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14. ABSTRACT

The overarching objective of this study is to characterize abnormalities of vestibulo-ocular reflexes (VOR) in Autism Spectrum Disorder (ASD). Specific Aim 1: Characterize 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: Characterize 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: Characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback. The present report covers the second year following award initiation. Complete research data have been obtained from one child with ASD and 18 typically developing children.

15. SUBJECT TERMS

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: Characterize 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: Characterize 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: Characterize in ASD vertical VOR and torsional VOR, both without optokinetic feedback. Because neither of these aspects of the VOR have been described previously in ASD, Aim 3 is exploratory.

The present report covers the second year following award initiation on 15 May 2010. Limited data were obtained during Year 1 due to equipment issues that were not solved until Year 1 Month 12. During the second year, it was possible to obtain complete research data from one child with ASD and 18 typically developing children. A second child with ASD began the data collection during the second year but did not complete testing until May 25, 2012 (third year). Five additional typically developing children began but did not complete data collection during the second year. One such typically developing child was excluded on the basis of oculomotor abnormality discovered during testing, and the other four typically developing children completed data collection early during year 3. One child with ASD provided partial data during the second year but has not yet been scheduled to complete his testing One further child with ASD has not yet shown up for scheduled appointments for testing.

Overall, the VOR data obtained from typically developing children seem entirely consistent with norms from the literature, as further described below. This correspondence leads us to have confidence that the equipment is operating properly and that our procedures are representative.

The VOR data from one case of ASD had highly unusual features also further described below. Specifically, as hypothesized, we observed in the ASD child vertical eye movement intrusions during horizontal nystagmus which were time-locked to the horizontal nystagmus, and which differed from voluntary saccades. Unexpectedly, the two eyes moved dysconjugately during the vertical intrusions.

In order to increase recruitment of participants with ASD during the next grant year, we will propose increasing the upper limit of age for inclusion in the study from 12 to 17 years old. Normative data (Casselbrant et al., 2010) published since the proposal was written support that the VOR normally does not change over this age span, so this change of age range appears warranted.

BODY

Statement of Work: Abnormal Vestibulo-Ocular Reflexes in Autism: A Potential Endophenotype

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 in April 2010, renewed in April 2011 and in April 2012, and granted by ARO Human Research Protections Office (HRPO Log No. A-16019). Minor changes to the protocol, such as creation of a phone screening form for recruitment, have been approved by the UF IRB and HRPO on several occasions. Milestone #1 is on track.

Subtask 1b. Research assistants trained in administration of Autism Diagnostic Observation Schedule (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 and renewed as used up. Tana Bleser, Graduate Research Assistant, and Jill Weish, Graduate Research Assistant, completed ADOS training. Milestone #2 is on track.

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). Milestone #3 is presently on track. Approximately 2000 recruitment fliers were distributed in public and private schools before the school year ended, and several therapy centers also have fliers. Participants are currently responding to those fliers. We distribute new fliers periodically and visit premises to refresh permissions as needed.

Task 2. Acquire eye tracking apparatus and set it up on site integrated with existing equipment (year 1, months 1 - 9). The existing equipment had to be replaced during year 1 which cuased several months of delays .

Subtask 2a. Establish appropriate levels of infrared illumination for high frame rate eye tracking while maintaining low visibility to the participants. This has been achieved during year 2.

Subtask 2b. Synchronize eye tracking data acquisition and rotary device motion control computers. This has been achieved during year 2.

Subtask 2c. Create data base structures to link eye tracking data and rotary motion data to neuro-psychological results. This work has been achieved during year 2.

Subtask 2d. (year 1, months 1 - 9) Research assistants trained in administration of vestibulo-ocular reflex tests, data entry procedures, and data quality control/quality assurance. This work has been achieved during year 2.

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

Task 3. Recruitment and testing of 8 pilot study participants (year 1, months 6 - 12). Recruitment of research participants is from Alachua County public schools, local therapy centers having ASD clients, and the UF Center for Autism and Related Disorders (CARD). All research testing and laboratory work takes place at the University of Florida. Task 3 commenced Year 1 Month 12, having been delayed by equipment issues explained above.

