Dynamic Changes in Heart Rate and Cerebral Blood Flow

During Acute Vagal Nerve Stimulation

by

Peter Hillebrand

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2019 by the
Graduate Supervisory Committee:

Jeffrey Kleim, Chair
Stephen Helms Tillery
Jit Muthuswamy

ARIZONA STATE UNIVERSITY

May 2019

ABSTRACT

Vagal Nerve Stimulation (VNS) has been shown to be a promising therapeutic technique in treating many neurological diseases, including epilepsy, stroke, traumatic brain injury, and migraine headache. The mechanisms by which VNS acts, however, are not fully understood but may involve changes in cerebral blood flow. The vagus nerve plays a significant role in the regulation of heart rate and cerebral blood flow that are altered during VNS. Here, the effects of acute vagal nerve stimulation using varying stimulation parameters on both heart rate and cerebral blood flow were examined. Laser Speckle Contrast Analysis (LASCA) was used to analyze the cerebral blood flow of male Long–Evans rats. In the first experiment, results showed two distinct patterns of responses to 0.8mA of stimulation whereby animals either experienced a mild or severe decrease in heart rate. Further, animals that displayed mild heart rate decreases showed an increase in cerebral blood flow that persisted beyond VNS. Animals that displayed severe decreases showed a transient decrease in cerebral blood flow followed by an increase that was greater than that observed in mild animals but progressively decreased after VNS. The results suggest two distinct patterns of changes in both heart rate and blood flow that may be related to the intensity of VNS. To investigate the effects of lower levels of stimulation, an additional group of animals were stimulated at 0.4mA. The results showed moderate changes in heart rate but no significant changes in cerebral blood flow in these animals. The results demonstrate that VNS alters both heart rate and cerebral blood flow and that these effects are dependent on current intensity.

TABLE OF CONTENTS

Page

LIST OF FIGURES iii

BACKGROUND 1

MATERIALS AND METHODS 4

Subjects 4

Cuff Implantation and Vagal Nerve Stimulation 4

Laser Speckle Contrast Analysis (LASCA) 6

Pilot Procedures 7

Experimental Design 8

RESULTS 10

Regular Current Intensity – 0.8mA Stimulation 10

Reduced Current Intensity – 0.4mA Stimulation 13

DISCUSSION 15

REFERENCES 21

LIST OF FIGURES

Figure Page

Figure 1: Cuff Implanted On The Vagus Nerve 5

Figure 2: VNS Cuff (Left), VNS Cuff Design (Right) 6

Figure 3: Perimed PSI System (right) and Example of LASCA Image (left) 7

Figure 4: Perimed App Output, Including Blood Flow Graph (Top), Perfusion Image (Bottom Left), Intensity Image (Bottom Middle), Subject Photo (Bottom Right) 7

Figure 5: Vagal Nerve Stimulation Design; Experiment 1, Top (10 Minutes No Stimulation, 10 Minutes 0.8mA Stimulation (Black Bars), 10 Minutes No Stimulation), Experiment 2, Bottom (10 Minutes No Stimulation, 10 Minutes 0.4mA Stimulation, 10 Minutes No Stimulation)... 8

Figure 6: Heart Rate Averages for Control and Vagal Nerve Stimulation Groups 12

Figure 7: Cerebral Blood Flow Averages for Control and Vagal Nerve Stimulation Groups.. 12

Figure 8: Heart Rate Averages for Control and Mild and Severe VNS Groups............... 13

Figure 9: Cerebral Blood Flow Averages for Control, Mild, and Severe VNS Groups ... 13

Figure 10: Heart Rate Averages for Control, Mild, Severe VNS, 0.4 Stimulation Groups 15

Figure 11: Cerebral Blood Flow Averages for Control, Mild, Severe VNS, 0.4 Stimulation Groups 15

Figure 12: Potential Role of the Vagus Nerve in Modulating Cerebral Blood Flow and Heart Rate. RVLM: Rostral Ventrolateral Medulla, CVLM: Caudal Ventrolateral

Medulla, DMV: Dorsal Motor Nucleus of the Vagus Nerve, NA: Nucleus Ambiguous,

NTS: Nucleus Tractus Solitaries, BV: Blood Vessels 20

BACKGROUND

Neuromodulation, including non-invasive brain stimulation and cranial nerve stimulation, has become a promising therapeutic approach for the treatment of several neurological conditions. While the earliest known use of Vagal Nerve Stimulation (VNS) was in 1883, research into its mechanisms and possible uses are just beginning. The Food and Drug Administration (FDA) initially approved it for use in patients with epilepsy in 1997. It has since been cleared by the FDA for use in depression, cluster headaches, and migraines, and number published articles exploring it per year have increased dramatically in recent years (3).

The specific mechanisms by which VNS acts to treat these conditions are largely unknown. The right and left vagus nerves exit from the brainstem, and go through the neck (in the carotid sheath between the carotid artery and jugular vein), upper chest (along the trachea), lower chest and diaphragm (along the esophagus), and into the abdominal cavity (1). Additionally, in the brainstem, the sensory afferent fibers terminate in the nucleus tractus solitarius, which then sends fibers that connect directly or indirectly to different brain regions. These regions include the dorsal raphe nuclei, locus coeruleus, amygdala, hypothalamus, thalamus, and orbitofrontal cortex (2). The vagus nerve also interfaces with parasympathetic control of the heart, lungs, and digestive tract such that it regulates heart rate, blood pressure (3), the gag reflex and satiation following consumption of food (4).

Animal studies have shown that VNS causes a number of physiological changes. For example, VNS modulated the dendritic cell profile by decreasing CD103+ dendritic cell population in the posthemorrhagic shock mesenteric lymph, which mediates the inflammatory response (5). It has also been shown to attenuate inflammatory response to

endotoxin (6), and macrophage activation (7). Vagal nerve stimulation has been used as a therapeutic technique for many diverse conditions and diseases. For stroke, it has been used to improve functional outcomes in rehabilitation (8-9) and also has been shown to reduce infarct size if stimulation is applied 30 minutes after a stroke for of 1 hour (10). VNS has most effectively been used to treat epilepsy (11-12) and depression (12-13). Additionally, VNS has shown positive effects in the recovery of traumatic brain injury in humans (14). Studies in our own lab have suggested that VNS may enhance motor recovery and reduce infarct volume in an animal model of stroke (unpublished results). However, it did not seem to alleviate motor impairments in an animal model of Parkinson's Disease (unpublished results).

One potential mechanism by which VNS acts is by altering cerebral blood flow. According to previous studies, high and low levels of stimulation both increase and decrease cerebral blood flow in humans in certain regions of the brain (15-19). One study, though, found that standard VNS is less potent in modulating cerebral blood flow as microburst VNS (20). Decreases in the left and right lateral orbitofrontal cortex and left inferior temporal lobe and significant increases in the right dorsal anterior cingulate, left posterior limb of the internal capsule/medial putamen, the right superior temporal gyrus, and the left cerebellar body (21). In other studies, however, it was shown that VNS has induced inconsistent patterns of activation and deactivation. This inconsistency may reflect differences in the methods used to measure CBF measurement (SPECT or PET), the time point of measurement relative to the initiation of VNS and the kind of stimulation, (chronic, frequency, output current, stimulation time) (33).

The specific mechanisms by which the brain modulates cerebral blood flow are unclear, but several key mechanisms have been proposed (22). The brain has the ability to maintain relatively constant blood flow, known as autoregulation. Autoregulation occurs when cerebral perfusion pressure (CPP) is between 50 and 150 mmHg. A second mechanism that has been identified is flow-metabolism coupling, by which cerebral blood flow varies with cerebral metabolism. Several molecules have been shown to have an effect on neuronal activity in the context of the regulation of cerebral blood flow, namely potassium and hydrogen as a result of their involvement in synaptic transmission (22).

