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Development of novel methods for analysis of autonomic balance and gamma coherence in autism.

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DEVELOPMENT OF NOVEL METHODS FOR ANALYSIS OF AUTONOMIC BALANCE AND GAMMA COHERENCE IN AUTISM

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DEVELOPMENT OF NOVEL METHODS FOR ANALYSIS OF AUTONOMIC BALANCE AND GAMMA COHERENCE IN AUTISM

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ABSTRACT

INTRODUCTION: Autism spectrum disorder (ASD) is a developmental disorder that is characterized by difficulty in social interactions, limited range of interests, and repetitive behaviors. ASD has been shown to negatively affect both ambient vision and the autonomic nervous system (ANS). The objectives of this thesis were to determine the efficacy of two novel therapies, visuo-motor training with ambient prism lenses and repetitive transcranial magnetic stimulation (rTMS), on autonomic balance and EEG gamma coherence. Heart rate variability (HRV) and skin conductance level (SCL) were used as indicators of autonomic function. HRV is an excellent indicator of autonomic balance because it has a low frequency (LF) component, representative of combined parasympathetic and sympathetic inputs, and a high frequency (HF) component, representative of parasympathetic only inputs. SCL is controlled solely by sympathetic inputs and is therefore indicative of sympathetic tone. Gamma coherence refers to the co-activation of different regions of the brain during completion of a task.

DATA COLLECTION: Autonomic measures of heart rate (HR) and SCL were collected for both the prism lens study and the rTMS study. Patients enrolled in the prism lens study were fitted with an appropriate pair of lenses and performed daily visuo-motor exercises for six months. Autonomic data was collected twice: once at the baseline (before treatment) and again after completing the prism lens training course. Patients enrolled in the rTMS study underwent 18 weekly sessions of TMS. Autonomic data was collected during each session. EEG data was collected twice for both the prism lens and

rTMS study, once before and after the course of therapy. Behavioral checklists such as the Aberrant Behavior Checklist (ABC) and the Repetitive Behavior Checklist (RBS) were completed by the subjects' parents before and after therapy.

DATA PROCESSING AND ANALYSIS: HR and SCL data collected as the patients watched scenes from *The Lion King* (prism lens study) or as patients received TMS (rTMS study) was exported into Microsoft Excel to begin pre-processing. Statistical analyses were performed using SPSS 14.0 software. HR data was further analyzed using Kubios HRV software to calculate heart rate variability (HRV) measures. EEG data was exported into a software program called Brain Electrical Source Analysis (BESA) for analysis of gamma coherence.

RESULTS: Results from the prism lens study show that average HR among participants decreased significantly after completion of prism lens therapy. There was also a decrease in LF power, an increase in the percentage of HF power, a decrease in the ratio of LF to HF power (cardiac autonomic balance index), and a decrease in SCL. No significant changes were observed in gamma coherence following prism lens therapy. Results from the rTMS study show changes in several measures that reached significance: increased RR interval (time lapse between successive heartbeats), increased SDNN (standard deviation of RR interval), increased HF component of HRV, decreased LF/HF ratio, and decreased SCL. In addition, there was increased gamma coherence between frontal-temporal regions and frontal-parietal regions following TMS therapy.

CONCLUSIONS: ASD has been shown to present symptoms of disrupted autonomic balance resulting from excess activity of the sympathetic branch and suppressed parasympathetic activity. In this thesis, autonomic balance, as indicated by HRV and SCL, was used as a measure of the effectiveness of two novel therapies for the treatment of ASD: prism lenses and rTMS. Both therapies resulted in an improvement of autonomic balance through the activation of the parasympathetic branch and/or by reducing activity of the sympathetic branch. Prism lens therapy did not improve gamma coherence; however, TMS therapy was shown to increase gamma coherence between frontal-parietal and frontal-temporal regions during processing target stimuli.

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I. INTRODUCTION

A. Prevalence of Autism and Clinical Significance

ASD is characterized by difficulty in social interactions, lack of communication, and a restricted range of interests. ASD can be detected very early, even as young as 3 months of age. It was estimated by the Centers for Disease Control and Prevention (CDC) that in 2010 ASD affected approximately 1 in 68 children (Baio, 2014). According to several recent theories sensory processing abnormalities may play an important role in impairments of perception, cognition, and behavior in individuals with ASD. Among these sensory abnormalities visual distortion may contribute to such typical symptoms of ASD as relying on peripheral vision, strabismus, and manifestation of stereotypic behaviors (Kaplan, 2006; Dombroski et al., 2014).

It is also thought that ASD can manifest itself in abnormalities of the autonomic nervous system (ANS). The ANS is the non-voluntary part of the nervous system. It consists of two branches that work as antagonists to control organ function. In most cases the sympathetic branch acts to stimulate organ function. During times of stress the sympathetic system increases heart rate, skin conductance, blood pressure, and respiration, but also significantly decreasing activities not essential to immediate survival, such as digestion. Therefore, the sympathetic branch plays an important role in the "fight or flight" response. The parasympathetic branch typically acts as an inhibitor and operates when conditions are normal and the individual is at ease. In individuals without ASD a continuous balance exists between these two branches. However, in individuals with ASD there may be inappropriate activation of the sympathetic system even when

there are no stress inducers, leading to decreased autonomic control and a general state of anxiety. This autonomic imbalance may result in symptoms such as lack of blood pressure and temperature regulation, increased heart rate and skin conductance, and digestive issues.

B. The Autonomic Nervous System: HRV and SCL

Heart rate variability (HRV) is defined as the variation in the time interval between heartbeats, and is an important indicator of autonomic function (Berntson et al., 1997). Numerous studies have shown there are significant differences in HRV between individuals with anxiety disorders and those without, suggesting that abnormalities in the ANS play an important role in such disorders. In a study by Thayer et al. the researchers compared responses of three groups on patients to laboratory stressors: panickers, blood phobics, and nonanxious controls. Results demonstrated that panickers had the highest rates combined with the lowest HRV, while controls showed the greatest HRV. Blood phobics had higher level of vagal heart rate control than panickers, but less than the nonanxious controls (Friedman & Thayer, 1998).

Currently, HRV is used regularly as clinical indicator for the risk of myocardial infarction and as a marker of diabetic neuropathy. However, as the study by Friedman and Thayer described above suggests, HRV is becoming increasingly popular as a measurement tool for ANS function and overall health. HRV includes many indices that are described below (Kleiger et al., 2005):

Time-domain measures of HRV include:

1. Mean RR: Average of the R-R intervals for the entire session

2. STD RR (SDNN): Standard deviation of all normal to normal R-R (NN) intervals
3. Mean HR: Average of heart rate for the entire sessions
4. STDHR: Standard deviation of heart rate
5. RMSSD: Square root of the mean of the squares of successive NN interval differences (or the average change in interval between beats)
6. NN50: The number of NN intervals differing by >50 ms from the preceding interval
7. pNN50: The percentage of intervals >50 ms different from preceding interval

Frequency-Domain Results (Fast Fourier Transform) of HRV include:

1. High frequency (HF) component of HRV (0.15 – 0.40 Hz) is representative of parasympathetic cardiac control
2. Low frequency (LF) component of HRV (0.04 – 0.15 Hz) is an index of combined parasympathetic and sympathetic control
3. LF/HF ratio compares the powers of the LF and HF frequency bands and is considered a cardiac autonomic balance index

The HF component of HRV is often referred to as respiratory sinus arrhythmia (RSA) and is assumed to be the non-invasive index of parasympathetic influences on the heart (Sohn et al., 2001). As the HF component is indicative of parasympathetic function, it is thus also associated with vagal tone. Several studies have supported the hypothesis that increased vagal tone is associated with better social functioning. Studies involving

infants found a positive correlation between vagal tone and emotional reactivity to positive, novel, and negative situations (Fox, 1989; Calkins, 1997). Infants with high vagal tone were more sociable (Fox, 1989) and better adjusted to new social environments (Fox and Field, 1989). Another study on HRV found that preschool children with inhibited temperament, a potential precursor of anxiety in school-age children, had reduced HRV measures. This finding suggests that reduced parasympathetic activity may contribute to the association between elevated HR and anxiety in school-age children (Kagan et. al, 1987).

The LF component of HRV has been linked to sympathetic nervous system activity and sympathovagal balance by numerous studies (Malliani et al., 1994; Pagani et al., 1986). However, other studies have shown that the LF variability is rather a reflection of both sympathetic and vagal influences related to baroreflex mechanisms (Berntson et al., 1997). It is thought that changes in blood pressure may cause a vagally-mediated baroreflex response and also changes in LF variability.

Electrodermal activity (EDA) refers to changes in the electrical activity of the skin, specifically the activity of eccrine sweat glands. EDA is controlled by sympathetic branch of the ANS (Boucsein, 2012), and is therefore often used as a non-invasive measure of autonomic function. EDA can be measured in terms of skin conductance response (SCR) magnitude, non-specific skin conductance response (NS.SCR) frequency, and skin conductance level (SCL). The magnitude of EDA measures has been shown to correspond to the strength of an elicited emotion (Boucsein, 1999, 2012). Typically, EDA increases during stressful situations as the sweat glands become more active. In fact, EDA is such a reliable measure of stress that it is often used as one of the components of

the lie detecting apparatus (Gamer, 2011). Several studies have shown that age has an effect on EDA measures. For example, Plouffe and Stelmack (1984) reported decreased basal SCL with increased age. A study by Shields (1983) found that young subjects exhibited a larger magnitude of SCR than adults when presented with sensory stimuli. EDA measures can also be used to distinguish between different emotions.

C. Autism and the Autonomic Nervous System

Stress plays a major role on the ANS as seen by changes in autonomic variables. Autistic individuals are vulnerable to high levels of stress due to difficulty with communication and adapting to new environments (Jansen et al., 2006). For instance, de Bruin et al. (2007) reported that over 55% of sampled children with ASD met criteria for at least one anxiety disorder. Similar results were reported in another current study (McPheeters et al., 2011). Recent years have seen an increasing number of studies dedicated to understanding the autonomic nervous system abnormalities in ASD. Several types of autonomic dysfunctions have been reported in autism, including increased basal sympathetic tone (Hirstein et al., 2001), as well as reduced baseline parasympathetic activity in association with increased baseline sympathetic tone (Ming et al., 2011; Porges, 2001). Reduced HRV, specifically the attenuated power of high frequency (HF) component of the HRV is an indicator of limited psychophysiological flexibility. A recently published paper by Ming et al. (2012) reported evidence of reduced baseline parasympathetic activity and increased sympathetic tone in children with ASD. Another study by Bal et al. (2010) used respiratory sinus arrhythmia (RSA) as a measure of cardiac vagal tone and compared RSA values between children with and without ASD.

The study found that children with ASD had significantly lower RSA values and faster heart rate than those without ASD, which suggests decreased vagal regulation in autism. The clinical implications of chronic increased sympathetic activity and decreased vagal tone are poor control of HR and tendency for tachycardia (Berntson et al., 1997, 2008; Coronoa et al., 1998; Friedman & Thayer, 1998).

Studies dedicated to the relationship between ASD and the autonomic nervous system have also found manifestations of increased sympathetic activity in EDA measures. Palkovitz and Weisenfeld (1980) studied SCL in response to auditory stimuli in children with and without ASD. They did not find differences in electrodermal reactivity to auditory stimulation compared to controls, but reported that the autistic group had a higher baseline SCL. In addition, Angus (1970) found that children with ASD displayed more fluctuations in SCL compared to controls. SCR studies in autistic children have shown a lack of the normal SCR habituation to the same stimulus over time (Toichi & Kamio, 2003). It has also been reported that children with autism had blunted autonomic arousal to visual or auditory social stimuli (Hirstein et al., 2001). SCL is controlled solely by the sympathetic inputs (Boucsein 2012; Williams et el. 2004); therefore, the above findings are indications of high sympathetic tone and low selectivity of ANS responses in autism.

D. Gamma Coherence in Autism

EEG oscillations can be separated into several different frequency bands: delta (0.5 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 13 Hz), beta (13 – 30 Hz), and gamma (30 – 80 Hz). The frequency band of most interest in this thesis is gamma, specifically from 30 –

45 Hz. Gamma frequencies in particular have been linked to sensory processing, working memory, attention, and other cognitive domains (Jensen et al. 2007; Ward 2003). Two gamma oscillations are typically observed after a stimulus. The first, evoked gamma, appears within the first 200 ms post-stimulus. The second, induced gamma, typically occurs within the region of 300 – 600 ms post-stimulus. Evoked gamma tends to be fixed in its onset, or latency, and is therefore associated with sensory processing and does not vary from task to task. Conversely, induced gamma oscillations vary in latency and are thought to be involved in higher cognitive processes, such as pattern recognition or memory (Tallon-Baudry, 2003). Coherence is a measurement, varying between zero and one, of correlating gamma activity between two regions of the brain. Coherence suggests how well the different regions are working together to complete a common task. Previous studies have found that gamma coherence abnormalities are present in ASD, including deficiencies in long-distance connectivity and increased connectivity between local regions (Casanova et al., 2013)

E. Novel Therapies for the Treatment of Autism

This thesis describes two novel therapies, visuo-motor training using prism lenses and repetitive transcranial magnetic stimulation (rTMS), and their effects on autonomic balance and gamma oscillations coherence. These two studies were carried out separately. The efficacy of both treatment methods was determined by measuring autonomic variables (HRV and SCL) and gamma coherence.

