Effects of Trigeminal Nerve Stimulation on ANS and Proprioception

High Frequency TNS Reduces Proprioceptive End-point Error

by

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ABSTRACT

Previously accomplished research examined sensory integration between upper limb proprioception and tactile sensation. The active proprioceptive-tactile relationship points towards an opportunity to examine neuromodulation effects on sensory integration with respect to proprioceptive error magnitude and direction (Rincon-Gonzalez et al., 2011). Efforts to improve focus and attention during upper limb proprioceptive tasks results in a decrease of proprioceptive error magnitudes and greater endpoint accuracy (Singer et al., 1994). Increased focus and attention can also be correlated to neurophysiological activity in the Locus Coeruleus (LC) during a variety of mental tasks (Aston-Jones et al., 2005). Through non-invasive trigeminal nerve stimulation, it may be possible to affect the activity of the LC and induce improvements in arousal and attention that would assist in proprioceptive estimation. The trigeminal nerve projects to the LC through the mesencephalic nucleus of the trigeminal complex (Sasa et al., 1973; Shiozawa et al., 2014), providing a pathway similar to the effects seen from vagus nerve stimulation (Hulsey et al., 2017). In this experiment, the effect of trigeminal nerve stimulation (TNS) on proprioceptive ability is evaluated by the proprioceptive estimation error magnitude and direction, while LC activation via autonomic pathways is indirectly measured using pupil diameter, pupil recovery time, and pupil velocity (Murphy et al., 2014; Josh et al., 2016; Yamaji et al., 2000). TNS decreases proprioceptive error magnitude in 59% of subjects, while having no measurable impact on proprioceptive strategy. Autonomic nervous system changes were observed in 88% of subjects, with mostly parasympathetic activation and a mixed sympathetic effect.

ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

1.1 *Trigeminal Nerve Stimulation*

Cranial nerve stimulation offers a promising route of neuromodulation for a variety of applications. Vagus nerve stimulation (VNS) has been effective in offering an acute abortive effect on epileptic seizures as well as decreasing the frequency and duration of seizures. VNS has been used clinically to treat depression, epilepsy, obesity, PTSD, and to affect athletic performance (Howland, 2014; Elliott et al., 2011; Pardo et al., 2007; George et al., 2008; Hool, 2019). A few key experiments have shown similar performance between cuff electrodes and transcutaneous stimulation for VNS, offering a less invasive method of stimulation while retaining some of the benefits of VNS (Yuan et al., 2016). Finding methods of altering brain function and physiology without using invasive surgery can offer a first-step treatment option for symptomologies that do not immediately warrant the risks of surgical intervention. While VNS has been an important route of investigation for new treatment methods for these clinical applications, many other easily accessible cranial nerves have not been nearly as well-researched with regards to potential clinical uses involving stimulation, including the trigeminal nerve. The vagus nerve also controls a number of autonomic functions, including projections to the gastrointestinal tract, heart, and lungs, which stimulation may alter. In addition, the location of the vagus nerve under muscles in the neck necessitates a stimulator closer to the nerve itself for maximum current delivery, requiring surgical implantation.

Given the magnitude of effect transcutaneous alternating current stimulation (tACS) has had on measures of attention, working memory, and somatosensory perception, the exact method of action warrants further investigation (Tavakoli et al., 2017). There is a close proximity between placement of tACS electrodes and the trigeminal nerve, whose branches extend across the front of the scalp and face and it is possible that some of the effects of tACS can be traced back to transcutaneous AC stimulation of the trigeminal nerve. A more targeted approach in stimulating the branches of the nerve directly may yield further advantages when considering the anatomical placement of electrodes on the nerve.

1.2 *TNS Anatomy and Waveform*

The three branches of the trigeminal nerve reportedly have different neuromodulation effect. The ophthalmic branch remains the primary target for trigeminal nerve stimulation given its effectiveness in treating epilepsy, with little information available on the comparison between TNS of each of the three branches. The literature regarding ophthalmic branch TNS and epilepsy shows a direct, robust connection between stimulation and wide-reaching effects on cortical function and physiology, which could affect ANS activity and proprioception. Most other applications of TNS have had success with ophthalmic branch stimulation (DeGiorgio et al., 2013). Given the ophthalmic branch's proximity to many successful tACS neuromodulation experiment's electrode placement on the forehead, this branch is a clear candidate for targeted TNS. The mastoid is chosen as the site for the ground electrode due to its bony mass able to dissipate charge evenly. This prevents the stimulation from affecting a confounding cranial nerve such as the vagus nerve that also responds to transcutaneous stimulation and runs close to the neck.

Research into the current approaches to TNS yielded information on electrode placement and stimulation waveform parameters. Most attempts at neuromodulation via transcutaneous stimulation for either VNS or TNS use a biphasic waveform, with equal cathodic and anodic pulse widths. This stimulation train is repeated at a predetermined frequency, with preliminary data and several prior experiments pointing towards 3000 Hz and 50 us pulse width as an ideal waveform. Some anti-epileptic waveform generators using TNS found that a 30s on, 30s off cycling stimulation setup was incredibly successful in positively affecting vital epilepsy metrics. In addition, unpublished research on TNS in rhesus macaques shows that this cycled stimulation has a compounding, cumulative effect on firing rate in the locus coeruleus (LC), an area heavily involved in the reward pathway and motivation (Tanner, 2019; Aston-Jones et al., 2005).

1.3 *The Pupillary Light Reflex*

LC activation can be measured in a variety of ways, including fMRI, brainstem single-electrode neurophysiology, or microelectrode array implants. Most non-invasive methods of recording, such as EEG, cannot be used due to the anatomical location of the LC in the brain, as the LC is located deep within the brainstem. The feasibility of any of these techniques with regard to cost and safety for human subjects precludes most of these methods from being realistically approved as safe or effective. Therefore, a new method of measuring LC activity in response to TNS must be used. While a more direct method of recording LC activity exists, the factors intended to draw conclusions from can be measured in a few other ways non-invasively.

There are a variety of physiological changes that result in response to ANS changes, including the galvanic skin response, facial flushing, reaction time, vasodilation, breathing rate, heart rate, and certain factors related to digestion. While most of these changes can be influenced by conscious attention or only one branch of the ANS, either parasympathetic or sympathetic, metrics related to the dilation and contraction of the pupil are not consciously controllable and involve both branches of the ANS. In addition to being influenced by both parasympathetic and sympathetic activity, certain derived measures of the pupil's response to a flash of light are influenced by only one branch of the ANS. The initial pupillary contraction in response to a bright stimulus is regulated entirely by the parasympathetic nervous system, while the dilation of the pupil after the bright stimulus has disappeared is regulated entirely by the sympathetic nervous system. In recording the pupil diameter throughout a series of light flashes, an accurate and non-invasive metric of ANS activity can be obtained with few confounds and uncontrolled variables (Yamaji et al., 2001).

A specific experiment found mathematically-determined metrics that correlated with inhibition or activation of either the parasympathetic or sympathetic nervous system using this pupillary light reflex (Yamaji et al., 2000). This interpretation was given additional support by the introduction of known sympathomimetic or parasympatholytic agents into a subject's bloodstream. These metrics changed significantly, specifically the recovery amount during the third phase of the PLR and the maximum velocity of contraction during the first phase of the PLR. The recovery amount (RA) is defined as the percent difference between the baseline pupil diameter before the flash and the pupil diameter at near-zero acceleration during redilation. The point at which the pupil diameter acceleration nears zero during redilation varies from individual to individual and marks the start of phase three of the PLR. A metric of parasympathetic control is identified in the maximum contraction velocity of the pupil diameter during contraction after the bright stimulus (Vcmax).

The two metrics described here are almost entirely separated in terms of parasympathetic versus sympathetic control. The scaling of the RA is determined almost exclusively via sympathetic control, while the scaling of the Vcmax is controlled almost exclusively via parasympathetic control. A high degree of sympathetic activation decreases the RA, while a high degree of sympathetic inhibition increases the RA. For

Vcmax, the more parasympathetic activation, the lower Vcmax is and vice versa for inhibition. It is important to note that any reduction in activation will appear indistinguishable from an increase in inhibition and vice-versa for both branches of the ANS according to these metrics. There will be no observable PLR difference between sympathetic inhibition and any potential effects of TNS lowering an underlying method of sympathetic activation.

1.4 *Proprioceptive Map*

A previous study reports that spatial estimation of the hand in two dimensional space is improved with the addition of tactile information delivered to the fingertip (Rincon-Gonzalez et al., 2011; Orthlieb et al., 2018). Subjects estimated their forefinger positions after a blinded passive movement to a location on a horizontal flat grid. Subjects were guided towards a point on the grid in front of them while their vision was occluded and the forefinger was guided to a stationary point for five seconds and returned to a resting state. The points that subjects were actually located was recorded as well as the point that subjects estimated they were without visual confirmation. The subject's recorded forefinger position forms the base of a vector with the head defined as the subject's estimated position. A vector field of error direction and magnitude expresses the dynamic change of proprioceptive error across the x and y axes in front of the subject. These vector fields resist conventional statistical analysis and require a test that accounts for both the magnitude and directional components of the vectors at each point of the constructed proprioceptive map.

A statistical test of angle distribution between the hover and touch conditions yielded statistically similar error vector angle distributions for each subject's tactile conditions yet was not consistent between subjects, pointing toward a stable and idiosyncratic proprioceptive error map across all tactile inputs. These results persist across measures of handedness and gender.

In another study with a similar experimental paradigm, the effect of fingertip electrotactile and vibrotactile feedback on proprioceptive estimation error were investigated. At the target location, subjects received one of four stimuli to the fingertip: null (hover), applied tactile feedback, electrotactile stimulation, or vibrotactile stimulation. In an ANOVA comparison of these conditions, hovering over the grid produced significantly higher error than every other condition. Vibrotactile and electrotactile stimulation reduced error over hover, but not as much as normal. Errors under either vibrotactile or electrotactile stimulation were significantly worse than contact with the surface, which produced the lowest magnitude of error. Statistically, it appears electrotactile stimulation has marginally increased error compared to normal tactile feedback, while vibrotactile stimulation has an even greater magnitude of error. Potentially, the latter activates primarily rapidly adapting mechanoreceptors, while the former would indiscriminately activate local afferents - providing more diverse peripheral “information”. The difference in error magnitude between the two indicates a shift in sensory integration. This shift can be explained as an increase in sensory input increasing priority in proprioceptive processing, as a result of minute shifts in posture providing proprioceptive feedback, or as a result of stochastic resonance. Any changes to the distribution of the angles after TNS may indicate a fundamental shift of strategy or perception such that the general estimation of upper limb proprioception is altered. This work indicates that vibrotactile and electrotactile sensory substitution will effectively support necessary multisensory integration beyond simply hovering over a set location.

