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**Vagus Nerve Stimulation (VNS) and Rehabilitation in the Treatment of TBI**

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**14. ABSTRACT**
Prior studies have found that VNS is effective in accelerating and enhancing recovery of function following traumatic brain injury (TBI). The main limitation to these earlier results is that VNS treatment was initiated at either 2 hr or 24 hr after TBI. Present findings indicate that VNS treatment is effective for recovery of function after TBI even when the initiation of VNS treatment is delayed until two weeks after injury. In addition, present evidence demonstrates that, in general, practice, or a rehabilitation regimen, that is conducted across consecutive days is more effective when done in conjunction with VNS treatment. In conjunction with VNS, a regimen of delayed practice (every two days) was found to be less effective than consecutive practice for functional recovery. These results provide heretofore unknown answers regarding the potential utility of VNS as a clinical treatment for human TBI.

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**Introduction:**

Vagus nerve stimulation (VNS) has been previously shown to facilitate recovery of function when treatment is initiated within 2hr or 24hr following experimental brain injury in rats (Smith et al. 2005, 2006). The main purpose of the current study was to assess the effectiveness of VNS treatment in a clinically relevant manner. For VNS to be useful as a translational device in the treatment of TBI, investigations into the optimal window of opportunity first had to be conducted. Further, for similar reasons, the duration of the treatment that is necessary for the maintenance of maximal functional recovery was also investigated. In addition, the frequency of rehabilitative training that was required to maximize functional recovery with concurrent VNS treatment was assessed. Two studies assessing these issues were conducted in the period April 1st, 2008 – March 31st, 2009. Each study comprised 3 groups of animals. A total of 80 animals were subjected to surgical procedures, treatment, and behavioral assessment. Preliminary data analysis involving close data inspection for homogeneity of injury effects resulted in a total of 54 animals (9 per group) that were included for final data analysis. The remaining animals were excluded based on stringent criteria that have been previously established in our laboratory based on many years of experience in conducting this particular line of research.

**Body:**

In accordance with the approved SOW, the experimental protocol was conducted as follows: 1) Animal purchase and handling, 2) TBI surgery, 3) VNS surgery, 4) Initiation of VNS treatment, 5) Behavioral Assessments, 6) Sacrifice, 7) Histology, 8) Data Analysis. Two experiments were conducted. At this time, the data for both experiments are in the final stages of data analysis.

**Experiment 1.** In the first experiment, 3 groups of animals underwent either sham surgery or experimental TBI induced by Controlled Cortical Impact. A brain injury was induced over the left hemisphere at the following coordinates: Epicenter: Midpoint between Bregma and Lambda; ML: 2.5mm. A 6mm craniotomy was created. The exposed cortical surface was lesioned using a 5 mm cortical impact tip at 40 PSI, 250 ms duration, 2.5 mm depth of penetration. Four days following the induced brain injury, the animals underwent a battery of behavioral assessments. Ten days following the first surgery, all the animals were subjected to another surgery to implant the VNS device and electrode. On day 14 post-TBI, behavioral assessment was again conducted. Following the end of the behavioral assessments for that day, VNS treatment was initiated. To keep the experimenters blind to the group assignments, every animal was implanted with a bipolar helical nerve electrode and the accompanying VNS device. Each device was programmed either to provide an intermittent electrical pulse ( 0.75mA, 20 Hz, 30 sec duration) repeated every 30 minutes, or sham stimulation (0.0mA). Stimulation continued for a period of 32 days. Beginning on day 28 post-TBI, behavioral assessments were conducted for 10 consecutive days. The battery of behavioral assessment comprised the Beam Walk, Forelimb Flexion, Inclined Plane, Locomotor Placing, and Vibrissae-Forelimb Placing. On days 42 and 43 post-TBI, the

animals were run in the Morris Water Maze (8 trials a day, 4 trials per quadrant, inter-trial interval interval was 15 min). All the animals were sacrificed on day 47 post-TBI. After standard perfusion methods, the brains were harvested and subsequently submitted to frozen sectioning using a microtome. 40 micron slices were saved throughout the brain lesion. Sections were selected out of every 0.5 mm to 1.0 mm region of the lesion, stained with Cresyl Violet, mounted on slides and imaged. The images were then submitted to lesion analysis where the area of remaining cortex was calculated using the Calivieri method to assess the size of the lesion. In addition, remaining frozen sections were stained for GFAP, imaged and astrocytic cell counts were conducted.