The effect of vagal nerve stimulation on heart rate has also been researched extensively to help explain the mechanisms of the vagus nerve. VNS has been found to have the potential to effectively and rapidly to decrease heart rate, in acute settings (23-24). In the context of VNS, heart rate is often analyzed by measuring heart rate variability. Heart rate variability has been shown to both increase and decrease depending on individual response as well as stimulation parameters, but no direct correlation has been found (25). It has also been shown that in dogs, stimulation of the right vagus caused a greater reduction in heart rate than stimulation of the left vagus (26). In the present study, we investigated how acute VNS alters cerebral blood flow and heart rate in rats.

It is also important to make note of the effects of differing levels of stimulation already discovered in literature. When VNS has been used as a therapy for epilepsy, seizure frequency has been shown to increase at lower output currents (27). Additionally, it has been shown that different fibers of the vagus nerve respond differently to different types of stimulation, as vagal A-fibers have the lowest amplitude-duration threshold required for VNS to excite action potentials and B-fibers have higher excitation thresholds whereas the

highest excitation thresholds belong to the narrow, unmyelinated C-fibers (28). VNS. Increasing VNS intensity to resulted in tachycardia during the on-phase of VNS, and further dampened the sinus bradycardia with no change in average heart rate, and then eventually induced the expected bradycardia during the on-phase of VNS (29). Recent studies have shown that Reducing the current intensity and number of stimulations (Fast VNS) resulted in robust cortical plasticity, using 6 times fewer VNS pairings than the Standard protocol. However, reducing the current too much, under identical circumstances will insufficient in driving cortical plasticity (30, 31).

MATERIALS AND METHODS

Subjects

Nineteen adult (80+ days old) male Long–Evans hooded (330–500g) were used in the study Controls (n=9) and VNS (n=10). They were group housed (two animals/cage) in standard laboratory cages on a 12:12 hour light/dark cycle throughout the experiment. The animals were food restricted prior to surgery to ensure the appropriate response to isoflurane, xylazine, and ketamine. Rats were initially anesthetized via a 5% induction of isoflurane gas and maintained at 1.5%). After cuff implantation, a craniotomy over the frontal cortex was made (-0.5-4.0mmAP; 0.0-4mmML Bregma) under xylazine (5mg/kg) and ketamine (100mg/kg) anesthesia. The dura was removed and the cisterna magna was punctured to alleviate edema.

Cuff Implantation and Vagal Nerve Stimulation

Vagal Nerve Stimulation was applied via custom-made bipolar stimulating nerve (5-6 kOhms impedance) cuffs that were built in the Kleim Lab based on a protocol from the Kilgard Lab at University of Texas at Dallas. First, two strands of platinum iridium

wire were cut 7.5cm long and stripped on each end. Care was taken to ensure that just the necessary amount of bare wire was exposed, as extra bare wire could cause complications during stimulation. One end of each wire was then soldered into a gold pin and UV glue was added to cap the solder material and cured with UV light. Next, 3mm Micro-Renathane tubing with a diameter of 1.5mm was cut and an anterior cut was made into which the nerve would be placed. The PI wires with gold pins were then threaded through holes made in the tubing and the entire cuff was inspected under a microscope and UV glue was added where necessary to ensure that no bare wires were exposed. The cuffs were placed on the nerve in the opening that was made near the throat of the rat, as is shown below. The pins were left outside the rat and attached to the Isolated Pulse Stimulator. Stimulation consisted of a 500ms train of pulses at 30Hz. For the first experiment, each biphasic pulse was 0.8mA and 100µs in phase duration. For the second experiment, , each biphasic pulse was 0.4mA and 100µs in phase duration.

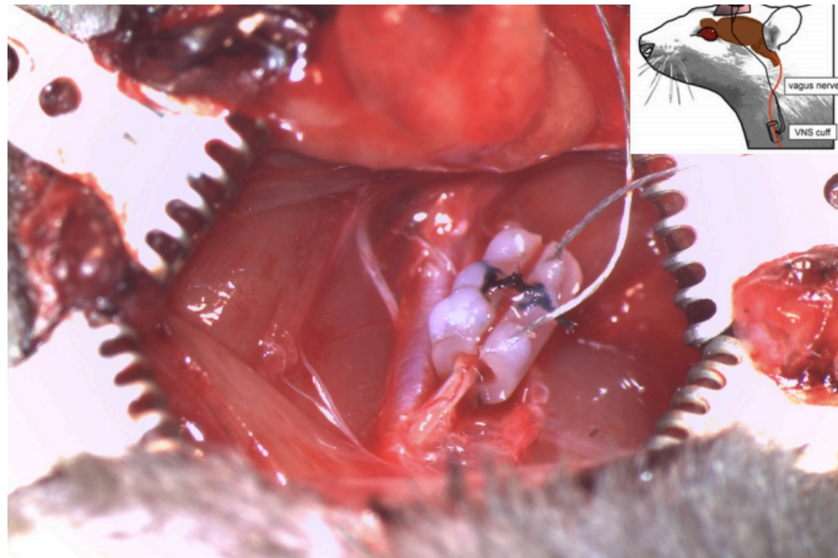

Figure 1: Cuff Implanted On The Vagus Nerve

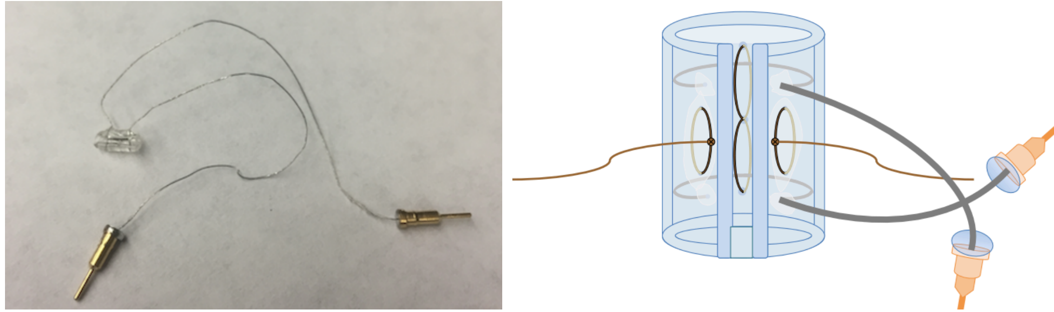

Figure 2: VNS Cuff (Left), VNS Cuff Design (Right)

Laser Speckle Contrast Analysis (LASCA)

Prior to the experiment, a number of pilot animals were to tested to optimize the LASCA system. The camera used was a PeriCam PSI System by Perimed and had been previously used to determine large scale changes in blood flow observed after endothelin 1 application to the frontal cortex. We then tested it to see if it could be used to test subtler differences observed during VNS. The PeriCam PSI System works by using Laser Speckle Contrast Analysis (LASCA) on a defined region of interest. LASCA visualizes tissue blood perfusion by illuminating the region of interest with laser light and collecting the resulting "speckle pattern" that is backscattered. This speckle pattern is sensitive even to blood flow movements, and can thus be used to measure blood perfusion. The PeriCam PSI System records the blood perfusion using the arbitrary units, Perfusion Units (PU) by measuring the speckle contrast, which is defined as the ratio between the standard deviation and mean of intensity. The camera was placed at approximately 10.0cm above the region of interest so that the entirety of the opened skull is within the region.