Trigeminal nerve stimulation offers a unique opportunity to affect attention, the ANS, and proprioception through the mesencephalic nucleus and LC non-invasively.

Using the PLR as a measure of sympathetic and parasympathetic activity can reveal immediate changes as a result of TNS, while a proprioceptive estimation task can identify changes in proprioceptive error and strategy. Given previous research, TNS could decrease proprioceptive error without changing general estimation shape and activate the sympathetic nervous system without affecting the parasympathetic branch. The results indicate that while TNS does decrease proprioceptive error without altering overall shape, the stimulation induces mixed effects on the sympathetic nervous system between subjects along with parasympathetic activation.

CHAPTER 2

MATERIALS AND METHODS

2.1 *General Overview*

In order to accurately and effectively draw conclusions between the application of TNS and changes to proprioceptive ability and the ANS, we have combined the techniques outlined above with two different sets of TNS parameters and stimulation paradigms that would potentially elicit the greatest response. All programs used to deliver stimuli and record data are custom built using MATLAB (Mathworks, Natick, MA, USA). A survey to measure handedness is administered to analyze its relevance, especially with relation to the proprioceptive components of the rest of the experiment. The experiment is then explained fully to the subject and time is given to answer questions and do some trial runs of the proprioceptive map task. In order to assess pre-stimulation proprioceptive error magnitude and map shape, the hover and touch conditions are both evaluated . Pupil diameter is recorded using a Gazepoint GP3 camera (Gazepoint, Vancouver, BC, Canada).

First, the time until phase three onset is measured in order to gauge when to record the recovery amount. A full range-nonlinearity is then established to record ANS states before stimulation and to compare against a complex relationship of baseline pupil diameter, recovery amount, and maximum contraction velocity. Amplitude tolerability is determined to ensure an equal comparison of stimulation current amplitude with regards to pain and discomfort between subjects. The pupillary light reflex task is then administered with five minutes of pre-stimulation data collection, ten minutes of data collection during stimulation, and ten minutes of data collection after stimulation in order to show the ANS state before, during, and after stimulation. Tracking ANS changes minute by minute also allows for the estimation of onset and persistence of stimulation effects.

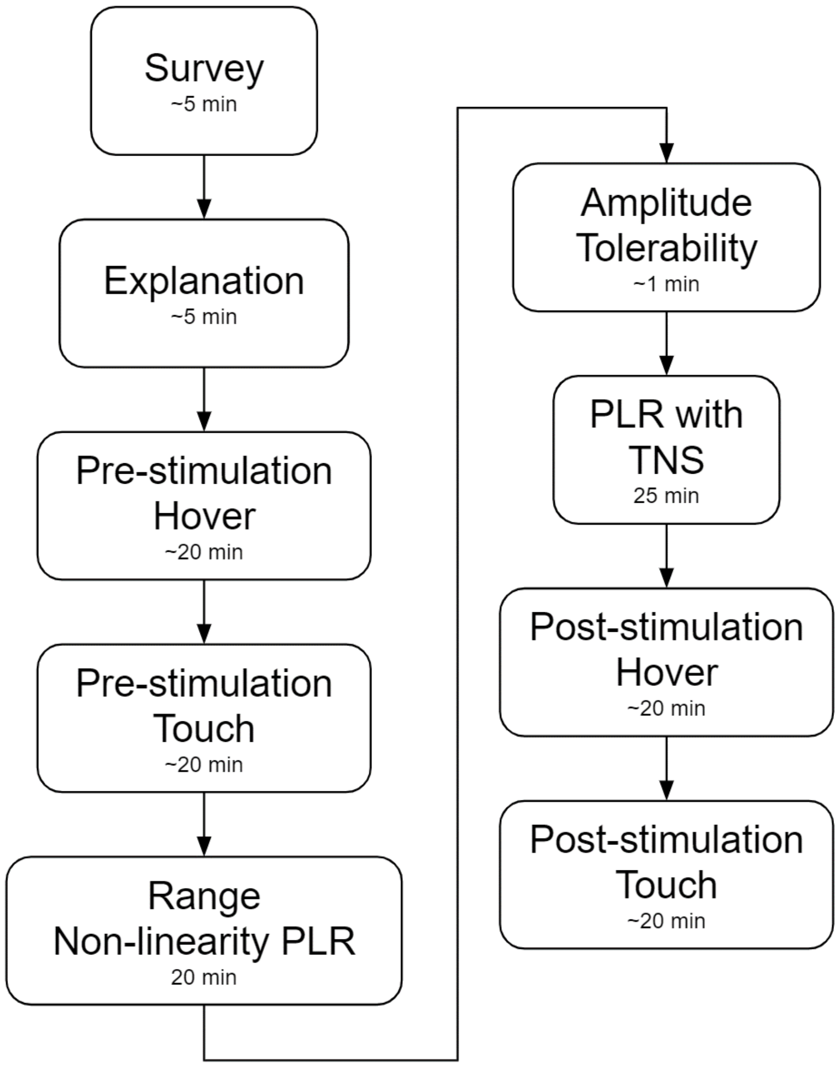

Figure 2.1: Overview of Experiment

2.2 *Proprioceptive Estimation Task*

Subjects are first instructed to take a handedness questionnaire based on the Edinburgh Inventory to gain a numerical approximation of subject handedness, as that was proven to be a variable of interest in previous papers (Rincon-Gonzalez et al., 2011). Subjects then take part in two 75-trial proprioceptive map tasks as described above, with the first set hovering over the grid and the second set touching down on the grid. This pre-stimulation data can later be compared to the hover and touch conditions during stimulation. The coordinates for the 75 trials are chosen semi-randomly, with an algorithm ensuring that the task tests an even distribution across the map to better approximate the subject's 2D proprioceptive map using polynomial regression during analysis. Throughout the total of four sets of 75 trials each, pre-stim hover, pre-stim touch, post-stim hover, and post-stim touch, the same exact coordinates are randomly ordered and used between the sets of 75 trials in order to ensure an accurate comparison between conditions.

Subjects are centered in a chair in front of the grid as seen in figure 2.2, with a set distance between their torso and the grid. The grid ranges from rows A-G and columns 1-10, with letter - number coordinates containing four colored dots in a square. When the subject reports where they estimate their fingertip to be, they are required to not reach back to the coordinate but instead list these set of identifiers, e.g. A-3-red. Before starting, they are asked to trace the perimeter of the grid with their finger to ensure that the subject does not have to lean to reach certain sections of the grid, which may confound results given the displaced torso. At least one practice trial is explained and administered, depending on the subject's self-reported confidence with being able to understand and accomplish the task. Across a sampling of the grid in front of the subject, a 4th-order polynomial regression of the x and y error are calculated separately and used to interpolate data points between each recorded hand movement trial.

A two-tailed t-test is performed on the interpolated vector magnitudes between pre-stimulation hover versus touch, post-stimulation hover versus touch, pre-stimulation hover versus post-stimulation hover, pre-stimulation touch versus post-stimulation touch, and all pre-stimulation conditions versus all post-stimulation conditions. In this way we can see if there is a decrease in error between hover and touch, an important indicator of sensory feedback that is well established by previous literature (Rincon-Gonzalez et al., 2011; Orthlieb et al., 2018). If this relationship no longer exists after stimulation, it may indicate a fundamental shift in proprioceptive estimation strategy. In addition, comparisons between the same condition pre stimulation and post stimulation can reveal changes in error magnitude, which would indicate a more accurate proprioceptive map for the same type of feedback.

Due to the effects of stimulation potentially carrying over after termination of stimulation, it was not possible to randomize the order of the set of trials with TNS and the set of trials without TNS. The proprioceptive estimation task must be completed without stimulation before the section with stimulation after the RNL to avoid any effects of stimulation carrying over to a control condition. While the individual coordinates remained the same throughout all conditions, they were randomized between each condition to prevent pattern recognition or learning. Given that there is no opportunity for error feedback during the set of proprioceptive estimations trials, it is not possible for any subject to decrease error magnitude as a result of adjusting their baseline proprioceptive strategy based on error feedback. There would be no difference between performing the set of hover trials after the touch trials given this setup, and no subjects had significantly different error magnitudes when comparing the first half of any condition to the second half of the same condition. Randomizing the order of hover versus touch conditions is therefore irrelevant. It is possible for the subject to improve during the first condition of the session, e.g. becoming more comfortable with the task and more confident in their answers or becoming bored as time goes on and more careless with their estimates. Despite this, the breaks in between each condition should address the boredom and the practice trials before the beginning of the first task should address the lack of confidence.

2.3 *The Kolmogorov-Smirnov Test*

To compare multiple maps against each other meaningfully, a t-test or ANOVA between the flattened 1D magnitudes of the vectors is performed. In order to accurately compare directional variations of the vector field between conditions, the two compared vector fields are overlaid on top of each other in a 1:1 comparison across the map. The difference in angle is calculated as the smallest angle that can be formed between any two vectors with the same point of origin. The same process of finding the smallest angle between vectors is repeated with the shuffled positions of the vectors of the overlaid map. These two sets of data, unshuffled and shuffled angle differences across the 2D map, are compared using a non-parametric distribution test. The Kolmogorov-Smirnov (KS) test is a non-parametric test that compares the cumulative distribution of the two datasets and takes the largest difference at any point along the ordered samples in the distribution, as seen in figure 2.3. A large distance at any point in the cumulative distribution indicates a fundamental shift in the shape of the proprioceptive map's distribution. A significant result from the comparison between shuffled and unshuffled distributions indicates that the difference in distribution can be explained by random chance. The shuffling changes the order of the points on the map such that angles differences that were once small because the two vectors had similar directions became much larger due to the similar shapes of the two compared maps. A non-significant result cannot prove that the two maps have a different overall shape or contour. A significant result indicates that the two compared maps have a similar distribution of angles. As shown in figure 2.3, the two compared maps' KS test p-value is much less than 0.05, indicating that the two maps' shapes are significantly similar over the 2-dimensional space.

