroke, 60% continuously struggle with deficits that affect daily functioning (Alaverdashvili and Whishaw, 2013). In the event of a spinal cord injury that preserves sensory but no motor function below the level of the injury, only 54% of individuals recover substantial strength below the site of injury (McDonald and Sadowsky, 2002). Thus, discovering new methods of treating motor disability due to neurological injury is crucial to improving the recovery of those affected.

**Modeling Ischemic Stroke**

Stroke is a major cause of disability both in the United States and worldwide (Ward and Cohen, 2004; Sicard and Fisher, 2009). Among the major types of stroke (ischemic, intracerebral, and subarachnoid), 88% of cases are ischemic, resulting from vascular obstruction within the brain (Alaverdashvili and Whishaw, 2013). The incidence of ischemic stroke has led to the development of several rodent models that can effectively reproduce certain deficits seen in human patients (Alaverdashvili and Whishaw, 2013). One such method involves the injection of endothelin-1 (ET-1), an endogenous peptide that restricts blood flow, which induces an ischemic lesion (Adkins et al., 2004). Ischemic lesions in the motor cortex produce motor impairments to the contralateral side of the body. Additionally, ET-1 injections can be localized to the left rostral and caudal forelimb regions of the motor cortex, regions analogous to the human primary motor cortices, producing ischemic damage to the right forelimb (Khodaparast et al., 2014). Rodent models, although imperfect, can replicate the main features of a human stroke and allow for the study of the behavioral deficits resulting from stroke and possible therapies that can be implemented in human patients (Alaverdashvili and Whishaw, 2013).

**Modeling Spinal Cord Injury**

Effectively modeling spinal cord injury has important implications in analyzing the aftereffects of common, yet devastating occurrences such as car accidents or contact sports injuries. There are currently three main experimental models that induce spinal cord injury (SCI): contusion,
compression, and transection, which comprise approximately 90% of spinal cord injury studies (Field-Fote, 2009). Transection requires the experimenter to tear at the spinal cord with a sharp object. This method of delivering an SCI reduces secondary damage caused by the injury, but also completely removes some of the neural connections within the spinal cord (Field-Fote, 2009). Compression and contusion injuries involve the use of a blunt object to damage the spinal cord. In these models, some of the axonal connections in the spinal cord remain intact. One main advantage of using either the contusion or compression model is that it is easy to study how neurons grow and change in response to the injury (Field-Fote, 2009). Location of the injury is also critical. Injury at the cervical level produces impairments in the forelimb, whereas injury at regions below the cervical region affects the lower limbs (Field-Fote, 2009). For the purpose of investigating loss of forelimb strength and the potential for VNS as an effective therapy, a contusion will be given to the subjects at the cervical level. The rat model is used to model SCI based on the similar changes that occur biologically and functionally in rats and humans (Onifer et al., 2007).

**Knob Task**

Motor deficits seen in ischemic stroke patients include decreased supination behavior. According to previous experiments, rodents receiving focal motor cortex stroke show less supination than control animals (Farr and Whishaw, 2002). Previous methods to measure supination in the rodent model have been qualitative in nature and inefficient in time and cost (Vergara-Aragon et al., 2003). In order to better study decreased supination activity in stroke subjects, we have utilized a new task involving a weighted spherical knob. The rodent must first reach through an aperture in the behavioral chamber. Then, the subject must grasp and turn a sphere-shaped knob by a preset amount (as measured in angles) via supination. To encourage this behavior, a food pellet is allotted per successful trial in which the rat meets the required turn threshold. This knob task provides an automated quantitative measure of supination loss and allows for efficient motor data acquisition.

**Isometric Pull Task**
In spinal cord injury, loss of strength is a serious and common deficit experienced by patients (McDonald and Sadowsky, 2002). To better study the effect of VNS as a therapeutic tool for recovery in spinal cord injury, the isometric pull task was used to assess forelimb strength prior to and following experimentally induced spinal cord injury. The isometric pull task is also a valuable tool in gathering quantitative measurements of forelimb function. In this task, rodents are trained to reach outside the behavioral cage through a narrow opening, grasp a handle, and pull with certain minimum amount of force. This task is completely automated and allows for easier collection of data in assessing multiple aspects of forelimb motor behaviors (Hays et al., 2013a).

