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14. ABSTRACT
The overarching objective of this study was to characterize abnormalities of vestibulo-ocular reflexes in Autism Spectrum Disorder (ASD). Specific Aim 1: To characterize aberrations in horizontal VOR post-rotary nystagmus without optokinetic feedback using a velocity step test. We hypothesize that in ASD vertical eye movement intrusions during horizontal nystagmus will occur more frequently than normal, will be time-locked to horizontal nystagmus, and will differ from voluntary saccades. Specific Aim 2: To apply a linear systems analysis based method for testing horizontal VOR without optokinetic feedback using sinusoidal oscillation tests. We hypothesize that gain and phase lag of horizontal VOR will differ in children with ASD compared to controls. Specific Aim 3: To characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback, using velocity step tests.

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Vestibulo-ocular reflex, autism

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INTRODUCTION

The overarching objective of this study is to characterize abnormalities of vestibulo-ocular reflexes (VOR) in Autism Spectrum Disorder (ASD). Specific Aim 1: To characterize aberrations in horizontal VOR post-rotary nystagmus without optokinetic feedback using a velocity step test. We hypothesize that in ASD vertical eye movement intrusions during horizontal nystagmus will occur more frequently than normal, will be time-locked to horizontal nystagmus, and will differ from voluntary saccades. Specific Aim 2: To apply a linear systems analysis based method for testing horizontal VOR without optokinetic feedback using sinusoidal oscillation tests. We hypothesize that gain and phase lag of horizontal VOR will differ in children with ASD compared to controls. Specific Aim 3: To characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback, using velocity step tests.

This Final Report covers the period from award initiation on 15 May 2010 to conclusion on 14 May 2014, a total of sixteen quarters including no-cost extension of four quarters. This Final Report will require future revisions as data analyses are completed and publications of these analyses folloe.

Equipment problems identified during the first three quarters of year one were addressed during the fourth quarter. Preliminary data collection using ten college student participants generated data in line with norms, at the end of the fourth quarter. Recruitment of children as partiipants commenced during the fifth quarter, and continued for eight more quarters resulting in 24 participants with ASD and 32 typically developing (control) participants. Since the fourtteenth quarter, efforts were devoted to data analysis. Data analysis has been completed for one PhD dissertation (Tana Bleser-Carson) and for one Master's thesis (Bradley Wilkes) which were defended. A second PhD dissertation (Jill Welsh) will likely result from this data analysis. Four papers have been presented at professional meetings and one paper has been submitted (to date) for journal publication.

KEY WORDS

Vestibulo-ocular reflex
Eye movement
Austism Spectrum Disorder
Autism

OVERALL PROJECT SUMMARY

Statement of Work: Abnormal Vestibulo-Ocular Reflexes in Autism:
A Potential Endophenotype
{**Bold** font: modifications of original Statement of Work}

Task 1. Activities preparatory to research (year 1, months 1 - 6)

Subtask 1a. Submit protocol for human research participation to UF Institutional Review Board.

Milestone #1: Human research participation approval by UF Institutional Review Board was granted on 12 April 2010, and approval by the ARO Human Research Protections Office (HRPO Log No. A-16019) was granted on 21 Oct 2010. UF IRB reapproved the protocol for the second year on 9 April 2011 and reapproved for the third year on April 9, 2012.
Milestone #1 has been met.

Subtask 1b. Research assistants trained in administration of ADOS (certification required) and other testing administration.
Milestone #2: Neuropsychological and vestibulo-ocular reflex tests ready to be administered by research assistants. Neuropsychological testing kits and materials have been acquired. Two Graduate Research Assistants have been certified for clinical and research administration of ADOS tests, and have been training undergraduate research assistants to aid in the administration of neuropsychological tests which do not require an advanced degree or special certifications to administer. **Minor changes were made to the neuropsychological tests package**, which were approved by the University of Florida IRB and have been forwarded to ARO HRPO.
Milestone #2 has been met.

Subtask 1c. Submit protocol for recruitment of research participants to Alachua County School District, to local therapy centers having ASD clients, and to UF Center for Autism and Related Disorders (CARD).
Milestone #3: Permission granted to recruit on premises (schools, therapy centers) or via a contacts database (CARD). Subtask 1c has been completed now that Task 2 (testing equipment) has also been completed.
Milestone #3 has been met. Permission has been granted by the Alachua County School Board to contact individual schools for permission from the school's Principal. We now have permissions to recruit from the Principals of several elementary schools. We have permission to recruit from local therapy centers and from CARD.

During the eighth quarter we also gained permission to recruit at the weekly child psychiatry clinic held for children with ASD. During the ninth quarter I contacted colleagues at the child psychiatry clinics at the University of South Florida in Tampa, potentially to recruit from those clinics. However, no recruits were obtained from Tampa.

Task 2. Acquire **equipment/software system from Neuro Kinetics, Inc.,** set up on site (year 1, months 1 - **11**).

Subtask 2a. Establish appropriate levels of infrared illumination for high frame rate eye tracking while maintaining low visibility to the participants. **(included in Neuro Kinetics, Inc. system)**
Subtask 2b. Synchronize eye tracking data acquisition and rotary device motion control computers. **(included in Neuro Kinetics, Inc. system)**

Subtask 2c. Create data base structures to link eye tracking data and rotary motion data **(included in Neuro Kinetics, Inc. system)** to neuropsychological results.
Subtask 2c was met during the sixth quarter. Separate databases maintain the eye tracking and rotary motion records, and the results of neuropsychological testing. Records can be exported from either database into a common Excel spreadsheet for examining correlations between eye movement/rotary motion and neuropsychological variables..

Milestone #4: Equipment and software ready for testing human participants. Milestone #4 has been met.

Task 3. Recruitment and testing of 8 pilot study participants (originally proposed for year 1, months 10 - 12)

VOR testing has been completed on 29 typically developing children and four ASD children.

Subtask 3a. Compare results from 4 non-ASD pilot participants to the literature for comparability. Subtask 3a has been met with 29 typically developing participants showing data within published norms. Example data were included in the seventh quarterly report.

Subtask 3b. Compare results from 4 ASD pilot participants to preliminary findings cited in the proposal for consistency. Subtask 3b has been met in the ninth quarter. Each of the four ASD participants had some vertical eye movements when tested for horizontal VOR in the dark.

Subtask 3c. Correct inefficiencies, if found, in test administration procedures or software for data structures. Subtask 3c has been met.

Subtask 3d. Prepare abstract of pilot test findings for presentation at national meeting. In fact, we have drafted a manuscript for submission to the journal *Autism*. We have not yet submitted an abstract for presentation at a meeting.

Milestone #5: Pilot study supports launch of formal research protocol. Milestone #5 has been met.

Task 4. Prepare annual report of grant activities with pilot study results and tentative conclusions from these pilot results (year 1, month 12)

Task 4 was completed on schedule except for the previously incomplete data from Task 3. The first annual report was subsequently revised and resubmitted to correct formatting issues. The second and third annual reports were also submitted and found acceptable.

Subtask 4a. Annual renewal of IRB human research participation approval was obtained on March 12, 2014, and was reported separately to Army Research Office Human Research Protections Office. The university IRB made the determination that the project has become exempt from further IRB oversight, because contact with human participants has concluded and no further participants will be recruited. Please see the attached copy of the IRB Exemption letter. With the exemption it will be possible to continue data analysis and publication of results.

General Summary of Research Protocol

Detailed methods are described in documents placed in the Appendices (section 11 of this report). Generally, each participant responded to a number of psychological tests in order to more fully characterize those who had been diagnosed with Autism Spectrum Disorder (experimental group) and those who had undergone typical development (control group). Each participant's eye movements were observed with high speed video recordings, both under conditions of making voluntary controlled movements and under conditions of making vestibulo-ocular reflex movements during or after whole-body rotation (angular acceleration). The eye movements recorded during voluntary controlled movements mainly served to rule out the presence of pervasive oculomotor anomalies. The eye movements recorded during or after whole-body rotation in the horizontal plane served to test hypotheses as given in the Specific Aims 1 and 2 of the proposal. The equipment purchased from Neuro Kinetics, Inc. was not designed for testing vertical or torsional nystagmus, therefore Specific Aim 3 could not be explored.

The completions of specific parts of the research protocol by participants are detailed below:

Psychological Tests

Autism Diagnostic Observation Schedule: 21 out of 24 participants in the ASD group completed the ADOS, while 3 were unable to perform the assessment due to behavioral problems* (A008, A019, and A020; one child (A012) who completed the ADOS was found to not have ASD, and was not asked to return to complete the remainder of the study. Children in the typically developing control group did not perform the ADOS.

*Here and below, "behavioral problems" encompasses refusal to wear the eye tracker goggles, defiance at not getting to play more games, or at not being allowed to do only a certain desired type of spinning, and similar lack of compliance with instructions from experimenters. Often such problems were transient but rarely were so persistent that partial data losses happened as noted above and belowl.

Leiter Non-verbal IQ Test: 21 out of 24 participants in the ASD group completed the Leiter; 2 were unable to perform the assessment due to behavioral problems (A019 and A020), and the child found not to have ASD (A012) was not asked to perform the Leiter after being excluded due to their ADOS. All 32 typically developing controls that participated in the study completed the Leiter.

Physical And Neurological Evaluation of Soft Signs: 19 out of 24 participants in the ASD group completed the PANESS. 4 were unable to perform the assessment due to behavioral problems (A008, A018, A019, and A020), while the child found not to have ASD (A012) was not asked to perform the PANESS after being excluded due to their ADOS. All 32 typically developing controls that participated in the study completed the PANESS.

S**ensory Profile**: The parent report obtained for the Sensory Profile was completed for 20 out of 24 participants in the ASD group. Those parents of children who had behavioral problems often attempted to help the child calm down or follow instructions, and were not filling out questionnaires during that time. Parents did not fill out the sensory profile for 4 children in the ASD group (A008, A012, A019, and A020). For the typically developing control group, the sensory profile was completed for 22 out of 32 participants. Many of the participants whose parents did not complete the Sensory Profile were lost to contact and did not participate in VOR testing during Session 2 (T007, T008, T009, T010, T017 and T018). Some children completed VOR testing, but their parents did not complete the Sensory Profile (T002, T003, T013, and T014), and these parents did not respond to further attempts to gather that information.

Social Communication Questionnaire: The parent report obtained for the Social Communication Questionnaire was completed for 19 out of 24 participants in the ASD group. Those parents of children who had behavioral problems often attempted to help the child calm down or follow instructions, and were not filling out questionnaires during that time. Parents did not fill out the sensory profile for 5 children in the ASD group (A001, A008, A012, A019, and A020). For the typically developing control group, the Social Communication Questionnaire was completed for 30 out of 32 participants. The two control children whose parents did not fill out the SCQ did complete the rest of the testing protocol (T002 and T003), but parents did not respond to further attempts to gather that information.

Vineland Adaptive Behavior Scale: The parent report obtained for the Vineland was completed for 18 out of 24 participants in the ASD group. Those parents of children who had behavioral problems often attempted to help the child calm down or follow instructions, and were not filling out questionnaires during that time. Parents did not fill out the Vineland for 6 children in the ASD group (A001, A008, A012, A018, A019, and A020). For the typically developing control group the Vineland was completed for 25 out of 32 participants. Many of the typically developing participants whose parents did not complete the Vineland were lost to contact and did not participate in VOR testing during Session 2 (T007, T008, T009, and T010). Three children whose parents did not fill out the Vineland completed the rest of the testing protocol (T002, T003, and T004), but parents did not respond to further attempts to gather that information.

Restrictive and Repetitive Behavior Scale - Revised: The parent report obtained for the RSB-R was completed for 21 out of 24 participants in the ASD group. Parents did not fill out the RBS-R for 3 children in the ASD group (A008, A012, and A019), all of which were unable to complete a majority of the testing protocol due to behavioral problems. For the typically developing control group, the RBS-R was completed for 29 out of 32 participants. One

participant was lost to contact (T009), while two completed the rest of the testing protocol (T002 and T003), but parents did not respond to further attempts to gather that information.

Eye Movement Tests

Saccades: 18 out of 24 participants in the ASD group completed horizontal and vertical saccade testing. 6 participants in the ASD group were unable to perform the task; 4 due to behavioral problems (A008, A018, A019, and A020), one due to failure to comprehend task (A004), and one due to exclusion from the study (A012). For the typically developing control group, 24 out of 32 participants completed Horizontal Saccade testing. 8 typically developing participants did not complete saccade testing; 6 of which were lost to contact and did not participate in any oculomotor or VOR testing (T007, T008, T009, T010, T017, T018), one who was found to have severe oculomotor abnormalities (T027), and one whose oculomotor data was lost due to computer error (T029).

Horizontal and Vertical Smooth Pursuit (0.10 Hz and 0.50 Hz): 15 out of 24 participants in the ASD group completed all smooth pursuit testing. One participant was able to complete all pursuit tasks except for vertical smooth pursuit at .10 Hz (A009); the child was nonverbal and despite having completed the same task in horizontal direction, would not maintain attention on the stimulus in the vertical direction at that frequency. 8 Participants in the ASD group were unable to perform the task; 5 due to behavioral problems (A008, A018, A019, A020, and A024), one due to failure to comprehend the task (A004), one due to repeated failure to attend the stimulus during the task (A002), and one due to exclusion from the study (A012). For the typically developing control group, 24 out of 32 participants completed smooth pursuit testing. 8 typically developing participants did not complete smooth pursuit testing; 6 of which were lost to contact and did not participate in any oculomotor or VOR testing (T007, T008, T009, T010, T017, T018), one who was found to have severe oculomotor abnormalities (T027), and one whose oculomotor data was lost due to computer error (T029).

Gaze Evoked Nystagmus (central, horizontal ±10º, and vertical ±10º): 11 out of 24 participants in the ASD group completed all gaze evoked nystagmus tasks. 3 participants provided partial data; A010 was only able to the complete central gaze task, while A006 and A007 were able to complete central and horizontal, but not vertical. 5 ASD participants did not complete the task due to behavioral problems (A008, A018, A019, A020, and A024), 2 due to failure to comprehend the task (A004 and A009), and one due to exclusion from the study (A012).

For the typically developing control group, 24 out of 32 participants completed smooth pursuit testing. 8 typically developing participants did not complete gaze evoked nystagmus testing; 6 of which were lost to contact and did not participate in any oculomotor testing (T007, T008, T009, T010, T017, and T018), one who was found to have severe oculomotor abnormalities (T027), and one whose oculomotor data was lost due to computer error (T029).

Trapezoidal Rotational VOR Testing: 16 out of 24 participants in the ASD group completed all trapezoidal rotational testing. 3 participants with ASD provided partial data for trapezoidal testing. One participant was developing defiant behavior problems and only trapezoidal testing

in the dark was performed before the child would not continue (A015). One participant completed light and dark testing, but failed to comprehend instructions for the suppression condition and would not maintain focus on the suppression stimulus (A004). 5 participants did not complete any VOR testing procedures due to behavioral problems (A008, A018, A019, A020, and A024), and one due to exclusion from the study (A012).

For the typically developing control group, 16 out of 32 participants completed all trapezoidal rotational testing. 6 control participants provided partial data for trapezoidal testing. 5 of these participants were tested under an earlier protocol before the suppression condition was added, and thus only have light and dark rotational data (T001, T002, T003, T005, and T011). One participant (T006) completed all trapezoidal tests except for suppression in the counter-clockwise direction. The first 2 control participants tested (T004 and T012) were used to help refine the rotational device, and resulted in modifications to the head position and support; as such their data are not comparable to other participants. 6 control participants were lost to contact after their first session and did not participate in any VOR testing (T007, T008, T009, T010, T017, and T018). One control participant was found to have severe oculomotor abnormalities (T027) and was not included for VOR testing. One control participant's data is not able to be interpreted due to computer error and loss of data (T029).

Sinusoidal Harmonic Acceleration VOR Testing (Dark and Visual Suppression at 0.05 Hz, 0.10 Hz, and 0.50 Hz): 15 out of 24 participants with ASD completed all SHA testing. 3 children became fatigued and developed behavioral problems after prior testing and did not want to continue with SHA (A004, A014, and A015). 5 participants did not complete any VOR testing procedures due to behavioral problems (A008, A018, A019, A020, and A024), and one due to exclusion from the study (A012).

For the typically developing controls, 17 out of 32 participants completed all SHA testing. One participant has partial data, including all SHA except the .10 Hz dark condition, not collected due to researcher error. 6 participants were tested under an earlier protocol with different SHA frequencies than those used for the remainder of the study, and as such their data are not comparable (T001, T002, T003, T004, T011, and T012). 6 control participants were lost to contact after their first session and did not participate in any VOR testing (T007, T008, T009, T010, T017, and T018). One control participant was found to have severe oculomotor abnormalities (T027) and was not included for VOR testing. One control participant's data is not able to be interpreted due to computer error and loss of data (T029).

Results

A complete analysis of the findings has not yet been completed. As detailed above, many of the possible comparisons will have unequal numbers of experimental versus control obsrvations. In no cases are the numbers of independent observations adequately large to justifiably assume that the law of large numbers supports the use of parametric inferential statistics. Accordingly, a more painstaking analysis with non-parametric inferential statistics is in progress.

Serendipitous findings (i.e., not in the Specific Aims of the proposal) within the incomplete analyses have already stood out and have been reported (see documents in the Appendices).

These serendipitous findings are: (1) that the exceptional ability of spatially stationary visible stimuli to effect abnormally rapid suppression of vestibule-ocular reflexes in ASD, originally reported by Ritvo et al. in 1968 and subsequently replicated, reverses with small centrally-located targets; and (2) albeit that voluntary horizontal eye movements are indistinguishable between ASD and typically developing children, as found by other investigators, we find that their rarely studied vertical eye movements can differ.

Hypothesized differences between the ASD group and the control group, as listed in Specific Aims 1 and 2 of the proposal, are as follows, with discussion of the results thereunto appertaining:

"Specific Aim 1: To characterize aberrations in horizontal VOR post-rotary nystagmus without optokinetic feedback using a velocity step test. We hypothesize that in ASD vertical eye movement intrusions during horizontal nystagmus will occur more frequently than normal, will be time-locked to horizontal nystagmus, and will differ from voluntary saccades."

Findings: We cannot yet conclude whether or not vertical eye movement intrusions during horizontal VOR post-rotary nystagmus are more frequent in ASD versus control participants. Unexpectedly complex features in the vertical eye movement records (i.e., dysconjugate vertical eye movements made by ASD) have forced us to adopt a new and more complex algorithm to classify the eye movement records for group analysis. The said algorithm is still under development.

We found by chance unexpected evidence of dysconjugate vertical eye movement intrusions during horizontal VOR for one ASD participant, as previously detailed in a quarterly report. The significance of this unexpected finding by chance is as follows: (1) Normal eye movements consist of version types, saccades and smooth pursuit, in which the eyes move in the same direction in a yoked conjugate fashion, as well as (2) vergence types, in which the two eyes move separately. A typical human eye movement toward a stationary target away from one's initial visual axis will typically elicit conjugate version movements and non-conjugate vergence movements, which differ in their time courses of initiation as well as their speeds of execution, and which respectively rely upon somewhat separated neural circuitry. Voluntary eye movements and reflex eye movements share a great deal of common neural circuitry. It is thus very surprising that stimulation designed to elicit horizontal version eye movements additionally elicits in ASD children both vertical version and vertical vergence movements, in addition to the expected horizontal version reflex movements. This finding, if representative, suggests highly abnormal "crosstalk" within sub-cortical systems for oculomotor control.

Specific Aim 2: To apply a linear systems analysis based method for testing horizontal VOR without optokinetic feedback using sinusoidal oscillation tests. We hypothesize that gain and phase lag of horizontal VOR will differ in children with ASD compared to controls.

Findings: This analysis is presently inconclusive.

Specific Aim 3: To characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback, using velocity step tests.

Findings: We were unable to purchase commercially available apparatus capable of eliciting in isolation vertical VOR and torsional VOR. Research apparatus we had initially planned to use for these experiments suffered catastrophic irreparable failure during grant year one.

The best commercially available equipment for replacement of the failed hardware, purchased from Neuro Kinetics, Inc. (NKI) was unable to provide the necessary rotational stimulations to elect vertical or torsional VORs. This the exploratory studies of Specific Aim 3 were abandoned by the and of grant year 1.

In addition, The NKI software had limitations on its ability to resolve accurately torsional eye movements, because this was a novel requirement for NKI. It is currently unknown whether an algorithm will eventually be discovered to extract useable data from the existing records of ocular torsion movements as they were captured by this NKI equipment.

The reasons why it will be scientifically important to understand the torsional components of eye movements are twofold: (a) the midbrain locations of the final common pathway for torsional eye movements, the trochlear cranial nerve nuclei, are nearby the midbrain locations of the "vertical gaze centers" innervating the inster-stitial nuclei of Cajal and the anterior nuclei of the medial longitudinal fasciculi; and (b) proximity of the aforementioned midbrain region to the Edinger-Westphal nuclei affecting pupillary autonomic responses is suggestive of differential responses of the pupillary reflexes in ASD. Albeit that pupil size was not proposed as an independent variable in the funded proposal, these findings suggest that analysis of pupil size dynamics should be obtained from the acquired data.

Key Research Accomplishments

(1) Children diagnosed with ASD have anomalous vestibulo-ocular reflex eye movements. These anomalies manifest in several forms:

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a. Visible surroundings profoundly affect the eye movements of ASD children.

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b. When tested in the dark (surroundings not visible), ASD children can have prominent vertical components present in their ongoing horizontal vestibulo-ocular reflex eye movements, whereas typically developing children do not.

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c. When tested in the dark (surroundings not visible), ASD children can have dysconjugate vertical eye movements during horizontal vestibulo-ocular reflex tests, whereas typically developing children do not.

Conclusion

Due to small sample sizes no definitive conclusion is possible at the present time, pending non-parametric group data analyses.

Publications, Abstracts, and Presentations

Wilkes, B.J., Bleser-Carson, T.M., Patel, K.P., Lewis, M.H. and White, K.D. (2014) Oculomotor Performance in Children with High-Functioning Autism Spectrum Disorders. Submitted to *Research in Developmental Disabilities* (in review).

Carson, T.B., Wilkes, B., Patel, K., Radonovich, K., and White, K. (2011) Abnormal Vestibulo-Ocular Reflexes in Autism Spectrum Disorders: Pilot Data, 2011 Association for Research in Otolaryngology Mid-Winter Meeting, Baltimore, MD.

Carson, T.B., Wilkes, B., Patel, K., Radonovich, K., and White, K. (2013) Abnormal Visual Suppression of Rotary Vestibulo-Ocular Reflexes in Autism, 2013 Association for Research in Otolaryngology Mid-Winter Meeting, Baltimore, MD.

Wilkes, B., Bleser-Carson, T., Patel, K., Ko, J., Bodfish, J., Newell, K. and Lewis, M. (2014) Abnormal vestibulo-ocular reflex eye movements in autism spectrum disorders. . Poster # 380. University of Florida College of Medicine Celebration of Research (March 31st, 2014), Gainesville, FL.

Wilkes, B. (2014) Oculomotor and Vestibulo-ocular reflex function in children with high-functioning ASD. Spoken presentation to Autism Interdisciplinary Meeting (April 2, 2014), Gainesville, FL

Bleser-Carson, T., Wilkes, B., Patel, K. Welsh, J., Lewis, M. and White, K. (2014) Abnormal Vestibulo-Ocular Reflexes and Possible Link to Cerebellar Deficits in Autism. Poster # 108.072, International Meeting for Autism Research (May 14 - 17, 2014), Atlanta, GA

Wilkes, B., Bleser-Carson, T., Ko, J., Bodfish, J., Newell, K. and Lewis, M. (2014) Abnormal Vestibulo-Ocular Reflexes in Autism Spectrum Disorders. Poster # 158.064, International Meeting for Autism Research (May 14 - 17, 2014), Atlanta, GA

Inventions, Patents and Licenses

Nothing to report.

Reportable Outcomes

Pending non-parametric group data analyses.

Other Achievements

Nothing to report.

References

Please see Appendices.

Appendices

IRB Exemption letter, 1 page
Wilkes et al. Res. in Developmental Disabilities, 24 pages
Wilkes Master's Thesis, 21 pages
Bleser-Carson Dissertation, 175 pages

Oculomotor Performance in Children with High-Functioning Autism Spectrum Disorders

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Abstract

There is a high prevalence of sensory abnormalities in Autism Spectrum Disorders (ASD). However, compared to investigations of diagnostic criteria there is a relative lack of sensorimotor research in ASD. Despite this, there is evidence for abnormal visual processing and motor control in ASD. In this study, we assessed oculomotor performance among children with high functioning ASD and typically developing controls, ages 6-12 years. Children with ASD exhibited greater horizontal saccade latency, as well as greater phase lag in vertical smooth pursuit among. Saccades and smooth pursuit are complex sensorimotor behaviors that involve several spatially distant brain regions and long-fiber tracts between them, many of which have evidence of abnormality among individuals with ASD. Oculomotor behaviors are modifiable, and treatment of oculomotor abnormalities in children with ASD could have a positive functional impact.

Keywords: autism, oculomotor, saccade, smooth pursuit.

Oculomotor Performance in Children with High-Functioning Autism Spectrum Disorders

Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders characterized and diagnosed by deficits in communication and social skills, as well as the presence of restricted and/or repetitive behaviors (DSM–IV–TR; American Psychiatric Association, 2000). Although not currently part of the diagnostic criteria, sensorimotor abnormalities have also been identified in ASD, such as deficits in postural stability (Minshew, Sung, Jones, and Furman, 2004), hyper-responsive tactile reflexes (Baranek, 1997), vestibulo-ocular reflex abnormalities (Ritvo, Ornitz, Eviatar, Markham, Brown, and Mason, 1969; Ornitz, Constance, Kaplan, and Westlake, 1985). Estimates of the prevalence for sensory abnormalities in ASD range between 42-88% (Tomcheck and Dunn, 2007).

Although there is a high prevalence of sensory abnormalities in ASD, there is a relative lack of sensorimotor research in ASD, as compared to investigations of diagnostic criteria or other comorbid problems (e.g. hyperactivity, anxiety). Sensory integrative and sensory based therapies are a common intervention for individuals with ASD, yet there is a lack of evidence based approaches for such interventions. Sensorimotor interventions, such as sensory integration therapy, are performed with the goal of modifying arousal and abnormal responses to sensory stimuli. However, these interventions often employ a broad set of treatments, without specific evaluation of the functional impact of these treatments. Ideally, sensorimotor intervention could be targeted to address issues specific to each patient, in order to create a positive functional impact on daily living (for a review see Case-Smith and Arbesman, 2008). Before such issues can be adequately addressed, there is a need for thorough characterization of sensory systems and sensorimotor behaviors in ASD.

Much of the current research regarding sensorimotor systems in ASD deal with highly complex sensory perceptions (e.g. face processing) or gross motor output (e.g. balance and gait). While findings from such investigations may seem to be more easily translated to overt impairments in ASD, the complexity of such phenomena can make intepretation of experimental findings and the mechanisms by which they occur difficult. The oculomotor system is a sensorimotor network which controls volitional eye movements by incorporating visual information into appropriate motor outputs to the extraocular muscles. Oculomotor assessments are useful in the study of neurodevelopmental disorders, as abnormal outcomes measures can provide insights into aberrant neural circuitry in these populations (Sweeney, Takarae, Macmillan, Luna, and Minshew, 2004). The developmental trajectory and neural circuitry underlying the oculomotor system have been thoroughly characterized in typical individuals and methodologies for objective quantification of this system early in life are reliable and well established (for a review see Luna, Velanova, and Geier, 2008). Oculomotor outcomes and their potential associations with core features of ASD could provide insights into the underlying features of these phenomena and provide targets for sensorimotor interventions among individuals with ASD.

Two of the most common oculomotor assessments are those of saccades and visual smooth pursuit. A saccade is a quick, darting eye movement that occurs for both eyes in unison, typically to center a target on the retina. Saccades are characterized by fast acceleration to high velocity, followed by a quick deceleration of equal magnitude. Two parameters of saccades which are commonly studied are gain and latency. Saccade gain is the degree of displacement of the eye as compared to the degree of displacement for the target visual stimulus. Saccade latency

is the time lag between appearance of a target visual stimulus and onset of saccadic eye movement to that stimulus.

Visual smooth pursuit entails a slower, gradual eye movement that serves to stabilize images on the retina during object motion. Smooth pursuit eye movements have much slower acceleration and velocity than do saccades, and cannot be performed in the absence of a moving visual stimulus. Two parameters of smooth pursuit which are commonly studied are gain and phase lead/lag. Smooth pursuit gain is the maximum degree of displacement of the eye as compared to the maximum degree of displacement of the target visual stimulus, while smooth pursuit phase lead/lag (referred to hereafter as just "phase") is the mean degree of displacement by which the eye leads or lags behind the target stimulus during a trial.

Oculomotor assessments among individuals with ASD have yielded mixed results. Rosenhall, Johansson, and Gillberg (1988) reported saccade hypometria (reduced gain) in 6 of 11 young adolescent participants with autism. However, Minshew, Luna, and Sweeney (1999) reported no difference in saccade gain or latency among young adults with high-functioning ASD. More recently Takarae, Minshew, Luna, Krisky, and Sweeney (2004) as well as Takarae, Luna, Minshew, and Sweeney (2008) found that participants with high-functioning ASD do not have significantly different sacade gain from control participants, although ASD participants did have greater variance in saccade gains. In addition, Kemner, van der Geest, Verbaten, and van Engeland (2004) report that children with high-functioning pervasive developmental disorder not otherwise specified (PDD-NOS) have typical saccade function.

Takarae et al. (2004; 2008) have demonstrated that participants with high-functioning ASD display significant abnormalities in visual smooth pursuit gain and latency (similar to

phase, except measured in time as opposed to degrees). Rosenhall et al. (1988) reported that although smooth pursuit function was found to be normal in 4 out of 11 participants with autism, 7 could not perform the task as instructed. Scharre and Creedon (1992) also attempted to assess voluntary smooth pursuit in children with autism, but 29 out of 34 participants displayed a series of saccades, rather than smooth pursuit, while tracking the target. Only 5 out of 34 children were able to successfully perform the task, but those data were not reported by the authors. These reports seem to indicate that smooth pursuit tasks are challenging for children with ASD who have intellectual disability, since many participants were unable to complete the task as instructed. The difficulty participants with autism had completing visual smooth pursuit tasks in Rosenhall et al. (1988) and Scharre and Creedon (1992) could have been related to intellectual disability, in that they had trouble understanding and following instructions. However, in light of the findings from Takarae et al. (2004; 2008), it could be that those participants were performing the task to the best of their ability and saccadic intrusions in the visual smooth pursuit task are part of the ASD population's oculomotor deficits, rather than a confound of low IQ reflecting gross central nervous system damage.

Together these studies show that high-functioning adults with ASD have relatively typical, if more varied saccade function, but abnormal visual smooth pursuit. However, it is unclear the extent to which *children* with high-functioning ASD have altered saccade and smooth pursuit function, as these studies have focused on adult populations with high-functioning ASD, or child populations without controlling for intellectual disability. It may be that saccade hypometria is present in those with ASD at younger ages, and that this deficit resolves through maturation. The difficulty participants with autism had completing visual smooth pursuit tasks in Rosenhall et al. (1988) and Scharre and Creedon (1992) could have been

related to intellectual disability, in that they had trouble understanding and following instructions. However, in light of the findings from Takarae et al. (2004; 2008), it could be that those participants were performing the task to the best of their ability and saccadic intrusions in the visual smooth pursuit task are part of the ASD population's oculomotor deficits, rather than a confound of low IQ reflecting gross central nervous system damage.

Oculomotor systems are optimal targets for sensorimotor research in ASD because they can be reliably assessed, have well understood neural circuitry, and well developed methodologies for investigation. Oculomotor behaviors are established early in development and remain modifiable. Training paradigms targeting oculomotor deficits in human subjects have shown positive outcomes (Ciuffreda, Han, Kapoor, and Ficarra, 2006). As such, these systems are amenable to early identification and intervention, which has been shown to significantly improve the prognosis of children with ASD among other types of interventions (Dawson, Rogers, Munson, Smith, Winter, Greenson, Donaldson, and Varley, 2010; Rogers, 1998). Saccade and visual smooth pursuit behaviors are also functional at birth and can be reliably assessed in infants at 4 weeks of age (Roucoux, Culee, and Roucoux, 1983).

The focus of this investigation was to assess saccade and smooth pursuit function in children with ASD, but without intellectual disability (IQ>70). A number of neuropsychological assessments commonly used among individuals ASD were also performed, and we explored possible relationships between oculomotor function and neuropsychological assessments. In addition to replicating recent investigations of oculomotor function in high-functioning adults with ASD (Takarae et al., 2004; 2008), the present work extended studies of oculomotor function into the vertical plane, which has not previously been performed in a population with developmental disability.

Methods

Participants

Participants included 16 children with ASD and 24 typically developing controls. Children were between 6 to 12 years of age at the time of testing. Children were recruited through advertisements distributed to Alachua county public schools, local clinics, and the University of Florida Center for Autism and Related Disabilities (CARD). Diagnoses were confirmed with the Autism Diagnostic Observation Schedule (ADOS; Lord, Risi, Lambrecht, Cook, Leventhal, DiLavore, Pickles, and Rutter, 2000) and Social Communication Questionnaire (SCQ; Rutter, Bayley, Lord, and Berument, 2003) administered by a clinical psychologist. Exclusion criteria for the ASD group were diagnosis of Fragile X, Rett Syndrome, tuberous sclerosis, seizure disorder or fetal cytomegalovirus infection. Exclusion criteria for the control group were any current or past history of psychiatric disorders. None of the children included in the study were prescribed medications known to alter oculomotor function (e.g. Risperdal). Mean age for the ASD group was 104(±23) months, and mean IQ was 104(±20), with one high outlier of 159. For typically developing controls, mean age was 110(±21) months, and mean IQ was 106(±14). Children with an IQ below 70 were not included in this sample, in order to control for intellectual disability as a potential confound. See Table 1 for a summary of participants.

Experimental Setting: Space Theme

Given the number of assessments performed and their duration, some degree of participant attrition was expected. In an attempt to reduce participant attrition, we sought to increase the children's compliance and motivation to participate by providing a stimulating experimental setting. Walls in the room where oculomotor testing were carried out were painted with a space theme, with the planets of the solar system and a space-shuttle. Children generally

reported enjoying the experience, and parents described that children were excited to return if an additional session was needed to complete testing.

Neuropsychological Assessment

For children included in the ASD group, diagnoses were confirmed with the ADOS administered by a clinical psychologist, and SCQ completed by a parent or primary caregiver. A parent or primary caregiver of the participants in both groups was asked to complete the Repetitive Behaviors Scale-Revised (RBS-R; Bodfish, Symons, Parker, and Lewis, 2000) and the Sensory Profile Caregiver Questionnaire (Dunn, 1999). Although there were several outcome variables derived from the questions in the Sensory Profile Caregiver Questionnaire, only the Visual Processing sub-score was used in this investigation. See Table 1 for a summary of neuropsychological outcome measures.

Equipment

Video-oculography (VOG) was performed with the I-Portal® system from Neuro-Kinetics, Inc. (NKI). This system included VOG goggles, computer hardware, and VEST ™ 6.8 software for acquisition and analysis. This system sampled from both eyes, in real-time, at a rate of 100 Hz. Visual stimuli were presented as a red laser-light, generated by NKI Pursuit Tracker® laser. The Pursuit Tracker® laser was mounted on the underside of the circular platform to which the seating apparatus is mounted, and projected on a cylindrical arc of height 48 inches, with a radius of 76.5 inches. The chair and seating arrangement were created by the authors for pediatric use and include a padded chair, safety harness, and head stabilizers with occipital head rest and support arms placed on the temporal region of the participant to prevent head movement during testing.

Calibration and Oculomotor Tests

Participants were individually calibrated to the testing equipment by projecting a laser stimulus onto the cylindrical screen and providing a fixed target at ± 10º in both the horizontal and vertical directions (e.g., first 10º to the left, then right, then up and then down). Calibration values were averaged across two trials. Two oculomotor tasks were then performed, saccades and smooth pursuit, which are described in the next section.

During saccade testing, participants were instructed to focus on the red laser stimulus projected onto the cylindrical screen, and to re-focus on the stimulus when it appeared at another location. Saccade testing included one trial of horizontal saccades with no vertical component, and one trial of vertical saccades with no horizontal component. In both horizontal and vertical conditions, 30 saccades were generated with random position between ± 25º and duration between 1-2 seconds; the same randomly generated set of saccades was used for each participant. Mean saccade gain and latency for each participant were determined using VEST™ 6.8 software.

During visual smooth pursuit testing, participants were instructed to "look at the red light" stimulus projected onto the cylindrical screen, and to "keep looking at the red light while it is moving." The stimulus for smooth pursuit testing moved in an oscillatory fashion; trials were performed at .10 Hz and .50 Hz for 6 cycles, between ± 10º. At each frequency, a trial was performed once in the horizontal direction and once in the vertical direction. Gain and phase lag/lead were determined for each smooth pursuit trial using VEST™ 6.8 software.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 21 software. T-tests were used to compare group differences in saccade gain, saccade latency, smooth pursuit gain, and smooth pursuit phase lead/lag. Subsequently, a stepwise linear regression was used to investigate whether outcome variables from neuropsychological assessments could predict oculomotor parameters that were found to be significantly different between groups. A significance level of $\alpha = .05$ was used for both t-tests and linear regressions.

