Traumatic brain injury (TBI) continues to be a major cause of death and disability. Vagus nerve stimulation (VNS) has been shown to reduce proinflammatory responses in TBI models. We assessed the effect of a non-invasive VNS (nVNS) on reducing the lesion volume in a rat model of TBI.

**MATERIALS AND METHODS**

Male Wistar rats were randomly assigned to three different study groups: 1) Control (TBI with sham stimulation treatment), 2) lower dose (TBI with five 2-minute) nVNS, and 3) higher dose (TBI with five 2x2-minute) nVNS.

We used a standard controlled cortical impact (CCI) model. Cranietomy was performed over the left motor cortex area using a micro drill with a 5-mm trephine. Next, a CCI device (Impact one, Leica Microsystem, Buffalo Grove, IL) with a 3-mm flat impactor tip was centered at 2.5 mm lateral and 3.0 mm posterior from bregma to induce a moderate injury with the following parameters: 5 m/s speed, 100 ms dwell-time, and 2 mm deformation depth. After inducing the injury, the skin was closed.

A modified version of the commercial gammaCore (electroCore, Inc., NJ, USA) with miniaturized electrodes affixed to a velcro-collar was used to deliver non-invasive electrical stimulations (24 V, 60 mA, and 1 ms duration bursts of 5 kHz sinewaves, repeated at 25 Hz). We initiated nVNS therapy 30 minutes post-TBI with five nVNS treatments at the highest intensity/amplitude (24 V) with two treatment durations (lower dose [five 2-minute] or higher dose [five 2x2-minute], 10 minutes apart), see Figure 1.

We performed neurobehavioral assessments to assess the impact of nVNS therapy on improving the outcomes. For balance end motor function, we used rotarod and beam-walking assessments. For anxiety assessment, we used elevated plus maze (EPM) and modified beam walking (MBW). After a training period before injury, the assessments were performed on day 1 and 7 post-injury. Magnetic resonance imaging studies were performed 1 and 7 days post-injury to confirm lesion volume.

We observed smaller brain lesion volume in the lower dose nVNS group compared with the Control group on days 1 and 7. The lesion volume for the higher dose nVNS group was also significantly smaller compared with the lower dose nVNS and the Control groups on days 1 and 7 post-injury, see Figure 2.

The observed injuries on H&E slides for the treatment groups confirmed observed brain damage observed on MRI, see Figure 3. Voxel-based morphometry analysis revealed an increase in the ipsilateral cortical volume in the Control group due to tissue deformation and swelling. On day 1, these abnormal volume changes were 13% and 55% smaller in the lower dose and higher dose nVNS groups, respectively, compared with the Control group. By day 7, nVNS dampened cortical volume loss by 35% and 89% in the lower dose and higher dose nVNS groups, respectively, compared with the Control group, see Figure 4. Our TBI Control group showed significant deficits in rotarod, anxiety, and modified beam walking performance. Rotarod, beam walking, and anxiety performances were significantly improved in the higher dose nVNS group on day 1. The anxiety indices were also improved on day 7 post-injury compared with the Control and the lower dose nVNS groups, see Figure 5. Overall, the higher dose of nVNS therapy (five 2x2-minute stimulations) reduced brain lesion volume and was associated with improvement in motor function and anxiety.

**RESULTS**

**CONCLUSION**

Our study on the use of higher dose (five 2x2-min) nVNS demonstrates a marked reduction in the lesion volume observed by MRI studies, translating into improved motor function and anxiety. However, the reduction in the neurobehavioral deficits was not seen in our lower dose (2-minute) nVNS group. Should nVNS therapy be proven effective in clinical settings, it could dramatically change the landscape and potential for TBI therapy in civilian and military populations. The advantage of the nVNS treatment is that it can be potentially applied to a TBI victim (due to its small portable nature) at an earlier stage (as early as when the paramedic attends the patient) than is currently possible.

**Funding:** This research was funded by the Center for Brain Recovery and Repair (NIH P20GM109098, Pilot PI: Divani) and US Army Medical Research Acquisition Activity (USAMRIID, Award Number: W81XWH-17-2-0053, PI: Divani). Also, Department of Radiology start-up funding to Dr. Taylor.