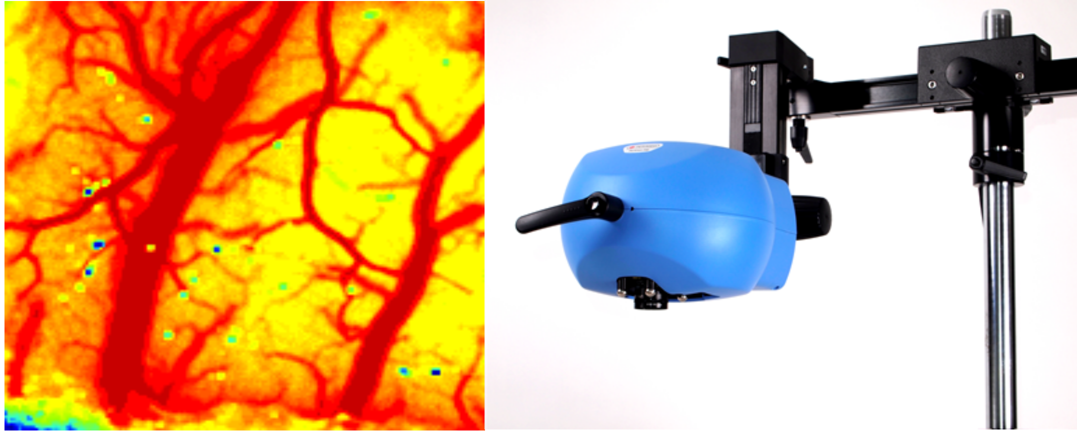

Figure 3: Perimed PSI System (right) and Example of LASCA Image (left)

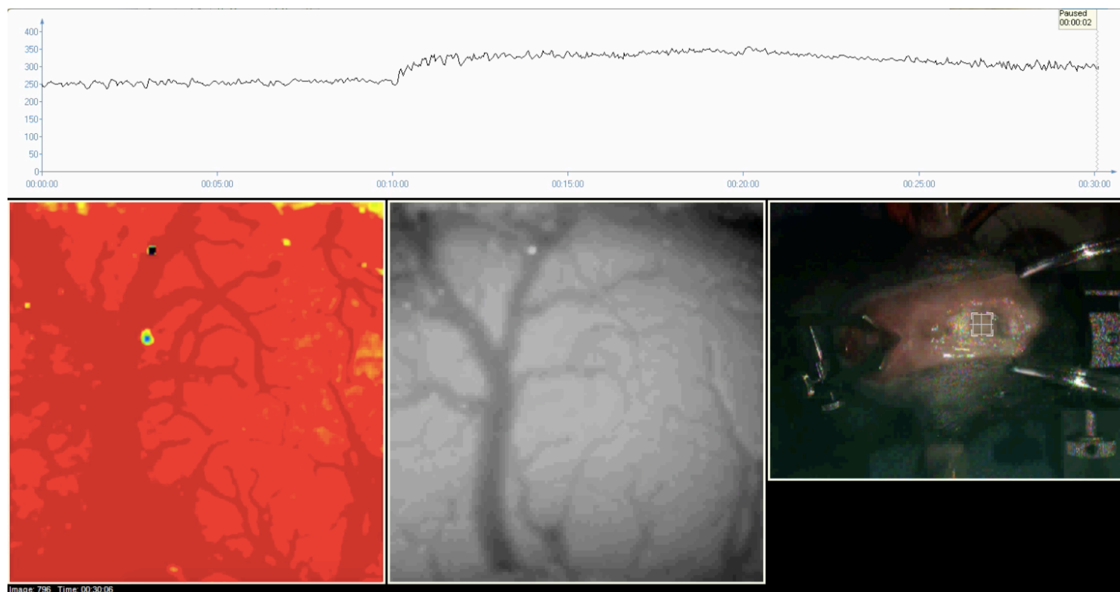

Figure 4: Perimed App Output, Including Blood Flow Graph (Top), Perfusion Image (Bottom Left), Intensity Image (Bottom Middle), Subject Photo (Bottom Right)

Pilot Procedures

During a test surgery, several important parameters were analyzed and finalized. For one, it was determined whether or not cerebral blood flow could be adequately measured without fully exposing the brain. If this were the case, the subject could be kept under gas the entire surgery. It was discovered that this was not the case and, as a result, it

was decided to inject the subject with ketamine and xylazine to keep them anesthetized during the opening and measurement of cerebral blood flow. Additionally, the parameters of the PIMSoft software (distance of camera, size of region of interest, frame rate) were tested and analyzed as well as the practical functions of each directive option provided by the software. Once this was done, cerebral blood flow was measured on this test animal. To ensure that the blood flow was being adequately measured, an agent that causes blood flow to dramatically decrease, endothelin was placed onto the region of interest. The camera immediately registered a severe drop in blood flow, and the functionality was confirmed.

Experimental Design

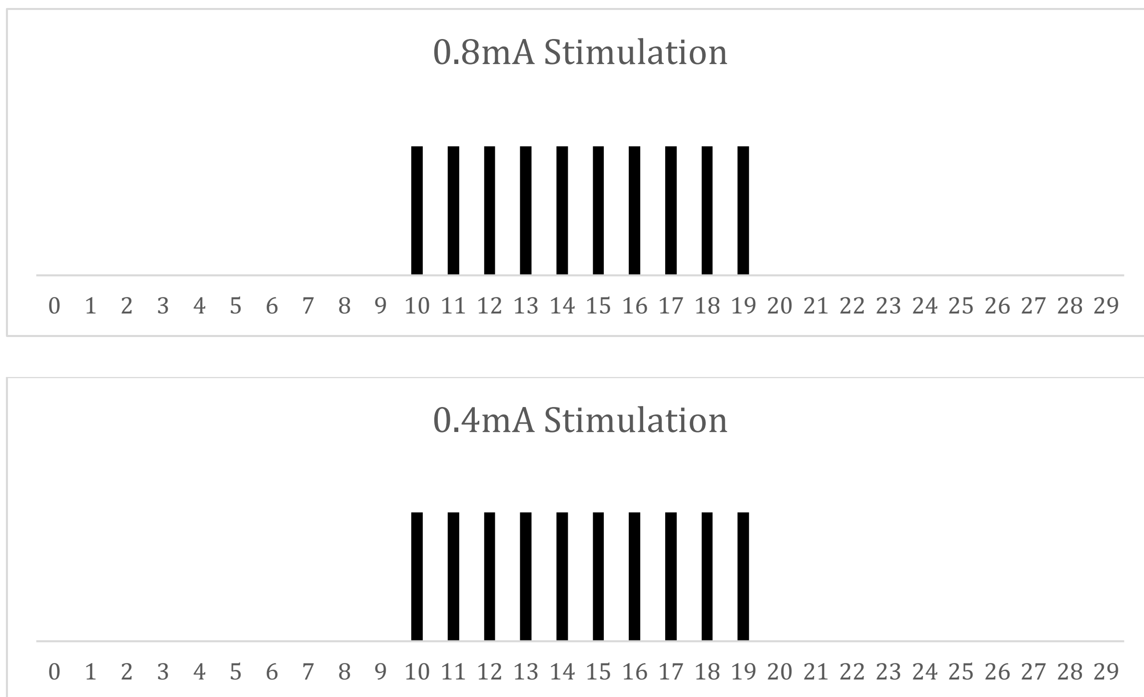

Figure 5: Vagal Nerve Stimulation Design; Experiment 1, Top (10 Minutes No Stimulation, 10 Minutes 0.8mA Stimulation (Black Bars), 10 Minutes No Stimulation), Experiment 2, Bottom (10 Minutes No Stimulation, 10 Minutes 0.4mA Stimulation, 10 Minutes No Stimulation)

While under, the vagus nerve was isolated by making an incision slightly left of midline and above the sternum. The vagus nerve was then isolated from the carotid artery in order to place the cuff around the nerve. After finding the nerve and ensuring that the correct nerve had been located, the cuff was implanted on the nerve and tested in three ways. Firstly, there had to be no error light on the stimulator, which would denote whether or not the current was actually passing through the electrode. Secondly, it was ensured that the electrode was not stimulating any other part of the body as it was noted that surrounding muscle tissue could be stimulated if the cuff was not placed properly. Finally, the heart rate was monitored using a small animal pulse oximeter and compared with the vagal nerve stimulation on and off. After these tests were done, the cuff was secured in various ways: chiefly suturing, dental acrylic, and wex-cels. Some of these methods were discovered throughout the experimental process and will be discussed further in the discussion section.