**Vagus Nerve Stimulation and Plasticity**

The vagus nerve is responsible for activating non-voluntary neural activity such as parasympathetic responses including heart rate and intestinal functions. This nerve also communicates a great amount of information from the periphery to the central nervous system (Hays et al., 2013b). This communication with the periphery has been shown to have effects on memory by changing levels of neuromodulators in the cortex. Specifically, the left cervical branch of the vagus nerve contains afferent fibers that synapse on neurons that project to the locus coeruleus and basal forebrain (Hays et al., 2013b). Therefore, VNS triggers the release of neuromodulators acetylcholine and norepinephrine, which promotes plasticity (Engineer et al., 2011; Hays et al., 2013b). Plasticity occurs due to reorganization of connections in neural tissue. Particularly in the case of injury, plasticity is thought to be an important factor in recovery of neural functioning (Kleim, 2011). This neural rewiring can be important in directing learning and memory in healthy individuals or in reforming synaptic connections in those affected by injury. For example, VNS paired with auditory tones in rats presenting behavioral and physiological deficits resembling tinnitus has shown to enhance plastic changes in the auditory cortex (Engineer et al., 2011). VNS has also been shown to drive plasticity in the motor cortex. Pairing VNS with a motor task reorganizes the primary motor cortex, resulting in a larger cortical representation of the movement (Porter et al., 2012). Previous
studies evaluating the effect of VNS in subjects suffering from epilepsy, tinnitus, ischemic and hemorrhagic stroke have shown that VNS enhances recovery of function in each case by driving cortical plasticity (Krahl, 2011; Lehtimäki et al., 2013; Cai et al., 2014). Thus, we hypothesize that VNS can improve recovery in a novel motor task (knob task) following a previously studied injury (ischemic stroke) and that VNS can also improve recovery in an established motor task (pull task) following a new injury (spinal cord injury).

**Materials and Methods**

**Subjects**

The University of Texas Institutional Animal Care and Use Committee oversaw and approved care, training, and surgical procedures used throughout the stroke and spinal cord injury studies.

**Stroke Subjects**

Female Sprague-Dawley rats (n=7) were trained on the behavioral task. Subjects were food deprived 5 consecutive days per week to effectively utilize food pellets as incentive during the behavioral task. All animal weights were maintained above 85% of optimum body weight during the study (average weight 250g). The animals were placed on 12:12 reversed light cycle.

**Spinal Cord Injury Subjects**

A total of 23 female Sprague-Dawley rats were included in the SCI study. All subjects received a spinal cord injury, but 4 were placed on rehabilitation, and 7 were given VNS with rehabilitation. Subjects were cared for and housed in the same manner as the subjects in the stroke study, with food deprivation 5 days per week, maintenance of at least 85% normal body weight, and a 12:12 reversed light cycle environment.

**Behavioral Training**

**Stroke study**
Subjects were trained 5 days per week, twice a day, in 30-minute sessions. Initially, pellet dust was placed on or near the knob in order to help the subject associate the knob with a positive reward. The knob was initially placed within the cage but was moved gradually outside the chamber in \( \frac{1}{4} \)" increments to a final location of \( \frac{3}{4} \)" from the inner cage wall. In beginning stages, a successful trial was defined as turning the knob 5 degrees. Upon completion of a successful trial a chocolate pellet (45mg, dustless chocolate precision pellet, BioServ, Frenchtown, NJ) was dispensed from custom pellet dispensers (Vulintus, LLC, Dallas, TX) to the bottom left corner of the cage. Once the rat associated turning the knob with receiving the pellet, the rat advanced to adaptive training. Adaptive training involves calculation of the median peak turn angle of the previous 10 trials and sets this angle as the new threshold the subject must surpass in order to achieve a successful trial and receive a pellet reward. Rats were trained until a maximum threshold of 60 degrees was reached. Once the subject reached the 60 degree threshold on adaptive stages, the subject was then placed on a fixed 60 degree threshold stage and was required to turn the knob at least 60 degrees for the trial to be considered successful. At this fixed stage, if the rat could perform 5 consecutive days or 10 consecutive sessions with at least an 80% success rate, the rat was deemed eligible to receive an ischemic lesion.