We have had great success in recruiting and testing 18 typically developing children , exceeding the goal for subtask 3b below. We have had more limited success in recruiting ASD children for subtask 3a below.

Subtask 3a. (year 1, months 6 - 12) Recruitment of 4 ASD research participants from Alachua County public schools, local therapy centers, and UF Center for Autism and Related Disorders (CARD). Administration of the following questionnaires to each set of parent(s)/guardian(s): Children's Communication Checklist-2, Repetitive Behavior Scale-Revised, Vineland-II, and the ShortSensory Profile. Questionaire responses are scored and entered into database. Administration of neuropsychological tests to each ASD child: Autism Diagnostic Observation Schedule (ADOS) and Leiter test of non-verbal problem solving. Scoring and validation of neurpsychological tests and entry into database. Administration of vestibulo-ocular reflex tests to each ASD child. Individual-level analysis made of eye movements to insure valid data capture of the reflexes (data quality control). Individual-level audits made of database record integrity (quality assurance). This subtask is currently in progress. . There have been minor changes to the specifc forms of neuro- psychological tests to be administered, as approved by UF IRB and HRPO.

We have completed testing one child with ASD during year 2, and have just completed testing a second chikld with ASD ealy in year 3.

Subtask 3b. (year 1, months 6 - 12) Recruitment of 4 non-ASD control participants from Alachua County public schools. This subtask will not begin until 10 ASD participants (50% of the target ASD sample size in Subtask 4a) have been recruited, to allow for the selection of controls who are (as group averages) age-and gender-matched to the ASD participants. Administration of the following questionnaires to parents: Children's Communication Checklist-2, Repetitive Behavior Scale-Revised, Vineland-II, and the Short Sensory Profile. Questionaire responses are scored and entered into database. Administration of Leiter test of non-verbal problem solving to each non-ASD (control) child, which is scored and scores entered into the database. Administration of vestibulo-ocular reflex tests to each non-ASD age- and gender-matched control child. Individual-level analysis of eye movements made to insure valid data capture of the reflexes (data quality control). Individual-level audits made of database record integrity (quality assurance). This subtask is currently in progress. There have been minor changes to the specifc forms of neuro- psychological tests to be administered to child participants, as approved by UF IRB and HRPO.

We have completed testing of 18 non-ASD typically developing children during year 2, and have just completed testing of an additional 4 non-ASD children early in year 3.

Subtask 3c. (year 1, month 12) Compare results from 4 non-ASD pilot participants to the literature for comparability. This subtask cannot be carried out at the present time. I believe it will be delayed until roughly Year 3 Month 3 to Month 6.

Subtask 3d. (year 1, month 12) Compare results from 4 ASD pilot participants to preliminary findings cited in the proposal for consistency. This subtask cannot be carried out at the present time. I believe it will be delayed until roughly Year 3 Month 3 to Month 6.

Subtask 3e. (year 1, month 12) Correct inefficiencies, if found, in test administration procedures or software or data structures. This subtask has in effect been carried out by testing a larger than planned sample of non-ASD children.

Subtask 3f. (year 1, month 12) Prepare abstract of pilot study findings for presentation at a national professional meeting. The PI has been encouraged by his neurologist colleagues to consider making a case report of the unusual vertical eye movements observed in the one ASD child who completed testing during year 2.

Milestone #5: Pilot study of 8 participants supports launch of formal research protocol by the end of grant year 1. Milestone #5 has not been met as originally proposed, but has instead been met in an alternative fashion by testing much larger sample of non-ASD participants (18) than the 4 originally proposed. Combined with successful testing of 2 children with ASD (although the second ASD child did not complete testing until May 25, a few days into year 3) we are reasonably confident that no changes will be needed in the protocol. We anticipate that two more ASD children may be fully tested in the near future allowing Milestone #5 to be completed as proposed.