**Experiment 2:** In the second experiment, the same experimental protocol was used with the exception of the days of behavioral testing. Instead of 10 consecutive training days, the animals were given 10 sessions of behavioral training, once every other day.

**Data analysis:**

For both experiments, the behavioral data was first inspected to ensure homogeneity of severity of injury. The criteria for inclusion into final data analysis have been previously established in our laboratory. It is crucial for our purpose that we establish homogeneity of injury because we did not want to increase the risk of committing a Type 1 error.

Inclusion criteria was based on the Beam walk assessment which assesses the animal's ability to traverse an elevated beam on a scale of 1 to 7 where 1 indicated an inability to maintain balance on the beam and 7 is the ability to traverse the beam with no more than 2 foot slips. The data collected on the beam walk on days 4 and 14 post-TBI prior to the initiation of VNS treatment were used to assess the level of injury sustained by the animal.

**Shams:** All shams needed to receive a rating of no less than 5 on day 4 and no less than 6 on day 14. Based on our extensive prior experience in behavioral assessments and experimental neurotrauma, any other score received would have been indicative of inadvertent injury to the brain as a possible result of surgery.

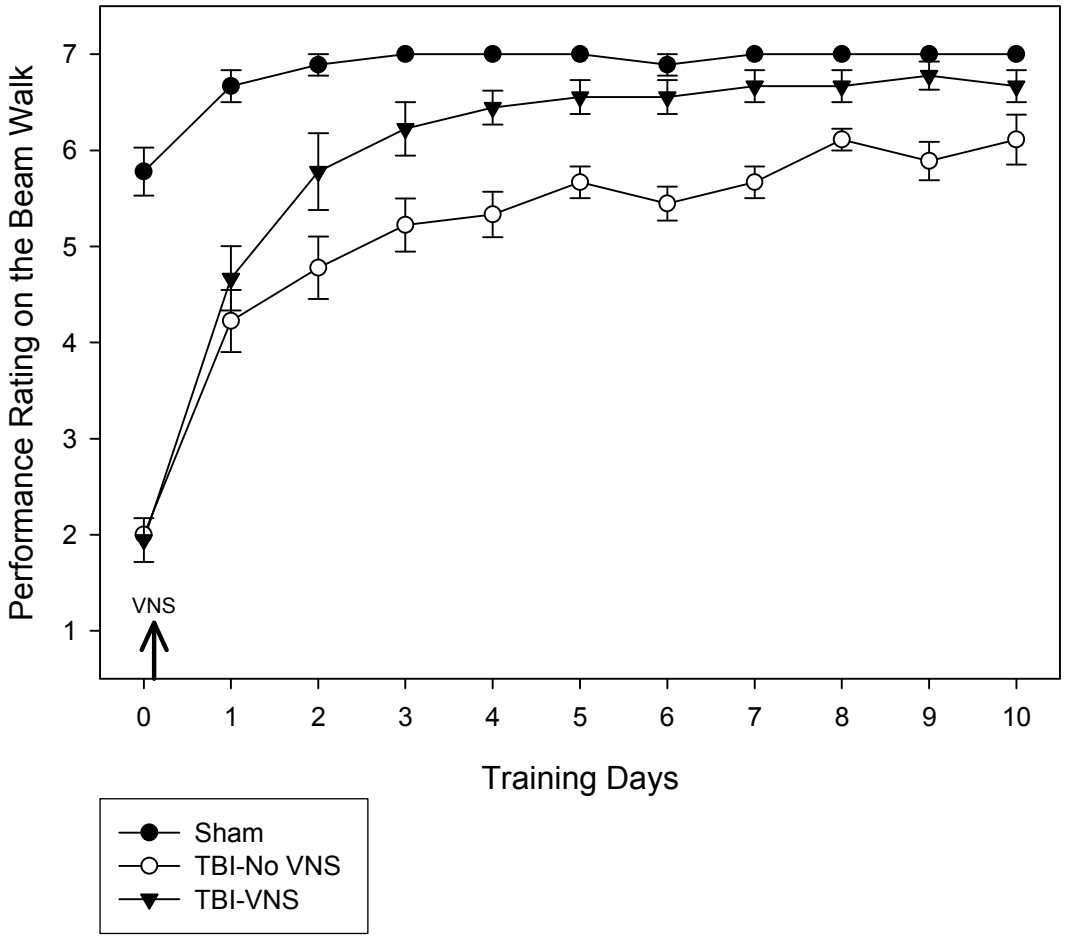
**Injured animals:** All animals that received a brain injury were required to have a rating of no more than 3 on day 4 post-TBI and no more than 4 on day 14 post-TBI. Any rating above that would have indicated that the animal did not sustain an injury that can be considered moderate – severe.