Results

Saccades

A significant group difference was identified for horizontal saccade latency (see Figure 1) [$t(25.130) = -2.32$, $p<0.05$]. No significant difference was identified between groups for vertical saccade latency, or saccade gain in either horizontal or vertical conditions. A stepwise linear regression was performed among ASD participants to investigate whether horizontal saccade latency could be predicted by ADOS communication plus social composite score, ADOS repetitive behavior composite score, ADOS combined score, RBS-R score, SCQ score, or Sensory Profile visual processing sub-score. From this regression, it was determined that SCQ score was a significant predictor [$\beta = -0.70$, $t(12)=3.288$, $p< .05$] of horizontal saccade latency among participants with ASD. For this model with SCQ score as a predictor, the adjusted $R^2 = .45$. Thus, about 45% of the variation in horizontal saccade latency among the ASD group could be explained by SCQ score. Higher SCQ scores, which indicate greater deficits, predicted lower horizontal saccade latency, where lower saccade latency typically indicates more appropriate saccade function.

Smooth Pursuit

A significant group differences was identified for vertical smooth pursuit phase at 0.10 Hz [$t(14.88) = 2.22$, $p< 0.05$] (see Figure 2). There was a trending group difference for vertical smooth pursuit phase at 0.50 Hz [$t(20.06) = 1.86$, $p= 0.08$] (see Figure 2). No significant group differences were identified for horizontal smooth pursuit gain or phase at either .10 Hz or .50 Hz. A stepwise linear regressions were performed among ASD participants to investigate whether either vertical smooth pursuit phase at .10 Hz could be predicted by ADOS communication plus social composite score, ADOS repetitive behavior composite score, ADOS combined score, RBS-R score, SCQ score, or Sensory Profile visual processing sub-score. This regression did not find any significant predictors for vertical smooth pursuit at.10 Hz among the included predictor variables.

Discussion

This study was the first to use VOG to investigate saccade and smooth pursuit function in children with high-functioning ASD, without intellectual disability (IQ>70). By controlling for intellectual disability, results from this experiment reflect neurodevelopmental abnormalities specifically relevant to ASD, rather than general intellectual deficits. In addition, this study is the first to characterize vertical saccade and smooth pursuit in the ASD population. We found evidence of abnormal visual smooth pursuit phase in high-functioning children with ASD. We also found evidence that high-functioning children with ASD have a greater latency for initiating saccades.

We found participants with ASD had significantly greater mean phase lag for vertical smooth pursuit at .10 Hz. We did not find significant differences in mean gain or phase for

horizontal smooth pursuit conditions. In light of previous findings of reduced gain during pursuit tasks among ASD participants older than 16 years (Takarae et al., 2004), it is not surprising that we found no group difference for pursuit gain in our sample, since all participants in this investigation were between 6 and 12 years of age.

Our findings regarding pursuit phase are similar to those of Takarae et al. (2004; 2008) of pursuit latency (similar to phase, except measured in time as opposed to degrees). Their work suggests that differences in pursuit latency were only detectable after controlling for language delay (i.e. those with language delay have lower pursuit latency). Our findings of greater variance among ASD participants for horizontal smooth pursuit phase, but no group difference in *mean* horizontal smooth pursuit phase makes sense, as we did not control for language delay in this study.

Our finding of greater mean vertical smooth pursuit phase among children with ASD are novel as we are, to our knowledge, the first investigators to measure vertical smooth pursuit in children with developmental disability. This pronounced difference in group means for phase in vertical, but not horizontal conditions, could relate to the relatively infrequent use of visual pursuit in the vertical plane. In other words, both populations have less experience tracking objects with continuous motion in the vertical plane, as moving stimuli with motion in the vertical plane are less common than those with motion in the horizontal plane in daily experience.

Smooth pursuit is a complex sensorimotor behavior that involves several spatially distant brain regions such as the frontal eye fields, lateral intra-parietal area, medial superior temporal area, caudate, superior colliculus, cerebellar vermis, brainstem premotor nuclei, and vestibular

nuclei (for a review see Krauzlis, 2004). Several of these structures have evidence of abnormality among individuals with ASD (for a review see Courchesne, Pierce, Schumann, Redcay, Buckwalter, Kennedy, and Morgan, 2007). There is also evidence that individuals with ASD have abnormalities in long-fiber tracts between distal brain regions (Courchesne, Redcay, Morgan, and Kennedy, 2005). Local abnormalities in the above brain regions implicated in smooth pursuit, or aberrations in long-fiber tracts connecting them could both play a role in abnormal smooth pursuit function among some individuals with ASD.

For some ASD participants pursuit phase lag was 10º in horizontal, or up to 20º in vertical conditions. Interestingly, the group difference in horizontal smooth pursuit phase variance and vertical smooth pursuit mean phase were more pronounced at the slower frequency of .10 Hz, where pursuit is typically more accurate (i.e. less phase difference between eye and target). This implies that for some participants the fovea was not on target, but rather, that the target was in the periphery. Participants from this study were part of a larger investigation characterizing vestibulo-ocular reflex function, performed with the same equipment. Remarkably, ASD participants were unable to significantly suppress post-rotary nystagmus with a foveal target stimulus displayed inside otherwise dark VOG goggles, but *were* able to suppress post-rotary nystagmus normally when the entire room was visible (Bleser-Carson et al., unpublished). Taken together, these results may indicate that foveal and peripheral visual streams may be utilized differently in people with ASD. Such difficulties in integrating foveal and peripheral visual information meshes well with sensory integration difficulties described in ASD (Tomcheck and Dunn, 2007). Future investigations among individuals with ASD to directly ascertain whether foveal and peripheral visual streams can be utilized in an integrative fashion, comparable to neurologically typical individuals, seem warranted.

It is possible that abnormalities in smooth pursuit could cause functional impairments among individuals with ASD, such as altered spatial awareness in environments with moving stimuli. Indeed, previous work has shown that ASD participants with language delay had poorer performance than controls on a visual motion discrimination task, correlated with visual smooth pursuit performance (Takarae et al., 2008). It has been demonstrated that adaptive changes in smooth pursuit can be induced by training in non-human primates (Fukushima, Wells, Yamanobe, Takeichi, Shinmei, and Fukushima, 2001) and in humans following brain injury (Ciuffreda et al., 2006). The adaptive benefits of smooth pursuit training could also potentially improve deficits in visual motion discrimination in the ASD population.

Regarding saccades, this investigation found evidence that children with high-functioning ASD have a significantly greater latency for initiating horizontal saccades. This group difference in mean saccade latency did not extend to the vertical plane. Children with ASD had similar saccade latency for both horizontal and vertical conditions, whereas typically developing controls initiated saccades more quickly in the horizontal condition. These findings are in contrast with previous work indicating typical saccade function among high-functioning individuals with ASD (Minshew et al., 1999; Kemner et al., 2004). Although these previous investigations used random order of presentation for saccade stimuli, each included only three potential target locations, mirrored on each side, and a constant duration of presentation for each stimulus. In the current investigation, each saccade had a unique, randomly generated position and duration of presentation. Perhaps the greater difficulty of the saccade task in this investigation revealed mild deficits in latency to initiate a horizontal saccade in children with ASD that would not be detectable with more predictable stimuli.

Deficits in latency to initiate saccades in monkeys occurred after lesions to the frontal eye fields (Lynch, 1992). Among individuals with ASD, there is evidence of reduced functional connectivity from frontal eye fields and dorsal anterior cingulate cortex (Kenet, Orekhova, Bharadwaj, Shetty, Israeli, Lee, Agam, Elam, Joseph, Hämäläinen, and Manoach, 2012), both of which are regions critical in oculomotor control. Perhaps horizontal saccade latency deficits observed in this study are a reflection of neurodevelopmental abnormalities in frontal eye fields, or efferent targets. As to why these latency deficits did not extend to vertical saccades, it may be that the circuit implicated in vertical saccades includes some structures that do not overlap with the circuit involved in horizontal saccades, and that these are unaffected in this population. Work in monkeys show that lesions to the superior temporal poly-sensory area, which receives indirect afferents from the frontal eye fields via the medial pulvinar, negatively affected horizontal saccade latency but not vertical saccade latency (Scalaidhe, Albright, Rodman, and Gross, 1995).

We performed stepwise linear regression to investigate whether neuropsychological outcome measures could predict those oculomotor parameters found to be significantly different between groups. SCQ score was found to be a significant predictor of horizontal saccade latency, explaining 45% of the variation in horizontal saccade latency among participants with ASD. However, the direction of this relationship was unexpected as higher SCQ scores, which indicate greater deficits, predicted lower horizontal saccade latency, where lower latency typically indicates more appropriate saccade function. Perhaps it is adaptive for individuals with greater social and communication deficits to develop shorter latency for initiating visually guided saccades, in order to derive more information about their surroundings from visual stimuli.

Other neuropsychological measures from this investigation were not found to be good predictors for those oculomotor parameters found to be significantly different between groups. It

may be that the degree of severity of ASD diagnostic symptoms, indexed by ADOS scores, does not directly affect performance on these oculomotor parameters. Many of the neuropsychological measures included in these regression models were completed with the aid of parents or caregivers, and thus may not be sensitive enough to function as predictor variables for those oculomotor parameters found to be abnormal in this study.

Future studies of oculomotor function in ASD should seek to confirm findings from this investigation regarding saccade and smooth pursuit function in the vertical plane. Although the current investigation focused on individuals with high-functioning ASD in order to control for general intellectual disability, the inclusion of participants with lower-functioning ASD will help to elucidate whether such oculomotor abnormalities generalize to all individuals with ASD. Clinicians could also develop targeted therapeutic interventions for patients with ASD who show smooth pursuit phase lag in order to improve smooth pursuit function. Furthermore, the selectivity of oculomotor abnormalities to ASD can be addressed by future studies that include comparisons of oculomotor abnormalities among other developmentally disabled populations (e.g. Down Syndrome) alongside individuals with ASD.

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Mean and standard deviations for age, IQ, and neuropsychological assessments scores of participants			
Measure		Group	
		ASD	TD
Age [a]	Mean	104	110
	STDEV	23	21
IQ [b]	Mean	104	106
	STDEV	20	14
SCQ [f]	Mean	21	2
	STDEV	7	2
RBS-R [g]	Mean	38	3
	STDEV	24	4
Sensory Profile (visual) [h]	Mean	32	40
	STDEV	6	4
ADOS (CS) [c]	Mean	11	-
	STDEV	5	-
ADOS (R) [d]	Mean	2	-
	STDEV	2	-
ADOS (total) [e]	Mean	13	-
	STDEV	7	-

Table 1: Mean and standard deviations for age, IQ, and neuropsychological assessment scores of participants. Typically developing participants were not given the Autism Diagnostic Observation Schedule. Three participants were missing SCQ or RBS-R data, due to parents not completing these questionnaires.

a. Age in months

b. Leiter non-verbal intelligence quotient

c. Autism diagnostic observation schedule subscore (communication + social).

d. Autism diagnostic observation schedule subscore (repetitive behavior).

e. Autism diagnostic observation schedule (total score).

f. Social Communication Questionnaire.

g. Repetitive Behaviors Scale – Revised.

h. Sensory Profile visual processing subscore.

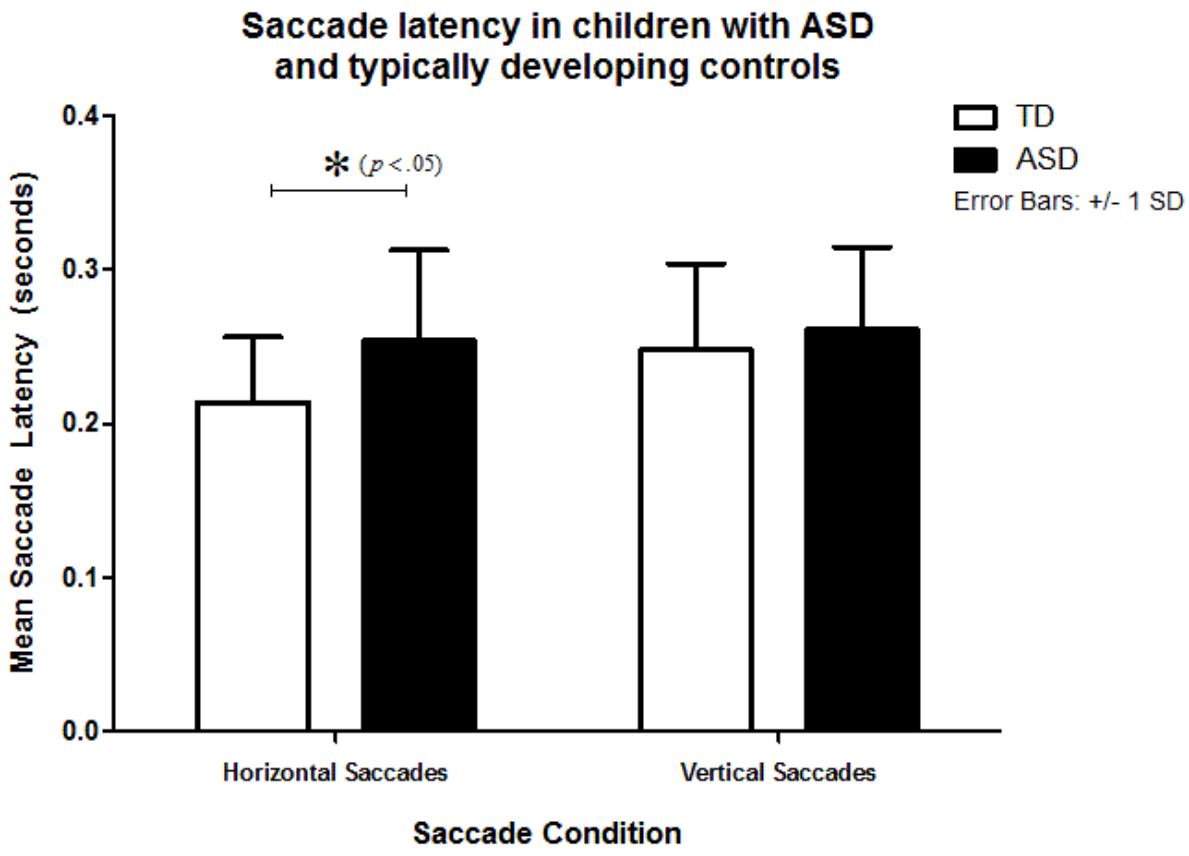

Figure 1. Mean latency (in seconds) for horizontal and vertical saccades. A significant group difference was identified between groups for horizontal, but not vertical saccades.

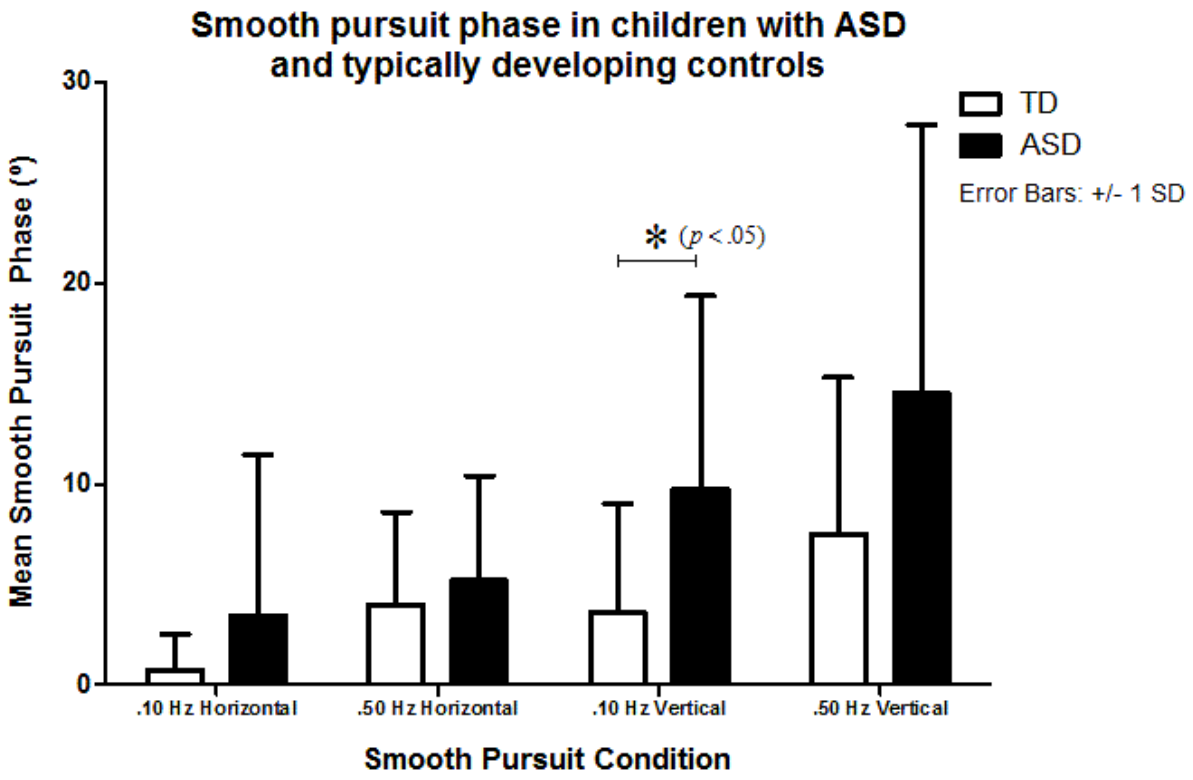

Figure 2: Mean phase (in degrees) for horizontal and vertical visually guided smooth pursuit at .10 Hz and .50 Hz. A significant group difference was identified for vertical smooth pursuit at .10 Hz.

OCULOMOTOR PERFORMANCE IN CHILDREN WITH HIGH-FUNCTIONING AUTISM SPECTRUM DISORDERS

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ABSTRACT

There is a high prevalence of sensory abnormalities in ASD. However, compared to investigations of diagnostic criteria there is a relative lack of sensorimotor research in ASD. We assessed oculomotor performance among children with high functioning ASD and typically developing controls, ages 6-12 years. We found evidence of greater horizontal saccade latency, as well as greater phase lag in vertical smooth pursuit among children with ASD. Visually guided saccades and smooth pursuit are complex sensorimotor behaviors that involve several spatially distant brain regions and long-fiber tracts between them, many of which have evidence of abnormality among individuals with ASD. Oculomotor behaviors are modifiable, and treatment of oculomotor abnormalities in children with ASD could have a positive functional impact.

INTRODUCTION

Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders characterized and diagnosed by deficits in communication and social skills, as well as the presence of restricted and/or repetitive behaviors (DSM–IV–TR; American Psychiatric Association, 2000). Although not currently part of the diagnostic criteria, sensorimotor abnormalities have also been identified in ASD, such as deficits in postural stability (Minshew et al., 2004), hyper-responsive tactile reflexes (Baranek et al., 1997), and vestibulo-ocular reflex abnormalities (Ritvo et al., 1969; Ornitz et al., 1985). Estimates of the prevalence for sensory abnormalities in ASD range between 42-88% (Tomcheck and Dunn, 2007).

Although there is a high prevalence of sensory abnormalities in ASD, there is a relative lack of sensorimotor research in ASD, as compared to investigations of diagnostic criteria or other comorbid problems (e.g. hyperactivity, defiant behavior). Sensory integrative and sensory

based therapies are a common intervention for individuals with ASD, yet there is a lack of evidence based approaches for such interventions. Sensorimotor interventions, such as sensory integration therapy, are performed with the goal of modifying arousal and abnormal responses to sensory stimuli. However, these interventions often employ a broad set of treatments, without specific evaluation of the functional impact of these treatments. Ideally, sensorimotor intervention could be targeted to address issues specific to each patient, in order to create a positive functional impact on daily living (for a review see Case-Smith and Arbesman, 2008). Before such issues can be adequately addressed, there is a need for thorough characterization of sensory systems and sensorimotor behaviors in ASD.

One particular sensorimotor reflex with some evidence of abnormality in the ASD population is the vestibulo-ocular reflex (VOR; Ritvo et a., 1969; Ornitz et al., 1985). VORs serve to provide stable vision during movement of the head by providing compensatory eye movements. Abnormalities in VORs may provide insights to disturbances in other related systems such as balance, postural stability, and visual perception during head movement. Among children with hearing-impairment, VOR abnormalities appear to be an important predictor of impairment in motor performance (De Kegel et al., 2012). Furthermore, it has been shown that degree of motor impairment is negatively associated with degree of socialization among children with ASD, so that those with greater motor impairment display poorer socialization skills (Sipes et al., 2011). It seems then, that among children with ASD, abnormalities in VOR may correspond to motor impairments, which in turn affect socialization in this population. The neural circuitry underlying VORs have been thoroughly characterized in typical individuals (Leigh and Zee, 2006) and methodologies for investigating them are reliable and well established (Clarke, 2010). Despite reports of abnormal VORs among individuals with ASD (Ritvo et al.,

1969; Ornitz et al., 1985), VORs have not been characterized in this population. Characterization of abnormal VORs in ASD could provide useful insights for targeted sensorimotor interventions, and aid further understanding of specific aberrant neural circuits in this population. Our lab sought to perform an investigation further characterizing VORs in children with ASD.

In order to characterize VORs in children with ASD, we first performed oculomotor assessments in order to rule out gross oculomotor dysfunction as a factor contributing to potentially abnormal VORs. Oculomotor assessments are useful in the study of neurodevelopmental disorders, as assessments are relatively easy to perform and abnormal outcomes measures can provide insights into aberrant neural circuitry in these populations (Sweeney et al., 2004). Two of the most common oculomotor assessments are those of saccades and visual smooth pursuit.

A saccade is a quick, darting eye movement that occurs for both eyes in unison, typically to center a target on the retina. Saccades can be intentional, or reflexive to visual stimuli, and are characterized by fast acceleration to high velocity, followed by a quick deceleration of equal magnitude. Two parameters of saccades which are commonly studied are gain and latency. Saccade gain is the degree of displacement of the eye as compared to the degree of displacement for the target visual stimulus. Saccade latency is the time lag between appearance of a target visual stimulus and onset of saccadic eye movement to that stimulus.

Visual smooth pursuit entails a slower, gradual eye movement that serves to stabilize images on the retina during object motion. Smooth pursuit eye movements have much slower acceleration and velocity than do saccades, and cannot be performed in the absence of a moving visual stimulus. Two parameters of smooth pursuit which are commonly studied are gain and

phase lead/lag. Smooth pursuit gain is the maximum degree of displacement of the eye as compared to the maximum degree of displacement of the target visual stimulus, while smooth pursuit phase lead/lag (referred to hereafter as just "phase") is the mean degree of displacement by which the eye leads or lags behind the target stimulus during a trial.

Studies of these oculomotor behaviors in ASD have yielded mixed results. Minshew et al. (1999) reported no difference in visually guided saccade gain or latency among young adults with high-functioning ASD. More recently Takarae et al. (2004; 2008) demonstrated that participants with high-functioning ASD display significant abnormalities in visual smooth pursuit gain and latency (similar to phase, except measured in time as opposed to degrees). Although performance on saccade tasks was not significantly different from control participants, ASD participants did display greater variation in saccade gain. In addition, Kemner and colleagues (2004) report that children with high-functioning pervasive developmental disorder not otherwise specified (PDD-NOS) have typical saccade and smooth pursuit function.

Rosenhall and colleagues (1988) reported saccade hypometria (reduced gain) in 6 of 11 young adolescent participants with autism. These investigators also reported that although smooth pursuit function was found to be normal in 4 out of 11 participants with autism, 7 could not perform the task as instructed. Scharre and Creedon (1992) also attempted to assess voluntary smooth pursuit in children with autism, but 29 out of 34 participants displayed a series of saccades, rather than smooth pursuit, while tracking the target. Only 5 out of 34 children were able to successfully perform the task, but those data were not reported by the authors.

These studies indicate that high-functioning adults with ASD have relatively typical, if more varied saccade function, but abnormal visual smooth pursuit. However, it is unclear the

extent to which *children* with high-functioning ASD have altered saccade and smooth pursuit function. It may be that saccade hypometria is present in those with ASD at younger ages, and that this deficit resolves through maturation. The difficulty participants with autism had completing visual smooth pursuit tasks in Rosenhall et al. (1988) and Scharre and Creedon (1992) could have been related to intellectual disability, in that they had trouble understanding and following instructions. However, in light of the findings from Takarae et al. (2004; 2008), it could be that those participants were performing the task to the best of their ability and saccadic intrusions in the visual smooth pursuit task are part of the ASD population's oculomotor deficits, rather than a confound of low IQ reflecting gross central nervous system damage.

This project was designed to expand the growing body of sensorimotor investigations in ASD, which has previously received little attention. Reflex systems are optimal targets for sensorimotor research in ASD because they can be reliably assessed, have well understood neural circuitry, and well developed methodologies for investigation. Reflex systems are often modifiable, which make them amenable to interventions. VORs and oculomotor behaviors are established early in development and remain modifiable. As such, these systems are amenable to early identification and intervention, which has been shown to significantly improve the prognosis of children with ASD among other types of interventions (Dawson et al., 2010; Rogers et al., 1998). The vestibular system is functional at birth, and VORs can be reliably measured in infants at 6 months of age (Phillips and Backous, 2002). Saccade and visual smooth pursuit behaviors are also functional at birth and can be reliably assessed in infants at 4 weeks of age (Roucoux et al., 1983). Further characterization of VOR and oculomotor abnormalities in ASD will provide useful insight into aberrant neural circuitry, and contribute to an evidence base for targeted sensorimotor interventions.

The current investigation of oculomotor function was part of a larger project examining vestibulo-ocular reflex (VOR) function in children with ASD. The oculomotor tests described here were used as a screening tool to control for oculomotor dysfunction before a participant could enter the VOR phase of the project. The focus of this investigation was to assess visual smooth pursuit and visually guided saccade function in children with ASD, but without intellectual disability (IQ>70). A number of neuropsychological assessments commonly used among individuals ASD were also performed, and we explored possible relationships between oculomotor function and neuropsychological assessments. In addition to systematically replicating recent investigations of oculomotor function in high-functioning adults with ASD (Takarae et al., 2004; 2008), the present work extended studies of oculomotor function into the vertical plane, which has not previously been performed in a population with developmental disability.

METHODS

Participants

Participants included 16 children with ASD and 24 typically developing controls. Children were between 6 to 12 years of age at the time of testing. Children were recruited through advertisements distributed to Alachua county public schools, local clinics, and the University of Florida Center for Autism and Related Disabilities (CARD). Diagnoses were confirmed with the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and Social Communication Questionnaire (SCQ; Rutter, Bayley and Lord, 2003) administered by a clinical psychologist. Exclusion criteria for the ASD group were diagnosis of Fragile X, Rett Syndrome, tuberous sclerosis, seizure disorder or fetal cytomegalovirus infection. Exclusion criteria for the control group were any current or past history of psychiatric disorders. None of

the children included in the study were prescribed medications known to alter oculomotor function (e.g. Risperdal). Mean age for the ASD group was 104(±23) months, and mean IQ was 104(±20), with one high outlier of 159. For typically developing controls, mean age was 110(±21) months, and mean IQ was 106(±14). Children with an IQ below 70 were not included in this sample, in order to control for intellectual disability as a potential confound. See Table 1 for a summary of participants.

Experimental Setting: Space Mission

Given the number of assessments performed and their duration, some degree of participant attrition was expected. In an attempt to reduce participant attrition, we sought to increase the children's compliance and motivation to participate by providing a stimulating experimental setting. Walls in the room where oculomotor and vestibulo-ocular reflex testing were carried out were painted with the planets of the solar system and a space-shuttle. The assessments performed were incorporated into the modifiable storyline of a space-themed mission, with breaks and optional games for the participants between assessments. Children generally reported enjoying the experience, and parents described that many were excited to return when additional testing sessions were required.

Neuropsychological Assessment

For children included in the ASD group, diagnoses were confirmed with the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) administered by a clinical psychologist, and Social Communication Questionnaire (SCQ; Rutter, Bayley and Lord, 2003) completed by a parent or primary caregiver. A parent or primary caregiver of the participants in both groups was asked to complete the Repetitive Behaviors Scale-Revised (RBS-R; Bodfish et. al., 2000) and the Sensory Profile Caregiver Questionnaire (Dunn, 1999). Although there were

several outcome variables derived from the questions in the Sensory Profile Caregiver Questionnaire, only the Visual Processing sub-score was used in this investigation. See Table 1 for a summary of neuropsychological outcome measures.

Equipment

Video-oculography (VOG) was performed with the I-Portal® system from Neuro-Kinetics, Inc. (NKI). This system included VOG goggles, computer hardware, and VEST ™ 6.8 software for acquisition and analysis. This system sampled from both eyes, in real-time, at a rate of 100 Hz. Visual stimuli were presented as a red laser-light, generated by NKI Pursuit Tracker® laser. The Pursuit Tracker® laser was mounted on the underside of the circular platform to which the seating apparatus is mounted, and projected on a cylindrical arc of height 48 inches, with a radius of 76.5 inches.

Participants were seated in chair mounted on top of a rotary platform, constructed by NKI. The chair and seating arrangement were created by the authors for pediatric use and include a padded chair, safety harness, and head stabilizers with occipital head rest and temporal stabilizing arms to prevent head movements.

Calibration and Oculomotor Tests

Participants were individually calibrated to the testing equipment by projecting a laser stimulus onto the cylindrical screen and providing a fixed target at $\pm$ 10º in both the horizontal and vertical directions (e.g., first 10º to the left, then right, then up and then down). Calibration values were averaged across 2 trials. Two oculomotor tasks were then performed, saccades and smooth pursuit, which are described in the next section.

During saccade testing, participants were instructed to focus on the red laser stimulus projected onto the cylindrical screen, and to re-focus on the stimulus when it appeared at another location. Saccade testing included one trial of horizontal saccades with no vertical component, and one trial of vertical saccades with no horizontal component. In both horizontal and vertical conditions, 30 saccades were generated with random position between $\pm$ 25º and duration between 1-2 seconds; the same randomly generated set of saccades was used for each participant. Mean saccade gain and latency for each participant were determined using VEST™ 6.8 software.

During visual smooth pursuit testing, participants were instructed to focus on the red laser stimulus projected onto the cylindrical screen, and to maintain focus on the stimulus while it was moving. The stimulus for smooth pursuit testing moved in an oscillatory fashion; trials were performed at .10 Hz and .50 Hz for 6 cycles, between $\pm$ 10º. At each frequency, a trial was performed once in the horizontal direction and once in the vertical direction. Gain and phase lag/lead were determined for each smooth pursuit trial using VEST™ 6.8 software.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 21 software. T-tests were used to compare group differences in saccade gain, saccade latency, smooth pursuit gain, and smooth pursuit phase lead/lag. Subsequently, a stepwise linear regression was used to investigate whether outcome variables from neuropsychological assessments could predict oculomotor parameters that were found to be significantly different between groups. A significance level of $\alpha = .05$ was used for both t-tests and linear regressions.

RESULTS

Saccades

A significant group difference was identified for horizontal saccade latency (see Figure 1) [$t(25.130) = -2.32$, $p<0.05$]. No significant difference was identified between groups for vertical saccade latency, or saccade gain in either horizontal or vertical conditions. A stepwise linear regression was performed among ASD participants to investigate whether horizontal saccade latency could be predicted by ADOS communication plus social composite score, ADOS repetitive behavior composite score, ADOS combined score, RBS-R score, SCQ score, or Sensory Profile visual processing sub-score. From this regression, it was determined that SCQ score was a significant predictor [$\beta = -0.70$, $t(12)=3.288$, $p< .05$] of horizontal saccade latency among participants with ASD. For this model with SCQ score as a predictor, the adjusted $R^2 = .45$. Thus, about 45% of the variation in horizontal saccade latency among the ASD group could be explained by SCQ score. However, the direction of this relationship was unexpected, as higher SCQ score predicted lower horizontal saccade latency.

Smooth Pursuit

Levene's test for equality of variances indicated that there were significant differences in variance between groups for horizontal smooth pursuit gain at .10 Hz [$F = 9.51$, $p< 0.05$], horizontal smooth pursuit phase at .10 Hz [$F = 40.10$, $p< 0.05$], horizontal smooth pursuit gain at .50 Hz [$F = 8.25$, $p< 0.05$], vertical smooth pursuit phase at .10 Hz [$F = 4.11$, $p = 0.05$], and vertical smooth pursuit phase at .50 Hz [$F = 9.49$, $p< 0.05$]. T-tests revealed significant group differences for mean vertical smooth pursuit phase at 0.10 Hz [$t(14.88) = 2.22$, $p< 0.05$] (see Figure 2). There was a trending group difference for vertical smooth pursuit phase at 0.50 Hz [$t(20.06) = 1.86$, $p= 0.08$] (see Figure 2). No significant group differences were identified for

horizontal smooth pursuit gain or phase at either .10 Hz or .50 Hz. A stepwise linear regressions were performed among ASD participants to investigate whether either vertical smooth pursuit phase at .10 Hz could be predicted by ADOS communication plus social composite score, ADOS repetitive behavior composite score, ADOS combined score, RBS-R score, SCQ score, or Sensory Profile visual processing sub-score. This regression did not find any significant predictors for vertical smooth pursuit at.10 Hz among the included predictor variables.

DISCUSSION

This study was the first to use VOG to investigate saccade and smooth pursuit function in children with high-functioning ASD, without intellectual disability (IQ>70). By controlling for intellectual disability, results from this experiment reflect neurodevelopmental abnormalities specifically relevant to ASD, rather than general intellectual deficits. In addition, this study is the first to characterize vertical saccade and smooth pursuit in the ASD population. We found evidence of abnormal visual smooth pursuit phase in high-functioning children with ASD. We also found evidence that high-functioning children with ASD have a greater latency for initiating saccades.

Regarding visually guided smooth pursuit, we found a significant group difference in mean phase for vertical smooth pursuit at .10 Hz. We did not find significant differences in mean gain or phase for horizontal smooth pursuit conditions. In light of previous findings that mean gain during pursuit tasks were significantly different only among ASD participants older than 16 years (Takarae et al., 2004), it is not surprising that we found no group difference for pursuit gain in our sample, since all participants in this investigation were between 6 and 12 years of age. Our

finding of a significantly larger variance for horizontal smooth pursuit gain among children with high-functioning ASD is novel. This finding suggests that in addition to an adolescent maturational end-point for horizontal smooth pursuit gain, there is a subset of individuals with high-functioning ASD who have congenital or early maturational deficits in pursuit gain.

Our findings regarding pursuit phase are similar to those of Takarae et al. (2004; 2008) of pursuit latency (similar to phase, except measured in time as opposed to degrees). Their work suggests that differences in pursuit latency were only detectable after controlling for language delay (i.e. those with language delay have lower pursuit latency). Our findings of greater variance among ASD participants for horizontal smooth pursuit phase, but no group difference in *mean* horizontal smooth pursuit phase makes sense, as our study included children with *and* without language delay.

Our finding of greater mean vertical smooth pursuit phase among children with ASD are novel as we are, to our knowledge, the first investigators to measure vertical smooth pursuit in children with developmental disability. This pronounced difference in group means for phase in vertical, but not horizontal conditions, could relate to the relatively infrequent use of visual pursuit in the vertical plane. In other words, neither population has much experience tracking objects with continuous motion in the vertical plane, because such stimuli are relatively uncommon in daily experience. On the other hand, tracking of objects with continuous horizontal motion is fairly common. Because of the typical lack of experience tracking objects in the vertical plane, visual pursuit testing in this plane may reveal more about the naïve state of the underlying circuitry without as much influence through experience.

Smooth pursuit is a complex sensorimotor behavior that involves several spatially distant brain regions such as the frontal eye fields, lateral intra-parietal area, medial superior temporal area, caudate, superior colliculus, cerebellar vermis, brainstem premotor nuclei, and vestibular nuclei (for a review see Krauzlis, 2003). Several of these structures have evidence of abnormality among individuals with ASD (for a review see Courchesne et al., 2007). There is also evidence that individuals with ASD have abnormalities in long-fiber tracts between distal brain regions (Courchesne et al., 2005). Local abnormalities in the above brain regions implicated in smooth pursuit, or aberrations in long-fiber tracts connecting them could both play a role in abnormal smooth pursuit function among some individuals with ASD.

For some ASD participants pursuit phase lag was 10º in horizontal, or up to 20º in vertical conditions. Interestingly, the group difference in horizontal smooth pursuit phase variance and vertical smooth pursuit mean phase were more pronounced at the slower frequency of .10 Hz, where pursuit is typically more accurate (i.e. less phase difference between eye and target). This implies that for some participants the fovea was not on target, but rather, that the target was in the periphery. Participants from this study were part of a larger investigation characterizing VOR, performed with the same equipment. Remarkably, ASD participants were unable to significantly suppress post-rotary nystagmus with a foveal target stimulus displayed inside otherwise dark VOG goggles, but *were* able to suppress post-rotary nystagmus normally when the entire room was visible (Bleser-Carson et al., unpublished). Taken together, these results may indicate that foveal and peripheral visual streams may be utilized differently in people with ASD. Such difficulties in integrating foveal and peripheral visual information meshes well with sensory integration difficulties described in ASD (Tomcheck and Dunn, 2007). Future investigations among individuals with ASD to directly ascertain whether foveal and

peripheral visual streams can be utilized in an integrative fashion, comparable to neurologically typical individuals, seem warranted.