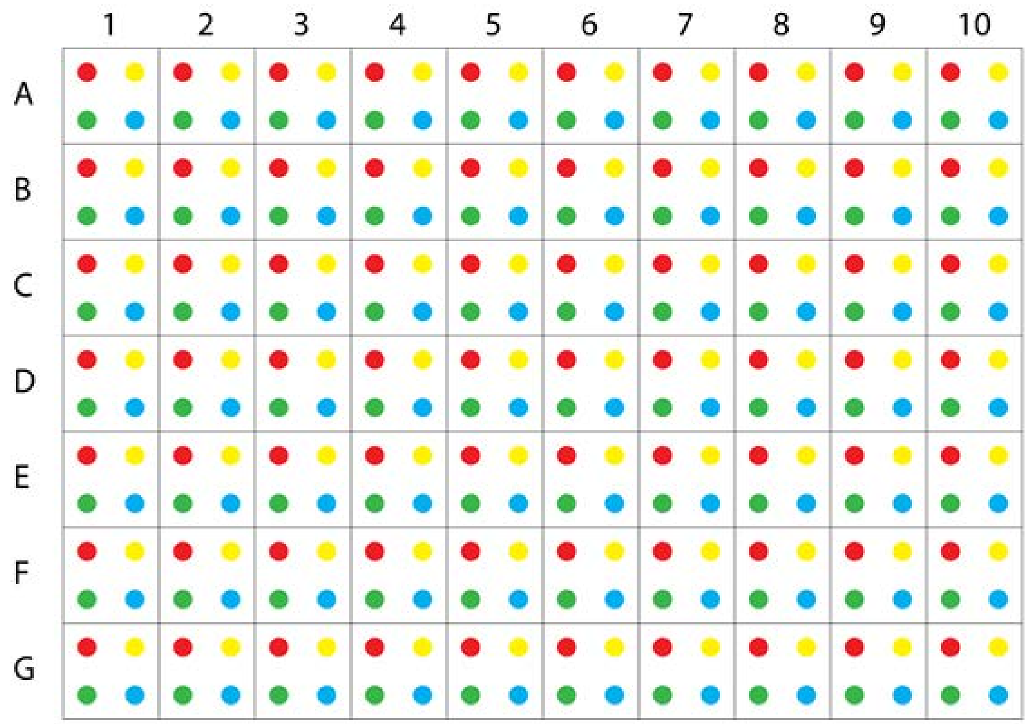

Figure 2.2: The 2-dimensional grid used for proprioceptive estimation

The notable criticisms of this method of statistical analysis are few but worth consideration. While this test is a useful and powerful tool to compare maps with a varied distribution of angles, if any one subject's map is uniform for any particular angle, the test will erroneously record the difference between it and any other map as being explained by random chance even if the maps are obviously different. A vector field consisting of entirely twenty degree angles will always appear statistically significant, even if the other field is observed to have a completely opposite and varied field from the first. Each field's overall uniformity, therefore, must be evaluated before applying the revised KS test to any other field. A cursory visual inspection in combination with statistical bounds should ensure the distribution of each field is not weighted to any particular angle.

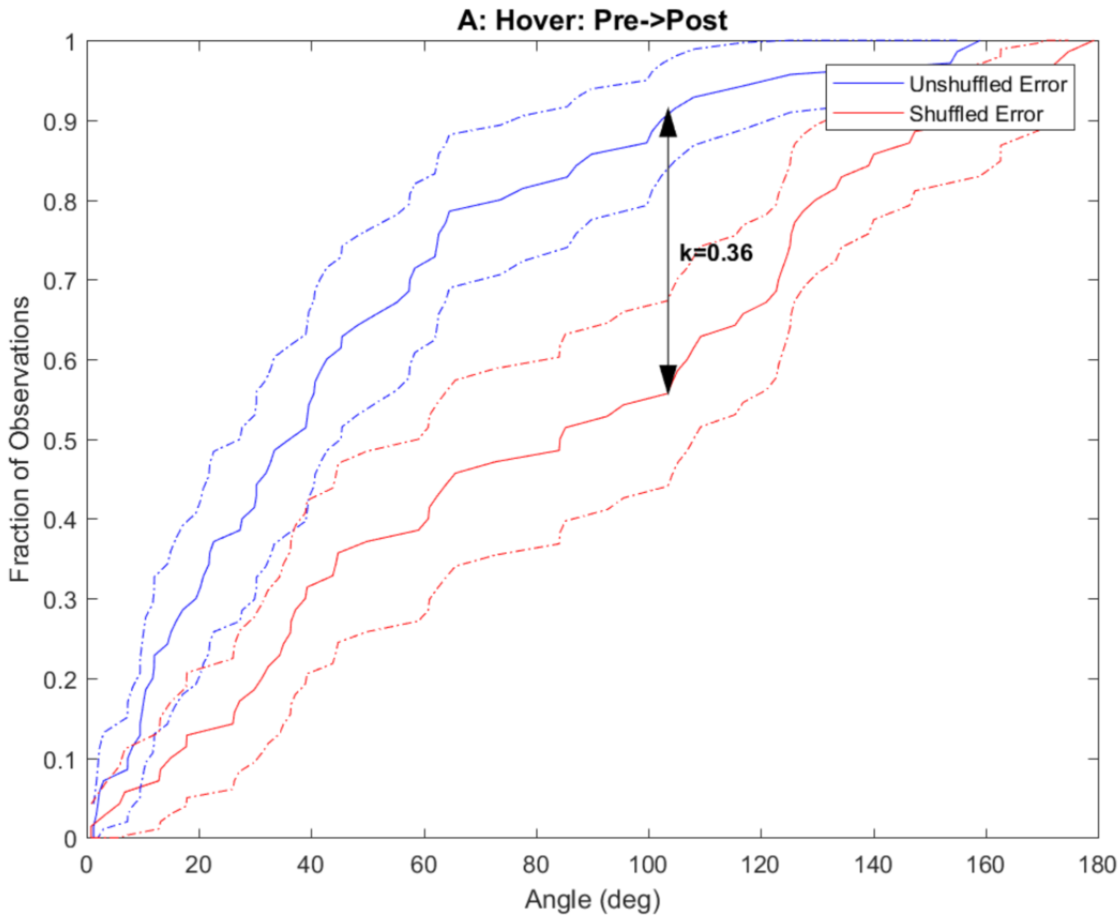

Figure 2.3: The Kolmogorov-Smirnov test statistic k, defined as the largest distance between the cumulative distributions

Upon further testing and deliberation, any field with more than fifty percent of its angles distributed in a section of ten degrees cannot be reasonably expected to draw any useful conclusions and is excluded from experimental analysis. Every vector's angle from zero degrees is measured and ordered from smallest to largest. If more than 36 consecutive angles are within a ten degree section, the subject's proprioceptive data is regarded as unusable. While no subject crossed this threshold, this method ensures that the statistical conclusions derived from comparisons between vector fields are meaningful given the likelihood of the KS test to evaluate any compared map as significantly similar in shape when shuffled.

2.4 *Range Non-Linearity for Pupillary Light Reflex*

Before conducting any kind of PLR experiment, a range-non linearity measure is necessary in order to establish a baseline response across a range of initial pupil diameters. The subject is positioned in front of the screen and camera until the distance meter on the Gazepoint eye-tracking and pupillometry camera indicates an optimal length from camera to pupils. If possible, the camera is angled to exclude the subject's earlobes from view of the IR camera, given that the camera may mistake the subject's earlobes for pupils and invalidate sections of data collection. The participant is directed to focus their gaze on a small square in the center of the upper half of the screen during any of the PLR tasks. The subject is then exposed to the gap determination test in order to find the time at which phase three of the PLR begins, which may differ from subject to subject. Ten flashes of light are spaced apart evenly, with the phase three time for each averaged together. This phase three time is then recorded and used in the next two PLR tasks to calculate the RA. The next step to compare stimulation data against is the collection of the RNL for the PLR. The background brightness is varied using a screen in front of the subject, with increasing values of background brightness throughout the RNL for a total of 30 different settings to compare against. The subjects are instructed to keep their eyes open for the duration of the set of three flashes and for one second both before and after the set of flashes. While subjects are encouraged to blink freely for any other period during the test, they are told that if blinking is necessary during the set of three flashes, to do so quickly and evenly spaced rather than slowly and less frequently.

This is necessary to ensure the best and most accurate interpolation of the pupil diameter before calculating the RA or Vcmax.

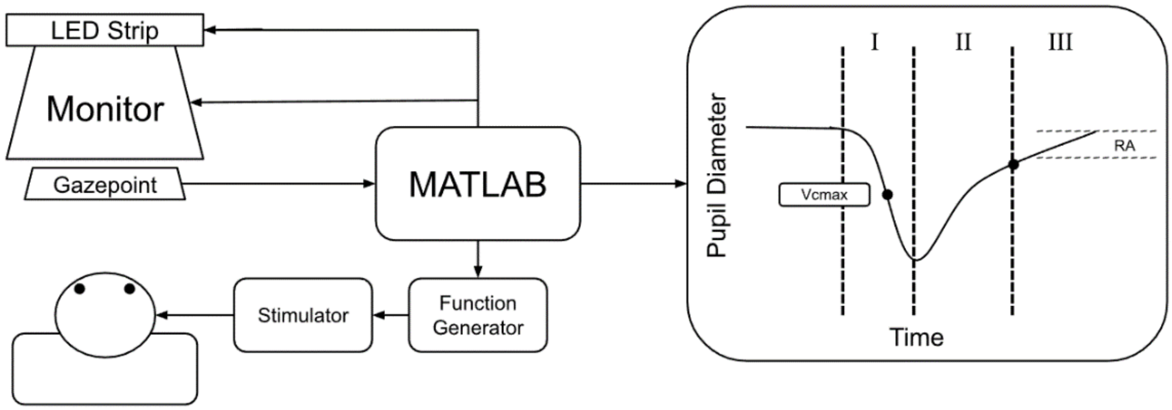

Figure 2.4: Pupillary light reflex task and setup

2.5 *Trigeminal Nerve Stimulation Delivery and Setup*

After the RNL is completed, the subject's amplitude tolerability range is determined. Neuromodulatory stimulation is delivered using a Digitimer DS8R (Digitimer, Herfordshire, UK), triggered by a Tektronix AFG1022 function generator (Tektronix CITY,STATE,USA). After swabbing area of the mastoid process and ophthalmic branch of the trigeminal nerve to lower impedance, 1.25" PALS Neurostimulation Electrodes (Axelgaard, Fallbrook, CA, USA) are adhered to the skin on these areas and secured using medical tape. The ground electrode is placed directly on the mastoid process, while the positive electrode is placed immediately superior to the supraorbital termination of the trigeminal nerve. Starting from the lowest setting of 2mA for the parameter combination of 3 kHz and 50 us pulse width, the subject is instructed to inform the researchers when the stimulation is detectable and then again when the stimulation is painful or intolerable. The amplitude is increased by 1 mA as long as the subject does not report a painful sensation until either the amplitude reaches 10mA or the subjects does report a painful sensation. The stimulation is then lowered by 1mA until the subject reports the sensation as no longer painful.