The data for all animals included in the final analysis were submitted to a General Linear Model analysis for repeated measures (SPSS 16.0). Post hoc analysis was conducted using Tukey's HSD. A significance level of 0.5 was used.

**Preliminary Results:**

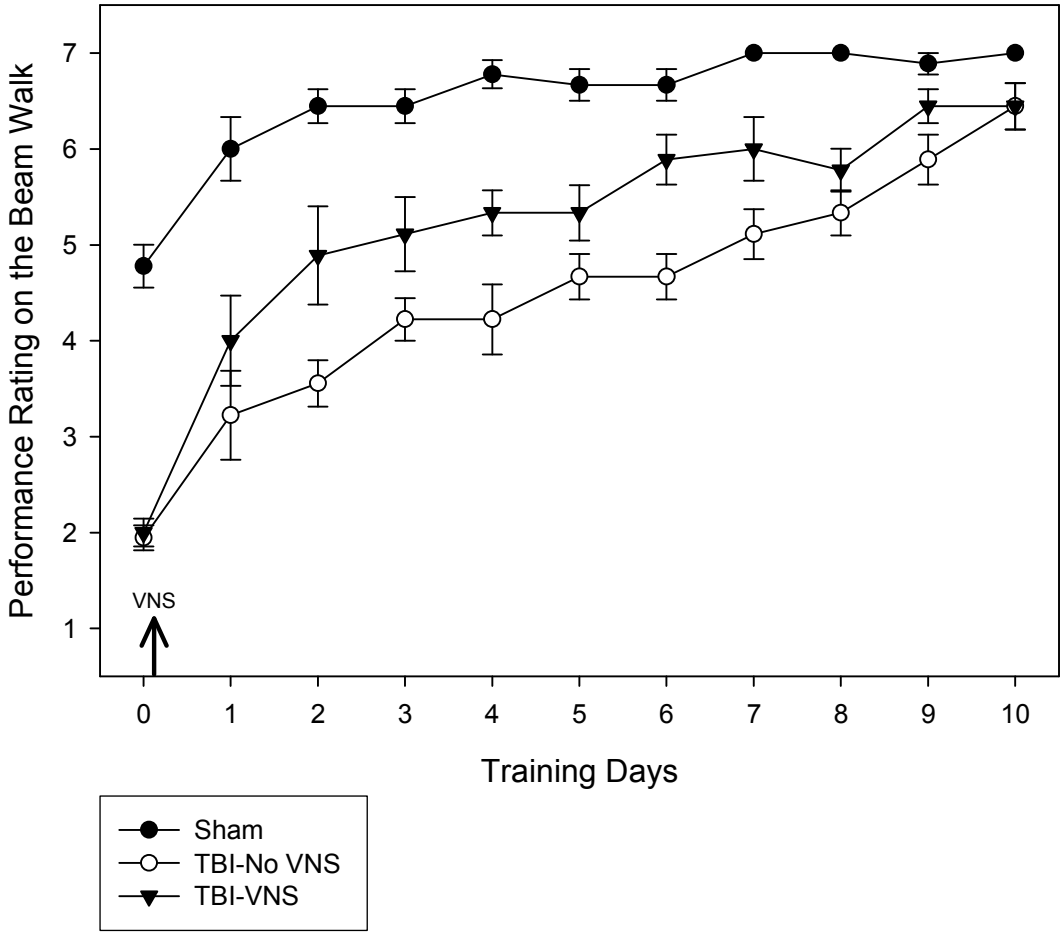
**Experiment 1:** Data from the Beam walk test was analyzed. Three groups of animals (n=9 per group) were included in the final analysis. The graph below (Fig. 1) summarizes the results obtained on the beam walk test. The results indicate that VNS treatment is effective in facilitating recovery of function on the beam walk after experimental brain injury. Animals receiving VNS treatment displayed less severe impairment in their ability to traverse the beam and a slightly accelerated rate of attaining ceiling performance.