It is possible that abnormalities in smooth pursuit could cause functional impairments among individuals with ASD, such as altered spatial awareness in environments with moving stimuli. Indeed, previous work has shown that ASD participants with language delay had poorer performance than controls on a visual motion discrimination task, correlated with visual smooth pursuit performance (Takarae et al., 2008). In monkeys, it has been demonstrated that adaptive changes in smooth pursuit can be induced by training (Fukushima et al., 2001). The adaptive benefits of smooth pursuit training may extend to human subjects as well, and could perhaps improve deficits in visual motion discrimination as well.

Regarding saccades, this investigation found evidence that children with high-functioning ASD have a significantly greater latency for initiating horizontal saccades. This group difference in mean saccade latency did not extend to the vertical plane. Children with ASD had similar saccade latency for both horizontal and vertical conditions, whereas typically developing controls initiated saccades more quickly in the horizontal condition. These findings are in contrast with previous work indicating typical saccade function among high-functioning individuals with ASD (Minshew et al., 1999; Kemner et al., 2004). Although these previous investigations used random order of presentation for saccade stimuli, each included only 3 potential target locations, mirrored on each side, and a constant duration of presentation for each stimulus. In the current investigation, each saccade had a unique, randomly generated position and duration of presentation. Perhaps the greater difficulty of the saccade task in this investigation revealed mild deficits in latency to initiate a horizontal saccade in children with ASD that would not be detectable with more predictable stimuli.

Deficits in latency to initiate saccades in monkeys occurred after lesions to the frontal eye fields (Lynch, 1992). Among individuals with ASD, there is evidence of reduced functional connectivity from frontal eye fields and dorsal anterior cingulate cortex, both regions critical in oculomotor control (Kenet et al., 2012). Perhaps horizontal saccade latency deficits observed in this study are a reflection of neurodevelopmental abnormalities in frontal eye fields, or efferent targets. As to why these latency deficits did not extend to vertical saccades, it may be that the circuit implicated in vertical saccades includes some structures that do not overlap with the circuit involved in horizontal saccades, and that these are unaffected in this population. Work in monkeys show that lesions to the superior temporal poly-sensory area, which receives indirect afferents from the frontal eye fields via the medial pulvinar, negatively affected horizontal saccade latency but not vertical saccade latency (Scalaidhe et al., 1995).

We performed stepwise linear regression to investigate whether neuropsychological outcome measures could predict those oculomotor parameters found to be significantly different between groups. SCQ score was found to be a significant predictor of horizontal saccade latency, explaining 45% of the variation in horizontal saccade latency among participants with ASD. However, the direction of this relationship was unexpected, as higher SCQ score predicted lower horizontal saccade latency. Perhaps it is adaptive for individuals with greater social and communication deficits to develop shorter latency for initiating visually guided saccades, in order to derive more information about their surroundings from simple visual stimuli.

Other neuropsychological measures from this investigation were not found to be good predictors for those oculomotor parameters found to be significantly different between groups. It may be that the degree of severity of ASD diagnostic symptoms, indexed by ADOS scores, does not directly affect performance on these oculomotor parameters. Many of the neuropsychological

measures included in these regression models were completed with the aid of parents or caregivers, and thus may not be sensitive enough to function as predictor variables for those oculomotor parameters found to be abnormal in this study.

Future studies of oculomotor function in ASD should seek to confirm findings from this investigation regarding saccade and smooth pursuit function in the vertical plane. Although the current investigation focused on individuals with high-functioning ASD in order to control for general intellectual disability, the inclusion of participants with lower-functioning ASD will help to elucidate whether such oculomotor abnormalities generalize to all individuals with ASD. Furthermore, the selectivity of oculomotor abnormalities to ASD can be addressed by future studies that include comparisons of oculomotor abnormalities among other developmentally disabled populations (e.g. Down Syndrome) alongside individuals with ASD.

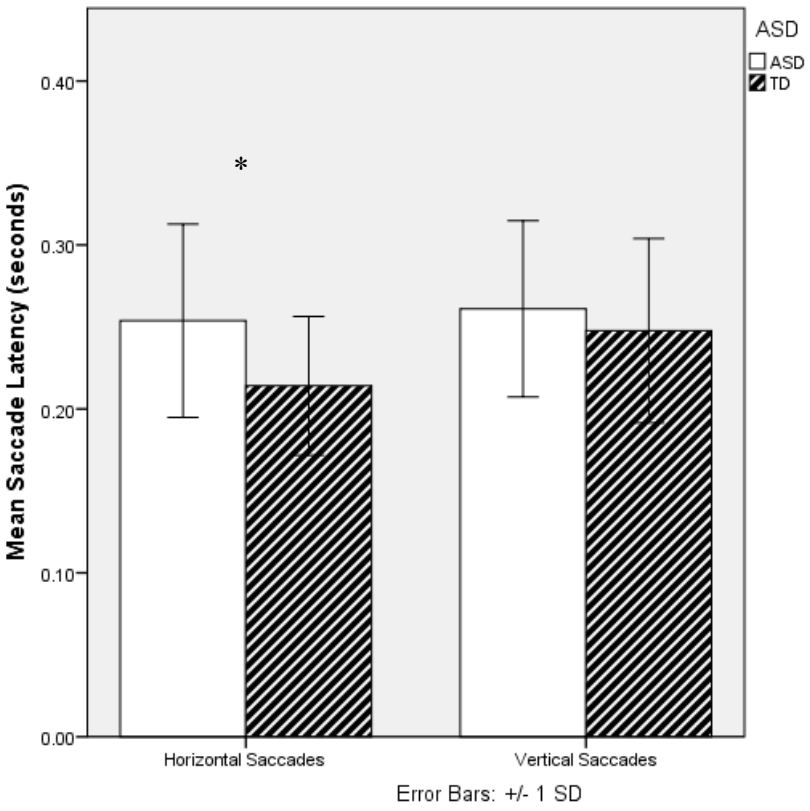

Figure 1: Mean latency (seconds) for horizontal (left) and vertical (right) saccades. A significant group difference was identified between groups for horizontal, but not vertical saccades.

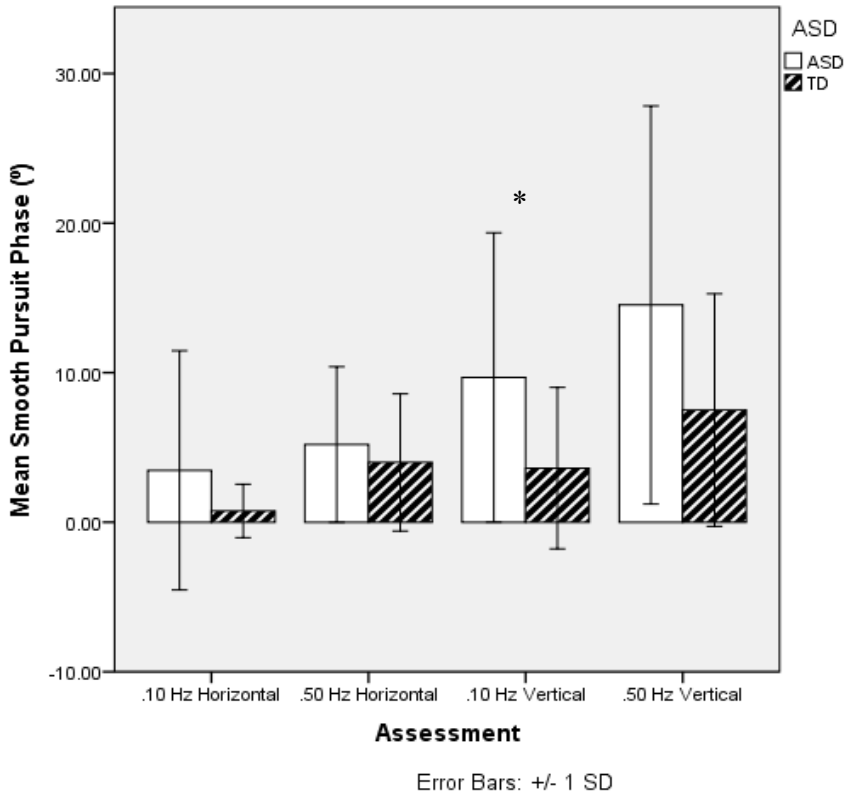

Figure 2: Mean phase (degrees) for horizontal (left) and vertical (right) visually guided smooth pursuit. A significant group difference was identified for vertical smooth pursuit at .10 Hz.

Participant ID	Age[a]	IQ[b]	ADOS (CS)[c]	ADOS (R)[d]	ADOS (total)[e]	SCQ[f]	RBS-R[g]	Sensory Profile (visual)[h]
A001	120	85	6	2	8	.	38	28
A002	90	87	9	2	11	29	62	22
A003	107	115	7	1	8	18	56	35
A005	99	111	9	2	11	19	6	30
A006	74	111	19	6	25	6	2	41
A007	125	102	25	4	29	19	25	39
A010	99	71	16	6	22	24	15	22
A011	145	87	8	0	8	24	27	28
A013	153	85	13	1	14	32	.	23
A014	98	100	7	1	8	16	49	41
A015	84	102	12	2	14	22	62	33
A016	102	115	7	2	9	31	77	30
A017	74	115	7	1	8	21	62	35
A021	94	159	10	5	15	24	31	33
A022	118	105	7	1	8	14	49	.
A023	89	107	7	2	9	9	5	36
T001	79	135	.	.	.	7	4	.
T002	90	100	.	.	.	.	.	.
T003	108	93	.	.	.	.	.	.
T004	105	102	.	.	.	1	5	36
T005	122	107	.	.	.	2	3	39
T006	138	124	.	.	.	2	3	34
T011	106	105	.	.	.	7	1	35
T012	122	119	.	.	.	3	0	43
T013	96	98	.	.	.	3	4	.
T014	144	107	.	.	.	3	20	.
T015	111	111	.	.	.	0	0	45
T016	111	95	.	.	.	3	5	35
T019	120	109	.	.	.	4	0	44
T020	149	89	.	.	.	0	0	45
T021	77	95	.	.	.	3	0	43
T022	111	102	.	.	.	1	0	45
T023	101	129	.	.	.	1	3	41
T024	73	111	.	.	.	0	2	39
T025	92	103	.	.	.	2	1	43
T026	129	129	.	.	.	0	0	41
T028	120	77	.	.	.	0	2	32
T030	117	89	.	.	.	3	1	41
T031	143	91	.	.	.	6	1	39
T032	85	113	.	.	.	2	0	39

Table 1: Age, IQ, and neuropsychological scores from participants. TD participants were not given the ADOS. Three participants are missing SCQ or RBS-R data, due to parents not completing these questionnaires.

a. Age in months

b. Leiter non-verbal IQ score

c. ADOS communication + social subscores

d. ADOS repetitive behavior subscore

e. ADOS combined score

f. Social Communication Questionnaire

g. Repetitive Behaviors Scale - Revised

h. Sensory Profile visual processing subscore

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March 12, 2014

TO: Keith D. White, PhD; Krestin Radonvich; Tana Bleser
PO Box 112250
Campus

FROM: Ira S. Fischler, PhD; Chair
University of Florida
Institutional Review Board 02

SUBJECT: **Exemption of Protocol #2010-U-0049**
Abnormal Vestibulo-Ocular Reflexes in Autism: A Potential Endophenotype

SPONSOR: Department of Defense: Congressionally Directed Medical Research Programs, Autism Idea Award

Because the portion of this research that involved human participant contact has been completed and no further participants will be recruited, it is exempt from further review by this Board as not human subjects research in accordance with 45 CFR 46.102(f) *Human subject means a living individual about whom an investigator (whether professional or student) conducting research obtains: (1) Data through intervention or interaction with the individual, or (2) Identifiable private information.*

Should the nature of your study change or if you need to revise this protocol in any manner, please contact this office before implementing the changes.

IF:dl

VESTIBULO-OCULAR REFLEX FUNCTION IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

By

TANA BLESER CARSON

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2013

To my cousin, Cheryl, my friend and inspiration

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LIST OF ABBREVIATIONS

ASD	autism spectrum disorders
EOG	electro-oculography
HFA	high functioning autism
IDD	intellectual developmental disability
IQ	Intelligence quotient
PANESS	physical and neurological exam of subtle signs
PDD-NOS	pervasive developmental disorder- not otherwise specified
RBS-R	restricted repetitive behavior scale revised
SCQ	social communication questionnaire
SHA	sinusoidal harmonic acceleration
SP	sensory profile
TD	typically developing
TCD	time constant of decay
VNG	video nystagmography
VOG	video-oculography goggles
VOR	vestibulo-ocular reflex
rVOR	rotational vestibulo-ocular reflex
tVOR	torsional vestibulo-ocular reflex

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

VESTIBULO-OCULAR REFLEX FUNCTION IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

By

Tana Bleser Carson

December 2013

Chair: Mark Lewis
Major: Psychology

Autism spectrum disorders (ASD) are characterized by social and communication skill deficits and excessive restricted/repetitive behaviors (APA, 2000; DSM-IV TR). Additional features commonly noted in ASD are deficits in sensory processing and motor control. Some motor control differences reported in ASD include decreased postural stability, decreased muscle tone, altered vestibulo-ocular reflexes (VORs) and delayed motor milestones (Fournier et al., 2010; Ben-Sasson et al., 2009; Bhat et al., 2011; Ornitz et al., 1985). These deficits may share a common dependence upon processing of vestibular sensory input. Therefore a better understanding of the extent to which vestibular sensory processing and related motor control is compromised in ASD would be beneficial for studying the underlying neurobiology of ASD. The VOR is useful for studying vestibular related sensory motor processing in this population as it involves a relatively simple reflex system, amenable to study in very young children. The rotational vestibulo-ocular reflex (rVOR) functions to maintain stable vision by generating oculomotor responses to angular rotation head movements. Our understanding of these VOR differences in ASD is currently hindered by a paucity of studies investigating this reflex. In the current studies, children ages 6 – 12 diagnosed

with autism spectrum disorders observed three main differences in rVOR metrics including: (1) increased time constant of decay of post-rotary nystagmus during velocity step tests in the dark and with fixation suppression (Chapter 2); (2) increased per-rotary nystagmus gain during velocity step tests in the dark and increased gain during sinusoidal harmonic acceleration (SHA) tests in the dark as well as fixation suppression (Chapter 2 and 3 respectively); and (3) increased phase lead during SHA tests at 0.5Hz frequency cycle (Chapter 3). Several functional measures were found to correlate with rVOR time constant differences in ASD such as: adaptive skills and balance deficits (Chapter 4). Additional aberrations in the quality of rVOR such as increased slow phase irregularities are described and methods for further analysis of these differences are discussed (Chapter 5). The current findings suggest vestibular processing deficits in the rVOR that are consistent with reports of cerebellar deficits in the ASD population and warrant further study.

CHAPTER 1
INTRODUCTION

Vestibular Sensorimotor Processing in Autism Spectrum Disorders

Abnormal sensory processing and motor control have been frequently reported in autism spectrum disorders (ASD or referred to as “autism” collectively hereafter). Motor deficits include reports of decreased postural stability, decreased muscle tone, and delayed motor milestones (see Fournier et al., 2010; Ben-Sasson et al., 2009; Bhat et al., 2011 for reviews). Acquisition of such motor skills as the ability to maintain upright posture, the development of skeletal muscle tone and coordination of locomotion are dependent on normal vestibular function (Phillips & Backous, 2002). Studies of dynamic posturography, a sensory-selective method for evaluating the functional integration of these systems, have shown that maintaining postural stability is significantly more difficult for children with ASD, particularly when vision is occluded and proprioceptive cues are diminished, forcing participants to rely on vestibular sensation (Minshew et al., 2004; Molloy, Dietrich & Bhattacharya, 2003). The vestibular system contributes to a body-centered spatial coordinate system onto which sensory modalities such as proprioception and vision are mapped in the posterior parietal cortex (Anderson, 1997). Therefore, successful maintenance of posture is dependent on sufficient integration of vestibular, visual, and proprioceptive sensory input with appropriate motor output via brainstem, cerebellum, and parietal lobe circuitry and may be deficient in individuals with ASD. Recent imaging, histological, and behavioral studies show abnormalities in these regions of the brain in individuals with ASD (see DiCicco-Bloom et al., 2006 for review), which also correlate with sensory processing deficits observed in this population (Jou et al., 2008). In summary, abnormal processing of vestibular sensory

input could contribute to several of the sensory motor processing deficits commonly observed in ASD.

The Vestibulo-ocular Reflex

In addition to postural responses, the vestibular system also drives oculomotor responses known as vestibulo-ocular reflexes (VORs). VORs are important for maintaining stable vision during movement of the head and body and have been very well studied in both intact and lesioned animal experiments and human studies. The VORs are compensatory eye movements that occur in response to head movement (see diagram in Figure 1-1 for an example of the simplest form of VOR circuitry). Several forms of VOR exist including translational and rotary. Translational VOR (tVOR) produces compensatory eye movements in response to otolith stimulation from linear motion of the head in the forward-aft, side-to-side or up and down directions. Ocular counter-roll, a second type of VOR occurs in response to the otolith organs sensing a change in the static relationship between the head and gravity. For example, when the head is tilted to one side the VOR allows the eye to counter-roll or roll in the opposite direction of head movement in order to compensate for static head tilt (Leigh & Zee, 2006). The current study will focus on rotational VOR (rVOR), a compensatory eye movement that occurs in response to angular rotation of the head in one of three planes of movement: yaw, pitch or roll (see Appendix A for related glossary of terms). For example, when the head rotates in yaw to the left, the vestibular system translates information about that movement such as the acceleration and direction and commands a compensatory eye movement to occur in the opposite direction (i.e., yaw to the right). This oculomotor response to head movement allows maintenance of stable visual fixation during movement of the head/body.

After prolonged rotation of the head (e.g., when a child spins continuously on a merry-go-round), the oculomotor response is prolonged and occurs in the form of repetitive eye movements or nystagmus. Vestibular nystagmus eye movements are composed of two phases: a slow phase eye movement occurs in the opposite direction of head rotation followed by a quick phase eye movement that “resets” the eye back to center. These two phases occur in a repetitive, oscillating fashion (Figure 1-2).

Two methods of rVOR testing will be used in the current study: velocity step tests and sinusoidal harmonic acceleration tests (SHA). For the current studies, these tests will be conducted with chair rotations about an earth vertical axis, providing rotational vestibular stimulation in the yaw plane (Figure 1-3). Velocity step tests include a rapid acceleration, “step”, up to a constant velocity of rotation for a set duration of time followed by a rapid deceleration to stop (see Figure 1-4 for an illustration of this type rotary chair motion profile; see Chapter 2 for detailed methods). SHA testing includes side to side, oscillating rotations back and forth with a set peak velocity. This test included several trials conducted at a range of frequency cycles of rotation (see Figure 1-5 for an illustration of this type of rotary chair motion profile; see Chapter 3 for detailed methods).

There are two types of vestibular nystagmus that occur as a result of the continuous rotation of the head and body *en bloc* (i.e., when spinning the whole body); per- and post- rotary nystagmus. During rotation, the slow phase moves in the opposite direction of head rotation followed by a quick/fast phase in the same direction of head rotation, thus resetting the eye to center. This nystagmus that occurs during rotation is called per-rotary nystagmus. For example, during clock-wise yaw rotation, the slow

phase moves counter clock-wise to compensate for moving away from the previous visual stimuli and the fast phase moves clock-wise to quickly reset the eye to center. As rotation reaches a constant velocity, the cerebellum stores this velocity information in an effort to increase the efficiency of the rVOR response during low frequency stimulation such as this and prolongs per-rotary nystagmus for a measurable amount of time known as the time constant of decay (TCD; Raphan, Matsuo & Cohen, 1979). After rotation has stopped, a second form of nystagmus called post-rotary nystagmus will occur. Although the body and head have stopped rotating, the fluid in the semicircular canals continues to move until friction against the membrane of the canals eventually slows the fluid down. Therefore, the vestibular system is excited in the opposite direction and, when visual cues are omitted, may give the individual the perception that he/she is now spinning in the opposing direction even though he/she is stationary at this time. Post-rotary nystagmus also has a measurable time constant of decay. The TCD reflects the cerebellum's ability to modulate vestibular nuclei velocity storage mechanisms (Waespe & Henn, 1977; Raphan et al., 1979). Normative data for TCD in children have been reported to range from 13 to 17 seconds in children ages 4-12 years (Horak et al., 1988; Casselbrant et al., 2010).

Another measurable feature of rVOR is the gain. Gain is a measure of the accuracy of the rVOR system reactions to changes in head position. To do so, the rVOR must compensate for head movements with eye movements that are equally matched in velocity to head movements in order to prevent instability of the retinal image. Therefore, gain is measured as the ratio of peak eye velocity to peak head velocity. If eye and head velocity are perfectly matched the rVOR gain is 1.0. If there is an error in

this system as small as 2 degrees / second, cerebellar modulation of the vestibular nuclei will detect and correct such errors by modifying vestibular nuclei output. For example, a head movement of 100 degrees/second would require that the rVOR gain must be at least 0.98 to prevent retinal image slip and blurred vision (Hain and Helminski, 2007). In individuals with cerebellar deficits (Thurston et al., 1987), gain may be increased reflecting the inability for the cerebellum to detect and correct for differences between eye and head velocity. Since cerebellar deficits have been one of the most consistent neurobiological findings in ASD, gain is certainly a measure of interest in the ASD population.

The rVOR is an excellent example of sensory and motor processing that holds considerable promise for studying such processes in ASD. The extensive body of literature outlining both healthy and aberrant rVOR function provides an excellent platform from which to study aberrations in brainstem, cerebellar and cortical sensorimotor processing in ASD. However, the number of studies that have systematically investigated the characteristics of rVOR in this population is very limited.

Vestibulo-ocular Reflex Studies in Autism

Three studies have reported aberrations in rVOR in ASD (Ritvo et al., 1969; Ornitz et al., 1974; Ornitz et al., 1985) and two studies have reported some typical characteristics of rVOR in this population (Ornitz et al., 1985; Goldberg et al., 2000). According to these reports, aberrations include: (a) decreased duration of post-rotary nystagmus when tested in conditions where light and/or visual stimuli are available (Ritvo et al., 1969; Ornitz et al., 1974), (b) decreased frequency of post-rotary nystagmus beats (Ornitz et al., 1985, Ornitz et al., 1974), (c) differences in per- and post-rotary time constants of decay (Ornitz et al., 1985), (d) increased incidences of

abnormal slow phase excursion (Ornitz et al., 1985) and (e) increased within-subjects variability in the frequency of rVOR nystagmus beats as a function of the duration of rVOR (Ornitz et al., 1974) compared to typical controls.

Two characteristics of rVOR in ASD have been shown to be no different from controls. The first characteristic is gain. Only one study examined gain in ASD (Ornitz et al., 1985) and found no difference between ASD and typical controls on this measure when tested in the dark. Healthy gain suggests that the peripheral vestibular organ itself is functioning properly in ASD and that other aberrant characteristics of rVOR observed in the study were likely attributable to central nervous system rather than peripheral pathology.

The second typical feature of rVOR examined in the ASD population is tilt suppression. Tilt suppression is a phenomenon that results in a decrease in rVOR time constant of decay in response to a change in head position (i.e., tilting or leaning forward) after continuous rotation. In a study of children with high functioning autism (HFA) there was no difference between HFA and typical controls (Goldberg et al., 2000). Normal tilt suppression in HFA suggests that vestibulo-cerebellar function in this sub-group of ASD may be spared. However, up to 68% of individuals with ASD have been estimated to have intellectual disability (Yeargin-Allsopp et al., 2003) and tilt suppression in individuals who are lower functioning has not been investigated. Questions remain as to whether or not cerebellar related tilt suppression deficits may exist in the lower functioning individuals with ASD.

Thus, the nature of and the neural mechanisms responsible for rVOR abnormalities in ASD and whether or not these aberrations exist in ASD as a whole or

within sub-groups with low IQ only remains unclear. There are several advantages to studying rVORs in an effort to answer such questions. First, rVORs are well studied in both human and animal models and provide a wealth of normative data as well as clinical data for comparison. Second, rVORs can be measured in low functioning individuals as well as young infants; thus, this reflex lends itself well to being studied across the spectrum of functional levels under the umbrella of ASD diagnoses as well as offering potential biobehavioral markers for further study in young children at risk for ASD. Third, rVORs are highly modifiable, so by better characterizing rVOR dysfunction in ASD, we may be better able to development rVOR sensorimotor interventions for this population.

Specific Aims

The overall objective of the present studies was to provide a comprehensive, detailed and systematic evaluation of rVOR function from the horizontal semicircular canals via rotational stimuli in children with ASD. The studies described in Chapters 2, 3, 4 and 5 were developed to achieve the following specific aims:

Specific Aim 1: To identify alterations in function from the horizontal semicircular canals via rotational stimuli in ASD. We aimed to replicate and extend previous findings of horizontal rVOR aberrations including decreased frequency of nystagmus beats and increased slow phase eye movement aberrations in children with ASD compared to typically developing controls. Two standard rotary chair tests were conducted: (1) velocity step test and (2) sinusoidal harmonic acceleration (SHA) tests. Velocity step tests have been conducted in ASD previously; however, those studies did not use a video-oculography goggle (VOG) system, therefore, our studies aimed to extend previous findings with the use of these methods (see Chapter 2 for detailed methods).

SHA has never been studied in ASD and were conducted to explore whether aberrations in SHA measures exist in the ASD population (see Chapter 3 for detailed methods).

> *Hypotheses: Velocity step tests will show significantly altered rVOR response in ASD as evidenced by decreased number of nystagmus beats and increased frequency of slow phase errors. SHA tests will be explored for possible differences in gain, phase and symmetry between groups. Based on preliminary results, it is expected that ASD groups will exhibit increased frequency of vertical slow phase eye movements in both tests.*

Specific Aim 2: To determine differences in fixation suppression of rVOR in ASD and TD groups. Children with ASD have previously demonstrated greater than normal suppression of rVOR time constants when experiencing velocity step testing in a lighted room (Ritvo et al., 1969) or when presented with various visual stimulus conditions after rotation (Ornitz et al., 1974). To replicate and extend these findings velocity step tests were conducted in light, dark and fixation suppression conditions. The fixation suppression condition visual stimulus was provided by an LED visual stimulus within the VOG system during and after rotation to test whether children with ASD exhibit any differences in fixation suppression of rVOR. Fixation suppression during SHA tests have never been conducted in ASD and were conducted to explore possible differences between groups in fixation suppression for this test.

> *Hypothesis: Based on previous findings, it is expected that children with ASD will demonstrate significantly greater rVOR suppression compared to controls as evidenced by significantly decreased time constants of decay in both the light and fixation suppression conditions and no difference in time constants in the dark conditions.*

Specific Aim 3: To identify correlations between rVOR and functional measures in ASD. If alterations in rVOR are correlated with functional ability (i.e., IQ) or other

measures of sensory/motor function, then rVOR may hold promise as an early marker or risk factor for the development of ASD.

> *Hypothesis: We predict that functional measures such as: (1) the severity of ASD symptoms,(2) functional ability (intelligence quotients and adaptive scales), (3) vestibular processing measures may be correlated with alterations of rVOR performance in ASD.*

Overview of Studies Presented in this Dissertation

Chapter 2: Effect of Visual Conditions on Rotational Vestibulo-ocular Reflex Function in Autism Spectrum Disorders. Chapter 2 reports the results of velocity step testing results conducted in three conditions including: light, dark and fixation suppression in ASD compared to TD controls. This study addresses two of the specific aims listed above as follows: (1) to identify alterations in function from the horizontal semicircular canals via rotational stimuli in ASD and (2) to determine differences in fixation suppression of rVOR in ASD and TD groups.

Chapter 3: Sinusoidal Harmonic Acceleration (SHA) Tests of Vestibulo-ocular Reflex Function in Autism Spectrum Disorders. Chapter 3 describes the results of SHA testing conducted in dark and fixation suppression conditions in ASD compared to TD controls. This study addresses two of the specific aims listed above as follows: (1) to identify alterations in function from the horizontal semicircular canals via rotational stimuli in ASD and (2) to determine differences in fixation suppression of rVOR in ASD and TD groups.

Chapter 4: Functional Correlates of the Vestibulo-ocular Reflex in Autism Spectrum Disorders. Chapter 4 describes correlation analyses between time constant of decay during velocity step test results in the dark and suppression conditions and other measures such as intelligence, autism severity and vestibular processing. This

study addresses specific aim (3): to identify correlations between rVOR measures and functional measures in ASD.

Chapter 5: Abnormal Quality of the Rotational Vestibulo-Ocular Reflex in Autism Spectrum Disorders. Chapter 5 describes qualitative differences in rVOR between groups and provides a preliminary analysis of methods for comparing the temporal dynamics of rVOR between ASD and TD groups. This study addresses specific aim (1): to identify alterations in horizontal rVOR in ASD.

General Methods

Participants and Recruitment

For the studies presented in Chapters 2-5, 16 children with ASD and 17 typically developing (TD) children, ages 6-12, were recruited to participate in functional assessments including ASD diagnostic assessments, neuropsychological assessments, as well as assessments of sensory processing and motor function in addition to tests of rVOR function. Data from the same children with ASD and TD children are presented across all chapters; therefore, the participant data presented in each chapter do not represent different sets of subjects or repeated testing.

Participants were recruited from the University of Florida Center for Autism and Related Disabilities as well as local community resources such as schools and medical centers within Alachua county, Florida. The ASD group included individuals with one of the following diagnoses: Autism, Asperger's syndrome, or Pervasive Developmental Disorder - Not Otherwise Specified. Diagnoses were confirmed by a clinical neuropsychologist. Exclusionary criteria include diagnoses of Fragile X, Rett Syndrome, tuberous sclerosis, seizure disorder or fetal cytomegalovirus infection. Exclusionary criteria for control participants included parent report of any current or past history of

psychiatric or neurologic disorder or any immediate family with a history of autism, schizophrenia, developmental disorder, mood disorder or anxiety disorder, or if they were taking any psychiatric medications. These exclusionary criteria were selected in an effort to minimize confounding variables and to better define the ASD and TD groups by excluding disorders with known etiologies such as Fragile X, Rett Syndrome, tuberous sclerosis, and fetal cytomegalovirus infection and to reduce the risk of any harm to participants as seizures have been reported to be elicited by vestibular stimulation. Since children with ASD frequently take psychiatric medications for concurrent disorders, medication status and concurrent disorders were documented for participants in the ASD group for subsequent analyses. All demographic and neuropsychological test results are presented in Chapter 2, Table 2-1.

General Procedure

All four studies were conducted using the same study paradigm (Figure 1-5) composed of three main steps. Each of the four studies (Chapters 2, 3, 4 and 5) present data collected on the same cohort of children with the exception of a few participants that completed some, but not all tests. Data are presented for those studies that participants were able to complete; therefore a minor difference in the number of subjects exists for each study. The entire study required that participants attend the lab for 2 to 3 testing sessions, as needed to complete all tests.

During Step 1 of the general study (Figure 1-5; Step 1), participants were recruited through community centers with flyers. Interested parents called or emailed the lab and were asked to complete a phone screening questionnaire to assess whether or not their child was eligible to participate in the studies (see Participants and Recruitment section above for inclusion/exclusion criteria).

If the child was eligible to participate they were scheduled to attend their first testing session (Figure 1-5; Step 2). During the first testing session, each child participated in neuropsychological testing and oculomotor screening. For neuropsychological testing children from both groups participated in the following two assessments: (1) the Leiter International Performance Scale – Revised (Leiter-R) Brief IQ test (Roid, Miller, & Leiter, 1997), a non-verbal intelligence assessment that allows children to indicate their responses through gestures or manual selection and (2) the Physical and Neurological Examination for Soft Signs (PANESS), a standardized pediatric neurological assessment of motor control (Denckla, 1974) where children completed motor tasks such as rapid finger tapping or walking heel-to-toe. Children from the ASD group only participated in the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), a semi-structured play-based assessment of social and communication skills and restricted repetitive behaviors. During these assessments, parents/guardians of participants in both groups completed the following questionnaires: the Repetitive Behavior Scale-Revised (RBS-R; Bodfish et. al., 2000), the Sensory Profile Caregiver Questionnaire (Dunn, 1999) and the Vineland-II Adaptive Scales (Sparrow & Cicchetti, 1985; Sparrow, Cicchetti, & Balla, 2005). Parents from the ASD group also completed the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003) a diagnostic screening tool for ASD. The combination of ADOS and SCQ was used to confirm diagnoses of children recruited into the ASD group. A combination of Vineland-II Adaptive Scale and Leiter-R IQ results was used to determine presence or absence of intellectual disability. The final part of Step 2 included calibration of the VOG system and oculomotor screening for each participant (see Chapter 2 for

methods). This first testing session took approximately 60 to 120 minutes to complete depending on group assignment, participant compliance and endurance for tests.

If participants were able to successfully complete the first testing session (i.e., if there was no aversion to wearing the VOG goggles, no gross oculomotor impairments and no behavioral or compliance barriers to participation), they were scheduled to return to the lab within 1 to 8 weeks for a second testing session (Figure 1-5; Step 3). The second testing session included velocity step and SHA tests of VOR function (see Figure 1-6 for schematic of rVOR testing sequence). Velocity step tests were conducted first and included 2 trials (one in each direction) within 3 conditions as follows: (i) light, (ii) dark, and (iii) fixation suppression (see Chapter 2 for detailed methods) resulting in a total of 6 trials of velocity step tests. SHA tests followed velocity step testing and were conducted within two conditions, first in the dark condition, then in the suppression condition. Within each condition one trial was conducted at each of 3 frequency cycles as follows: 0.05, 0.10 and 0.50Hz resulting in a total of 6 trials of SHA testing (see Chapter 3 for detailed methods). The rVOR testing session took approximately 90 to 120 minutes to complete. Several children in the ASD group required the vestibular testing session to be broken up into two shorter sessions. In this case, these children attended the lab for a total of 3 testing sessions in order to complete all tests. Upon completion, participants were provided with a $50 gift card to Wal-Mart or Target stores for their participation whether they completed all tests or not.

Innovation

Conceptual Innovation. It is clear that the neural processes which result in autism spectrum disorders (ASD) occur very early in development before the presentation of the classic symptoms of the disorders such as deficits in

social/communication skills and excessive restricted, repetitive behaviors. If the abnormal eye movements observed in this study are specific to ASD, they could provide a novel as well as simple and reliable, early identifier of risk for ASD. In addition, this aberrant reflex could provide a novel approach to patient-treatment matching for specific balance and visual rehabilitation treatment methods. Finally, if these eye movement abnormalities are present in only a sub-group of ASD, then rVOR may be a novel way to differentiate among sub-groups on the autism spectrum.

Methodological Innovation. Although sinusoidal harmonic acceleration (SHA) tests and SHA tests with fixation suppression are standard rVOR assessments, these have not been evaluated in ASD to date. SHA fixation suppression testing may help improve our understanding of the large effect of significantly decreased post-rotary nystagmus in ASD when tested when visual feedback is presented (Ornitz et al., 1974). It is possible that visual hyper-vigilance observed in children with ASD may allow them to visually suppress nystagmus to a greater extent compared to typically developing children. Conducting SHA tests with a controlled visual stimulus such as the light emitting diode (LED) stimulus provided in the fixation suppression tests represents an innovative method to better understand this phenomenon in ASD.

Technical Innovation. The novel pediatric rotary chair equipment developed for the current study is a combination of a customized pediatric chair designed in our lab specifically for use with children with ASD and a standardized videonystagmography and computer controlled motor system from NeuroKinetics, LLC., an international manufacturer of high quality clinical vestibular assessment equipment. The customized pediatric chair was designed in our lab to allow children with ASD to be tested

independently, rather than requiring these children to sit on an adult's lap. The chair was also designed to resemble a flight chair for a space mission theme to increase compliance and comfort for children with the testing procedures. The lab environment, rotary chair and testing protocol have been integrated into a space themed mission with graded activities during breaks to keep children engaged and to minimize anxiety.