Task 4. Prepare annual report of grant activities with pilot study results and tentative conclusions from these pilot results (year 1, month 12). Accomplished June 2011 and June 2012.

Subtask 4a. (year 1, months 11 - 12) Annual renewal of human research participation approvals (UF IRB and HRPO). Accomplished April 2011 and April 2012.

Summary:

Task 3 and Milestone #5 are currently in progress. Potential child participants are currently responding to recruitment fliers to be scheduled for testing. Delay in meeting Milestone #4 has in turn delayed meeting Milestone #5. Task 4 has been accomplished.

By 15 May 2011, the start of award year 2, the equipment had been installed and founf to be operational. During grant year 2, eighteen typically developing children and one child with ASD have completed all neuropsychological, oculomotor, and VOR testing. Five additional typically developing children and three additional children with ASD began but did not complete testing during grant year 2. Four of the typically developing children and one child with ASD completed their testing early in grant year 3. One typically developing child was excluded due to oculomotor abnormality. Two children with ASD have not yet been scheduled to complete their testing.

Representative Findings from non-ASD typically developing children

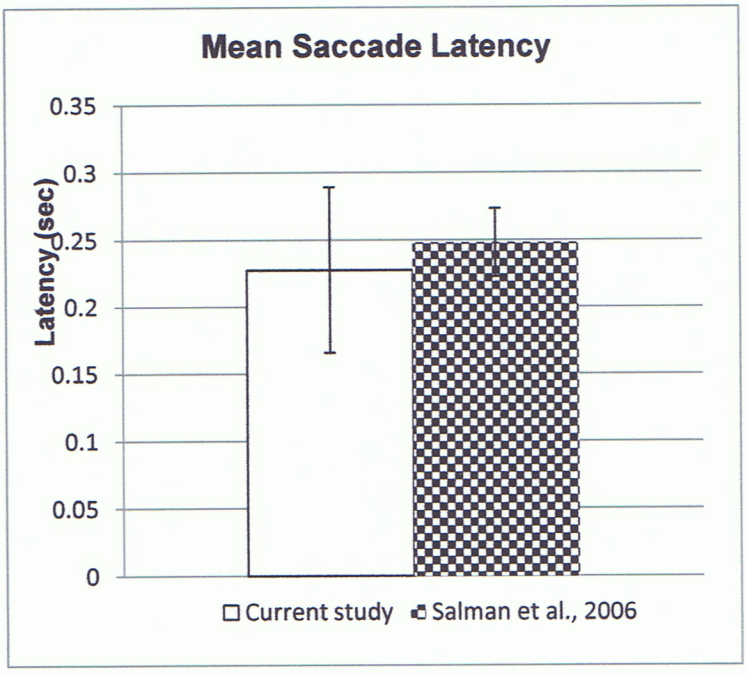

Figure 1. Mean saccade latency (msec) comparing 17 typically developing children who have participated in the current study during year 2 (open bar) to similarly aged children studied by Salman, et al. (2006) depicted in the checked bar. One participant was excluded due to average saccade latency more than 5 standard deviations outside the group mean. There is a good correspondence between the current study measures and those from the literature. In both studies these oculomotor tasks were performed while participants' heads were still.