Once a tolerable amplitude has been determined, the subject is designated randomly to undergo either cyclic or continuous stimulation throughout the next period of PLR testing as well as the second set of hover and touch proprioceptive map trials. Cyclic stimulation is defined as 30 seconds of stimulation followed by 30 seconds of no stimulation, repeated for the duration of the experiment. Continuous stimulation is only interrupted when the subject switches from the PLR task back to the proprioceptive map task, upon which the stimulation is resumed. The stimulation type is determined by assigning whatever type has the least number of subjects or by random chance if it is equal. The same set of three flashes is administered throughout the PLR after the initial RNL, with the first five minutes of the task without stimulation, the next ten minutes with the stimulation protocol as determined previously, and the last ten minutes without stimulation for a total of 25 additional minutes of PLR after the RNL.

After the RNL and stimulation PLR, the subject completes the second set of 75 trials of hover and touch each for the proprioceptive map portion of the experiment while undergoing either cyclic or continuous stimulation. In all, the experiment takes around 2.5-3 hours to complete, with the error over time graphed for each subject to ensure fatigue does not confound results.

In order to compare the data from the RNL to the data acquired from the experimental data during stimulation, the RNL is plotted with baseline pupil diameter on the x-axis and the RA on the y-axis. For each minute of either pre-stimulation, during-stimulation or post-stimulation, all of the data for that minute of the PLR is graphed in the same manner with the total RNL data. This normalization of comparisons using the baseline pupil diameter is due to the inherently non-linear relationship of the

RA and the baseline pupil diameter. The RA tends to be generally higher under dark conditions than under brighter conditions as a result of the upper and lower limits of pupil diameter. This means that any changes to baseline pupil diameter that the stimulation elicits must be controlled for by comparing to pre-stimulation data for that same baseline pupil diameter. For any given window of one minute, the lowest and highest baseline pupil diameter data during the stimulation PLR is used as the lower and upper bounds of RNL data to compare with. Thus, all pre-stimulation RNL data that falls within these baseline pupil diameter bounds can be compared against the test data from the PLR during stimulation.

CHAPTER 3

RESULTS

3.1 *Edinburgh Handedness Inventory*

All subjects except for two were self-reported right-handed. One left-handed subject was assigned to each stimulation type group, either cycling or continuous stimulation. Figure 3.1 shows the handedness scores for each subject. All subjects were evaluated as the same handedness as the self-reported handedness, although some subjects fell below the 0.5 or -0.5 threshold for mixed handedness with a preference for the corresponding hand. Six subjects of the total 17 fell into this mixed handedness category, including one of the left handed subjects, although previous research does not indicate any kind of difference in error magnitude or ANS changes in relation to handedness.

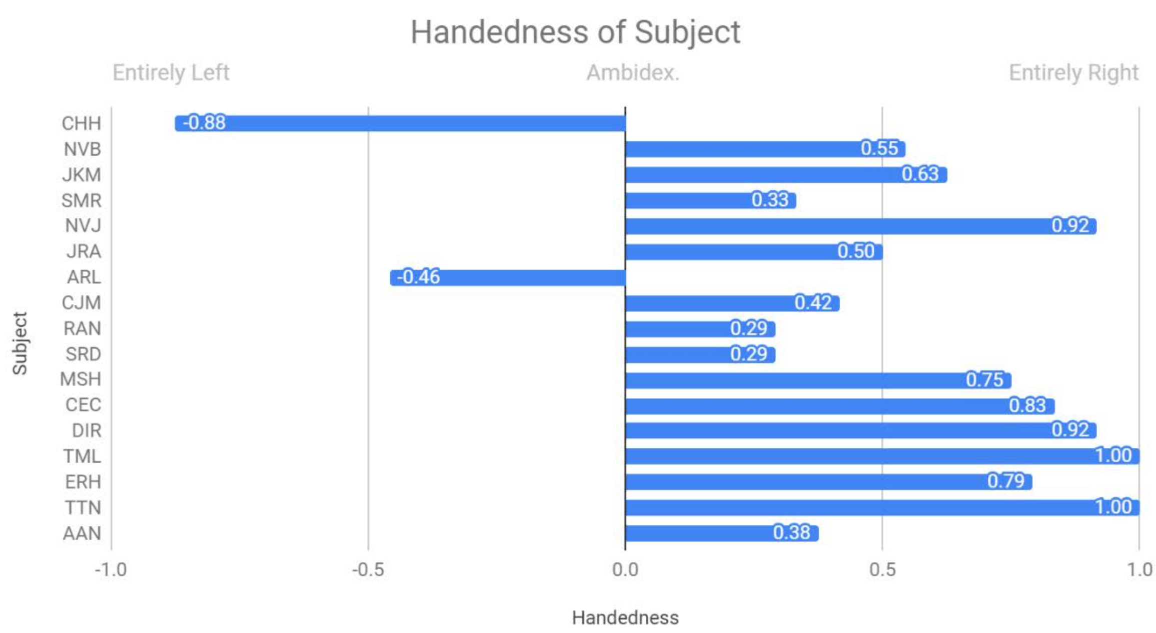

Figure 3.1: Handedness scores of all subjects from the Edinburgh Handedness Inventory

3.2 *Proprioceptive Map Results*

After interpolating data from the proprioceptive estimation task, either hover or touch, the complete proprioceptive map is graphed across all points on the map, with both the directional and magnitude components of the vectors, as seen in figure 3.2.

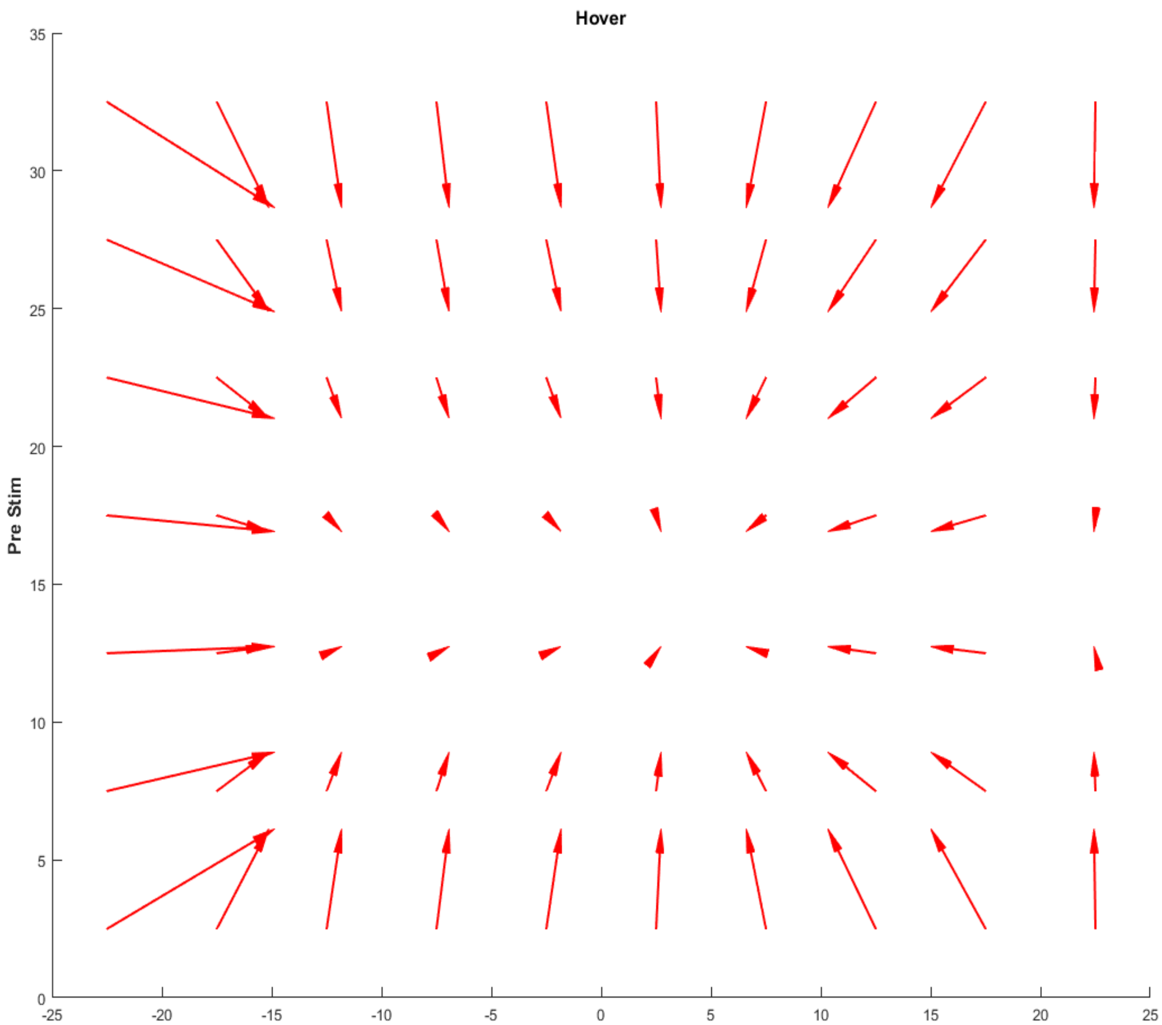

Figure 3.2: The interpolated proprioceptive map, with the focus of least proprioceptive error near the center of the map.

In order to compare the different conditions of hover, touch, pre-stimulation, and post-stimulation, the maps are overlaid and compared using the KS test method described above, as well as an overall test for error magnitude between the maps. As seen in figure 3.3, the two proprioceptive maps are tested for both shape change as well as any overall changes in error magnitude. In this example case, the second proprioceptive map in black has significantly higher error magnitudes, while the maps retain significantly similar shape between the conditions as determined by the KS test.