II. CASE I: PRISM LENS THERAPY

A. Background Information

The sense of vision is used to gather information about our surroundings, read directions, recognize familiar faces, and numerous other activities. Vision can be classified into two main types. The first, focal, is conscious and allows us to see objects in high-resolution color. At the doctor's office it is focal vision that is tested and given a score, sometimes resulting in the prescription of corrective lenses for near- or farsightedness. The other type of vision is known as ambient vision, which is largely non-conscious and is used in spatial orientation and depth perception. Unlike focal vision, which is restricted to two degrees of visual field, ambient vision involves the entire visual field and integrates with other sensory systems to determine one's position in space. Deficits in this ambient vision system could clearly cause for confusion, disorientation, and stress. Such deficits have been linked to autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and other developmental disorders by previous studies. In fact, some of the stereotypical behaviors of ASD are actually the individual's attempts to determine his or her orientation in space (Kaplan, 2006; Dombroski et al., 2014). Prism lenses have the ability to improve ambient vision, thus reducing the stress in trying to decipher one's environment. In a prior study by Kaplan et al. (1998) the behavior, attention, and orientation of two groups of autistic individuals were evaluated over a period of 4 months. One group wore prism lenses and the other wore placebo lenses. At the 1.5 month mark, individuals in the prism lens group showed a decrease in

behavior problems compared to the placebo group. A slight loss of these benefits was observed at the 3 and 4 month mark.

B. Protocols and Data Collection

1. Subject Demographics

Fourteen out of 24 children enrolled in the study completed the whole course of prism lenses treatment and visuo-motor training, along with baseline and post-treatment assessments. Mean age of participants was 13.1 years (SD=3.37 yrs), range varied within 8-19 years, and 4 of them were girls. All participants' ASD diagnosis was made according to the DSM-IV-TR (APA, 2000) and further ascertained with the Autism Diagnostic Interview – Revised (ADI-R) (Le Couteur et al., 2003). They also had a medical evaluation by a developmental pediatrician. All subjects had normal hearing based on past hearing screens. All autistic participants were high-functioning persons with full scale IQ > 80 assessed using the Wechsler Intelligence Scale for Children (WISC-IV; Wechsler, 2004). Four out of 14 participants had a diagnosis of Asperger's Disorder. Study participants were recruited through advertisement posted at local autism advocacy organizations (e.g., Louisville Chapter of FEAT) and some were recruited from existing pool of children with ASD seeking treatment.

2. Determining each subject's prism lens prescription

Dr. Melvin Kaplan from the Center for Visual Management (Terrytown, NY) visited the University of Louisville twice and on-site observed each patient's head tilt,

body posture, facial expressions, and breathing during visual pursuits, ball catching, and watching TV to determine which base direction of ambient lenses should be prescribed for each patient (Figures 1 and 2). Prism lenses were manufactured at the optic center in the state of New York following Dr. Kaplan's prescription to each participant.

FIGURE 1- Dr. Kaplan conducting evaluations to prescribe prism lenses

3. Daily vision exercises

At the beginning of each month, each study participant along with prescribed lenses received a packet of visuo-motor exercises program that were to be done for Week 1, Week 2, Week 3 and Week 4. Each week's exercises were designed to build neurosensory and neuromuscular skills from one week to the next. A description of the materials, lenses, setup, procedure, observations and time expected to be spent on each exercise were all included in the instructions. The prescription prism lenses, the dysruptive lenses and the red/green lenses were all provided by the Center for Visual Management. The materials needed involved a combination of household items and a few items that were relatively inexpensive to buy or build on your own, such as a balance board and a chalk board. At the end of each month, patients came into the lab at the

University of Louisville for a post-assessment evaluation and video recording of selected procedures chosen by the Center for Visual Management. The video recordings were mailed to the Center for Visual Management for oversight in determining each patient's progress throughout the study.

Month 1: The purpose behind the exercises assigned for month 1 was to disrupt and reintegrate the visual system. Observational changes could be seen in the patient's stress level, ease or difficulty in reorganizing visual perception and balance to the base direction, level of comfort or discomfort in completing the exercise, sustained visual attention, awareness of double vision, visual tracking, hand-eye coordination, smooth or uncoordinated movements, visual convergence, proper posture, and improved perception of distance and speed. In addition to the prescription prism lenses, the disruptive lenses and/or the red/green lenses, other equipment used in these exercises included a balance board, a trampoline and a yoga mat.

Month 2: The visuo-motor exercises assigned for the second month utilized a visual-vestibular technique to enhance body schema and self-awareness, develop visual-vestibular integration, coordinate balance and visual attention to self, and engage binocular fusion. Additionally, patients practiced sustaining visual feedback to a focal target, while increasing ambient awareness and reorganizing current visual perceptual patterns and visual fields which may have been previously compressed prior to wearing prism lenses and visuo-motor therapy. Additional equipment used in some of these exercises included a full length mirror, a "Star" chart, and a chalkboard.

Month 3: The purpose of this month's exercises was to engage bilateral movements and visual-auditory integration as well as increase visual attention and eye movement skills including tracking and coordination. Other objectives included the introduction of fixation jumps to expand the visual eye field while incorporating proprioception of body movement and auditory input to reorganize the patient's current visual perceptual patterns as well as promote smooth tracking and breathing skills. New equipment introduced during this month's exercises included a metronome, bongos, a checkerboard, a circle and square chart, and a Hart chart. Figure 2 illustrates one of the subjects performing these exercises in the lab.

FIGURE 2 – Subject performing visuo-motor exercises

Month 4: During month 4 exercises, patients were asked to read before and after visuo-motor therapy and trainers were instructed to observe differences in speed, reading fluency and comprehension as well as changes in breathing and posture, including

whether or not the patient appeared relaxed or tense. The primary goal of this month's exercises was to integrate two visual tracts, both focal and ambient vision while also creating depth perception, increasing smooth eye movements, and increasing visual attention and tracking ability. Additional equipment during this month's exercises included a pie tin, a slinky on a dowel, two matchbox cars or tennis balls, and bubble blowing solution with a wand.

Month 5: A constant theme throughout visuo-motor therapy is to increase visual attention, eye movements and tracking ability. For this month's exercises, several previously learned procedures were repeated, but now combined with other procedures to create depth perception, increase smooth eye movements, develop visual-vestibular integration and increase visual processing ability. Much of the same equipment was reused during this month's exercises; however, a walking rail was added to incorporate balance and depth perception with an exercise that was previously done sitting or standing in a stationary position.

Month 6: The final month's exercises for this study highlighted the fusion of vision and auditory integration with controlled breathing, increased proprioceptive input, visual attention, convergence skills and depth perception. This study concluded with the integration of visual-verbal sequences in which the patient was directed to name items such as animals or fruits in alphabetical order or simply count or recite the alphabet while bunting the ball. The ability to provide a correct verbal response while visually attending to and physically making contact with the ball is the direct result of cortical reorganization of the vision system.

4. Psychophysiological reactivity tests

Data was collected while the patients watched emotionally arousing video excerpts taken from the classic Disney film *The Lion King* (Figure 3). Each episode (high and low emotional context block) was 2 minutes long, while a total of 4 episodes were used, thus making the period of watching video up to 10 minutes. The order of movie episodes was counterbalanced across all subjects. HR and SCL was monitored and collected during the session. Physiological activity was recorded using C-2 J&J Engineering Inc (Poulsbo, WA) real-time physiological monitor with USE-2 Physiodata software while watching *The Lion King* episodes with prism lenses. The test was conducted twice, at the baseline (before treatment) and after completion of the prism lenses treatment course.

FIGURE 3 – Low Emotional (Left) and High Emotional (Right) scenes selected from the classic Disney film, *The Lion King*.

5. Behavioral Checklists

Participants were also evaluated using the following questionnaires: Aberrant Behavior Checklist (ABC) (Aman & Singh, 1994) and Repetitive Behavior Scale (RBS) (Bodfish et al., 1999).

6. Posner Spatial Attention Task

EEG data, to be used for analysis of gamma coherence, was collected before and after the six months of prism lens therapy while subjects completed a cued Posner spatial attention task. In this task, subjects pressed a button to indicate on which side of the screen the stimulus "X" appeared. Before each stimulus, subjects were presented with a pre-cue red square. In the congruent condition the "X" appears in the red square, while in the incongruent condition the "X" appears in the opposite square. This task consisted of two blocks each 10 minutes long. In one block the stimuli are oriented horizontally, as in the left side of Figure 4; in the other block the stimuli appear in the corners (diagonal), as shown in the right side of Figure 4.

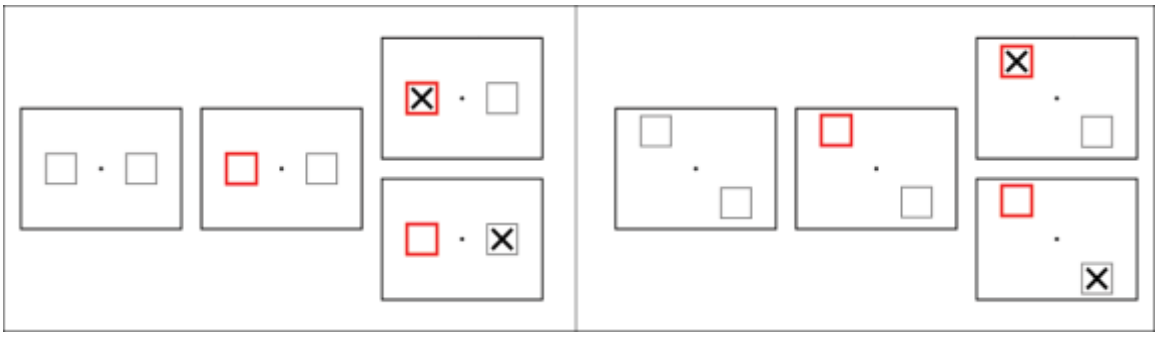

FIGURE 4 – Cued Posner Spatial Attention Task

C. Data Analysis

1. Heart Rate Variability

Analysis of HRV began with a recording (usually 5-10 minutes) of an individual's electrocardiogram (EKG). The USE-2 Physiodata software program then derived HR from the EKG signal by detection of adjacent QRS complexes. After export of the data,

applying a simple conversion to HR yielded the time between heartbeats, known as the RR interval. The RR interval was then opened in Kubios HRV software (Helsinki, Finland) for artifact removal and calculation of HRV measures. Figure 5 (top) shows the RR interval graphed over the entire recording session. The two noticeable peaks are artifacts, possibly due to subject movement, that can distort the results of HRV measure calculation. Figure 5 (bottom) shows the same RR data with the artifacts removed. Only the highlighted yellow region was used in calculation of HRV measures for this particular session.

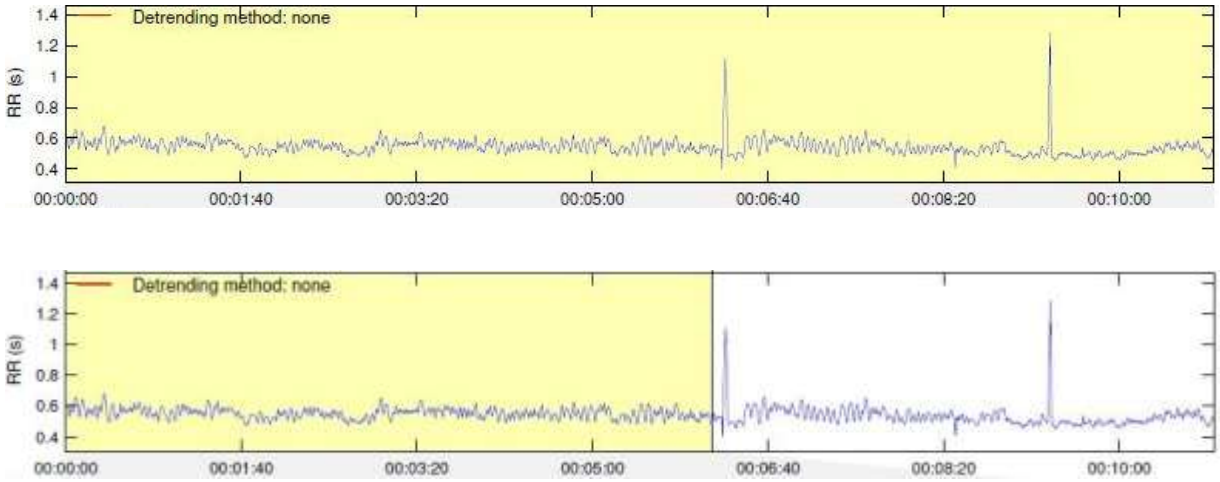

FIGURE 5 – Representation of artifact removal in Kubios HRV

Kubios HRV uses the selected region of RR data to calculate measures such as mean RR, SDNN, mean HR, STDHR, RMSSD, NN50, and pNN50. Calculations of a few of these time-domain measures are shown below in Equations 1-3. Table 1 shows example time-domain results for one subject.

$$SDNN = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N}(RR_j - \overline{RR})^2} \quad (1)$$

$$RMSSD = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N-1}(RR_{j+1} - RR_j)^2} \quad (2)$$

$$pNN50 = \frac{NN50}{N-1} \times 100\% \quad (3)$$

Equations 1-3: Calculations for time-domain HRV measures

TABLE I

EXAMPLE TIME-DOMAIN HRV RESULTS

Measure	Units	Value
Mean RR	ms	691.2
SDNN	ms	112.6
Mean HR	bpm	88.64
STDHR	bpm	11.76
RMSSD	ms^2	129.9
NN50	count	124
pNN50	%	29.9

To obtain frequency-domain results, a power spectrum density (PSD) was calculated using a Fast Fourier Transform (FFT). The frequency-domain measures were then extracted from the PSD. The absolute, relative, and peak powers were reported for each frequency band. Integration of the spectrum over the respective regions allowed for calculation of the absolute power of each frequency band (Tarvainen & Niskanen, 2012). Another option for derivation of the frequency-domain measures is to use autoregressive (AR) modeling. These methods, FFT and AR, are the two most commonly used for spectral analysis. FFT spectral analysis includes all data in the series, whereas the AR

method uses a model-fitting approach to identify the more significant peaks from which the final spectrum is derived (Porges & Bohrer, 1990; Berntson et al., 1997). Data that does not fit the model is considered to be noise and is either partially or completely removed. In addition, the AR modeling technique requires an estimation of the model order, for minimization of prediction error, prior to spectral analysis. There are several different criteria for the estimation of model order, and many theories about which combination of criteria yields the most accurate model order. (Boardman et al., 2002). The AR technique is recommended when the number of data points is low, since the frequency resolution of the AR-derived spectrum is not as dependent as the FFT method on the length of the recording (Parati et al., 1995). The number of data points in this experiment was large, therefore, the FFT method was selected as optimal. Figure 6 shows an example of a PSD derived using the FFT method, and Table 2 is an example of the extracted frequency-domain measures for the VLF, LF, and HF frequency bands.