Following the craniotomy, the cuff was again tested to ensure that there was no error light, no unnecessary stimulation, and a significant drop in heart rate. Once each of these things were assured, the VNS was set in the "off" position, and the camera was placed over the brain so that the entirety of the "target" region included the exposed brain. This was done by making incremental movements of the camera until the entire region had either red or yellow pixels, which would later average out to a more defined image of the brain. Once this was completed, all the lights in the room were turned off and the recording button was pressed. Two groups were planned for Experiment 1. In the first group, ten minutes of data was collected with no stimulation, and then the VNS was turned on and ten more minutes of data were collected. Lastly, the VNS was turned off again and ten minutes of data were collected. In control animals, no stimulation was given for the entire

thirty-minute period. For Experiment 2, ten minutes of data was collected with no stimulation, and then the VNS was turned on at 0.4mA and ten more minutes of data were collected. This was again followed by 10 minutes of no stimulation and then the VNS was turned on again, this time at 0.8mA and ten minutes of data were collected. Throughout the recordings, both the heart rate and oxygen saturation levels were recorded every two minutes. Additionally, the initial drop in heart rate was noted at its minimum to keep track of the total initial drop. Any injection or disturbance throughout the thirty minutes was registered. Due to variability in both heart rate and blood flow, all measures were normalized to baseline (0-10 minutes).

RESULTS

Regular Current Intensity – 0.8mA Stimulation

A Oneway Repeated measures ANOVA with Group as a between subject factor and Time as a within subject factor revealed a significant Group X Time interaction [$F(14,238)=5.68$; $p<0.001$) on relative cerebral blood flow. VNS animals had significantly lower mean blood flow measures compared to controls across minutes 10 through 11:40, normalized at minutes 12-14 and then significantly higher through minute 30 (Figure 7). A similar analysis conducted on heart rate showed a significant Group X Time interaction [$F(14,38)=3.678$; $p<0.01$) on heart rate. VNS animals showed a significant decrease in heart rate compared to controls immediately after VNS was turned on that remained lower until minute 24 (Figure 6).

It was noted that there were two very distinct responses in VNS rats. Half of the animals showed a severe drop in heart rate (60% or more; mean decrease of 148.4) and half showed a mild drop (20%; mean decrease of 56.8bpm). A subsequent analysis was conducted to analyze changes in cerebral blood flow and heart rate in these subsets of groups. A significant Group X Time interaction [$F(30,240)=15.197$; $p<0.001$) was found for heart rate with the Severe VNS animals showing a significantly lower mean heart rate in comparison to both the controls and Mild VNS animals immediately after VNS onset that returned to Mild VNS levels through minutes 14-20. Heart rate levels then were significantly higher than both groups the duration of VNS. Mild animals were significantly lower than Controls until minute 28. Further a significant Group X Time interaction [$F(38,304)=9.19$; $p<0.01$) was found for cerebral blood flow. Severe VNS animals showed a decrease in CBF as compared to both the Controls and Mild VNS animals immediately within the first 40 seconds after VNS was turned on (Figure 9). Cerebral blood flow then began to increase and stayed higher than control levels from minute 16 and then throughout the recording time. Mild VNS animals on the other hand showed a progressive increase in blood flow as compared Controls through minute 30. Thus two different patterns of cerebral blood flow were observed within the VNS animals that appeared to coincide with differences in the initial decrease in heart rate.

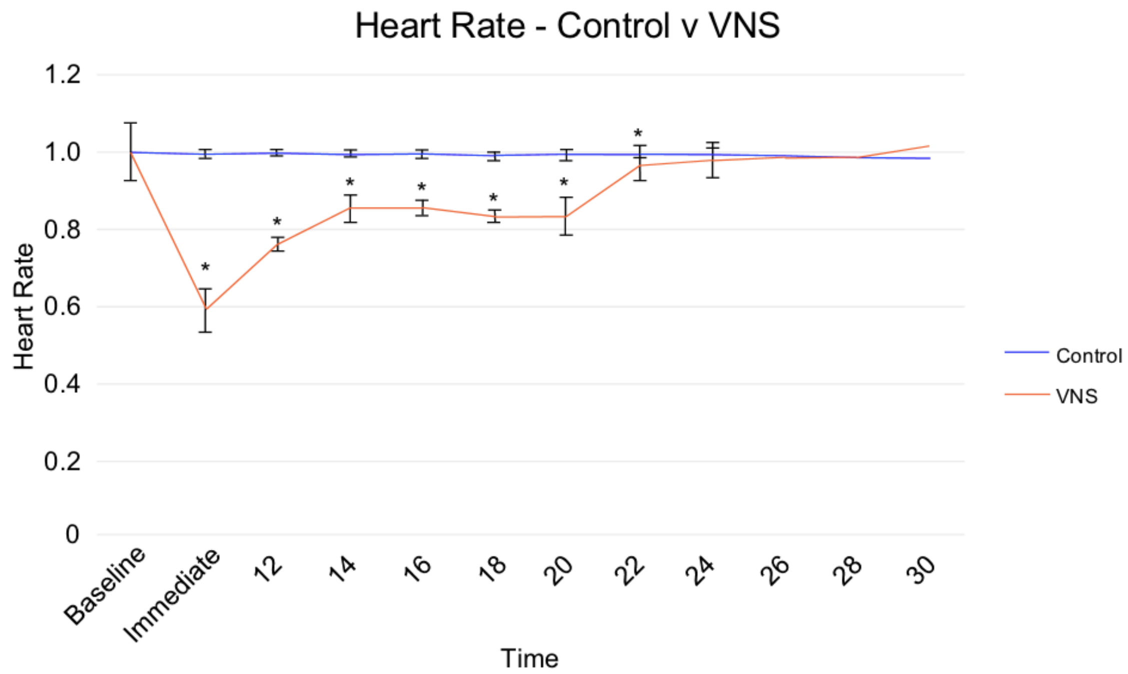

Figure 6: Heart Rate Averages for Control and Vagal Nerve Stimulation Groups

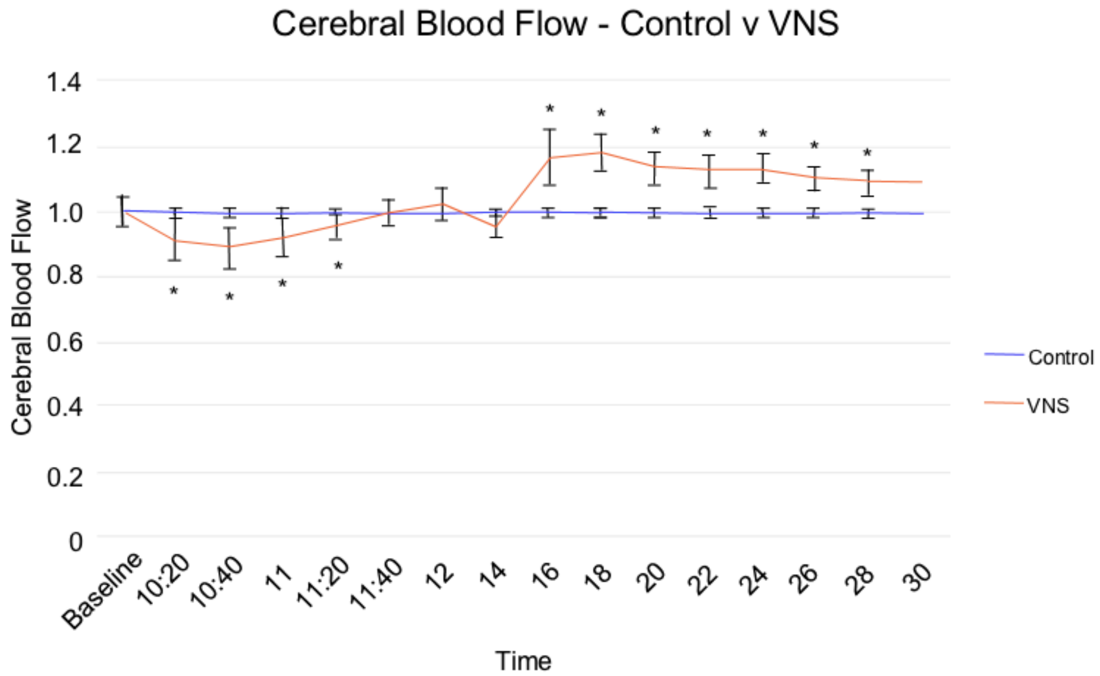

Figure 7: Cerebral Blood Flow Averages for Control and Vagal Nerve Stimulation Groups