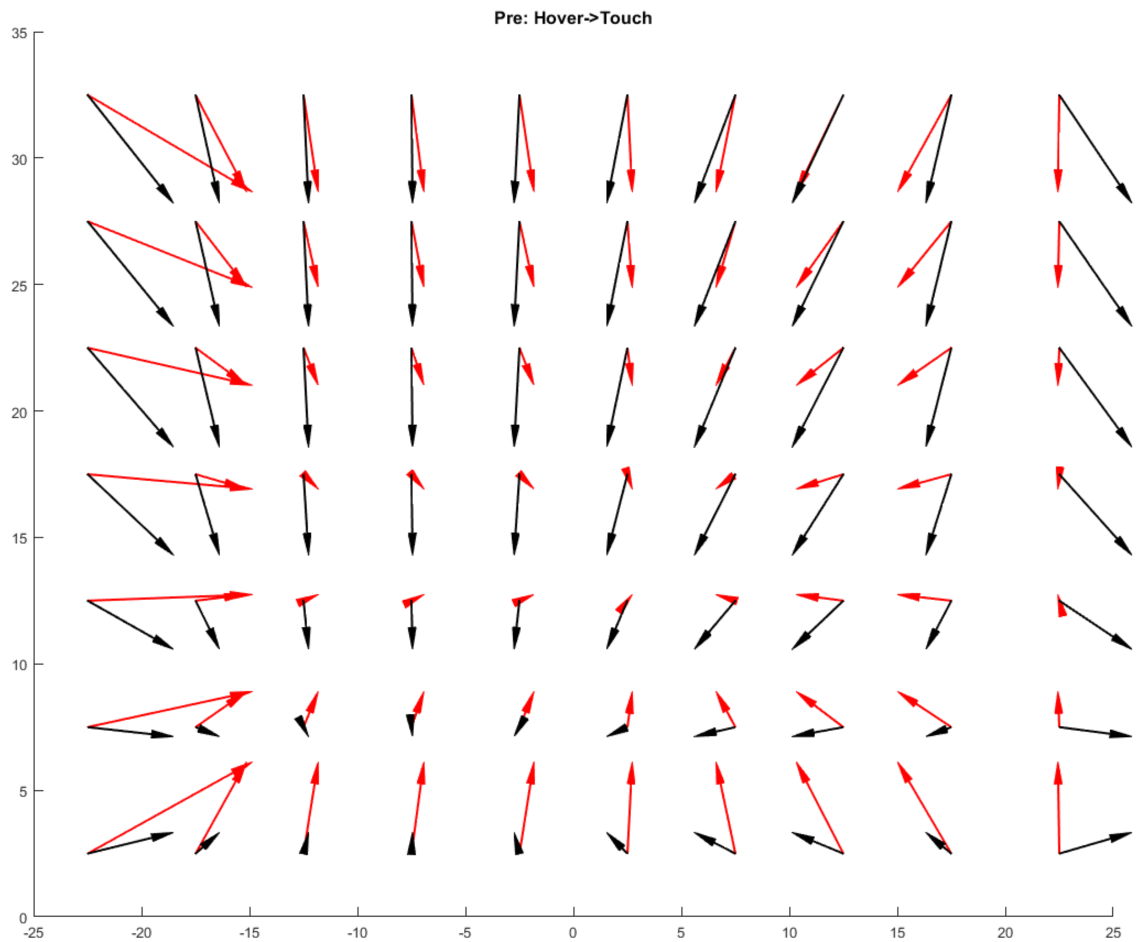

Figure 3.3: Two proprioceptive maps compared against one another

This process is repeated between all conditions for a single subject, comparing pre-stimulation hover versus touch, post-stimulation hover versus touch, pre versus post-stimulation hover, and pre versus post-stimulation touch. In doing so, any proprioceptive changes in the 2-dimensional space as a result of a change in tactile condition or the introduction of stimulation can be observed and categorized. In figure 3.5, the error magnitude decreases between hover and touch for both conditions, and decreases after stimulation for this particular subject.

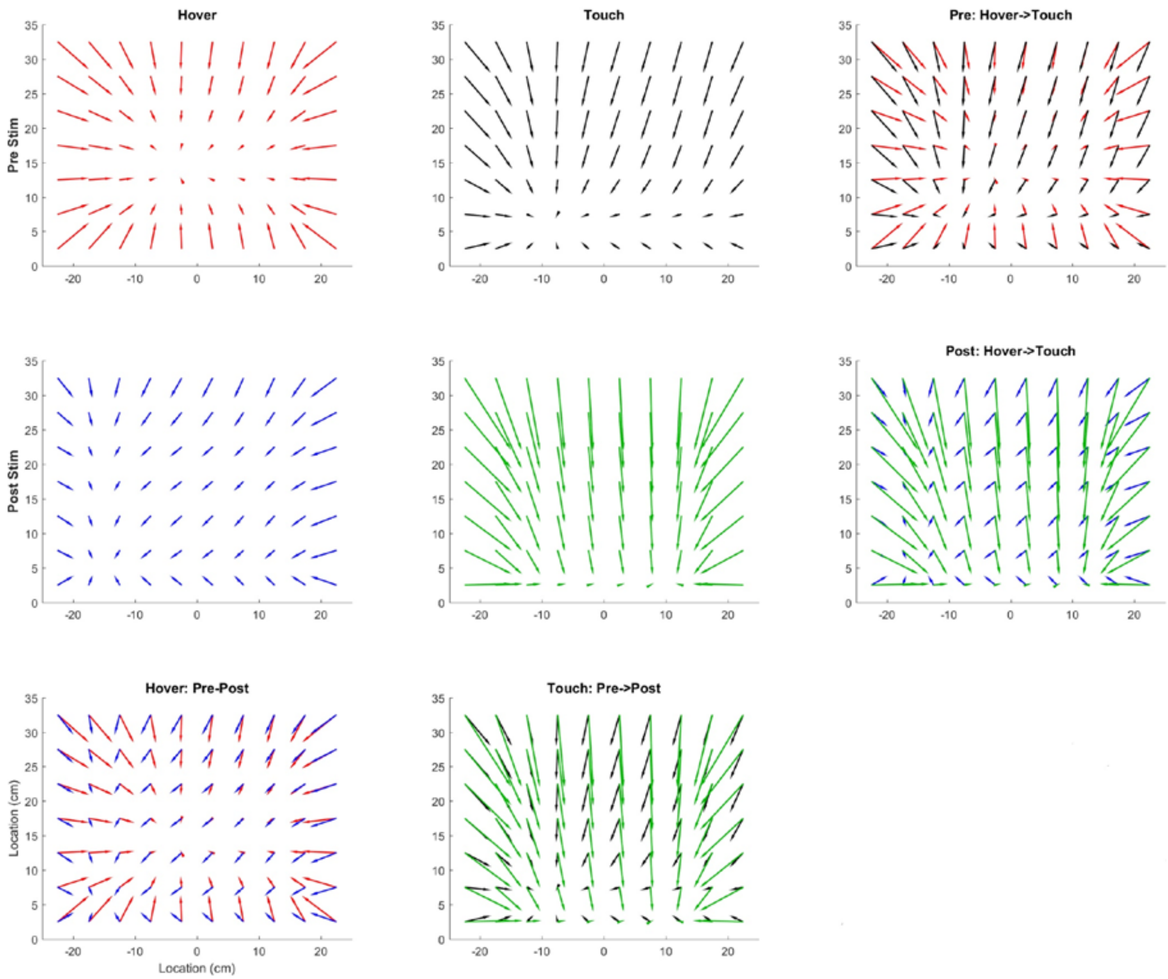

Figure 3.4: Comparison of all four conditions for a single subject

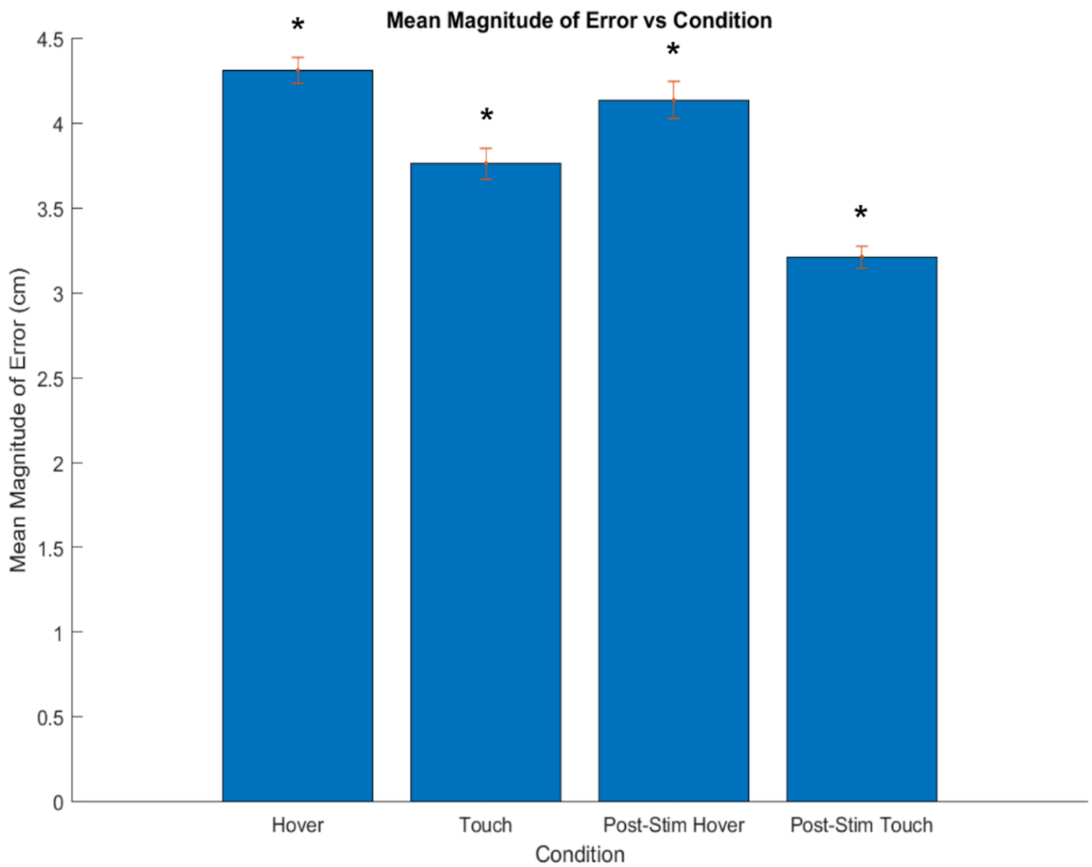

Figure 3.5: Error magnitude for all conditions of a single subject

As seen in previous research, almost all of the proprioceptive maps of right-handed subjects had the lowest magnitude of error centered around the upper right-hand corner of the field with the vector field pointing towards this central point. All of the subjects showed significantly similar proprioceptive maps across all conditions as determined by the KS test. When comparing hovering over the grid versus touching down on the grid before stimulation, 59% of subjects had decreased error, consistent with previous research. After stimulation, 53% of subjects showed this decrease of error between hover and touch. Comparing the hover condition pre-TNS and post-TNS, 59% of subjects had decreased error, with 12% increasing error magnitude post-stimulation. In a pooled comparison of all trials before stimulation versus all trials after stimulation, error magnitude decreased in 59% of subjects, had no change in 29% of subjects, and increased in 12% of subjects.