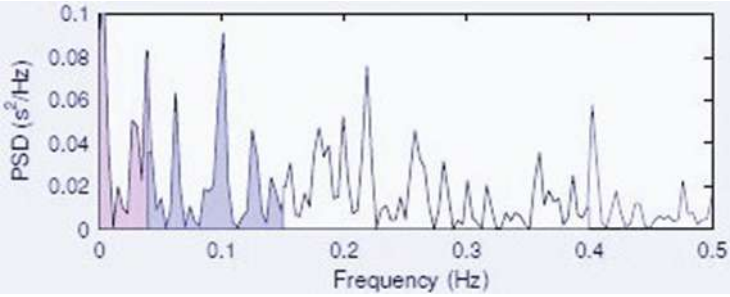

FIGURE 6 - Example PSD derived using the Fast Fourier Transform method

TABLE II

EXAMPLE FREQUENCY-DOMAIN HRV RESULTS

Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u)
VLF (0-0.04 Hz)	0.0039	1595	20.5	
LF (0.04-0.15 Hz)	0.1016	2249	28.9	36.3
HF (0.15-0.4 Hz)	0.2166	3939	50.6	63.7
Total		7783		
LF/HF		0.571		

2. Skin Conductance

Skin conductance data was exported into Microsoft Excel. All statistical analysis (t-test and regression) was done using SPSS Statistics software.

3. Gamma Coherence

Raw EEG data was recorded as a time signal using EEG electrodes and opened in Brain Electrical Source Analysis (BESA) for analysis. Figure 7 below depicts an example of raw EEG data in BESA software.

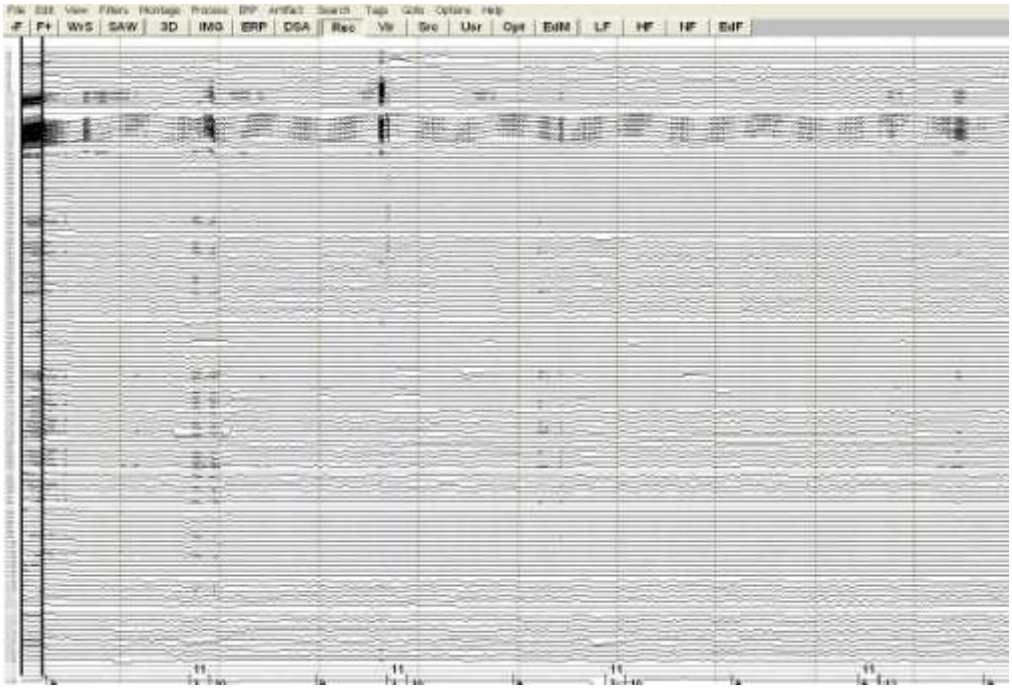

FIGURE 7 – Raw EEG opened in BESA software

An issue regarding calculation of coherence is that there two forms of coherence: scalp and source. The EEG above is referred to as a scalp waveform. Scalp waveforms can become distorted by overlap between electrode signals on the scalp. Therefore, these waveforms cannot be used to distinguish between coherence due to propagation (scalp coherence) and true coherence (source coherence). Montages are user-defined programs in BESA that allow for computation of coherence between selected regions of the brain, and based on the montage design, it can also be used to eliminate or reduce the effects of scalp coherence. The first attempted montage involved 18 different brain regions, shown below in Figure 8.

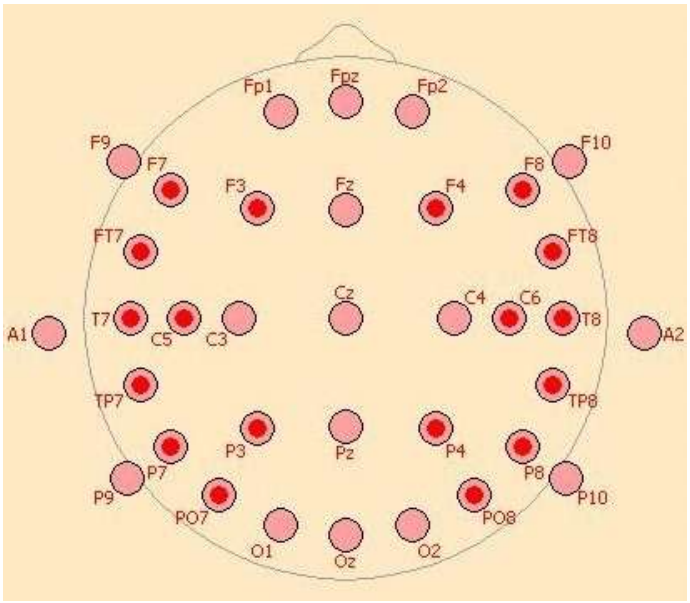

FIGURE 8 – Coherence montage involving 18 channels

The disadvantage of this montage was that it did not take into consideration the effect of scalp coherence, which is especially pronounced between regions located near each other, for example, F3 and F4. Many studies in EEG coherence have used the common reference (Cz) located at the vertex (Mathewson et al., 2012) in calculation of coherence coefficients. Specifically, source coherence is calculated by averaging the cross-spectral density of one reference channel with all other channels over trials (BESA Research Tutorial, 2010). Therefore, the next montage was designed to reference each electrode to Cz in order to reduce effects of signal propagation between electrodes. The revised montage is shown below in Figure 9.

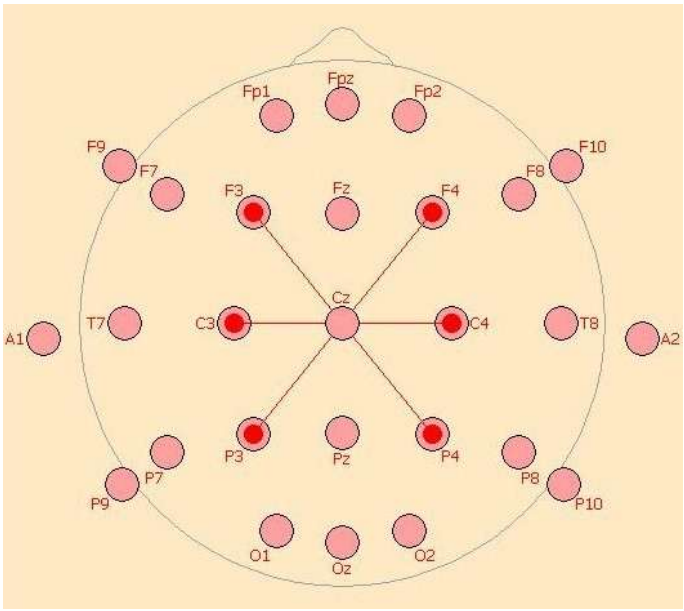

FIGURE 9 – Revised montage with centrally located reference (Cz)

The number of channels to be analyzed was also reduced in the revised montage. Selection of these channels was based on the following factors. Frontal lobe channels F3 and F4 were included because the frontal lobe is known to regulate problem solving and emotion. Channels C3 and C4 were included since they are located over the sensory motor cortex. Lastly, parietal lobe channels P3 and P4 are involved in visual processing. The new montage was applied to raw EEG data. The resulting source waveform is shown in Figure 10.

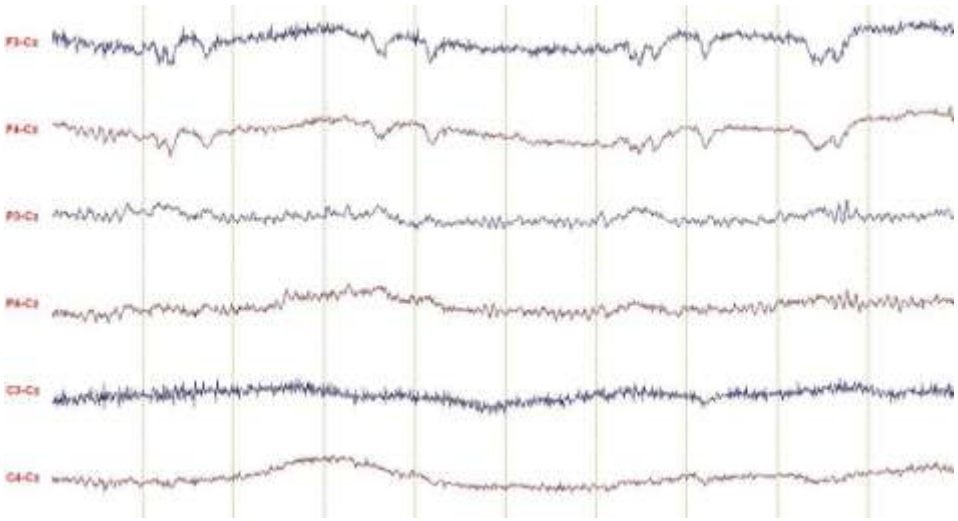

FIGURE 10 – Source waveform after application of the montage

The next step was to remove artifacts caused by eye blinks or movement from the waveform. BESA generated a 2-dimensional diagram (Figure 11) organized with channels on the y-axis and trials on the x-axis, sorted by amplitude of the signal. Certain artifacts were removed if their amplitude exceeded that defined as the threshold.

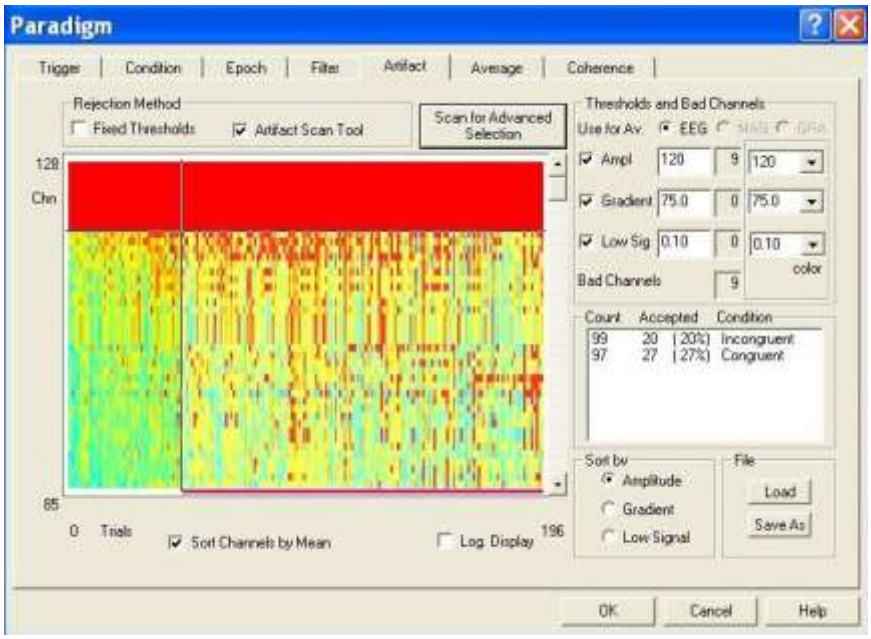

FIGURE 11 – Waveform artifact removal in BESA

Once artifacts have been removed, the source waveform was then used in calculation of true coherence, which is defined as the oscillatory activity between two coupled brain regions. Two regions are considered to have coupled oscillatory activity if the oscillations are correlated in both amplitude and phase. As Figure 12 shows below, coupled oscillations can have a phase difference but the two regions must have a constant phase relation.

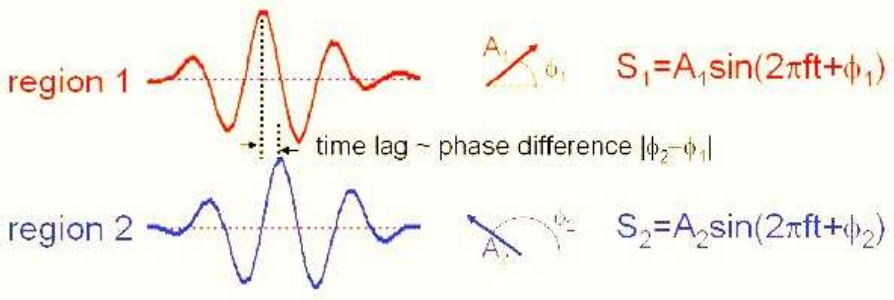

FIGURE 12 – Phase relationship between two coupled oscillations

Oscillations between two regions are measured over a number of trials, and several criteria must be met for coherence to be assumed. These criteria are (BESA Tutorial, 2010):

1. Same frequency in coupled regions within each trial
2. Possible variation of frequency and duration across trials
3. Similar phase difference across trials
4. Similar amplitude fluctuations across trials

Figure 13 shows an example of an analysis between two regions that is accepted as coherence, and an analysis that does not qualify as coherence.