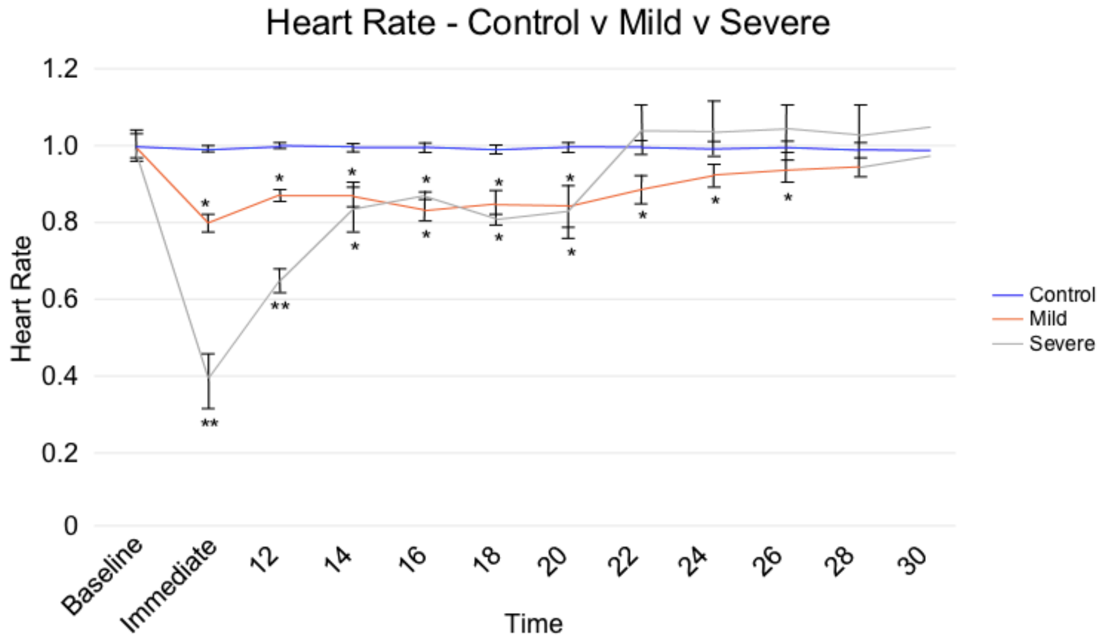

Figure 8: Heart Rate Averages for Control and Mild and Severe VNS Groups

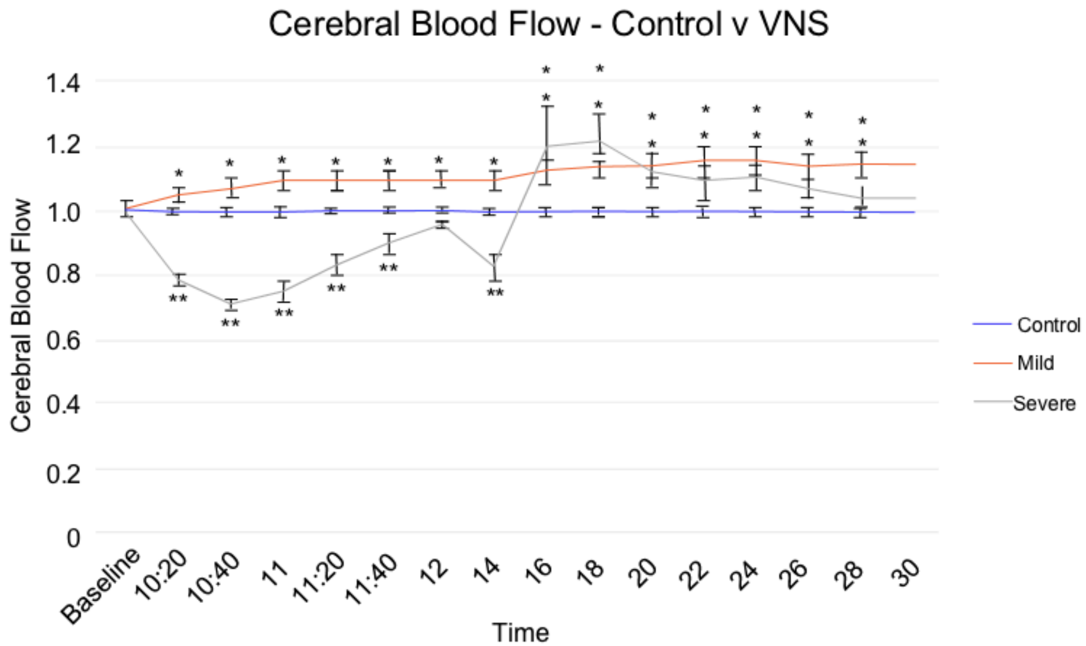

Figure 9: Cerebral Blood Flow Averages for Control, Mild, and Severe VNS Groups

Reduced Current Intensity – 0.4mA Stimulation

To examine the effects of reducing current intensity on the variable responses observed above, an additional group of animals was added. Stimulation again consisted of a 500ms train of pulses at 30Hz, but each biphasic pulse was now 0.4mA and 100µs in phase duration. A Oneway Repeated measures ANOVA with Group as a between subject factor and Time as a within subject factor revealed a significant Group X Time interaction [$F(45,330)=3.53$; $p<0.001$) on relative cerebral blood flow. Subsequent multiple comparisons (Fisher's LSD) showed the 0.4mA animals did not significantly differ from Controls at any point but did differ from the Mild and Severe animals at the same time points (Figure 11). A Oneway Repeated measures ANOVA with Group as a between subject factor and Time as a within subject factor revealed a significant Group X Time interaction [$F(30,2200)=17.08$; $p<0.001$) on relative heart rate. Subsequent multiple comparisons showed the 0.4mA animals did have significantly lower heart rates than the Controls and Mild animals at the immediate, 12,14,16 and minute time points (Figure 10)

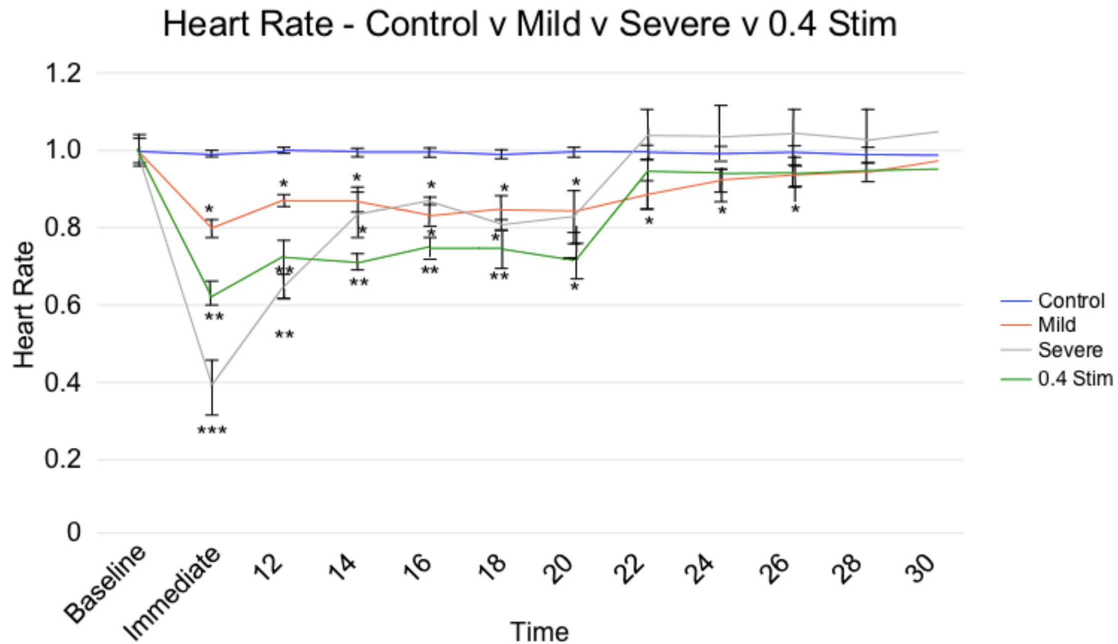

Figure 10: Heart Rate Averages for Control, Mild, Severe VNS, 0.4 Stimulation Groups