*Spinal Cord Injury Study*

Subjects were also trained 5 days a week and twice per day in 30-minute sessions. Pellet dust was placed near the pull handle to help the subject associate the handle and the food reward. The pull handle was initially placed \( \frac{3}{4} \)" inside the behavioral cage but also withdrawn in \( \frac{1}{4} \)" increments until the handle was \( \frac{3}{4} \)" outside the cage wall. At beginning stages, the minimum threshold of a successful trial was 10 grams. For each trial throughout the study, the rat had 2 seconds to pull with a force equal to or greater than the set threshold. Initially, once a rat pulled the handle with at least 10g of force, a chocolate pellet (identical to the pellets described above) was dispensed. After the rat made the association between the handle and the reward, the rat was moved to adaptive stages,
where the threshold for a successful trial depended on the rat’s performance on the previous 10 trials. In the adaptive stages, the minimum threshold to accomplish a successful trial was 10g and changed to the median of the previous 10 trials, as the rat continued the task. When the subject maintained an adaptive threshold of 120g of force on adaptive stages, the subject was moved to static training, with a minimum and unchanging threshold of 120g for a successful trial. If a subject achieved an 85% success rate for 10 successive training sessions at this static stage, the animal was considered proficient and ready for a spinal cord lesion.

Behavioral Apparatus

Behavioral training for both the stroke and spinal cord injury experiments took place in a clear acrylic cage measuring 12” x 4” x 10”. The clear acrylic material allowed for subject surveillance during the task. A 1cm x 2in opening located near the bottom right quadrant of the cage forced the subject to use the right forearm only to interact with either the knob or pull handle. The knob was spherical, grooved to improve grip, and weighted with a 5.9-gram counterweight to enforce a consistent clockwise rotation of the device. Knob accuracy could be measured to 0.25 degrees. The pull handle was made of aluminum and resembled a thin, rectangular rod with a small handle portion bent perpendicular to the ground. Pull measurements were accurate within 1 gram. In both studies, software in MATLAB was used to display, control file writing, and analyze data.

Figure 1. Schematic depiction of the behavioral apparatus for the knob and pull tasks.
A). Figure above illustrates the behavioral cage and knob device. Aperture on the right side limits animals to train only the right forelimb to perform the task. B). Pull task animals in the SCI study were also trained in a similar apparatus with the exception of a handle attachment in the place of the knob device. Image has previously been published in another experiment utilizing the isometric pull task (Hays et al., 2013a).

**Figure 2.** Photos illustrate the progression of movement required of the subject in order to perform the knob task. A female Sprague Dawley rat is shown to first extend the right forelimb, then grasp the grooved knob, and finally rotate the forelimb to successfully turn the knob. Images were captured of a subject prior to receiving a lesion.

**Figure 3.** Pre-lesion rat demonstrating pull task. Images show subject engaging with pull handle by employing right forelimb reach, grasp, and pull. Photos were taken from a previous study conducted under the same laboratory conditions with identical behavioral parameters (Khodaparast et al., 2013).
Ischemic Stroke Surgery