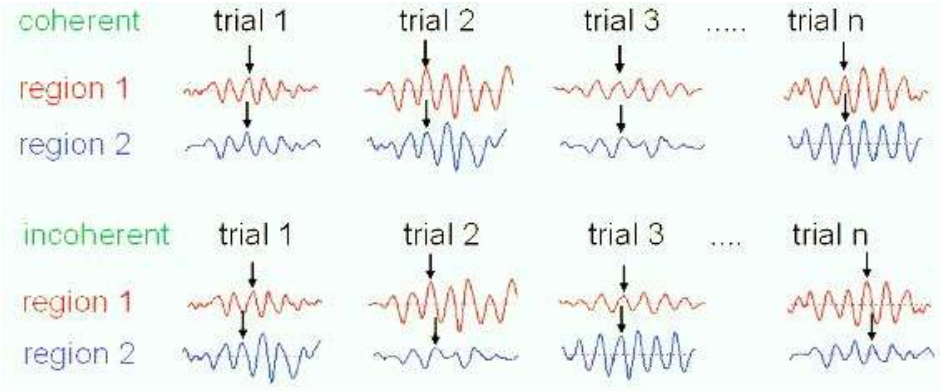

FIGURE 13 – Coherent vs. incoherent oscillations

Each oscillation is represented as a time-frequency signal S(f, t) which is the amplitude of a signal at a given frequency and time post-stimulus. Two time-frequency signals are used to compute the coherence between the respective brain regions (Equation 4).

$$\mathrm{Coh}(f,t) = \frac{\left|\sum_n S_{1,n} \cdot S^*_{2,n}\right|^2}{\sum_n \left|S_{1,n}\right|^2 \cdot \sum_n \left|S_{2,n}\right|^2} \quad (4)$$

Equation 4: Formula for the calculation of coherence

BESA uses the calculated coherence values to produce coherence maps, specifically for coherence within the gamma frequency band. These maps are color-coded (blue = low coherence, red = high coherence) to display coherence of all designated regions of the brain relative to one region. The region of interest can be interchanged by selecting a different region. This creates a new set of coherence maps relative to the new region.

D. Goals and Expectations

Our hypothesis was that six months of daily visual-motor exercises with prism lenses would lead to decreased overall HR, increased STDHR, increased HF and/or decreased LF, decreased SCL, improvement in behavioral checklist scores, and increased long-distance gamma coherence during the Posner task.

E. Results

Post-treatment, a significant decrease was observed in average HR among the participants from the baseline 94.6 ± 2.99 beats/min to post-sessions 89.6 ±3.65 beats/min, with paired-sample t-test showing significant change (t=-2.52, p=0.025). There was a decrease in LF power from 60.2± 2.81 power units to 48.6 ± 3.57 units (t= -2.38, p=0.033) and an increase in the percentage of HF power from 24.8± 2.16 % to 37.6 ± 4.61 % (t=2.42, p=0.03). VLF component of HRV (another measure of sympathetic activity) tended to decrease from 37.8 % down to 29.7%, but the change was not statistically significant (t=-1.69, p=0.11). Lastly, the LF/HF power ratio decreased from 1.70± 0.22 to 1.06 ± 0.13 (t=-2.52, p=0.025). Figure 14 below shows how the proportions of HF and LF power changed before and after treatment with prism lenses.

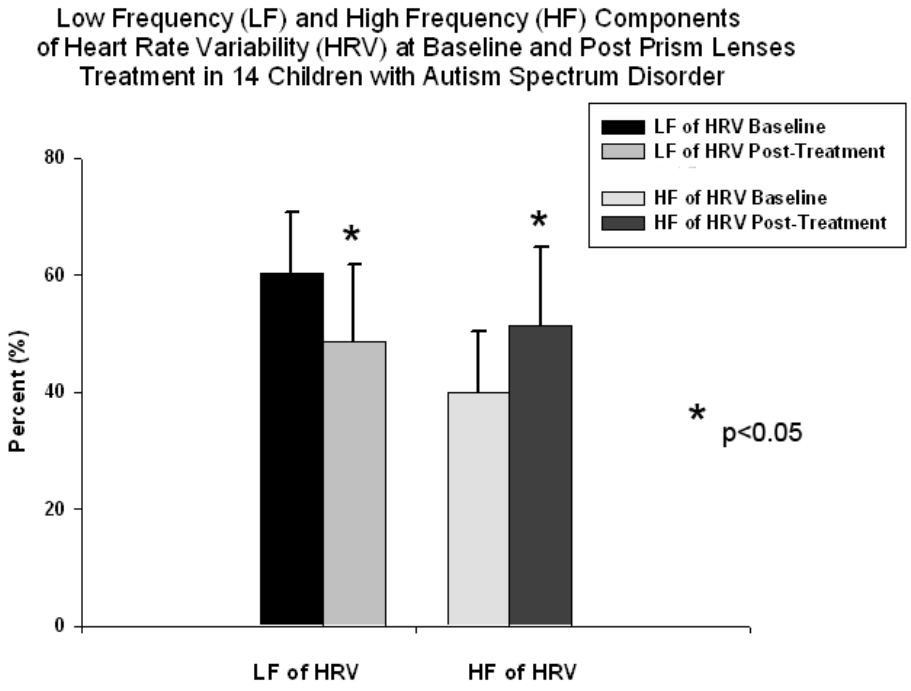

FIGURE 14 – LF and HF components of HRV pre- and post-treatment

A significant decrease in SCL was observed post-treatment with prism lenses (Figure 15). The pre-treatment average among subjects was 18.3 µS and post-treatment average was 7.9 µS ($t = -2.824$, $p = 0.037$).

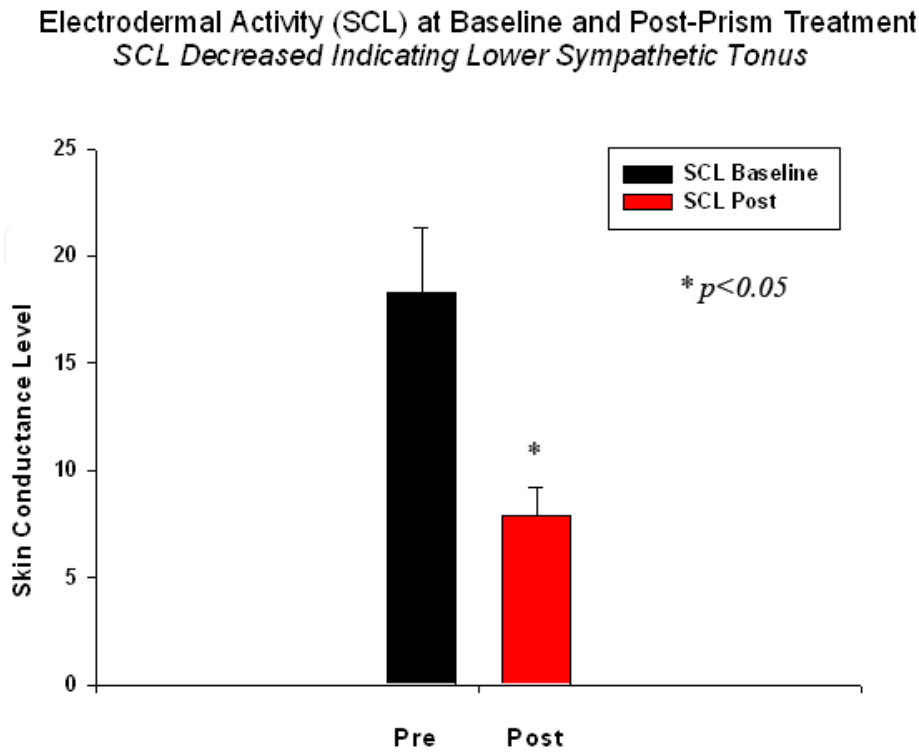

FIGURE 15 – SCL pre- and post-treatment

In addition, empirical behavioral data outcomes were reported using the Aberrant Behavior Checklist (ABC) (Figure 16) and Repetitive Behavior Scale (RBS) (Figure 17). In the ABC, three out of five scaled scores were reduced. In particular, scores tended to be decreased in Lethargy ($t = 2.09$, $p = 0.058$) and Inappropriate Speech ($t=2.06$, $p= 0.06$) while significantly decreased in Hyperactivity ($t= 2.39$, $p = 0.034$) compared to baseline scores. According to the Repetitive Behavior Scale (RBS), three out of six subscale scores were significantly different. These subscale scores included: Stereotypic Behavior ($t = 3.47$, $p = 0.01$), Compulsive Behavior ($t= 3.49$, $p = 0.01$) and overall Total Score ($t = 2.70$, $p = 0.031$) compared to baseline scores.

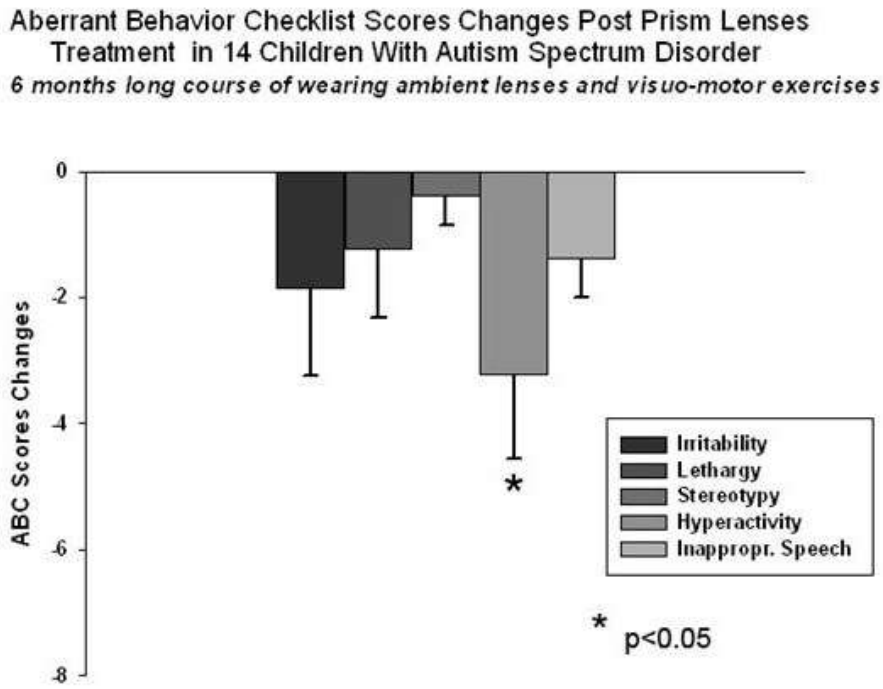

FIGURE 16 – Outcomes of Aberrant Behavior Checklist questionnaires showing decreased Hyperactivity score post prism lenses treatment

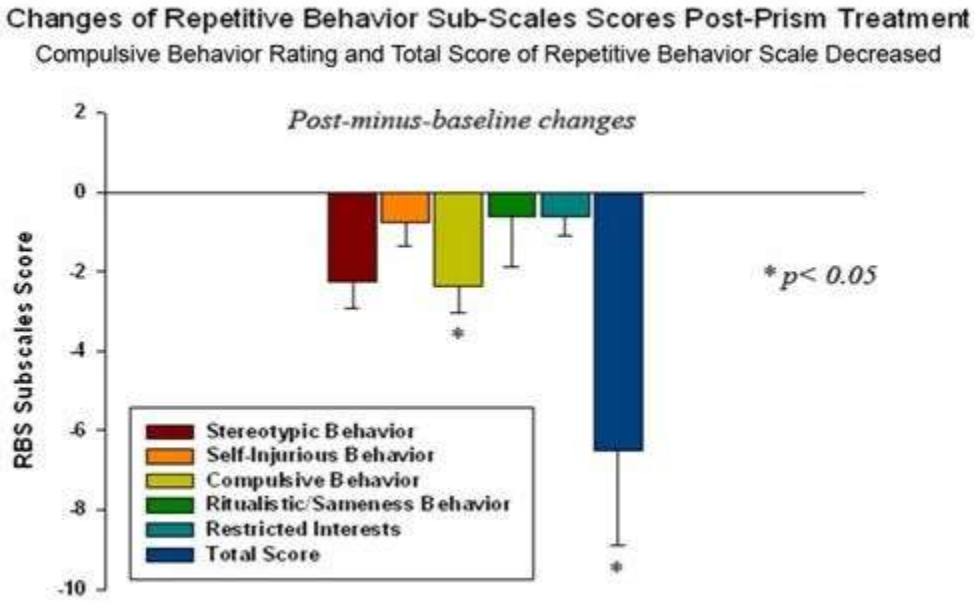

FIGURE 17 – Outcomes of Repetitive Behavioral Scale questionnaires (RBS) showing decreased Compulsive Behaviors Score and Total Score post prism lenses treatment

Lastly, results of the Posner cued spatial attention task show no significant differences in gamma coherence or latency (time post-stimulus at which coherence was observed) between pre- and post-prism lens treatment. However, analysis of coherence between individuals with and without ASD (controls) shows significant differences in both coherence and latency. Table 3 compares mean coherence coefficient values between controls and autistic subjects at four different brain region pairings. For example, the first row in Table 3 describes induced gamma coherence between two parietal regions (P3 and P4) in the congruent condition, while the second row describes induced coherence between a frontal and a parietal region (F3 and P3) in the incongruent condition of the Posner task.

TABLE III

POSNER TASK COHERENCE COEFFICIENT VALUES: CONTROL & ASD

Pair	**Mean Coh: Control**	**Mean Coh: ASD**	**F**	**p-value**
P3-P4 Induced, Congruent	.496	.628	4.288	.045
F3-P3 Induced, Incongruent	.552	.692	4.586	.039
P3-P4 Evoked, Incongruent	.586	.798	13.725	.001
P3-P4 Induced, Incongruent	.682	.818	6.970	.012

Controls and individuals with ASD also displayed differences in coherence in response to congruent and incongruent conditions. The results in Table 4 and Figure 18 below indicate that both controls and subjects with ASD had a similar coherence response to the congruent condition, but that those with ASD had an excessive fronto-parietal coherence response (0.692) to the incongruent condition compared to controls (0.553).

