Long-term Effect of VNS on Seizure Burden in an Animal Model of Chronic Epilepsy

Ioannis Vlachos1,2, Balu Krishnan1,4, Rashaad Sidique1, Edward Tobin1, Vinay Venkataraman1, Aaron Faith1, Shashank Prasanna1, Ashfaqe Shafique3, Kostas Tsalakis3, Leon Iasemidis1,2,3, Steve Marsh5, David Teirman5, Shivkumar Sabesan6, Steve Maschino6

1School of Biological and Health Systems Engineering, Arizona State University, 2Center for Biomedical Engineering Rehabilitation Science, Louisiana Tech University, 3School of Electrical, Computer and Energy Engineering, Arizona State University, 4Cleveland Clinic Foundation, Ohio, 5Laboratory for Translational Epilepsy Research, Barrow Neurological Institute, Phoenix, Arizona, 6Cyberonics, Inc., Houston, TX

Introduction
In a longitudinal animal study, we investigated the effect of Vagus Nerve Stimulation (VNS) on seizure burden employing the lithium-pilocarpine model of epilepsy in rats. We tested the hypothesis that the seizure burden is reduced upon stimulation of the vagus nerve over a comparatively long time. This hypothesis was tested by utilizing a protocol with VNS alternatively ON and OFF for 2 weeks at a time. The plasticity of the effect of VNS was investigated too.

Methods
Three male Sprague-Dawley rats were induced to chronic epilepsy according to the lithium-pilocarpine model of epilepsy [1].

Rats were implanted with a total of 8 tungsten microwires for long-term EEG recording from thalamus, hippocampus and cortex, and with two leads (Cyberonics Inc., Houston, TX) wrapped around the left vagus nerve in the ventral cervical region for electrical stimulation by externally positioned, programmable stimulators (AM Systems Inc., Sequim, WA). The VNS was delivered periodically (60 sec ON, 300 sec OFF). During the ON phase, a 30 Hz train of pulses, each of 220 μsec in duration and 0.5 mA in amplitude, was delivered. The long-term EEG was recorded and analyzed over five separate periods of the experiment that each lasted for at least 2 weeks: Baseline 1 (no stimulation), Baseline 2 (VNS stimulation), Baseline 3 (no stimulation). Seizures’ occurrence and duration were detected by retrospective analysis of the continuously recorded EEG via in-house developed software based on Teager energy [2]. Seizure burden was defined as the percentage of recording time a rat spent in ictal state, combining both seizure frequency and duration information.

Results
Average seizure frequency, seizure duration and seizure burden were estimated every 6 hours providing 56 to 70 values for each period.

All three rats showed reduction of seizure burden during VNS stimulation periods when compared with their preceding baselines. Similar behavior with small variations was observed for seizure frequency and duration. In addition, a trend towards reduction of seizure burden over time (from Baseline 1 to Baseline 3) was observed in two rats. In the third rat (rat #2), increase of the seizure burden was observed from Baseline 1 to 2, and saturation from Baseline 2 to 3.

Statistical analysis with the Wilcoxon rank-sum test [3] showed that the changes were statistically significant only for rats 1 and 2 at significance level α=0.05 (see table).

<table>
<thead>
<tr>
<th>Rat</th>
<th>Baseline 1</th>
<th>Baseline 2</th>
<th>Baseline 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL1-ST1</td>
<td>(0.02)</td>
<td>(0.01)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>BL1-ST2</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>ST2-BL2</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>BL1-BL3</td>
<td>(0.07)</td>
<td>(0.07)</td>
<td>(0.07)</td>
</tr>
</tbody>
</table>

Results from the Wilcoxon rank-sum test for comparison of seizure frequency, duration and burden between the different experiment periods. The arrows indicate increase or decrease with respect to the first of the two tested periods; the p-value of the test is given inside the parentheses. Bold are the changes that were statistically significant.

Conclusion
VNS was found to be effective in significantly reducing the seizure burden in the limited number of rats with chronic epilepsy that participated in this longitudinal study. Furthermore, plasticity of the VNS effect was observed in the majority of the rats. These results provide supporting evidence for the longitudinal use of VNS in reducing seizure burden.

References

Acknowledgements
This study was funded by Cyberonics, Inc., Houston, TX.