Fig. 1. Beam Walk
Consecutive Days of Rehabilitative Training



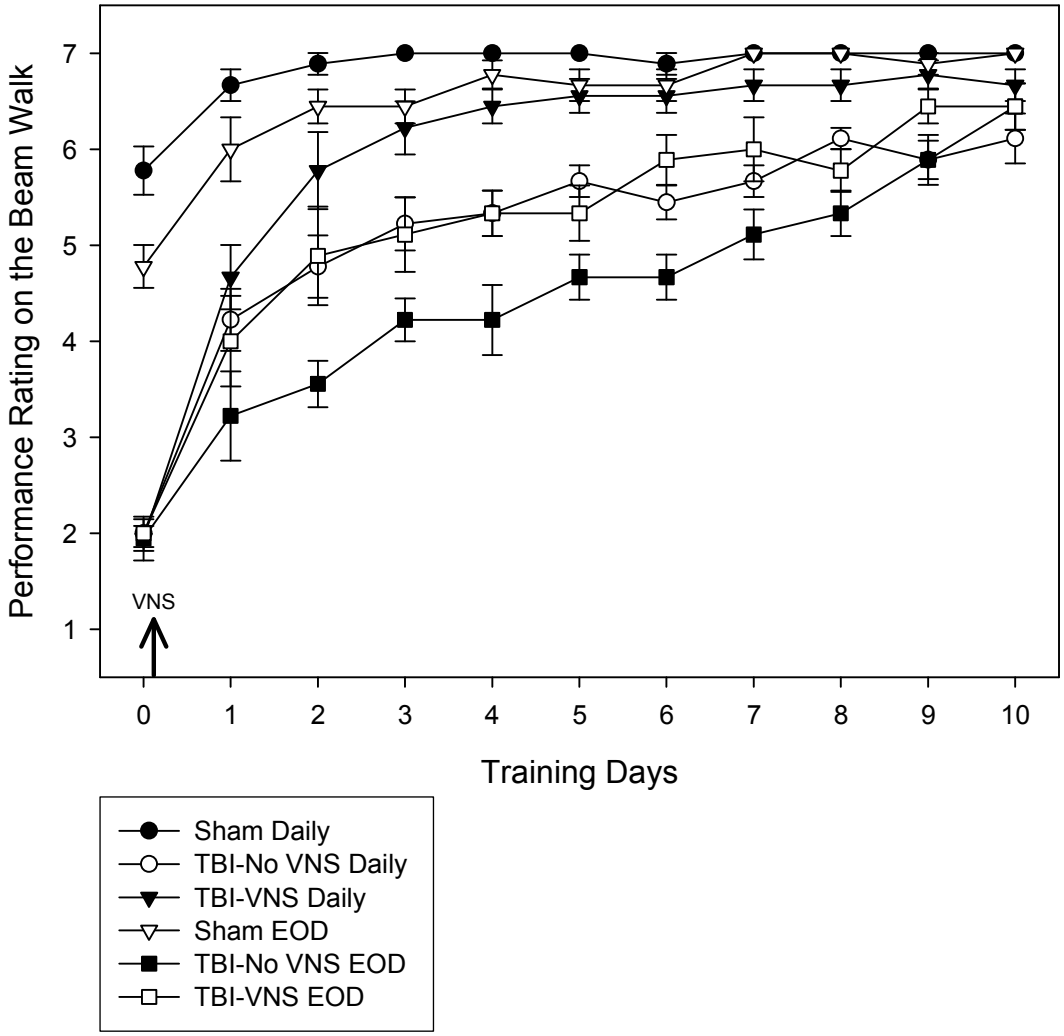
**Experiment 2:** Similar to Experiment 1, VNS treated animals performed better than non-treated injured animals on the beam walk. The graph below (Fig. 2) summarizes the results of this test. However, it appears that when rehabilitative training is delayed and occurs only every other day, the rate at which ceiling performance is attained is only slightly better than animals not receiving VNS.