Subject	Stim	Hover vs Touch Pre	Hover vs Touch Post	Pre vs Post H	Pre vs Post T	Shape Change
CC	Continuous	None	Decrease	Decrease	Decrease	None
CH	Continuous	None	Decrease	Increase	None	None
JM	Continuous	Decrease	Decrease	Decrease	Decrease	None
MH	Continuous	Increase	Decrease	Decrease	Decrease	None
NB	Continuous	Increase	None	Increase	None	None
SR	Continuous	Decrease	Decrease	Decrease	Decrease	None
TL	Continuous	Decrease	Decrease	Increase	None	None
TN	Continuous	Decrease	Increase	Decrease	Increase	None
AL	Cycle	Increase	Decrease	Increase	Decrease	None
AN	Cycle	Decrease	Increase	Decrease	None	None
CM	Cycle	Decrease	Increase	None	None	None
DR	Cycle	Decrease	Increase	Decrease	Increase	None
EH	Cycle	Decrease	Decrease	Decrease	None	None
JA	Cycle	None	Increase	None	Increase	None
NJ	Cycle	Decrease	None	Decrease	None	None
RN	Cycle	Decrease	Decrease	Decrease	None	None
SD	Cycle	None	None	None	None	None

Table 3.1: Summary of proprioceptive error magnitude and shape changes

3.3 *Pupillary Light Reflex Results*

Figure 3.6 below shows a simulated response of the pupil diameter to a bright flash. For every set of three contraction cycles, the maximum contraction velocity and recovery amount is determined and logged, with five data points for each minute of the PLR. This set of five points is compared to the original range non-linearity obtained previously.

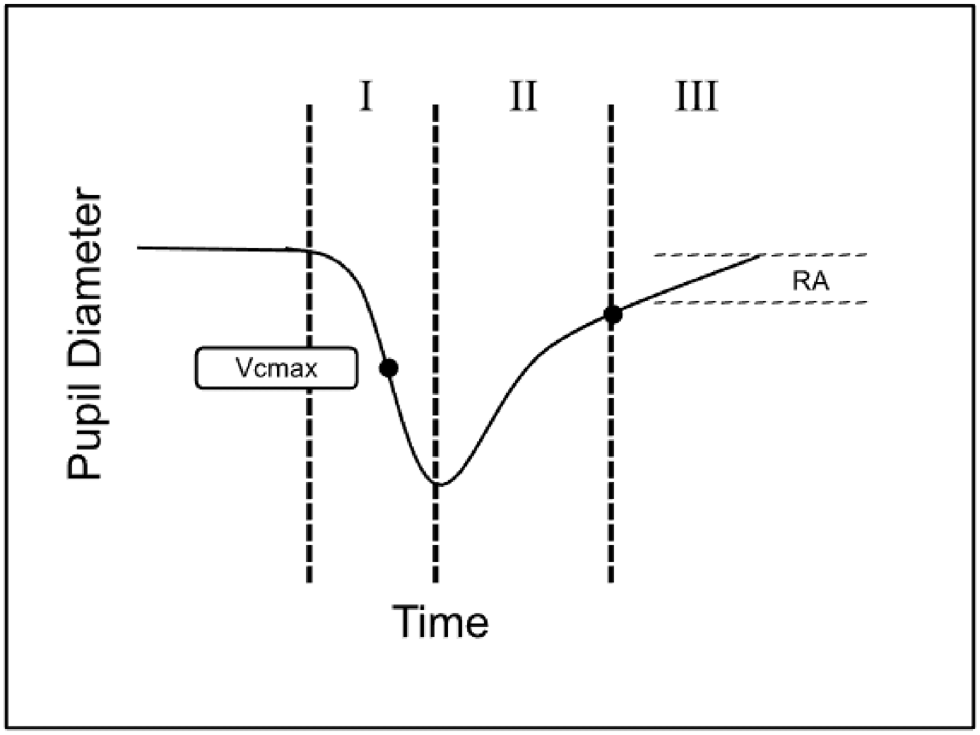

Figure 3.6: Pupil diameter after a bright flash, with the relevant metrics of maximum contraction velocity and recovery amount

The figure 3.7 below shows the compiled data from a single minute of 15 flashes. The recovery amount graphed on the left is compared using a wilcoxon rank sum test to any RNL points within the same baseline pupil diameter range in the x-axis. In this way, only sections of the RNL that have similar starting baseline pupil diameter are compared against the test cluster for each minute of TNS. Due to the non-linear relationship between baseline pupil diameter and recovery amount, it is necessary to make one-to-one comparisons with regards to pupil diameter. The statistical range of the test cluster for any given minute defines the bounds of which horizontal section of the RNL to use when comparing against the test cluster. The red or green color indicates a significant change for that minute of data. If the significant change can be explained by activation of either ANS branch, the plot is colored green. A red background corresponds to a change in the metric that can be explained by inhibition of the specific ANS branch.

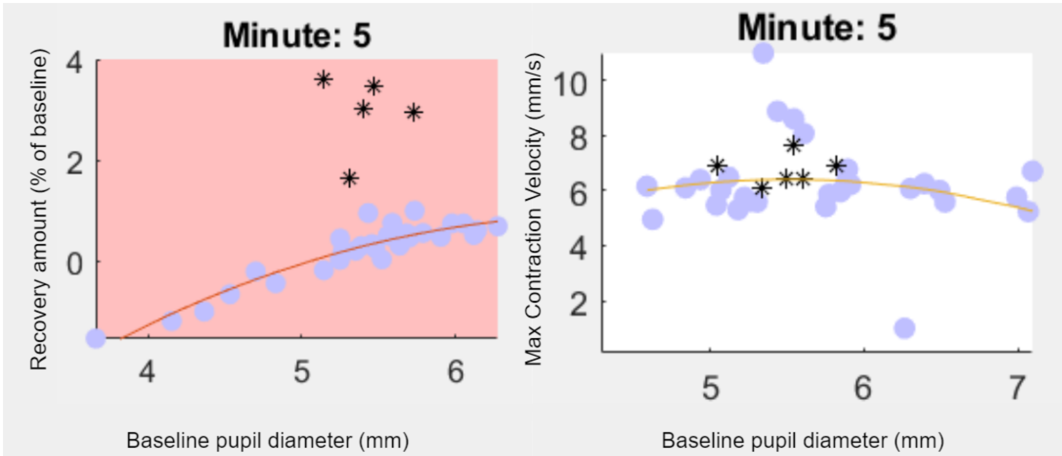

Figure 3.7: Recovery amount (Left) and Vcmax (Right) with RNL in blue, test date in black stars, and the overall trend line for the RNL in orange

Figure 3.8 below show a subject's data for the 25 minutes of the PLR. For the RA, any set of points significantly greater than the RNL shows sympathetic inhibition, while a cluster of points significantly lower than the RNL shows sympathetic activation. The same evaluation is used when comparing the Vcmax during the RNL versus each minute of the PLR, with a Vcmax above the RNL showing parasympathetic activation and a Vcmax below the RNL showing parasympathetic inhibition.

