Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** University of Pittsburgh – Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 6/01/2009 – 5/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):**
   Nancy J. Minshew, MD

4. **Grant Contact Person's Telephone Number:**
   412-246-5485

5. **Grant SAP Number:** 4100047862

6. **Project Number and Title of Research Project:** 01 - Deciphering Altered Brain Connectivity in ASD to Improve Intervention

7. **Start and End Date of Research Project:** 06/01/2009 – 5/31/2013

8. **Name of Principal Investigator for the Research Project:** Nancy J. Minshew, MD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 2,997,245.33

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	Institution	% of Effort on Project	Cost
Bahorik	Graduate Student Assistant	University of Pittsburgh	YR03: 4% YR04: 55%	$14,440
Baker	Data Manager	University of Pittsburgh	YR01-03: 20% YR04: 5%	$3,769
Beck	Student Research Assistant	University of Pittsburgh	YR03: 5%	$2,341
Becker	Tester	University of Pittsburgh	YR01-04: 10%	$23,814
Behrmann	Co-Investigator	Carnegie Mellon University	YR01-YR04: 20%	$99,946
Bonato	Tester	University of Pittsburgh	YR03 50% YR04 50%	$37,169
Bowser	Research Coordinator	Penn State University	YR03: 100%	$54,722
Brown	Tester	University of Pittsburgh	YR04 30%	$18,075
Carter	Project Manager	University of Pittsburgh	YR04 20%	$9,726
Christ	Administrative Liaison	University of Pittsburgh	YR01-03 25%	$43,652
Conrad	Systems Administrator	University of Pittsburgh	YR03-04 20%	$21,604
Crawford	Outreach Coordinator	University of Pittsburgh	YR02: 15% YR03: 30%	$10,856
Dandar	Data Manager	University of Pittsburgh	YR01-03: 20% YR04: 19%	$12,010
Day	Tester	University of Pittsburgh	YR03-04: 45%	$41,829
Dayton	Administrative Assistant	University of Pittsburgh	YR03-04: 25%	$29,810
Eack	Co-Investigator	University of Pittsburgh	YR01-YR04: 25%	$13,658
Gigliotti	Research Assistant	University of Pittsburgh	YR03: 12%	$5,197
Greenwald	Clinician	University of Pittsburgh	YR02-03 15% Y4: 50%	$36,288
Hogarty	Clinician	University of Pittsburgh	YR01-YR04: 30%	$141,837
Hwang	Programmer	Penn State University	YR04: 32%	$20,571

Johnson	Research Assistant	Carnegie Mellon University	YR02: 50%	$32,305
Litschge	Clinician	University of Pittsburgh	YR02: 1% YR03-04: 33%	$49,564
Lorenzi	Research Coordinator	Carnegie Mellon University	YR01: 100%	$38,144
Markiewicz	Data Manager	University of Pittsburgh	YR01-04: 20%	$40,887
McAniff	Tester	University of Pittsburgh	YR03: 30% YR04: 75%	$58,341
McCarroll	Program Manager	University of Pittsburgh	YR03-04: 50%	$81,764
McFadden	Co-Investigator	University of Pittsburgh	YR01-YR04: 65%	$502,269
Minshew	Project Director	University of Pittsburgh	YR01: 20% YR02: 14% YR03: 32% YR04: 32%	$297,843
Munoz	Research Assistant	Carnegie Mellon University	YR02: 50%	$33,161
Perrin	Recruiter	University of Pittsburgh	YR03-04 50%	$83,958
Plaut	Consultant	Carnegie Mellon University	YR01-YR04: 10%	$94,391
Rush	Research Assistant	University of Pittsburgh	YR03-YR04: 25%	$4,043
Scherf	Co-Investigator	CMU 2008-2011 and Penn State 2011-2013	YR01-YR04: 25%	$68,840 $81,743
Sowers	Research Assistant	University of Pittsburgh	YR03-YR04: 25%	$4,988
Wang	Research Associate	University of Pittsburgh	YR01-YR04 100%	$198,623
Webber	Senior Administrator	University of Pittsburgh	YR03: 10% YR04: 0.5%	$8,958
Whyte	Research Coordinator	Penn State University	YR04: 15%	$7,210
Wilson	Data Manager	University of Pittsburgh	YR03: 21% YR04: 96%	$36,247
Winkowski	Tester	University of	YR04: 25%	$8,774

		Pittsburgh		

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).
% of effort needs listed even if they are unpaid

Last Name, First Name	Position Title	Institution	% of Effort on Project
Record	Undergraduate Student	Carnegie Mellon University	20%
Elbich	Research Assistant	Penn State University	30%
Picci	Graduate Student	Penn State University	10%
Garcia	Graduate Student	Penn State University	5%
Legault	Graduate Student	Penn State University	5%
Unger	Undergraduate Student	Penn State University	10%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Evidence-Based Cognitive Rehabilitation to Improve Functional Outcomes for Young Adults with Autism-Spectrum Disorders	☐NIH ☐ Other federal (specify:_____ _____) ☒ Nonfederal source (specify: Autism Speaks)	February 2009*	$300,000	$300,000
A Randomized Clinical Trial of Cognitive Enhancement Therapy for Adults with Autism Spectrum Disorders	☐NIH ☒ Other federal (specify: Department of Defense) ☐ Nonfederal source (specify: _____)	July 2010	$1,412,388	$1,412,388
Adapting Cognitive Enhancement Therapy for ASD	☒ NIH ☐Other federal (specify: Depart. of Defense) ☐ Nonfederal source (specify: _____)	May 2010	$644,278	$644,278

*Note: The notice of award, which was received in early February, 2009, of the DOH support contributed significantly to the positive decision by Autism Speaks to contribute funding for this effort. Each of the above grants was requested to add a new aspect to the DOH supported study of Cognitive Enhancement Therapy (CET). The DOH funding started the CET effort moving forward.

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

A detailed description of our plans for each of our aims is provided under each aim's progress report. In summary, Kathryn McFadden, MD, has a grant under review at NIH to extend and expand on the research of Aim R1. Once the papers are published reporting the original data from this project, she will submit a larger grant application to NIH to move this work to the next stage. K. Suzanne Scherf, PhD has three post-doctoral fellowship grant applications submitted to Autism Speaks (2) and to Autism Science Foundation (1) to support fellows to continue the publication work of Aim R2. Once the original data from this aim has been published, Dr. Scherf will submit an R01 grant to NIH to extend and expand on Aim R2 work. For Aim HT2, Shaun Eack, PhD, and Dr. Minshew will submit an R01 early next year proposing a multisite controlled trial of CET for adults with Autism Spectrum Diagnosis (ASD) to expand the evidence for efficacy of this treatment and to demonstrate reliable dissemination of this treatment across sites with good fidelity.

12. Future of Research Project. What are the future plans for this research project?

We will continue to pursue and expand each of the three research studies initiated under this DOH grant.

We anticipate that Aim R1 research will lead to identification of a new facet of the developmental neurobiology of autism and new risk genes for ASD. These advances will contribute to earlier and more accurate identification of ASD through microarray analysis and will guide the development of biological approaches to restoring brain development. The principal investigator (PI) of this study, Dr. McFadden, will devote the next two years to publishing manuscripts reporting her findings in major journals in her field with advice from Peter Strick, PhD, at the University of Pittsburgh. Once these articles are in press, she will apply to NIH for grant funding to move this study to the next stage.

The PI of Aim R2, Dr. Scherf, will likewise devote the next two years to preparing and submitting publications of her extensive database from this period of DOH support; she is currently seeking funding for three graduate student fellowships to participate in this process.

Dr. Scherf also plans to begin work to convert her intervention to a gaming format that can be widely used by individuals with ASD.

The PIs of Aim HT2, Drs. Eack and Minshew, will continue publication of data generated by the controlled trial that is in progress and will pursue R01 funding next year to support a multisite controlled trial. They will also pursue their studies to understand the neural and cognitive basis of heterogeneity among the adults with ASD who participated in this treatment study, which we anticipate will lead to the development of new therapies and combinations of therapy to address the deficits experienced by each individual with ASD. We have considerable hope for repetitive transcranial magnetic stimulation (rTMS) methods, followed by neurocognitive intervention, to integrate enhanced abilities into their general function. We are also focusing on new methods for treating emotion dysregulation and anxiety in ASD. Lastly, Drs. Minshew and Eack are working with Russell Johnson, PhD, to introduce a new cognitive-based treatment for ASD and anxiety disorder in area high schools beginning this fall. The DOH support has enabled these collaborations, which we hope may lead to changes in the standard of care for ASD in Pennsylvania within the next five to eight years. Generally, two to three controlled trials are needed to provide sufficient evidence of efficacy for Medicare to approve reimbursement for a new treatment practice. Once that standard is achieved, we can focus on dissemination through schools, community agencies, and parents.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	3		0	
Female	3		4	
Unknown	0		0	
Total	**6**		**4**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic	0		0	
Non-Hispanic	6		4	
Unknown	0		0	
Total	**6**		**4**	

	Undergraduate	Masters	Pre-doc	Post-doc
White	0		0	

Black	6		4	
Asian	0		0	
Other	0		0	
Unknown	0		0	
Total	**6**		**4**	

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes____X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    This health research project greatly expanded and enhanced our capacity to pursue these three new, innovative research efforts in autism spectrum disorder. None of the research that we report would have been possible in the absence of this award. This health research project enabled us to add three new researchers to this field of research- Drs. McFadden, Scherf, and Eack- and three new lines of research. Each has made remarkable progress that will lead to major advances in research, diagnosis, and help them establish productive careers in ASD research. In addition, support for our faculty enabled them to expand their autism research efforts even beyond the DOH goals and to pursue ancillary projects. An important example of this multiplicative effect is that participants recruited for two treatment studies (Aims R2 and HT2) also participated in other ASD research studies. Hence, we were able to refine the definition of the cognitive and neural mechanisms underlying autism manifestations in very important ways. For example, these research participants also took part in studies that demonstrated that neither attention abnormalities nor abnormalities in the brain's exhibition-inhibition balance were responsible for autism manifestations. Both of these deficits have been widely hypothesized to be causing autism, however. In addition, studies of sensory perception by Marlene Behrmann, PhD, demonstrated that there is inconsistency in sensory perception at a cortical level in individuals with autism that may contribute to neural signal decay or distortion. Likewise, with DOH support, we had the infrastructure by way of a clinical trials expert, Dr. Eack, to design a project to test another cognitive intervention for high school students with ASD; funding was obtained from the state for this project, which will launch this fall. Third, Dr. Eack was able to compare the

data collected in study participants with ASD to data from participants with schizophrenia who participated in a separate CET trial, demonstrating important commonalities in these two disorders, which are often clinically confused. This is very important because most verbal adolescents and adults with autism are misdiagnosed with schizophrenia later in life and medications used are very different. Publications from our group reflect the multiplicative effect of the critical infrastructure support provided by this DOH grant.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X_____ No_____

If yes, please describe the collaborations:

Dr. Scherf (Aim R2) was recruited to Penn State University, creating a new research effort in autism in the middle region of the Commonwealth. In her new faculty position, she has developed many collaborations with faculty at Penn State. Drs Eack and Minshew have developed new collaborations with Robert Mason, PhD, (Carnegie Mellon University), who does research in repetitive Transcranial Magnetic Stimulation (rTMS) and J. David Cresswell, PhD, (Carnegie Mellon University) who does research on mindfulness meditation and its potential for changing cognition and related brain circuitry. We anticipate developing new treatments for ASD that combine rTMS with a neurocognitive approach.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes___X_____ No_____

If yes, please describe involvement with community groups that resulted from the research project:

We have begun working more closely with two parent groups in Pittsburgh—Autism Connections of Pennsylvania and the Pittsburgh chapter of the Autism Society of America. We are also working with groups that are directly involved in services/activities for adults with ASD. One group, Cranberry Cares, is located in Cranberry Township, north of Pittsburgh, and the other group is a new vocational program in Massachusetts

that is being established by John Elder Robison, a well-known and successful adult with Asperger's syndrome.

Cranberry Cares has developed a strong collaboration with an internationally renowned artist, William Rock, who is extremely talented at interacting and promoting the growth of young adults with ASD using art as a nonverbal medium for self-expression and self-esteem building. Mr. Rock is also an expert on meditation and will likely begin working with Drs. Cresswell and Minshew in the next year to develop mindfulness meditation for adults with ASD.

Mr. Robison, who has authored several books on ASD, has established the first vocational training program for ASD high school students at a factory site, e.g., his automobile repair and restoration business. His wife, Maripat Robison, is working with him on establishing this program and is also an expert in meditation. The Robisons met with Mr. Rock and with Drs. Mason and Cresswell at a recent event in Pittsburgh organized and hosted by Dr. Minshew. Our tentative plans are to introduce Luminosity brain training to promote cognitive improvement and Toastmasters clubs to promote competent social communication at both community sites (Cranberry Cares and the Robisons' new vocational program in Massachusetts.) Once these community programs have developed successfully, we will then work with the researchers to investigate the cognitive and neural basis of the improvements in teens and adults with ASD to establish an evidence base to justify dissemination throughout Pennsylvania, Massachusetts, and elsewhere.

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. <u>Provide detailed results of the project.</u> Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a <u>DETAILED</u> report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Aim R1: Neuropathology & Genetics of Brain Connectivity: Altered Axonal Pathfinding

Aim R1 Timeline, Study 1

Period	Milestones	Research Activities
6/1/09-6/30/09	• Aim R1, study 1 initiated	• Tissue proposal to National Institute of Child Health and Human Development Brain Tissue Bank (NICHD BTB)• Initiation of in-house tissue collection • Initiation of optimization of antibodies, in-situ probes and PCR primers
7/1/09-6/30/10	• Aim R1, study 1 • Aim R1, study 1 enhancement initiated	• In-house tissue collection • Optimization of antibodies, in-situ probes and PCR primers • Initiation of RT-PCR on mid-gestational samples • Initiation of new immunohistochemistry protocol with TSA amplification on mid-gestational and adult samples • Optimization of new immunohistochemical protocol on PEG-embedded ATP tissue
7/1/10-6/30/11	• Aim R1, study 1 • Aim 1, study 1 enhancement	• In-house tissue collection • RT-PCR completed for 6 LRRs on 15 mid-gestation brains • Immunohistochemistry on collected tissue and PEG-embedded ATP tissue
7/1/11-6/30/12	• Aim R1, study 1 • Aim 1, study 1 enhancement completed	• In-house tissue collection • Immunohistochemistry on collected tissue and PEG-embedded ATP tissue completed • Initiation of characterization of developmental expression of LRRs (data collection from immunohistochemical staining).
7/1/12-5/31/13	• Aim R1, study 1 completed	• Data collection of developmental expression of LRRs completed

The overall goal of Aim R1 has been to assess the plausibility of the hypothesis that alterations in certain Leucine-rich repeat (LRR) proteins (Table 2) implicated by Autism Genome Project linkage, association and/or copy number variation (CNV) data, confer risk for ASD by altering normal axonal guidance. Because LRR proteins almost certainly play an important role in modulating neurite growth, we suspect that alterations in the expression and/or function of a subset of these LRR genes generates risk for autism. Specifically, the proposed studies were designed to ascertain whether LRR candidate molecules: 1) are expressed in a meaningful temporospatial pattern and/or 2) potentially participate in axonal outgrowth/guidance, and were modeled on previous approaches to assess ASD candidate genes (e.g. MET receptor and Neurexin 1). Such studies, while often considered basic, are exceedingly important as the underlying developmental mechanisms implicated in the pathogenesis of autism are largely unknown, as are the specific functions of the majority of the significantly expanding list of genetic candidates.

Aim R1 - Study 1. Confirmation of Appropriate Spatial/Temporal Expression of Candidate Gene Products

Introduction:
Our model predicts that the LRR gene products of our set of candidate genes will be: 1) expressed during critical time periods of axonal outgrowth and remodeling, and 2) expressed in the brain areas most frequently affected clinically and anatomically in autism. We therefore assessed the developmental patterns of LRR candidate gene expression by measuring gene specific mRNA and protein levels, as well as protein localization across development. For each LRR of interest, we considered three possible outcomes: 1) selective temporospatial brain expression/ localization, 2) absence of detectable brain expression during development (indicating the initial genetic signal was a false positive or due to gene linkage disequilibrium), or 3) ubiquitous and constitutive brain expression (indicating mechanistic linkage to a more temporally selective but unknown protein). Only LRR candidates showing selective temporospatial brain expression/ localization were considered for subsequent mechanistic studies (i.e. Study 2).

Methods:
Tissue: As outlined in the Research Proposal, our original intent was to obtain frozen and formalin fixed tissue samples from seven association cortical/limbic brain areas from individuals from three developmental time frames known to be relevant to neuritic/axonal outgrowth, guidance, and remodeling, i.e. midgestation (18-25 gestational weeks), infancy (3-18 months) and late childhood/early adolescence (ages 7-15 years). Samples were to be obtained from the National Institute of Child Health and Human Development (NICHD) Brain and Tissue Bank (BTB) Baltimore, Maryland). We proposed (outlined in detail in the Research Proposal), to assess the developmental patterns of LRR candidate gene expression in these samples by measuring gene specific mRNA (by reverse transcriptase PCR [RT-PCR] and *in situ* hybridization, as well as protein levels (by Western blot) and protein localization (by immunohistochemistry).

Due to significant tissue shortages, particularly for our age groups of interest, the BTB was unable to provide our requested samples which became evident during the tenth month of the study. This necessitated a number of changes to our methodology and timeline very early in the research period:

1. All study tissue was obtained from our institution, specifically the University of Pittsburgh Health Sciences Tissue Bank and the neuropathology autopsy service. We had already initiated this process at the onset of the study in order gain enough material to optimize antibodies, primers and probes, thereby saving time and preserving the valuable, banked tissue. We increased our in-house collection efforts significantly during the second year of the study.
2. Therefore, instead of having acquired the complete set of materials at the onset of the research period, sample collection occurred on an ongoing basis as appropriate tissue became available. This necessitated prolongation of Study 1 expression studies beyond originally expected (i.e. to the end of the third year of the research period).