Fig. 2 Beam Walk
Delayed (every other day) Rehabilitative Training



**Combination of results:** When the data from Experiment 1 and 2 are analyzed together, the combined effects of VNS and rehabilitative training are shown more clearly. As indicated in the graph below (Fig. 3), the brain injured animals that received both VNS treatment and consecutive days of rehabilitative training showed a faster rate of functional recovery.

Fig. 3 Beam Walk
Combined graph of Daily and Delayed (Every Other Day) Rehabilitative Training




Data analysis for the remaining behavioral assessments, including the data assessing cognitive performance, lesion size and cell counts is at this point incomplete, but it is expected that the results will be finalized in the next 2 months.

**What's New:**

1) The present results are exciting because they represent the first evidence that the initiation of VNS can be significantly delayed following brain injury and still be effective. Previously published results showed VNS to be an effective treatment for experimental brain injury when VNS was initiated at either 2hr or 24 hr post-TBI (Smith et al., 2005, 2006). Our findings extend the window of opportunity for VNS treatment from the acute period 2 hr or 24 hrs post-TBI) to the chronic stage (14 days post-TBI). Clinically, these results greatly enhance the attractiveness of VNS as a potential treatment for use in brain injured patients. Clearly, it is highly unlikely that a prosthetic device such as VNS would be implanted in a brain injured patient while he or she is still in the acute stage post-injury. Indeed, VNS treatment would not be suggested as a first in line treatment for brain injury. Instead, VNS is a potentially very promising adjunctive treatment after the initial phase of the trauma is complete and the patient is ready for rehabilitation.

2) The present findings also present the first evidence of the effectiveness of VNS coupled with rehabilitative training. In previously published studies by Smith et al. (2005, 2006) a regimen of pre-injury training followed by 7 sessions of behavioral assessments were administered every two days up to day 14 post-TBI. In the current study, the animals were not trained prior to brain injury. Further, the current experiments demonstrate that when training is conducted on consecutive days, the rate of functional recovery is enhanced. Further analysis of the histological data will be conducted to assess if there are any detrimental or positive effects of training on consecutive days versus every other day. The analysis is not complete at this time.

3) The current experiments also extended the duration of VNS from 14 days (Smith et al., 2005, 2006) to 32 days. In addition, rehabilitative training was begun at day 28 post-TBI. There is a general paucity of experimental neurotrauma literature that conducts behavioral assessments at this late a time after brain injury. Smith et al. (2005, 2006) completed all behavioral assessments within the first two weeks after brain injury. In the present experiments, behavioral assessments are begun almost one month after brain injury and continued for almost another 3 weeks thereafter. This represents an attempt to assess the effects of rehabilitative training in the chronic / recovery period after brain injury in humans.

**Problems:**

Several unanticipated problems were encountered during these two experiments. We did not foresee the complications of keeping the implanted prosthetic device in the animal.

1) Several animals developed excessive fluid buildup or seroma within the subcutaneous pocket that was created to house the device. Although the general health of the animals were not affected by the seroma (as verified by the institutional veterinarian) a frequent need to aspirate the region is not experimentally feasible for future studies.

2) Due to the extended duration (36 days instead of 14 days) that the device and electrodes were implanted, some animals began to scratch excessively at the scar where the sutures had healed and created open sores. These sores were fastidiously treated and when necessary, the area was sutured again.
3) Also due to the extended duration of the implant, tissue re-growth around the site of the helical coil of the electrode made extraction and re-use of the electrodes extremely difficult. As a result, several electrodes are no longer functional.