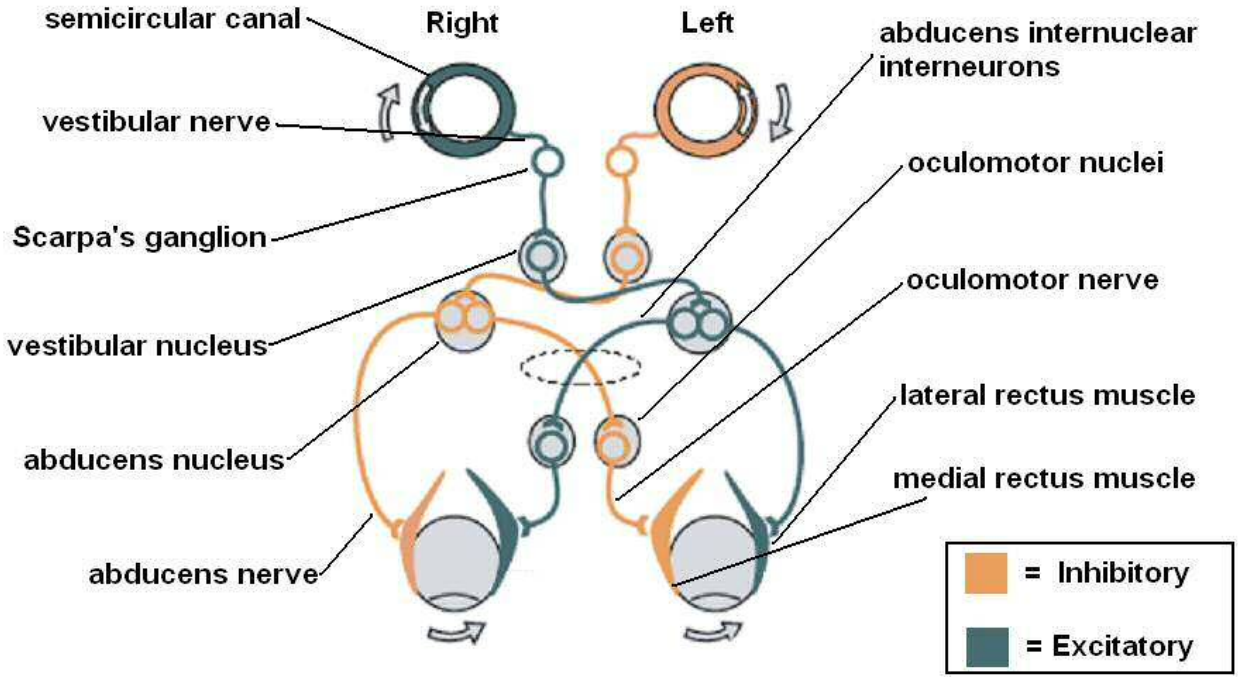

Figure 1-1. Basic circuitry of the rVOR. This image illustrates rVOR slow phase eye movement response to clock-wise head rotation in the yaw plane. Sensory hair cells within the two semicircular canals (top of image) are stimulated when the fluid lags the head rotation. This stimulation sends an ipsilateral (right sided) excitatory signal to the vestibular nuclei. Excitatory neurons project to the contralateral abducens nucleus which signals the right oculomotor nucleus to initiate contraction of the right medial rectus muscle as well as the left eye lateral rectus, thus counter-rotating the both eyes to the left in order to compensate for the clock-wise yaw head rotation. The left semicircular canal provides an inhibitory response along the parallel pathway and inhibiting the opposing ocular muscles and allowing the slow phase eye movement to occur and maintain a stable visual field. Figure provided by User: Mikael Häggström (Image:ThreeNeuronArc.png) [GFDL (http://www.gnu.org/copyleft/fdl.html) or CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0/)], via Wikimedia Commons on July 11, 2013.

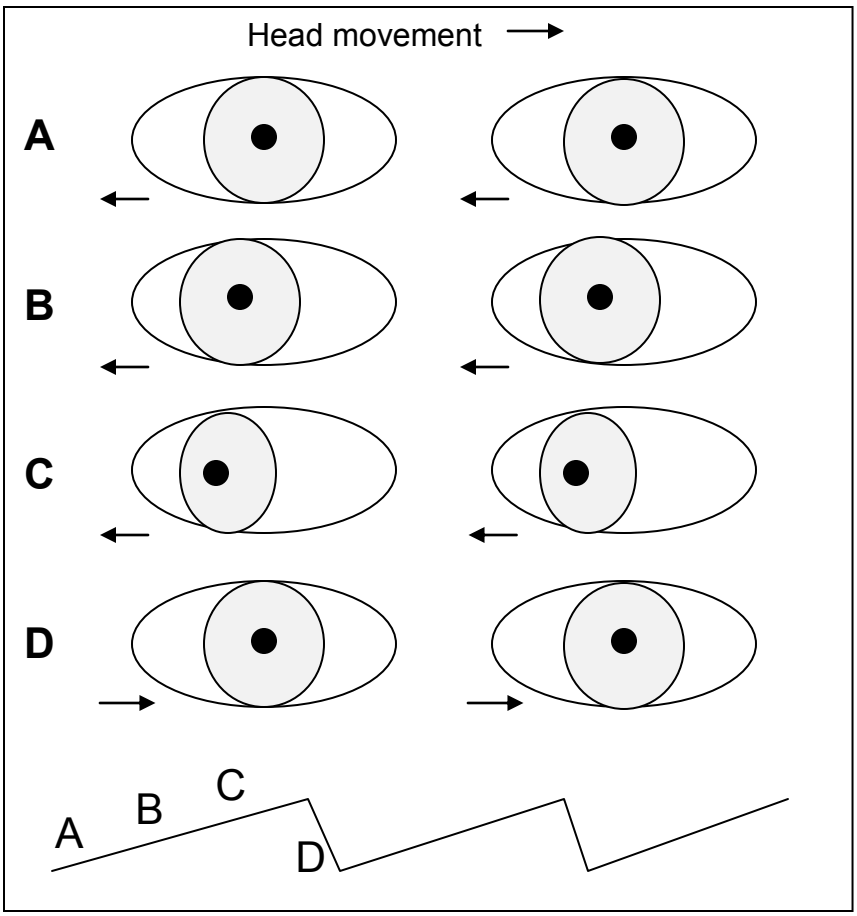

Figure 1-2. Illustration of a Nystagmus Beat. If the subject is facing you and rotating his/her head in the yaw plane to the left, their eye would exhibit a slow phase eye movement to the right (A-C) followed by a quick/fast phase eye movement to the left (D). The tracing below the eye diagram is an example of the eye movement tracing provided by video-oculography wherein the software tracks the pupil as it moves through this nystagmus beat. The tracing is labeled according to the eye movement diagram above (A-D). Rightward eye movements are reflected as a positive or upward change in position and leftward eye movements are reflected as negative or downward change in position.

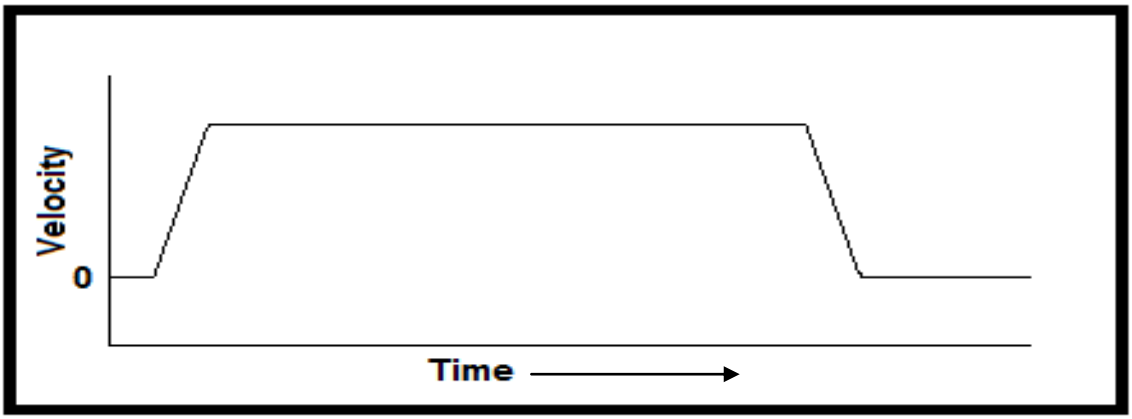

Figure 1-3. Illustration of Velocity Step Rotary Chair Motion Profile. This diagram depicts the motion of the rotary chair during a velocity step testing trial. The chair begins at 0º/sec and rapidly accelerates rotation in one direction (clock-wise or counter clock-wise) to a constant velocity (e.g., 100º/sec) which is maintained for a set duration of time, followed by rapid deceleration back to 0º/sec. Per-rotary nystagmus is observed during this constant velocity rotation and post-rotary nystagmus is observed after rotation has stopped.

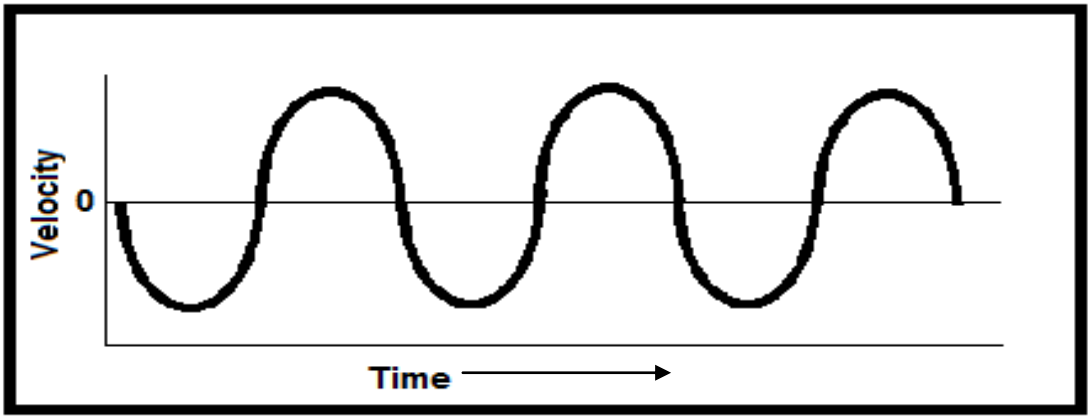

Figure 1-4. Illustration of SHA Rotary Chair Motor Profile. This diagram depicts the motion of the rotary chair during a single SHA testing trial. The chair begins at 0º/sec and accelerates rotation in one direction to a set peak velocity (e.g., 60º/sec) followed by deceleration back to 0º/sec. This same acceleration and deceleration profile is then repeated in the opposite direction, resulting in a sine wave velocity profile and a back and forth motion experience by the participant.

Figure 1-5. General Lab Procedure Schematic. Step 1: Participants were recruited and parents participated in a phone screener to evaluate whether or not they're child was eligible to participate in the current studies; Step 2: Participants attended the lab for testing session 1 where the child participated in neuropsychological testing and oculomotor screening while parents completed questionnaires; Step 3: Participants returned to the lab within 1 to 8 weeks to complete VOR tests and including velocity step tests and sinusoidal harmonic acceleration tests .

Figure 1-6. rVOR Testing Session Schematic. rVOR testing included two types of tests: Velocity Step and SHA tests. Velocity step tests were conducted in 3 conditions (light, dark and fixation suppression) with one trial each of clock-wise and counter clock-wise rotation completed in each condition for a total of 6 trials of velocity step testing. SHA tests were conducted in two conditions (dark and fixation suppression) with one trial at each of the 3 frequency cycles (0.05, 0.10 and 0.50 Hz) for a total of 6 trials of SHA testing.

CHAPTER 2
EFFECT OF VISUAL CONDITIONS ON ROTATIONAL VESTIBULO-OCULAR REFLEX FUNCTION IN AUTISM SPECTRUM DISORDERS

Introduction

The rotational vestibulo-ocular reflex (rVOR) is an oculomotor response to angular vestibular stimulation (i.e., rotation of the head) and is required to maintain stable vision during head movement. The rVOR response is modulated by brainstem and cerebellar structures, two areas consistently reported to have neurobiological differences in ASD (Amaral, Schumann & Nordahl, 2008) and may provide useful insights as to the function of these structures in ASD.

Rotary chair testing is considered the gold standard for assessing rVOR function. Velocity step tests, one method of rotary chair testing, involves the participant seated on a motorized rotary chair that provides a step in acceleration to a constant velocity in order to provide continuous whole body rotational vestibular stimulation. The rVOR is characterized by nystagmus beats or repetitive sequences of slow followed by quick eye movements that occur in response to continuous rotation. During rotary chair tests, eye movements are recorded during and after rotation to assess three primary measures used in evaluating rVOR function: gain, symmetry and time constant of decay. Gain is the ratio of head movement to eye movement and reflects the peripheral sensory organ and eighth cranial nerve function. Time constant of decay, the time it takes the rVOR post-rotary nystagmus response to decrement to 37% of its peak velocity, is a measure of the velocity storage mechanism or central processing of rVOR including brainstem and cerebellar function. Symmetry is a measure comparing the response of the eyes to leftward and rightward rotation (i.e., stimulating left and right vestibular systems respectively) to test for unilateral deficits (Brey, McPherson & Lynch, 2008).

Children with ASD have been reported to demonstrate significantly greater ability to suppress their rVOR response after rotary chair velocity step testing compared to typically developing controls. Ritvo et al. (1969) tested horizontal rVOR in a sample of children with “autism or early childhood schizophrenia” in two conditions: (1) in the light with surroundings visible to the participant and (2) in the dark with surroundings not visible to the participant (i.e., children were blindfolded). In the light, the children with autism showed significantly decreased duration of post-rotary nystagmus compared to typically developing children, whereas in the dark, there was no difference in the duration of post-rotary nystagmus between the two groups (Ritvo et al., 1969). The authors suggested that children with autism were able to use optokinetic feedback for inhibiting post-rotary nystagmus to a greater extent than the typically developing children and further suggested that aberrations in brainstem or cortical input may be responsible for this difference.

A subsequent study exploring the effects of various visual fixation stimuli on rVOR in ASD failed to replicate this finding of decreased post-rotary nystagmus in the light (Ornitz et al., 1974). They also reported no difference between ASD and controls when tested in the dark. They did report significantly decreased post-rotary nystagmus response in ASD, however, when various visual fixation stimuli were provided after rotation in the dark including: (i) a standard visual field with a fixation object, (ii) a pinpoint of red light in otherwise complete darkness, (iii) frosted goggles that admitted light but prevented fixation and (iv) frosted goggles with lower light intensity to further prevent fixation (Ornitz et al., 1974). These reports, although contradictory in the light

condition, suggest that individuals with ASD respond differently than controls to visual fixation suppression of rVOR.

Ornitz et al., (1985) reported increased time constants of decay during per-rotary nystagmus in the dark (Ornitz et. al., 1985). These results, combined with those reported by Ritvo et al. (1969) and Ornitz et al. (1974) indicate that aberrations in rVOR may arise in regions of the central nervous system that modulate rVOR and oculomotor control such as the brainstem nuclei and cerebellar velocity storage modulation of rVOR.

Visual fixation suppression testing provides information regarding midline cerebellum and vestibular nuclei modulation of rVOR (Brey et al., 2008b). A previous study by Ornitz et al. (1974) attempted to determine visual suppression differences in ASD, however, they did not provide fixation stimuli during rotation. They conducted velocity step tests in the dark and after rotation stopped, participants were provided with visual fixation stimuli. To date, fixation suppression testing with fixation stimuli provided during and after rotation has not yet been conducted with children with ASD and may help to improve our current understanding of visual fixation suppression of rVOR and may shed light on brainstem and cerebellar function in this population.

The objective of the current study was to replicate and extend the earlier findings of Ritvo et al. (1969) and to determine differences in fixation suppression of rVOR in ASD and TD groups using pediatric rotary chair tests. Significantly greater than normal suppression of rVOR in ASD has been demonstrated during velocity step testing in a lighted room (Ritvo et al., 1969) and with various visual fixation stimuli (Ornitz et al., 1974). Velocity step tests will be conducted in light and dark conditions to replicate and

extend previous findings. Based on previous findings, it is expected that children with ASD will demonstrate significantly greater rVOR fixation suppression (i.e., decreased time constants of decay in the light and visual fixation suppression conditions) and increased time constants of decay in the dark condition compared to controls.

Methods

Participants and Recruitment

For this study, 16 children with ASD and 17 typically developing children ages 6-12 were recruited from the University of Florida Center for Autism and Related Disabilities (UF CARD), schools and medical centers within Alachua county. This lower age limit was selected for the current study to be confident in the diagnosis of ASD (Table 2-1). Participant medical history was obtained by parent report at the time of informed consent. The ASD group included individuals with one of the following diagnoses: Autism, Asperger's syndrome, or Pervasive Developmental Disorder - Not Otherwise Specified. Diagnoses were confirmed by the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003). Children were excluded from the study if they had a diagnosis of Fragile X, Rett Syndrome, tuberous sclerosis, seizure disorder or fetal cytomegalovirus infection. Control participants were excluded if parents reported any current or past history of psychiatric or neurologic disorder or related medications. These exclusionary criteria were selected in an effort to minimize confounding variables and to better define the ASD and TD groups by excluding disorders with known etiologies such as Fragile X, Rett Syndrome, tuberous sclerosis, and fetal cytomegalovirus infection and to reduce the risk of any harm to participants as seizures

have been reported to be elicited by vestibular stimulation. Demographic data are presented in Table 2-1.

Neuropsychological Testing and Standardized Rating Scales

Children in the ASD group participated in the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000). The combination of ADOS and SCQ scores were used to confirm ASD diagnoses. The ADOS is a semi-structured play-based assessment of social and communication skills and restricted repetitive behaviors. During Neuropsychological testing, both groups were administered the Leiter International Performance Scale – Revised (Leiter-R) Brief IQ test (Roid, Miller, & Leiter, 1997), a non-verbal intelligence assessment where children are allowed to indicate their responses through gestures such as pointing or manual selection. Both groups also participated in the Physical and Neurological Examination for Soft Signs (PANESS; Denckla, 1974), a standardized pediatric neurological assessment of motor control where children were asked to complete motor tasks such as rapid finger tapping or walking heel-to-toe while being video recorded for later scoring. The PANESS sum of balance errors subscale has been previously shown to distinguish typically developing children from children with ASD (Jansiewicz et al., 2006), therefore this subscale was chosen as a variable of interest for the current study.

While children participated in the assessments listed above, parents/guardians from both ASD and TD groups completed four questionnaires as follows: (1) the Repetitive Behavior Scale-Revised (RBS-R; Bodfish et. al., 2000), a parent report measure that is used to index the range of these behaviors in ASD, (2) the Sensory Profile Caregiver Questionnaire (Dunn, 1999), a parent report of non-adaptive behavioral responses to various vestibular sensory stimuli encountered in daily activities

and (3) the Vineland-II Adaptive Scales (Sparrow & Cicchetti, 1985; Sparrow, Cicchetti, & Balla, 2005), a measure of communication, daily living, socialization and motor skills for daily living. Parents/caregivers from the ASD group were also asked to complete the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003), a parent report measure of social and communication skills. The RBS-R includes 6 subscales as follows: (1) stereotyped behavior, (2) self-injurious behavior, (3) compulsive behavior, (4) ritualistic behavior, (5) sameness behavior and (6) restricted behavior. Each item within these subscales is rated on a 4-point Likert scale from 0 (behaviors do not occur) to 3 (behaviors do occur and are a severe problem).The sum of all six subscales was used for comparison between groups. The Sensory Profile includes statements of non-adaptive responses to sensory stimuli. Parents rate these statements on a Likert scale of 1 (always) to 5 (never) occurring for their child. The higher the Sensory Profile score the more adaptive the behavior and the lower the score the less adaptive. The adaptive behavior composite score of the Vineland-II Adaptive Scales was used for the current study. The combination of ADOS and SCQ was used to confirm diagnoses of children recruited into the ASD group and a combination of Vineland-II Adaptive Scale and Leiter-R Brief IQ results was used to determine presence or absence of intellectual disability in both groups. The entire neuropsychological testing session took approximately 90 to 120 minutes for the ASD group to complete and 60 to 90 minutes for the TD group to complete.

Testing Equipment

Our lab used a computer-controlled, motorized rotary chair and video-oculography goggle (VOG) system for VOR tests. The motor, controls, VEST^{TM} 6.8 Software and VOG I-Portal^{TM} Video-Oculography Goggle (VOG) System were

manufactured by Neuro-Kinetics Inc. (NKI) and are comparable to commercially produced NKI vestibular assessment systems used in clinics around the world. On top of the rotary platform we added a seating system specifically for use in pediatrics including: an enlarged rotary platform, a small chair, safety harnesses, and a head stabilizing system (Figure 2-1).

Procedure

VOG System Calibration. Prior to testing each participant, calibration of the VOG system was completed. For both calibration and oculomotor testing, a laser visual stimulus was provided via an NKI motor controlled laser mounted below the rotary chair projecting a small moving laser onto a smooth black screen. The black screen had a 60º arc with a radius of 76.5 inches so that movement of the laser was congruent with the arc of eye movement rotation that occurs when tracking the laser. During calibration, the participant was seated on the rotary chair, wearing the VOG head set, with their head fixed by the head stabilizing system. The rotary chair remained stationary and the participant was asked to visually track a laser visual stimulus in complete darkness as the laser moved + 10º from center first to the left, then to the right, then up and finally downward. This process was repeated two times and results were averaged for calibration. This same calibration value was later used for the oculomotor tests as well as vestibular tests.

Oculomotor Screening. Since the behavior of interest in rVOR is the oculomotor response to vestibular stimluli, it was important to screen for oculomotor deficits prior to interpreting rVOR oculomotor responses. Therefore, oculomotor assessments were conducted as a screening tool to rule out the confounding variable of oculomotor disorders such as spontaneous nystagmus or gross oculomotor differences

not previously reported in ASD. Additionally, children who could not tolerate wearing the goggles or remaining seated in the rotary chair for calibration of the VOG system or oculomotor screening tests, were not asked to return for vestibular testing. Three oculomotor assessments were conducted as follows: saccade, smooth pursuit and gaze evoked nystagmus test. Each oculomotor test was explained to the child prior to testing so that he/she understood the requirements of each task. Children were instructed to follow the red glowing dot with their eyes. *Saccade* tests were completed using a laser stimulus moved horizontally and vertically to elicit 30 horizontal and 30 vertical saccades with pseudo-randomly assigned location and timing of each trial ranging from 0º to 24º for target locations and 1 to 2 second delays between trials. *Smooth Pursuit* testing included moving the laser stimulus in the horizontal plane and in the vertical plane at two different frequencies and velocity profiles as follows: at a frequency of 0.1Hz and 4º /second and at a frequency of 0.5Hz and 20º/second. *Gaze Evoked Nystagmus* testing included presenting the laser visual stimulus at -10º in the horizontal plane for 5 seconds and then turned off while the participant was instructed to continue to focus on that spot for 15 seconds. This process was repeated at +10º in the horizontal plane, and then again the entire process was repeated +/- 10º in the vertical plane. The average slow phase velocities of eye movements as they drift away from the target location were recorded.

Velocity Step Testing. The entire testing procedure was themed as a “space mission”, including space related stories and games for the participants during breaks and between each trial. Participants completed a 3-part battery of velocity step tests in three different conditions as follows: (1) *in the light* with the goggle cover off and room

lights on, (2) *in the dark* with the goggle cover on to prevent visual stimulation and with room lights off and (3) with a *fixation suppression* LED turned on inside the goggle cover in the dark with room lights off. Participants were asked to count aloud with researchers to prevent drowsiness and control for attention to task and were monitored for signs of drowsiness such as excessive blinks. Trials where participants indicated feeling drowsy were repeated. Each trial was followed by a short break (1-2 minutes) including activities to increase alertness such as a variety of games and activities with varying levels of difficulty, Velocity step test trials were completed in both clock-wise and counter clock-wise directions in each condition with a ramp-up time of 1.2 seconds and a peak velocity of 100º/ second. Per-rotary recordings were taken for 60 seconds, followed by post-rotary recordings that lasted as long as nystagmus was occurring up to 60 seconds. Each test was completed once in each direction. If one of these two trials was disrupted by excessive blinking, talking, head movement or the child requesting a break, however, the entire trial was repeated with the participant's assent. The light condition was presented first because young children are often fearful of the dark, thus, presenting the velocity step test in the light initially helped the children to become accustomed to this testing protocol and decrease anxiety. After the light condition, the dark condition was introduced. Finally, velocity step testing was conducted in the suppression condition (in the dark with suppression LED on inside the VOG). During suppression testing, the participants were instructed to keep their eyes focused on the small glowing red dot (also referred to as a star/planet depending on the child's preference for the space theme) as they are riding on the "space ship". The entire vestibular testing session lasted approximately 60 minutes.

Statistical Methods

Oculomotor screening. For oculomotor screening tests of saccades, smooth pursuit and gaze evoked nystagmus tests, group means comparisons were conducted using independent samples t-tests with Bonferroni corrections for experiment-wise alpha using IBM SPSS Statistics software v. 21. Saccade test group means were compared for four measures including: vertical and horizontal saccade latency as well as vertical and horizontal saccade gain. Smooth pursuit test group means were compared for eight measures including: vertical and horizontal gain at frequencies of 0.1Hz and 0.5Hz in each direction as well as vertical and horizontal phase at frequencies of 0.1Hz and 0.5Hz in each direction. Finally, gaze evoked nystagmus test group means were compared for eight trials with target conditions including target LED turned “on” or “off” and target locations of up, down, right or left.

Velocity step test rVOR analysis. Comparisons of rVOR metrics for both per- and post-rotary nystagmus including gain, symmetry, and time constant of decay were conducted with mixed ANOVAs using IBM SPSS Statistics software v. 21 with group (ASD or TD) as a between-subjects factors, and visual condition: (i) light, (ii) dark and (iii) fixation suppression conditions as within-subjects factors. No difference was found between symmetry comparisons (i.e., clock-wise vs. counter clock-wise trials) of gain and time constant of decay for both groups for per-rotary or post-rotary nystagmus. Therefore, clock-wise and counter clock-wise values for time constants and gain were averaged for each participant.

Results

Demographics and Neuropsychological and Rating Scale Assessments

Table 2-1 provides a summary of participant demographics including age and gender as well as neuropsychological and standardized rating scale assessment results. One control participant demonstrated spontaneous vertical nystagmus during oculomotor screening and was then excluded from the study. Independent samples t-tests were run to determine if there were differences in demographic or assessment data between groups. There was no significant difference in age (months) between the ASD group and controls, $t(31) = -.41, p = .69$ (means and SDs are presented in Table 2-1). There was also no significant difference in Leiter Brief IQ score between ASD and controls, $t(31) = -.59$, $p = .56$. There were statistically significant between group differences for all other neuropsychological and rating scale assessments as follows: Vineland-II Adaptive Scores were lower in ASD compared to controls, $M = -20.62$, 95% CI [-30.83, -10.43], $t(37.998) = -4.12$, $p < .005$; SCQ scores were higher in the ASD group compared to controls, $M = 18.66$, 95% CI [14.48, 22.84], $t(31) = 10.07$, $p < .005$; Vestibular processing subsection scores from the Sensory Profile were lower in the ASD group compared to controls, $M = -7.92$, 95% CI [-11.60, -4.24], $t(31) = -4.40$, $p < .005$; RBS-R scores were higher in the ASD group compared to controls, $M = 33.65$, 95% CI [21.15, 46.16], $t(31) = 5.87$, $p < .005$; and PANESS balance sum of error scores were higher in the ASD group, $M = 3.57$, 95% CI [1.74, 5.40], $t(31) = 4.11$, $p = .001$. The ADOS was only conducted as a diagnostic assessment for participants with ASD, therefore, no comparison tests were conducted between groups on this measure. Homogeneity of variance was assessed by Levene's Test for Equality of Variances for each measure. There was homogeneity of variances for age ($p = .90$), Leiter Brief IQ

scores (p = .22), Vineland-II Adaptive Scales (p = .63) and Vestibular Processing subsection scores from the Sensory Profile (p = .18). Homogeneity of variances was violated for SCQ scores (p< .005), RBS-R scores (p< .005) and PANESS balance sum of error scores (p = .001), so for these measures separate variances and the Welch-Satterthwaite correction were used.

Oculomotor Screening

Saccade gain. There was no significant difference in saccade gain between groups in either direction, horizontal, t = -1.62, p = 0.120 or vertical, t = 0.45, p = 0.657 based on a Bonferroni adjusted experiment-wise alpha of 0.025 for the two comparisons completed within this test (Figure 2-2).

Saccade latency. The ASD group demonstrated significantly greater horizontal saccade latency (M = 260ms, SD = 60) than the TD group (M = 210ms, SD = 40), t = 2.65, p = 0.012. No significant difference was observed between groups for vertical saccade latency, t = 0.85, p = 0.405, however. These results are based on a Bonferroni adjusted experiment-wise alpha of 0.025 for the two comparisons completed within this test (Figure 2-3).

Smooth pursuit gain. No significant difference was observed in smooth pursuit gain between groups at any of the movement frequencies (0.1 Hz or 0.5 Hz) or directions (horizontal or vertical) as follows: 0.1Hz horizontal, t = 0.38, p = 0.704; 0.5Hz horizontal, t = -0.67, p = 0.511; 0.1 Hz vertical, t = 0.42, p = 0.680 and 0.5Hz vertical, t = -1.32, p = 0.200. These results were based on a Bonferroni adjusted experiment-wise alpha of 0.0125 for the four comparisons completed within this test (Figures 2-4 and 2-6).

Smooth pursuit phase. No significant difference was observed between groups for smooth pursuit phase for any of the movement frequencies (0.1 Hz or 0.5 Hz) or directions (horizontal or vertical), based on a Bonferroni adjusted experiment-wise alpha of 0.0125 for all four comparisons completed (Figures 2-5 and 2-7). However, a noteworthy and systematic trend was observed with the ASD showing higher smooth pursuit phase lag than the TD group, particularly during vertical smooth pursuit ($p <$ 0.03; see Table 2-2 for a summary of t-test statistics).

Gaze Evoked Nystagmus. The gaze evoked nystagmus tests were the most challenging for children as they required compliance to more complex instructions than the other two tests. Therefore, only 9 participants in the ASD group and 16 in the TD group were able to complete this portion of the oculomotor screening battery. There was no statistically significant difference in mean eye movement excursion between ASD and TD for gaze evoked nystagmus tests at any of the target statuses ("on" or "off) or target directions ("right, left, up or down"; Figure 2-8) and no nystagmus was elicited by the gaze evoked nystagmus test in any subjects included in this study.

Velocity Step Tests

Gain

Per-rotary nystagmus gain. For per-rotary nystagmus, there was a statistically significant main effect of condition on per-rotary nystagmus gain, F (1,31) = 374.55, $p<$ .005, partial η^2 = .924, with both groups displaying lower gain in the suppression condition and higher gain in the dark condition. The main effect of group showed that there was a statistically significant difference in per-rotary gain between groups F(1, 31) = 11.158, p = .002, partial η^2 = .265, with the ASD group showing higher gain than the TD group. There was no statistically significant group by condition (i.e., light, dark, and

fixation suppression) interaction for per- rotary nystagmus gain, $F(1,31) = .450$, $p < .508$, partial $\eta^2 = .014$ (Figure 2-9).

Post-rotary nystagmus gain. There was a statistically significant main effect of condition on post-rotary nystagmus gain, $F(2,62) = 90.69$, $p < .005$, partial $\eta^2 = .745$, with lower gain for both groups in the light and suppression conditions and higher gain in the dark condition (Figure 2-10). No statistically significant difference in post-rotary gain was found between groups $F(1, 31) = 1.734$, $p = .198$, partial $\eta^2 = .053$. No statistically significant group by condition interaction for post-rotary nystagmus gain was observed, $F(2,62) = .520$, $p < .597$, partial $\eta^2 = .016$.

Time Constant of Decay.

Per-rotary nystagmus time constant of decay. For per-rotary nystagmus, there was a main effect of condition on per-rotary time constant of decay, $F(1, 31) = 4.32$, $p = .046$, partial $\eta^2 = .122$, with both groups demonstrating lower per-rotary time constants during the suppression condition and higher time constants in the dark condition (Figure 2-11). No statistically significant difference in per-rotary time constant of decay was observed between groups $F(1, 31) = 3.453$, $p = .073$, partial $\eta^2 = .100$. There was no statistically significant group by condition interaction for time constant of decay, $F(1,31) = .328$, $p = .571$, partial $\eta^2 = .010$.

Post-rotary nystagmus time constant of decay. There was a statistically significant main effect of condition, $F(2,62) = 5.919$, $p < .005$, partial $\eta^2 = .592$, where both groups demonstrated lower time constants in the light condition and higher time constants in both dark and suppression conditions. There was a statistically significant group by condition interaction for post-rotary time constant of decay, $F(2,62) = 5.919$, $p = .004$, partial $\eta^2 = .160$. There was a significant difference between groups for post-

rotary time constant of decay in both the dark condition, $F(1,31) = 6.195$, $p = .018$, partial $\eta^2 = .167$ and suppression condition, $F(1, 31) = 20.125$, $p < 0.005$, partial $\eta^2 = .394$, with the ASD group demonstrating higher time constants in both conditions. There was no significant difference, however, between groups for post-rotary time constant in the light condition, $F(1, 31) = .379$, $p = .543$, partial $\eta^2 = .012$ (Figure 2-12). The greatest difference between groups was in the suppression condition.

Discussion

Neuropsychological Tests and Standardized Rating Scales

There was no significant difference in age or IQ scores between the ASD group and controls, indicating that the samples were relatively well matched for age and IQ. Every effort was made to recruit high functioning children into the ASD group to better match the two samples; however, one out of the 16 ASD participants had IQ below 70, whereas none of the 17 TD group participants had IQ below 70. As expected, based on previous studies of these measures in ASD, the ASD and TD groups differed significantly on all other neuropsychological measures. Vineland-II Adaptive Scores result from parent reports of the child's functional adaptive skills and were lower in ASD compared to controls.

The Social Communication Questionnaire (SCQ) was used in combination with the ADOS to confirm diagnoses for the ASD group, but was also conducted to screen for any ASD characteristics in the TD group (SCQ scores > 15 are clinically relevant for ASD). SCQ scores were significantly higher in the ASD group and well above the ASD cut off score compared to controls, whose scores were well below the ASD cut off, indicating that there were no significant ASD characteristics of concern in the TD group.

The ASD group demonstrated significantly lower scores on the vestibular processing subscale of the Sensory Profile than the TD group, indicating less adaptive behavioral responses to vestibular stimuli. According to the scoring form for the Sensory Profile Caregiver Questionnaire the ASD group mean reflects a “definite difference” in vestibular sensory processing and the TD group mean reflects “typical performance”.

The PANESS Physical and Neurological Exam for Subtle Signs (PANESS; Denckla , 1974) is a motor assessment that is useful for pediatric assessment of neuromotor control deficits. The PANESS has been shown to be useful for distinguishing differences in motor control between children with ASD and typically developing children as well as in distinguishing high functioning children with autism from children with Asperger’s syndrome (Jansiewiwcz et al., 2006). The balance subscore presented in this study was found, as expected, to be significantly different between groups, with the ASD group having a higher score, indicating greater number of errors made such as loss of balance during the balance assessments.

Oculomotor Screening

Oculomotor control deficits have been well documented in the ASD population. In the current study we observed significantly increased horizontal saccade latency in the ASD group compared to typically developing controls, but no difference in gain between groups. These findings are inconsistent with previous findings of no difference between autism, Asperger’s syndrome or control groups in horizontal saccade latency (Takarae, Minshew, Luna & Sweeney, 2004). Previous studies have also shown that individuals with high functioning autism show increased deficits in horizontal saccade accuracy, but no difference in saccade metrics such as gain or latency (Johnson et al., 2012).

Additionally, we observed a noteworthy trend in ASD towards increased smooth pursuit latency, particularly in the vertical direction (p <0.03). Although this was not statistically significant based on the Bonferroni adjusted experiment-wise alpha (p < 0.0125) used in the current study, this was an interesting systematic pattern worthy of note. Increased vertical smooth pursuit latency has not been previously reported in ASD. However, increased horizontal pursuit latency in ASD has been previously observed in subgroups of ASD without a history of language delay. Those with and without a history of language delay have been shown to make a greater number of catch-up saccades during pursuit tasks (Takarae et al., 2008), indicating that regardless of language development, some level of horizontal smooth pursuit deficit does exist in ASD. Our study extends these previous findings to suggest the possibility that vertically directed smooth pursuit eye movements may also display increased latency in this population and could contribute to functional visual deficits.

Velocity Step Test: Per-rotary rVOR

Per-rotary Gain. For per-rotary nystagmus, both groups displayed a trend of lower per-rotary rVOR gain during the suppression condition and higher gain in the dark condition. This effect of condition is expected due to the suppressive effect of the fixation stimulus provided in the fixation suppression condition but lacking in the dark condition. This change in gain for both groups suggests that both groups were attending to the fixation suppression visual target during rotation and benefitted from the suppression effects. Even though the ASD group demonstrated the same trend of decreasing gain and time constants in the suppression condition compared to the dark condition, in both conditions the ASD group demonstrated significantly higher per-rotary

nystagmus gain compared to controls. This may indicate possible deficits in cerebellar inhibitory modulation of rVOR in ASD.

Per-rotary Time Constant of Decay. For per-rotary nystagmus, there was no difference in TCD between groups. This is inconsistent with the findings reported by Ornitz et al. (1985) of increased per-rotary TCD in children with ASD. It is not clear why we observed no difference in TCD during rotation, but did observe a difference after rotation and why Ornitz et al. (1985) found the opposite pattern of increased TCD in their participants.

Velocity Step Test: Post-rotary rVOR

Post-rotary Gain. For both groups, the light and fixation suppression conditions resulted in the lowest gain and the dark condition resulted in higher gain (approaching 1.0). This suppression of rVOR gain in the light and fixation suppression conditions is likely due to the visual fixation stimuli provided by the visible surroundings after rotation has stopped in the light condition as well as the fixation suppression target stimuli provided both during and after rotation in the fixation suppression. These visual fixation stimuli provide visual feedback useful for suppressing nystagmus gain. Again, this effect of condition and decrease in gain for both groups suggests that both groups were attending to the visual fixation stimuli available in both the light condition and the fixation suppression condition after rotation and benefitted from the suppression effects. There was no difference between groups; however, for post-rotary nystagmus gain in any of the three conditions (light, dark or suppression).