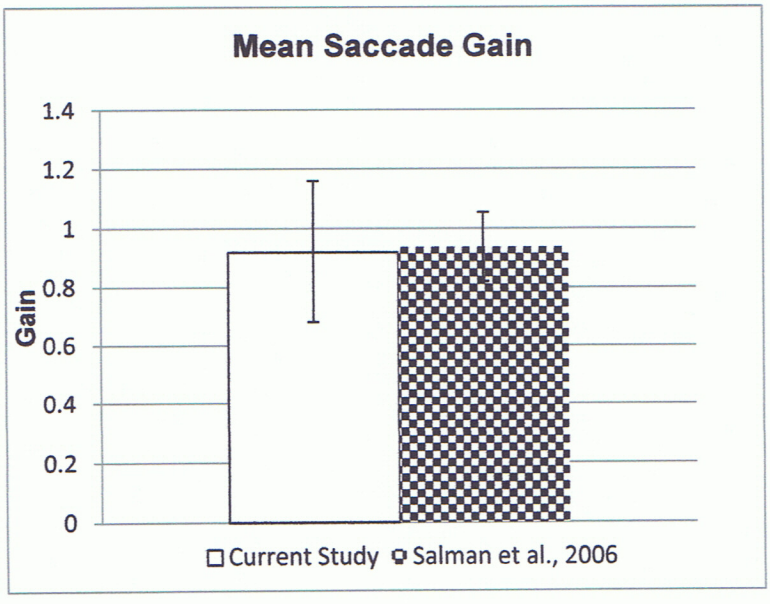

Figure 2. Mean saccade gain, ratio of eye movement displacement to target displacement, comparing 18 typically developing children who have participated in the current study during year 2 (open bar) to similarly aged children studied by Salman, et al. (2006) depicted in the checked bar. There is a good correspondence between the current study measures and those from the literature.