3. Reliance on our own, more limited, resources also limited which types of expression studies were feasible. Specifically, adequate samples could not be obtained for the late childhood/early adolescence time period (Table 1) and only limited amounts of frozen tissue were available for 28 midgestation and 8 adult brains. Therefore, while in-lab and commercial RT-PCR oligos were obtained for all LRRs of interest, and in situ hybridization probes developed for three, these studies were very circumscribed (results described below). Western blot studies were not carried out.
4. On the other hand, due to the relative abundance of formalin-fixed, paraffin-embedded (FFPE) tissue available, immunohistochemical studies were quite successful with one significant methodological modification involving Tyramide Signal Amplification (TSA) (see methods for Revised Aim R1-Study 1). Immunohistochemical studies proved superior for detecting protein localization to neuronal processes quite distant from the nuclear site of mRNA production. As all Aim R1 Study 1 results pertaining to immunohistochemistry were obtained after substantial changes to the sample composition and research methodology within the first year of the research period, these are outlined in the methods section under Revised AimR1.

qRT-PCR: Tissue samples from 15 mid-gestation brains (15 to 23 gestation weeks (GW)) were homogenized and ribonucleic acid (RNA) prepared. Levels of gene specific messenger RNAs (mRNAs) were measured by quantitative real-time PCR (qRT-PCR) using an APPLIED BIOSYSTEMS StepOne Thermocycler. The mRNA expression level was quantified as delta cycle threshold (CT) normalized to the CT of the housekeeping gene GAPDH (glyceraldehyde 3-phosphate dehydrogenase).

In Situ Hybridization: Ribonucleotide probes corresponding to unique portions of LINGO1 and AMIGO1 were transcribed with T7 to generate antisense transcripts using oligonucleotide probes designed to unique genomic areas. These were tested on a limited sample of control tissue to determine their specificity. Control sense probes were generated and hybridized to adjacent sections. Briefly, tissue sections were prehybridized then hybridized overnight in hybridization buffer containing 35S labeled probe. Sections were subsequently washed and exposed to P-max Hyperfilm (Amersham) for 7 days and developed.

Results:
qRT-PCR: qRT-PCR was completed for six candidate LRRs for all 15 specimens. All candidates showed significant expression during mid-gestation which was in general agreement with the immunohistochemical data. Two candidates, LRRN1 and Amigo1 appeared to increase over the represented time span but this interval was too narrow for statistical significance.

In Situ Hybridization: In frozen tissue sections of mid-gestational brain, both probes detected clear, ubiquitous expression in cortical neurons. In situ hybridization using these probes on postmortem, FFPE tissue did not yield convincing results.

Conclusions/Discussion:
As adequate frozen tissue was not available for other early age groups, qRT-PCR and *in situ* hybridization studies were necessarily limited as we were unable to detect statistically significant dynamic changes in expression of our candidates. However, all were clearly expressed during mid-gestation, a developmental period marked by significant axonal extension and growth cone migration.

Revised Aim R1- Study 1:

Introduction:
For our circumstances and purposes, immunohistochemical studies were quite successful, and actually superior for detecting protein localization to neuronal processes quite distant from the nuclear site of mRNA production. This allowed us to examine more specifically LRR protein expression during critical time periods of axonal outgrowth and remodeling in our cortical areas of interest. As qRT-PCR (above) demonstrated brain expression during development for all LRRs of interest, we evaluated initial immunohistochemical results for two potential outcomes: 1) selective temporospatial localization, and ubiquitous/constitutive brain expression. Only LRR candidates showing selective temporospatial brain expression were considered for more detailed expression studies or subsequent mechanistic studies (i.e. Study 2).

Methods:
Brain Tissue Samples: In addition to the previously described mid-gestational frozen brain tissue, we obtained perinatal FFPE brain tissue from the University of Pittsburgh Health Sciences Tissue Bank, with appropriate maternal consent and approval by the Institutional Review Board (IRB) of the University of Pittsburgh. Pediatric (40GW to 6 yrs) and adult (20 yrs to 66 yrs) FFPE brain tissue was obtained from the neuropathology autopsy service with approval by the University of Pittsburgh Committee for Oversight of Research Involving the Dead (CORID). Overall, tissue from 74 individuals was obtained for our studies (Table 1). For the analyses, both association cortical areas (dorsolateral and medial prefrontal, orbitofrontal and occipital visual association) and limbic areas (anterior cingulate cortex, fusiform gyrus and amygdala) were analyzed.

Immunohistochemistry: While commercial antibodies were available for all candidate LRRs under examination, most were for use on frozen tissue and had to be optimized for immunohistochemistry on postmortem FFPE brain tissue. This was accomplished primarily by employing TSA, with excellent results. Specifically, tissue samples were paraffin embedded and sectioned at 5 microns. Immunohistochemical investigation was performed on adjacent sections using a standard avidin-biotin-peroxidase technique and immunofluorescence. Antigen retrieval was performed using Target Retrieval Solution (Dako) at 95º for 30 minutes. Sections were then incubated with serum-free protein block (Dako) for 30 minutes followed by primary antibody (Table 2) at a dilution of 1:1000 overnight. Sections were then incubated with biotinylated secondary antibody followed by TSA amplification (Elmer Perkins) for all antibodies. This was followed by standard ABC incubation and DAB detection. Sections were counterstained with 0.5% cresyl violet (Sigma) and examined by transmission light microscopy. In the last month of

this research project, selected DAB sections were scanned as virtual slides using an Aperio scanner and visualization/photomicrographs performed with a WebScope viewer. This system has allowed for superior photomicrographs (particularly at lower powers) and data sharing. Sections from other LRR staining have been submitted for scanning as well. For fluorescence labeling, FITC, Cy3 and Cy5-conjugated secondary antibodies (Jackson ImmunoResearch, 1:250) were used. Fluorescent sections were visualized on a Nikon Eclipse E400 fluorescent microscope and photomicrographs captured with a Photometrics Cool Snap $E5^2$ camera and NIS Elements 4.1 software.

Results:

We conducted basic immunohistochemistry studies on human tissue for all of the originally proposed candidate LRRs. A summary of results is provided in Table 2. Over the course of the research period, based on ongoing results, we have pared this list significantly to the most interesting candidates (i.e. LINGO1, LRRN1, LRRN3, and LRRTM3) for more detailed study. For the purposes of manageability (the data derived from this study are predominately visual and descriptive), we present select results below. The emphasis is on LRRN3, the candidate providing the most intriguing data which we have currently chosen to pursue in greater depth in terms of both expression and function.

In the earliest fetal ages examined (Figure 1), LRRN3 was expressed by scattered cells in the ventricular zone (1B) as well as migrating neurons in the subventricular (1A) and intermediate zones. Staining was particularly strong in the leading process of these cells. Staining was additionally seen in neuronal processes (likely primary apical dendrites) of upper cortical layers (1C) and in scattered larger neurons in the fetal subplate (1D). These findings were very consistent with a role for LRRN3 in neuritic process extension and guidance. However, what was truly shocking was the persistence of strong subplate expression of LRRN3 after at least 24 GW (Figure 2), when practically all other cortical staining became undetectable. Large, intensely staining, LRRN3-positive neurons were observed in the superficial cortical white matter in all regions examined from 24 GW through 66 years of age (Figures 2, 3B, 4B, 5B). The antibody labeled the neuronal body and principle neurites (Figure 3B). None were seen in either the deep white matter compartment, although a rare positive neuron was observed in the cortical plate proper (Figure 4A) and a few glial nuclei and processes also stained (not shown). Strongly staining, beaded-appearing, LRRN3-positive processes were observed to extend superiorly from the subplate into the cortex, many reaching Layer 2 (Figures 3A, 5A). Finally, scattered large LRRN3-positive neurons and processes were observed in the caudate and putamen (Figure 6). To date, no subplate-specific marker has ever been reported in humans. Also, given what is known about the function of the fetal subplate in human brain development, its potential connection to autism is particularly intriguing. Subplate neurons are some of the earliest generated neurons, appearing before establishment of the cortical plate, and are the first cortical neurons to mature functionally, differentiating into diverse subpopulations in terms of morphology, molecular markers and neurotransmitter identity. Throughout subsequent fetal development, the subplate serves as a transient target for numerous cortical afferents originating in the basal forebrain, thalamus, brainstem, as well as other cortical areas, both near and distant. Subplate neurons serve as transient synaptic partners for these afferents as well as guides for

their ingrowth by means of an extracellular matrix rich in axon guidance molecules. Incoming axons make temporary connections and subsequently wait to be routed to their final cortical destinations.

In perinatal to early postnatal life, the subplate undergoes significant reorganization characterized by a gradual dissolution and eventual disappearance of the subplate zone by apoptotic cell death. Neurons surviving the dissolution of the subplate are incorporated into the subcortical white matter and persist well into adulthood as so called "interstitial neurons" (reviewed in Suárez-Solá et al., 2009). This process begins earlier in primary motor and sensory cortices and later in association areas (e.g. prefrontal cortex and operculum) and appears to be associated with the development of gyral complexity. In primates, a huge, mixed population of interstitial neurons (INs) may be seen in the superficial subcortical white matter throughout the lifespan. They remain more numerous in frontal and prefrontal areas relative to more caudal regions, e.g. visual cortex. The postnatal persistence of the subplate in frontal association areas has been related to the ongoing growth of short-range cortico-cortical connections and it is hypothesized that abnormal axonal connectivity during fetal life may cause, or be reflected by, abnormalities in the numbers and/or distribution of interstitial neurons that persist into adulthood.

To date, multiple studies have found abnormalities in numbers and/or distribution of INs in a variety of conditions such as schizophrenia and ASD. A particularly common finding in neuropathologic and structural MRI studies of ASD is relatively poor differentiation of the gray-white junction associated with seemingly excess superficial white matter neurons (Cassanova et al., 2002; Bailey et al., 1998; Hutsler et al., 2007; Simms et al., 2009; Wegiel et al. 2010). This has been widely attributed to cortical migrational abnormalities despite very limited findings of laminar abnormalities within the cortex proper (Avino and Hutsler 2010) and so could also be explained by the presence of excess INs, caused by either abnormal proliferation or reduced developmental apoptosis (Chun and Shatz 1989; Avino and Hutsler 2010).

Because of this intriguing finding, we decided to examine the genetic evidence in more detail as outlined in the Conceptual Framework section of the Aim R1 Research Proposal. LRRN3 is a nested gene positioned within the third intron of the *IMMP2L* (inner mitochondrial membrane peptidase-like) gene located in 7q31.1. Linkage studies have consistently implicated this region as harboring a risk locus (IMGSAC 1998, 2001; Lamb et al. 2005; Barrett et al. 1999; Risch et al. 1999; Philippe et al. 1999; Badner et al. 2002; and Trikalinos et al. 2006). A recent study of association by Sousa et al. (2010), which investigated genetic variation in and around four genes encoding leucine-rich repeat proteins found significant evidence for association of variation around *LRRN3* and risk for ASD. Finally, a small set of studies implicate the region by CNV events (usually deletions) and with a range of phenotypes: dyslexia (PMID: 3213131); ADHD (Elia et al. 2010), autism (Maestrini et al. 2010; Pagnamenta et al. 2010), and Tourette syndrome, which shows some clinical overlap with ASD (Petek et al. 2001; Kroisel et al. 2001).

We then performed a more in-depth evaluation of results from the recent Autism Genome Project analysis, as outlined in the Conceptual Framework section of the Aim R1 Research Proposal, and

found an interesting concentration of association, albeit not of genomewide significance, in *IMMP2L*/ *LRNN3*, particularly regarding transmission from fathers (Figure 7). This association could be meaningful if the variation affects expression of *LRRN3*, and is consistent with other results suggesting that common genetic variants exert only small effects on risk for autism (e.g. Anney et al. 2012). It is intriguing that the strongest association emerges from paternal transmission of alleles, because the nesting of genes is a hallmark of genomic imprinting. It must be noted, however, there is no direct evidence at this time that *LRRN3* is imprinted.

In the course of manuscript preparation (ongoing) detailing this study of the developmental human expression of LRRN3, it was deemed necessary to better characterize the subtype identity and dynamics of LRRN3-positive subplate/interstitial neurons. Although not explicitly stated in the original research proposal for Study 1 (as we could not have foreseen such subtype-specific expression for any of our candidates), this more detailed characterization is considered basic to delineating overall temporospatial expression as is the stated goal for Study 1. To this end, we applied the methods for determining neuronal subtype in cultured neurons (outlined in Study 2) to tissue sections. Double immunofluorescence staining at different ages and in neurons differentiated from mouse neural stem cells (see Aim R1, Study 2 Revised, below) confirmed these to be glutamatergic projection neurons (Figure 8), which had been originally suspected based on their multipolar morphology. Double staining with MAP2, and detailed cell counting, further confirmed the LRRN3-positive subplate/interstitial neurons to comprise approximately 45% of all large, multipolar, white matter neurons in the pre-and perinatal periods, and approximately 30% of this subpopulation in postnatal periods.

In the earliest fetal ages examined (16 GW, Figure 9A, B, and C), LRRN1 was expressed in a vaguely similar pattern as LRRN3 in the same age group, i.e. cytoplasmic positivity in cells of the ventricular zone, subventricular, and intermediate zones (not shown). Relatively strong staining was evident in neuronal processes of upper cortical layers (9A) in a more granular (synaptic appearing) pattern than that seen for LRRN3 (9B). Staining in the striatum was also marked (9C). By 23 GW, superficial cortical staining had declined but scattered, large, LRRN1-positive pyramidal neurons were seen in layers 3 and 5 (9D and E). LRRN1-positive neurons in the fetal subplate were polymorphous (both small and large, multipolar and bipolar) and were more numerous than LRRN3-positive subplate neurons (9F). By 2 months of age, superficial cortical staining had disappeared, but selective cortical pyramidal, striatal, and subplate staining remained, and persisted into adulthood (not shown). LRRN1 is located at 3p26.2, a candidate region for non-syndromic intellectual disability (ID) and mild ID with epilepsy. While common alleles of LRRN1 not associated with increased risk for ASD, functional LRRN1 mutations have been found in one pedigree with syndromic autism.

LINGO1 appeared to be expressed in the cytoplasm and processes of most cortical neurons in the fetus (Figure 10A, B, and C). The highest expression appeared to be myelinating or myelinated fibers (10D) and, thus, varied in an age-dependent manner. Antibody expression was only barely discernible in white matter past the age of 2 months (not shown). In P6 mouse pup brains, Liingo-1 expression was also visualized in neuritic processes of most neuronal populations of the cortex (10E), hippocampus, and thalamus. There was also particularly strong staining of large

white matter tracts such as the corpus callosum (10F) and internal capsule (not shown). Cytoplasmic staining of LRRTM3 was visualized in scattered pyramidal neurons of cortical layers 2, 3, and 5 by 23 GW (Figure 11A), a pattern which persisted into adulthood (11B) and was essentially replicated in P6 mouse pup cortex (11C).

Conclusions/Discussion:
Furthermore, LRRN3 shows unusual expression in fetal subplate, a structure increasingly implicated in the pathogenesis of cortical wiring abnormalities in ASD reflected by increased numbers of postnatal interstitial neurons. What role these neurons may play in postnatal brain function is unknown, although it is hypothesized that abnormal axonal connectivity during fetal life may cause, or be reflected by, abnormalities in the numbers and/or distribution of interstitial neurons that persist into adulthood. The presence of excess interstitial neurons in ASD could potentially be explained by either abnormal proliferation early in embryonic life or reduced developmental apoptosis in the later fetal/perinatal period. These alternatives will be fruitful to explore.

Aim R1 – Study 1 Enhancement:

Introduction:
At the request of an early reviewer of our Research Proposal, this proposal was revised to include examination of our candidate LRRs in a limited sample of tissue derived from autistic individuals (thereafter designated Study 1 Enhancement). We therefore examined LRR expression in postmortem brain tissue from the orbitofrontal and dorsolateral prefrontal cortices of 3 ASD subjects (5–29 years of age) and 5 age-matched controls (2 –32 years of age) obtained from the ATP Brain Atlas Project.

Methods:
Immunohistochemical methods are essentially identical to those outlined in Aim R1 – Study 1 Revised, except for longer incubation times required for adequate staining in this thicker tissue.

Results:
Only one candidate, LRRN3, yielded discernibly different (albeit very intriguing) results in ASD versus control tissue (Figure 12). Noticeably more numerous LRRN3-positive INs were observed (Figure 12A) as well as scattered, large tangles of LRRN3-positive processes (12B).

Conclusions/Discussion:
We consider this to be suggestive but (very) preliminary evidence of the potential involvement by LRRN3 in the etiology of ASD. These results will only be verified with an increased sample size.

Aim R1 - Study 2. In Vitro Confirmation of a Functional Role in Axonal Growth/Guidance

Aim R1 Study 2 Timeline

Period	Milestones	Research Activities
6/1/09-6/30/09		
7/1/09-6/30/10		
7/1/10-6/30/11	• Aim R1, Study 2 initiated	• Mouse cortical cell cultures established • Immunohistochemical studies on cell cultures initiated
7/1/11-6/30/12	• Aim R1, Study 2	• Immunohistochemical studies on cell cultures • Optimization of siRNA constructs
7/1/12-5/31/13	• Revised Aim R1, Study 2 initiated and completed	• Mouse neural stem cell cultures established • Immunohistochemical studies on stem cell cultures initiated and completed • siRNA studies on neural stem cell cultures initiated and completed • Data collection of immunohistochemistry and knock-down experiments completed

Introduction:

Aim R1-Study 1 and revised Study 1 analyses above were designed to determine which of the LRR candidate genes have anatomic and developmental temporal patterns of expression that are consistent with a role in modulating axonal targeting and connectivity in development windows important in autism. They do not directly demonstrate a mechanistic role. Therefore, *in vitro* expression studies and standard neurite outgrowth assays were performed in mouse neural cell cultures for each of the LRR gene products considered good candidates, i.e. expressed in neuronal processes *in vivo* (Revised Aim R1-Study 1), for further study. These were determined to be AMIGO1, LINGO1, LRRN1, LRRN3, and LRRTM3. We first stained cultured cells with antibodies to these LRRs of interest to determine if cells *in vitro* expressed them. Second, we co-labeled with certain specific neuronal markers (see below) to analyze protein localization. Based on the results of these experiments, we refined our candidate list for further knock-down studies to those observed to be expressed in cultured neuronal processes (LRRN1, LINGO1, LRRN3, LRRTM3).

Methods:

Immunohistochemistry: Cortical tissue was harvested from E10.5 mouse embryos and dissociated mechanically. Cells were plated without a glial base on poly-d-lysine and laminin coated coverslips in 24-well plates. Cells were collected at 1 Day In Vitro (DIV), stage 1), 2 DIV (stage 2,3), 4 DIV (stage 4), 8 DIV (intermediate stage to assess developmental maturity), 14 DIV (stage 5 early), 18 DIV (stage 5 late) and 20 DIV, and fixed with 4% paraformaldehyde. At each time point, 12 coverslips were fixed and processed for fluorescence immunohistochemical staining (methods outlined in report for revised Study 1). We stained for AMIGO1, LINGO1, LRRN1, LRRN3, and LRRTM3, as well as co-labeled for Tuj1 to identify primary neurites and Map2 to identify dendrites. Glut1, Glut2, GABA, synaptophysin were used to identify projection neurons, interneurons, and synapse formation, respectively. Fixed and stained cell preparations were visualized on a Nikon Eclipse E400 fluorescent microscope and photomicrographs captured with a Photometrics Cool Snap E52 camera and NIS Elements 4.1 software.