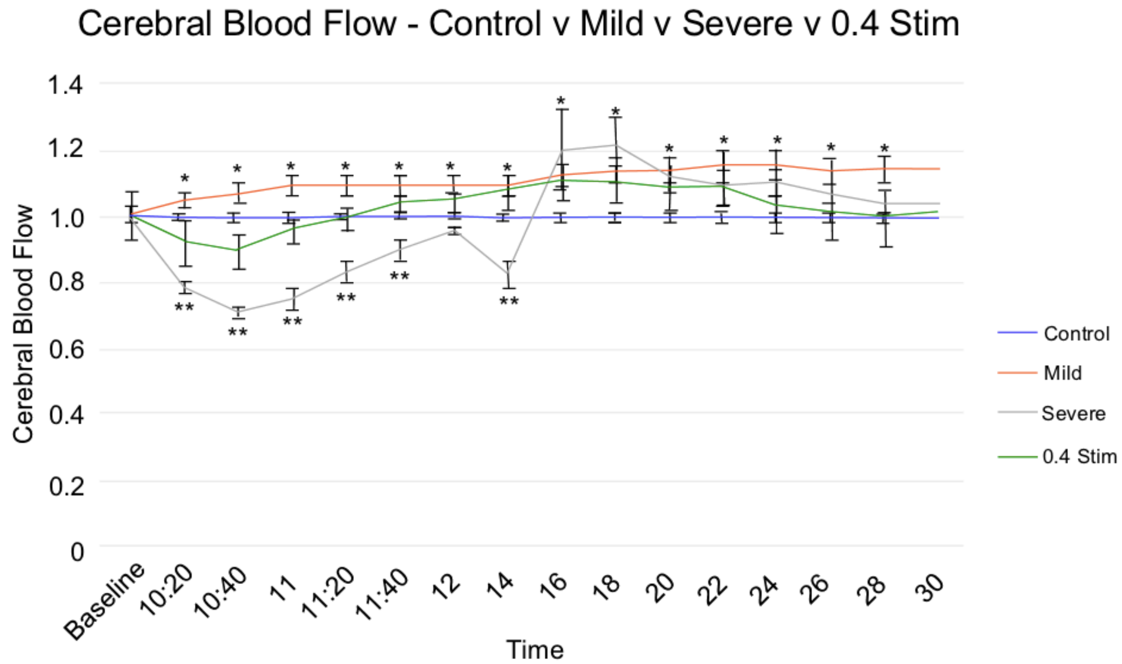

Figure 11: Cerebral Blood Flow Averages for Control, Mild, Severe VNS, 0.4 Stimulation Groups

DISCUSSION

The results of our experiments indicate that application of vagal nerve stimulation at 0.8mA results in two different patterns of acute changes in both cerebral blood flow and heart rate. All VNS animals showed an immediate decrease in heart rate that was limited to the duration of VNS and increases in cerebral blood flow that continued after VNS. Further, there were two different patterns of responses within the VNS animals. "Mild" animals showed a mild decrease in heart rate (20% decrease) while the "Severe" showed a severe decrease (60%). In the case of the "mild" VNS group, these effects appeared to be prolonged even after VNS was switched off, and in the case of the "severe" VNS group, these effects appeared to revert back to the norm.

Exactly why there were two different types of response to VNS is unknown but may be related to issues with the electrode cuffs themselves leading to differences in their efficacy for driving the vagus nerve. The VNS cuffs were constructed according to a protocol provided by another lab researching VNS in rats. While this protocol appeared to be working properly, there were challenges. Specifically, the width of the cuff was too wide and could result in poor contact with the nerve. We changed to smaller in diameter cuffs, and the wires were stripped with far more precision, and the cuff was inspected thoroughly under a microscope and UV-Glue was applied when appropriate with surgical precision to ensure that there were no potential sources of error. Once they were implanted and tested, many of the cuffs had a propensity to fill with fluid and shift within the body during different times of transfer on the surgery table. As a result of this, there were times during the early experimentation when a cuff would no longer be working by the time it was necessary to apply the stimulation and measure blood flow. These animals were used as controls. The new cuff design helped to solve this problem to some degree, but the issue of fluid within the cuff may have reduced the charge density at the contact site. One way this was combatted was by placing absorption sponges, which were sectioned wex-cels, in and around the cuff. Additionally, dental acrylic was often used to seal the cuff to prevent fluid from entering. These methods were largely successful but there is the possibility that there were differences in how well the cuffs were driving the vagus nerve. This is one potential reason that we observed two different patterns of responses.

If we assume that the differences in responses between the Mild and Severe groups were due to issues with electrode construction and therefore efficacy in stimulating the vagus nerve, then it is interesting to postulate how those differences may manifest as

changes in cerebral blood flow and heart rate. We hypothesize that VNS drives both the feedback systems that control heart rate and cerebral blood flow and that the responses of these systems varies as a function of how much stimulation is being delivered.

The parasympathetic vagus nerve is made up of 80% afferent fibers and has major cardiac branches that allow the vagus nerve to send information to the brain and have some neural control of heart rate (23). It has been found that there is significant variance in the induced changes in heart rate. Some correlation has been found between frequency of stimulation and change in heart rate (32). If, we were delivering different stimulation intensities we may have been differentially driving the neural circuitry regulating heart rate and cerebral blood flow.

Studies measuring cerebral blood flow in relation to VNS have had varied results throughout the years. In early studies, it was found the VNS increased rCBF in the brainstem, hypothalami, thalami, and insular cortices and decreased rCBF bilaterally in the hippocampus, amygdala and POC (15). As has been noted, though, other studies have shown that VNS has induced inconsistent patterns of activation and deactivation. This inconsistency was decided to be the result of many possible factors: the technique of rCBF measurement (SPECT or PET), the time point of measurement relative to the initiation of VNS and the kind of stimulation, which could be acute, chronic, frequency, output current, stimulation time) (33). In our experiment, many of these factors were kept consistent: the VNS was always acute, stimulation time was kept exactly consistent, and output current was not altered during experimentation. During each procedure, the cuff would be tested after implantation and the heart rate would be observed. During some procedures, a rat would show a drop in heart rate that was consistent with the “severe” VNS, but by the time

the rat was ready for CBF measurements, the VNS induced would only be consistent with the "mild" VNS parameters. The reason for the change, then, can only be ascribed to the changes in contact during transfer. This change could have resulted in less consistent frequency measures or dampened stimulation that would result in a change in cerebral blood flow. This is supported by the findings of Henry et al., who found differing responses of different brain areas to high and low stimulation levels (15).

Another question is related to the blood flow measurements in the "severe" VNS group. After the initial severe drop in CBF and heart rate, the blood flow spiked up to above the control value and eventually dropped down again to a value that was closer to the original value of perfusion. It is possible that this is due to the autoregulation of the brain, which is able to occur within certain cerebral pressures. It seems possible that the extreme change in CBF and heart rate prompted the autoregulation to be more acutely activated.