Given these problems, the investigators of this study do not feel that it would be advisable to continue using the VNS prosthetic devices in the final year of funding. It should be noted that at this time, these investigators feel that we have conducted a thorough and systematic series of experiments that provide sufficient evidence and justification for VNS to be tested in clinical trials, beginning with the series of published experiments in 2005 and 2006.

It is also crucial to understand that the problems we experienced in our current experiments are not of significance in clinical trials since VNS has been used as an FDA approved adjunctive therapy for refractory epilepsy since 1997 and for depression since 2005. While it appears that we experienced several surgical complications due to the implantation of the device (the size of the VNS device is rather large in proportion to the size of a standard laboratory rat), the side effects of VNS therapy in humans is minimal. Human side effects are limited mainly to sore throat and hoarseness that diminish over time.

**Recommended changes:**

At this time, we are seeking approval to make some changes to the previously approved SOW. Given the compounding issues we encountered with the VNS device, we are seeking to focus instead on the rehabilitative aspect of training in the chronic / late stages following TBI. We believe that it would be very beneficial to the field of experimental trauma if we were to further investigate the effects of rehabilitative training by systematic changes in the frequency of training provided, and expand the research to assess levels of cortisol as an index of anxiety and depression. Clearly, at this time, news reports indicate a disturbing trend in the increase of suicide rates and depression experienced by returning military personnel. It is also known in the literature that depression and anxiety are often co-morbid disorders following TBI. Since VNS has been shown to be an effective treatment for both epilepsy and depression, it is a natural extension of our investigations to more carefully assess the long-term effects of TBI. Few experimental studies conducted in rats have looked at the long-term effects of TBI on motor and cognitive behavior in conjunction with rehabilitative training and cortisol levels. It is proposed that rehabilitative training may play a role in decreasing cortisol levels, as an index of lowered anxiety.

In addition to the current battery of behavioral assessments we currently use, we would like to add the Rotor-Rod system (San Diego Instruments). We feel that the rotorod would better mimic the commonly used treadmill in terms of repetitive motor and coordination motions in human physical rehabilitation. Apart from the obvious use of assessing motor and coordination, we are also proposing that untreated brain injured animals are more likely than shams to be resistant to moving on the rotorod as a function of "depression" or "anxiety" and may choose instead to fall off, akin to the human element of suicide. Further, we would like to add a microplate reader to perform corticosterone ELISA to measure stress/PTSD following injury (Kohda et al., 2007). We seek approval at this time for the purchase of the Rotor-rod system and the microplate reader, in addition to the change in focus of the intial SOW. We believe that with the funds already received and the upcoming installment, we will have sufficient funds to purchase these two additional pieces of equipment and to conduct a comprehensive and careful investigation into the long-term effects of TBI, rehabilitative training, and anxiety/depression.

**Key research accomplishments:**

- Expanded window of treatment opportunity for VNS therapy in TBI (from 24 hr post-TBI to 14 days post-TBI)
- Established optimal frequency of rehabilitative training regimen in conjunction with VNS following TBI (daily vs alternate days)
- Expanded duration of behavioral assessments and treatment to better understand long term outcomes of TBI (out to 46 days post-TBI).

**Reportable outcomes:**

The results of this research will be used to generate 2 manuscripts (one focused on the VNS findings and the other focused on the effects of rehabilitative training). Data from this research will also be presented at either the Annual Meeting of the National Neurotrauma Society (2009) or at the Annual Meeting of the Society for Neuroscience (2009).

**Conclusion:**

Although data analysis is not complete at this time, the preliminary results obtained are exciting. The window of opportunity for VNS therapy in the treatment of TBI has now been extended to 2 weeks post injury in the rat. Further, it has been found so far that frequent, daily, consecutive rehabilitative training, when initiated

at the late stage (day 28) following TBI is more beneficial than intermittent (every other day) training. Further, this research lays the groundwork for future investigations into the effects of rehabilitation, depression/anxiety, and functional recovery in the later or chronic periods of TBI. The present results confirm and extend previous findings that VNS, an FDA-approved adjunctive therapy for refractory epilepsy and depression, is now, more than ever before, a potentially highly efficacious adjunctive treatment for long-term effects of TBI.

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**Appendices:** None

**Supporting Data:** None