TABLE IV

INDUCED COHERENCE COEFFICIENT VALUES (F3-P3) OF CONTROL & ASD GROUPS FOR CONGRUENT AND INCONGRUENT CONDITIONS

Group	Mean Coh: Congruent	Mean Coh: Incongruent	p-value
Control	.403	.553	.029
ASD	.396	.692	

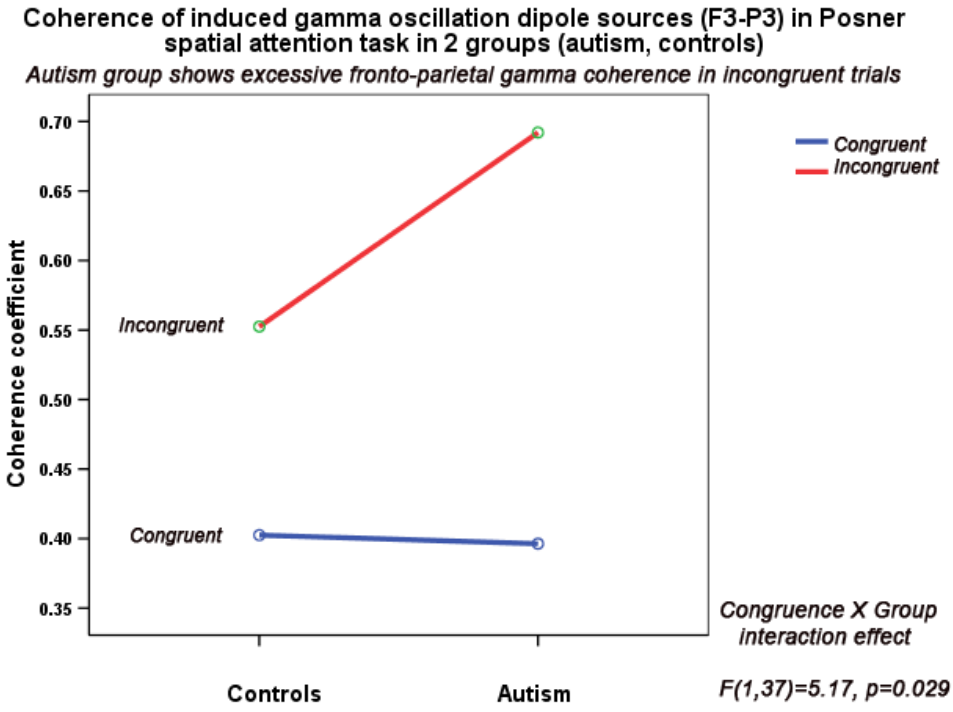

FIGURE 18 – Induced gamma coherence responses to congruent and incongruent conditions for individuals with and without ASD

In addition to differences in coherence, controls and autistic individuals also displayed differences in latency, the time post-stimulus in milliseconds at which gamma coherence occurs. Table 5 below shows the differences in latency for three different brain region pairs. These values suggest that control subjects have a shorter latency period to

stimuli in the congruent condition, while those with ASD have a shorter latency period for stimuli in the incongruent condition.

TABLE V

POSNER TASK LATENCY VALUES: CONTROL & ASD

Pair	**Mean Latency: Control (ms)**	**Mean Latency: ASD (ms)**	**F**	**p-value**
F4-P4 Evoked, Congruent	138.4	161.9	4.690	.037
F3-F4 Evoked, Incongruent	164.8	134.9	6.675	.014
F4-P4 Evoked, Incongruent	168.9	145.0	5.914	.020

F. Discussion

The analysis steps successfully transformed the raw data, either EKG or EEG, into a usable form for interpretation of results. Derivation of the RR interval from the EKG and subsequent artifact removal allowed for calculation of HRV measures in Kubios HRV software. It was determined that for the large number of data points collected for each session of autonomic monitoring, FFT was the preferred method of spectral analysis, as opposed to AR modeling. AR modeling is beneficial for frequency-domain calculations if only a limited number of data points are available. In order to analyze EEG data, the proper montage was first created in BESA to compute coherence and reduce effects of propagation between electrodes on the scalp (scalp coherence). Application of this montage and artifact removal created a waveform suitable for calculation of source coherence.

The results of the prism lens study are encouraging in that they support the hypotheses that ambient prism lenses in combination with therapy vision exercises had the desired effect on HRV variables and SCL, reflecting improved autonomic balance in children with ASD. The first result supporting the improved autonomic balance hypothesis was the decrease in average heart rate. Second, the decrease in LF power and the increase in HF power were changes in the desired direction toward improved autonomic balance. This is also seen in the decreased LF/HF power ratio. In addition, the decrease in SCL suggests a withdrawal of sympathetic tone, since SCL is controlled solely by sympathetic inputs. These results are in favor with increased parasympathetic and decreased sympathetic tone, a significant improvement from ANS activity in autism. Behavioral measures also reflect improvements in hyperactivity and repetitive behaviors.

No significant differences were observed in gamma coherence or latency times in autistic subjects before and after prism lens therapy. Results did show, however, some significant differences in both coherence and latency between controls and autistic subjects. Specifically, those with ASD appeared to show higher coherence in response to the incongruent condition compared to control subjects. Autistic subjects also displayed shorter latency periods in response to the incongruent condition. These findings suggest that those with ASD may have decreased sensitivity in their responses to congruent and incongruent conditions. While prism lenses were effective in producing desired effects on HR, HRV, and SCL, the results of the Posner task indicate a different therapy may be more effective in improving gamma coherence in ASD.

III. CASE II: TMS THERAPY

A. Background Information

TMS is a non-invasive neuromodulation technique with the potential to increase fronto-limbic inhibition known to be deficient in ASD. The limbic system is a complex network of structures central to anxiety and mood regulation (Mayberg, 2003; Sokhadze et al., 2009). Few studies have investigated the effects of rTMS on the autonomic nervous system, despite the fact that many frontal cortical areas are involved in autonomic nervous system control (Czeh et al., 2002; Filippi et al., 2000). The proposed mechanism of post-TMS effects on autonomic arousal may include improved tonic fronto-limbic inhibitory influences known to be deficient in autism (Loveland et al., 2008). The aim of this research project was to observe the effects of 18 sessions of rTMS in improving autonomic functions in children with ASD.

The organization of neurons in the cortex is based on cellular minicolumns. Previous research suggests that there is a greater number of minicolumns in the cortex of an individual with ASD, accompanied by increased neuronal density and reduced neuropil space (periphery of the minicolumn) (Casanova et al., 2002; Casanova et al., 2006). This lack of neuropil space is thought to reduce inhibitory effects, leading to dysfunction of sensory processing and overexcitement of the neurons. TMS applied to the DLPFC can improve excitability/inhibition of neural circuits, specifically by increasing cortical inhibitory neurons (Sokhadze et al., 2010). There are two forms of TMS: slow and fast. “Slow” TMS falls in the range of 0.3 – 1 Hz, while “fast” TMS is above 1 Hz. Slow TMS was selected as its effect is to activate inhibitory cells surrounding the

minicolumns (Sokhadze et al., 2010; Hedeker & Gibbons, 2006; Pascual-Leone et al., 2000). TMS is generally regarded as a safe procedure. Reported side effects have included mild, tension-type headache following stimulation and discomfort due to the sound of the pulses. However, there is a certain risk for inducing seizure (Wasserman et al., 1996). Therefore, patients with epilepsy or a family history of epilepsy are generally excluded from receiving TMS.

TMS has already shown to be an effective therapy tool. In a paper by Udupa et al. (2007) researchers compared rTMS with antidepressant therapy to address the autonomic imbalance associated with depression. The authors found that rTMS not only produced antidepressant effects, but also corrected the autonomic imbalance. The researchers used HRV measures as evidence that rTMS did in fact reduce the sympathetic/parasympathetic ratio thus improving the sympathovagal balance.

TMS is also becoming increasingly prevalent as a potential therapy for ASD (Oberman et al., 2013). In previous studies by the Cognitive Neuroscience Lab at the University of Louisville, slow rTMS was shown to improve both evoked gamma activity and error processing in individuals with ASD. Baruth et al. (2010) compared evoked gamma activity in the early stages of visual processing was between individuals with ASD and those without ASD (controls) using Kanizsa illusory figures. In autistic individuals, evoked gamma activity was not discriminative of stimulus type, whereas control subjects displayed early gamma-power differences between target and non-target stimuli. Individuals with ASD underwent 12 sessions of rTMS and repeated the Kanizsa test. Results show improvement in discriminatory gamma activity between target and non-target stimuli, as well as improvement in responses on behavioral questionnaires. In

a study by Sokhadze et al., TMS was used to improve error processing in children with ASD, as measured by event-related potentials (ERP) associated with response to errors, such as error-related negativity (ERN). Post-TMS results show significant differences in the response-locked ERP such as ERN, as well as behavioral response monitoring measures indicative of improved error monitoring and correction function (Sokhadze et al., 2012).

B. Protocols and Data Collection

1. Subject Demographics

Eighteen children (14 boys and 4 girls) were enrolled in this study (mean age 13.1 years, SD=2.2). Participants were recruited through the University of Louisville Weisskopf Child Evaluation Center (WCEC). Diagnosis was made according to the DSM-IV-TR and further ascertained with the Autism Diagnostic Interview – Revised (ADI-R) (LeCouteur et al., 2003) by Dr. Lonnie Sears, who also did pre- and post-TMS clinical evaluations. All participants were high-functioning children with ASD and with full-scale IQs >80 assessed using the Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV; Wechsler 2004). Two subjects were excluded for bradycardia. For another two subjects the data could not be retrieved due to high number of artifacts. Therefore, data was reported for 14 children.

2. TMS Specifications

The TMS instrument used was the Magstim Model 220 (Magstim Corp., Wales, UK) with a 70-mm wingspan figure-eight coil. Each session of TMS typically lasts

between 20 – 30 minutes. Patients were seated in a comfortable chair and fitted with a swim cap to help indicate the TMS coil position. First, the motor threshold (MT) was determined for each patient. MT refers to the intensity of the pulse delivered to the motor cortex necessary to elicit a noticeable motor response. The motor response was monitored by applying a sensor to the hand muscle opposite the site of stimulation. The output of the TMS coil was increased by 5% until a 50 μV deflection of electromyogram (EMG) was seen on the monitor, or until a visible muscle twitch was observed. Once the MT was established, the coil was moved to the site of stimulation (DLPFC) and the pulse intensity was adjusted based on the MT. Eighteen sessions of TMS were administered once per week to patients enrolled in the study. EKG and SCL were recorded during each session through an EKG-based C-2 J&J monitor. Figure 19 illustrates placement of the TMS wand over the DLPFC, and Figure 20 details the process of TMS application and data collection during one session. Figure 21 shows where the EKG, SCL, and EMG sensors were placed on the subject.

FIGURE 19 – Administration of rTMS over the DLPFC with physiological monitoring

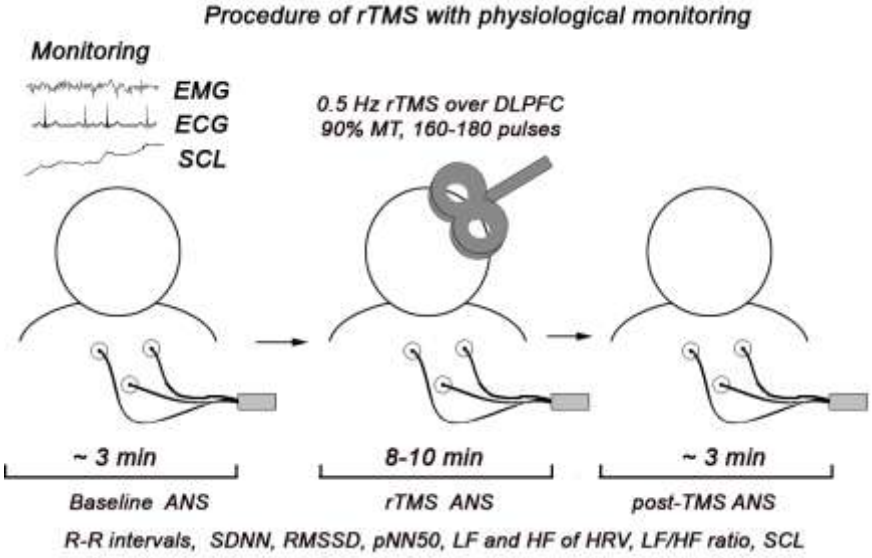

FIGURE 20 – Schematic of TMS application and autonomic data collection

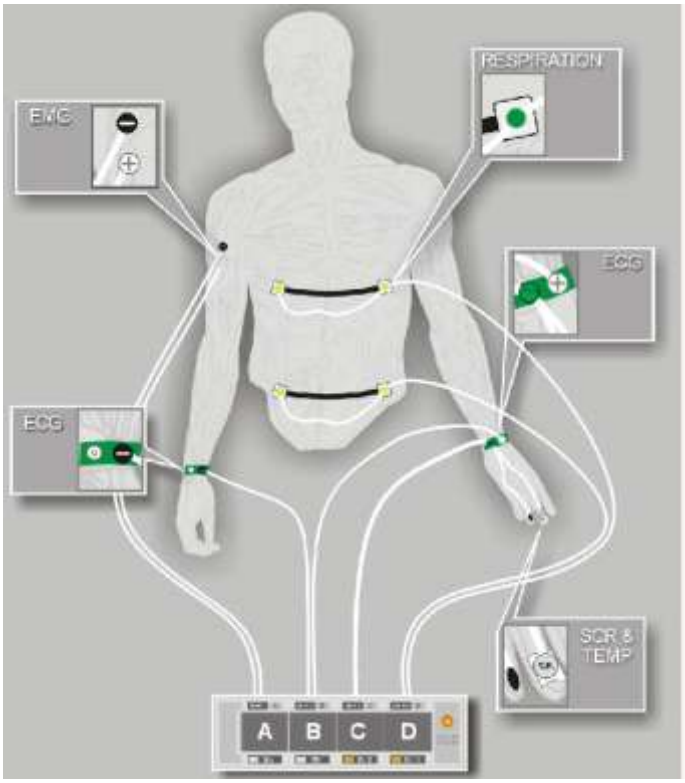

FIGURE 21 – Placement of sensors for autonomic data collection

3. Kanizsa Visual Oddball Task

EEG data was collected twice – once before beginning the course of TMS, and again after completing 18 sessions. Each patient was fitted with an EEG net containing 128 electrodes (Figure 22). While wearing the net, the patient was seated at a computer and completed a Kanizsa visual oddball task in which he or she was asked to identify certain images. The task involved pressing a button when a rare (25% probability) Kanizsa square (target condition) appears as opposed to Kanizsa triangles (non-target condition) and non-Kanizsa figures. The raw data was exported into the Brain Electrical Source Assessment (BESA) software package where coherence analysis was carried out.