Animals were administered left motor cortex ischemic lesions as outlined in previous studies (Khodaparast et al., 2014; Adkins et al., 2004). Prior to administration of the lesion, rats (n=7) were anesthetized with ketamine (60 mg/kg), xylazine (6 mg/kg), and acepromazine (6 mg/kg) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). A craniotomy and durotomy was performed to expose the forelimb area of the left motor cortex (3mm to -1mm anterior-posterior and 2mm to 4mm mediolateral relative to bregma). To deliver the Endothelin-1 (ET-1) and induce cortical ischemia, a 26-gauge, blunted Hamilton needle attached to a stereotaxic arm was used to deliver the peptide to the cortical tissue. Eight injections (anterior-posterior: 2.5 mm, 1.5mm, 0.5mm, and -0.5mm, and mediolateral 2.5mm and 3.5mm from bregma) were delivered. At each injection site, the Hamilton syringe was filled with 1µL of ET-1, lowered 2mm into the cortex, and raised 0.2mm so the syringe was ultimately 1.8mm below the cortical surface. Every 30 seconds, 0.2µL of endothelin was administered to the cortex. After a total of 1µL of endothelin was delivered, injections were given an extra 3 minutes to infuse into the surrounding tissue. A ninth injection (3mm mediolateral and 0mm anteroposterior to bregma) was delivered 6mm below the surface of the cortex targeting the striatum. 1µL of Endothelin-1 was injected in the same manner as in previous injections. Once all nine injections were administered and given adequate time to infuse, kwikcast polymer followed by a thin layer of acrylic was placed on top of the cortex to fill the craniotomy. The incision was sutured with non-absorbable nylon sutures and antibiotic ointment was spread on the area to prevent infection. Post-operation, all animals were given Ringer's solution (subcutaneous, 2mL) and Baytril (subcutaneous, 10mg/kg).
Figure 4. Injection site locations for Hamilton syringe containing endothelin-1. Measurements are in millimeters and in reference to bregma. First 8 injections that are delivered 1.8mm below the cortical surface are in blue. In red is the ninth and final injection administered 6mm below the cortical surface.

Spinal Cord Injury Surgery

Pull-task proficient animals (n=23) received a right hemicord contusion at spinal level C5/C6. Rats were anesthetized with ketamine (60 mg/kg), xylazine (6 mg/kg), and acepromazine (6 mg/kg). Following incision at the spinal cord region and exposure of the vertebrae, a partial C5 laminectomy was performed. Using microforceps, the spinal C5 vertebra was firmly stabilized. To deliver the contusion, an impactor probe (Infinite Horizon Impact Device, Precision Systems and Instrumentation, Lexington, KY, impactor tip 1.25 mm in diameter) was lowered to the right side of C5. The probe delivered 175-225 kilodynes of force and displaced the spinal tissue 1600-1800 µm. After the lesion was delivered, the skin above the vertebrae was closed with surgical staples. Post-operation, subjects were given Buprenex (subcutaneous, 0.03 mg/kg), Baytril (subcutaneous, 10 mg/kg, once a day for 3 days after surgery), and Ringer’s solution (subcutaneous, 5 mL, once on the day of surgery). All animals were observed daily following surgery for infection or surgical complications.
**Figure 5.** Drawing of the C5 spinal cord contusion site. After the spinal cord is exposed, an impactor probe delivers the injury.

*Injury-only Control Group Assessment*

Subjects in both studies were randomly assigned to an injury only, rehabilitation only, or vagus nerve stimulation (VNS) and rehabilitation group. Injury-only animals were given one week following surgery to recover and then were assessed for 2 days, 2 sessions per day. These subjects did not return to daily behavioral training, but were instead assessed weekly, 2 consecutive days per week, for 4 weeks following recovery and the 2-day, post-operative assessment. During the assessment, rats underwent 2 successive adaptive sessions (day 1) and 1 static threshold session and 1 adaptive session (day 2).

*Rehabilitation*

Following one week of recovery after surgery, stroke rats were assessed on either the knob or pull task for 2 days to quantify the decrease in performance in the motor task after the lesion. Stroke rehab animals returned to behavioral training for 6 weeks following post-lesion assessment. Spinal cord injury rats returned to behavioral training 6 weeks following injury. Subjects did not perform the pull task during these 6 weeks of recovery, however were assessed in weeks 4 and 5 to determine pre-therapy performance. After 6 weeks of no behavioral training, rats returned to post-lesion training, or physical therapy, for another 6 weeks. As during pre-lesion training, subjects ran behavior in 30-minute sessions, twice per day, 5 days a week. Four days per week, subjects
completed the behavioral task on adaptive threshold stages to retrain the forelimb. The fifth day involved subjects performing the assigned motor task on static threshold stages, which was also when assessment took place.