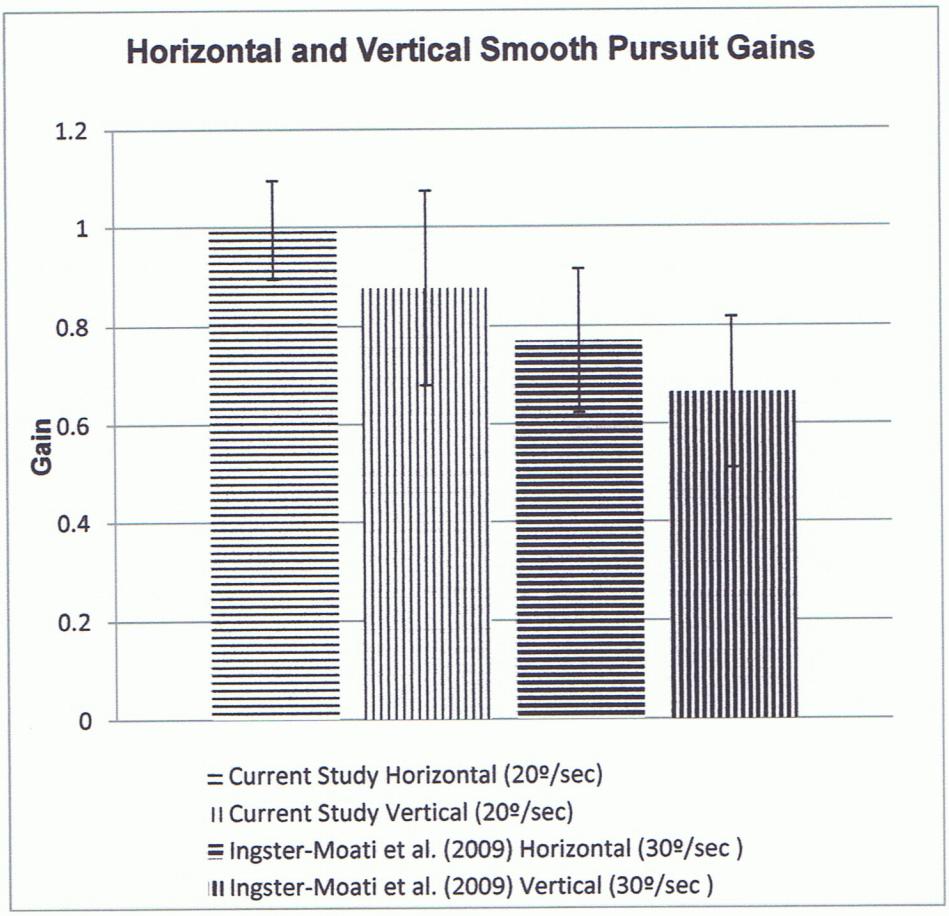

Figure 3. Mean smooth pursuit gain, ratio of eye movement velocity to target motion velocity, is shown for horizontal or vertical directions comparing 18 typically developing children who have participated in the current study during year 2 (lighter bars) to similarly aged children studied by Ingster-Moati et al. (2009) depicted in the darker bars. The current study used target motion velocity of 20 degrees per sec, while the study by Ingster-Moati et al. (2009) used target motion velocity of 30 degrees per sec. This task difference between studies is likely an important reason for the differences in gain between studies. Adult smooth pursuit typically has lower gain for target motion velocity of 30 degrees per sec than for 20 degrees per sec. In both studies these oculomotor tasks were performed while the participants' heads were still.

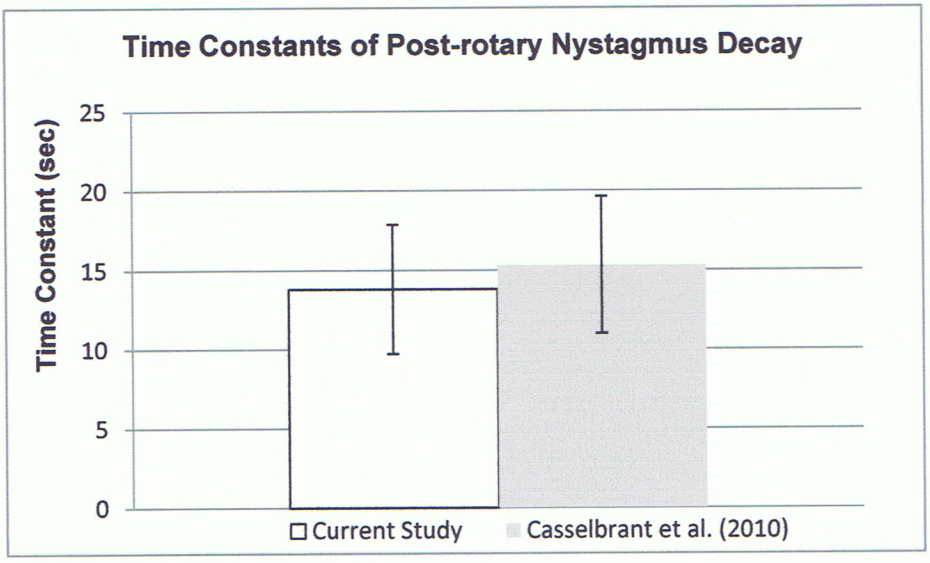

Figure 4. During trapezoidal rotational testing the head is undergoing angular accelerations that affect the semicircular canals and elicit vestibulo-ocular reflex (VOR) eye movements. The term trapezoidal describes the profile of angular velocity over time; namely, a gradual ramp up from zero to a desired angular velocity, which is then held at that constant angular velocity for a period of time, after which the velocity ramps back down to zero. Angular acceleration occurs during the ramp up portion, and deceleration occurs during ramp down. Soon after entering the ramp-up period the eye movements follow a stereotyped pattern of nystagmus. Nystagmus has slow-phase portions that will approximately match angular velocity of the eye to angular velocity of the head. The eye can only rotate to a certain extent before fast-phase movements return the eye roughly to primary position. Each slow-phase/fast-phase pair is called a beat, and the beats continue throughout the ramp-up to constant angular velocity. Upon reaching the constant velocity angular acceleration ceases. Nystagmus beats continue, with slow-phase eye velocity initially nearly equal to head angular velocity. As time at constant angular velocity continues, slow-phase eye velocity gradually diminishes in a fashion resembling exponential decay. This decay can be characterized by its time constant, or the number of seconds for the VOR response to decline to 1/e (about 37%) of its initial value. These time constants measured on 18 typically developing children in the current study during year 2 (open bar) are similar to those reported by Casselbrant et al. (2010) for typically developing children of similar ages.