As a control, EEG recordings were also taken from a group of patients who did not participate in TMS (waitlist). Patients on the waitlist were given the opportunity to receive TMS after the study was completed.

FIGURE 22 – Patient completing Kanizsa task while wearing 128-channel EEG net

4. Behavioral Checklists

Participants were also evaluated using the following questionnaires: Aberrant Behavior Checklist (ABC) and Repetitive Behavior Scale (RBS).

C. Data Analysis

The same data analysis methods for HRV, SCL, and gamma coherence were used for the rTMS study as in the prism lens study. However, the montage created in BESA was altered to replace C3 and C4 with two different channels. Channels C3 and C4 were appropriate for analysis of EEG data from the Poser spatial attention task because they are involved in motor function. The Kanizsa visual oddball task is based on recognition

of visual stimuli. Therefore, channels T7 and T8 were included in the montage for analysis of data from the Kanizsa task since functions of the temporal lobe include processing visual stimuli and memory (Eichenbaum et al., 2007).

D. Goals and Expectations

The objectives of the TMS study were to study the effects of 18 sessions on autonomic measures and gamma coherence in ASD. Expected autonomic outcomes were decreased overall HR, increased RR interval, increased STDHR, increased SDNN, increased RMSSD, increased pNN50, increased HF and/or decreased LF, decreased LF/HF ratio, and decreased SCL. Behavioral checklist scores were expected to improve. Lastly, TMS therapy was expected to improve gamma coherence between long-distance regions of the brain.

E. Results

Results of HRV analysis show several measures with significant differences between pre- and post-TMS therapy. Table 6 below shows the t-test results for RR interval, SDNN, HF component of HRV, LF/HF ratio, and SCL.

TABLE VI

T-TEST: PRE/POST TMS THERAPY HRV MEASURES

Pairs	Units	Paired Differences				t	df	p-value
		Mean	Std. Dev.	95% CI				
				Lower	Upper			
RR pre-post	ms	-39.08	53.61	-70.04	-8.13	-2.73	13	.017
SDNN pre-post	ms	-39.09	66.78	-77.65	-.54	-2.19	13	.047
HF pre-post	ms^2	-1249.3	1556.1	-2147.8	-350.8	-3.00	13	.010
LF/HF pre-post	N/A	.48	.81	.01	.95	2.23	13	.044
SCL pre-post	µS	4.37	5.65	1.11	7.64	2.89	13	.013

The table above shows that there was a significant increase ($p = .017$) in the RR interval from pre- to post-TMS treatment. This result can also be interpreted as a decrease in HR, since the RR interval is the time between successive heartbeats. The t-test also reveals significant increases in SDNN and HF power, as well as significant decreases in the LF/HF ratio and SCL.

Regression analysis was completed to observe trends during the entire 18 session TMS course. Table 7 shows the results of regression analysis.

TABLE VII

REGRESSION ANALYSIS: HRV MEASURES OVER 18 SESSIONS OF TMS

Measure	Units	t	p-value	R	R^2	Reg. Equation
RR	ms	3.524	.003	.661	.437	y = 2.696x + 684.57
SDNN	ms	3.38	.004	.645	.417	y = 2.098x + 52.28
HR	1/min	-2.88	.011	.584	.341	y = -.356x + 89.68
STDHR	1/min	3.10	.007	.613	.376	y = .105x + 6.244
RMSSD	ms	2.15	.047	.473	.224	y = 1.480x + 52.80
NN50	count	3.57	.003	.666	.443	y = 3.598x + 65.15
LF power	ms^2	-1.02	.323	.247	.061	y = -15.23x + 1775.4
HF power	ms^2	5.12	.000	.788	.621	y = 68.65x + 671.90
LF/HF ratio	N/A	-3.83	.001	.691	.478	y = -.028x + 1.619
SCL	µS	-3.719	.002	.681	.464	y = -.171x + 8.65

Regression analysis shows that the trend in each measure was significant for all analyzed, expect for LF power. Although there was a negative trend for LF power observed over the 18 sessions of TMS, it did not reach significance. NN50 (count of RR intervals differing by >50 ms from the preceding interval) does show a significant positive trend, however, because the length of each physiological recording session was not uniform, regression analysis results of NN50 are not a reliable reflection of this measure. pNN50, the percent of RR intervals differing by >50 ms from the preceding interval, did not show a significant positive trend. Figures 23 – 28 below show the regression analysis for individual measures.