*Vagus Nerve Stimulation*

Animals in the VNS and rehabilitation group underwent another surgery to insert a stimulation cuff and headcap, the equipment that helps deliver stimulation to the vagus nerve. Rats were again anesthetized with ketamine (60 mg/kg), xylazine (6 mg/kg), and acepromazine (6 mg/kg). A headcap (four-channel omnetics connector) was secured into the skull with 4 bone screws and acrylic surrounding the lambda skull suture. To insert the stimulation cuff, an incision to the left region of the neck was made. After blunt dissection of the muscle and surrounding fascia, the left cervical branch of the vagus nerve was exposed and the cuff (3mm) was placed so the tubing of the cuff would surround the nerve. The cuff was closed around the nerve via silk sutures within the cuff. Wire leads attached to the cuff and in contact with the vagus nerve were placed in a subcutaneous tunnel connecting the neck and head. The leads, soldered to metal pins, were then attached to the headcap. Any exposed wire was acryliced to the base of the headcap and the animal was sutured with non-absorbable nylon sutures. The animals were given one week to recover from surgery in which no behavioral training or assessment took place. After recovery, rats were assessed for 2 days by returning to the behavioral task to gauge the deficit after lesion administration. Following this post-lesion assessment, VNS with rehab animals returned to the behavioral task twice daily, 5 days per week, for 6 weeks. VNS was delivered through a cable attached to a commutator and the headcap. Once a successful trial was achieved during the behavioral session for the vagus nerve stimulation group animals, a set of 15 biphasic, 0.8 mA, 30Hz, 100µsec pulse-width pulses were administered by an isolated pulse stimulator (AM Systems, Isolated Pulse Stimulator, Sequim, WA). Animals in VNS and rehab group performed either the knob or pull task for 6 weeks following recovery. VNS was delivered for the first 5 weeks of rehab but
during the sixth week, subjects were not given any stimulation in order to evaluate any lasting effects of VNS.

Statistics

Data is reported as mean ± SEM. SEM is used in place of standard deviation to prevent representation of large shifts in spread due to a low n value. For stroke data, results are from a one-way repeated measures ANOVA. Bonferroni post hoc test compared pre-lesion performance to week 4 post-lesion. SCI-only data was from a one-way repeated measures ANOVA followed by a simple contrast to compare all post-SCI time points to pre-lesion performance. SCI therapy data is from a two-way repeated measures ANOVA followed by paired t-tests to compare VNS with rehab to rehab alone.

Results

Ischemic Stroke

Success Rate of Subjects at Knob Task Prior To Ischemic Lesion

To achieve a successful trial and receive a pellet reward, rats had to turn the weighted spherical knob by 60 degrees using the right forepaw. By the end of pre-lesion behavioral training, rats (n=7) had 84.0 ± 1.6% success rate at the knob task. Prior to receiving focal motor cortex ischemic stroke, average maximum turn angle of the knob apparatus was nearly 70 degrees (66.56 ± 1.51 degrees).
Figure 6a. Mean success rate (± SEM) of stroke only animals prior to and after focal motor cortex ET-1 ischemic stroke. Subjects displayed significant loss of knob task performance after ischemic injury. *** indicates p<0.001.

Figure 6b. Mean maximum turn distance (± SEM) of knob-trained rats receiving strokes only. Animals had greatest loss of turn ability immediately following ischemic stroke and recovery. The rats gradually showed improvement but remained significantly below pre-lesion turn performance throughout the 4 weeks after surgery recovery. * indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001.
**Figure 6c.** Mean trials per day (± SEM) of stroke subjects. Subjects completed significantly fewer trials per day during post-operative assessment but returned to achieving almost the same number of trials in weeks following surgery. * indicates p<0.05.