Knock-down experiments: Cortical tissue was harvested from E10.5 mouse embryos and dissociated mechanically. Cells were plated without a glial base on poly-d-lysine and laminin and transfected with the siRNA (Origene) for LRRN1, LINGO1, LRRN3, and LRRTM3 using Lipofectamine PLUS reagent (Life Technologies) 3 hours after plating. Cells were then collected at the following:

Stage 1-2: Neurons extend lamellipodia and immature neurites develop (5-hours to 1 DIV).
Stage 3: Neuron polarity is established, axon elongates (1-2 DIV)
Stage 4: The axon elongates further, dendrites elongate and branch (2-4 DIV)
Stage 5: Dendritic spines form. (14-18 DIV)

Cells were fixed with 4% paraformaldehyde, washed three with PBS, and visualized and photographed as above. We examined and analysed branching of neuronal processes, dendritic length, as well as primary, secondary and tertiary dendritic branch lengths at 4,8 and 20 DIV using the concentric multiple level sphere intersections method (Uylings and van Pelt, 2002).

Results:
As the results for Aim R1-Study 1 are identical to and greatly expanded on in Revised Aim R1-Study 1, below, the results are presented in that section. Quantitative analyses of the mechanistic studies for both are in progress.

Revised Aim R1- Study 2: In Vitro Confirmation of a Functional Role in Axonal Growth/ Guidance

Introduction:
While we originally performed these experiments in primary cortical neurons cultured from mouse embryos, the observed *in vivo* (Revised Aim R1-Study 1) expression of various LRRs outside of the fetal cortical plate proper (i.e. ventricular zone cells, migrating neuroblasts, and/or glia) prompted us to employ primary neural stem cell cultures isolated from the cerebral cortex of E10.5 mouse embryos in the final year of the study. While somewhat time consuming to establish, this experimental model has allowed us to better examine the effects of our LRRs on neural specification as well as earlier neurite out-growth, neuronal maturation and synapse formation, as neural stem cells grown as spheres in culture (neurospheres) can be induced to differentiate into radial glia, neurons, glia and oligodendrocytes in culture.

Methods:
Cortical tissue was harvested from E10.5 mouse embryos and dissociated mechanically (Figure 13). Dissociated cells were cultured in the presence of growth factors to select for neural stem cells which proliferated as neurospheres. Neurospheres were re-dissociated and re-cultured, i.e., passaged once (P1). Neural cell differentiation was then induced by growth factor withdrawal for various time periods, i.e., days of division (DD). Markers of neuronal differentiation were usually observable within 5 hours of growth factor withdrawal. We examined the cell-type and localization expression patterns of our candidate LRRs in the context of the stages of neuronal

maturation outlined in the methods for Aim R1-study 2, above; Stage 1-2, Stage 3, Stage 4, and Stage 5. Cells were fixed and stained with Nestin and Sox2 to identify progenitor cells, RC2 and Glutamine synthetase to identify radial glial cells, Tuj1 to identify primary neurites, Map2 to identify dendrites, S100 to identify glia, and Olig2 and MBP to identify oligodendrocyte progenitors or mature oligodendrocytes, respectively. Glut1, Glut2, GABA, synaptophysin were also used to identify projection-type neurons, interneurons, and synapse formation, respectively. Fixed and stained cell preparations were visualized on a Nikon Eclipse E400 fluorescent microscope and photomicrographs captured with a Photometrics Cool Snap E52 camera and NIS Elements 4.1 software.

For initial inhibition studies, P1 neurospheres were differentiated in the presence of an anti-LRR antibody or in the presence of IgG or heat inactivated LRR antibody as controls. Cultures were grown for 1, 2, 3, 5, 12 days. Cells were fixed and, as above, were stained with cell-specific antibodies to determine if inhibition of each LRR influenced cell type specification or synapse formation. We also examined branching of neuronal processes, dendritic length, as well as primary, secondary and tertiary dendritic branch lengths at 4, 8 and 20 days in vitro using the method of concentric multiple level sphere intersections to quantify dendritic properties (Uylings and van Pelt, 2002). These experiments were then repeated using transfected LRR siRNA (Origene) and analyzed in the same manner. While we are still in the process of analyzing these results, preliminary data are shown below.

Results:

LRRN3 expression was seen in radial glia differentiated early from mouse neural stem cells in a pattern remarkably consistent with ventricular zone staining in the 16 GW fetus. Figure 14A shows characteristically elongated cells showing co-expression of LRRN3 with RC2 (a specific radial glial marker) and glutamine synthetase (14B, a more general glial marker). Much of this expression is in a perinuclear, cytoplasmic distribution (single arrows). Interestingly, there is a strong focus of LRRN3 positivity (arrow) at the terminal end of the growing radial glial process (double arrow). LRRN3 was also observed to be expressed in later developing glial cells (Figure 15), again in a perinuclear (and possibly nuclear) distribution. LRRN3 was also present throughout the neuron at the earliest stages of neuronal differentiation (Figures 15 and 16). Expression was particularly marked in the principle dendrite (Figure 16, double arrow), in a manner consistent with cortical staining in early midgestation, and, even more gratifyingly, the axonal growth cone (single arrow). In mature neurons (16C), expression became restricted to distinct puncta in the cytoplasm of growing neurites. This staining pattern is reminiscent of microtubule staining leading to the tantalizing speculation that LRRN3 may be associated or trafficked with microtubules during neurite outgrowth. SiRNA knock down of LRRN3 produced a striking (almost 70%) reduction in neurite extension in early developing neurons (Figure 17) with practically no long axonal processes in later stages (not shown).

LRRN1 was similarly expressed in early developing neurons and glia, but quickly acquired a finely granular, synaptic pattern of staining in mature neurons similar to that seen in tissue staining of the fetal brain. Synaptic co-localization was confirmed by double staining for synaptogranin (Figure 18). LRRTM3 was also expressed in both early developing neurons and

glia (Figure 19) and, finally, LINGO1 was expressed only in growing axons of glutamatergic neurons (Figure 20) and early oligodendroglia (not shown). Again, this pattern was consistent with that seen in tissue sections. Early knockdown experiments conducted on these candidates indicate both LRRN1 and LRRTM3 are required for normal neuritic extension (and branching), and that LINGO1 is particularly required for axonal elongation (not shown). These data are still being tabulated.

Conclusions/Discussion:
The overall goal of Aim R1-Study 2 and Revised Aim R1-Study 2, as stated in the Research Proposal, was to assess the mechanistic plausibility of the hypothesis that alterations in expression of certain LRR proteins, implicated by genetic data, confer risk for ASD by dysregulating normal axonal outgrowth and/or guidance. We have provided initial evidence that at least four (LINGO1, LRRN1, LRRN3, and LRRTM3) participate in this process, at least *in vitro*. One candidate in particular, LRRN3, is expressed differentially in extending dendrites and the axonal growth cone.

Published Abstract:
McFadden, K., & Monaghan-Nichols, A.P. (2013). "Novel subplate expression of LRRN3, An autism spectrum disorder candidate." *Journal of Neurology and Experimental Neuropathology*, 72(6):570. Abstract presented at the 89th annual meeting of the American Association of Neuropathologists, Charleston, SC, June 2013.

Grant Proposal Under Review:
R21 *"LRRN3, an ASD Candidate, is Involved in Subplate Development"*. Under review.

Plans for Publishing Results:
"Developmental Brain Expression of LRRN3, an Autism Spectrum Disorder Candidate". Expression studies of ASD candidate LRRN3, in pre- and postnatal human brain (funded in whole by the Pennsylvania Department of Health, acknowledged in manuscript). Unprecedented specific cortical subplate/interstitial (white matter) neuron expression. Only known specific subplate marker in human brain. Additional expression in migrating neuroblasts and early cortical neuritic processes. Findings to be re-submitted after additional experiments to Cerebral Cortex 2/14.

"LRRN3 is Necessary for Neuritic Outgrowth in Developing Cortical Neurons". Expression and functional studies of LRRN3 in differentiated mouse neural stem cells. LRRN3 expressed in leading process of radial glia and growth cone of early developing neurons. Marked reduction in neuritic outgrowth in developing neurons transfected with LRRN3 siRNA. Findings to be submitted to Developmental Neuroscience 04/14. (funded in whole by the Pennsylvania Department of Health, which will be acknowledged in manuscript).

"*LRRN3 and the Subplate in ASD*". Preliminary evidence of excess numbers and altered neuritic process morphology in LRRN3-positive interstitial neurons in ASD post-mortem cortex versus age and site matched controls. Currently seeking to expand sample size of ASD and control post-mortem brain tissue (funded in part by the Pennsylvania Department of Health, which will be acknowledged in manuscript)

"*LRRN3 and the Subplate in Periventricular Leukomalacia*" Evidence for significant loss of large, projection neurons from the fetal subplate in mild cases of PVL. Findings to be submitted to Pediatric and Developmental Pathology 3/14. Expression and functional studies of ASD candidate LRRN1, in pre- and postnatal human brain and differentiated mouse neural stem cells. Explorations of possible interaction with LRRN3 (funded in part by the Pennsylvania Department of Health, which will be acknowledged in manuscript).

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Table 1: Tissue Samples Examined in Aim R1 Study1 and Revised Aim R1 Study1

Group	*Age Range*	*# Formalin-Fixed*	*# Frozen*
Midgestation	18-25 gestational weeks	14	28
Perinatal	26 – 40+ gestational weeks	13	0
Infant	Birth – 2 years	11	0
Childhood	2-7 years	7	0
Late childhood/ adolescence	7-18 years	0	0
Adult	18-66 years	11	8

Note: In total, brain tissue from 74 individuals was obtained for Study 1 analyses. Perinatal brain tissue was derived from the University of Pittsburgh Health Sciences Tissue Bank and pediatric (40GW to 6 yrs) and adult (20 yrs to 66 yrs) tissue from the neuropathology autopsy service.

Table 2: Summary of Immunohistochemistry Results of Initial Candidate Leucine Rich Repeat (LLR) Proteins (Revised Aim1 Study 1) in Human Tissue Samples

LRR Antibody	*Species*	*Manufacturer*	*Results*
LINGO1	Goat polyclonal	R&D Systems	Cytoplasmic neuronal and process staining in frontal cortex. In fetus, highest expression in myelinating long tract fibers Low level, white matter expression in adult tissue.
LINGO2	Goat polyclonal	R&D Systems	Ubiquitous/non-specific
LRRN1	Rabbit polyclonal	Sigma	Cytoplasmic neuronal and axonal staining in frontal cortex in processes of superficial cortex and later in scattered neurons of layers 3 and 5. Additionally in hippocampus and germinal matrix of fetus.
LRRN3	Rabbit polyclonal	Sigma	Early ventricular zone staining and in migrating neuroblasts. Cytoplasmic neuronal and axonal staining in early frontal cortex later confined to white matter neurons. Also striatum.
LRRTM3	Rabbit polyclonal	Sigma	Most cortical layers in fetal to adult frontal cortex –highest in layer 2.
AMIGO1	Rabbit polyclonal	Abcam	Cytoplasmic neuronal and axonal staining in frontal cortex. In fetus, highest expression in myelinating long tract fibers. Low level, white matter expression in adult tissue.
AMIGO 2	Rabbit polyclonal	Abcam	Ubiquitous/non-specific.
LRIG1	Goat polyclonal	R&D Systems	Cytoplasmic neuronal and axonal staining

			in frontal cortex. Similar to LINGO1.
SLIT1	Rabbit polyclonal	Abcam	Cell bodies of mid to lower (layers IV, V and VI) cortical projection neurons in the adult cortex. Frontal > occipital.

Note: Summarized results of immunohistochemistry results of Revised Aim1 Study 1 on tissue samples summarized in Table 1.