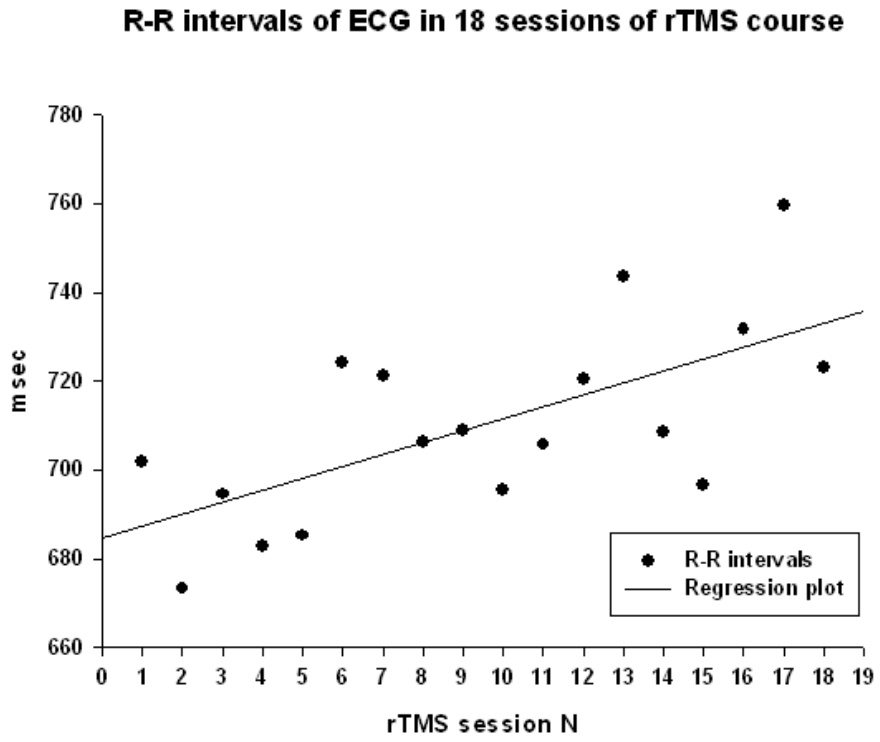

FIGURE 23 – Regression trend of RR interval in 18 sessions of rTMS

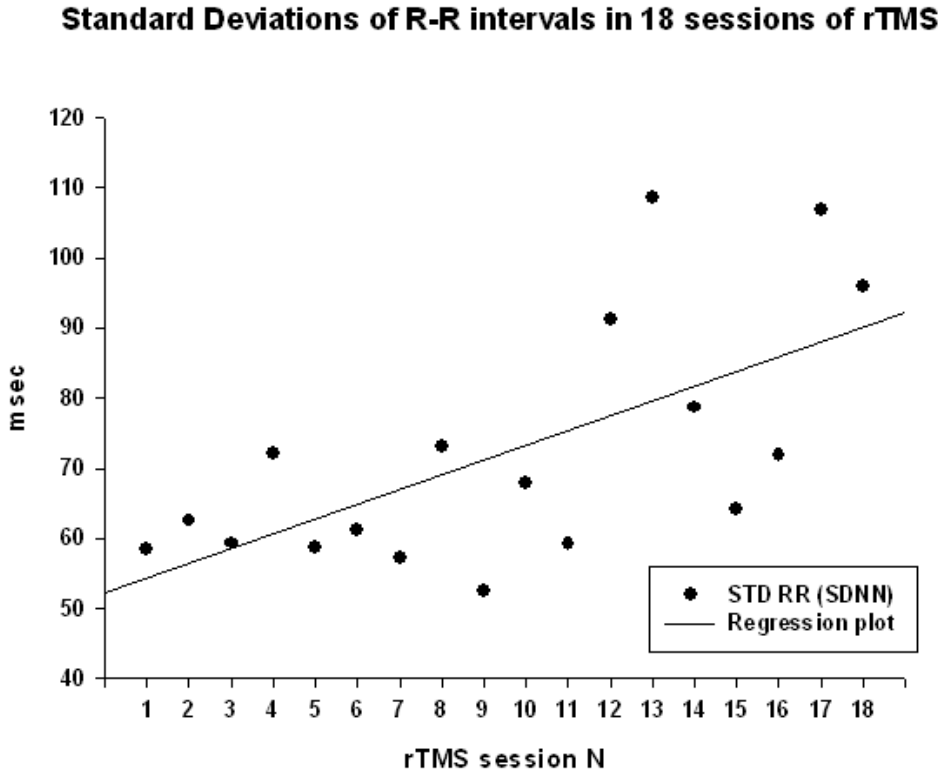

FIGURE 24 – Regression trend of SDNN in 18 sessions of rTMS

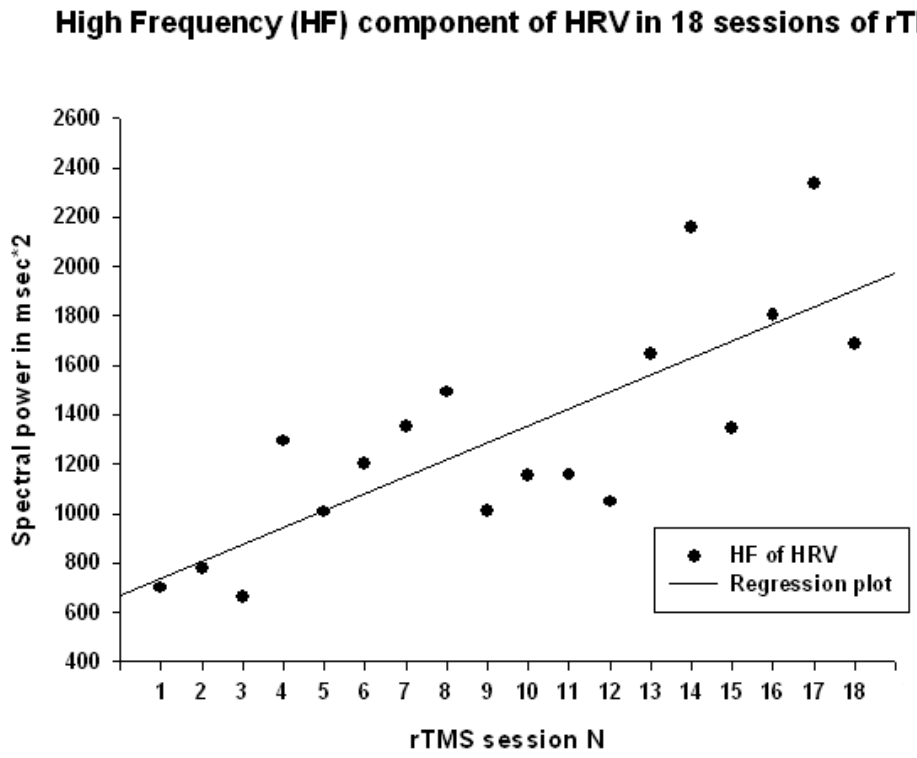

FIGURE 25 – Regression trend of HF component of HRV in 18 sessions of rTMS

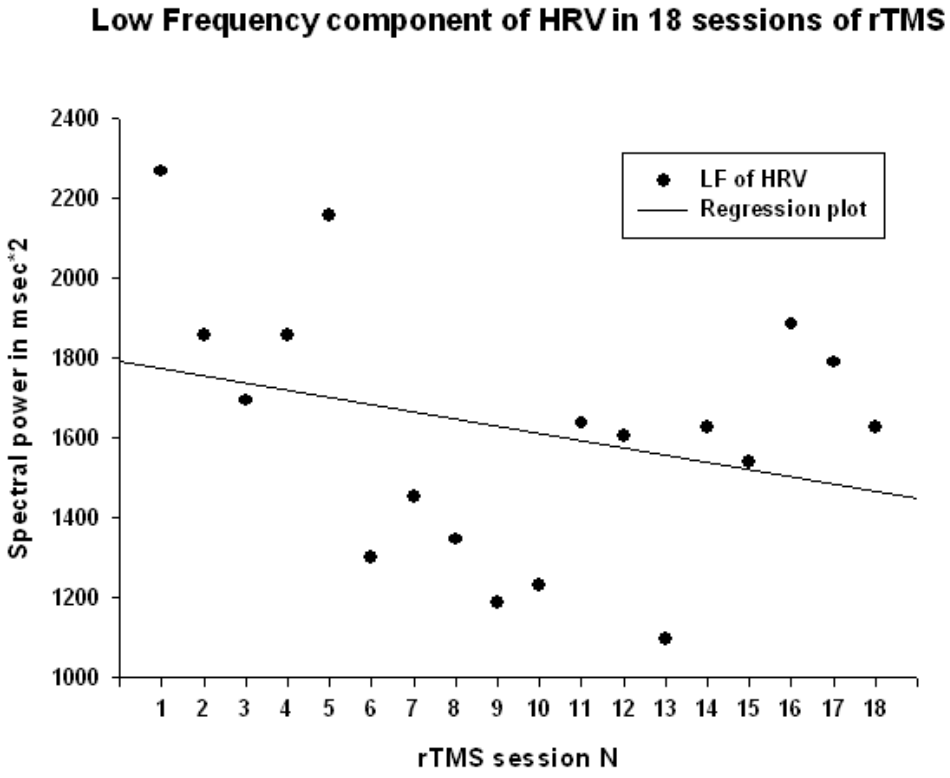

FIGURE 26 – Regression trend of LF component of HRV in 18 sessions of rTMS

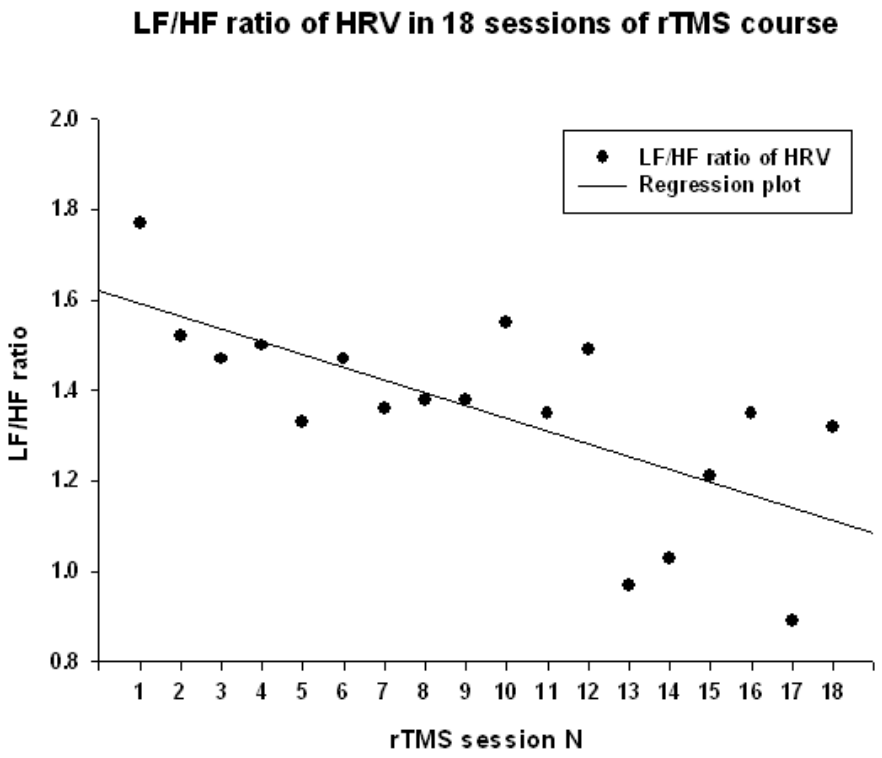

FIGURE 27 – Regression trend of the LF/HF ratio in 18 sessions of HRV

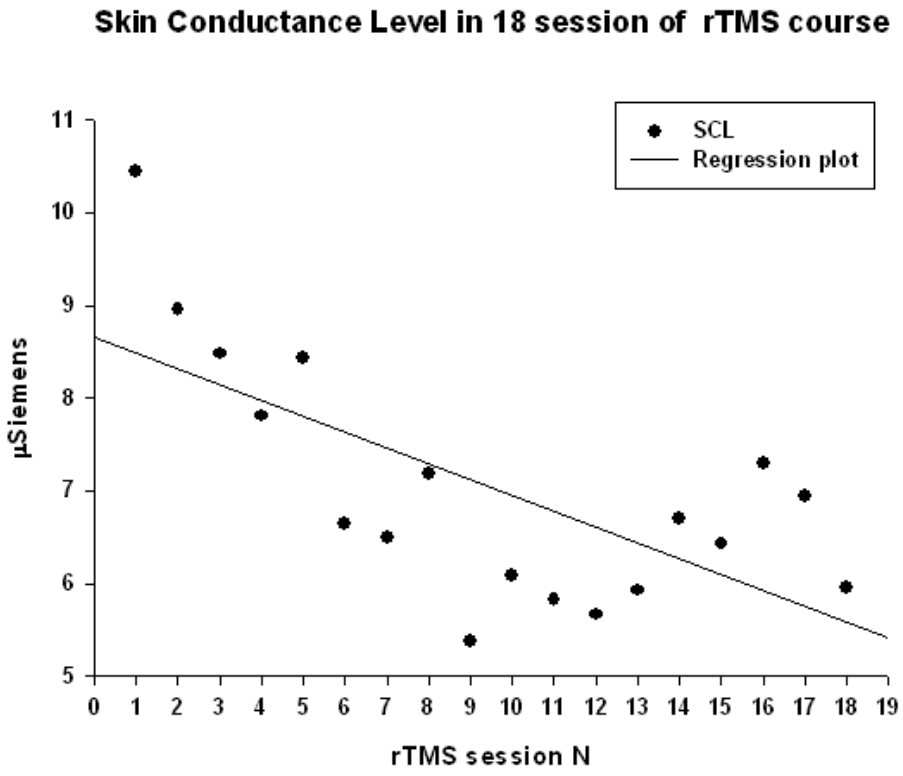

FIGURE 28 – Regression trend of SCL in 18 sessions of rTMS

The ABC and RBS behavioral checklists show significant improvements in several areas. Specifically, ABC scores show improvement in irritability, lethargy, and hyperactivity post-rTMS, as seen in Figure 29. RBS scores show improvement in stereotypic behaviors and compulsive behaviors following rTMS treatment, as seen in Figure 30.

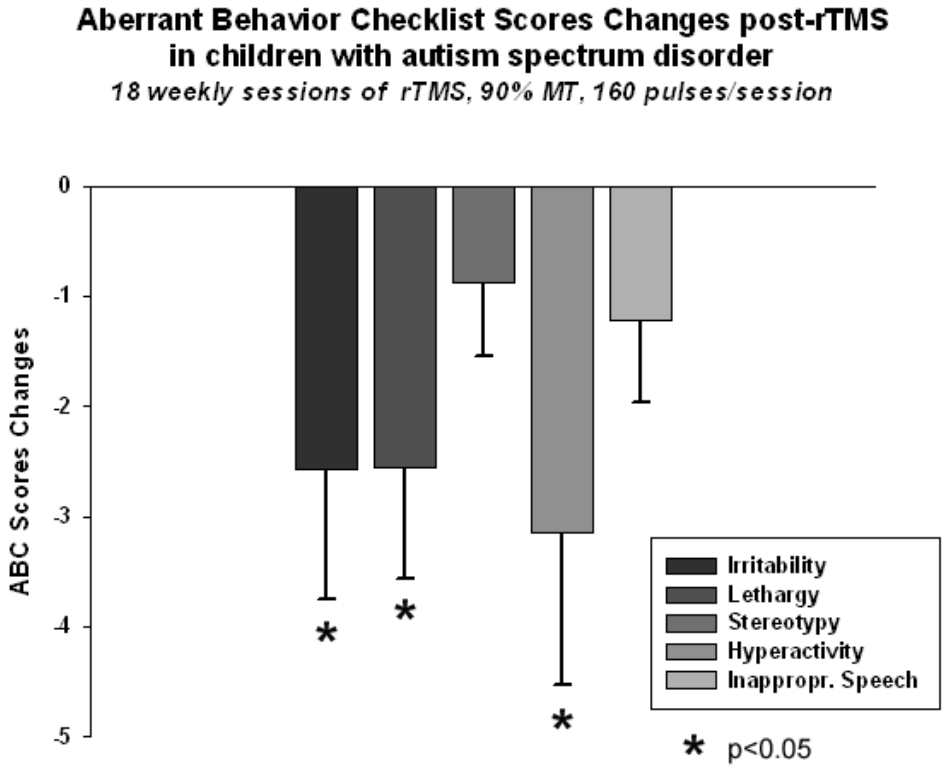

FIGURE 29 – ABC scores changes post-rTMS

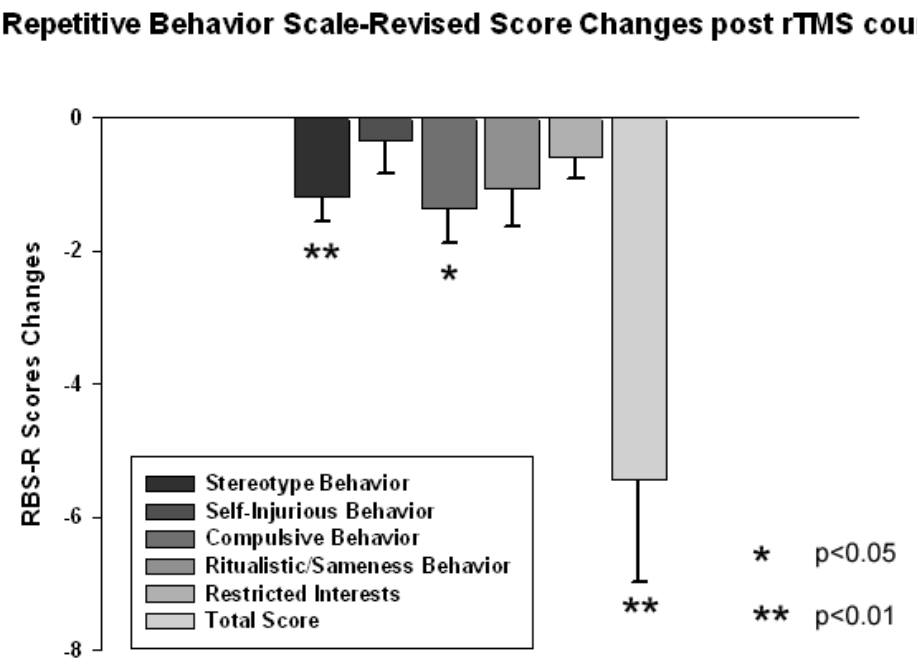

FIGURE 30 – RBS scores changes post-rTMS

Results of gamma analysis from the Kanizsa visual oddball task reveal several significant differences in coherence between pre- and post-TMS treatment. Table 8 shows the regions where increased gamma coherence was observed following 18 sessions of TMS.

TABLE VIII

KANIZSA TASK COHERENCE COEFFICIENT VALUES: PRE- AND POST-TMS TREATMENT

Pair	**Mean Coh: Pre-TMS**	**Mean Coh: Post-TMS**	**F**	**p-value**
F3-T7 Evoked, Target	.394	.566	6.939	.013
F3-T7 Induced, Target	.457	.596	4.605	.040
F4-P4 Evoked, Target	.156	.278	5.084	.032
F3-T7 Induced, Non-target	.419	.589	6.771	.014

Figure 31 below shows an example of a coherence map for one subject pre- and post-18 sessions of TMS. These maps show target coherence between the F3 and T7 regions. Blue represents little coherence (close to 0), while red represents more coherence (close to 1). The light orange color represents and intermediate amount of coherence. As indicated in Table 8 above, coherence to the target condition between F3 and T7 improved in both the evoked region (100 – 200 ms) and in the induced region (300 – 600 ms). Figure 31 confirms this as there is much more coherence visible in the post-TMS map compared to the pre-TMS map.

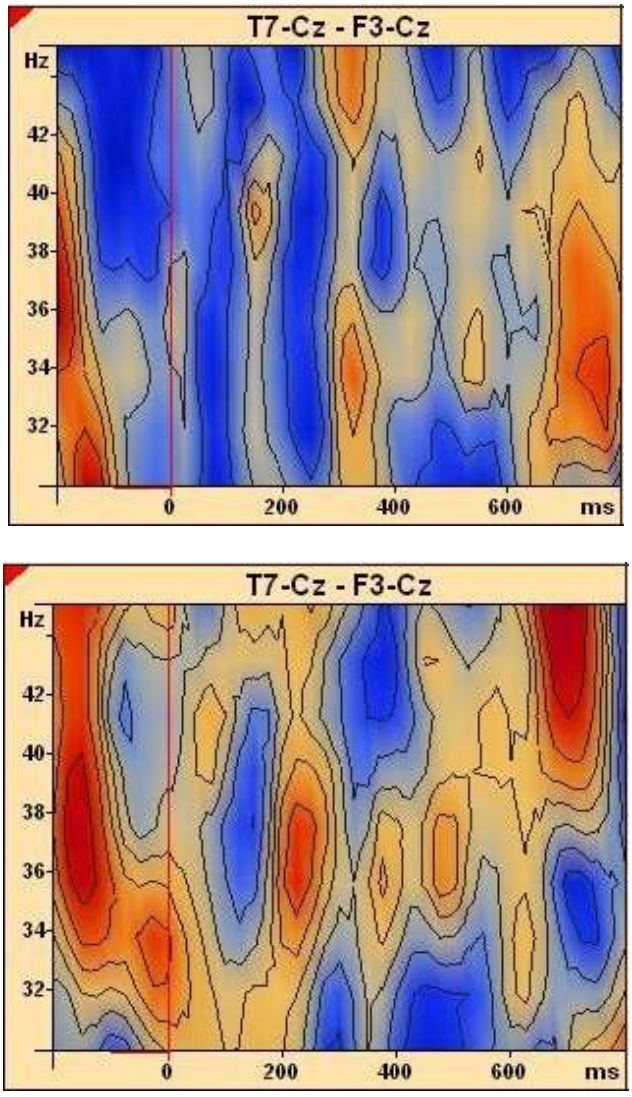

FIGURE 31 – Pre- (top) and post-TMS (bottom) maps for coherence between F3-T7 to the target condition

In addition to improvement in coherence between pre- and post-TMS, differences were also observed in the subjects' responses to target and non-target stimuli following TMS therapy. Analysis of evoked gamma coherence between F4 and T8 to both target and non-target stimuli indicate that before treatment, non-target coherence was .428 and target coherence was .451, fairly similar values. However, after completion of TMS therapy target coherence increased to .559 and non-target coherence decreased to .424.