Post-rotary Time Constant of Decay. The ASD group exhibited significantly increased post-rotary TCD in the dark and fixation suppression conditions compared to typically developing controls, but no difference in the light condition. Based on previous

studies reporting decreased duration of post-rotary nystagmus in the light (Ritvo et al., 1969) and with visual fixation stimuli provided after rotation (Ornitz et al., 1985), it is surprising that the ASD group showed no difference in TCD in the light and increased TCD in the fixation suppression condition in the current study. Neither of the previous studies provided visual fixation stimuli both during and after rotation; therefore, the difference in methods and presentation of visual fixation stimuli may have resulted in the differences observed in the current study. Since both groups demonstrated an effect of condition where gain was decreased in the light and fixation suppression condition compared to the dark condition, it is reasonable to assume that the ASD group was able to follow directions during the suppression condition and was attending to the visual target after rotation. Therefore, it is reasonable to suspect that these results reflect a true difference in rVOR function between groups rather than a difference merely in ability to follow directions or attend to fixation stimuli.

The time constant of decay serves to increase the efficiency of the rVOR response to low-frequency stimulation, as was provided in the current study. This is accomplished through vestibulo-cerebellar modulation of the velocity storage mechanism in the brainstem (Leigh & Zee, 2006). Lesions in the nodulus and uvula (i.e., velocity storage mechanism of the cerebellum) can result in increased time constant of decay (Waespe et al., 1985). Such increases in time constant of decay have been observed in ASD previously when tested in the dark (see Chapter 3; Ornitz et al., 1985) and are consistent with the current findings.

The differences between the visual stimuli in the fixation suppression condition and light condition may be key to understanding why these differences occurred in the

suppression condition but not in the light condition for the ASD group. First, during the visual fixation condition, an LED visual target stimulus is presented in the right eye piece of the videooculography goggle. This visual stimulus follows the participant as they rotate and places demands on both the smooth pursuit system as well as the brainstem neural integrator circuitry for maintaining gaze stability and fixation. In the light condition, during rotation the full-field visual stimuli of the surrounding environment remains fixed as the participant rotates creating retinal slip and providing optokinetic visual feedback that should facilitate per-rotary nystagmus. When rotation stops, this full visual field also provides visual feedback that motion has ceased and helps to inhibit post-rotary nystagmus. Second, during the visual fixation task, the participant was asked to fixate on the visual LED target, therefore the target is present within the foveal visual field; whereas, in the light condition the visual stimuli is presented within the full visual field including both foveal and peripheral visual fields. Therefore, there may be a difference between these two conditions based on differing requirements of smooth pursuit and optokinetic systems.

Conclusions

Taken together, these results of increased time constant of decay in the dark and during fixation suppression conditions suggest that there may be deficits in cerebellar modulation of velocity storage as well as visual fixation. The increased gain during per-rotary nystagmus and increased time constant of decay of post-rotary nystagmus in the dark indicates a lack of inhibition from the cerebellum to the brainstem velocity storage system in ASD. This lack of inhibition may be related to similar findings of pre-natal and post-natal neuropathological processes suspected in the cerebellum in ASD such as Purkinje cell loss (Ritvo et al., 1986; Bailey et al., 1998; Kemper & Bauman, 2002;

Purcell et al., 2001; Lee et al., 2002; Palmen et al., 2004).. The increased time constant of decay for post-rotary nystagmus during visual fixation suppression suggests that there is a possible deficit in visual fixation or smooth pursuit. Although no significant difference was found in the smooth pursuit oculomotor screening tests, there was a consistent trend toward higher smooth pursuit latencies in the ASD group. The current study may not have had enough statistical power to identify significant differences in this measure. Additionally, the individuals with ASD recruited into the current study were relatively high functioning and therefore, may have more subtle differences in oculomotor results. Oculomotor differences within ASD have been previously shown to depend on presence or absence of language delay, which was not recorded in this sample (Takarae et al., 2004; Takarae et al., 2008).

Future Studies

Optokinetic nystagmus (OKN) and optokinetic after nystagmus (OKAN) are repetitive eye movement responses to visual motion stimuli rather than vestibular stimuli as with per- and post-rotary nystagmus is to rVOR. Like rVOR however, both OKN and OKAN are also dependent upon normal functioning of the nodulus and uvula. Horizontal OKAN is prolonged with damage to these structures (Angelaki & Hess, 1994; Wearne et al., 1998). OKN and has been briefly described in the literature to differ from controls in one oculomotor study (Scharre & Creedon, 1992). However, these tests were conducted by visual observation of OKN only and actual measurements of gain and time constant of decay were not possible. The authors reported that 83% of the ASD group exhibited atypical OKN, defined as having a “latency or response greater than 2 seconds, duration of the response less than 5 seconds, gaze avoidance, and/or stereotypic behavior”. Some of these characteristics are associated with the ASD

phenotype rather than OKN such as stereotypic behavior and gaze avoidance and there may have been some bias towards the ASD population to exhibit these types of "atypical" OKN. Therefore, it is unclear as to what percentage of atypical OKN reported was due to abnormal OKN responses or ASD related behaviors. OKAN has not been studied in this population to date. Since horizontal rVOR is prolonged in ASD, it is reasonable to expect that OKAN might also be prolonged in this population and may further provide evidence for deficits in nodular/uvular function in ASD.

Due to the differences in the type of visual stimuli provided during the "light" condition and fixation suppression condition, possible differences may exist in the ability of individuals with ASD to process central/foveal vs. full-field vision for optokinetic feedback for gaze stabilization. Rotary chair testing with OKN full visual field used as the fixation suppression stimulus during rotation (Brey, McPherson & Lynch, 2008b) may help to determine whether or not there are differences in visual processing of full field vs. foveal fixation suppression of rVOR in ASD.

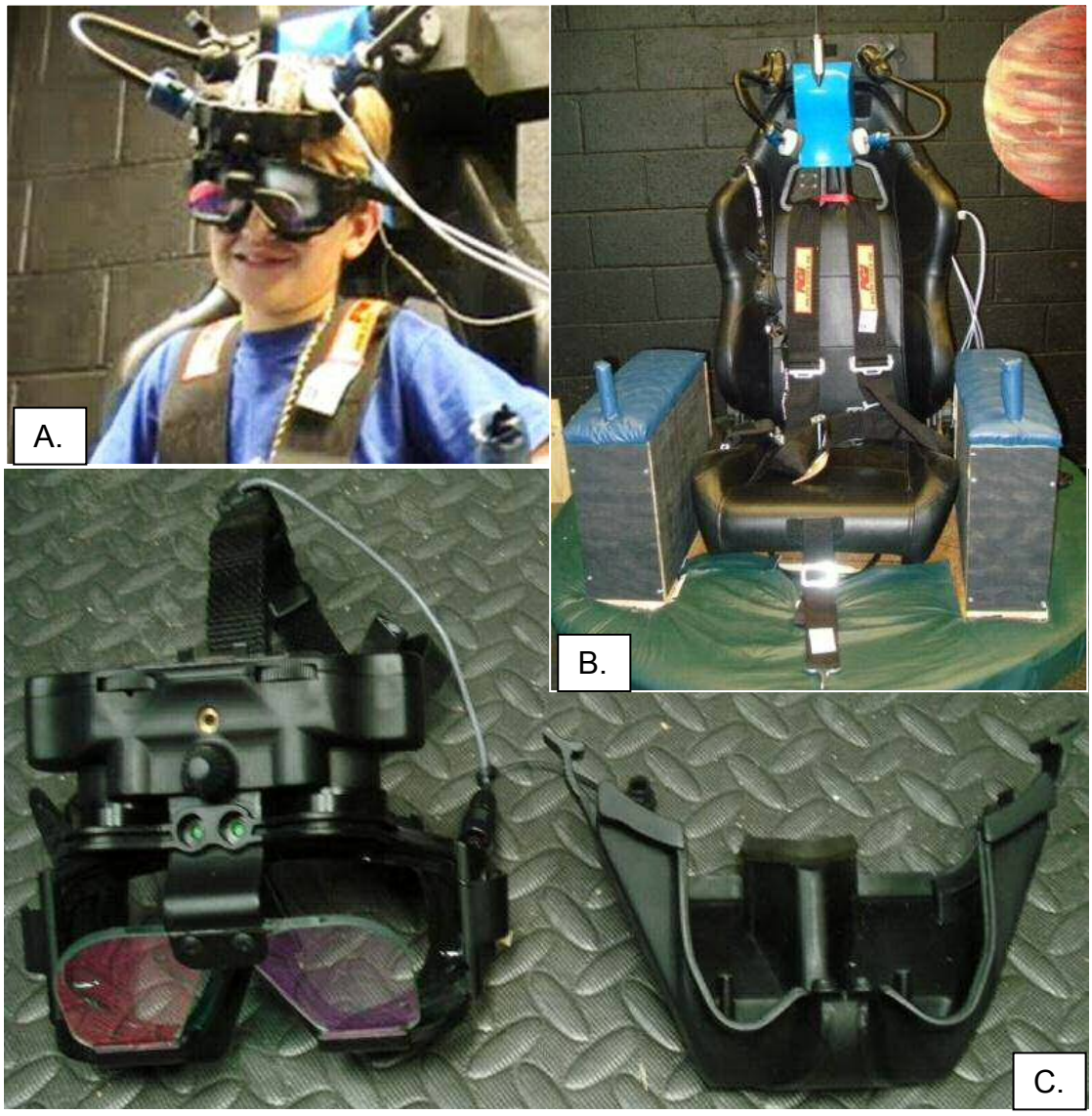

Figure 2-1. Pediatric Rotary Chair. A) child seated in rotary chair wearing goggles without cover, head stabilizer arms and safety harness, B) 'Space ship" themed rotary chair secured to motorized platform, armrests equipped with "control" joysticks, C) video-oculography goggle system and goggle cover.

Table 2-1. Summary of Group Mean (SD) for Demographics and Neuropsychological Assessments

	ASD (n = 16)			Controls (n = 17)				
	Mean	SD	n	Mean	SD	n	*t*	*p*
Age (months)	106.63	24.51	16	110.06	23.76	17	-.409	0.69
Leiter Brief IQ	100.19	24.23	16	104.24	14.61	17	-.585	0.56
Vineland-II Adaptive Score	81.31	13.68	16	101.94	14.97	17	-4.12	<0.005
ADOS	10.25	5.39	16	N/A	N/A	17	N/A	N/A
SCQ	20.60	7.43	15	1.94	1.71	17	9.50	<0.005
Vestibular Processing (SP)	42.75	6.03	16	50.67	3.60	15	-4.40	<0.005
RBS-R	36.13	23.15	16	2.47	4.78	17	5.70	<0.005
PANESS Balance errors	4.69	3.11	16	1.12	1.73	17	4.04	0.001

N/A = ADOS testing only conducted with the ASD group.

Table 2-2. Summary of Smooth Pursuit Comparisons for Phase (degrees)

Target condition	Group	*n*	*M*	SD	*T*	Sig.
Horizontal 0.1 Hz	ASD	14	4.25	9.53		
	TD	17	0.85	1.42	1.37	0.193
Horizontal 0.5 Hz	ASD	15	6.71	8.11		
	TD	17	4.32	4.22	1.06	0.296
Vertical 0.1 Hz	ASD	15	10.03	9.94		
	TD	17	3.37	5.86	2.32	0.028
Vertical 0.5 Hz	ASD	15	14.93	13.71		
	TD	17	6.30	8.00	2.34	0.029

Results based on a Bonferroni adjusted p-value of $p < 0.0125$ for the four comparisons made in this test

Figure 2-2. Saccade Gain. There was no statistically significant difference between groups in saccade gain for either horizontal or vertical saccades.

Figure 2-3. Saccade Latency. The ASD group showed significantly increased horizontal saccade latency. No difference was observed between groups for vertical saccade eye movement latency (error bars indicate standard deviations; *p < 0.025).

Figure 2-4. Horizontal Smooth Pursuit Gain. No difference in horizontal smooth pursuit gain between groups at either frequency of target movement (error bars indicate standard deviations).

Figure 2-5. Horizontal Smooth Pursuit Phase. No statistically significant difference in horizontal smooth pursuit phase between groups; however, there is a noteworthy increase in the phase lead in the ASD group at both movement frequencies. There is also a noteworthy difference in variability between groups, with the ASD group showing greater variability (error bars indicate standard deviations).

Figure 2-6. Vertical Smooth Pursuit Gain. No significant difference in vertical smooth pursuit gain between groups at either frequency of target movement (error bars indicate standard deviations).

Figure 2-7. Vertical Smooth Pursuit Phase. No statistically significant difference in vertical smooth pursuit phase between groups; however, there is a noteworthy increase in the phase lag in the ASD group at both movement frequencies. There is also a noteworthy difference in variability between groups, with the ASD group showing greater variability (error bars indicate standard deviations).

Figure 2-8. Gaze Evoked Nystagmus Mean Eye Excursion. No significant difference between groups in any direction of target movement or with target on/off. Also, no nystagmus beats were elicited by this test, which is normal (error bars indicate standard deviations).

Figure 2-9. Velocity Step Test Per-Rotary rVOR Gain. Both groups displayed decreased mean gain in the suppression condition, as expected, due to the suppression effects of fixation during and after rotation. However, the ASD group showed higher per-rotary nystagmus gain in both dark and fixation suppression conditions (error bars indicate standard deviations; $^{**}p < 0.01$).

Figure 2-10. Velocity Step Test Per-rotary rVOR TCD. Both groups displayed decreased time constant of decay in the suppression condition, as expected, due to the suppression effects of fixation during and after rotation. There was no difference between groups in either condition.

Figure 2-11. Velocity Step Test Post-Rotary rVOR Gain. Both groups displayed decreased mean gain in the light and suppression conditions, as expected, due to the suppression effects of visual stimuli after rotation. There was no difference between groups in any of the three conditions (error bars indicate standard deviations).

Figure 2-12. Velocity Step Test Post-rotary rVOR TCD. The typically developing (TD) control group displayed decreased time constant of decay in the light and suppression condition, as expected, due to the suppression effects of visual stimuli during and after rotation. However, the ASD group demonstrated a significantly increased post-rotary time constant of decay in the fixation suppression condition, which is unexpected. The ASD group also displayed a higher post-rotary time constant of decay in the dark condition (error bars indicate standard deviations; *$p < 0.05$; **$p < 0.01$).

CHAPTER 3
SINUSOIDAL HARMONIC ACCELERATION TESTS OF VESTIBULO-OCULAR FUNCTION IN AUTISM SPECTRUM DISORDERS

Introduction

Rotary chair tests are considered the gold standard for rotational vestibulo-ocular reflex (rVOR) assessments (Fife et al., 2000). A rotary chair assessment battery typically includes at least two types of tests: the velocity step test and the sinusoidal harmonic acceleration test (SHA). Velocity step testing has been previously reported to be abnormal in children with autism spectrum disorders (ASD; Ritvo et al., 1969; Ornitz et al., 1974; Ornitz et al., 1985); however, SHA tests have never been conducted in this population.

The rationale behind SHA testing is that the horizontal rVOR can be challenged by a range of frequencies of rotation at a moderate velocity of 50 - 60 degrees/second. Typically, throughout everyday movement, head movements occur between 0.5 and 5.0Hz (Leigh & Zee, 2006). The frequency range tested during SHA is anywhere from 0.01Hz to 0.64Hz; sometimes up to 2 Hz, depending on the type of testing equipment. Therefore, another benefit to SHA testing is that it can be conducted at higher frequencies (0.5 – 2 Hz) that more closely approximate natural ranges of motion than other forms of testing (Brey, McPherson & Lynch, 2008b). The ability to test at a range of frequencies is important because deficits in rVOR responses at high or low frequencies can be useful in determining the origin of vestibular symptoms (i.e., whether they are the result of central vs. peripheral dysfunction).

The rVOR is a remarkably fast reflex with just 7 – 15 ms latency that responds most efficiently to high frequency acceleration stimuli, such as that resulting from quick

rotational movements of the head. Abnormal responses to high frequency cycle SHA testing can reflect peripheral horizontal semicircular canal dysfunction. In order to maintain the efficiency and accuracy of this compensatory oculomotor response at lower frequencies, the velocity storage mechanisms in the brainstem and cerebellum work together to prolong the raw vestibular signal from the peripheral vestibular organ into what is known as the time constant of decay, thus making rVOR responses to low-frequency stimuli more efficient (Leigh & Zee, 2006). Therefore, deficits in the rVOR response to low frequency cycles of stimulation may reflect differences in the velocity storage mechanism of rVOR.

SHA testing can also be useful for determining whether or not there is a unilateral neurological deficit or lesion. Three primary measures are obtained from SHA testing including: gain, phase and symmetry. Gain is the ratio of head and eye velocity. Gain is an excellent indicator of how well the peripheral vestibular system is responding to angular acceleration. Phase is the timing relationship between the initiation of head movement and subsequent compensatory eye movement. Abnormally increased phase lead may be due to either central nervous system damage to vestibular nuclei or peripheral damage to vestibular organ nerve (Shepard & Telian, 1996). Symmetry is the comparison of slow-phase velocity between the two directions of movement (i.e., to the right vs. to the left) and indicates whether one or the other peripheral vestibular systems is not functioning properly.

SHA testing is typically conducted in the dark to prevent any visual fixation during rotation. However, a fixation suppression condition can also be conducted in combination with SHA protocols to evaluate the ability for visual fixation during

oscillation to inhibit rVOR eye movements. For this test of visual suppression, an LED visual stimulus is provided within the video-oculography goggle (VOG) system and the participant is directed to maintain visual fixation on the stimulus while they are rotating back and forth. This visual stimulus during rotation provides a target for visual fixation that moves with the subject and, in healthy subjects, should suppress rVOR gain during rotation, dampen the amplitude of nystagmus beats and allow the eye to remain fixed on the visual target. This response relies on a healthy connection between the primary vestibular processor or vestibular nuclei of the brainstem, and the adaptive processor, the midline cerebellar structures (Hain & Helminski, 2007; Brey et al., 2008b).

Previous studies of velocity step tests have reported findings of significant differences in rVOR responses in ASD compared to typically developing controls when visual stimuli in various forms are made available to the participants at different times during rotary chair velocity step testing (i.e., during or after rotation). Ritvo et al. (1969) found that children with ASD have significantly decreased rVOR post-rotary nystagmus response when provided full-field standard visual surroundings during and after rotation, however, this effect was not replicated in a recent study of children with ASD that displayed no difference in time constant of decay when tested in the light (see Chapter 2 of current studies). Ornitz et al. (1974) reported that children with ASD show no difference in rVOR post-rotary nystagmus response compared to controls when provided a blank, white visual field after rotation in the dark. When a visual fixation suppression target is provided via VOG goggles both during and after rotation (current studies, Chapter 2), the ASD group demonstrated increased time constants of decay. Whereas, if they rotated in the dark and *then* were presented with the visual fixation

suppression target after rotation has ceased, they exhibited decreased post-rotary nystagmus duration compared to controls (Ornitz et al., 1974). Since each of the above studies provide visual stimuli at different time points during testing, questions remain as to the consistency of rVOR fixation suppression deficits in ASD. Furthermore, questions remain as to the source of rVOR deficits in ASD, whether they are of peripheral or central origin. To date, no SHA testing has been reported in ASD with or without a fixation suppression condition.

The current study aimed to determine whether peripheral or central VOR processing deficits exist in ASD by conducting SHA tests in the dark over a range of high and low frequency cycles of rotation. The second aim of the current study was to explore the possibility that visual fixation suppression stimuli provided during SHA tests would produce differences in rVOR gain or phase in the ASD group. Symmetry is expected to be typical in children with ASD, as there is no evidence of unilateral deficits in ASD in any of the previous studies of rVOR function mentioned previously. The SHA fixation suppression testing will provide the LED suppression stimulus both during and after rotation, since this is the classic method for conducting this test and will allow comparisons to a wealth of basic science as well as clinical rVOR literature for interpreting any abnormal responses. Based on previous studies conducted by the authors (Chapter 2), it is expected that the fixation suppression will affect gain of rVOR for both groups, but there may not be a difference in fixation suppression gain between groups. Since phase has not been previously explored in this population, it is exploratory as to any differences that may arise between groups in the phase of rVOR

in ASD. However, if central velocity storage deficits in this population exist, it is conceivable that phase differences will be observed in the ASD group.

Methods

Participants and Recruitment

For this study, 15 children with ASD and 17 typically developing children ages 6-12 were recruited from the University of Florida Center for Autism and Related Disabilities (UF CARD), Alachua County elementary and middle schools and pediatric therapy centers within Alachua county. ASD diagnoses included: Autism, Asperger's syndrome, or Pervasive Developmental Disorder - Not Otherwise Specified and were confirmed by the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003). Exclusionary criteria include diagnoses of Fragile X, Rett Syndrome, tuberous sclerosis, seizure disorder or fetal cytomegalovirus infection. Exclusionary criteria for control participants included parent report of any current or past history of psychiatric or neurologic disorder or any immediate family with history of autism, schizophrenia, developmental disorder, mood disorder or anxiety disorder, or if they were taking any psychiatric medications. These exclusionary criteria were selected in an effort to minimize confounding variables and to better define the ASD and TD groups by excluding disorders with known etiologies such as Fragile X, Rett Syndrome, tuberous sclerosis, and fetal cytomegalovirus infection and to reduce the risk of any harm to participants as seizures have been reported to be elicited by vestibular stimulation.

Testing Equipment

A computer-controlled, motorized rotary platform, I-Portal^{TM} Video-Oculography Goggle (VOG) System and VEST 6.8 Software were used to record eye movements

during sinusoidal harmonic acceleration testing. On top of the rotary platform, padding, a chair, safety harnesses, and a head stabilizing system were added by the authors to adapt seating for pediatric use (Figure 2-1, Chapter 2).

Procedure

VOG System Calibration. The VOG system was calibrated prior to testing each participant. A laser visual stimulus was projected onto a black screen with a 60º arc 76.5 inch radius so that movement of the laser was congruent with the arc of eye movement. The laser visual stimulus was projected onto the screen in complete darkness at ± 10º from center, first to the left, then right, then up and down. This process was repeated twice and results were averaged.

Oculomotor Screening. Oculomotor screening tests included: saccade, smooth pursuit and gaze evoked nystagmus tests. Participants were instructed to follow the red glowing dot through a series of movements for each specific test (see methods section in Chapter 2 for further detail).

Sinusoidal Harmonic Acceleration (SHA) in the Dark. SHA tests were conducted under two conditions as follows: (1) *in the dark* with goggle cover on and room lights off and (2) with *fixation suppression* LED turned on inside goggle cover in the dark with room lights off. During SHA testing in the dark condition, the participant experienced acceleration to a peak velocity of 60º/sec followed by deceleration to 0º/sec in one direction, after which, the same acceleration and deceleration profile was repeated in the opposite direction, resulting collectively in a back and forth motion experience by the participant. This procedure has been performed with children age 3 to 9 previously with a frequency cycle profile of 0.02Hz, 0.05Hz, 0.1Hz and 0.5Hz (Casselbrant, 2010). However, the 0.02Hz condition takes up to (1/0.02) 50 seconds per

cycle. Generally, a minimum of 3 cycles are completed and averaged at each frequency (Brey, McPherson & Lynch, 2008a). Therefore, the 0.02Hz frequency trial takes 150 seconds or 2.5 minutes to complete 3 rotations. This is a very slow movement and it can be difficult to maintain children's attention for the 2.5 minutes of sitting quietly and rotating slowly. Therefore, it is not uncommon for studies to drop this frequency from their protocol. Thus, only 0.05, 0.10 and 0.50 Hz frequencies were conducted in this study.

Sinusoidal Harmonic Acceleration (SHA) with Fixation suppression. SHA testing in the fixation suppression condition was conducted as above with the addition of an illuminated fixation LED presented on the inner surface of the right eye view in the goggle cover. This LED provided a visual fixation stimulus during and after rotation and allowed visual feedback to inhibit the duration of the per- and post- rotary VOR.

Statistical Methods

Comparisons of rVOR metrics including gain, phase, symmetry, and an estimated time constant of decay were conducted with mixed ANOVAs using IBM SPSS Statistics software v. 21with group (e.g., ASD or TD) as a between-subjects factors, and visual condition: (1) light, (2) dark and (3) fixation suppression conditions as within-subjects factors. Time constant of decay was estimated using an equation to calculate time constant during SHA testing at 0.05Hz as described by Brey et al. (2008a).

Results

Oculomotor Screening

The results of the oculomotor screening revealed significantly greater horizontal saccade latency in the ASD group (Figure 2-2). No other significant differences between groups were discovered (refer to Chapter 2 Oculomotor Results). However, a

systematic trend of increased smooth pursuit phase was observed in ASD that is noteworthy and may be related to fixation suppression.

Sinusoidal Harmonic Acceleration (SHA)

SHA dark testing

Gain. The assumption of homogeneity of variances was met for all analyses, as assessed by Levene's Test of Homogeneity of Variance at each of the three frequencies tested ($p > .05$).The assumption of homogeneity of covariances was violated, as assessed by Box's test of equality of covariance matrices ($p = .039$). There was a statistically significant main effect of frequency on SHA gain in the dark, $F(2,60) = 3.574$, $p = .034$, partial $\eta^2 = .106$, where gain increased for both groups as the testing frequency increased for both groups. There was also a significant main effect of group on SHA gain in the dark, $F(1,30) = .196$, $p = .024$, partial $\eta^2 = .106$, where ASD participants showed higher gain than TD participants at each of the three frequencies tested. There was no statistically significant interaction between group and frequency on SHA gain in the dark, $F(2,60) = .604$, $p = .550$, partial $\eta^2 = .020$ (Figure 3-1).

Phase. The assumption of homogeneity of variances was met for all analyses, as assessed by Levene's Test of Homogeneity of Variance at each of the three frequencies tested ($p > .05$).The assumption of homogeneity of covariances was met, as assessed by Box's test of equality of covariance matrices ($p = .094$). There was a statistically significant main effect of frequency on SHA phase in the dark $F(2,60) = 95.361$, $p = .000$, partial $\eta^2 = .761$, where phase decreased as the frequencies of movement increased for both groups. There was no significant main effect of group on SHA phase in the dark $F(1,30) = 1.662$, $p = .207$, partial $\eta^2 = .053$ as well as no statistically

significant interaction between group and frequency on SHA phase in the dark, $F(2,60) = .814$, $p = .448$, partial $\eta^2 = .026$ (Figure 3-2).

SHA suppression testing

Gain. The assumption of homogeneity of variances was met for 0.1Hz ($p = .197$) but not for 0.05 Hz ($p = .005$) and 0.5 Hz ($p = .002$), as assessed by Levene's Test of Homogeneity of Variance ($p > .05$). The assumption of homogeneity of covariances was not met, as assessed by Box's test of equality of covariance matrices ($p = .025$). There were no statistically significant main effects of frequency, $F(2,60) = .124$, $p = .883$, partial $\eta^2 = .004$ or group on SHA gain with fixation suppression, $F(1,30) = 3.666$, $p = .065$, partial $\eta^2 = .109$. There was also no statistically significant interaction between group and frequency on SHA gain with fixation suppression, $F(2,60) = 1.753$, $p = .187$, partial $\eta^2 = .055$ (Figure 3-3).

Phase. The assumption of homogeneity of variances was met for all analyses, as assessed by Levene's Test of Homogeneity of Variance at each of the three frequencies tested ($p > .05$).The assumption of homogeneity of covariances was met, as assessed by Box's test of equality of covariance matrices ($p = .761$). There was a statistically significant main effect of frequency on SHA phase with fixation suppression, $F(2,60) = 14.23$, $p = .000$, partial $\eta^2 = .322$, where phase decreased as frequency increased for both groups. There was no significant main effect of group on SHA phase with fixation suppression, $F(1,30) = .433$, $p = .515$, partial $\eta^2 = .014$. There was, however, a statistically significant interaction between group and frequency on SHA phase with fixation suppression, $F(2,60) = 3.213$, $p = .047$, partial $\eta^2 = .097$, where the ASD group exhibited increased phase lead compared to the TD group in the 0.50 Hz frequency condition only (Figure 3-4).

Discussion

SHA in the Dark

As the testing frequency increased from 0.05 to 0.5Hz, the gain increased and phase lag decreased for both groups. The increase in gain indicates improved accuracy of the peripheral rVOR response and the decreased phase lag means that the eyes were able to better keep up with changes in head movement direction at higher frequencies. This improved efficiency of the rVOR at higher frequencies reflects healthy peripheral vestibular function in both groups since the rVOR responds best to high frequency cycle rotation, similar to that experienced by daily head movements. Although both groups followed the same increasing trend in increasing gain over the increasing frequencies (increasing x 3), the gain in the ASD group was significantly higher than controls at each of the three frequencies tested. This increased gain may reflect decreased cerebellar modulation of rVOR and is consistent with velocity step test findings of increased per-rotary gain and increased post-rotary time constant of decay in both dark and suppression conditions in ASD (see Chapter 2 of the current studies). There was no difference in phase between groups at each frequency. Overall, in the dark, the ASD group followed the same trend as controls of improved efficiency of gain at higher frequencies of rotation. The significant increase in gain compared to controls in the dark, however, may be related to hyper-responsivity to vestibular stimulation in ASD or to a lack of inhibition from the cerebellum.

The interpretation that this result reflects hyper-responsivity has interesting implications for clinical use considering that children with ASD have been noted to either seek or avoid vestibular sensory stimuli to an extent that is different from age-appropriate behavior (see Chapter 2) indicating children with ASD do not tend to

respond adaptively to vestibular stimulation in their activities of daily living. It seems reasonable to suspect that the sample of participants that volunteered for the current study would have an affinity toward or tolerance of vestibular stimuli. Therefore, it is surprising that these participants demonstrate such a heightened response during these tasks.

SHA with Fixation Suppression Testing

There was no difference between groups on gain during fixation suppression SHA testing at any of the frequencies tested and both groups demonstrated a reduction in gain compared to dark SHA tests. This reduction in gain across groups indicates that both groups were able to attend to the visual fixation stimulus and that both groups benefited from visual fixation suppression of rVOR during rotation by being able to suppress their rVOR nystagmus. Again, for both groups phase decreased as frequency increased. However, at the highest frequency tested (0.5Hz frequency), the ASD group exhibited increased phase lag compared to controls (Figure 3-4).

Conclusions

The ASD group exhibited significantly greater rVOR gain in the dark condition across all frequencies. Although this followed a typical trend, the increase in gain compared to controls indicates abnormal SHA response in this population that may be indicative of a lack of cerebellar inhibitory control of rVOR in this group. There was no difference between groups for phase in the dark condition; however, the ASD group exhibited greater mean rVOR phase lead compared to the TD group at 0.5Hz (the higher frequency) during the fixation suppression condition. Increased phase lead may indicate either peripheral vestibular differences or central deficits at the brainstem level in vestibular nuclei function (Shepard & Telian, 1996). Further studies are warranted to

discern whether these differences are due to perturbations in the peripheral or central nervous system.

Limitations and Future Studies

Several questions remain regarding rVOR performance during SHA tests in this population. Typically time constant of decay is estimated only for SHA tests conducted with a peak velocity of 50 - 60°/sec at the slowest frequency cycle (usually 0.01 or 0.02Hz). Both groups in the current study, however, would not tolerate testing at 0.01 or 0.02Hz frequency cycles and were tested instead at 0.05 Hz as the lowest frequency cycle. Although, the current study provided rotational stimuli at an appropriate peak velocity (60º/sec), the frequency cycle of 0.05 Hz was not low enough to calculate TCD from the current SHA test results Future studies should be conducted with SHA at 60°/sec peak velocity at a lower frequency cycle such as 0.01 or 0.02Hz in order to estimate TCD for comparison in ASD. It would be beneficial to calculate the TCD during SHA tests both in the dark and in fixation suppression conditions to better compare the results of these tests to velocity step tests conducted in ASD. However, the TCD estimate for SHA testing is directly related to the SHA phase. Based on the current results, increased phase lead in ASD at the 0.5Hz frequency cycle with fixation suppression indicates that at least for that condition and frequency cycle, TCD in the ASD group may be decreased compared to controls. This would be contradictory to velocity step test findings of increased TCD during fixation suppression in ASD. This contradictory finding is worthy of further investigation.

To extend the current findings of differences in fixation suppression, SHA fixation suppression testing should be explored using a full visual field as the fixation suppression stimulus during rotation (Brey et al., 2008b). A full visual field will stimulate

the optokinetic nystagmus system and may help to determine whether or not there are further differences in visual processing for full field vs. foveal fixation suppression of rVOR in ASD. If differences arise in response to full-field stimuli, it may be related to optokinetic system dysfunction; whereas, if differences with foveal fixation stimuli, differences may be related to gaze fixation or smooth pursuit deficits.

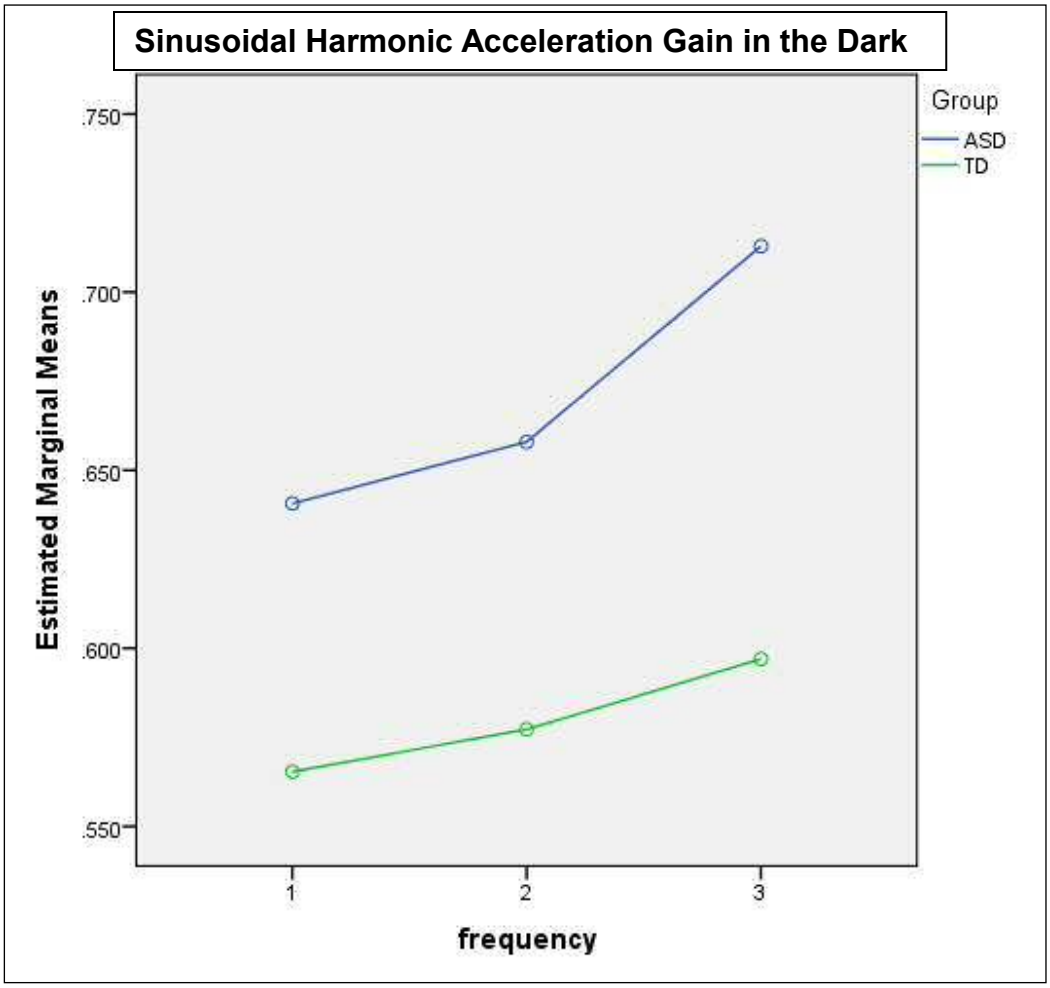

Figure 3-1. Mean gain for each group during SHA testing in the dark condition at cycle frequencies of (1) 0.05Hz, (2) 0.1Hz and (3) 0.5Hz. Gain was significantly higher for the ASD group across all frequencies ($p < 0.05$).