Figure 1: LRRN3 Expression 16 Gestational Weeks

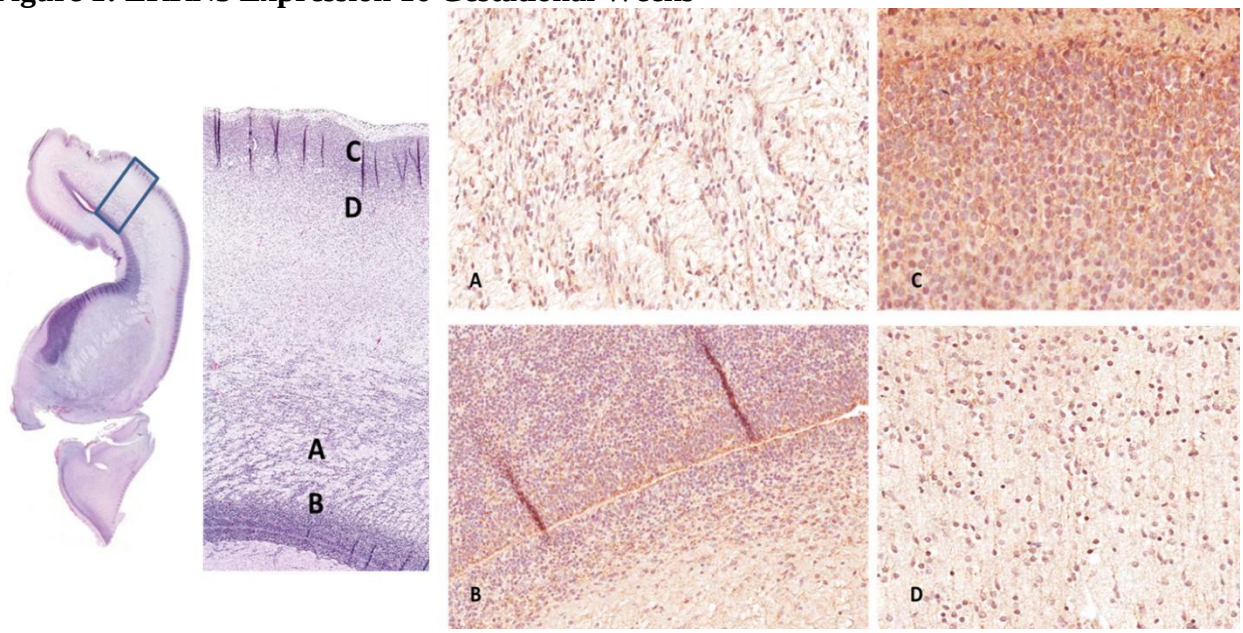


Figure 2: LRRN3 Expression 24-28 Gestational Weeks

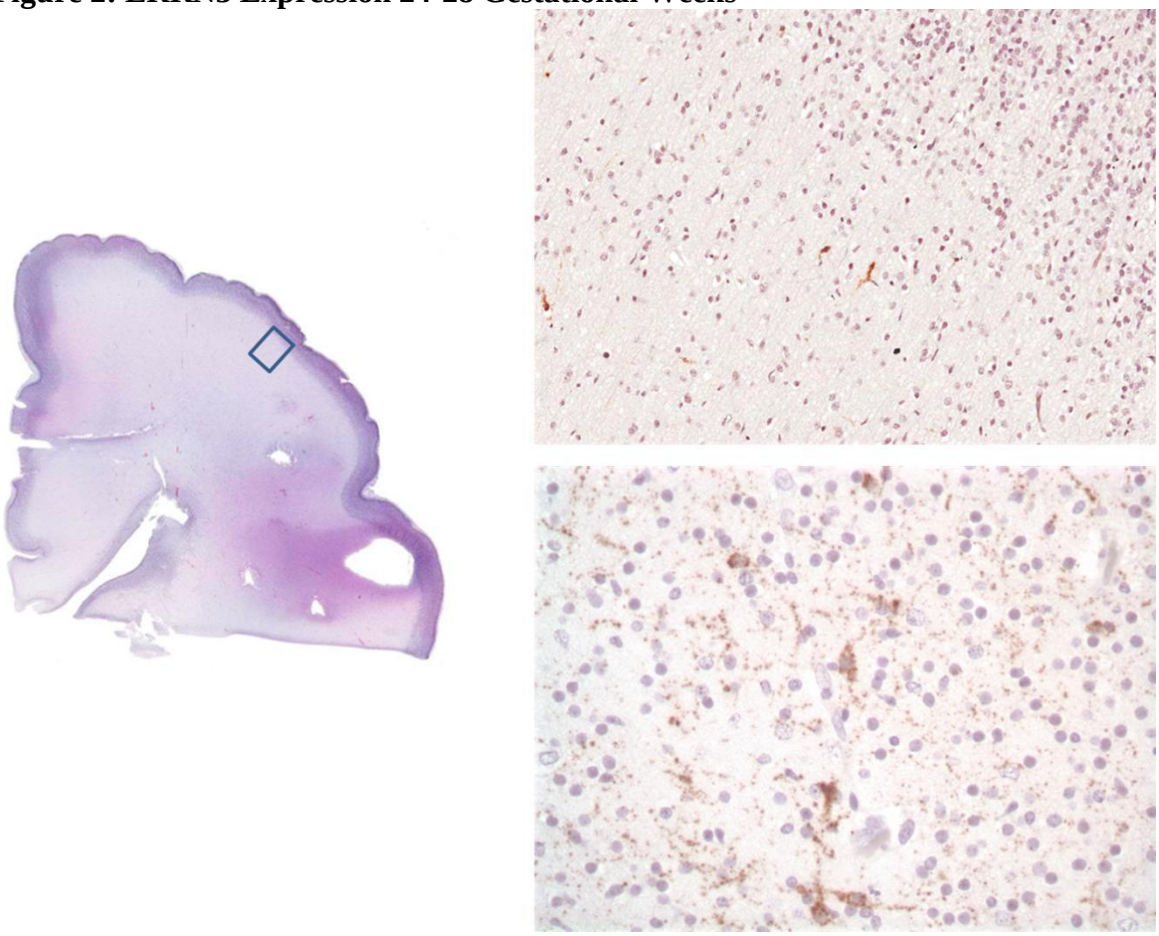


Figure 3: LRRN3 Expression 32-40 Gestational Weeks

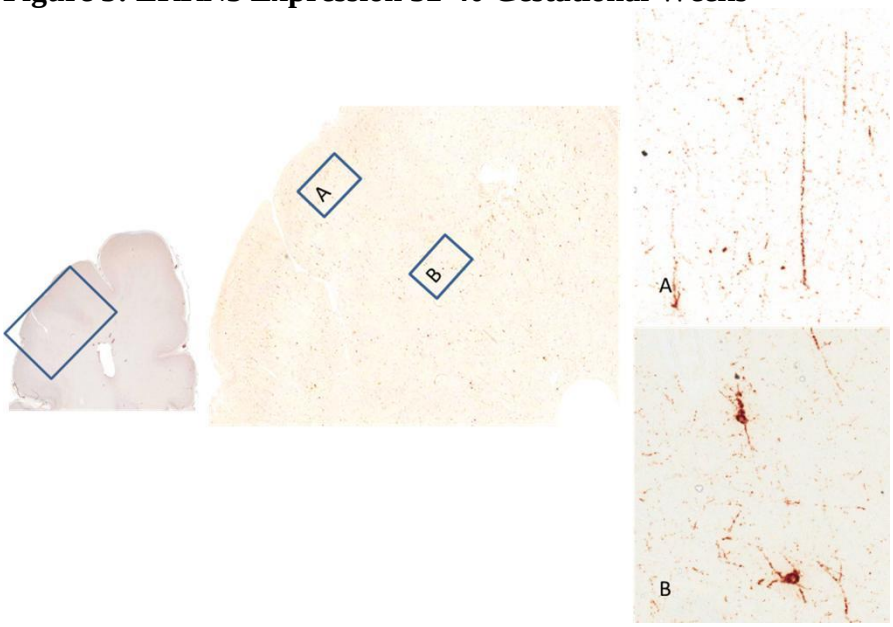


Figure 4: LRRN3 Expression 9 Weeks

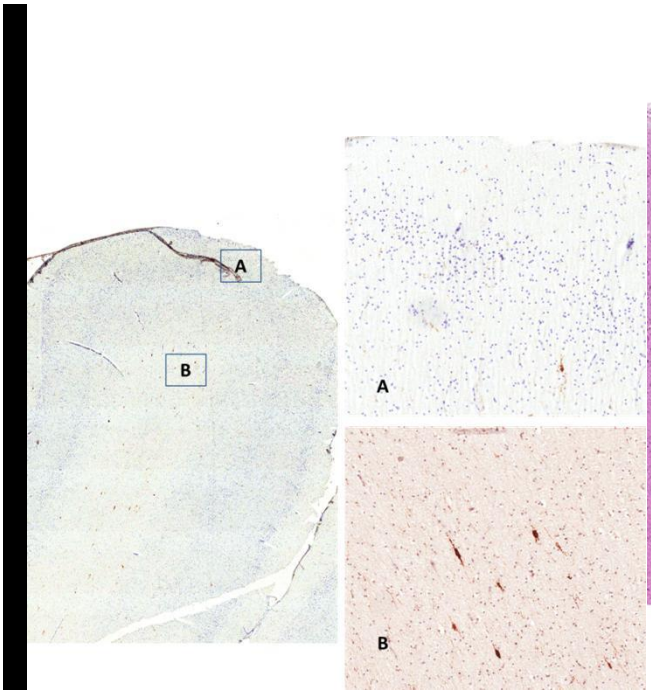


Figure 5: LRRN3 Expression Adult

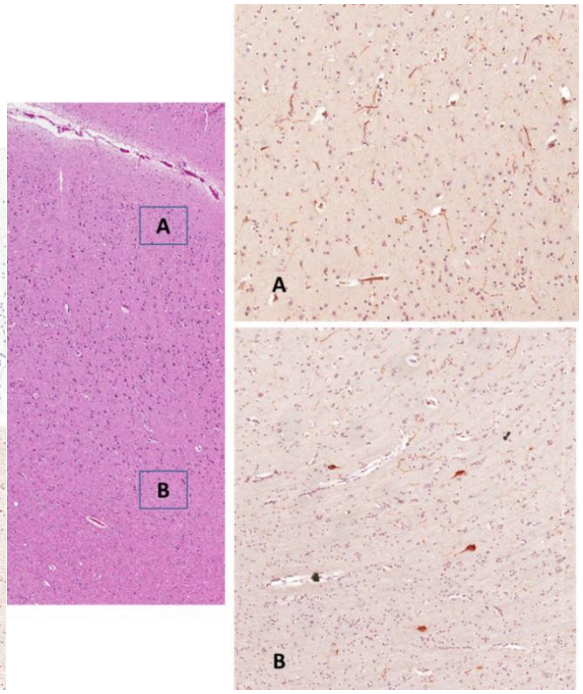


Figure 6: LRRN3 Expression Caudate 32-40 Gestational Weeks

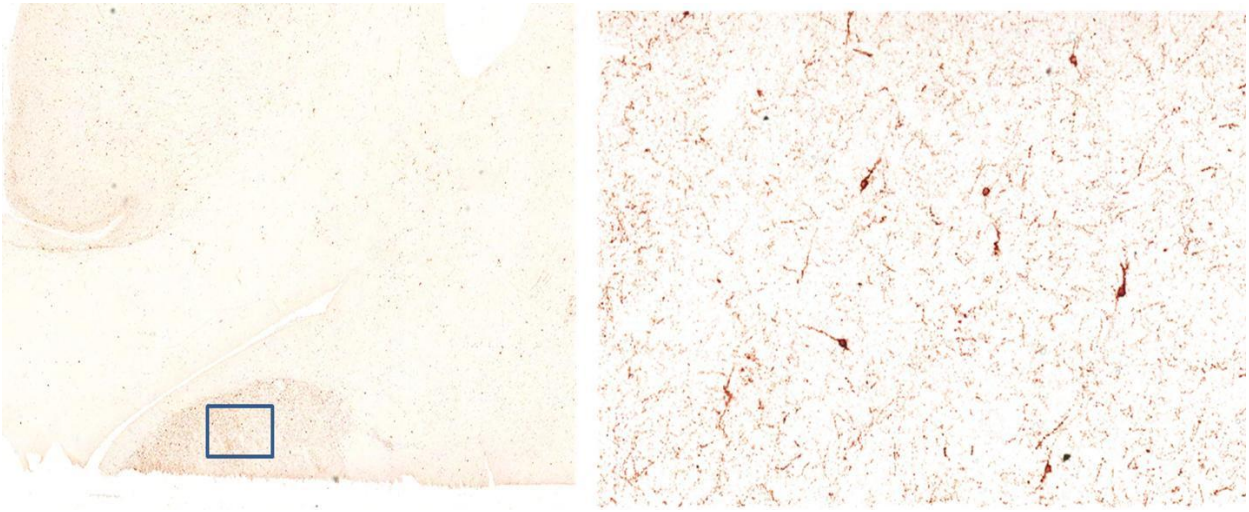
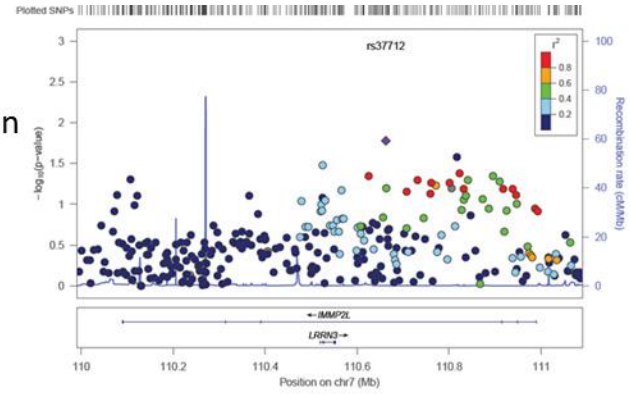
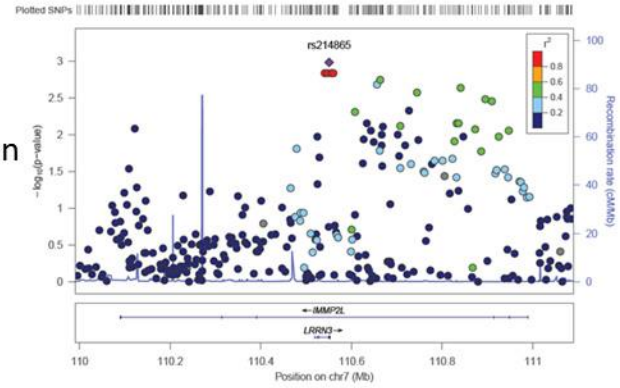


Figure 7: SNP Analysis (LRRN3 Region) Autism Genome Project Genome-Wide Association Scan

Autism, European (All)



Autism, European (Fathers)



Autism, European (Mothers)

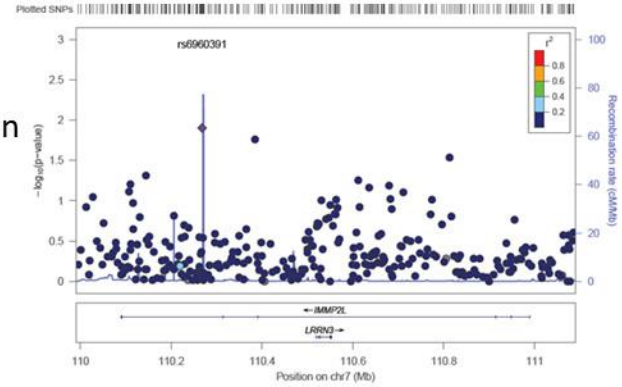


Figure 8: Confirmation of Glutamatergic Subtype of LRRN-3-Positive Subplate/Interstitial Neurons in 28 GW Human Prefrontal Cortex and Mouse Neural Cultures P1 D3

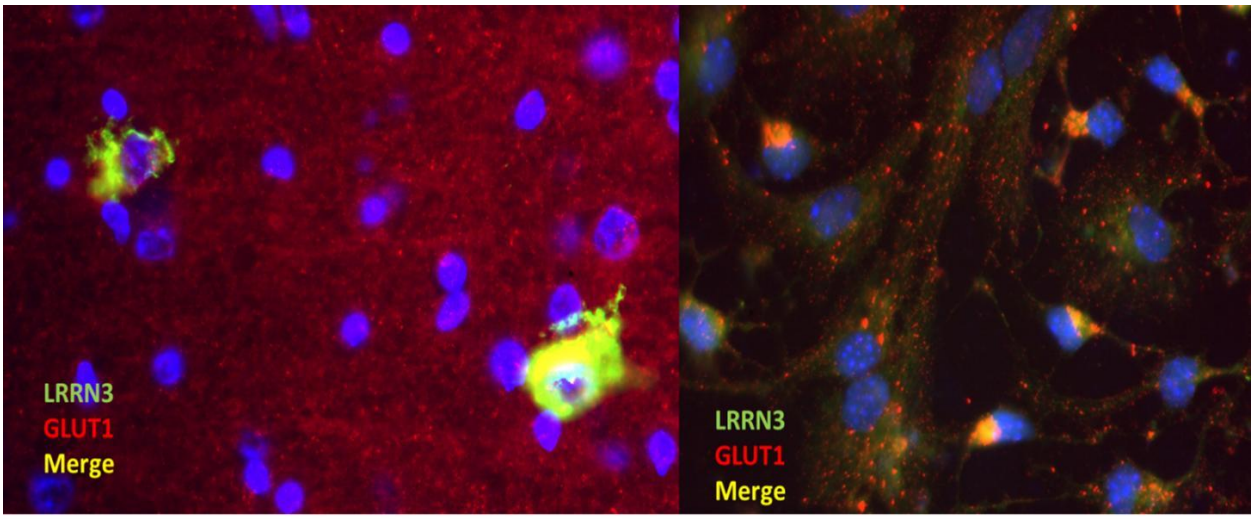


Figure 9: LRRN1 Expression, Human 16 and 28 GW (without counterstain)