Noteworthy Findings in ASD participant A001

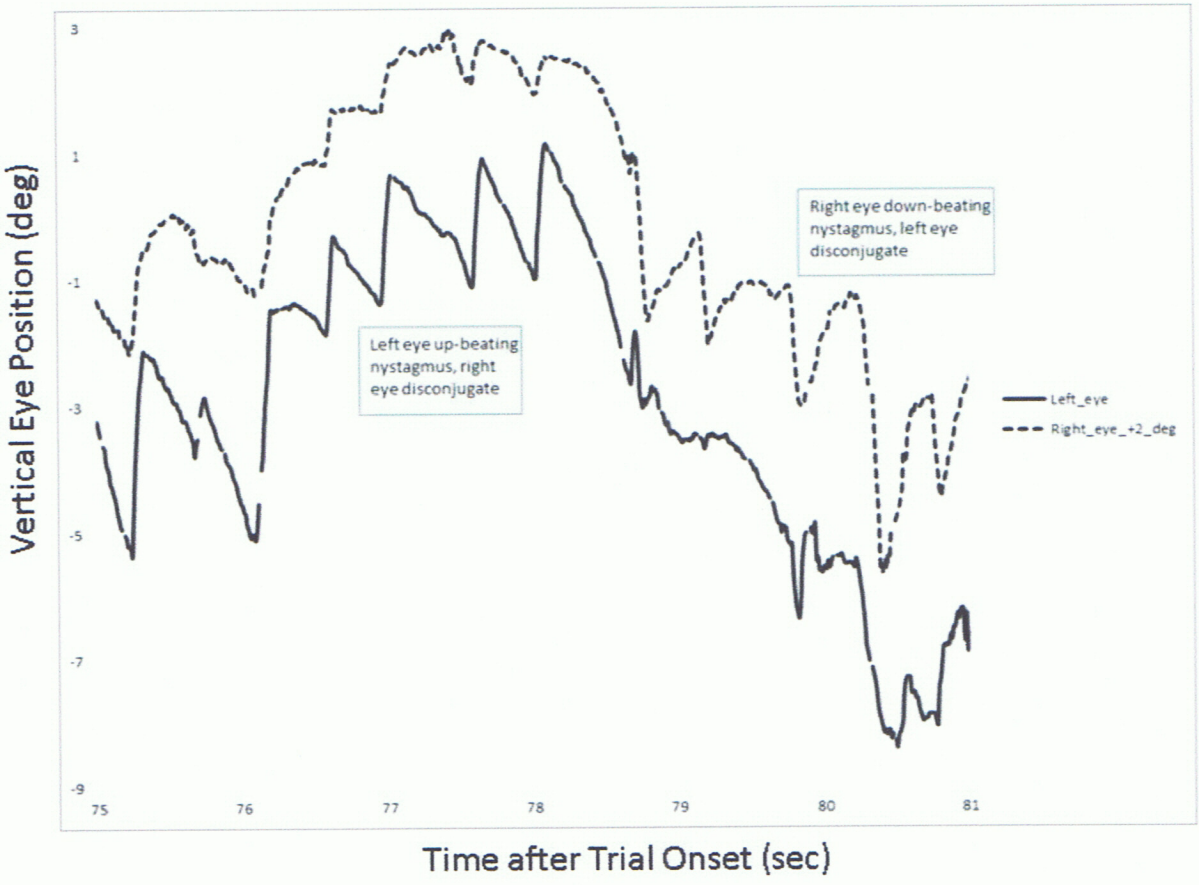

Figure 5 shows the vertical components of eye movements made by ASD participant A001 during a re-test of horizontal vestibulo-ocular reflexes (VOR) conducted in the dark. The record shows a selected six second period from 75 to 81 sec after the trial began. The left eye record (solid line) was displaced downward by 2 deg for clarity. Near the beginning of the period shown, above a text box added to the Figure, A001's left eye made up-beating nystagmus-like motions that his right eye (dotted line) did not faithfully follow. A few seconds later, below a second text box added to the Figure, his right eye made down-beating nystagmus-like motions that his left eye did not faithfully follow. These vertical components of eye movements were disconjugate and are believed to be involuntary. These vertical nystagmus-like movements are approximately time-locked to A001's horizontal VOR (the typical and expected responses) as shown in Figure 6 below. The qualitative differences in the trajectories of the two eyes cannot be readily explained as artifacts of differential magnifications of the two eyes' images nor as slippage of the recording cameras relative to the eyes. Motions of the head gear were monitored by both three-axis gyroscopic and three-axis linear accelerometer sensors, and none of these six time courses predicted the vertical eye motion time courses shown. The PI has carefully studied books by Leigh and Zee (1999) and by Straube and Buttner (2007) to find literature documentation of similar dysconjugate vertical eye movements, without success. My colleague, Dr, Kenneth Heilman, a neurologist, sent these data to Dr. David Zee seeking his comment but Dr. Zee has not yet replied.