Another source of difficulty during the implantation of the cuff was the bundle of nerves of which the vagus nerve is a part. It was discovered that there were always several nerves that ran alongside the vagus nerve that could have drastically different effects on the body. At times, both the vagus nerve and another one of the nerves in the bundle could be stimulated simultaneously, and in other cases, the wrong nerve was stimulated altogether. After this was realized, this was accounted for in future surgeries

All of these changes can be possibly explained by referring to the anatomy of the vagus nerve and its connections in both the brain and the heart, briefly described in Figure 13. Vagal nerve stimulation directly stimulates two of the pathways: the pathway between the heart and the dorsal motor nucleus of the vagus nerve (DMV) and nucleus ambiguous (NA) and the pathway between the nucleus tractus solitaries (NTS) and the baroreceptors.

These are both a part of the loop that regulates heart rate. This is connected to the regulation of cerebral blood flow by way of the caudal ventrolateral medulla (CVLM), which connects to the rostral ventrolateral medulla (RVLM), which is part of the loop that regulates cerebral blood flow by informing the dilation of blood vessels (BV) which connects to the trigeminal ganglion, which increases cerebral blood flow. It is clear that some stimulation has a distinct effect on heart rate, and that this stimulation also affects the cerebral blood flow. It is our hypothesis that the feedback loop that plays a significant role in the regulation of cerebral blood flow, including the trigeminal ganglia and rostral ventrolateral medulla, is only activated when the stimulation is significant enough to warrant autoregulation of cerebral blood flow. In the case of the severe stimulation, there is an initial drop in heart rate and blood flow. Then the blood flow spikes for a time before returning slowly returning to more normal levels, normalizing completely when VNS is turned off. This correlation between dramatic decrease in heart rate and an equally dramatic drop in cerebral blood flow is clearly related. This dramatic change and higher stimulation results in the activation of the feedback loop, resulting in an attempt by the brain to return to previous levels. Because the autoregulation feedback loop has been activated, by way of the NTS-CVLM-RVLM connection, it remains primed when VNS is switched off, and CBF is able to return back to normal levels. In the case of the mild VNS, the heart rate drop is not significant enough to result in a change in cerebral blood flow, and the stimulation is not enough to activate the feedback loop. As a result, the VNS results in a bump in cerebral blood flow, and this increase is sustained because the autoregulation feedback loop has not been activated.

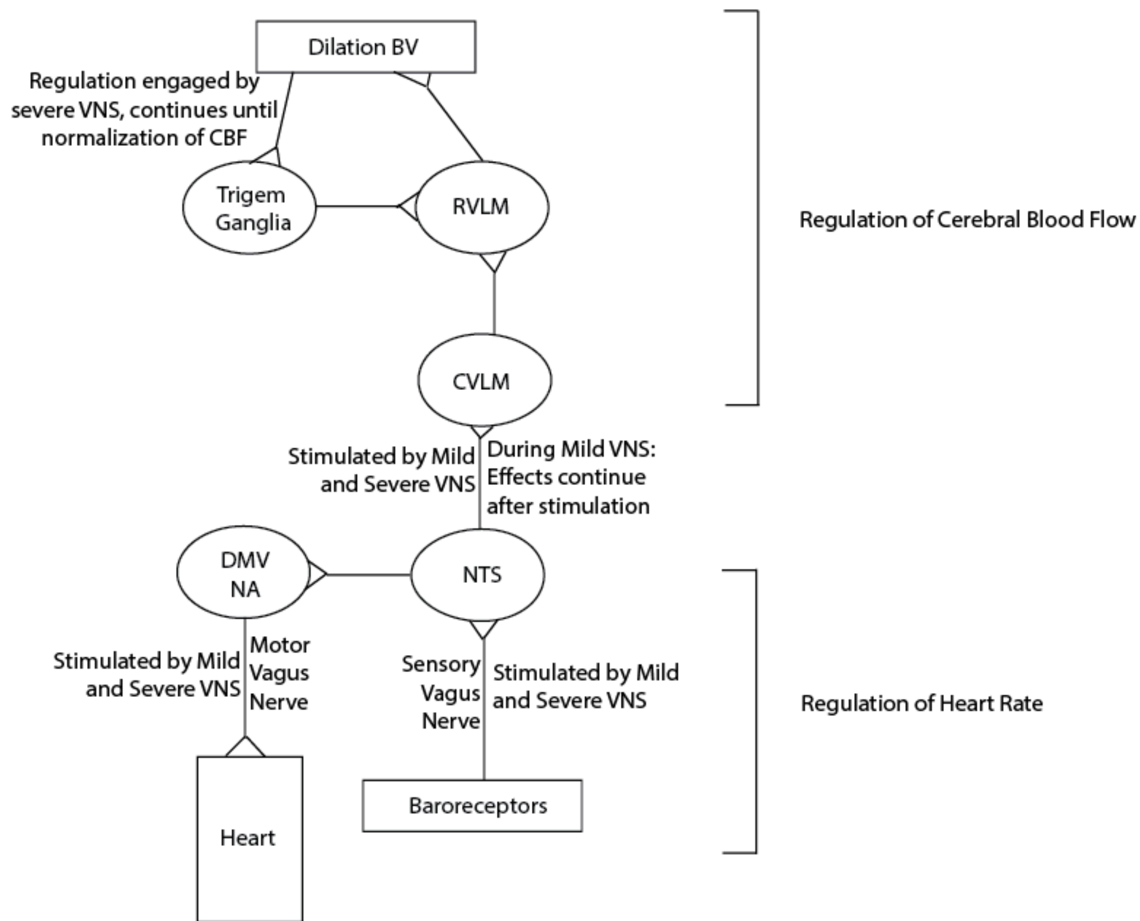

Figure 12: Potential Role of the Vagus Nerve in Modulating Cerebral Blood Flow and Heart Rate. RVLM: Rostral Ventrolateral Medulla, CVLM: Caudal Ventrolateral Medulla, DMV: Dorsal Motor Nucleus of the Vagus Nerve, NA: Nucleus Ambiguous, NTS: Nucleus Tractus Solitaries, BV: Blood Vessels

The results of the lower levels of stimulation were equally interesting. It is clear that there is a significant difference in cerebral blood flow in response to differing levels of vagal nerve stimulation. This is clear when comparing the 0.4mA data to the previously collected 0.8mA data. A change in cerebral blood flow still occurs under lower stimulation parameters, but this change is altogether different from both previously determined “mild” and “severe” VNS groups that occurred as a result of stimulation at 0.8mA. The difference is also evidenced by comparing cerebral blood flow in the same animal at two different

time points stimulating at differing levels. When a subject receives the 0.4mA level of stimulation, the response is shown statistically to be unique to the cerebral blood flow response when that same subject is later stimulated at 0.8mA. The exact responses are not always consistent—in fact, differences in age of the animal, effects of injected drugs, and efficacy of the cuff seal have all shown potential to affect the responses to the differing levels of stimulation.

It is still clear, though, that this small change in stimulation results in a drastic change in the response of cerebral blood flow to vagal nerve stimulation. This alteration of CBF response could have significant therapeutic consequences, as the brain is reacting in a significantly different way even within the same animal. More research is needed to discover what changes have the largest impact, and what considerations need to be taken to result in the most beneficial therapeutic parameters for vagal nerve stimulation.

REFERENCES

[1] S. E. Krahl, "Vagus nerve stimulation for epilepsy: A review of the peripheral mechanisms," Surg Neurol Int, vol. 3, no. Suppl 1, pp. S47–S52, Jan. 2012.

[2] R. H. Howland, "Vagus Nerve Stimulation," Curr Behav Neurosci Rep, vol. 1, no. 2, pp. 64–73, Jun. 2014.