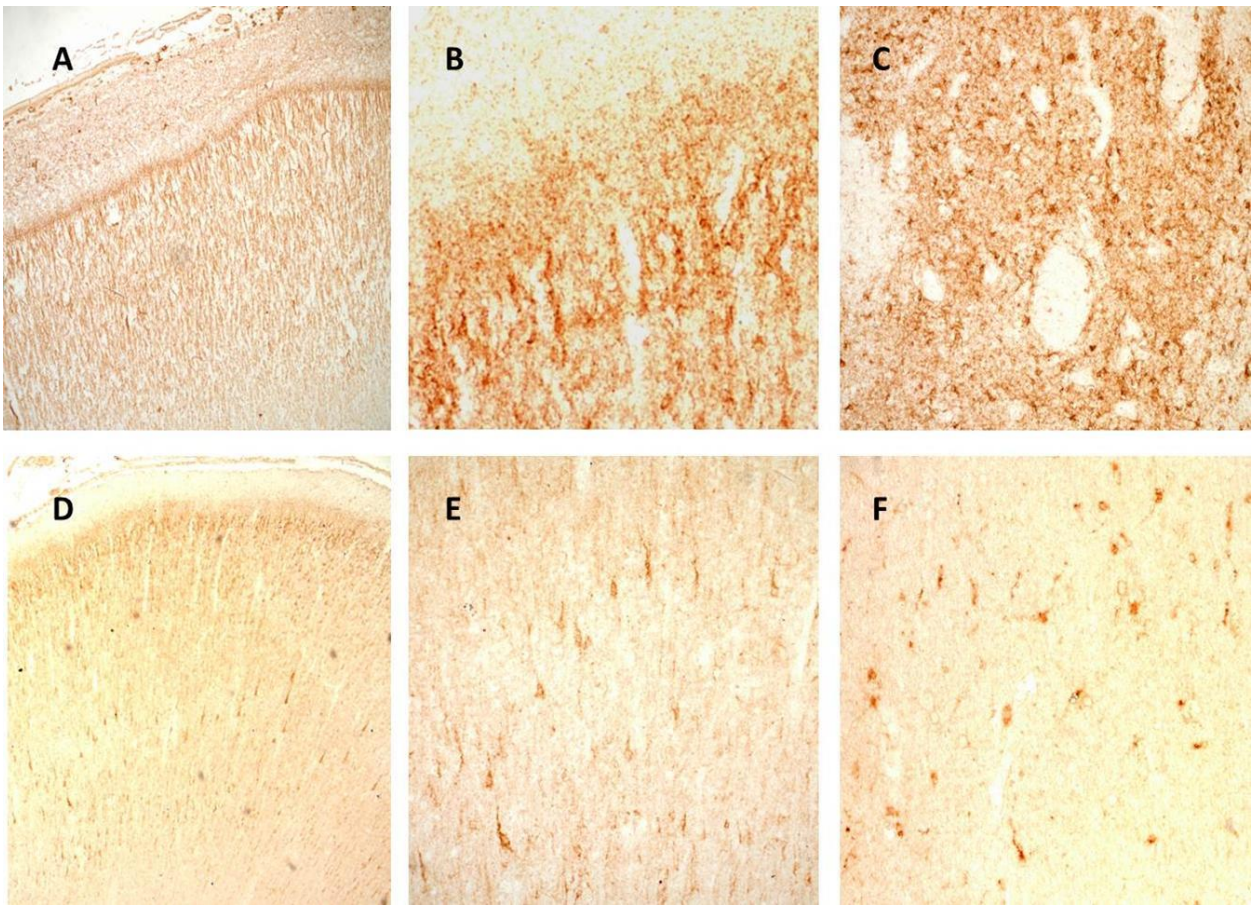


Figure 10: LINGO1 Expression, Human 28 GW and Mouse E16 and P6

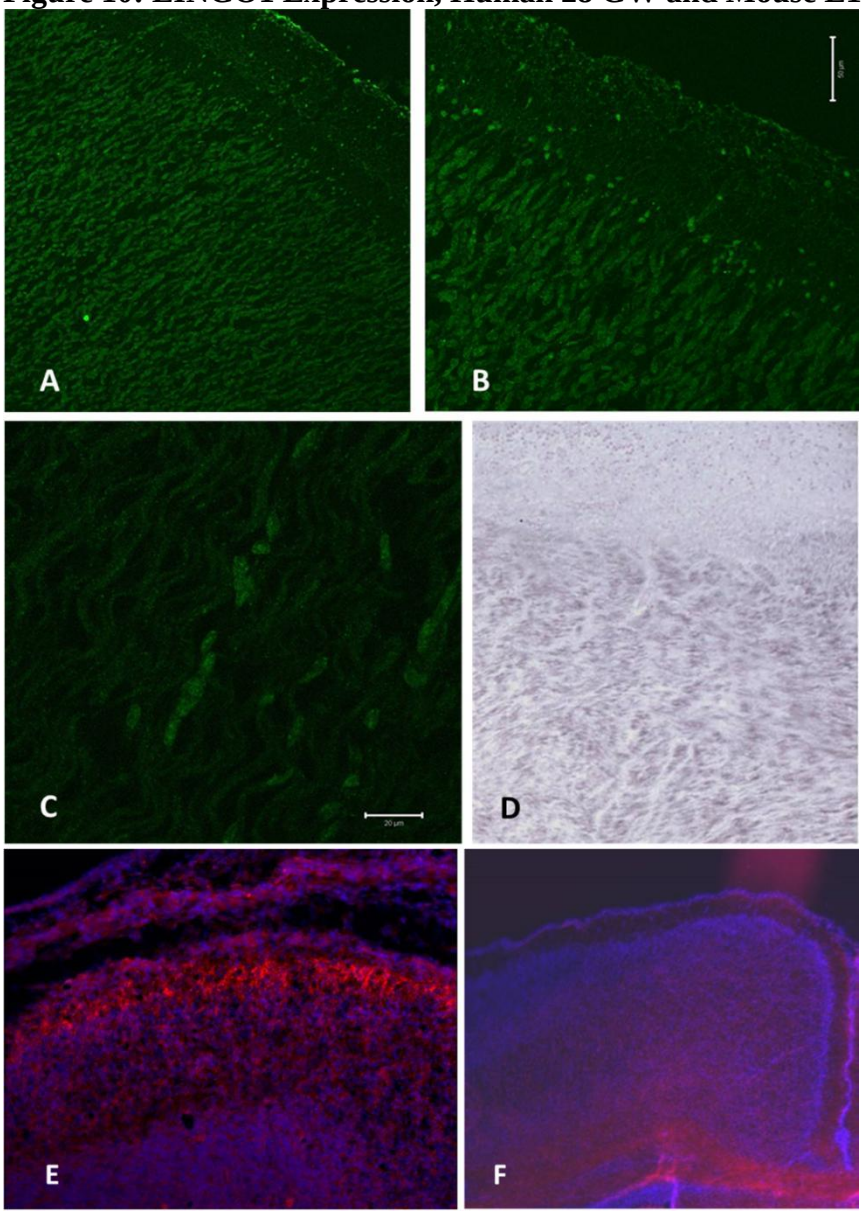


Figure 11: LRRTM3 Expression 40GW, Adult, P6 Mouse

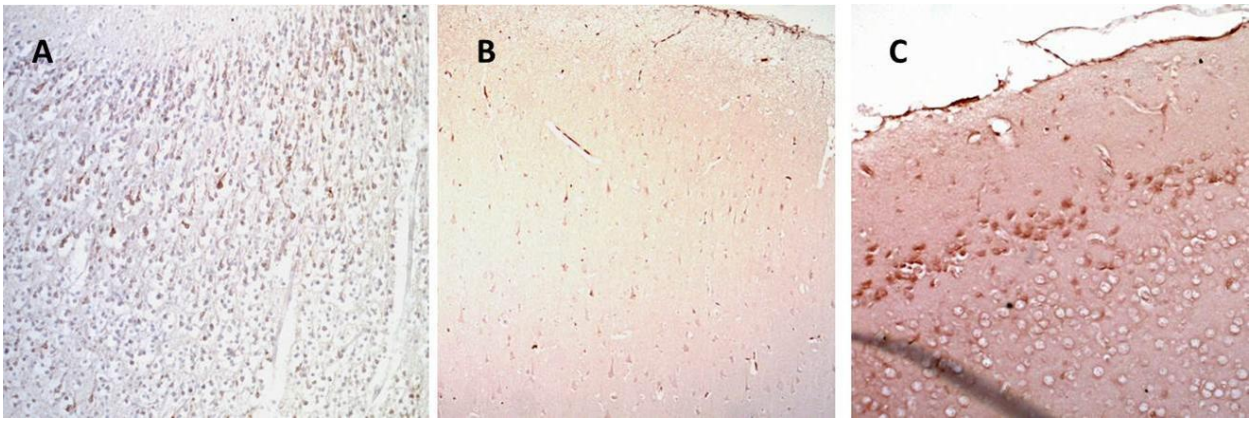


Figure 12: LRRN3 Expression ASD Cortex

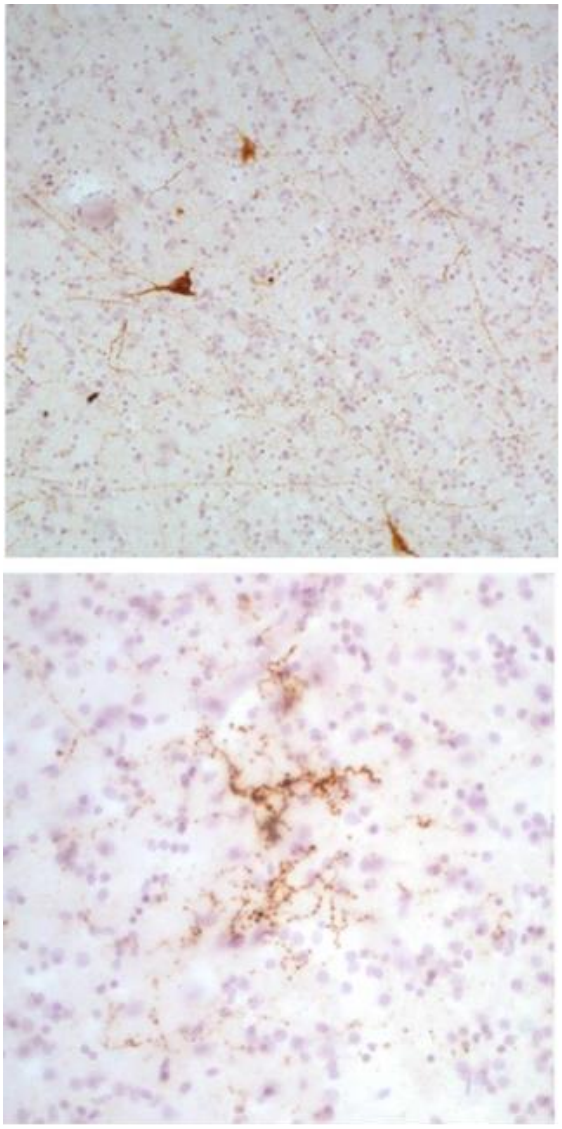


Figure 13: Mouse Neural Stem Cell Experimental Model

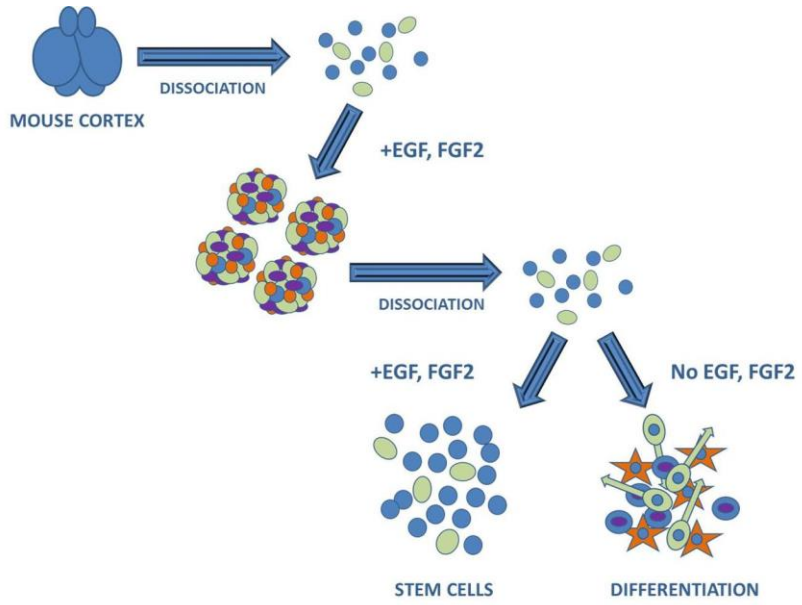


Figure 14: LRRN3 is Expressed by Developing Radial Glia

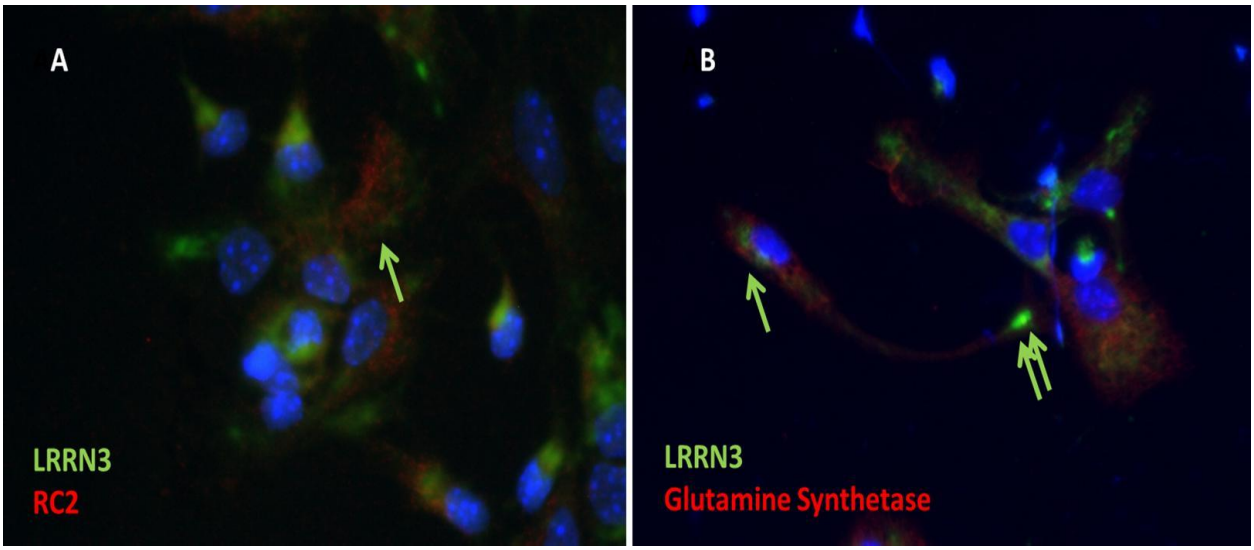


Figure 15: LRRN3 is Expressed by Developing Neurons and Glia

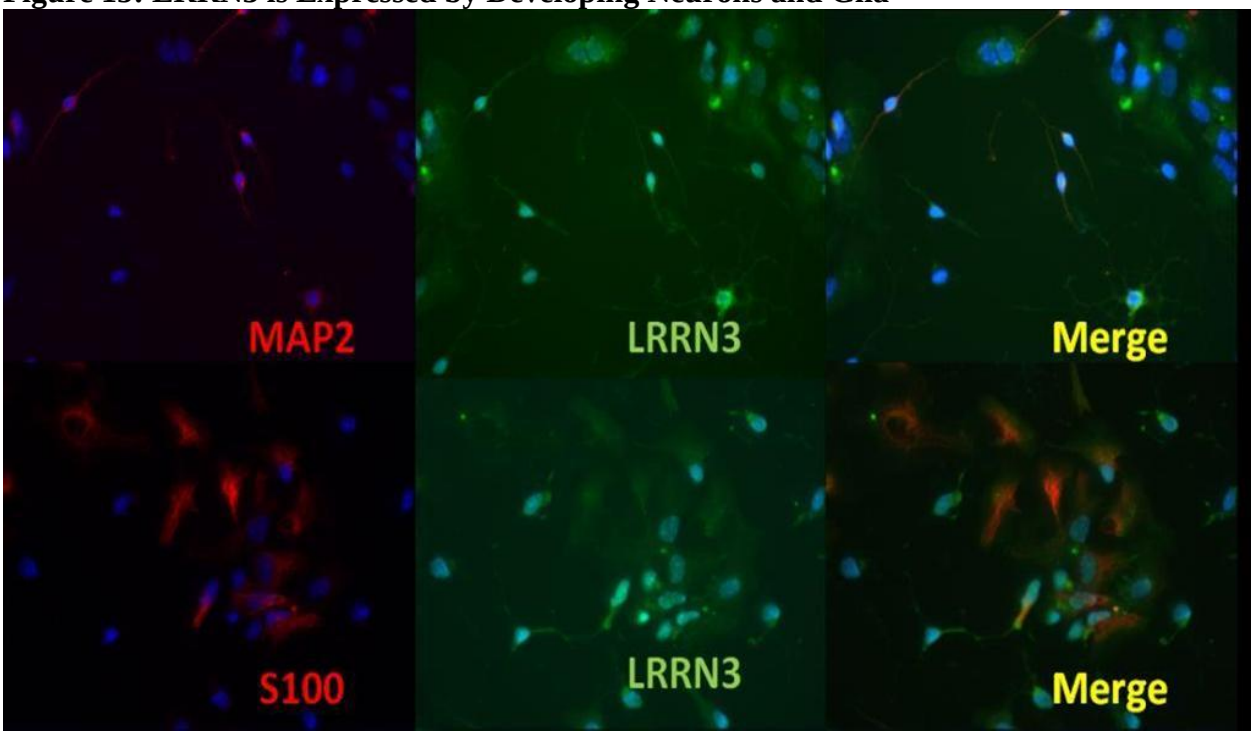
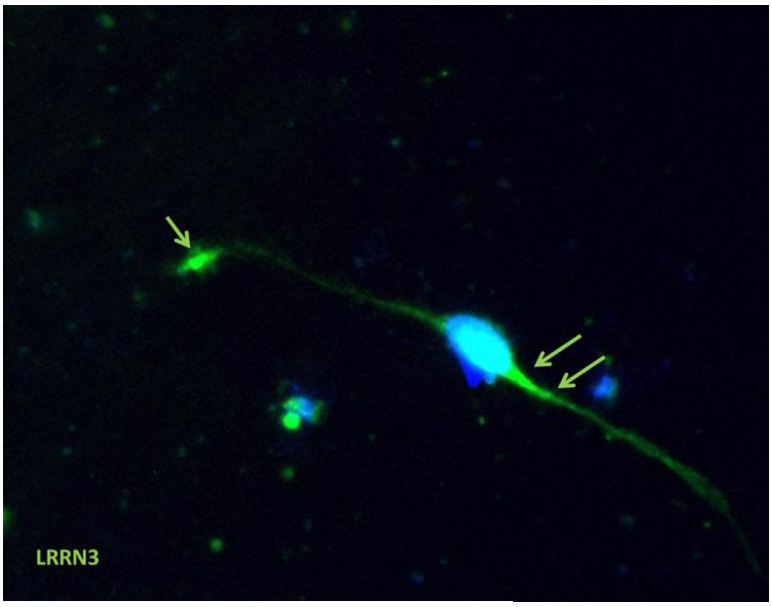


Figure 16: LRRN3 is Expressed by the Growth Cone and Principle Dendrite of Early Developing Neurons and in a Punctate Pattern in Later Developing Neurons