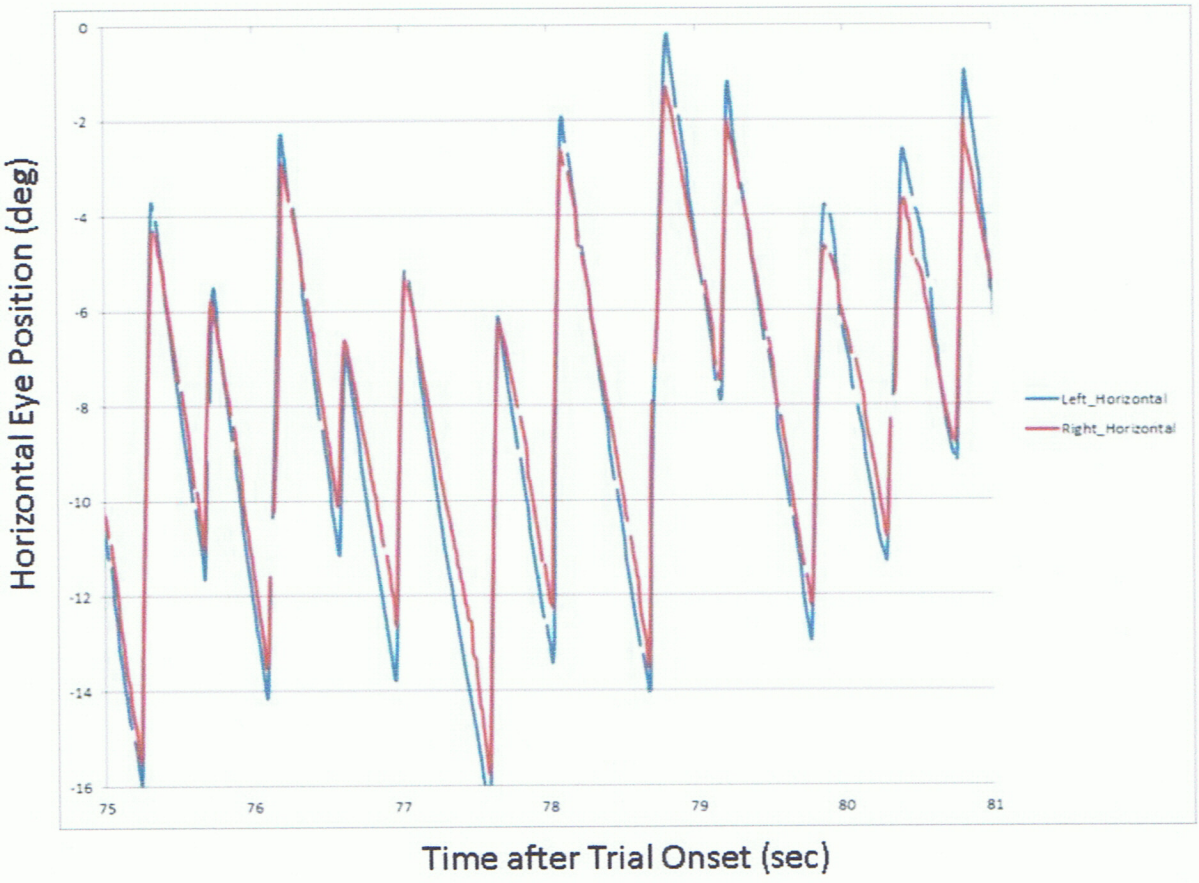

Figure 6 shows the horizontal components of eye movements made by ASD participant A001 during a same re-test of horizontal VOR for which the vertical components of eye movements were shown in Figure 5. Left eye horizontal components are shown in blue, while right eye horizontal components are shown in red. The two traces are highly similar although not identical. It cannot be ruled out that the left eye's image may have had a very slightly greater magnification on its recording camera than did the right eye's image, leading to consistently slightly larger recorded horizontal excursions for the left eye. The horizontal eye movements shown in Figure 6 are clearly more qualitatively similar for the two eyes, than are the vertical eye movements shown in Figure 5.

Albeit that Figures 5 and 6 each depict data from only a few seconds of observations, they are suggestive that very unusual features may exist in the vertical eye movements made by the ASD participant while the horizontal eye movements made at the same time were unremarkable.

KEY RESEARCH ACCOMPLISHMENTS

- Equipment needed to study vestibulo-ocular reflexes is fully operational with data integrated into databases for comparing VOR findings to corresponding neuropsychological test scores and demiographics.

- Eighteen non-ASD typically developing child participants were tested fully with this equipment, and the data obtained were of good quality and within norms.

- One ASD child participant was tested fully, and the data obtained were of good quality and of great interest.

REPORTABLE OUTCOMES

Bleser, Tana Marie. Masters of Occupational Therapy, University of Florida, December 2012.

CONCLUSION

Year 1 was delayed significantly by equipment failure and the acquisition and installation of new equipment. The new equipment was functional as of Year 1 Month 12, at which time we had obtained representative VOR data from 10 young adult participants. During year 2 we have completed testing of 19 child participants, only one of whom has ASD diagnosis.