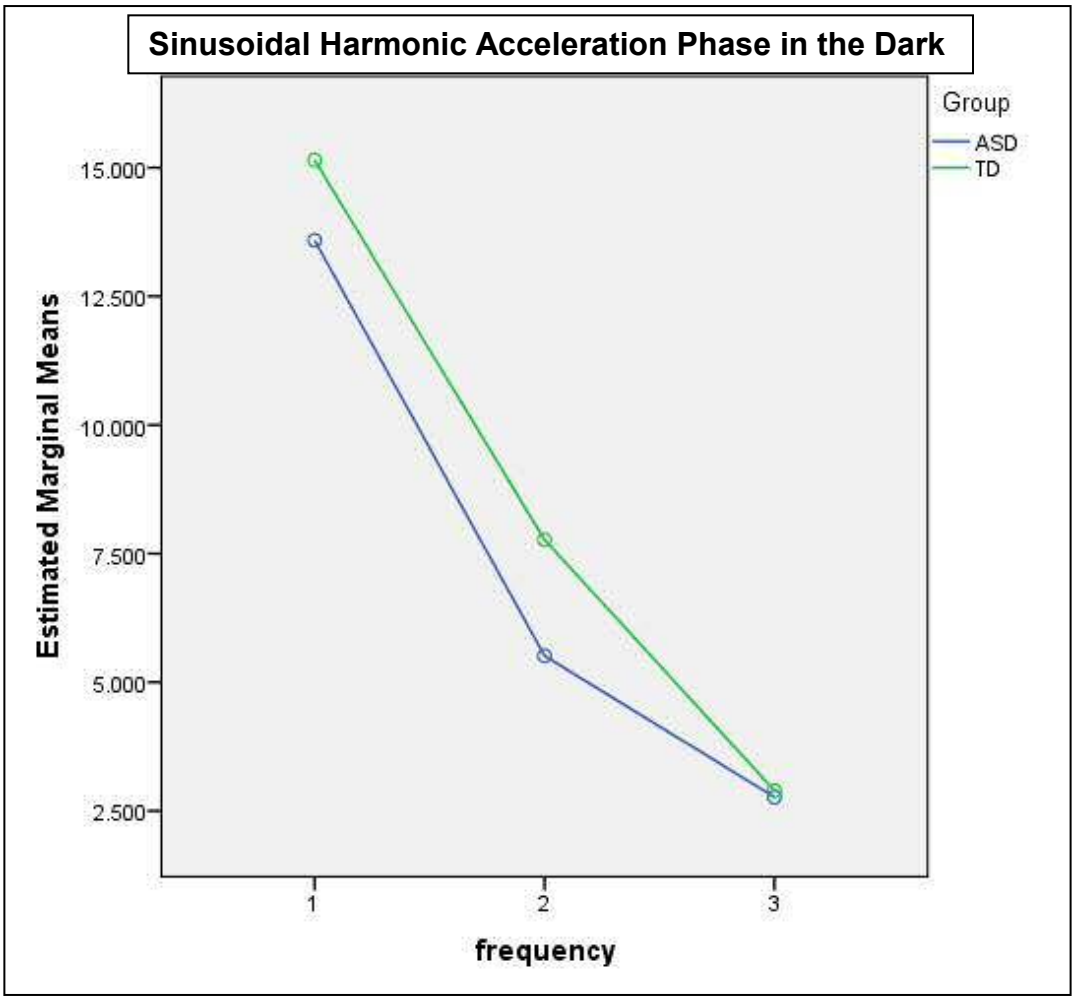

Figure 3-2. Mean phase for each group during SHA testing in the dark condition at cycle frequencies of (1) 0.05Hz, (2) 0.1Hz and (3) 0.5Hz. There was no significant difference between groups across conditions; however, there was a main effect of condition with both groups exhibiting a decrease in phase as frequency conditions increased.

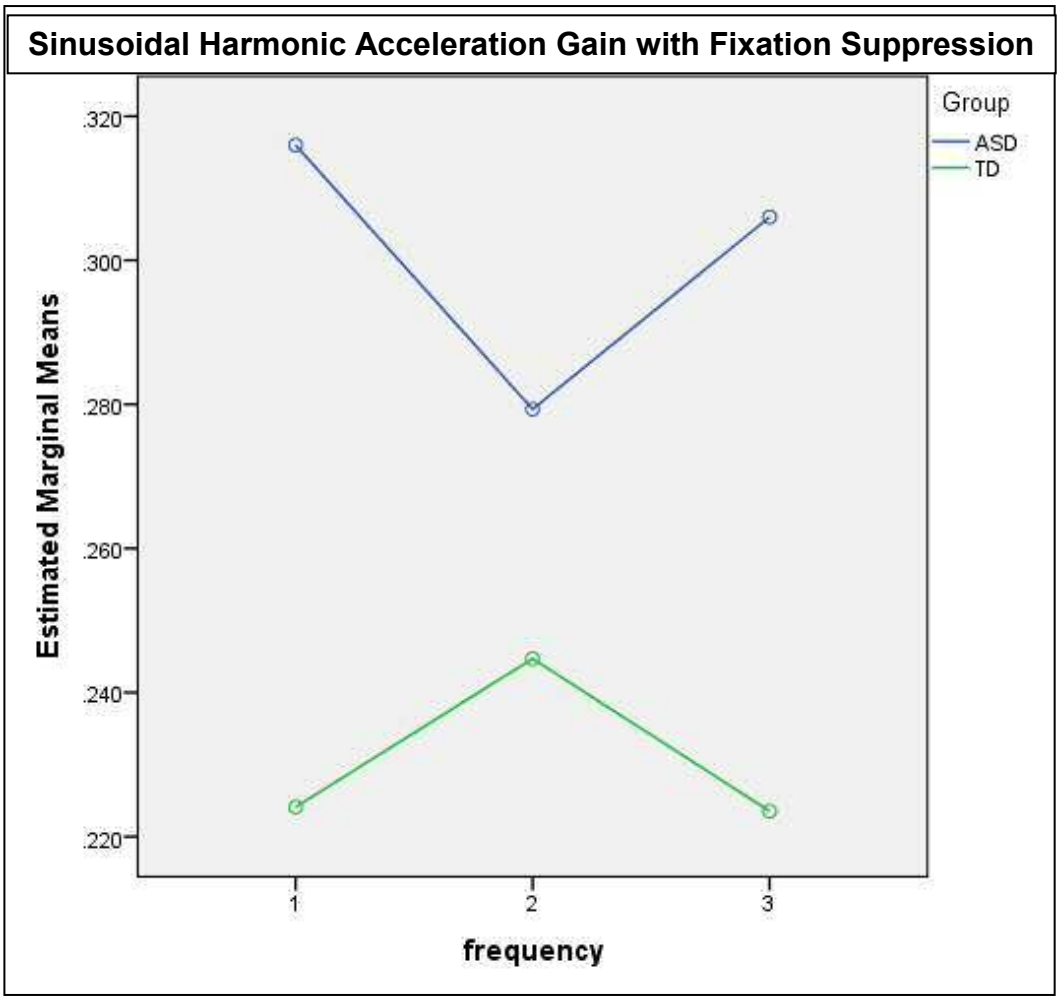

Figure 3-3. Mean gain for each group during SHA testing with fixation suppression LED at cycle frequencies of (1) 0.05Hz, (2) 0.1Hz and (3) 0.5Hz. It is important to note that the y-axis scale for mean gain in the fixation suppression condition above (0.22 to 0.32) is much lower than that in the dark condition (0.55 to 0.75) illustrating that both groups demonstrated a reduction in gain with fixation target present. There was no significant difference between groups for SHA gain across all frequency conditions.

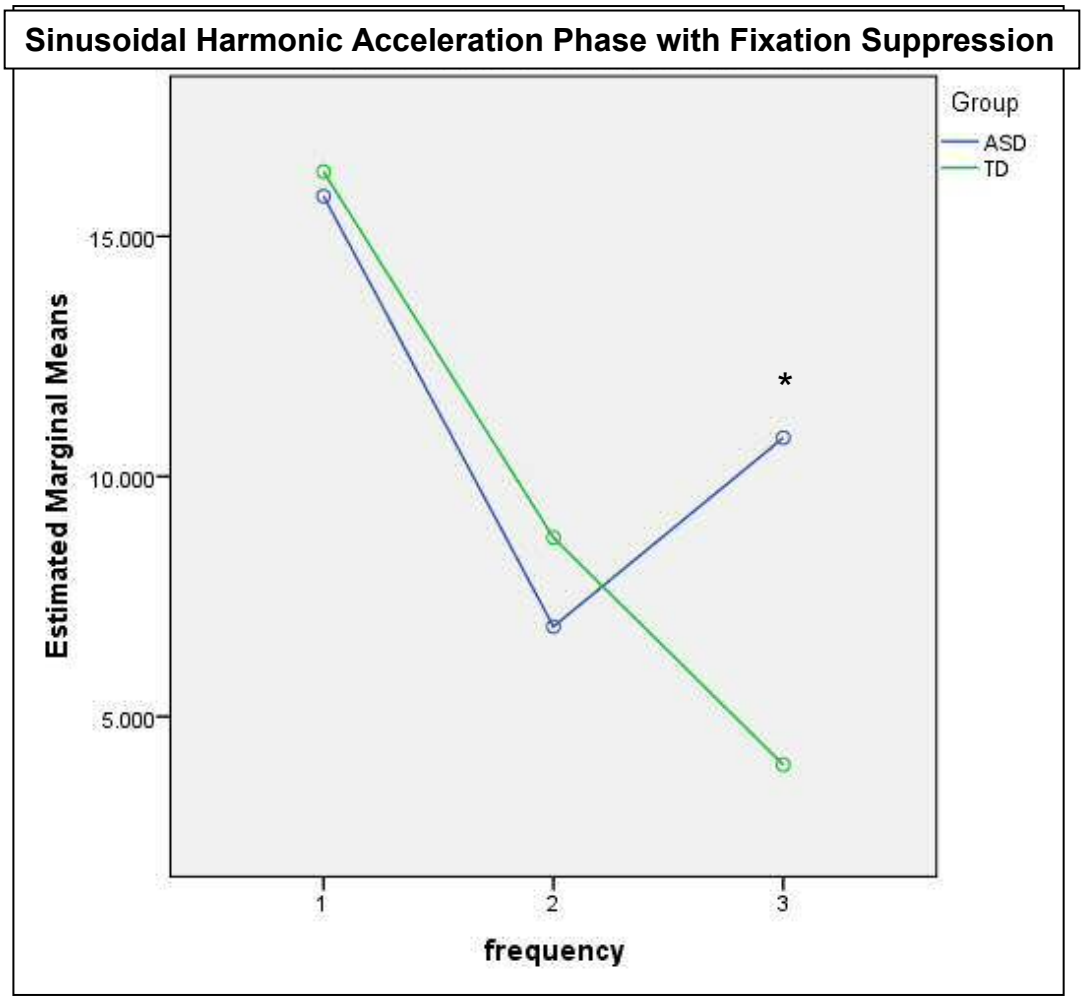

Figure 3-4. Mean phase for each group during SHA testing with fixation suppression LED at cycle frequencies of (1) 0.05Hz, (2) 0.1Hz and (3) 0.5Hz. The ASD group demonstrated increased phase lead at the 0.5Hz frequency cycle condition only (*$p < 0.05$).

CHAPTER 4
FUNCTIONAL CORRELATES OF THE VESTIBULO-OCULAR REFLEX IN AUTISM SPECTRUM DISORDERS

Introduction

Autism spectrum disorders (ASD) are developmental disorders characterized by deficits in communication skills, social skills and restricted repetitive behaviors (APA, 2000; DSM-IV TR). Additional features commonly reported in ASD are deficits in vestibular processing related motor functions such as postural control (Minshew et al., 2004; Molloy, Dietrich & Bhattacharya, 2003) and vestibulo-ocular reflex function (Ritvo et al., 1969; Ornitz et al., 1984; Ornitz et al., 1985). These sensory/motor deficits are of particular interest in ASD for both their functional implications, such as balance, motor planning/coordination, sensory perception and visual stability, as well as their usefulness in pinpointing neural substrates for pathology in ASD.

The most consistent finding of rotational vestibulo-ocular reflex (rVOR) aberration in ASD is a significant increase in the time constant of decay of the rVOR post-rotary nystagmus response following continuous rotation compared to typically developing controls (Ritvo et al., 1969; Ornitz et al., 1974; Ornitz et al., 1985; Chapter 2 of the current studies). The time constant is a prolongation of the nystagmus that occurs during rVOR. The time constant of decay functions to improve the efficiency of rVOR in response to low-frequency stimulation and is dependent upon brainstem and cerebellar modulation of rVOR (Leigh & Zee, 2006). Both brainstem and cerebellar structural differences have been consistently noted in ASD (Bauman &Kemper, 2005) and both structures are important for modulating vestibular sensory input and appropriate vestibulo-spinal or vestibulo-ocular motor output. It has been postulated that there is a general vestibular processing difference in ASD (Maurer & Damasio, 1979; Ornitz et al.,

1985; Kern et al., 2007). If there is, indeed, some common level of central vestibular processing dysfunction in ASD, it is reasonable to suspect that there may be a correlation between vestibulo-spinal (i.e., postural control/balance) and rVOR function as well as a relationship between vestibular function measures and parent reports of behavioral responses to vestibular stimuli in this population.

rVOR time constant of decay differences in ASD were observed with the use of sophisticated laboratory testing equipment and provided an objective index of rVOR deficits. Questions remain, however, as to their functional significance and the relationship of these reflex measures to functional abilities and deficits in children with ASD. If deficits in rVOR responses reflect neurological deficits in ASD, they may also be correlated to other functional measures in ASD such as the severity of ASD symptoms and ability measures.

The current study aimed to determine whether relationships exist between aberrant rVOR findings reported in Chapter 2 of the current studies and other functional performance measures in ASD. Specifically, it was anticipated that the following relationships would exist: (1) rVOR time constants of decay should be positively correlated with PANESS balance error measures indicating that a relationship exists between vestibulo-ocular and vestibulo-spinal measures in ASD; (2) parent reports of vestibular processing via the Sensory Profile should be negatively correlated with direct tests of vestibular function such as rVOR time constant of decay and/or balance errors such that as parent reports of adaptive vestibular processing behaviors improves, time constants and balance errors also improve; (3) Functional ability measures such as IQ and parent report of adaptive function should be negatively correlated with rVOR time

constants in ASD such that as IQ and adaptive scales improve, rVOR time constants will improve as well; and (4) If rVOR deficits are related to the symptoms of ASD, then rVOR time constants should be positively correlated with ASD symptom severity (i.e., as rVOR time constant increases ADOS, SCQ and RBS-R scores will also increase).

Methods

Participants and Recruitment

For this study, 16 children with ASD ages 6-12 were recruited from the University of Florida Center for Autism and Related Disabilities (UF CARD), Alachua County elementary and middle schools and pediatric therapy centers within Alachua county. ASD diagnoses included: Autism, Asperger's syndrome, or Pervasive Developmental Disorder - Not Otherwise Specified and were confirmed by the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003). Exclusionary criteria include diagnoses of Fragile X, Rett Syndrome, tuberous sclerosis, seizure disorder or fetal cytomegalovirus infection. These exclusionary criteria were selected in an effort to minimize confounding variables and to better define the ASD group by excluding disorders with known etiologies and to reduce the risk of any harm to participants as seizures have been reported to be elicited by vestibular stimulation.

Testing Equipment

A Neuro-Kinetics, Inc. (NKI) motorized, computer-controlled, rotary system was adapted for pediatric use for the current study. The authors added a seating system attached to the top of the rotary platform that was designed specifically for use with this pediatric population. This seating system included: padding, a chair, safety harnesses,

and a head stabilizing system (Figure 2-1 in Chapter 2). NKI I-Portal™ Video-Oculography Goggle (VOG) System and VEST 6.8 Software were used to record eye movement during whole-body continuous rotation velocity step tests.

Procedure

General procedure

Participants attended 2-3 testing sessions for participation in this study. For the first testing session, children and parents participated in various neuropsychological assessments. Some were conducted with direct observation of the child and others were questionnaires completed by parents/caregivers. At the end of the first testing session, participants were screened for gross oculomotor deficits and the videooculography (VOG) goggle system was calibrated. VOR tests were conducted during the second session and third session, if needed. Each session lasted approximately 1 to 2 hours.

Neuropsychological assessments

Children participated in four assessments as follows: (1) the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), (2) the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003), (3) the Leiter-R Brief IQ test (Roid, Miller, & Leiter, 1997), and (4) the Physical and Neurological Examination for Soft Signs (PANESS; Denckla, 1974). While children participated in the assessments listed above, parents/guardians completed three questionnaires as follows: (1) the Repetitive Behavior Scale-Revised (RBS-R; Bodfish et. al., 2000), (2) the Sensory Profile Caregiver Questionnaire (Dunn, 1999) and (3) the Vineland-II Adaptive Scales (Sparrow & Cicchetti, 1985; Sparrow, Cicchetti, & Balla, 2005). For the current studies, the combination of ADOS and SCQ was used to confirm diagnoses of children recruited into

the ASD group and a combination of Vineland-II Adaptive Scale and Leiter-R Brief IQ results was used to determine presence or absence of intellectual disability.

Autism Diagnostic Observation Schedule. The Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) is a semi-structured play-based assessment of social and communication skills and restricted repetitive behaviors. The ADOS is considered the gold standard in ASD diagnostic assessments and provides a method for direct observation and scoring of all three behavioral domains. The ADOS also provides a classification of autism, autism spectrum disorder and non-spectrum and thus, a measure of severity of autism symptoms ranging between ASD cut-off score (lower score, less severe) and autism cut-off score (higher score, more severe). This assessment takes approximately 60 - 120 minutes to administer.

Social Communication Questionnaire. The Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003) is a parent report measure of social and communication skills. The SCQ is a valid first-level screening tool for identifying school-age children who are at risk for ASD and would benefit from further ASD diagnostic testing (Eaves, Wingert, Ho & Mickelson, 2006; Chandler et al., 2007). In the current study, the SCQ was used in conjunction with ADOS scores to confirm diagnoses within the autism spectrum.

Leiter International Performance Scale - Revised (Leiter-R) Brief IQ. The Leiter-R Brief IQ test (Roid, Miller & Leiter, 1997) is a non-verbal intelligence assessment that allows individuals age 2 to 21 years to indicate their responses through gestures or manual selection. It is generally recommended that the Leiter-R be used for children with ASD due to deficits in communication that would preclude or prevent

completion of other forms of intelligence testing (Tsatsanis et al., 2003). This test takes approximately 60 minutes to administer.

The Physical and Neurological Examination for Soft Signs (PANESS). The PANESS is a standardized pediatric neurological assessment of motor control where children complete motor tasks such as rapid finger tapping or walking heel-to-toe (Denckla, 1974). The PANESS includes a subset of measures of balance such as single leg stance and hopping on one foot. The PANESS subscale sum of balance errors has been previously shown to distinguish typically developing children from children with ASD, but not children with high functioning autism from children with Asperger's syndrome (Jansiewicz et al., 2006). Balance is a measure of vestibulo-spinal function, therefore, the balance sum of errors subscale from the PANESS was chosen as a variable of interest for the current study to compare with other measures of VOR function. This test takes approximately 20 minutes to administer.

Repetitive Behavior Scale-Revised: Stereotypy Subscale. The Repetitive Behavior Scale-Revised (RBS-R; Bodfish et. al., 2000) is an index of restricted repetitive behaviors in ASD. The RBS-R includes 6 subscales as follows: stereotyped behavior, self-injurious behavior, compulsive behavior, ritualistic behavior, sameness behavior and restricted behavior. Each item within these subscales is rated on a 4-point likert scale from 0 (behaviors do not occur) to 3 (behaviors do occur and are a severe problem). The stereotyped behavior subscale of the RBS-R was selected for the current study rather than total RBS-R scores because 3 out of 5 of the questions in the stereotypy subscale include movement stereotypies that generate vestibular stimulation

such as (1) body rocking or body swaying, (2) rolling, nodding, or turning the head and (3) turning in circles, whirling, jumping or bouncing.

Sensory Profile: Vestibular Subscale. The Sensory Profile Caregiver Questionnaire (Dunn, 1999) is a tool for evaluating a child's sensory processing ability and adaptive responses to sensory stimuli based on parent report of the child's behavior. The Sensory Profile provides information about a child's ability to process and respond adaptively to six sensory processing domains including the domain of vestibular processing. The vestibular processing subsection of the Sensory Profile provides a parent report of non-adaptive behavioral responses to various vestibular sensory stimuli encountered in daily activities. Question prompts include examples such as "avoids playground equipment or moving toys" or "seeks all kinds of movement and this interferes with daily routines". Parents rate these statements regarding their child's behavior on a Likert scale of 1 (always) to 5 (never). Thus, higher scores reflect more adaptive responses and the lower scores reflect less adaptive responses.

Vineland-II Adaptive Scales. The Vineland-II Adaptive Scales (Sparrow & Cicchetti, 1985; Sparrow, Cicchetti, & Balla, 2005) is a measure of communication, daily living, socialization and motor skills for daily living. The Vineland-II is widely used clinically in combination with IQ to evaluate functional intelligence and has been shown to have test-retest reliability, internal consistency (Sparrow, Cicchetti & Balla, 2005) and concurrent validity (Perry et al., 1989).

VOG system calibration

The VOG system was calibrated prior to testing each participant using a laser stimulus projected onto a black screen (60º arc with 76.5 inch radius). The laser provided a visual target that moved to specified locations $\pm$ 10º from center (e.g., first

10º to the left, then right, then up and then down). Results were averaged across two trials.

Oculomotor screening

Oculomotor screening tests were conducted to rule out any neurological impairment related to oculomotor function that would preclude interpreting rVOR eye movements. These tests included: saccade, smooth pursuit and gaze evoked nystagmus tests. VOG recordings were taken of the participants' eye movements as they followed the red laser stimulus through a series of movements for each test (see methods section in Chapter 2 for further detail).

Velocity step testing general procedure

Velocity step tests were completed in both clock-wise and counter clock-wise directions with a ramp-up time of 1.2 seconds and a peak velocity of 100º/sec. This protocol was repeated across two conditions: (1) in the dark with no visible surroundings and (2) in the dark with a visual fixation suppression LED target provided inside the VOG system during and after rotation. Per-rotary recordings were taken for 60 seconds, followed by post-rotary recordings that lasted as long as nystagmus was occurring up to 60 seconds. Each test was completed once in each direction. However, if one of these two trials was disrupted by excessive blinking, talking, head movement or the child requesting a break, the entire trial was repeated with the participant's assent.

Data Analysis

Pearson product moment correlations were conducted using IBM SPSS Statistics software v. 21 to test for relationships between rVOR time constant in the dark condition or rVOR time constant in the fixation suppression condition and the following functional measures: (1) the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000);

(2) the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003); (3) the Leiter-R Brief IQ test (Roid, Miller, & Leiter, 1997); (4) the Physical and Neurological Examination for Soft Signs sum of balance errors (PANESS; Denckla, 1974); (5) the Repetitive Behavior Scale-Revised (RBS-R; Bodfish et. al., 2000); (6) the Sensory Profile Caregiver Questionnaire vestibular processing subscale (Dunn, 1999) and (7) the Vineland-II Adaptive Scales (Sparrow & Cicchetti, 1985; Sparrow, Cicchetti, & Balla, 2005). For the rVOR time constants in each of the conditions tested, data from both clock-wise and counter-clockwise trials were pooled for each ASD participant, since there was no significant difference between these conditions within subjects (paired t-tests, $p > 0.05$).

Results

A Pearson's product moment correlation was conducted to assess the relationship between neuropsychological assessments of autism severity, functional ability and vestibular function as well as the variables of age and rVOR time constant of decay in two conditions: dark and fixation suppression. All variables included in the analyses were observed to be approximately normally distributed, as tested by Shapiro-Wilk's test ($p > .05$), except for three measures including: (1) PANESS balance sum of errors ($p = 0.01$) which displayed a somewhat bimodal distribution with scores falling between either 0 to 2 total errors or 5 to 8 total errors (see Figure 4-8); (2) rVOR TCD in the dark ($p = 0.03$) which was slightly positively skewed (i.e., skewed towards more errors and longer time constants) and (3) ADOS scores ($p < .05$) which were slightly negatively skewed. Since Pearson's correlation analysis is somewhat robust to deviations from normality these variables were included in the analysis, but deviations from normality are considered in the interpretation of related results. A summary of

Pearson's correlation statistics are provided in Table 4-1. Figures 4-1 to 4-8 provide scatterplots of all neuropsychological and rating scale assessments to illustrate their relationships with time constant of decay in the dark condition as well as time constant of decay with fixation suppression.

Dark Condition rVOR Time Constants

The only neuropsychological assessment that demonstrated a significant relationship with rVOR time constant in the dark was the PANESS subscales for balance errors. There was a positive correlation between the sum of balance errors scores on the PANESS and time constant of decay for rVOR in the dark, $r(14) = .587$, $p < .017$.

Fixation Suppression Condition rVOR Time Constants

Only one significant association was observed between neuropsychological and rating scale measures and the time constant of rVOR in the fixation suppression condition. A significant negative correlation was observed between rVOR time constants during fixation suppression and Vineland-II Adaptive Scales, $r(14) = -.545$, $p = .029$. Non-significant but marginal correlations included: (1) a negative correlation with Leiter Brief IQ, $r(14) = -.434$, $p = .093$; (2) a negative correlation with RBS-R Stereotypy Subscales, $r(14) = -.442$, $p = .099$ and (3) a positive correlation with the sum of balance errors on the PANESS, $r(14) = .445$, $p = .084$.

Associations among Neuropsychological and Standard Rating Scale Assessments

Significant positive correlations were found between the three following pairs of neuropsychological assessments: (1) Sensory Profile and ADOS, $r(14) = .546$, $p = .029$; (2) Vineland-II and IQ, $r(14) = .597$, $p = .015$; and (3) RBS-R and SCQ, $r(12) = .565$, $p =$

.035. Significant negative correlations were observed between the four following neuropsychological assessments: (1) Age and IQ, $r(14) = -.592$, $p = .016$; (2) Vineland-II and Age, $r(14) = -.587$, $p = .017$; (3) RBS-R and ADOS, $r(13) = -.534$, $p = .040$ and (4) a very strong negative correlation between Sensory Profile and RBS-R, $r(13) = -.835$, $p < .005$. Vineland-II and SCQ exhibit a marginal negative correlation that approaches but does not reach significance, $r(14) = -.470$, $p = .077$;

Discussion

Laboratory tests of rVOR deficits (Ritvo et al., 1969; Ornitz et al., 1974; Ornitz et al., 1985; Chapter 2 of current studies) and postural control (i.e., vesitublo-spinal) deficits (Minshew et al., 2004; Molloy, Dietrich & Bhattacharya, 2003) have been reported in ASD. However, the implication of these findings and their relationship to other functional abilities of children with ASD remains unclear. For the current study, it was anticipated that significant relationships would be evident between laboratory test results of rVOR aberrations in ASD and the following functional measures: vestibulo-spinal function tests of balance, parent report of vestibular processing and neuropsychological assessments of ASD symptom severity and functional ability. Relationships were observed between rVOR time constant of decay and vestibulo-spinal/balance function as well as measures of functional adaptive skills. These results indicate that there may be vestibular processing deficits in ASD that include vestibulo-ocular as well as vestibulo-spinal dysfunction and that these two functional deficits may be related in ASD. The current study also demonstrates that rVOR deficits in ASD may be related to other measures of functional ability such as adaptive function. It is interesting to note that rVOR time constant of decay in the dark and time constant of decay with fixation suppression were not correlated with one another in the current

study. Furthermore, it is interesting to note that the fixation suppression time constant was significantly correlated with Vineland-II Adaptive Scales and marginally correlated with Leiter-R Brief IQ, RBS-R and the PANESS balance scores, whereas, the dark time constant was only significantly correlated with PANESS balance error scores with no marginal correlations to any of the other measures. This may indicate that the fixation suppression condition would be a better candidate for further study as a potential bio-behavioral marker.

Vestibular Function Measures: rVOR Time Constants, PANESS Balance Errors and Sensory Profile Vestibular Processing Subscale

Vestibulo-ocular and vestibulo-spinal function

The Physical and Neurological Exam of Soft Signs (PANESS; Denckla, 1974) is a pediatric measure of neurologic motor function. In the current study, PANESS balance error scores were strongly positively correlated with time constants of decay for rVOR in the dark condition and marginally positively correlated with time constant during fixation suppression testing. As PANESS balance errors increased so did the rVOR time constant of decay (i.e., increasing time constants indicate central rVOR processing deficits). This connection between measures of vestibulo-spinal (i.e., PANESS balance errors) and VOR function (i.e., time constant of decay) in ASD indicate that there may be a global vestibular processing deficit affecting both the vestibulo-ocular and vestibulo-spinal function in ASD and may share a common neurobiological basis.

Parent report of vestibular processing and direct measures of vestibular function

Often in pediatric rehabilitation, the first step for evaluating sensory processing deficits is obtaining a parent report of the child's response to sensory stimuli. One measure, the Sensory Profile Caregiver Questionnaire (Sensory Profile; Dunn, 1999), is

commonly used as a screening tool to evaluate adaptive behavioral responses to sensory stimuli. The Sensory Profile provides an indication to therapists that sensory processing deficits exist and may warrant further testing. It is also used as a guide for sensory-integration treatment planning. The Sensory Profile provides information about a child's ability to process and respond adaptively to six sensory processing domains including the domain of vestibular processing. The vestibular processing subscale of the Sensory Profile provides a parent report of non-adaptive behavioral responses to various vestibular sensory stimuli encountered in daily activities.

Since this is often the first step in evaluating sensory processing deficits, it would be useful to know if parent report of Sensory Profile is correlated with direct measures of vestibular function in children with ASD such as postural control/balance or VOR function. According to the Sensory Profile scoring package, the group mean for the vestibular processing subscale in this sample of children with ASD indicates a "definite difference" in vestibular processing. Although the sensory profile is a parent report, it is often used clinically in Occupational Therapy as the first screening tool or indication that a sensory processing deficit exists. Therefore, it is surprising then that the current study found no statistically significant relationships between the vestibular processing subscale of the Sensory Profile and either of the rVOR measures or the balance measure. Although not statistically significant, it is noteworthy that the vestibular processing subscale of Sensory Profile was slightly positively correlated with the following three vestibular function measures: (1) the PANESS balance error subscale, $r(14) = .354$, $p = .178$; (2) the rVOR time constant of decay in the dark, $r(14) = .354$, $p = .179$; and (3) the rVOR time constant of decay in the fixation suppression condition,

$r(14) = .373$, $p = .155$. The lack of significant findings may have been due to a small sample size and lack of sufficient power to pick up on this relationship. However, in a previous study with the same sample of children all three of these vestibular processing related variables were significantly different between ASD and typically developing controls (Chapter 2). Additionally, a larger study with 103 children and adults age 3 to 43 with ASD showed a significant difference between ASD and typically developing controls on the vestibular processing subscale of the Sensory Profile (Kern et al., 2007). Thus, it appears that although the vestibular subsection of the Sensory Profile is able to demonstrate differences between ASD and controls, it may not be related to direct measures of vestibular function in this population. Further studies with a larger sample size should be conducted before ruling out the possible relationship between the vestibular subsection of the Sensory Profile and direct measures of vestibular function.

IQ and vestibular function

No significant correlations between IQ and rVOR were found. However, although not-significant, IQ was marginally negatively correlated with time constant of decay for rVOR fixation suppression. If such a relationship did exist, the direction of this correlation indicated that as IQ increased, fixation suppression rVOR time constant decreased, meaning that as level of intelligence increased, the ability to use visual fixation to suppress rVOR post-rotary nystagmus improved. This relationship may indicate that central processing deficits in rVOR are related to deficits in using higher order processing function in ASD.

It is also possible that those participants with lower IQ have greater difficulty attending to task and, therefore, fixating on the visual target. However, as previously described in Chapter 2, there was no significant difference in gain between the two

groups for this condition (i.e., both groups demonstrated decreased gain during fixation suppression). Therefore, we are fairly confident that both groups were indeed fixating on the visual target during testing, as such a decrease in gain would not occur otherwise.

Intercorrelations among Neuropsychological and Standardized Rating Scale Assessments

Symptom severity: ADOS, SCQ and RBS-R

Autism is diagnosed based on functional deficits within three behavioral domains: social skills, communication skills and restricted, repetitive behaviors. The ADOS is considered the gold standard for diagnosing ASD and the SCQ is often used as a parent report screening tool to aid in the diagnosis of ASD. The SCQ and total score from the RBS-R provide measures of the three diagnostic domains for ASD including: deficits in communication skills and social skills (SCQ) and excess restricted, repetitive behaviors (RBS-R).

In the current study, ADOS scores were negatively correlated with motor stereotypy subscale scores of the RBS-R. Both measures provide scores that increase as an index of greater dysfunction and would be expected to be positively correlated (i.e., severity of ASD increases as motor stereotypies severity increases). However, in the current study, the negative correlation indicates that as the severity of ASD symptoms increases, the severity of stereotypies decreases.

Similarly, ADOS scores were positively correlated with the vestibular subscale of the Sensory Profile. Higher Sensory Profile scores indicate more adaptive behavior (i.e., less dysfunction). Therefore, it is expected that as ASD severity increases, vestibular processing scores should indicate greater dysfunction (i.e., decrease). However, according to the positive relationship found in the current study, as autism severity

increases, the parent report of adaptive behavioral responses to vestibular stimuli improves.

These two unexpected directional relationships are not consistent with the literature that suggests that children who are more severely affected display a greater incidence of stereotypic behaviors (Bodfish, Symons, Parker & Lewis, 2000; Michelotti, Charman, Slonims & Baird, 2002; Morgan, Wetherby & Barber, 2008). It may be possible that there is a distinction between ASD severity and movement-related vs. cognitive-related restricted, repetitive behavior. Additionally, these findings could be an artifact of only using stereotypy subscale scores of the RBS-R and Sensory Profile rather than the full scales. Additionally, the current study recruitment targeted high functioning somewhat older children with ASD; therefore, it is possible that motor stereotypy sub-scales may be relatively low in this group compared to younger children with ASD. Among other things, this could lead to a truncated range for these subscales, which could possibly generate spurious correlations. Therefore, it is difficult to make any conclusions based on these results at this time.

Surprisingly, correlations between ADOS and SCQ scores were very low. It is unclear as to why a strong relationship between ADOS and SCQ was not observed since both of these diagnostic tools are primarily focused on the social and communication aspects of the ASD triad. Although the current study did not account for presence or absence of language delay, only 2 participants exhibited limited verbal communication skills. Therefore, it is possible that the current group was skewed towards the higher functioning range and/or had a high percentage of children with Asperger's who may have performed better on the communication domain. It is also

possible that the lack of correlation between these measures is related to the fact that the SCQ is a parent report of the child's behavior in the natural environment over the lifespan and the ADOS is a direct measure of a child's performance in a clinical or research setting over a few hours of observation.

The RBS-R and SCQ in combination provide information about all three of the ASD core diagnostic behavioral domains including restricted repetitive behaviors and social/communication skills respectively. In the current study, the stereotypy subscale of the RBS-R was found to be positively correlated with SCQ total scores, meaning that as movement stereotypies become more severe, so do social/communication skill deficits. These results may provide support for the combination of the RBS-R stereotypy subscale and full scale SCQ as a potentially useful parent report battery to index the severity of ASD symptoms. It is unclear at this time whether or not these relationships would exist between the full scale RBS-R and SCQ measures.

The strongest correlation observed in the current study arose between the vestibular subscale of the Sensory Profile and the stereotypy subscale of the RBS-R. Since the stereotypy subscale of RBS-R was designed to collect parent reports of restricted, repetitive behaviors specific to ASD, 3 out of 5 of which result in vestibular stimulation, it was reasonable to assume that vestibular stimulating movement stereotypies would be related to some measure of vestibular function. However, the current study did not find any significant relationship between the stereotypy subscale of the RBS-R and direct measures of vestibular function such as rVOR time constant or balance errors. It is possible that vestibular function has no bearing on these stereotypies and that these repetitive behaviors are propagated by another

physiological process. There was, however, a significant positive relationship between the two parent report measures: the Sensory Profile vestibular subscale and the stereotypy subscale of the RBS-R, demonstrating that there is some consistency within parent reports of vestibular related movement behavior in ASD. Taken together, these results suggest that the stereotypy subscale or the RBS-R and the Sensory Profile vestibular processing subsection are more related to vestibular related restricted, repetitive behaviors rather than actual vestibular function in this group. This finding is consistent with the current use and interpretation of the RBS-R stereotypy subscale, but is inconsistent with the current use of the Sensory Profile vestibular subscale as an indicator of vestibular sensory processing function.

Functional ability: Leiter Brief IQ and Vineland-II Adaptive Scales

Vineland-II Adaptive Scores and Leiter Brief IQ scores were positively correlated meaning that as IQ increased, so did functional adaptive skills. This relationship between these two measures was expected since the combination of these measures is often useful for determining presence or absence of developmental delays and intellectual developmental disabilities. Previous studies have also demonstrated a strong correlation between IQ and age with adaptive skills in ASD (Kanne et al., 2010).

Age and IQ as well as Age and Vineland-II Adaptive Scales were negatively correlated such that as age increased in these individuals with ASD, IQ and adaptive skills decreased. Although not significant, the Vineland-II Adaptive Scales and SCQ total scores also exhibited a moderate negative correlation. These relationships between the Vineland-II and these two measures follow an unexpected pattern of social and communication skills increasing while adaptive skills decrease. This unexpected pattern of findings may indicate that deficits in adaptive function fail to improve as development

continues in this population or that the deficits in adaptive function are magnified as development continues in this population. This concept has been presented previously in a larger study of children with ASD and measures of Vineland-II Adaptive Skills demonstrating that as age increases in ASD, the gap between mental age and adaptive skills increases; i.e., deficits become more apparent in this population (Kanne et al., 2010; Klin et al., 2007).