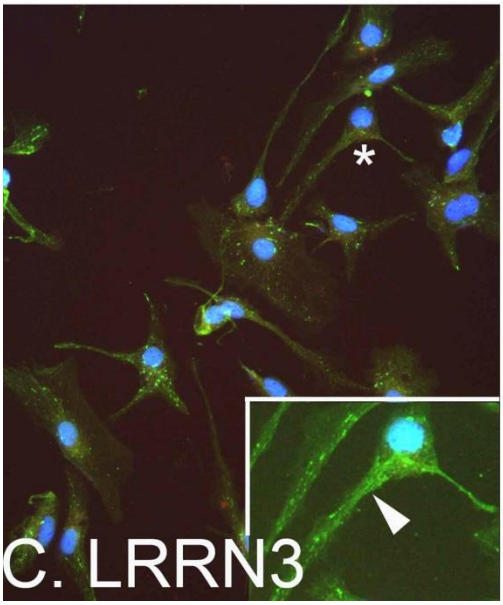


Figure 17: LRRN3 Inhibition Reduces Neuritic Outgrowth In Vitro

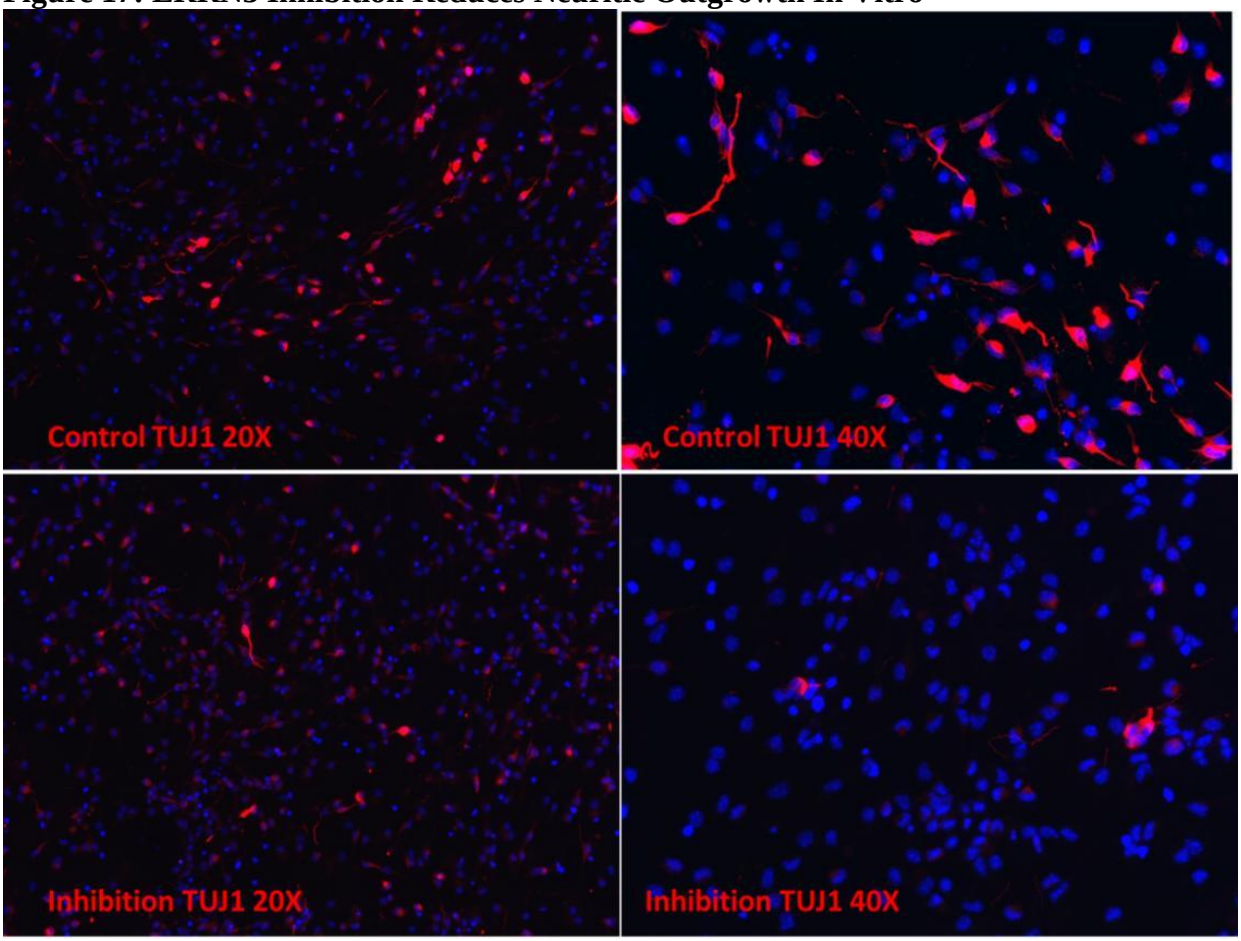


Figure 18: LRRN1 Acquires a Synaptic Localization In Vitro

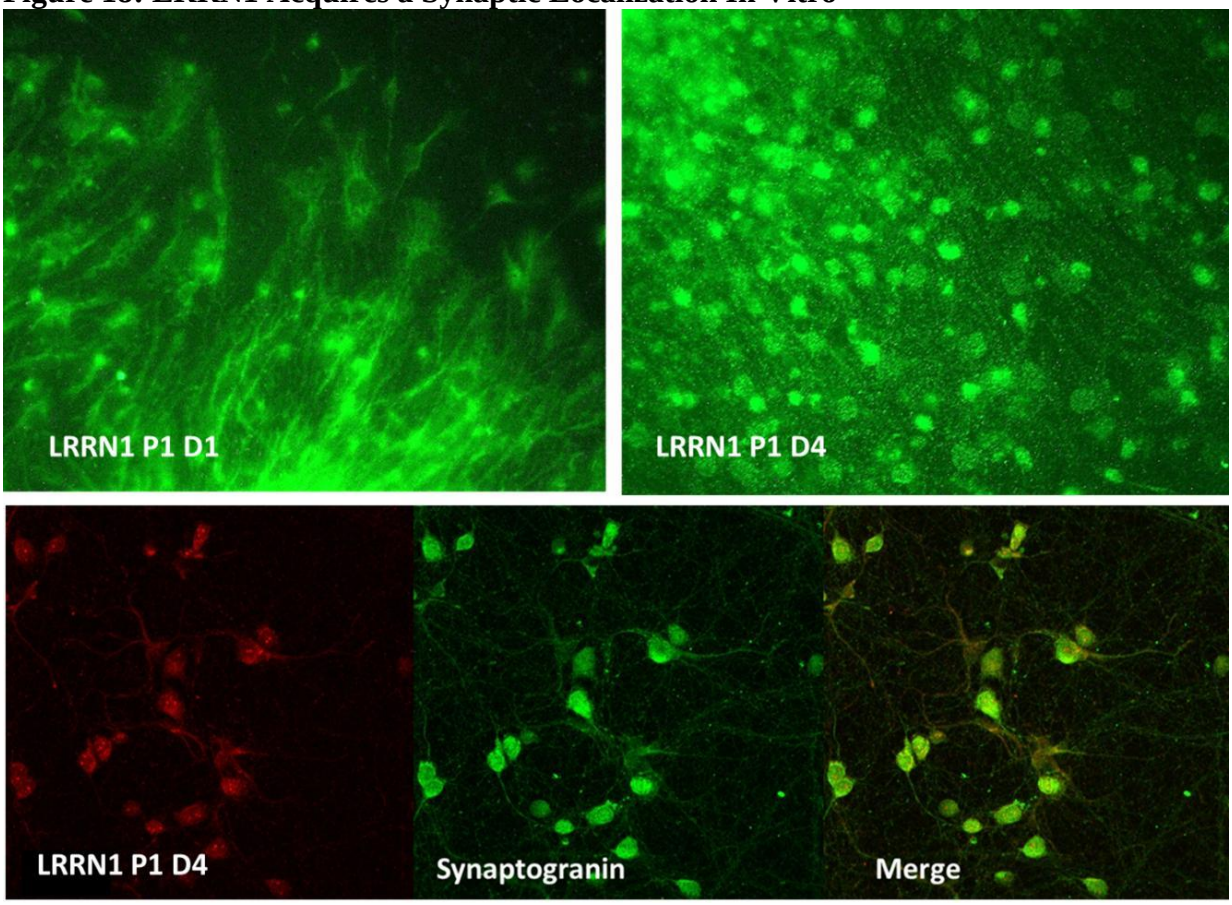


Figure 19: LRRTRM3 is Expressed by Developing Neurons and Glia

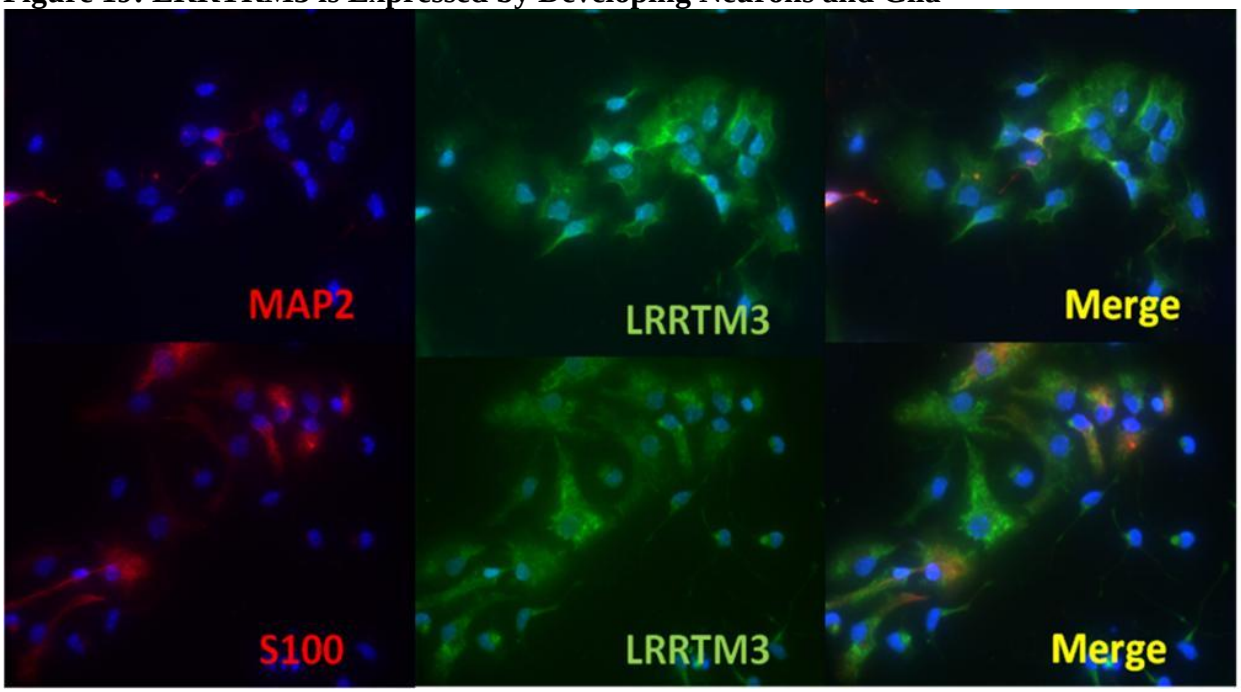
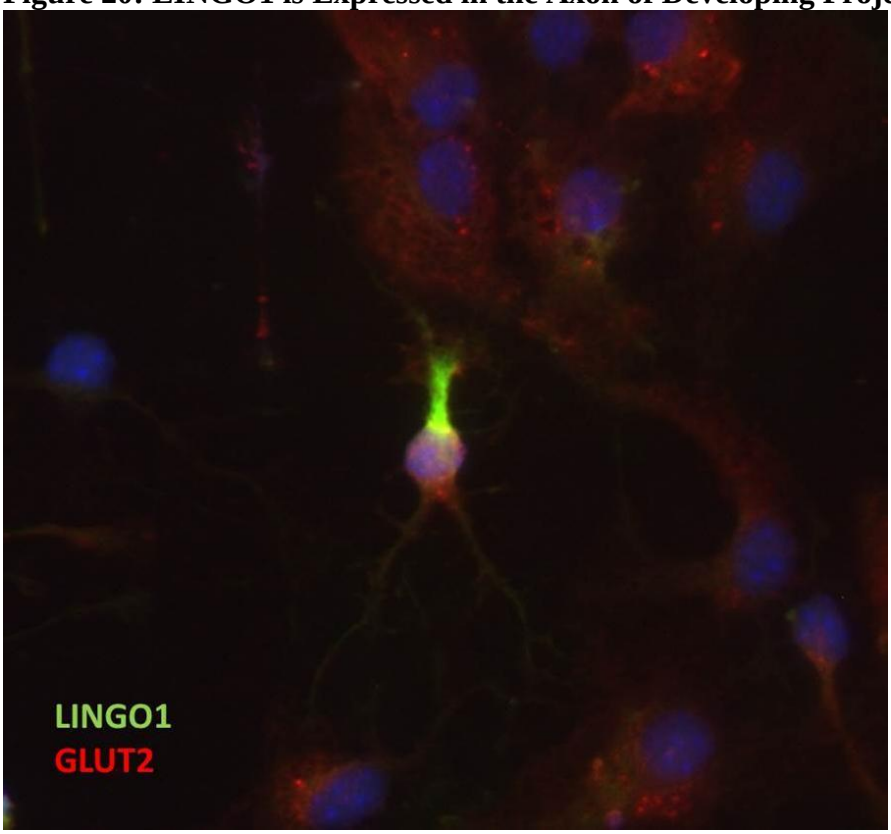


Figure 20: LINGO1 is Expressed in the Axon of Developing Projection Neurons



Aim R2. Inducing Plasticity in Cortical Connectivity via a Novel Intervention in ASD

Aim R2 Timeline

Period	Milestones
6/1/09-6/30/09	• Hire RAs and begin recruitment for Intervention Group A
7/1/09-6/30/10	• Conduct Intervention A – recruit 15 HFA adolescents
7/1/10-6/30/11	• Test Intervention Group A in 1-yr. follow-up Conduct Intervention B – recruit 15 HFA adolescents
7/1/11-6/30/12	• Test Intervention Group B in 1-yr follow-up • Initial testing of two Control groups – recruit 15 TD and 15 HFA adolescents
7/1/12-5/31/13	• Test two control groups in 1-yr follow-up

Aim R2.1 (original): Can adolescents with autism acquire visuoperceptual expertise for Greebles and does this generalize to improve face recognition?

Aim R2.2 (original*).* **Does training induce plasticity and reorganization in the functional topography of the ventral visual pathway in HFA adolescents?**

Aim R2.3 (original): Does training induce plasticity in the functional and structural connectivity among regions in the face-processing network?

The central goal of the intervention studies in Aim R2 was to evaluate whether a strategic intervention can improve object recognition abilities (with particular emphasis on face recognition) and the functional organization of the developing brain in adolescents with high-functioning autism (HFA). The intervention was designed to induce visuoperceptual expertise with a novel class of visual objects. Object and face recognition abilities as well as patterns of brain activation and functional and structural connectivity among brain regions was evaluated pre- and post-intervention and again a year later in a group of HFA individuals and was compared with those of a matched HFA group and a typically-developing (TD) group, neither of which received the targeted intervention. This work will have important impacts on the fields of autism research as well as developmental social and cognitive neuroscience more generally for several reasons.

Beneficial impacts of this work:

- The work conducted to satisfy Aims R2.1-2.3 are the first longitudinal studies of developing changes in the behavioral and neural foundation of face and object processing in both HFA and TD adolescents. This work is essential for identifying mechanisms (and alterations to such mechanisms) of developmental (not just age-related) change in both behavioral and brain development.
- The intervention results in the HFA adolescents will help delineate between two competing, although not mutually exclusive, theories for explaining face-processing deficits in autism (Aim R2.1).

- This work will fundamentally influence the content and design of future interventions to improve face processing, and social information processing more generally, in autism (Aim R2.1).
- This work also reflects on the potential usefulness of computer-based interventions for adolescents with autism (Aim R2.1).
- The neuroimaging findings (Aim R2.2) begin to elucidate how symptom severity, levels of adaptive functioning, as well as individual differences in behavioral performance are related to specific alterations in the social brain in autism.
- This work enabled us to document ongoing developmental changes in the organization of neural networks that support social information processing in adolescents with autism (Aim R2.2-R2.3). These results critically inform our models of brain development in autism to understand that regions in the social brain are not arrested in development; instead they exhibit ongoing plasticity.

Overall Summary and Changes to Original Proposal

As planned, we successfully completed our aims of having 7-10 participants complete the entire longitudinal protocol in each treatment group, in both interventions A and B (see Tables 3-4) (Aims R2.1 – R2.2). We were able to accomplish these aims in spite of experiencing several extenuating circumstances, including the temporary closure of the initial neuroimaging facilities and the transition of Dr. Scherf to a faculty position at Penn State University (PSU).

Briefly, we originally planned to scan participants at the Brain Imaging Research Center (BIRC). However, in February 2009, the Psychology Department at Carnegie Mellon University (CMU) was awarded an equipment grant from the National Institutes of Health for a state-of-the-art scanner, which also resulted in a plan to close the BIRC in June 2010. We made a strategic decision to delay running the full intervention on the first autism participants to Year 2 so that all the data points (including the one year follow up) from both the intervention and the control participants could be conducted on the scanner at CMU. Also, Dr. Scherf began a position as Assistant Professor of Psychology at Penn State University in the Fall of 2011 (the end of Year 2 of the grant). As a result, the team made another strategic decision to finish ongoing data collection for Intervention A at CMU, but to run Intervention B at PSU, given that Dr. Scherf was primarily responsible for conducting the intervention studies. Also, rather than recruiting an entirely new sample for Intervention B, we decided to treat the participants with autism who were randomized into the control group in Intervention A as a waitlist control group, and invited them to undergo Intervention B at PSU. We were able to enroll 90% of the waitlist control participants as Intervention B participants at PSU. We also required and tested 10 age-, IQ-, gender-, and handedness matched control TD adolescents for this Intervention. Importantly, instead of recruiting an additional sample of HFA control participants in Intervention B, we used the data from these very same participants when they were waitlist control participants in Intervention A.

The decision to move Intervention B data collection to PSU was instrumental in enhancing the capacity for research at the Social, Life, and Engineering Sciences Imaging Center (SLEIC) at PSU; this was the first developmental neuroimaging study conducted with special populations at

SLEIC and has now set a precedent and a set of protocols for doing so for the imaging community at large at PSU. Also, several of Dr. Scherf's undergraduate and graduate students as well as a post-doctoral fellow have now worked on this project, learning to collect and analyze both behavioral and brain imaging data. All of these students (see personnel) will be presenting findings from these studies at the annual meeting of the Society for Neuroscience in the Fall of 2013. Dr. Scherf has also formed new collaborations with faculty at PSU (Peter Molennar and Joshua Smyth) that will help address some of the core aims of the grant (specifically with respect to functional connectivity analyses with Dr. Molenaar) as well as direct future plans that expand this research to develop a more comprehensive "serious game" to help adolescents with autism learn to use eye gaze information appropriately (with Dr. Smyth).

In what follows, we outline the methods and analyses used to address each of the Specific Aims as well as figures that reflect the current status of the results.

Aim R2.1 (original): Can adolescents with autism acquire visuoperceptual expertise for Greebles and does this generalize to improve face recognition?

We proposed to test 30 HFA adolescents (ages 10-15) in two interventions, 15 in Year 1 (Intervention A) and 15 in Year 2 (Intervention B). The goal was to have 7-10 participants complete the entire protocol, which represents double the sample size of previous Greeble training studies with typical adults. In addition, two comparison groups, one HFA and one TD, matched on sex, age, and Full Scale IQ to the intervention groups, were proposed to be tested over the same time frame, but would not participate in the intervention. These comparison groups would provide a baseline of developmental change from which to measure effects of the intervention. We accomplished all of the milestones to fulfill this aim, including testing the three groups of individuals in Intervention A (See Table 3) and the HFA and TD adolescents in Intervention B (See Table 4).

Aim R2.1 - Intervention A (CMU) Methods: To evaluate this aim, 11 adolescents with high functioning autism (HFA) underwent a two-month long visuoperceptual expertise training protocol to learn to categorize and individuate a novel class of objects called Greebles (Gauthier and Tarr, 1997). Greebles were originally designed as a set of control stimuli for faces: they are perceptually homogenous and have an overall body shape combined with two horizontal and two vertically oriented appendages (i.e., features). The first goal of this aim was to evaluate whether HFA adolescents can learn to individuate Greebles and process them holistically. This would determine whether the visuoperceptual systems of adolescents with autism are fundamentally limited in their ability to do holistic processing as a likely result of their excessive focus on featural details. Importantly, previous research using Greebles has been criticized for selecting Greebles that can be easily discriminated based on featural differences (e.g., shape of appendages). Critically, we selected a set of Greebles for training that all had the same set of appendages and could only be distinguished based on the holistic analysis of the features in combination with the body shape. In so doing, we specifically prevented our participants from using a feature-based strategy, which they are quite good at using but that interferes with expert individuation, to learn the individual Greebles.

As proposed in the original grant application, we modified the training protocol from the standard model (Gauthier and Tarr, 1997) in a number of other ways to make it more engaging for the adolescents with autism and to ramp up difficulty in a manageable way during training. First, instead of introducing 4 novel Greebles to be learned on each of the first 5 days of training, we reduced that number to 2/day so that the adolescents in our study only had to learn a total of 10 individual Greebles, which were introduced in pairs every other session of training during the first 10 training sessions. Second, we chose to make the tasks in which participants were introduced to these new Greebles particularly easy so as not to discourage them early in training. Finally, we embedded the task in a narrative about a Greeble jewel thief. The participants listened to audio clips and viewed images describing the most recent escapades of the Greeble thief throughout the training. They were instructed to learn how to be a "Greeble detective" so that they could uncover the Greeble thief, who was revealed in the final session of training.

The training occurred at home on laptops that were customized specifically for this intervention project. Participants underwent 24 sessions of training. Our research coordinators monitored the progress of the participants remotely, but on a daily basis. During the first 10 sessions they were introduced to two new Greebles every other session, so that they learned to individuate (decided whether the name and Greeble image were correctly paired) a total of 10 Greebles. The remaining 14 sessions involved practicing individuating the Greebles as well as categorizing them into 1 of 5 families. In these practice trials, either a Family name (e.g., Family A) or an individual name (e.g., Kate) appeared above an image of a Greeble and participants had to decide whether the name and image were a correct match. The practice trials were embedded in a narrative about the need for participants to uncover the correct Greeble diamond thief. In total, participants executed more than 1500 trials individuating Greebles and 1000 trials categorizing them into families during training.

As proposed as part of R2.1, we designed an enormous battery of visual stimuli and experimental protocols to evaluate potential developmental- and intervention-related changes. For the three testing time points (baseline, post-intervention, follow-up), we designed two tests that evaluate different components of visuoperceptual expertise for faces, cars, and Greebles. The *sequential matching (SM) task* is a standard task of expert recognition and the *part-whole (PW) task* is a specific test of holistic processing for visual objects. Because we employed novel stimuli in each of these tasks at each time point, we created more than 300 stimuli in each visual category. In addition to these 18 experiments (3 visual categories x 3 time points x 2 tasks) in the testing battery, we also incorporated standard tests of basic visuoperceptual processing, including the *global-local* and the *embedded figures* tasks, and a standard test of face recognition, the *Cambridge Face Memory Test* (Duchaine & Nakayama, 2006). For intervention B, we also designed a composite task (full design) to test holistic processing of faces, Greebles, and cars. We analyzed accuracy, d-prime, reaction time for correct responses, as well as inverse efficiency (the amount of time it takes to make a correct response) for each participant across sessions. This thorough battery of tasks reflects the complexity and scope of the administration of this intervention. <u>Combined with the neuroimaging testing, this scope of longitudinal testing over the course of a year in both TD and HFA adolescents is unprecedented</u>.

Aim R2.1 - Intervention A Behavioral Results: Importantly, this intervention protocol was quite tolerable for our participants. Ten of the 11 enrolled participants completed the full training protocol. We experienced technical difficulties with one family that resulted in terminating the training. We had no adverse events or experiences reported to us by the participants or their families. In fact, several families reported to us (via the daily parent interviews) that the training was enjoyable for the participants, particularly in the beginning when the narrative was most integrated into the training.

The results overwhelmingly indicate that adolescents with HFA can learn to individuate Greebles, a novel class of perceptually homogenous objects (see Fig. 21). Separate repeated-measures general linear models (GLMs) with the factors of condition (individuation, categorization) and training session (1-24) as within-subjects factors reveal significant improvements in all four dependent measures, some of which varied by condition (see Figs 21a-21d). Specifically, there were main effects of session in accuracy $F(23, 184) = 3.2$, $p < .000$, and d-prime, $F(23, 184) = 2.5$, $p < .005$, reflecting improvements in performance across sessions. Additionally, there were condition x session interactions in all four dependent measures (all $p < .001$). To investigate the interactions, separate repeated-measures GLMs were run on each condition for each dependent variable. They revealed that increases in the accuracy and d-prime measures as well as decreases in the RT and inverse efficiency measures drove the interaction specifically in the family categorization, but not the individuation condition.