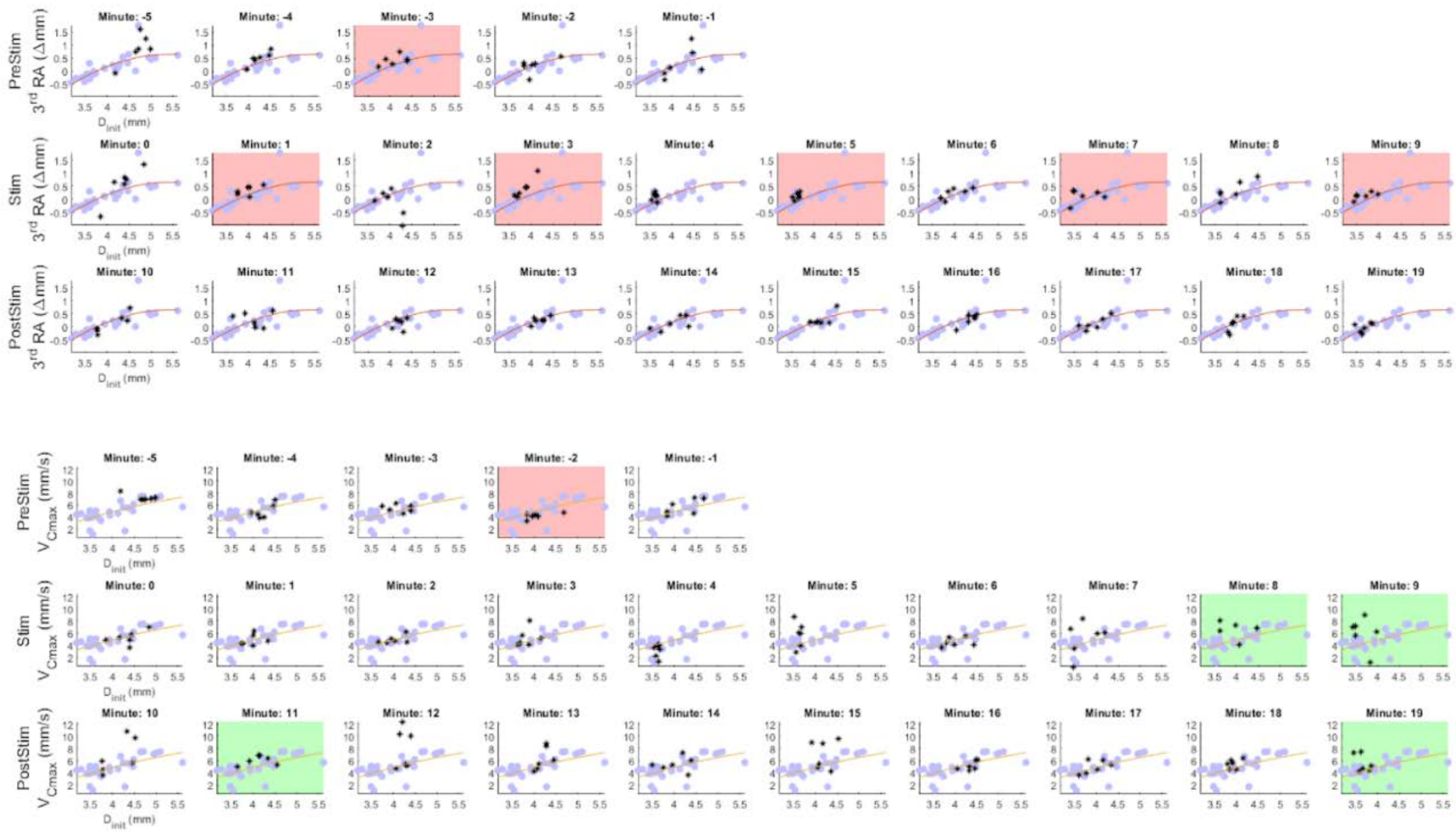

Figure 3.8: Example data of recovery amount and Vcmax for the 25 minutes of PLR

The table below shows the summarized results of all of the subjects' significant PLR changes in response to stimulation as well as changes in proprioceptive error magnitude. The number next to the indication of increase or decrease in the relevant metric denotes the number of minutes out of the twenty that were significantly different than the RNL. Consistency indicates the regularity with which the metric shows significance. In a subject with low consistency, statistically significant PLR changes can be found intermittently in less than five minutes cumulatively throughout the duration of the pupillary light reflex, both during and post stimulation. A high consistency would show significant PLR changes in five or more minutes of the total twenty.

Cycling the stimulation for 30s versus applying stimulation continuously has more PLR changes overall. Sympathetic changes are varied between individual subjects, with some subjects showing inhibition and others activation. Of the subjects that showed Vcmax changes in response to TNS, all but four subjects showed a significant increase in Vcmax. Given the nature of the two competing branches of the ANS, it is surprising that seven subjects showed contraindicating changes in RA and Vcmax at the same time. There was a large degree of variation of PLR changes between subjects for the same type of stimulation. The two types of stimulation, continuous and cycling, do not show wholly different effects on the PLR. However, 100% of subjects experienced some degree of PLR metric changes during cycling stimulation while 75% of subjects experienced changes during continuous stimulation. PLR and proprioceptive changes were observed for both low and high amplitude stimulation regardless of the type of stimulation.

Subject	Recovery Amount	Vcmax	Stim	Amplitude (mA)
TN	Decrease 13	Decrease 3	Continuous	4
JM	Increase 1	Increase 3	Continuous	5
CC	Increase 4	Decrease 2	Continuous	6
SR	None	Increase 1	Continuous	6
TL	Decrease 1	Increase 2	Continuous	6
NB	None	Increase 3	Continuous	8
MH	Increase 1	Decrease 1	Continuous	8
CH	Increase 8	Increase 3	Continuous	9
SD	Decrease 1	Increase 1	Cycle	5
DR	Increase 3	Increase 4	Cycle	6
AN	Decrease 1	Increase 4	Cycle	6
RN	Decrease 1	Increase 4	Cycle	6
AL	Increase 5	Increase 3	Cycle	7
NJ	Decrease 3	Increase 2	Cycle	7
EH	Decrease 6	Decrease 5	Cycle	8
CM	Increase 1	Increase 8	Cycle	9
JA	Decrease 3	Increase 1	Cycle	9

Table 3.2: Summarized results of all subjects for both proprioceptive error and PLR metric changes, ordered by stimulation type, with the number of minutes that were significant indicated numerically next to the RA or Vcmax metric change

Table 3.3 shows the minute by minute changes of Vcmax and RA for each subject for the entire PLR. This includes time periods before, during, and after stimulation. There is a high degree of PLR changes occurring before the onset of stimulation, with only three subjects showing no significant PLR changes before the onset of stimulation. Consistency of the PLR changes is low, with most of the changes lasting shorter than a minute and recurring sporadically. Four subjects of the seventeen showed both significant increase and decrease in at least one of the same metrics throughout the PLR. Approximately 17% of all minutes after the beginning of stimulation across all subjects were significantly different than the RNL. 22% of all minutes before the beginning of stimulation were significantly different than the RNL.

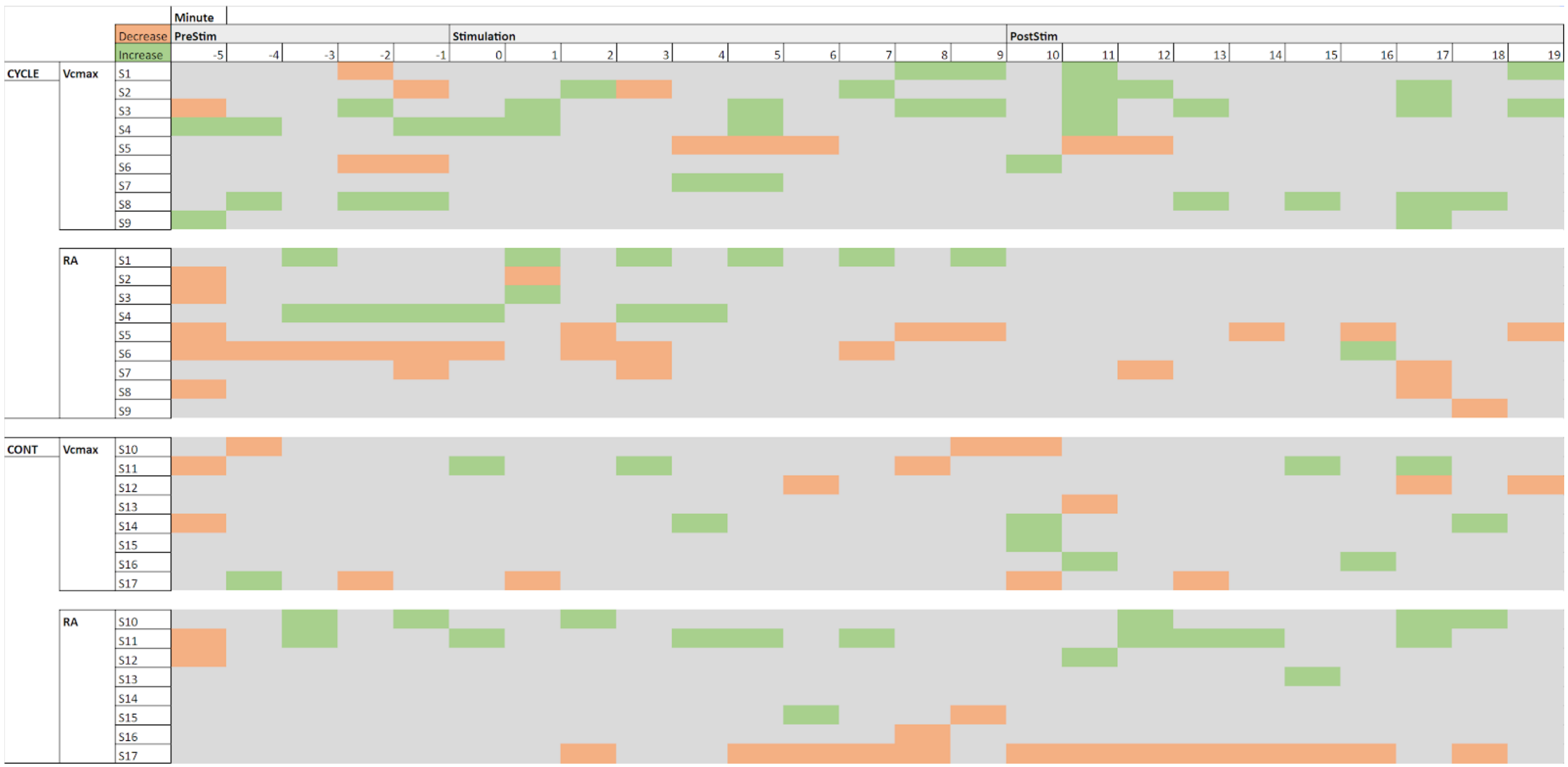

Table 3.3: Table with complete results of each subject's PLR data. Each minute of PLR with TNS is recorded, with no significant difference from the RNL in grey, significant decrease from RNL in orange, and significant increase from RNL in green

CHAPTER 4

DISCUSSION

4.1 *Conservation of Hover-Touch Error Decrease*

The decrease in error for hover versus touch in many of the subjects for both before and after stimulation confirms results seen in previous literature on tactile feedback and error magnitude. This replication of previous results lends credence to other observations on proprioceptive error as well as offering a comparison to how this sensory and attention relationship with error magnitude changes during TNS. The decrease is mostly preserved post stimulation and the contraindicative results of some subjects may be due to a lower limit of proprioceptive accuracy. Any decrease in error magnitude across all conditions during TNS that was bounded by this lower limit would appear similar in error post-stimulation. Given that the hover condition has a higher average error magnitude, it is much easier to make improvements on this high starting value than an already low touch condition. A minimum lower limit of proprioceptive ability that is constrained by factors like sensory noise, similar to the concept of tw0 point discrimination in the sensation of touch, would explain some of these results. This explanation is supported by the comparison of all pre-stimulation trials to all post-stimulation trials where most subjects show a decreased error magnitude. Error feedback or training could reduce this lower limit and offer insight into how TNS affects motor learning.