*Impaired Knob Task Performance Following ET-1 Stroke*

Administration of the cortical ischemic lesion significantly decreased success rate of subjects at the knob task. Initial post lesion assessment showed an average success rate of less than 20% (14.8 ± 4.2%, Bonferroni post-hoc test v. pre, p<0.001). By week 4, success rate barely improved and remained significantly below the success rate prior to lesion. Maximum turn angle performance also decreased to less than half of pre-lesion levels (29.81 ± 5.0 degrees) and only improved by less than seven degrees 4 weeks after the ischemic stroke was administered. Notably, the number of trials per day the rats performed significantly decreased during post-lesion assessment but returned to levels similar to pre-lesion performance one week later. Throughout assessment following the initial post-lesion training sessions, trials per day were not significantly lower than pre-lesion levels, indicating that lowered success rate was due to loss of supination function and not due to lower overall behavioral activity levels.

*(Expected) Recovery with VNS and Rehabilitation in Stroke Animals*
Because the stroke study is still in progress, data concerning the effect of vagus nerve stimulation and rehabilitation on motor performance at the knob task in rats is still in the process of being collected. Therefore, the effect of VNS and rehab in this experiment has yet to be determined. However, based on similar studies modeling the same stroke conducted under identical behavioral conditions but using the pull task instead of the knob task to measure loss of right forelimb strength, we expect that VNS and rehab rats will significantly improve motor function recovery.

Figure 7. Vagus nerve stimulation and rehab improves pull task hit rate performance in rats subjected to ET-1 focal motor cortex stroke in the left forelimb area. Data shows average success rate (±SEM) of VNS + rehab (n=6) verses rehab-only group (n=9). Success rate of subjects gradually improves through week 6. Vagus nerve stimulation group rats showed similar performance between weeks 5 and 6 even though stimulation was not delivered during the final week. Data is cited from previously published paper produced in the same laboratory conditions (Khodaparast et al., 2013). Based on these results, we anticipate similar significant recovery of supination function, as measured by the knob task, in VNS + rehab rats in the knob task study. We expect VNS + rehab rats to demonstrate significant improvements in success rate and maximum knob turn distance as compared to rehab only animals. We also predict that rats in the VNS group will show a consistent
success rate and maximum knob turn distance from week 5 to week 6 even if VNS is not delivered in week 6 because of the lasting effects of VNS on plasticity.

*Spinal Cord Injury*

**Successful Rate of Subjects at Pull Task Prior to Spinal Cord Injury**

Using the right forepaw, rats were required to pull on the pull handle with at least 120 grams of force to register a successful trial. Upon completion of pre-lesion behavioral training, subjects (n=12) had a successful trial rate of 86.8 ± 1.0%. Additionally, peak force during a pull task session was above 140 grams (156.7± 12.4 grams). Number of pulls per trial prior to lesion was approximately 5.5 ± 1.8 pulls.

![Hit Rate](image)

**Figure 8a.** Success rate (± SEM) of subjects (n=12) before surgery and after spinal cord injury. Following surgery, rats showed significant loss in pull task performance, even 4 weeks after spinal cord injury. Data represents average of the lesion only group. *** indicates p<0.001.
Figure 8b. Mean peak force (± SEM) of subjects in the injury only group. Subjects also showed a significant reduction of force during the pull task. There was slightly gradual recovery through week 4 after administration of the lesion, but rats remained unable to pull with the same peak force as prior to SCI. ** indicates p<0.01, *** indicates p<0.001.
Figure 8c. Mean number of pulls per trial (± SEM) during the pull task of SCI animals prior to and following injury. Pulls per trial decreased slightly, but not significantly, in the first week after spinal cord injury and gradually returned to pre-lesion levels within a month after injury.

Impaired Pull Task Performance After SCI

Following contusion at the cervical (C5) region of the spinal cord, animals exhibited significant decreases in pull task success rate as well as decreases in mean peak force. Success rate fell by more than 85 percent to 12.3 ± 15.9%. Peak force also decreased in the injury-only group, with maximum force dropping to approximately one-third of pre-lesion levels during the first week of post-lesion assessment and only recovering to less than half of pre-lesion levels one month after surgery and recovery.