Conclusions

Taken together, the results of the current study indicate that there was a strong correlation between measures of vestibulo-spinal (i.e., balance) deficits and rVOR TCD (only in the dark condition) in ASD indicating that central vestibular processing dysfunction does exist in this population and that these two systems may share common central processing deficits. The current study further demonstrated that IQ and adaptive function are also related to deficits in rVOR fixation suppression in ASD and that rVOR suppression deficits may be related to higher-order central processing deficits in ASD. A lack of any significant relationship between Sensory Profile vestibular processing subscales and direct measures of vestibular function is surprising and warrants further study. The RBS-R movement stereotypy subscale, however, did appear to be related to direct measures of rVOR fixation suppression deficits in ASD. The results of the current study may inform pediatric clinical rehabilitative practices for screening and assessment of vestibular processing deficits in ASD as well as future research studies of the neurological mechanisms underlying vestibular processing deficits in ASD.

The results of the current study may help to provide direct assessments that can accompany and strengthen current parent report measures of assessing sensory

processing in ASD. Alternatively, these findings may help to develop a vestibular processing parent report questionnaire with revised prompts to improve predictive validity for direct measures of vestibular function.

Limitations and Future Studies

Questions remain as to whether increased rVOR time constant of decay during the dark and visual fixation suppression conditions are specific to ASD as a whole, specific to subgroups within ASD, or specific to developmental disabilities in general. The current studies demonstrate that IQ and adaptive skills are positively correlated with both of these measures of deficits in rVOR function in ASD. Although the current study included one child with an IQ below 70, little is known about rVOR function in lower functioning individuals with ASD. It would be interesting to know whether lower functioning individuals would further strengthen this correlation and demonstrate a greater increase in rVOR fixation suppression.

One extension of the current findings could involve testing visual fixation suppression in lower functioning individuals with ASD. The available physiological research literature in this subgroup of individuals with ASD is limited, likely due to the added methodological challenges and reduced compliance associated with this population. Many of these challenges are due to communication deficits, however, and with added precautions can be ameliorated. Testing this population of individuals who are lower functioning or non-verbal may require modification of protocols such as the addition of augmentative alternative communication options for participants to use to indicate their assent to participate and whether or not they understand instructions for looking at the target during visual fixation or for tilting forward during tilt suppression testing. In other studies in our lab, the addition of a training video that visually illustrates

a participant going through the entire testing procedure has been proven to be helpful in preparing non-verbal or lower functioning participants for testing.

For clinical purposes, it is often assumed that a relationship exists between sensory processing subscale results on the Sensory Profile and the child's ability to process sensory input. The current study did not find a significant correlation between the vestibular processing subscale of the Sensory Profile and direct measures of vestibular dysfunction in ASD. Therefore, questions arise as to whether the vestibular subscale of the Sensory Profile is measuring behavioral characteristics or preferences of children or true sensory processing deficits. It is also interesting to note that the vestibular subscale of the Sensory Profile was negatively correlated with the movement stereotypy subscale of the RBS-R. Thus, the vestibular subscale of the Sensory Profile may be measuring behaviors that are more akin to motor stereotypies in ASD rather than symptoms of vestibular processing dysfunction per se. It remains a possibility that movement stereotypies in ASD are related to vestibular processing deficits, however, this relationship was not observed in the current study. Future studies should aim to investigate the level of face validity or predictive validity for sensory processing subscale results of the Sensory Profile by comparing each of these subscales to direct observations or functional measures of taste, tactile, auditory and visual processing and to clarify for practitioners whether definite differences in these areas are due to behaviorally-based or physiologically-based sensory processing deficits.

If the vestibular subscale of the Sensory Profile is truly not related to direct measures of vestibular function, future studies should aim to establish parent report measures of sensory processing that exhibit a clear relationship to physiological

assessments of sensory processing. An established connection between parent screeners and further physiological testing would be very useful to clinicians as a pediatric sensory processing evaluation tool. The rVOR is highly modifiable and amenable to rehabilitation. Thus, improvements in pediatric assessment and treatment planning in this area could have a large impact on clinical practice and functional outcomes for these children with ASD.

Pearson Correlation Statistics for Neuropsychological Assessments and Time Constant of Decay (TCD)

	Age	Leiter IQ	Vineland-II	ADOS	SCQ	RBS-R[1]	Sensory Profile[2]	PANESS[3]	TCD Dark	TCD Supp.
TCD Dark	-.253	-.229	-.087	.246	-.157	-.367	.354	.587*	____	
TCD Supp.	.407	-.434	-.545*	.154	-.196	-.442	.373	.445	.267	____

** Correlation is significant at the 0.05 level*

[1] RBS-R Stereotyped Movement subscale only

[2] Sensory Profile Vestibular Processing subscale only

[3] PANESS Balance sum of error scores only

Table 4-1. Pearson Correlation Summary Table for Neuropsychological Assessments and rVOR Time Constant of Decay in the Dark and with Fixation Suppression. Significant positive correlations are highlighted in yellow and significant negative correlations are highlighted in blue.

Pearson Correlation Statistics for Neuropsychological Assessments

	Age	Leiter IQ	Vineland-II	ADOS	SCQ	RBS-R[1]	Sensory Profile[2]	PANESS[3]
Age	____							
Leiter Brief IQ	-.592*	____						
Vineland-II	-.587*	.597*	____					
ADOS	.022	-.009	-.313	____				
SCQ	.396	-.202	-.470	-0.137	____			
RBS-R[1]	-.077	.192	.116	-.534*	.565*	____		
Sensory Profile[2]	-.009	-.277	-.227	.546*	-0.43	-.835**	____	
PANESS[3]	-.218	-.300	-.124	0.275	-0.174	-0.305	0.354	____

** Correlation is significant at the 0.05 level*

*** Correlation is significant at the 0.01 level*

[1] RBS-R Stereotyped Movement subscales only

[2] Sensory Profile Vestibular Processing subscale only

[3] PANESS Balance sum of error scores only

Table 4-2. Pearson Correlation Summary Table for Neuropsychological Assessments. Significant positive correlations are highlighted in yellow and significant negative correlations are highlighted in blue.

Figure 4-1. Scatterplots of age (in months) and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

Figure 4-2. Scatterplots of Leiter-R Brief IQ (composite scores) and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

Figure 4-3. Scatterplots of Vineland-II Adaptive Behavior Scales (composite scores) and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

Figure 4-4. Scatterplots of Autism Diagnostic Observation Schedule (ADOS) composite scores and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

Figure 4-5. Scatterplots of Social Communication Questionnaire (SCQ; total score) and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

Figure 4-6. Scatterplots of Restricted Repetitive Behavior Scale – Revised Stereotypy Subscale Total Score and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

Figure 4-7. Scatterplots of Sensory Profile (SP) Vestibular Processing Subscale Score and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

Figure 4-8. Scatterplots of Physical and Neurological Exam of Soft Signs (PANESS) Balance Error Subscale Total and time constant of decay (in seconds) in the dark condition and fixation suppression condition.

CHAPTER 5
ABNORMAL QUALITY OF THE ROTATIONAL VESTIBULO-OCULAR REFLEX IN AUTISM SPECTRUM DISORDERS

Introduction

The primary measures of the rotational vestibulo-ocular reflex (rVOR) include time constant of decay, gain and symmetry. Each of these parameters provides information about the functional ability of the peripheral vestibular anatomy or the central processing of vestibular sensory information via the brain stem and cerebellum. However, abnormalities in rVOR eye movements beyond these standard rVOR measures have been noted in children with autism spectrum disorders (ASD) and may be informative for better understanding the neurobiology of rVOR function and/or oculomotor function in this population. Evidence for such differences in rVOR are sparse in the available literature, however, and are often presented as a simple side note or post-hoc observations (Ritvo et al., 1969; Ornitz et al., 1974; Ornitz et al., 1985) or not mentioned at all (Goldberg et al., 2000). Therefore, further evaluation of the quality of rVOR eye movements is warranted to clarify if these abnormalities exist in this population and if so, to better characterize and define them.

Number of Nystagmus Beats

The first abnormality of rVOR that has been previously noted in ASD is a reduction in the number of post-rotary nystagmus beats, nystagmus that occurs after rotation has ceased. Two studies reported a decreased frequency of post-rotary nystagmus beats in ASD compared to controls (Ornitz et al., 1974; 1985). Ornitz et al. (1985) suggested that this decrease in the number of beats may be due to an observed “dysrhythmia” between the slow phase and quick phase eye movements of nystagmus in the ASD group.

Slow Phase Irregularity

The second abnormal feature of rVOR previously noted in ASD is an irregularity in the slow phase of nystagmus eye movements. The slow phase component of nystagmus is the most informative characteristic for identifying underlying disorders (Leigh & Zee, 2006). Ornitz et al. (1985) found no difference in rVOR gain between ASD and controls, suggesting that ASD participants demonstrated normal cerebellar flocculus modulation of rVOR. Nonetheless, they did report abnormal characteristics of rVOR nystagmus eye movements, such as aberrations in slow phase velocity and increased incidences of slow phases that failed to be followed by a quick phase in the opposite direction. The authors suggested that such “dysrhythmia” between slow and quick phases may be a result of brainstem dysfunction, particularly with regard to coordination between pontine reticular formation control of quick phases and vestibular nuclei control of slow phases (Ornitz et al., 1985). Since no follow up studies have been conducted it remains unclear as to whether or not these results are replicable and what neurobiological insights into ASD they may provide.

Vertical Eye Movement Intrusions

The third rVOR abnormality of vertical eye movement intrusions have not been reported by the previous studies mentioned above. These aberrations in rVOR have only been noted by our lab and were observed in a preliminary study conducted in preparation for the current project. In this pilot study with three children with ASD and three typically developing children age 7 to 10 years, we observed vertical eye movement intrusions occurring concomitantly with horizontal rVOR. These vertical intrusions occurred significantly more often in the ASD group (26.67 $\pm$ 4) than the typically developing controls, 9.61 $\pm$ 3; Mann Whitney-U, $p = 0.001$. However, no

significant difference in the occurrence of horizontal eye movements between groups was noted, Mann Whitney-U, p = 0.35. Furthermore, these vertical intrusions were clearly visible such that novice, blind observers (n=17) were able to correctly identify 4.88 (±1.23) out of 6 control videos as "normal" and 4.47 (± 1.09) of 6 ASD videos as "abnormal" (Figure 5-1).

The previously cited studies noting abnormalities in number of beats and slow phase duration were conducted using electro-oculography (EOG) to record eye movements. Often when using this technique, movements in the vertical channel are discarded as noise or eye blinks. Thus, vertical eye movements may have been present in previous studies, but not detected. Without a visual recording of the eye movements as a reference, these vertical eye movement excursions were eliminated as artifact. Therefore, it would be beneficial to measure these eye movements using videooculography (VOG) or video recordings.

The primary objective of the present study was to replicate and extend the earlier findings of aberrations in rVOR noted by Ritvo et al. (1969), Ornitz et al. (1974) and Ornitz et al. (1985) using videooculography techniques to better characterize differences in rVOR in the dark beyond standard measures of gain and time constant of decay. Based on prior studies (Ritvo et al., 1969; Ornitz et al., 1974; 1985), it was expected that the ASD group would exhibit fewer post-rotary nystagmus beats than the control group. Our preliminary study suggested that the ASD group would also exhibit a greater number of vertical eye movement intrusions and greater number of abnormal horizontal slow-phase eye movements. The current study also aimed to provide findings

useful for generating hypotheses about what neurobiological differences in this population may serve as the basis for such qualitative differences in rVOR.

Methods

Participants and Recruitment

For the current study, children diagnosed with autism spectrum disorders (n = 16) and typically developing children (n = 17) ages 6-12 were recruited from the University of Florida Center for Autism and Related Disabilities (UF CARD), public schools and pediatric therapy centers within Alachua county. ASD diagnoses included: Autism, Asperger's syndrome, or Pervasive Developmental Disorder - Not Otherwise Specified. Diagnoses were confirmed by assessments administered by a clinical psychologist including the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and the Social Communication Questionnaire (SCQ; Rutter, Bayley & Lord, 2003). Children in the ASD group with diagnoses of Fragile X, Rett Syndrome, tuberous sclerosis, seizure disorder or fetal cytomegalovirus infection were excluded from the study. Children in the typically developing group with any current or past history of psychiatric disorders were excluded from the study. Intelligence quotients were obtained for both groups via the Leiter-R Brief IQ test (Roid, Miller, & Leiter, 1997).

Testing Equipment

Rotary chair testing was completed using a computer-controlled, motorized rotary platform and binocular video-oculography goggle (VOG) system. The motor, control system, software and video oculography goggles were manufactured by Neuro-Kinetics Inc (NKI). The seating system was created by the authors for pediatric use and included a small, padded chair, safety harness and head stabilizers with occipital head rest and temporal stabilizing arms to prevent head movements and allow en bloc rotation of head

and body (Figure 2-1, Chapter 2).The I-Portal™ Video-Oculography Goggle (VOG) System was used to record rVOR eye movements during velocity step testing and VEST™ 6.8 Software was used to analyze oculomotor screening and rVOR testing eye movement data. Additionally, we used live screen shot video recordings to observe eye movements visually during testing. Such video documentation of the eye movements are not typically included with VOG recordings, however, due to the vertical intrusions previously noted in our pilot study, we elected to record that additional information.

Procedure

VOG System Calibration. The VOG system calibration was conducted prior to testing each participant. The calibration procedure involved projecting a laser stimulus onto a black screen (60º arc with 76.5 inch radius). The laser provided a visual target that moved to specified locations ± 10º from center (e.g., first 10º to the left, then right, then up and then down). Results were averaged across two trials. This procedure takes approximately 5 to 10 minutes to complete.

Oculomotor Screening. Following VOG calibration, oculomotor screening tests were conducted to rule out any neurological impairment related to oculomotor function that would confound interpretation of rVOR eye movements. These tests included: saccade, smooth pursuit and gaze evoked nystagmus tests. For these assessments the participant was seated in the rotary chair with their head fixed in place (see Figure 2-1) while wearing the VOG system to record their eye movements as they followed a red laser stimulus through a series of movements specific to each test (see methods section in Chapter 2 for further detail). This procedure took approximately 10-15 minutes with up to 5 scheduled breaks as needed for each participant.

Velocity Step Testing General Procedure. Velocity step tests were completed in both clock-wise and counter clock-wise directions in the dark. Participants were seated and secured to the pediatric rotary chair while wearing the VOG system to record eye movements. Each trial began with a ramp-up time of 1.2 seconds to a peak velocity of 100º/second for 60 seconds followed by a rapid deceleration to zero. Per-rotary eye movement recordings were taken for 60 seconds, followed by post-rotary recordings for up to 60 seconds or until nystagmus ceased. For each participant three phases of testing occurred. For phase 1, one trial was completed in the clock-wise direction. Phase 2 was a 60 second break which was provided while the participant remained seated in the rotary chair and was engaged in space themed games. Finally, phase 3 involved a second trial which was completed in the counter clock-wise direction. If one of these two trials was disrupted by excessive blinking, talking, head movement or the child requesting a break, a 60 second break was provided and the entire trial was repeated with the participant's assent. The velocity step test procedure including providing the participant with instructions, two trials of rotation and 1 to 2 scheduled breaks took approximately 10 to 15 minutes to complete.

Data Analysis

Methods for analyzing number of post-rotary nystagmus beats

Post-rotary nystagmus beats were counted manually by a research assistant using eye movement tracings provided by NeuroKinetics, LLC VEST™ 6.8 Software. A post-rotary nystagmus beat was defined as a slow phase eye movement in the direction of rotation for that trial (i.e., clock-wise or counter clock-wise) followed by a quick phase reset eye movement in the opposing direction. The number of post-rotary nystagmus beats for clock-wise and counter clock-wise trials were compared for each participant

using paired t-tests. This comparison was made to test for asymmetries between trials prior to pooling the data from these two trials for each participant. No difference between trials within subjects was found (p = .28), therefore clock-wise and counter clock-wise trials were pooled and a mean number of nystagmus beats was entered for each subject. An independent samples t-test was conducted to compare the mean number of post-rotary nystagmus beats between groups.

Development and testing of a quantitative method for analyzing temporal dynamics of slow phase irregularity and vertical intrusions

Tracings of post-rotary nystagmus in the horizontal plane for three TD children (see Figure 5-2) depict the rhythmicity or regularity of these eye movements. The slower excursions followed by relatively fast reset generates the saw-tooth pattern depicted in Fig. 5-2. Based on these tracings, we explored (and continue to assess) the application of several statistical models designed to uncover the temporal structure in these time series and provide estimates of periodicity or regularity. These measures can then be used to quantify differences in the temporal dynamics of eye movements between the ASD and TD groups. We hypothesized that the horizontal eye movements of children with ASD would be less regular or periodic compared to TD children.

Approximate Entropy (ApEn). One potentially useful statistical model to apply to these data would be Approximate Entropy or ApEn. The ApEn analysis is designed to provide an index of regularity that can be derived from relatively short and noisy time series (Pincus et al., 1991). ApEn requires at least 1000 data points for analysis and its output provides an index of the level of regularity of a time series that ranges from 0 to 1 with 0 indicating perfect periodicity to 1 being completely random. ApEn was explored

using one trial of velocity step testing from one participant from the ASD group and one from the TD group. This provided an exemplar of the utility of this statistical approach.

Spectral Analysis. A second approach to quantifying the temporal dynamics of these eye movements would be to use spectral analysis. Unlike ApEn, which is conducted in the time domain, spectral analysis involves a transformation to the frequency domain. Spectral analysis assesses the temporal dynamics of a time series by deconstructing the time series into a set of constituent frequency bandwidths and depicting how much of the total variance in the time series is accounted for by each frequency bandwidth. Data from one participant from each group was used to test each of these methods and the results from these preliminary analyses are presented. Spectral analysis was explored using comparison of peak frequency and f95 statistics (the range of frequency bandwidths needed to capture 95% of the variance in the time series) of one trial of velocity step testing from one participant from the ASD group and one from the TD group.

The presence of vertical eye movements during what should be essentially horizontal rVOR eye movements may result in interference of these horizontal eye movements and may lead to a disruption in the temporal dynamics of rVOR. For instance, what is typically a periodic series of subsequent nystagmus beats with a saw-tooth pattern of slow phase eye movement in one direction followed by a quick phase reset in the opposite direction in the horizontal plane becomes an irregular (or less regular pattern) of nystagmus beats with changes in the temporal dynamics extended by vertical eye movements occurring concomitantly. This hypothesis can be tested in

future analyses by applying variants of both ApEn (cross ApEn) and spectral analysis (cross spectral).

Results

Number of Post-rotary Nystagmus Beats

There was no statistically significant difference in the number of post-rotary nystagmus beats between groups, $p = 0.94$ (Table 5-1). Although both groups had similar mean number of beats (ASD = 46.25 and TD = 45.85), the ASD showed a larger standard deviation (ASD = 18.34 and TD = 10.90; see Table 5-1 for a summary of results).

Slow Phase Irregularity

Figure 5-2 depicts tracings of post-rotary nystagmus eye movements in the horizontal plane for three typically developing children. These tracings exemplify the repetitive pattern of slow phase followed by quick phase eye movements resulting in a saw-tooth pattern with roughly equal amplitude and linear acceleration slopes. Figure 5-3 depicts tracings of post-rotary nystagmus eye movements in the horizontal plane for three participants with ASD. These representative samples illustrate the irregular slow phase eye movements and disrupted horizontal rVOR nystagmus beat patterns seen in the ASD group. Slow phase irregularities were characterized by increased duration of slow-phase eye movements with altered slopes (i.e., arcing slopes with changing acceleration rather than flat lines indicating consistent acceleration). Figures 5-2 and 5-3 represent short periods (3 to 5 seconds) of the full post-rotary nystagmus sequence which may last up to approximately 20 to 30 seconds in the dark. Each image illustrates the position of the eye over time for a single participant. The x-axis represents the time domain and the y-axis represents the eye's position. Positive position values indicate

rightward movement and negative values indicate leftward movement. For trials of clock-wise rotation, the post-rotary nystagmus should present with slow phases to the right (i.e., up or positive direction) and quick phases to the left (i.e., down or negative direction) and vice versa for counter clock-wise trials where slow phases move to the left (i.e., down or negative direction) and quick phases to the right (i.e., up or positive direction).

Vertical Eye Movement Intrusions

The tracings for both horizontal and vertical recordings of post-rotary rVOR are presented in Figures 5-4 for the TD group and in Figure 5-5 for the ASD group. All of the examples in Figure 5-4 and 5-5 are taken from the same subjects as the examples provided in Figures 5-2 and 5-3 (i.e., horizontal eye movements only). The images in Figures 5-4 and 5-5 include four lines that run in two pairs: one pair of lines represents both eyes in the vertical plane and the other pair represents both eyes in the horizontal plane. The pair of lines representing the pair of eyes should be yoked or move in parallel as can be seen in each of these examples. When both directions of eye movement (horizontal and vertical) are plotted together in this manner, it is clear to see that vertical eye movements accompany the horizontal rVOR anomalies (see Figure 5-4 and 5-5).

Figure 5-5 illustrates the horizontal rVOR slow phase irregularities observed in the ASD group and the vertical excursions that were not observed in the TD group. Figure 5-5 also illustrates the potential influence of vertical eye movements on the periodicity, trajectory and duration of horizontal rVOR slow phase eye movements in the ASD group.

Preliminary Results from Temporal Dynamics Analysis

Approximate Entropy (ApEn). In order to assess the utility of Approximate Entropy (ApEn) to capture the regularity in the post-rotary horizontal eye movements, we subjected data from one TD group participant and one ASD group participant to this model for comparison purposes. As can be seen in Figure 5-6, the ASD participant demonstrated a higher level of complexity or irregularity (ApEn = 0.24) compared to the TD participant whose horizontal eye movements show a high degree of regularity (ApEn = 0.07; Figure 5-6).

Spectral Analysis. Spectral analysis was used as a second potential strategy for capturing group differences in the periodicity of horizontal eye movements. Therefore, we used the data from the same two subjects (one TD participant and one ASD participant) for spectral analysis that were used to calculate ApEn. Spectral plots for each participant's eye movements in the horizontal (Figure 5-7) and vertical (Figure 5-8) plane show that in both planes of movement, the variance in the time series of the ASD participant was distributed over a greater range of frequency bandwidths. In contrast, the variance in the time series associated with the TD participant was largely captured by a single frequency bandwidth indicating a much higher degree of periodicity in the eye movements. There was no difference between the ASD participant and the TD participants in the peak frequency of horizontal eye movements. The ASD participant, however, demonstrated a higher peak frequency (0.07) than the TD participant (0.02) for eye movements in the vertical plane (Figure 5-9). The ASD participant also had a higher f95 value (2.75), the frequency that accounts for 95% of the total power spectrum, than the TD participant (0.51; Figure 5-10) indicating that the ASD participant

required a much larger range of frequency bandwidths to account for 95% of the total variance in the time series.

Discussion

The present study sought to characterize key features of the eye movements associated with post-rotary nystagmus in TD and ASD participants. One aim was to establish the presence of aberrant vertical excursions in the eye movements of the ASD children. We also sought to establish the utility of two time series analyses to determine if they could capture salient features of these eye movements including their regularity or periodicity.

No difference was found between ASD and TD participants in the number of post-rotary nystagmus beats. There does appear to be a difference between groups, however, in the temporal dynamics of post-rotary nystagmus. Specifically, the TD participants exhibited a regular or periodic pattern in their horizontal eye movements whereas the ASD group exhibited considerably more irregular horizontal rVOR. This may be a result of the influence of vertical eye movements on horizontal rVOR. The difference in the regularity of the horizontal eye movements between ASD and TD children was captured nicely by both ApEn and spectral analyses.

Number of Post-rotary Nystagmus Beats

There is conflicting evidence of decreased nystagmus beats in ASD in the literature. Ornitz et al. (1985) found a significant decrease in the number of beats in the ASD compared to the TD group when tested in the dark. However, an earlier study by Ornitz et al. (1974) with varied lighting and visual conditions during rVOR testing found no difference in the number of nystagmus beats between the ASD and TD groups in the dark condition. They did find a significant difference in the number of beats between

groups in the presence of different visual fixation stimuli. It is difficult to discern the factor or factors that may account for the inconsistencies in results. Factors such as IQ or other participant demographic characteristics may account for some of the variability and the current study did not have enough power to investigate such differences.

Slow Phase Irregularity

Although Ornitz et al. (1985) found no difference in rVOR gain between ASD and controls, suggesting that individuals with ASD demonstrate normal cerebellar flocculus modulation of rVOR, they did report abnormal characteristics of rVOR nystagmus slow phase eye movements. The current study identified a potentially increased number of slow phase eye movement irregularities in ASD similar to that which Ornitz et al. (1985) described previously but were unable to quantify. Such irregularities included intermittent slow phases with greater amplitude than expected, slow phases that do not promptly result in a quick phase reset eye movement or slow phases with changes in velocity.

Changes in eye velocity during slow phase are of great interest. In Participant ASD – 3, the irregular slow phase is noteworthy in that it exhibits a negative exponential curve. Such decreasing changes in slow phase velocity during spontaneous nystagmus are indicative of a dysfunctional neural integrator (Leigh & Zee, 2006), the brainstem neural network that mathematically integrates velocity signals into position signals for gaze stabilization (Arnold & Robinson, 1997). In this case, however, the decaying slow phase velocity appeared to be intermittent (i.e., not evident in surrounding nystagmus beats) in this participant and therefore, may be caused by some other factor. It would be worth investigating whether such exponentially decreasing velocity profiles occur in other ASD participants within the study.

In light of the representative samples of rVOR tracings presented for comparison and preliminary ApEn and f95 spectral analyses, it is clear that in this small sample of participants, there is less regularity or periodicity in the horizontal rVOR in the participants with ASD. It is not yet clear however, whether this disorganization of the rVOR is due to vertical eye movement intrusions or abnormalities in slow phase eye movements or both. Variants of ApEn (cross ApEn) and spectral analysis (coherence spectra) may allow us in future studies to assess horizontal-vertical coupling of eye movements objectively and quantitatively. If irregular slow-phase eye movements do exist in the ASD group it would be interesting to know whether there are also similar smooth pursuit oculomotor control differences in this population.

Vertical Eye Movement Intrusions

Typically developing participants exhibited substantially less overall movement in the vertical plane than ASD participants. What vertical movement did exist in the TD participants occurred in phase with horizontal nystagmus beats and did not seem to disrupt them. By comparison, the ASD representative examples provided here exhibited greater amplitude of eye movements in the vertical direction and such eye movements occurred concomitantly with the irregularities of the horizontal slow-phase eye movements observed in these ASD participants. Therefore, it may be that vertical intrusions in ASD are responsible for influencing or disrupting the regular pattern of horizontal rVOR from occurring, whereas this does not appear to occur in the TD participants.

One hypothesis for cross-coupling of vertical and horizontal eye movements during horizontal rVOR is that there may be cross-talk or abnormal connectivity between the vertical and horizontal oculomotor neural integrators such as the interstitial nucleus

of Cajal (for vertical eye movements), nucleus prepositus hypoglossi, paramedian tracts and medial vestibular nucleus (for horizontal eye movements; Kheradmand & Zee, 2011). Although these sites have not been reported to show morphological differences in ASD, there have been reports of general patterns of abnormal cortical cellular growth pathology such as long range neuronal under-connectivity and short-range neuronal over-connectivity (Courchesne et al., 2005). This hypothesis would be consistent with short-range over-connectivity within the brainstem oculomotor neural integrators.

Alternatively, more evidence would suggest that the increased number of vertical eye movement intrusions noted in the ASD participants may be indicative of cerebellar damage in this population. Patients with diffuse cerebellar lesions can exhibit cross-coupling of vertical and horizontal slow phase eye movements during what should be purely horizontal rVOR (Walker & Zee, 1999; Kim et al., 2005; Moon et al., 2009) such as that observed in the ASD group in the current study. Specifically, cross-coupling such as this in response to low-frequency stimulation, as was provided in the current study, can specifically be indicative of dysfunction of the velocity-storage mechanism of the cerebellum located in the nodulus/uvula, vermian lobules IX and X (Kherandmand & Zee, 2011) .

Lesions in the nodulus and uvula (i.e., velocity storage mechanism of the cerebellum) can result in increased time constant of decay (Waespe et al., 1985). Such increases in time constant of decay have been observed in ASD previously when tested in the dark (see Chapter 3; Ornitz et al., 1985). Goldberg et al. (2000) showed a slight increase in time constant of decay in the ASD group, although not statistically significant in their sample, when tested in the dark without tilt suppression stimuli provided.

The most consistently reported neuroanatomical abnormalities in ASD are found in the cerebellum and the inferior olivary nucleus of the brainstem (Bauman &Kemper, 2005). Deficits in oculomotor and gross motor adaptation have been attributed to abnormal cerebellar-learning in ASD (Mosconi et al., 2013). Vermian lobules VI and VII have been shown to differ in size (hyperplasic or hypoplasic) resulting in a bimodal distribution of the ASD population (Courchesne et al., 1994). Many post-mortem studies have reported decreased size of the cerebellum and number of Purkinje cells in the cerebellum of individuals with ASD (Ritvo et al., 1986; Bailey et al., 1998; Kemper & Bauman, 2002; Purcell et al., 2001; Lee et al., 2002; Palmen et al., 2004). Decreased Purkinje cells may cause notable aberrations in the VOR including hypo- or hyper-metric nystagmus, as demonstrated by Hg toxicity in the cerebella of guinea pigs (Young, Chuu, Liu, Lin-Shiau, 2002). However, in ASD, intelligence or level of function has been shown to affect the presence or absence of some cerebellar functional or structural difference (Goldberg et. al., 2000; Holttum et. al., 1992; Piven et. al., 1992).

Age-related morphological changes in the inferior olivary nucleus of the brainstem in individuals with ASD have also been consistently reported (Bailey et. al., 1998; Palmen, van Engeland, Hof, Schmitz, 2004; Bauman & Kemper, 2005). Motor learning in the cerebellum has been proposed to occur at the synapses between Purkinje cells and the climbing fibers that extend from the inferior olive into the cerebellum (Leigh & Zee, 2006). Since the inferior olive plays an important role in oculomotor control and vestibular processing, future studies of rVOR adaptation in ASD may help to better understand the functional effects of such developmental changes in these nuclei of the brainstem.

Subtle dysmetria in saccade accuracy has also been observed in individuals with ASD, which may also be related to observations of cerebellar pathology (Takarae et al., 2004). Such abnormal eye movements have also, however, been correlated with abnormal activity in fronto-striatal circuitry in ASD (Takarae et al., 2007), areas of the brain that have been linked to the restricted and repetitive behavioral symptoms of ASD (Langen et al., 2007; Lewis et al., 2007). Thus, aberrations in the oculomotor output of the rVOR may be linked to multiple neurobiological areas of interest for ASD including the cerebellar, brainstem and fronto-striatal systems and could potentially serve as a model to better understand the functional effects of differences in these brain structures in ASD.

Future Studies

Future studies should be aimed at investigating rVOR and oculomotor function in an effort to determine whether deficits are diffusely dispersed throughout the cerebellum or limited to one region such as the nodulus and uvula. Future studies should also aim to identify whether such deficits are present within the broad autism spectrum or within smaller subgroups such as those with above or below average IQ or presence or absence of language delay.

High frequency, high acceleration head impulse tests can be conducted to investigate flocculus/paraflocculus function (Walker & Zee, 1999, 2005a; Shaikh et al., 2011). If children with ASD continue to exhibit cross-coupling of vertical and horizontal movements in response to high-frequency rather than low-frequency stimuli only (as provided in the current study), this would provide evidence that similar cerebellar deficits may exist within the nodulus/uvula as the flocculus and paraflocculus more diffusely throughout the vestibulocerebellum rather than in one specific region alone.

Furthermore, cross-coupling of vertical and horizontal eye movements can also occur during horizontal optokinetic nystagmus (OKN), nystagmus induced by visual motion, and optokinetic after nystagmus (OKAN), the after response of OKN driven by optokinetic velocity storage, with nodular/uvular damage (Walker & Zee, 1999). If vertical and horizontal cross-coupling is exhibited in the rVOR in ASD, it would be reasonable to expect that horizontal and vertical cross-coupling would exist in OKAN as well. It has been reported that in a sample of 34 children with ASD, 28 exhibited atypical OKN oculomotor response to a hand held rotary drum with stripes (Scharre & Creedon, 1992), however, a thorough analysis of these eye movements has not been conducted and OKAN has not assessed in this population to date. Further investigation of OKN/OKAN in ASD could help to clarify whether deficits in the nodulus and uvula do exist and to better characterize the oculomotor functions affected.

Further exploration of the observation made by Ornitz et al. (1985) describing slow phase eye movements that fail to be followed by a quick phase eye movement should also be explored. One possible explanation is increased horizontal saccade latency in ASD reported in Chapter 2 of the current studies. For each nystagmus beat a slow phase eye movement should be followed by a quick phase eye movement that resets the eye to center and prepares the eye for the next oncoming visual scene. The quick phase of each nystagmus beat is saccade-like meaning that it should have the same movement profile as a saccade and depends on the same circuitry as saccade eye movements. In the current study, the ASD group demonstrated significantly greater horizontal saccade latency (*M* = 260ms, *SD* = 60) than the TD group (*M* = 210ms, *SD* = 40). Typical reactive saccade latency is approximately 200ms and saccades typically

last anywhere from 20 to 200ms (Leigh & Zee, 2006). If the latency between the slow phase and the initiation of its quick phase outlasts the typical saccade duration (20-200ms), it is reasonable to suspect that this may result in the quick phase being surpassed by the initiation of the next nystagmus beat (i.e., initiation of the next slow phase). Presumably, this would result in a failure to reset the eye to center and may cause the initial nystagmus beat to have what appears to be an abnormally long excursion, as described by Ornitz et al., (1985). It would be beneficial to evaluate how many of these longer slow phases with failed quick phases occur in the ASD group and whether there is any correlation between horizontal saccade latency and the number of failed quick phase errors in ASD.

Lastly, to thoroughly explore the possibility of nodular/uvular deficits in at least a sub-group of the ASD population, tilt suppression tests should be conducted with lower functioning individuals with ASD. Tilt suppression is a phenomenon where pitching or tilting the head forward immediately following continuous rotation results in reduced post-rotary nystagmus time constant of decay. The nodulus and uvula are important for tilt suppression of rVOR (Hain et al., 1988). In high functioning individuals with ASD, tilt suppression has been shown to be normal (Goldberg et al., 2000). However, it has been estimated that individuals with lower than average IQ comprise approximately 68% of the ASD population (Yeargin-Allsopp et al., 2003). Since the Goldberg et al. (2000) study included only those individuals with high functioning (i.e., average to above average IQ) it is not clear whether a large percentage of the ASD population may indeed exhibit differences in tilt suppression. Although the current study included both children with above average, average as well as those with below average IQ, there is

not enough power in the current study to evaluate whether there are differences between functional subgroups within the ASD group. Thus, it would be beneficial to test both rVOR in the dark as well as during tilt suppression in low functioning individuals with ASD to evaluate whether there are consistencies in nodulus/uvular related rVOR deficits such as increased time constant in the dark and decreased tilt suppression in at this subpopulation of the autism spectrum.

Conclusion

The current study provided descriptive evidence of cross-coupling between vertical and horizontal eye movements resulting in measurable changes in the temporal dynamics of rVOR in ASD. Specifically, this abnormal coupling of vertical eye movements to horizontal rVOR may be indicative of deficient cerebellar modulation of rVOR in ASD. It is unclear at this point whether these deficits are limited to nodular/uvular function or are representative of more diffuse cerebellar deficits in ASD. It is also difficult to rule out with the current evidence whether such differences are specific to ASD as a whole or to subgroups within the spectrum. These results warrant further study of rVOR indicators of cerebellar sensory motor processing differences in ASD.

Table 5-1. Summary of Post-Rotary Nystagmus Number of Beats for Velocity Step Test

Group	N	Mean	SD	SE Mean	*p-value*
Autism Spectrum Disorders	16	46.25	18.34	4.59	
Typically Developing	17	45.85	10.90	2.64	0.94

Figure 5-1. Novice observer rating as “normal” or “abnormal” for videos of rVOR from three ASD and three TD participants. Seventeen novice observers, with no prior knowledge of rVOR or nystagmus ages 19 to 23 years were provided a brief training session including a written definition of normal rVOR, a sample video of normal nystagmus obtained from a subject not included in the study, and a rubric for distinguishing normal from abnormal nystagmus. Observers were blind to group assignment and were provided with two videos from each of the three ASD and three TD participants in random order. Each video included 20 seconds of per-rotary nystagmus followed by 20 seconds of post-rotary nystagmus. Observers then rated these videos as “normal” or “abnormal”.

Figure 5-2. Example of Post-Rotary Nystagmus Slow Phase Regularity in the TD Group. A) Participant TD-1: counter clock-wise velocity step post-rotary nystagmus; B) Participant TD-2: clock-wise velocity step test post-rotary nystagmus; C) Participant TD-3: counter clock-wise velocity step test post-rotary nystagmus. Note the regular saw-tooth pattern across all participants.