At first glance this seems to directly contradict the goals of the intervention to enhance expertise in individuation of novel perceptually homogenous objects. However, it is important to note that we implemented a strategic decision to make the individuation trials particularly easy in the early stages of training and gradually ramping up difficulty in this condition throughout the first 10 days of the training. Specifically, participants only had to individuate a total of 2 Greebles in sessions 1-2, 4 in sessions 3-4, 6 in sessions 5-6, and so on until session 10 when the last two Greebles were introduced. Figure 22 shows performance of the group for each Greeble to be learned across sessions. As new Greebles are introduced on sessions 3, 5, 7, and 9, performance is low for the newly introduced Greebles, but is relatively maintained for the previously learned Greebles. In other words, introduction of new Greebles during training did not interfere with representations of previously learned Greebles. This suggests that our modification to the training protocol for adolescents with HFA was successful and enabled us to ramp up difficulty during training (by introducing new Greebles), without overwhelming the participants and sacrificing overall performance. This finding will critically inform the design of other kinds of computer-based interventions for autism in the future.

In order to understand how individuation performance changes as a function of the number of learned Greebles, we scaled the performance measures by the number of learned Greebles in each session (see Figure 23). After scaling performance in this way, it becomes very clear that accuracy for individuation increased, and inverse efficiency decreased, linearly during the first 10 sessions of learning new Greebles and plateaus for the remainder of training. This is confirmed in main effects of session ($p < .001$) as well as Bonferroni corrected post-hoc tests comparing differences in accuracy between each session for both measures. Together, these

results show that HFA adolescents can learn to individuate and categorize a novel class of perceptually homogenous objects.

The second part of this aim (R2.1) was focused on understanding whether the learning acquired during training generalizes to facilitate individuation of novel Greebles and faces. The central idea is that the social demands on face processing may impair the acquisition of sensitivity to configural properties of faces in autism, but that learning about such properties in another class of perceptually homogenous objects that do not have the same social/affective demands may ultimately bootstrap learning about the configural properties of faces. To evaluate this aim, we developed a battery of tests that evaluate the visual strategies (i.e., feature and/or configural) participants use to recognize Greebles, faces, and a set of control objects (e.g., vehicles). We compared the performance of the HFA adolescents who underwent the intervention to a group of age-, FSIQ-, ADOS total score, handedness-, and gender-matched HFA adolescents as well as a similarly matched group of typically developing (TD) adolescents, who were only tested on the battery of tasks once (as compared to 3 times in Intervention A). Importantly, the pre- and post-intervention and 1-yr follow up tasks all included novel exemplars of objects from each of these visual classes since we were primarily interested in understanding the extent to which learning during training generalized. The one exception was the Cambridge Face Memory Task (CFMT, which is a standard task of unfamiliar face recognition/individuation (Duchaine and Nakayama, 2006) that has been used to evaluate face recognition performance in children, adolescents and adults with autism previously (O'Hearn et al., 2010). There is only one version of this task, which was administered at each time point.

Aim R2.1 - Generalization of Intervention A Learning: We experienced some unfortunate experimenter errors in the administration of the correct versions of the post-intervention tasks for approximately 1/3 of the participants. This included 2 HFA intervention, 3 HFA control, and 2 TD control participants. They received the baseline version of the task at the post-intervention testing session, which prevents us from using those data to evaluate generalization of learning. As a result, we have treated these data as missing data and used the expectation-maximization (EM) method to address missing data so that these participants could be included in the analysis (Little & Rubin, 2002).

Generalization of Intervention A Learning to Improve Novel Greeble Recognition:
Sequential Matching: We employed a sequential matching task to evaluate potential increases in the ability to discriminate and recognize increasingly similar novel exemplars of Greebles. In this task, participants are briefly presented with a stimulus, followed by a checkerboard mask, and a second stimulus. Participants have to decide if the stimuli are the same or different. When they differ, they do so at the more superordinate family level (where body shape and appendages differ) or at a more subordinate level (within the same family so that they have the same body shape and could have very similar appendages). Improvement in the more difficult condition reflects the ability to make increasingly fine-tuned discriminations between novel Greebles and relies more heavily on configural processing (detecting subtle differences in the second order spacing between features). Also, improvement over time in this task provides evidence of generalization of learning since the test items were novel at each time point.

Accuracy of responses at baseline was submitted to a repeated-measures ANOVA with the factors of condition and group. There was only a main effect of condition, $F(1, 26) = 59.3$, $p < .001$, and no main effect of group or interaction between condition and group. This reflects that all three groups were more accurate when making the more super- as compared to the sub-ordinate level discriminations, as to be expected when attempting to recognize a novel class of objects for the first time (see Fig 24). A separate repeated-measures ANOVA was used to evaluate change in performance on this task using the factors of condition, time (baseline, post-) and group. There was a main effect of condition ($p < .001$) as well as a main effect of time ($p < .025$), but no interactions among any of the factors (p = ns). This set of findings indicates that all groups improved in their recognition abilities for Greebles at the second time point and that the intervention participants did not show statistically bigger gains in Greeble recognition than did the other groups. The results were the same when comparing baseline and follow-up performance. Together these findings suggest that there was little generalization of Greeble learning for the HFA intervention participants.

Part-Whole Task: The Part-Whole task (Farah and Tanaka, 1993) was our primary task to measure holistic processing of Greebles, faces, and control objects (i.e., cars). In this task, participants study the faces of 10 individuals. Then they are required to decide which of two face parts is from a face that they learned. The face parts are either presented in isolation, embedded in another individual's face, or embedded in a new faces but the features are scrambled within the face. Holistic processing is indicated by faster decisions when the faces are embedded in new configuration as compared to when they are presented in isolation or in the scrambled condition (new configuration > face part condition or scrambled).

A repeated-measures ANOVA on the accuracy of responses in the Greeble Part-Whole task at baseline with the factors of condition (3) and group (3) revealed that there was no main effect of group, $F(2, 23) = 1.1$, p = ns, condition, $F(2, 46) = 1.9$, p = ns, or a group x condition interaction (p = ns). These results reflect that the groups performed comparably in this task at baseline and that none of the groups exhibited holistic processing for Greebles prior to training (see Fig 25). In the analyses comparing baseline and post-intervention performance, there was a main effect of time, $F(1, 23) = 6.0$, $p < .025$, but also a time x group interaction, $F(2, 23) = 4.2$, $p < .05$. There were no other main effects or interactions. To interpret the group x time interaction, we conducted a test of the simple effect of group at post-intervention. There was a main effect of group following the intervention, $F(2, 23) = 4.0$, $p < .05$. Post-hoc tests revealed that the HFA adolescent group was now more accurate than the HFA control group, but not more accurate than the TD control group.

Together, these findings provide initial evidence that the Greeble learning of the HFA intervention participants supported generalization of their knowledge, but they do not suggest that there was holistic processing of the Greebles. Finally, a comparison between the baseline and follow-up time points failed to reveal any main effects or interactions, suggesting that any intervention-related boost in performance was likely short-lived given that the intervention participants did not practice recognizing Greebles after the training finished.

Generalization of Intervention A Learning to Improve Face Recognition:
Cambridge Face Memory Task (CFMT): Results from the CFMT suggest that there were some group differences in the processing of faces over time (see Fig. 26). A repeated-measures ANOVA with time as the within-subjects factor and group as the between subjects factor on the accuracy of performance in the upright version of the CFMT revealed main effects of time, $F(2, 46) = 7.4$, $p < .005$, and group, $F(2, 23) = 3.9$, $p < .05$ (See Fig. 26a). Bonferroni corrected post-hoc tests showed that accuracy at baseline was lower than both the post-intervention ($p < .025$) and at the 1-year follow-up ($p < .01$) sessions. Also, accuracy on the post-intervention session was lower than on the follow-up session ($p < .01$) As expected, the HFA intervention group was less accurate across all sessions than the TD controls ($p < .05$). However, the HFA controls were NOT less accurate across sessions than the TD controls. Importantly, the HFA intervention and HFA control participants did not differ from one another across session. The omnibus ANOVA failed to show a significant group x session interaction; however, based on our strong a priori hypotheses, we evaluated improvement over time in the upright version of the CFMT in each group in separate repeated-measures ANOVAs. Only the HFA intervention participants evinced a trend for a linear improvement across sessions, $F(2, 16) = 3.6$, $p = .05$. The two control groups did not show this trend across session. These findings suggest that the intervention group experienced long-term improvement in face recognition abilities over the course of a year, whereas the two control groups only experienced improvement after taking the test a second time. These are promising results suggesting that the intervention may have helped to improve unfamiliar face recognition abilities in HFA adolescents.

Furthermore, this effect was specific to upright faces. Figure 26b shows the accuracy of performance for each group across testing sessions for the inverted version of the CFMT. There were no main effects of session or of group or an interaction between these variables in the analyses of these data (p = ns). Separate repeated-measures GLMS in each group also failed to reveal any evidence of a change in performance across time for any group.

Sequential Matching (SM): At baseline, all three groups performed near ceiling on the faces sequential matching task. There were no main effects of group or condition, or an interaction between group and condition (see Fig. 27). This indicates that the HFA participants were as good overall as the TD participants in recognizing faces in this task. Also, they were comparable to the TD adolescents when discriminating faces at the more difficult subordinate level even at baseline. Unfortunately, this high level of performance made it difficult to evaluate increases in performance for any of the groups, and particularly the intervention group. When we compared the changes in performance between baseline and post-intervention, there was still no main effect of group, indicating that all three groups performed comparably on this task at the second testing. However, there were main effects of time ($p< .05$) and condition ($p< .05$) as well as a condition x time interaction ($p<.05$). However, none of these factors interacted with group. This pattern of results suggests that there was a decrease in performance, particularly in the subordinate condition, across all three groups. This suggests that the subordinate trials in our post-intervention SM task were generally more difficult for all participants than were the comparable trials at baseline. There was a similar pattern of findings when we compared performance between baseline and follow-up. We will also conduct similar analyses of the d-prime data,

which may reveal important differences in performance after response biases are removed. Importantly, these results show that the intervention did not interfere with face recognition abilities in the adolescents with autism.

Part-Whole (PW) Task: The analyses of the accuracy of responses in the Faces PW task at baseline revealed a trend for a main effect of group, $F(2, 23) = 2.9$, $p = .075$. The HFA intervention participants tended to be less accurate in this task than the TD adolescents (p = .085), however, the HFA and TD controls performed similarly as did the HFA adolescents and controls (see Fig 28). There was also a main effect of condition, $F(2, 46) = 29.4$, $p < .001$, and no condition x group interaction (p = ns). Across all three groups of participants, accuracy was higher for the configuration condition than for both the parts and scrambled conditions, indicating that all 3 groups were processing faces holistically even at baseline. When comparing differences in performance between baseline and post-intervention, there was no longer a main effect of group (p = ns), nor was there a main effect of time (p = ns), nor an interaction between time and group (p = ns). There was still a main effect of condition $F(2, 42) = 41.5$, $p < .001$, and a time x condition interaction, $F(2, 46) = 38.3$, $p < .001$. However, there was no condition x time x group interaction (p = ns). Interestingly, all three groups improved in performance in the parts condition and slightly decreased in performance on the configuration condition. We interpret these results to reflect problems with the formation of the stimuli the post-intervention version of the task in both the configuration and parts conditions. The follow-up data look very similar to the baseline data, indicating that all three groups show strong holistic processing of faces.

Summary Intervention A Behavior (R2.1): In sum, we conducted the first longitudinal Greeble intervention for autism that includes a comprehensive battery of behavioral (and neuroimaging) tests for evaluating generalization of acquired visuoperceptual expertise in the objects of expertise, but also of faces, and other control objects. Our results indicate that home-based computer mediated interventions can be quite tolerable for families and adolescents with autism. Also, we have novel evidence that HFA adolescents can learn to reliably and accurately recognize Greebles over the course of a 2-month training protocol. Importantly, the HFA adolescents who underwent the intervention showed selective improvements in Greeble recognition, above and beyond either control group. This finding indicates that the Greeble training generalized to improve recognition of novel Greebles. However, we did not find evidence that the intervention participants learned to do holistic processing on the Greebles to support this increased recognition behavior. Given the way we selected the Greebles for training (all had same features), we suggest that it is unlikely that they used a feature-based strategy to improve recognition behavior. It may also be that the training was not sufficiently long or intensive to foster the emergence of holistic processing, an issue we addressed in Intervention B.

This work also provides initial evidence that the Greeble training may boost face-processing skills in adolescents with autism. There were group differences in the rate of change in performance on the upright unfamiliar face recognition task (CFMT) across the three time points. HFA adolescents who underwent the intervention experienced a linear increase in face recognition abilities across all three sessions, whereas the other two groups did not. Unfortunately, participants were nearly at ceiling on the sequential matching task of face

recognition at baseline, preventing us from being able to measure improvements in this task over time. Also, all three groups evinced strong holistic processing of faces in the faces PW task that did not change between baseline and follow-up. We think that performance on the post-intervention version of this task made the parts condition superficially easy for all three groups, preventing us from properly assessing the magnitude of their holistic processing of faces, another issue we addressed in Intervention B.

Aim R2.1 - Intervention B (PSU) Methods: In the original proposal, we argued that the design of Intervention B would be influenced by our findings of the feasibility and behavioral changes (or lack thereof) in Intervention A. As a result, Intervention B was designed to address some issues that were revealed in Intervention A, but also to make the training even more engaging as well as demanding to enhance learning and generalization. To do so, we hired a graphics technician who made animated movies of Greebles stealing jewels at museums and stores all around the world. These videos were designed to enhance the narrative of the training and thus its entertainment value, and, in so doing, we hoped to increase motivation and engagement of the participants as well. Given the lack of evidence from Intervention A for the emergence of holistic processing of Greebles as well as limited generalization of learning to recognize novel Greebles following training, we implemented several changes to the training so as to increase the likelihood of holistic processing and generalization of learning to novel Greebles following training. These included, 1) increasing the number of to-be-learned Greebles to 20, but still introduced them at a rate of 2/day; 2) eliminating the categorization task so as to focus exclusively on individuation; 3) driving more invariant representations of the training Greebles by requiring participants to recognize them from different viewpoints, and 4) dramatically increasing the number of training trials by a factor of 10 (approximately 15,000 trials). The trial structure was the same as in Intervention A, except that participants only did individuation trials (matching a first name with the image of a Greeble). The participants executed the intervention on a customized laptop at home over the course of a 2-month period of time, with daily monitoring by our research coordinator. Finally, we modified the testing battery to include a composite test of holistic processing for faces, Greebles, and cars, and to exclude the part-whole task. This was motivated by concerns about how natural the face and car stimuli looked in the configuration condition across the different versions of the Part-Whole task, which might explain why performance of all three groups was comparable on the configuration and parts conditions in the face Part-Whole task administered at the post-intervention time point.

Intervention B Results: Nine of the HFA control participants from Intervention A returned and enrolled as intervention participants at PSU in Intervention B, allowing us to fulfill the milestones related to recruitment and testing of individuals in Intervention B. As with Intervention A, Intervention B was quite tolerable for our participants, even with the dramatic increase in the number of training trials. All 9 of the enrolled participants completed the full training protocol. To accommodate the increase in the number of trials, we only asked the adolescents to train 2x/week as opposed to 3x/week in Intervention A. As a result, the time between pre- and post-intervention testing was a bit longer in Intervention B (more like 3 months), which prevented us from acquiring follow-up data from 2 of the participants one year after completing the training (time ran out on the grant). Again, we did not receive any reports of

adverse events or experiences during the training for any participants. In fact, they quite enjoyed the enhanced narrative and videos of Greeble thieves in Intervention B. Parents reported that they thought an expanded educational game built around this theme would be quite engaging for their children.

Importantly, we replicated and extended our findings from Intervention A showing that HFA adolescents can acquire visuoperceptual expertise for a class of novel perceptually homogenous objects. Figure 29 shows the learning curves for the intervention participants across training sessions separately for each dependent variable. As in Intervention A, we scaled the accuracy (Fig. 29c) and inverse efficiency (Fig. 29f) for the number of to-be-remembered individual Greebles and submitted these data to a repeated-measures ANOVA with the factor of session. The analysis revealed a main effect of session in the accuracy, $F(19, 144) = 146.0$, $p < .001$, and IE, $F(19, 144) = 27.9$, $p < .001$, data. Bonferroni corrected post-hoc tests revealed that accuracy continued to improve through session 8 and then plateaued for the remainder of training, while IE plateaued much earlier by Session 3.

Composite Task. The composite task (complete design, see Richler et al., 2009) was our primary tool for evaluating whether holistic processing for visual objects changes as a function of training in the adolescents with autism. In this task, holistic processing is indicated by an alignment effect; that is, less accurate responses to decide if the top halves of two stimuli are different when aligned with different bottom halves, compared to when the top and bottom halves of the stimuli are misaligned. In other words, holistic processing interferes with these decisions in the aligned, but not misaligned, conditions. Therefore, an alignment effect reflects holistic processing, whereas the lack of an alignment effect reflects no holistic processing. Figure 30 shows accuracy of performance in the Greeble composite task for HFA adolescents prior to undergoing the Greeble intervention and TD adolescents.

In the first analysis, we used a repeated-measures ANOVA with the factors of condition (aligned, misaligned) and session (pre, post, follow-up) to evaluate whether the magnitude of the alignment effect (in accuracy) changed with time in the intervention participants. The analysis revealed a main effect of session, $F(2, 12) = 8.2$, $p < .01$, but also a condition x session interaction, $F(2, 12) = 7.3$, $p = .01$, but no main effect of condition, $F(1, 6) = 0.2$ p = ns. The Bonferroni corrected post-hoc tests revealed that intervention participants improved their overall performance in this task from pre- to post-intervention testing ($p = .056$) but not from post-intervention to follow-up. More importantly, the condition x session interaction reveals the emergence of an alignment for novel Greebles following training. Prior to the intervention, the intervention participants tended to be more accurate in the aligned than misaligned condition, $t(8) = 2.1$, $p = .067$, indicating no use of holistic processing of Greebles (see Fig 30). However, <u>immediately following the intervention, these same participants showed strong evidence of holistic processing of Greebles</u>; they were significantly more accurate in the misaligned compared to the aligned condition, $t(8) = 3.4$, $p = .01$ (two-tailed). Interestingly, holistic processing of Greebles was not sustained at the follow-up testing, $t(8) = 0.8$, p = ns (see Fig 30). Importantly, <u>the TD control participants did not evince holistic processing of Greebles</u>, $t(9) = 0.1$, p = ns (see Fig 30), indicating their lack of visuoperceptual expertise for Greebles. These

results show that HFA adolescents acquire holistic processing of Greebles as a result of this visuoperceptual training protocol, but that this holistic processing is short-lived, presumably since the adolescents did not continue to practice individuating Greebles between the post- and follow-up testing.