[3] D. A. Groves and V. J. Brown, “Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects,” Neurosci Biobehav Rev, vol. 29, no. 3, pp. 493–500, May 2005.

[4] H.-R. Berthoud, “The vagus nerve, food intake and obesity,” Regul Pept, vol. 149, no. 1–3, pp. 15–25, Aug. 2008.

[5] K. Morishita, T. W. Costantini, B. Eliceiri, V. Bansal, and R. Coimbra, “Vagal nerve stimulation modulates the dendritic cell profile in posthemorrhagic shock mesenteric lymph,” J Trauma Acute Care Surg, vol. 76, no. 3, pp. 610–617; discussion 617-618, Mar. 2014.

[6] L. V. Borovikova et al., “Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin,” Nature, vol. 405, no. 6785, pp. 458–462, May 2000.

[7]W. J. de Jonge et al., “Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway,” Nat. Immunol., vol. 6, no. 8, pp. 844–851, Aug. 2005.

[8] N. Khodaparast et al., “Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke,” Neurobiol. Dis., vol. 60, pp. 80–88, Dec. 2013.

[9] S. A. Hays et al., “The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength,” Neuroreport, vol. 25, no. 9, pp. 676–682, Jun. 2014.

[10] “Vagus nerve stimulation reduces infarct size in rat focal cerebral ischemia: An unlikely role for cerebral blood flow.” [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3293221/. [Accessed: 12-Apr-2018].

[11] K. Vonck, P. Boon, M. D’Havé, T. Vandekerckhove, S. O’Connor, and J. De Reuck, “Long-term results of vagus nerve stimulation in refractory epilepsy,” Seizure, vol. 8, no. 6, pp. 328–334, Sep. 1999.

[12] A. H. Milby, C. H. Halpern, and G. H. Baltuch, “Vagus nerve stimulation for epilepsy and depression,” Neurotherapeutics, vol. 5, no. 1, pp. 75–85, Jan. 2008.
[13] K. J. Ressler and H. S. Mayberg, “Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic,” Nat Neurosci, vol. 10, no. 9, pp. 1116–1124, Sep. 2007.

[14] C. Shi, S. R. Flanagan, and U. Samadani, “Vagus nerve stimulation to augment recovery from severe traumatic brain injury impeding consciousness: a prospective pilot clinical trial,” Neurol. Res., vol. 35, no. 3, pp. 263–276, Apr. 2013.

[15] T. R. Henry et al., “Brain blood flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: I. Acute effects at high and low levels of stimulation,” Epilepsia, vol. 39, no. 9, pp. 983–990, Sep. 1998.

[16] T. R. Henry, R. A. E. Bakay, P. B. Pennell, C. M. Epstein, and J. R. Votaw, “Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. prolonged effects at high and low levels of stimulation,” Epilepsia, vol. 45, no. 9, pp. 1064–1070, Sep. 2004.

[17] T. R. Henry et al., “Acute blood flow changes and efficacy of vagus nerve stimulation in partial epilepsy,” Neurology, vol. 52, no. 6, pp. 1166–1173, Apr. 1999.

[18] K. V. Laere, K. Vonck, P. Boon, J. Versijpt, and R. Dierckx, “Perfusion SPECT Changes After Acute and Chronic Vagus Nerve Stimulation in Relation to Prestimulus Condition and Long-Term Clinical Efficacy,” J Nucl Med, vol. 43, no. 6, pp. 733–744, Jun. 2002.

[19] M. Kosel, H. Brockmann, C. Frick, A. Zobel, and T. E. Schlaepfer, “Chronic vagus nerve stimulation for treatment-resistant depression increases regional cerebral blood flow in the dorsolateral prefrontal cortex,” Psychiatry Res, vol. 191, no. 3, pp. 153–159, Mar. 2011.

[20] V. Martlé et al., “Regional brain perfusion changes during standard and microburst vagus nerve stimulation in dogs,” Epilepsy Res., vol. 108, no. 4, pp. 616–622, May 2014.

[21] C. R. Conway et al., “Brain Blood-flow Change with Acute Vagus Nerve Stimulation in Treatment-Refractory Major Depressive Disorder,” Brain Stimul, vol. 5, no. 2, pp. 163–171, Apr. 2012.

[22] E. C. Peterson, Z. Wang, and G. Britz, “Regulation of Cerebral Blood Flow,” Int J Vasc Med, vol. 2011, 2011.

[23] Buschman Hendrik P., Storm Corstiaan J., Duncker Dirk J., Verdouw Pieter D., van der Aa Hans E., and van der Kemp Peter, “Heart Rate Control Via Vagus Nerve Stimulation,” Neuromodulation: Technology at the Neural Interface, vol. 9, no. 3, pp. 214–220, Jul. 2006.

[24] Y. Wang and G. Li, “[Effect of vagal nerve stimulation on heart rate and heart rate variability in the toads],” Zhongguo Ying Yong Sheng Li Xue Za Zhi, vol. 26, no. 2, pp. 229–232, May 2010.

[25] M. G. Frei and I. Osorio, “Left vagus nerve stimulation with the neurocybernetic prosthesis has complex effects on heart rate and on its variability in humans,” Epilepsia, vol. 42, no. 8, pp. 1007–1016, Aug. 2001.

[26] W. C. Randall and J. L. Ardell, “Selective parasympathectomy of automatic and conductile tissues of the canine heart,” Am. J. Physiol., vol. 248, no. 1 Pt 2, pp. H61-68, Jan. 1985.

[27] C. Szabó, F. Salinas, A. Papanastassiou, J. Begnaud, M. Ravan, K. Eggleston, R. Shade, C. Lutz, and M. D. L. Garza, “High-frequency burst vagal nerve simulation therapy in a natural primate model of genetic generalized epilepsy,” Epilepsy Research, vol. 138, pp. 46–52, 2017. Ruffoli a, Filippo S. Giorgi b, Chiara Pizzanelli b, Luigi Murri b, Antonio Paparelli a, Francesco Fornai a,c,*

[28] R. Ruffoli, F. S. Giorgi, C. Pizzanelli, L. Murri, A. Paparelli, and F. Fornai, “The chemical neuroanatomy of vagus nerve stimulation,” Journal of Chemical Neuroanatomy, vol. 42, no. 4, pp. 288–296, 2011.

[29] J. L. Ardell, H. Nier, M. Hammer, E. M. Southerland, C. L. Ardell, E. Beaumont, B. H. Kenknight, and J. A. Armour, “Defining the neural fulcrum for chronic vagus nerve stimulation: implications for integrated cardiac control,” The Journal of Physiology, vol. 595, no. 22, pp. 6887–6903, 2017.

[30] K. W. Loerwald, E. P. Buell, M. S. Borland, R. L. Rennaker, S. A. Hays, and M. P. Kilgard, “Varying Stimulation Parameters to Improve Cortical Plasticity Generated by VNS-tone Pairing,” Neuroscience, vol. 388, pp. 239–247, 2018.

[31] K. W. Loerwald, M. S. Borland, R. L. Rennaker, S. A. Hays, and M. P. Kilgard, “The interaction of pulse width and current intensity on the extent of cortical plasticity evoked by vagus nerve stimulation,” Brain Stimulation, vol. 11, no. 2, pp. 271–277, 2018.

[32] B. H. Neely and F. Urthaler, “Quantitative effects of sympathetic and vagal nerve stimulations on sinus and AV junctional rhythms,” Journal of the Autonomic Nervous System, vol. 37, no. 2, pp. 109–120, Feb. 1992.

[33] A. Barnes, R. Duncan, J. A. Chisholm, K. Lindsay, J. Patterson, and D. Wyper, “Investigation into the mechanisms of vagus nerve stimulation for the treatment of intractable epilepsy, using 99mTc-HMPAO SPET brain images,” Eur. J. Nucl. Med. Mol. Imaging, vol. 30, no. 2, pp. 301–305, Feb. 2003.