Vagus Nerve Stimulation and Rehabilitation on Recovery of Performance on Pull Task

Figure 9a. Success rate (± SEM) of VNS + rehab (red, n=7) and rehab only (blue, n=4) groups. Animals in both groups showed improved recovery of motor performance at the knob task over injury-only animals. Significant improvements in recovery of VNS + rehab animals compared to rehab only animals are seen in the final stages of therapy, in weeks 4, 5, and 6. Note the consistent performance in VNS + rehab rats in week 6, although no VNS stimulation was delivered. Post 4/5 is
in reference to assessments in weeks 4 and 5 of recovery following injury while subjects were not regularly performing the pull task. * indicates p<0.05.

**Figure 9b.** Mean peak force of VNS + rehab (red, n=7) and rehab only subjects (blue, n=4). Peak force also improved in rehab and VNS + rehab animals as compared to the injury only group. In each week of therapy, VNS + rehab rats pulled with greater maximum force than rehab only rats. Peak force also increased between weeks 5 and 6 within the VNS + rehab group, even when VNS was not administered. In weeks 4, 5, and 6, rats in the VNS + rehab group pulled with significantly greater force than rehab-only rats. * indicates p<0.05.

Vagus nerve stimulation with rehabilitation and rehabilitation-only subjects both showed improvements in performance at the pull task as compared to rodents that received a spinal cord injury alone. By the end of therapy, animals receiving rehab alone had almost a 150% greater success rate over SCI-only animals, whereas rats treated with VNS paired with rehab achieved nearly a 300% increase in success rate at the pull task compared to injury only animals. VNS with rehab animals also showed a difference of approximately 26% in success rate above rehab-only animals. Mean peak force, another parameter measuring strength, also increased in rats that received either rehab or VNS with rehab therapy over injury-only animals. During weeks 5 and 6,
VNS with rehab animals showed the largest peak force difference over animals that were exposed to rehab only. At the end of therapy, VNS animals showed an increase of around 20.3 grams of peak pull force over rehab-only rats. In addition, VNS with rehab animals recovered nearly 80% of peak force pulled prior to spinal cord injury and rehab-only animals recovered around 65% of pre-lesion peak force. Finally, in both peak pull force and success rate assessments, VNS with rehab animals showed either better or nearly consistent performance in week 6, even though no vagus nerve stimulation was administered.

**Discussion**

Vagus nerve stimulation paired with rehab significantly improved recovery of motor function after injury, specifically after spinal cord injury. For the stroke study, stroke-only subjects showed significantly reduced supination function during the knob task as compared to pre-lesion levels. Currently, the study is still in progress, so we have not fully compiled the VNS with rehab or rehab-only data for the knob task animals. However, we anticipate significantly improved outcomes in the knob task after lesion when VNS is added to the therapy based upon previous studies examining the effect of VNS on ischemic stroke on similar forelimb tasks including a lever press or pull task (Khodaparast et al., 2013, 2014; Hays et al., 2014). Thus, vagus nerve stimulation may prove to be beneficial for supination function recovery after ischemic stroke. Subjects in the spinal cord injury study also showed significantly reduced strength in the pull task after injury alone. Importantly, during the last 3 weeks of therapy, VNS + rehab animals showed significantly greater improvements than rehab animals alone. This suggests that VNS may be an important therapeutic tool in recovery of strength in SCI. Also noteworthy was the improvements in motor function of VNS rats even after stimulation was no longer applied. This finding is consistent with prior studies and corroborates the long-lasting effects of VNS on plasticity. Because of the improvements in motor performance of VNS animals over rehabilitation only animals, utilization of targeted, plasticity-
driven therapy may be valuable in helping patients who currently cannot recover normal functioning with physical therapy alone. As of last year, approximately 60,000 patients have received VNS therapy for epilepsy or depression (Khodaparast et al., 2014). Some patients have reported minor side effects, but overall VNS is considered a safe intervention for several neurological disorders (George et al., 2000). Thus VNS may be a promising treatment for injuries including ischemic stroke and spinal cord injury.
References:


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