The p-value of the comparison of coherence for F4-T8 between target and non-target for both pre- and post-treatment was .044. These results are illustrated in Figure 32 below.

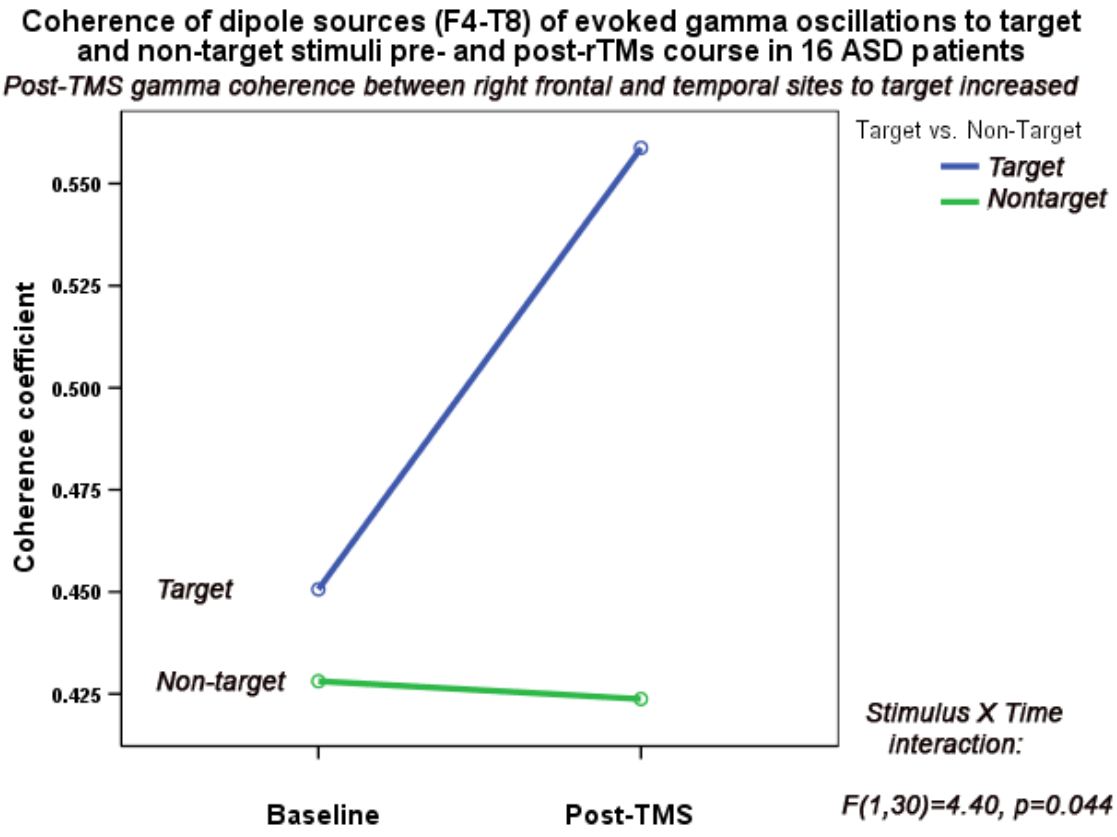

FIGURE 32 – Comparison of target and non-target evoked gamma coherence (F4-T8) pre- and post-TMS therapy

Another significant effect of TMS treatment was observed in evoked gamma coherence between F4 and P4. As in the case above, coherence in response to the target condition increased significantly following TMS, from .156 to .278. Coherence in response to the non-target stimuli increased only slightly after the TMS course, from .184 to .211, as seen in Figure 33. The comparison of mean coherence valuesfor F4-P4

between target and non-target stimuli pre- and post-TMS treatment reached significance (p = .019).

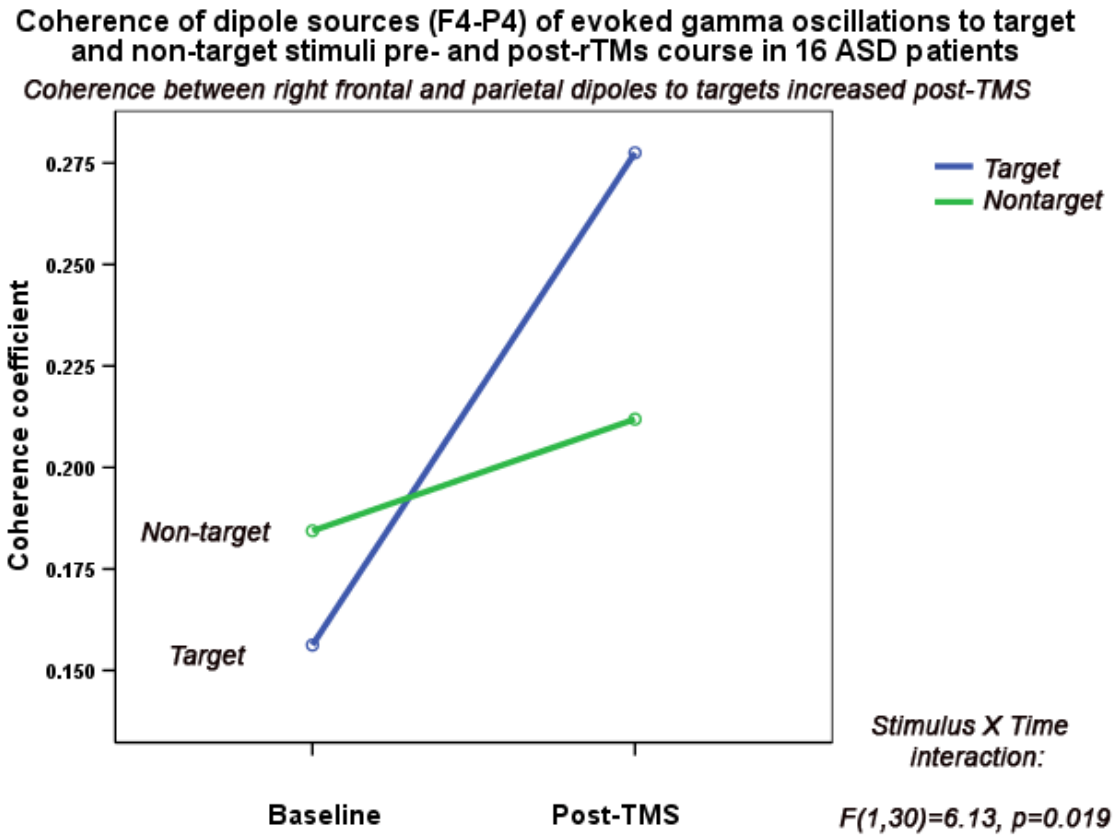

FIGURE 33 – Comparison of target and non-target evoked gamma coherence (F4-P4) pre- and post-TMS therapy

Subjects in the waitlist group also completed two Kaniza tasks but did not receive TMS treatment between the first and second Kanizsa task. Analysis of evoked gamma coherence between F4 and T8 for those in the waitlist group does not show significant differences in responses to target and non-target stimuli (p = .724). Similarly, no significant differences were observed in responses to target and non-target stimuli for evoked gamma coherence between F4 and P4 (p = .710).

F. Discussion

Measuring heart rate variability and electrodermal activity is a noninvasive and effective way to gather information about ANS functioning. Accelerated heart rate in association with lower HRV index and high electrodermal activity (SCL) found in children with ASD are indicators of excessive sympathetic and reduced parasympathetic activation in ASD resulting in a limited psychophysiological flexibility and in behavioral rigidity. Changes were investigated in autonomic activity during 18 rTMS sessions in children with ASD. Our hypothesis was that children with ASD would show improved HRV measures (decreased overall HR, increased RR interval, increased STDHR, increased SDNN, increased RMSSD, increased pNN50, increased HF and/or decreased LF, decreased LF/HF ratio), decreased SCL, improved behavioral outcomes, and increased gamma coherence between long-distance regions of the brain.

Similar data analysis methods were used for the rTMS study as in the prism lens study. Analysis of HRV began with detection of QRS complexes and producing a series of successive RR intervals. As in the rTMS study, it was decided that FFT was the optimal method for spectral analysis of HRV. The only difference in analysis of EEG coherence was replacing channels C3 and C4 in the montage with channels T7 and T8 to reflect the emphasis of the Kanizsa task on visual recognition.

Time-domain HRV results show significant increases in both RR interval and SDNN (standard deviation of the RR interval). The increased RR interval, indicating a decrease in HR, and the increased standard deviation in RR are promising because they suggest more prominent parasympathetic activity and more flexibility in heart rate overall. Within the frequency-domain of HRV, there was increased HF power, which is

also very important because this suggests parasympathetic tone was enhanced. As we did not observe a significant decrease in the LF component, it can be inferred that restoration of autonomic balance was achieved mainly through increased HF component of HRV, which correlates to parasympathetic (n. vagus) cardiac neural control. However, while the change in the LF component was not significant, we did observe a significant decrease in the LF/HF ratio over 18 sessions of TMS, which shows that some decrease in LF power did occur. There was also a decrease in SCL over the 18 sessions, which suggests a withdrawal of sympathetic tone as SCL is controlled solely by sympathetic inputs.

ABC and RBS scores also show that TMS therapy improved behavioral outcomes of irritability, lethargy, hyperactivity, and stereotypic and compulsive behaviors.

Lastly, gamma coherence analyzed from the Kanizsa task shows that TMS was effective in improving gamma coherence between frontal and temporal regions, and between frontal and parietal regions. The increase in frontal-parietal coherence is especially promising as it has been shown in previous studies that coherence between long-distance regions is decreased in ASD. In addition to increasing coherence, TMS therapy also affected the way subjects responded to target versus non-target conditions. Evoked gamma coherence in response to target stimuli increased significantly post treatment in both F4-T8 and F4-P4. However, coherence between F4-T8 and F4-P4 in the waitlist group showed no significant increase in response to the target condition. These findings indicate that TMS therapy has the potential to improve gamma coherence in ASD.

IV. CONCLUSION

Both studies presented in this thesis, prism lens therapy and TMS therapy, were selected as potential means to improve autonomic balance and gamma coherence in ASD. Prism lenses address these issues by altering one's ambient vision and perception of their surroundings, while TMS works by increasing cortical inhibition. The appropriate analysis steps were formulated for each type of collected data to transform from the raw EKG or EEG into a usable RR interval or source waveform, respectively.

Prism lenses proved to be more effective in producing the desired effects on the autonomic nervous system than on gamma coherence. ASD is thought to adversely affect ambient vision, thus making it difficult for the individual to interpret his or her surroundings. The results of this study suggest that wearing prism lenses did in fact help to improve ambient vision, which may have decreased stress, reflected by the observed decrease in HR, increase in HF power, decrease in SCL, and improved behavioral outcomes. Based on these findings, prism lenses show potential for improving autonomic balance in ASD.

TMS is now being considered for a number of applications, including treatment of depression (Udupa et al., 2007), improvement of gamma band activity in ASD (Baruth et al., 2010), and in the case of this study, improvement of autonomic function and gamma coherence in ASD. The mechanism of low-frequency TMS involves increasing inhibition of the stimulated cortex. For this study the stimulated region was the DLPFC, which is linked to ANS control. The findings of the study support the hypothesis that TMS applied to the DLPFC enhances autonomic balance in ASD through activation of parasympathetic tone and withdrawal of sympathetic tone. The findings of the TMS study

also suggest increased gamma coherence as a result of TMS therapy, specifically between frontal and temporal regions and frontal and parietal regions. Coherence between long-distance regions, such as frontal to parietal, is typically decreased in ASD. These findings support further investigation into the use of TMS as a means to improve both autonomic measures and gamma coherence in ASD.

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APPENDIX I: LIST OF ABBREVIATIONS

ABC – Aberrant Behavior Checklist

ADI-R – Autism Diagnostic Interview- Revised

ANS – Autonomic Nervous System

ASD – Autism Spectrum Disorder

DLPFC - Dorsolateral Prefrontal Cortex

DSM-IV – Diagnostic Statistical Manual of Mental Disorders, Fourth Edition

EDA – Elctrodermal Activity

EKG – Electrocardiogram

EMG – Electromyogram

HF – High Frequency component of HRV

HRV – Heart Rate Variability

LF – Low Frequency component of HRV

MT – Motor Threshold

NS.SCR- non-specific Skin Conductance Responses

RBS – Repetitive-Behavior Scale

rTMS – Repetitive Transcranial Magnetic Stimulation

SCL – Skin Conductance Level

SCR – Skin Conductance Response

SPSS – Statistical Package for the Social Sciences

TMS – Transcarnial Magnetic Stimulation

VFL – Very Low Frequency component of HRV

WCEC – Weisskopf Child Evaluation Center

VITA

I am from Louisville, Kentucky and went to went to high school at DuPont Manual. I received my B.S. in Bioengineering from the University of Louisville in August 2013, and am currently in my fifth year at the J.B. Speed School of Engineering. Notable honors and awards include graduating with my B.S. with Highest Honors and receiving the Mickey R. Wilhelm Achievement Award. During the past three years as a research assistant in Dr. Sokhadze's lab, I have been fortunate to work on a variety of projects and collaborate with esteemed researchers and professors. In addition, I have gained valuable experience in poster presentations and scientific writing. A few highlights include presenting posters at Neuroscience Day (2011, 2013, 2014) and meetings of the Society for Psychophysiological Research (2012 New Orleans, LA) and the Association for Applied Psychophysiology and Biofeedback (2014 Savannah, GA), with a supporting travelship grant from AAPB, as well as being a co-author for chapters in both Cutting Edge Therapies for Autism and Imaging the Brain in Autism. In 2011 I was a co-recipient of a research grant on prism lenses from the Autism Research Institute, and in 2014 received a research grant to study rTMS and heart rate variability from the Foundation for Education and Research in Biofeedback and Related Sciences (FERB). Both of these grants were instrumental in completion of this thesis. In addition, part of the work presented in this thesis is expected to be published in *Frontiers in Neuroscience* in 2014. I will be finishing my last semester in the Master of Engineering program this July, and will attend University of Louisville School of Medicine in the fall.