Recruitment of children with ASD is top priority for year 3. We have already completed testing of a second ASD child, and additional children with ASD are in process of being scheduled for testing.

Looking forward to year 3, we have determined that our proposed and currently approved upper limit of age for inclusion in the study (12 years old) may have been overly conservative. Evidence now exists that VOR responses normally become adult-like by age 12 (Casselbrant et al., 2010). Accordingly, we will seek IRB and HRPO approvals to raise the upper limit for inclusion to 17 years old. In this way, consent and assent processes will not need to be altered significantly but the pool of eligible participants will expand. We have had to turn away potential participants who were slightly too old for inclusion (teenagers). We do not intend to request any change for the current lower age for inclusion (6 years old).

REFERENCES

Casselbrant, M. L., Mandel, E. M., Sparto, P. J., Perera, S., Redfern, M. S., Fall, P. A., and Furman, J. M. (2010) Longitudinal posturography and rotational testing in children three to nine years of age: normative data. Archives of Otolaryngology Head and Neck Surgery 142, 708-714.

Ingster-Moati, I., Vaivre-Douret, L., Bui Quoc, E., Albuisson, E., Dufier, J-L., and Golse, B. (2009) Vertical and horizontal smooth pursuit eye movements in children: A neuro-developmental study. European Journal of Paediatric Neurology **13,** 362-366.

Leigh, R. J. and Zee, D. S. (1999) *The Neurology of Eye Movements.* Oxford Univ. Press, Oxford, UK.

Salman, M. S., Sharpe, J. A., Eizenman, M., Lillakas, L., Westall, C., To, T., Dennis, M., and Steinbach, M. J. (2006) Saccades in children. Vision Research 46, 1432–1439

Straube, A. and Buttner, U. (2007) *Neuro-Ophthalmology: Neuronal Control of Eye Movements.* Karger, Basel, Switzerland.