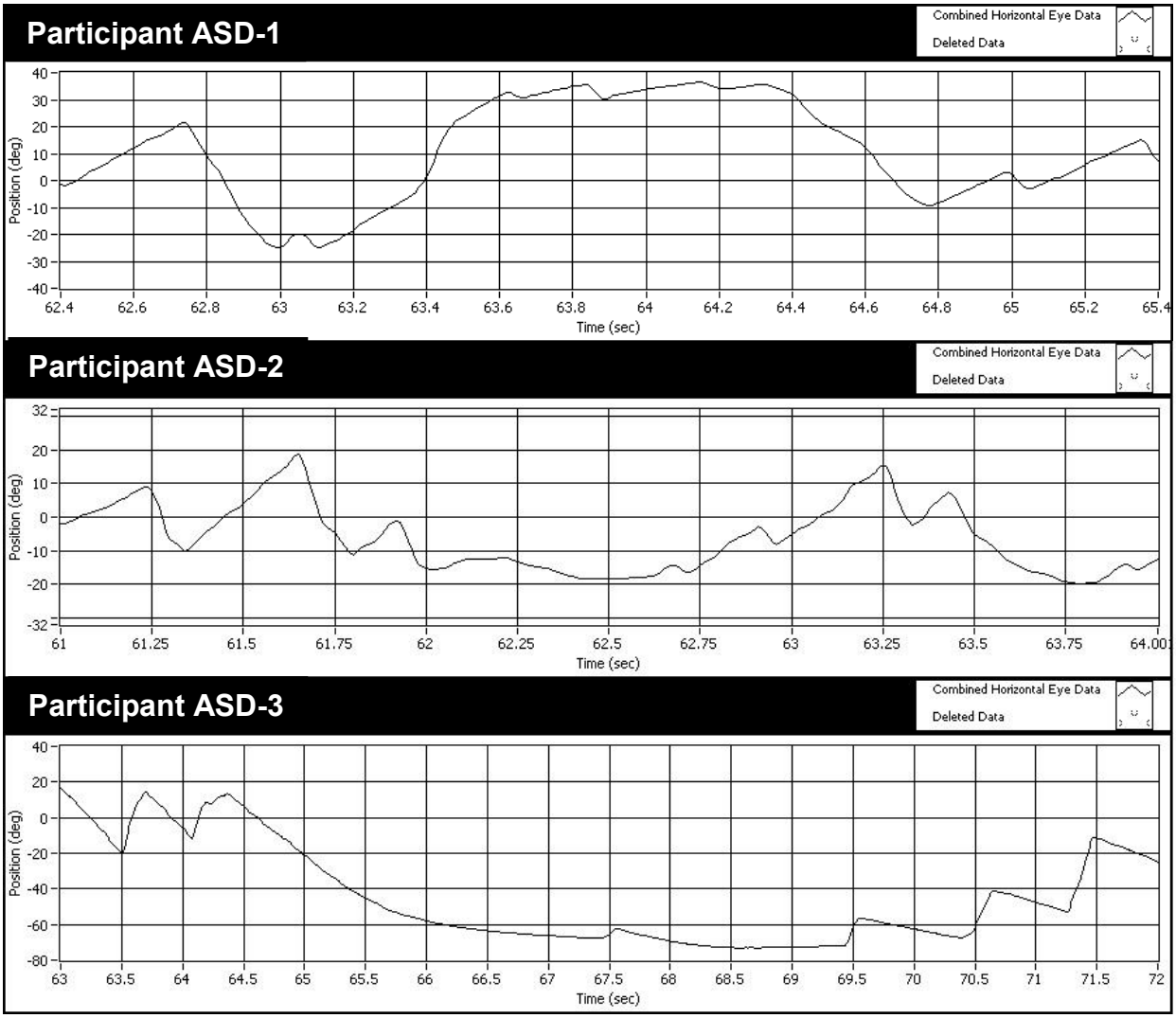

Figure 5-3. Example of Post-Rotary Nystagmus Slow Phase Irregularity in the ASD Group. A) Participant ASD-1: clock-wise velocity step post-rotary nystagmus with notable change in eye position and amplitude of nystagmus beats beginning at 63.4 seconds; B) Participant ASD-2: clock-wise velocity step test post-rotary nystagmus with a notable interruption in horizontal nystagmus beginning at 62 seconds; C) Participant ASD-3: counter clock-wise velocity step post-rotary nystagmus with a notable change in slow phase velocity beginning at 65.5 seconds and lasting for approximately 4 seconds.

Figure 5-4. Vertical and Horizontal Eye Movements during Post-Rotary Nystagmus in the TD Group. A) Participant TD-1: counter clock-wise velocity step post-rotary nystagmus; B) Participant TD-2: clock-wise velocity step test post-rotary nystagmus; C) Participant TD-3: counter clock-wise velocity step test post-rotary nystagmus. Note that there is minimal movement in the vertical plane for all three subjects.

Figure 5-5. Vertical and Horizontal Eye Movements during Post-Rotary Nystagmus in the ASD Group. A) Participant ASD-1: clock-wise velocity step post-rotary nystagmus; B) Participant ASD-2: clock-wise velocity step test post-rotary nystagmus; C) Participant ASD-3: counter clock-wise velocity step test post-rotary nystagmus. Note that there are large movements in the vertical plane that are coupled with horizontal slow phase irregularities.

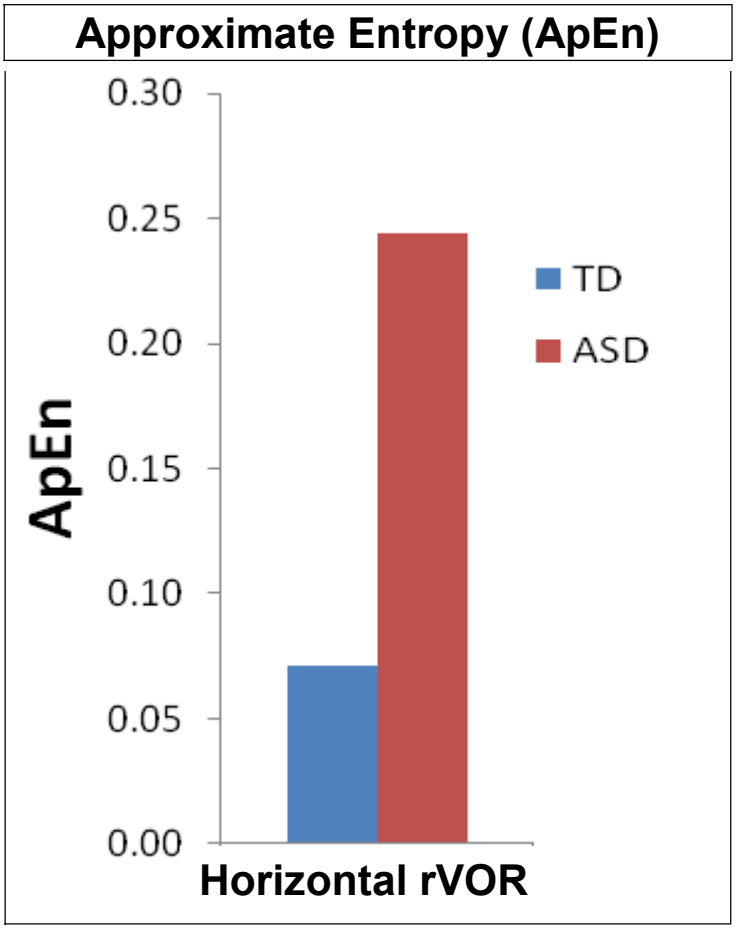

Figure 5-6. Approximate entropy (ApEn) of horizontal rVOR during velocity step testing in the dark presented for one ASD participant and one typically developing control. ApEn outcome values can range from 0 to 1; a “0” indicating perfect periodicity and “1” indicating completely random data. Thus, the higher ApEn value for the ASD participant indicates increased complexity in the ASD participant’s data.

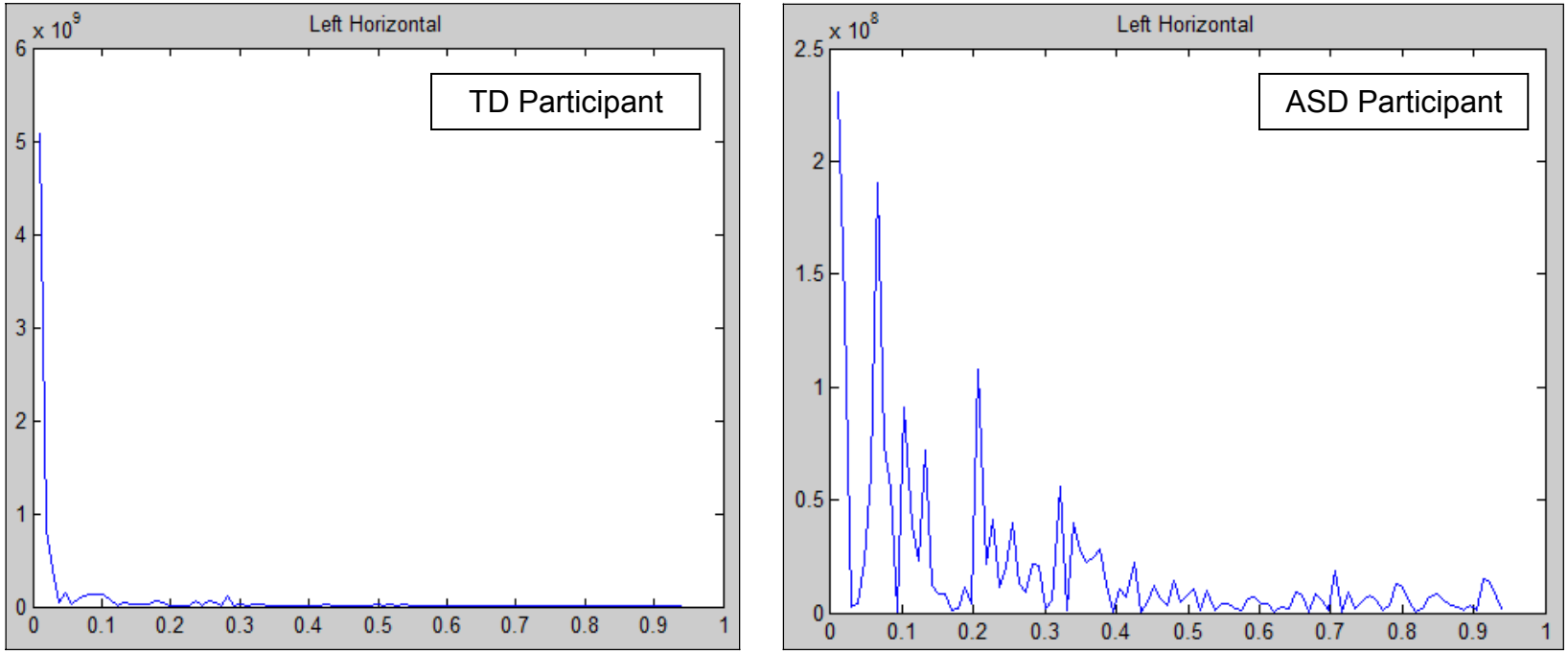

Figure 5-7. Spectral plot comparison of horizontal eye movements during a single trial of velocity step testing in the dark for one ASD and one TD participant. Note that the ASD participant exhibits a greater amount of noise across a wider range of frequencies indicating that the ASD data is less periodic.

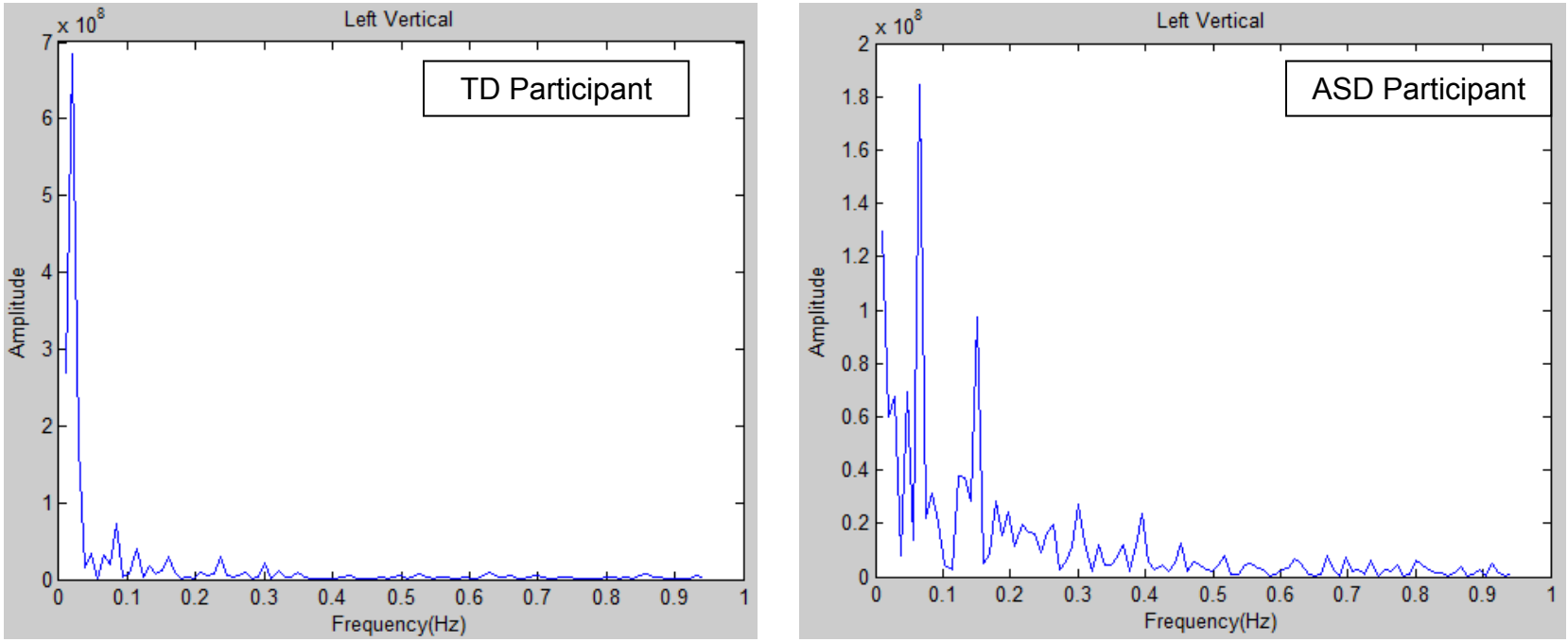

Figure 5-8. Spectral plot comparison of vertical eye movements during a single trial of velocity step testing in the dark for one ASD and one TD participant. Note that the ASD participant exhibits a greater amount of noise across a wider range of frequencies.

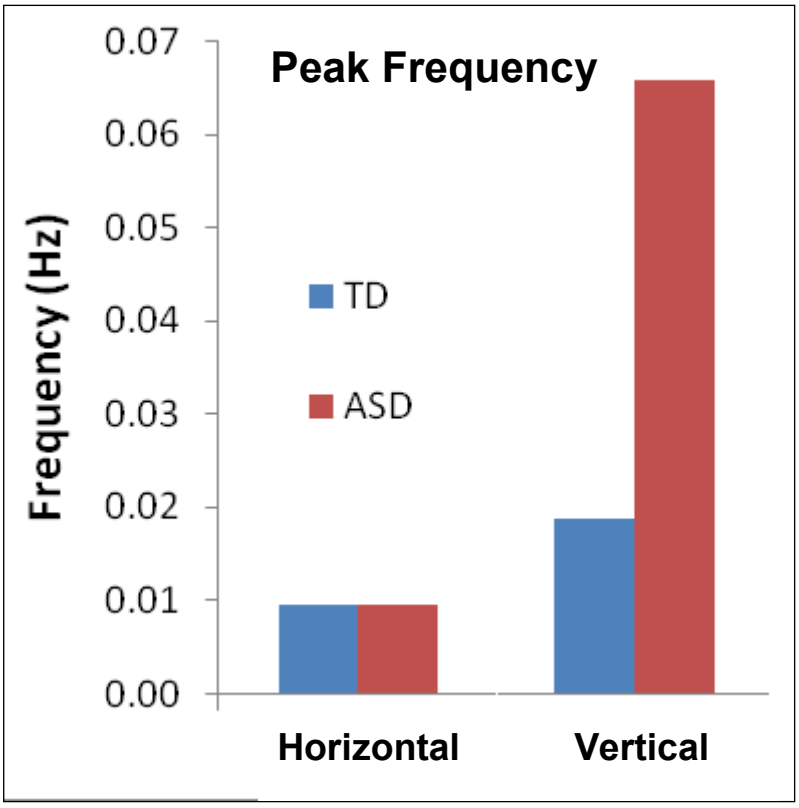

Figure 5-9. Peak frequency of horizontal and vertical rVOR measured in one ASD and one TD participant during velocity step testing in the dark. Note that there is no difference in the peak frequency between the TD and ASD for eye movements in the horizontal plane; however, there is a large difference for eye movements in the horizontal plane. The ASD participant exhibits a peak vertical frequency that is much higher than the horizontal frequency and higher than the TD participant's vertical peak frequency.

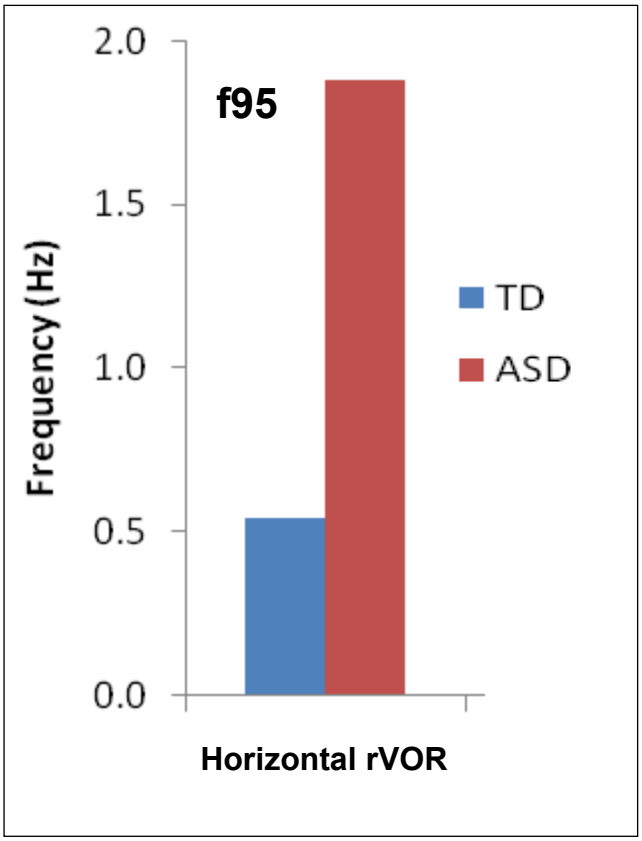

Figure 5-10. Comparison of the f95 or the frequency that accounts for 95% of the total power spectrum for one ASD and one TD participant during velocity step testing in the dark.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Objective

The overall objective of the current studies was to provide a comprehensive, detailed and systematic evaluation of horizontal rotational vestibulo-ocular reflex (rVOR) in children with autism spectrum disorders (ASD). The studies described in Chapters 2, 3, 4 and 5 were developed to achieve the following specific aims: (1) to identify alterations in horizontal rVOR in ASD; (2) to determine differences in fixation suppression of rVOR in ASD compared to typically developing children; and (3) to identify correlations between rVOR and functional measures in ASD.

Summary of Results

Fixation Suppression and Smooth Pursuit Deficits in ASD: Chapters 2 and 3

In the current studies, children ages 6 – 12 diagnosed with ASD demonstrated increased time constant of decay of post-rotary nystagmus during velocity step tests in the dark and with fixation suppression (Chapter 2). Increased time constants during velocity step tests in the dark have previously been reported in ASD Ornitz et al. (1985) and are consistent with the current findings.

The significantly increased time constant of decay of post-rotary nystagmus during velocity step tests with fixation suppression stimuli in ASD was surprising (Chapter 2). The cerebellum is important for visual suppression of rVOR, particularly the flocculus/paraflocculus (Zee et al., 1981; Belton and McCrea, 2000, 2002; Rambold et al., 2002). This finding of decreased visual suppression of rVOR in ASD is inconsistent with previous reports of significantly decreased post-rotary nystagmus duration following velocity step tests in the dark when various fixation suppression stimuli were presented

after rotation stopped. Additionally, there was no difference in time constant of decay in the light condition, which is inconsistent with a very early study that reported significantly decreased duration of post-rotary nystagmus following velocity step testing in a well lit room (Ritvo et al., 1969). Reasons for the differences between these two studies may include differences in participant demographics and methods. In the Ritvo et al. (1969) study 26 children ages 3 to 7 years were characterized as having infantile autism, up to16 of whom were considered “mute” and 5 of whom had “CNS pathology”. In the Ornitz et al. (1974) study included 21 children ages 3 to 5 years with infantile autism and all children were either “mute or echolalic”. The current study, however, was selective in recruiting only children with high functioning ASD whose diagnoses were confirmed using validated diagnostic assessment tools. Only a small proportion of our sample was non-verbal or had limited verbal abilities (n = 3 out of 16). Another possible explanation for the difference between studies may result from differences in testing protocols. The current study conducted velocity step tests with a ramp-up time of 1.2 seconds to a peak velocity of 100º/second for 60 seconds of rotation using a motorized rotary chair. Visual fixation suppression stimuli in the current study were provided both during and after rotation. Both previous studies conducted the standard Barany procedure including 10 revolutions within 20 seconds with a rotary chair operated by hand. Ornitz et al. (1974) conducted this rotation protocol in the dark and only provided visual stimuli after rotation had stopped. Thus, although the previous studies may have reached a constant peak velocity reaching nearly 180º/second, it is unclear as to the duration of time that the constant velocity was provided since the rotation was

conducted by hand. Thus, there may have been differences between studies both in participants as well as in the amount and duration of vestibular stimulation.

Even though the ASD group followed the same pattern of decreased gain and time constants in fixation suppression conditions compared to dark conditions, their gain was consistently higher than controls. Per-rotary nystagmus gain during velocity step tests as well as gain during SHA tests were higher in ASD in the dark as well as with fixation suppression (Chapter 2 and 3 respectively).

Taken together, the findings from Chapter 2 indicate that differences in rVOR time constant of decay in ASD are of central rather than peripheral origin. Increased time constants in the dark suggest cerebellar nodulus/uvula deficits in the ASD group. However, increased per-rotary gain suggests deficits in cerebellar flocculus modulation of rVOR. The lack of fixation suppression on the post-rotary time constant of decay of rVOR further supports the idea of cerebellar modulation deficits in ASD.

Although not statistically significant, trends were noted towards smooth pursuit differences in ASD (Chapter 2). Smooth pursuit deficits may explain lack of fixation suppression in ASD and cannot be ruled out at this time. Additionally, smooth pursuit deficits in ASD support the idea of vestibulo-cerebellar deficits both in the flocculus/paraflocculus (Zee et al., 1981; Belton et al., 2000, 2002; Rambold et al., 2002) as well as the nodulus/uvula (Heinen and Keller 1996; Walker et al., 2008b).

During SHA tests in the dark (Chapter 3), there was no difference in phase between groups at each frequency. The ASD group followed the same trend as controls of improved efficiency of gain at higher frequencies of rotation. However, the ASD group did show significantly increased gain compared to controls in the dark. An increase in

SHA gain may be related to a lack of inhibition from the cerebellum and to hyper-responsivity to vestibular stimuli in ASD (Robinson, 1976; Thurston, Leigh, Abel & Dell'Osso, 1987). Decreased cerebellar modulation of rVOR for SHA is consistent with the findings of increased time constants of decay for velocity step testing in the dark.

Fixation suppression SHA tests (Chapter 3) showed no difference in gain between groups at any frequency tested. Increased phase lag was observed in ASD only at 0.5Hz, the highest rotation frequency tested. Since this difference in phase lag was observed during fixation suppression testing, the difference in ASD may possibly be related to optokinetic or smooth pursuit system deficits. However, increased phase lead may also indicate either peripheral vestibular differences or central deficits at the brainstem level in vestibular nuclei function (Shepard & Telian, 1996). Further studies are warranted to discern whether these differences are due to perturbations in the peripheral or central nervous system and where they are located.

Neuropsychological Correlates to rVOR in ASD: Chapter 4

It is clear that a relationship exists between measures of rVOR and vestibulo-spinal function in ASD. This relationship may indicate a global vestibular processing deficit in this population. However, since the direct observations of vestibulo-spinal function provided by the PANESS balance error scores displayed a bimodal distribution, these results should be interpreted with caution as there may be subgroups within the autism spectrum based on presence or absence of a balance deficit. The relationship between balance and rVOR deficits may also be dependent on this bimodal difference in balance.

Sensory Profile vestibular processing sub-scores were not correlated with either measures of vestibulo-ocular or vestibulo-spinal function in the children with ASD in the

current study. However, the Sensory Profile vestibular subscale was correlated with the RBS-R stereotypy subscale and may reflect stereotypic behavior in ASD rather than vestibular processing dysfunction. A lack of any significant relationship between Sensory Profile vestibular processing subscales and direct measures of vestibular function was surprising and also warrants further study.

rVOR Qualitative Differences in ASD: Chapter 5

Statistical measures of regularity (ApEn and spectral analysis) appear to be promising methods of analysis for evaluating differences in rVOR quality in ASD. Based on preliminary approaches to the data with these methods, it appeared that there were increased slow phase irregularities in ASD that may be related to vertical eye movement intrusions. If cross-coupling between vertical and horizontal eye movements during rVOR in response to low-frequency stimuli can be demonstrated, this may indicate cerebellar deficits, possibly in the nodulus/uvula. However, increased saccade latency in ASD may also explain slow phase deficits in this population such as failure to execute a quick phase following slow phase eye movements, as previously noted by Ornitz et al., 1985. Further analysis of the qualitative differences in rVOR in ASD is warranted and may help provide further evidence for deficient cerebellar modulation of rVOR.

Conclusion

The present findings suggest that there were vestibular processing deficits in the ASD sample tested. These deficits may well be consistent with reports of cerebellar deficits in the ASD population and warrant further study. Specifically, the ability to suppress rotationally evoked nystagmus is a good indicator of connections between midline cerebellar structures and vestibular nuclei (Brey et al., 2008b). Since children with ASD demonstrate deficits in visual suppression of nystagmus in the current study,

these results may indicate that the connections between these two structures should be an area of interest for further neuroanatomical study.

Implications

Autism spectrum disorders (ASD) are currently diagnosed on the basis of abnormal behavior within three core domains: (1) social skills, (2) communication skills, and (3) restricted/repetitive behaviors (American Psychiatric Association, 2000). Beyond the three main behavioral domains of ASD, deficits in sensory processing and motor coordination have been observed in young children less than 2 to 3 years of age who were later diagnosed with ASD (Baranek, 1999; Karmel, 2010; Landa, Garrett-Mayer, 2006; Teitelbaum, P., Teitelbaum, O., Nye, Fryman, Maurer, 1998; Watson, Baranek, Crais, Reznick, 2007; Zwaigenbaum et. al., 2005).

Early identification and early intervention have been shown to have a significant effect on the prognosis for young children with ASD (Dawson et. al., 2001; Rogers, 1998). However, an early diagnosis of ASD has often been limited to children who are approximately 2 to 3 years of age (Lord, 1995; Moore, & Goodson, 2003; Rogers, 2000). Yet, it is clear that the abnormal neurobiological processes resulting in ASD occur during fetal development (Rodier, 2002; Rodier, Ingram, Tisdale, Nelson, & Romano, 1996) and/or infancy (Courchesne, Redcay, Morgan, Kennedy, 2005) long before the onset of the classic behavioral symptoms currently used to affirm a diagnosis. Thus, identification of a bio-behavioral marker that occurs early in development and is related to the neurobiology of ASD would be particularly useful both for earlier identification of risk and for understanding the neuropathological processes resulting in ASD. Thus, sensory and motor abnormalities may currently provide the earliest warning signs of risk for ASD.

The rVOR is a promising candidate for an early sensorimotor bio-behavioral marker of risk for ASD for several reasons. First, horizontal rVOR has been previously shown to be abnormal in young children with ASD (Ornitz et.al, 1985; Ritvo, 1969). Second, the rVOR involves integration of sensory and motor information at sites that have been shown to have morphological abnormalities in ASD such as the brainstem (Rodier, 2002; Jou et al., 2009), cerebellum (Scott, Schumann, Goodlin-Jones, Amaral, 2009), thalamus and parietal lobes (Baron-Cohen et. al., 2009; Teitelbaum, Teitelbaum, Nye, Fryman, Maurer, 1998).Third, the anatomy and physiology of the rVOR is one of the best studied of all the vestibular and postural reflexes which provides a solid foundation for studying this reflex and its related neurobiology in ASD. Fourth, the rVOR can be measured reliably in infants as young as 6 months of age (Phillips, Backous, 2002); hence, if an rVOR marker were selective for ASD, then this reflex may provide a promising means for early identification of ASD risk. Lastly, the rVOR is highly modifiable (Braswell, Rine, 2006; Schubert, Zee, 2010) therefore, it is reasonable to suspect that at least some of the deficits of rVOR in ASD could respond to vestibular rehabilitation interventions.

The results of these studies provide a comprehensive, detailed and systematic evaluation of horizontal rotational rVOR in children with ASD. Additionally, these results may aid the identification of neural substrates responsible for vestibular related sensorimotor deficits observed in ASD. If rVOR abnormalities are selective for ASD, then rVOR tests could potentially provide a promising method for early identification of ASD. As of yet, diagnoses on the spectrum cannot be reliably confirmed until 2-3 years of age (Rogers, 2000). However, the rVOR can be measured reliably in infants as young

as 6 months (Phillips & Backous, 2002). Furthermore, the vestibular system is fully developed by 9 weeks in utero; thus, aberrations in vestibular function may provide a critical time point for investigation of fetal vestibular development and related neural development. Alternatively, if rVOR abnormalities are present in a sub-population of ASD, then rVOR tests could potentially serve as a technique for patient-treatment matching with vestibular and oculomotor-related interventions for this population. Certain rVOR characteristics can be modified through experience, and therefore, may become a useful outcome measure for specific interventions. Thus, studying the nature of perturbations in the rVOR in ASD, holds promise to improve early identification of sensorimotor deficits, to inform sensorimotor intervention methods and to guide future studies of aberrant neural mechanisms underlying vestibular related sensorimotor deficits in ASD.

Limitations

Although the current study had sufficient power for the main comparisons of rVOR metrics, the relatively small sample size (n = 16 ASD participants and n = 17 TD participants) may not have had sufficient power to identify subtle oculomotor differences or neuropsychological assessment correlations and may have potentially lead to Type II errors or missing differences between groups.

Another limitation to the current study is that SHA tests were not conducted at sufficiently low frequency cycles to calculate time constant of decay such as 0.01 or 0.02Hz. Future studies should be conducted with SHA at such frequency cycles, if possible, in order to estimate time constants for comparison in ASD. Based on velocity step tests, it is expected that children with ASD would demonstrate higher time

constants of decay during SHA tests conducted in the dark and with fixation suppression.

Lastly, the current study aimed to recruit high functioning children with ASD in an effort to better match the typically developing control group on age and IQ. Therefore, the results of the current study cannot be extrapolated to individuals with ASD who are lower functioning. Based on correlations discovered between IQ and functional ability measures it would be reasonable to suspect that rVOR deficits would be even greater in individuals with ASD who are lower functioning.

Future Studies

Future studies will be required to address questions of specificity to ASD. The first question is whether differences in rVOR suppression are specific to ASD or simply to developmental disabilities in general. This question can be answered by comparing groups of children with ASD to other groups with developmental disabilities of known etiology that are not related to ASD, such as children with Down syndrome or cerebral palsy. One study of individuals with Down syndrome reported that during velocity step tests of rVOR, adults with Down syndrome exhibit decreased number of per- and post-rotary nystagmus beats when tested in the dark and reduced fixation suppression of rVOR gain. However, there was no mention of significant differences between Down syndrome and controls for time constant of decay in either the dark or fixation suppression conditions. Therefore, questions remain as to the specificity of the time constant and qualitative differences observed in ASD. One extension of the current work could compare rVOR metrics from velocity step tests in the dark and with fixation suppression between groups of children with ASD and Down syndrome. If rVOR metrics

were found to be specific to ASD such as time constant of decay, this information may help to differentiate cerebellar deficits between these two groups.

Optokinetic nystagmus (OKN) and optokinetic after nystagmus (OKAN) are repetitive eye movement responses to visual motion stimuli rather than vestibular stimuli as with per- and post-rotary nystagmus is to rVOR. Like rVOR however, both OKN and OKAN are also dependent upon normal functioning of the nodulus and uvula. Horizontal OKAN is prolonged with damage to these structures (Angelaki & Hess, 1994; Wearne et al., 1998). OKAN has not been studied in ASD; however, based on the results of the current study and possible indications that they involve damage to or deficits in nodulus/uvula function, it would be reasonable to suspect that OKAN would be prolonged in ASD. OKN has been briefly described in the literature to differ from controls in one oculomotor study (Scharre & Creedon, 1992), but it remains unclear as to the exact nature of these aberrations. A systematic examination of OKN and OKAN in ASD would be beneficial for better understanding cerebellar optokinetic differences and possible smooth pursuit differences in this population. Furthermore, since the current study found differences in ASD between full field visual stimuli in the light condition and foveal vision provided during fixation suppression, SHA rotary chair testing with OKN full visual field stimuli (Brey, McPherson & Lynch, 2008b) may also help to determine whether or not there are differences in visual processing of full field vs. foveal fixation for suppression of rVOR in ASD.

Future studies should aim to include lower functioning individuals with ASD to determine if they exhibit the same differences in rVOR as the individuals included in the current study who are considered high functioning. Several oculomotor deficits in ASD

have been linked to other measures of functional level such as presence or absence of language delay (Takarae et al., 2004; Takarae et al., 2008). Presence or absence of language delay is often used to distinguish autism from Asperger's syndrome. Although the current study did not record or compare history of language development, we did find a correlation with other measures of functional ability. Future studies should assess whether or not rVOR differences are dependent upon history of language development.

APPENDIX A
GLOSSARY OF TERMS RELATED TO VESTIBULO-OCULAR REFLEXES

Acceleration – degrees^2 / second

Frequency (Hz) – cycles / second.

Gain – the ratio of peak eye velocity (i.e., output) to peak head velocity (i.e., input); a measure of accuracy of the rVOR; perfect gain = 1.

Latency – the timing relationship between visual target onset and eye movement toward the target

Nystagmus (specifically vestibular nystagmus) – repetitive oscillating eye movements that occur in response to angular vestibular stimulation or optokinetic stimulation; includes two types of eye movements: a slow phase excursion followed by a quick/fast phase reset of the eye to physiological center.

Optokinetic Nystagmus (OKN) – nystagmus that occurs in response to optokinetic stimuli; specifically in response to continuous, repetitive movement of full-field (minimum of 90% of visual scene); for example, nystagmus that occurs in response to looking a moving car window at scenery passing by); primarily driven by smooth pursuit system; requires several seconds to build up response; will cease as soon as visual scene stops moving (Shepard & Schubert, 2008).

Optokinetic After Nystagmus (OKAN) – the prolonged nystagmus that occurs after OKN response to full-field visual scene motion; occurs only when subject is placed in the dark immediately following at least 30 seconds of exposure to full-field visual motion; primarily driven by optokinetic system (Leigh & Zee, 2006).

Per-rotary nystagmus – repetitive eye movements that occur during continuous *en bloc* whole body rotation.

Phase – the timing relationship between head velocity and eye velocity; eye movements should be 180 degrees offset from head movement (i.e., moving in the opposing direction).

Pitch – rotation around the pitch axis; interaural axis; y-axis; results in vertical eye movements.

Post-rotary nystagmus – repetitive eye movements that occur during continuous *en bloc* whole body rotation.

Roll – rotation around the roll axis; naso-occipital axis; x-axis; results in torsion eye movements.

Sinusoidal Harmonic Acceleration Test (SHA) - whole body *en bloc* rotary chair test with an oscillating side-to-side motion profile; typically conducted at peak

velocities of 50-60 degrees/second and at a range of frequencies from 0.01 up to 2.0 Hz; eye movement are recorded during rotation to measure gain, phase and symmetry of rVOR.

Symmetry – the comparison between the rVOR response for each direction of movement clock-wise or counter clock-wise; asymmetrical responses can reflect unilateral deficits.

Time Constant of Decay (TCD) – the time it takes rVOR slow phase velocity to decrease to 37% of its peak velocity.

Velocity Step Test – whole body *en bloc* rotation rVOR testing conducted with the participant seated on a rotary chair with a movement profile that includes a specified acceleration to a constant velocity for a set duration of time followed by rapid deceleration to stop; conducted in clock-wise and counter clock-wise directions with a minimum of 60 second break between trials; eye movement recordings during and after rotation measure gain, symmetry and time constant of decay of rVOR.

Velocity – degrees / second

Yaw – rotation around the yaw axis; rostral-caudal; z-axis; results in horizontal eye movements

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BIOGRAPHICAL SKETCH

Tana Bleser Carson was born in Key Largo, Florida. She attended Coral Shores High School, where she graduated in 2003. After high school, she attended the University of Florida for her undergraduate studies and graduated with a Bachelor of Science in Neurobiological Sciences in 2007. She continued at the University of Florida for her graduate studies in the Psychology PhD Program studying Behavioral and Cognitive Neuroscience with a research focus on sensory and motor processing in autism spectrum disorders. In 2009, she began a dual enrollment in Occupational Therapy Master's Program in the College of Health and Professions Nursing and Pharmacy also at the University of Florida. She graduated with her Masters in Occupational Therapy in December of 2011 and obtained licensure in February of 2012. She was awarded her PhD in Psychology in the area of Behavioral and Cognitive Neurosciences at the University of Florida College of Liberal Arts and Sciences in 2013.