4.2 *Error Decrease and Shape Changes During TNS*

The post-stimulation decrease of error for the pooled trials of both hover and touch shows potential for reduced proprioceptive error as a result of TNS. The lack of any shape changes during stimulation provides evidence that TNS does not fundamentally alter the general estimation strategy as a whole. While this lack of change was expected, it is important to note that the same kind of decrease in error without affecting map shape is also reflected in tactile feedback of many different types as well as efforts to increase attention and arousal. This may indicate that the error decreases are a result of ANS changes that alter attention, although the particular ANS changes observed during stimulation contradict this. When considering both the ANS changes and the decrease in error, a different explanation is that the decrease in error magnitude is not a direct effect of the ANS changes but instead is regulated by a factor that is affected by the stimulation in parallel to the parasympathetic activation. The stimulation could increase the salience of relevant proprioceptive or tactile information that makes proprioceptive estimation better. The resultant decrease in error could have benefits to many fine motor tasks, especially ones that cannot rely upon visual confirmation of proprioception.

While most subjects showed decreased proprioceptive error magnitude, some had increased error magnitude during stimulation. Many factors that could change the initial state of any individual subject's ANS were not accounted for, as detailed below. Because these initial states were not identified, increased sympathetic activation could have had opposing effects depending on these initial states. A subject with low arousal and sympathetic activity that received stimulation that increased sympathetic activation could improve at the proprioceptive estimation task while a different subject that started the experiment with mid to high levels of sympathetic activity would see a performance decrease, following the Yerkes-Dodson law of arousal. This effect could also explain some of the subjects that initially showed a decrease in error magnitude between hover and touch but showed no such difference during stimulation.

Due to the inherent complexity of any pathway, either anatomically or physiologically, between the stimulation at the trigeminal nerve and the subsequent decrease in error magnitude, it is unclear at what point along its course of effect the stimulation would alter underlying proprioceptive factors. Given the slight parasympathetic activation that the stimulation elicits, any theory involving a pathway connecting the stimulation's effects on the ANS directly to its decrease in proprioceptive error magnitude can be safely shelved. It is more likely that a different but parallel pathway, such as through the mesencephalic nucleus of the trigeminal nerve, is the source of any effects on upper limb proprioception. Anatomically, inputs from the trigeminal nerve run through the trigeminal nucleus and to the mesencephalic nucleus, which projects to the dorsal column-medial lemniscus pathway (DCML). Sensory information relating to proprioception passes through the brainstem at the dorsal column nuclei, where trigeminal nerve inputs are anatomically adjacent to proprioceptive inputs for the upper limbs. This proximity in no way implies interaction between the two, and as such any course of effect would have to exist in higher-level integration areas like the thalamus and sensorimotor cortex.

The integration of proprioceptive inputs to create a mental map of the 3d positions of each limb with respect to each other is a key component of proprioception and motor learning. Without the presence of vision, much of the normal proprioceptive integration is disrupted. No direct visual connection between where the eyes are located on the body to where the hands are visually located can be used to inform the position of the hand. TNS could improve proprioception of the head, which stabilizes and anchors the proprioceptive reference of the head and face without vision. In this way, the positional relationship between the head can be better estimated, leading to better estimation relative to the neck, to the shoulder and so on until the target at the forefinger. This hypothesis is strengthened by the specific experiment fixing the torso, which could potentially offer fewer degrees of freedom to estimate along the proprioceptive connections to the forefinger's position. Testing whether TNS affects head proprioception would be a first step in confirming or refuting this theory, as well as fixing versus freeing movement of each joint of the arm or shoulder to better examine the connection between each limb's proprioception.

4.3 *Autonomic Nervous System Changes*

Inhibition and activation is observed in both the sympathetic and parasympathetic systems during TNS. Some subjects experienced no ANS changes at all, while other experienced change in just one branch or both branches. This inconsistency may be a result of varied internal states. The initial state of each subject's ANS could limit any effect stimulation may have on parasympathetic and sympathetic systems. The large number of trials for both the proprioceptive estimation task and PLR task make comparisons between the same subject's data reliable, but comparisons between different subjects with these varied internal states indicates that neuromodulation effect is idiosyncratic. While subjects with recent caffeine intake and epilepsy or other neurological conditions were excluded, no measure of their initial state was taken. Time of day, average hours of sleep per night, hydration, and various other measures could provide insight into a subjects resting state - and therefore efficacy of neuromodulation.

The parasympathetic activation was unexpected, especially in conjunction with the decrease in proprioceptive error magnitude. The working theory of sympathetic activation leading to decreased error magnitude seems not to apply with this experimental paradigm. Parasympathetic activation could be useful to soothe anxiety or help induce sleep, although the data collected in this experiment is not directly related to these applications and warrants additional investigation. Sympathetic changes occurred at approximately the same rate as parasympathetic changes, although no clear activation or inhibition trend can be gleaned. A complex, idiosyncratic relationship between TNS and sympathetic nervous system changes can be explained by an uncontrolled factor or variable. This unseen third force could be the prior activation or inhibition as referenced earlier, but the high consistency of some of the subjects' sympathetic changes, including both activation and inhibition, points to a more detailed reason as the cause.

The overall low consistency in both activation and inhibition for both branches was expected. This method of measuring ANS activity is particularly conservative due to the multiple degrees of separation between the muscles of the pupil and the changing ANS states. Even when using strong sympathomimetic and parasympatholytic agents, the consistency and magnitude of the RA changes as compared to the RNL were low (Yamaji et al., 2000). Stimulation that affects the ANS to a lesser degree would appear even less consistent. There were a few subjects, including the one shown in figure 3.X, that show significant changes to Vcmax or RA before the onset of stimulation. Considering the testing for tolerable amplitude is conducted immediately before the 25-minute PLR task with TNS, it is possible that the small amount of stimulation may have prompted ANS changes that manifest as pre-onset significance. Onset of effect for other measures of the effects of TNS mark the time as less than 5 minutes, so the stimulation at the start of the tolerability determination test could eclipse the start of the 25-minute PLR and last long enough to affect multiple minutes of pre-onset PLR.

It is difficult to point to any obvious trends in the PLR results, especially for recovery amount and when considering the high degree of significance pre-stimulation. This may be a result of levels of ANS changes that are too low to be reliably measured in such a distant target as the pupils. There are many contributing structures between the skin above the trigeminal nerve and the pupils, which could also contribute to the variability between subjects. While the PLR was reliably correlated with ANS changes in the original paper by Yamaji, TNS may have a drastically different path of effect than the sympathomimetic and parasympatholytic pharmaceuticals used in the experiment.

4.4 *Stimulation Type and Amplitude*

It is difficult to conclude whether cycled stimulation elicited a larger decrease of error magnitude over continuous stimulation. While all subjects that underwent cycled stimulation had either no effect or decreased error magnitude, the rate at which this decrease occured is close to the rate that continuous stimulation provided. Other categories of comparison for proprioceptive error magnitude and shape change yielded similarly equal rates, with most of the differing effects between the stimulation types seen when examining the ANS changes rather than the proprioceptive changes. This lends further support to the idea that the pathway of effect for decreased proprioceptive error magnitude is different than the pathway of effect for the observed ANS changes. The process of increasing current amplitude to the high end of the tolerable range for each subject resulted in a fair amount of variability, from 4 - 9 mA. In each case, the subject reported the next 1 mA increase above this level as painful. It is important to note that the different stimulation types were verbally reported by the subjects as perceptibly the same sensation, except that cycling the stimulation had repeated onsets, which may reset the initial surprise that would usually dissipate during continuous stimulation. This sensation may have confounded the purpose of the stimulation paradigm by prompting an increase in arousal (Alamia et al., 2019). It is unclear whether increasing amplitude past the point of comfort would elicit greater ANS or proprioceptive changes.

4.5 *Future Direction and Applications*

The results of this experiment could be compared to other types of neuromodulation like VNS directly to see if the stimulation prompts ANS changes that are of a similar intensity and duration. In addition, the PLR task is easy to set up and requires no trained personnel to administer, offering opportunities to use the PLR as a screening test to see if TNS would have a neurological or ANS effect before recommending the use of a device to treat a disease.

TNS shows promising changes to both the ANS and proprioceptive error magnitude but the idiosyncracies between subjects, especially with regards to sympathetic activation or inhibition, mean that there are hidden states or alternative factors that contribute to these systems. Controlling or introducing some of these potentially relevant factors could reveal better opportunities to effectively and consistently regulate the ANS. An experiment that catalogues or controls for factors like sleep duration, time of day, food intake, temperature, and ambient light level would be able to refine the results seen here and more definitively make conclusions on the magnitude of ANS changes during and after TNS.

Comparing the effects of VNS on the PLR would offer insight into the benefits and potential differences that TNS versus VNS would have on modulating the ANS. The inconsistency of significance for the two metrics of the PLR could be better compared to other metrics of ANS activity such as galvanic skin response or heart rate. An experiment that introduced a positive control of caffeine would also offer a baseline for comparing TNS to a known sympathomimetic agent and how that affects proprioception and the PLR.

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APPENDIX A

EDINBURGH INVENTORY FOR HANDEDNESS

Have you ever had any tendency to left-handedness? *

- Yes
- No
- Unsure

Directions

Please indicate your preferences in the use of hands in the following activities by checking the "+" box in the appropriate column.

Where the preference is so strong that you would never try to use the other hand unless absolutely forced to, put a "++". If in any case you are really indifferent, check the "Neutral" box.

Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

Please try to answer all the questions, and only leave a N/A response if you have no experience at all of the object or task.

Figure A.1: Directions for the Edinburgh Inventory for Handedness

Activities *

	L (++)	L (+)	Neutral	R (+)	R (++)	N/A
Writing	☐	☐	☐	☐	☐	☐
Drawing	☐	☐	☐	☐	☐	☐
Throwing	☐	☐	☐	☐	☐	☐
Scissors	☐	☐	☐	☐	☐	☐
Toothbrush	☐	☐	☐	☐	☐	☐
Knife (without fork)	☐	☐	☐	☐	☐	☐
Spoon	☐	☐	☐	☐	☐	☐
Broom (upper hand)	☐	☐	☐	☐	☐	☐
Striking match (holding the match)	☐	☐	☐	☐	☐	☐
Opening box (holding the lid)	☐	☐	☐	☐	☐	☐
Which foot do you prefer to kick with?	☐	☐	☐	☐	☐	☐
Which eye do you use when using only one eye?	☐	☐	☐	☐	☐	☐

Figure A.2: Questions on the Edinburgh Inventory