Sequential Matching. As in Intervention A, we also employed a sequential matching task to evaluate potential increases in the ability to discriminate and recognize increasingly similar novel exemplars of Greebles. D-prime scores were submitted to a repeated-measures ANOVA with the factors of condition (aligned, misaligned) and time (pre, post, follow-up). There was a main effect of time, $F(2, 8) = 8.0$, $p < .001$. Bonferroni corrected post-hoc tests revealed that performance was lower prior to training than both immediately after training ($p < .05$) as well as at the follow-up session ($p < .01$) (see Fig 31). Performance did not increase from post-intervention to follow-up. These results provide strong evidence for generalization of learning during training to recognize novel Greebles. There was a main effect of condition, $F(1, 4) = 23.5$, $p < .005$, such that HFA adolescents were consistently more accurate when making superordinate compared to subordinate recognition decisions across all three time points. This pattern of performance did not change over time. There was no main effect of time nor an interaction between condition x time (all p = ns). However, the intervention participants also exhibited an increase in performance on the control object (i.e., cars) sequential matching task (see Fig 31). There was a main effect of time on d-prime scores in the car sequential matching task, $F(2, 10) = 17.3$, $p < .01$. Bonferroni corrected post-hoc tests showed that car performance at baseline was only lower than at follow-up ($p < .001$). There was also a main effect of condition, $F(1, 5) = 8.5$, $p < .05$, indicating that, as with Greebles, HFA adolescents were better at discriminating between cars at the more superordinate than subordinate level consistently across all testing time points. There was no interaction between condition x time. Together these findings suggest that either the intervention improved visuoperceptual processing for Greebles and cars simultaneously or that age-related changes in the visuoperceptual system were responsible for improvements in performance in these tasks. Comparison with the Face Sequential Match task will help delineate between these alternative explanations, which we describe in the next session.

Generalization of Intervention B Learning to Improve Unfamiliar Face Recognition

Composite Task. In contrast to when processing Greebles, the TD control adolescents did evince holistic processing for faces. They were more accurate on the misaligned compared to aligned trials, $t(8) = 2.8$, $p < .025$ (see Fig 32) even prior to the intervention. This finding was unexpected given previous work arguing that individuals with autism do not process faces holistically (Gauthier et al., 2009). The repeated-measures ANOVA with condition and time as factors on the intervention participant accuracy scores from the face composite task revealed a main effect of time, $F(2, 12) = 5.3$ $p < .025$, indicating improvement across sessions, as well as a main effect of condition, $F(1, 6) = 7.3$ $p < .05$, indicating consistently more accurate responses in the misaligned condition. However, the condition x time interaction was not significant, reflecting that the magnitude of the alignment effect for faces did not increase following training (see Fig 32). Interestingly, the magnitude of the alignment effect for faces at baseline and at follow-up in the HFA adolescents did not differ from the magnitude of the effect in the TD adolescents. Importantly, these intervention participants did not show similar holistic processing

for the control stimuli (cars), $t(9) = 0.4$, p = ns at any time during testing. These findings indicate that HFA adolescents processed unfamiliar faces holistically even prior to the intervention, and that this holistic processing of faces did not change over the course of testing.

CFMT. As in Intervention A, the HFA and TD adolescents performed upright and inverted versions of the CFMT. Prior to the intervention, the HFA adolescents were significantly worse on the upright, $t(17) = 1.9$, $p < .05$ (one-tailed), but not inverted, $t(17) = 1.1$, p = ns, version of the CFMT (see Fig 33) compared to the TD adolescents. Unlike in Intervention A, the HFA intervention participants did not evince an improvement in their performance on the upright (or inverted) version of this task with time (all p = ns). However, this analysis is limited by the fact that we only have data from 7 of the 9 participants at the follow-up time point. Importantly, when we compare performance of the HFA adolescents at this follow-up time point to the TD adolescents, they were no longer worse on the upright CFMT, $t(15) = 0.9$, p = ns. This finding also suggests that there was improvement in processing of unfamiliar upright faces in the HFA adolescents. These data provide some evidence that either the intervention or the natural development of the face recognition system (the participants were a year older in this Intervention) lead to subtle improvements in unfamiliar face recognition abilities in the HFA adolescents. Note that the repeated testing on this task is not the likely mechanism supporting subtle improvement on this task for the HFA adolescents since they did not show similar improvements in the inverted condition with repeated testing in either intervention.

Sequential Matching: A repeated-measures ANOVA on the d-prime scores generated from the Face Sequential Matching task with the factors of condition and time revealed a main effect of time, $F(2, 10) = 23.5$, $p < .005$. As with Greebles, performance prior to training tended to be better than immediately after the intervention ($p = .059$) and was significantly lower than at follow-up ($p < .05$), but performance did not differ between post-intervention and follow-up (see Fig. 34). These results are consistent with improvements in both the Greeble and car versions of the sequential matching task (see Fig. 31), and therefore are consistent with the idea that either the intervention boosted recognition and discrimination skills for all visual objects, or that age-related changes that are not specific to the intervention supported the improvement of recognition and discrimination abilities more generally. A comparison with the data from Intervention A will help sort out these interpretations.

Importantly, and different from performance in both the Greeble and car versions of the task, there was no main effect of condition, $F(1, 5) = 2.7$, p = ns, indicating that HFA adolescents in this study were quite good a making fine-grained discriminations between faces even at baseline, which is consistent with the performance on the Face Composite task as well. There was no change in this comparable performance on the superordinate and subordinate level trials across training; there was no condition x time interaction.

Summary of Findings from Aim R2.1 In sum, we were successful in enrolling and testing the expected number of participants in the two separate visuoperceptual training intervention studies. We have strong evidence that HFA adolescents can indeed learn to discriminate and recognize novel perceptually homogenous objects, by learning to process them holistically. This

knowledge generalizes to help them recognize novel exemplars as well, even 1 year after training. These findings represent a strong proof-of-concept that will greatly inform the current debate in the literature about inherent biases in the visuoperceptual systems of individuals with autism. Specifically, although there is clear evidence that the visuoperceptual systems of individuals with autism exhibit biases toward processing the features of visual stimuli (and particularly faces), this bias either 1) fails to interfere with the acquisition of holistic processing for novel perceptually homogenous objects, and/or 2) varies greatly across individuals with autism, with some individuals showing no difficulty in holistic processing of faces or other visual objects.

In contrast to our predictions, we did not find any evidence that the intervention boosted face-processing skills. In both Intervention A and B, we observed longitudinal improvements in upright face processing abilities of all groups on the CFMT (follow-up > pre-intervention). Given that we did not change the stimuli in this task at each time point, this could simply reflect test-specific learning. However, the improvements in the Face Sequential Matching test in both interventions, which did include separate exemplars at each time point, suggests that face recognition behavior was improving for all groups longitudinally. This is an exciting finding that demonstrates the ongoing development of this system through adolescence even in adolescents with autism.

There are several important caveats to consider about our lack of findings of intervention-related improvements in face processing behavior. First, the participants in Intervention B were particularly good at face recognition and evinced holistic processing of faces even before the Greeble training. This characteristic of our sample may have made it particularly difficult to observe improvements in holistic processing of faces. Future intervention studies would be advised to select participants for the study who evince impaired face processing behavior as part of the inclusion criteria for the study (as in Tanaka et al, 2012). Second, technical problems prevented us from acquiring the full battery of follow-up tasks with 3 of our 9 participants in Intervention B. This may have undermined our ability to observed subtle changes in the holistic processing of faces. Finally, at face value, this finding establishes that the Greeble training did NOT interfere with face processing abilities. This was a concern given previous work with an acquired prosopagnosic patient who was terminated from a Greeble training study because of decreasing performance in face recognition abilities (Behrmann et al., 2005) and other studies showing possible trade-off in face processing with increasing skill in another visual domain.

We will be presenting data from Intervention A in a talk and from Intervention B in a poster at the annual meeting of the Society for Neuroscience in October 2013. We are currently writing several manuscripts describing these results that will be submitted for publication in the next few months.

Aim R2.2 (original*)*. Does training induce plasticity and reorganization in the functional topography of the ventral visual pathway in HFA adolescents?

To address this aim, we developed an fMRI localizer task to map the topography of neural activation in both the core (i.e., visuoperceptual) and extended (e.g., social/affective/motivational) regions of the face processing network as well as in regions supporting processing of common objects and places. We also developed an additional task in which only blocks of faces and Greebles were presented. The blocks varied in the perceptually hetero- or homogeneity of the faces and Greebles. This task was designed to be analyzed using multivariate pattern analyses to evaluate changes in the patterns (as opposed to the topography) of activation as a function of the intervention and of development. The same fMRI tasks were used in both Interventions A and B. However, given that the data were collected on different scanners (CMU has a Siemens 3T Verio with a 32-channel head coil and PSU as a Siemens 3T Trio with a 12-channel head coil), the data needed to be analyzed separately.

Aim R2.2 - Methods and Analyses: As proposed, functional images were acquired across two runs of a 1-back localizer task, which included blocks of neutral faces, fearful faces, common objects, vehicles, houses, Greebles, and scrambled images. Each run lasted a total of 9 minutes and 12 seconds and began with a 20-second block of fixation and a 12-second block of patterns. Thereafter, blocks of stimuli were presented in a randomized order followed by intervening blocks of fixation (6 seconds). Within a block, 12 stimuli were each presented for 800 ms, followed by a 200 ms fixation. The order of the images was randomized within each block for each participant. Participants were required to indicate, by button press, when they detected a repeated image. There were two repeats in each of the stimulus blocks, the position of which was counterbalanced across blocks.

The neuroimaging data were analyzed using Brain Voyager QX v2.3. Preprocessing of functional images included 3D-motion correction, filtering low frequencies, and re-sampling the voxels to 1 mm^3. Runs in which participants moved more than 3 mm (1 voxel) were not included in the analyses. For each participant, the timeseries images for each brain volume were analyzed for category differences in a fixed-factor GLM. Each category was defined as a separate predictor and modeled with a box-car function adjusted for the delay in hemodynamic response. There was no spatial smoothing. The timeseries images were then spatially normalized into Talairach space, which is common practice in autism research. Selectivity for each visual category was defined in contrast to all other categories of interest (Scherf et al., 2010). For example, face selectivity was defined with the following balanced contrast: {[3*(neutral faces)+3*(fearful faces)] – [2*(common objects)+2*(houses)+2*(scrambled images)]}. As proposed, we conducted both individual ROI and group-level whole brain analyses of these data. Category selectivity was determined separately for each group by submitting the time-series images from each participant to a random-effects GLM in which category was a fixed factor and participant was a random factor. The group-level contrast maps were corrected for multiple comparisons using a Monte Carlo simulation separately on the TD and HFA group maps. To compare group differences in category-selectivity, the full set of timeseries data from all participants was submitted to a mixed-model ANOVA including Group and Category as fixed

factors and Subject as a random factor. We specifically evaluated Group x Category interactions in each voxel in a whole brain analysis based on the contrasts of interest. For example, to compare group differences in face-selective activation, we coded the following interaction: TD (faces-other) > HFA (faces-other). We used a Monte Carlo simulation to correct the resulting interaction maps for false positive activations.

We originally proposed to subject these data to a multivariate pattern analysis procedure as well as to explore whether there are changes evident in the different pre- to post-data associated with greebles, faces and objects at a more fine-grained level. We did design two runs of an fMRI task that were optimized for this particular analysis strategy. In these tasks, participants performed a 1-back memory task as they look at blocks of homogenous and heterogeneous Greebles and faces. We have preprocessed these data and analyzed them using univariate analyses, which were presented at the annual meeting of the Society for Neuroscience meeting. We have yet to conduct the multivariate analyses, but are actively developing the protocol to do so now.

Aim R2.2 - Intervention A fMRI Localizer Results Baseline: Prior to evaluating changes in the functional topography that result from participating in the intervention and from inherent developmental changes, we aimed to characterize group differences in this topography at baseline and to evaluate whether potential differences are due to individual differences in autism symptom severity, levels of adaptive social functioning, and or face recognition behavior. We have described these findings in a manuscript that is currently under review at *Brain* (see peer-reviewed manuscripts). We determined that HFA adolescents exhibit hypo-activation in the majority, but not all, of the core and extended face processing regions compared to TD controls. Specifically, although HFA adolescents, as a group, exhibited face-related activation in the pre-eminent fusiform face area (FFA) in both hemispheres; activation in the right, but not the left, FFA was significantly hypoactive compared to the TD adolescents (see Fig 35a). Also, face-related activation in the right and left occipital face area (OFA), right posterior superior temporal sulcus (pSTS) was hypoactive in the HFA group as well. Importantly, this hypo-activation was only evident during face processing. HFA adolescents exhibited comparable activation to TDs bilaterally in the lateral occipital complex (LOC) and hyper-activation in the precuneus during object-recognition (see Fig 35b), and comparable activation in the parahippocampal place area (PPA) during house-recognition (see Fig 35c).

We also report novel evidence that the magnitude of hypo-activation in the right FFA among the HFA adolescents is selectively related to the severity of autism symptoms as well as to lower levels of adaptive social functioning (see Fig. 36). Specifically, individuals with less severe autism symptoms and higher levels of adaptive social functioning exhibited *more* face-related activation in the right FFA and no other region. There were also no regions in which object- or house-related activation correlated with symptom severity or levels of social functioning. These highly selective results suggest that the right FFA is particularly vulnerable in autism and that activation in this region may be related to the success with which individuals with autism interact with the social world.

In summary, our findings identify the right FFA as a particularly vulnerable node in the broadly distributed face-processing network in adolescents with autism. Importantly, we show that it is *not the only* atypical node, indicating that the extent of impairment in the functional organization of neural regions supporting face processing in autism is much broader than previously reported. In general, most of the core and extended regions of this network are *hypoactive* in autism, including the right amygdala. Given recent findings that the amygdala processes the subjective salience and relevance of stimuli (Adolphs, 2010), our findings of hypo-activation in the amygdala during face processing are consistent with the notion that individuals with autism exhibit a diminished social interest in faces (Chevallier et al., 2012). At the same time, our results indicate possible preservation of some nodes in autism, including the left FFA and right anterior temporal lobe, particularly in individuals who exhibit better recognition behavior. This is a particularly exciting and informative finding that can potentially guide remediation strategies and targets for improving face and social processing in autism.

Intervention A fMRI Localizer Results Change Over Time: To evaluate developmental changes in face-, greeble-, and common object-related activation among the participants, we are relying primarily on individual-level analyses, given the small number of participants that we have in each of the groups and the individual differences in recognition behavior, autism symptom severity, and levels of adaptive functioning. These analyses are ongoing, but we have clear evidence of developmental change in HFA intervention participants in the topography of activation for each of these visual categories, particularly in the comparison between follow-up and baseline.

Figures 37 and 38 illustrate significant change in face (red), Greeble (pink) and object (blue) activation as a function of time for each of the intervention participants in Intervention A and B. These maps are corrected for each individual using the False Discovery Rate at $q < .01$. This means that fewer than 1% of the significantly active voxels could be false positives.

These figures 37 and 38 illustrate that there are impressive changes in many of the core and extended face processing regions of the intervention participants, particularly in the contrast between follow-up and baseline, which covers a full year of time. We are actively processing the same data from the control participants in each intervention and defining the regions of interest for our ROI-based analyses of developmental and intervention-related changes. When we finish with these analyses, we will be able to document the longitudinal development in the ventral visual pathway in adolescents with autism as well as TD adolescents. We will also be able to say whether the changes that are so visible in Figures 38 and 39 above are primarily due to the Greeble training, or to inherent developmental changes that are ongoing and independent of the training. We are currently finishing these analyses and preparing several manuscripts describing these results in each of the interventions.

Aim R2.3. Does training induce plasticity in the functional and structural connectivity among regions in the face-processing network?

<u>The functional connectivity, or coherence in activation, among regions supporting Greeble and face processing will be evaluated for intervention-related improvements.</u>
We originally proposed to use simple correlational analyses to investigate group differences in functional connectivity between regions of the face-processing network using Dynamic Causal Modeling (DCM) to evaluate differences in effective connectivity among these nodes. However, given the notion that DCM is a confirmatory approach and, therefore, requires a priori specification of the specific connections as well as their direction of influence to be tested, we had concerns about using this model given the limited research on functional connectivity within the face processing network in both TD and HFA adolescents and thus, our ability to generate such models *a priori*. As a result, we are currently using an approach that is more data-driven and exploratory in nature. As a result of our new collaboration with Drs. Peter Molenaar at PSU and Katie Gates at UNC Chapel Hill, we are exploring the use of a new method called Group Iterative Multiple Model Estimation (GIMME) that will enable us to conduct the functional and effective connectivity analyses simultaneously (Gates et al., 2010) and that addresses many of the deficits in the existing approaches for evaluating functional connectivity (as recently articulated by Smith et al., 2011). Specifically, the Molenaar/Gates model is data-driven and does not require a priori models to test. It also capitalizes on individual variability across participants when settling on a group model. This is especially critical when there are vast individual differences affecting the potential neural responses and patterns of functional organization, as is true in autism and in developmental populations. GIMME has exceptional validity when tested against simulated data (Gates et al., 2011).

The most difficult part of these analyses (for any kind of connectivity analyses) involves deciding how to define the nodes or ROIs from which to pull the time series data that are then submitted to the connectivity analyses. We are currently exploring the use of individually defined regions, which is optimum but often limits power since individuals without clearly defined regions cannot be included in the analyses. We are also exploring how defining ROIs based on group-level models (TD or HFA) can also be used. They enable all participants to be included in the analyses, but do not accommodate individual differences as well as the individually defined ROI approach does. We will perform analyses on both kinds of ROIs to understand potential differences in the functional organization of face-processing regions between the HFA and TD adolescents 1) at baseline 2) with respect to long-term developmental changes, and 3) as a result of the intervention. We will also evaluate potential relations between differences in the functional and structural organization of these networks (e.g., correlations between beta weights on functional connections between two regions and measures of structural properties of the fasciculi connecting these regions). We anticipate submitting multiple manuscripts describing these baseline, developmental, and intervention-related differences in the functional organization of the face-processing network.

Potential changes in the structural integrity of large fasciculi, or axonal bundles that relay information among cortical regions in the ventral visual pathway will be evaluated using diffusion tensor imaging.

To accomplish this aim, we needed to develop the protocol for extracting several fasciculi in the brain that relay information between nodes within the face processing network, including the inferior longitudinal fasciculus (ILF) and the inferior fronto-occipital fasciculus (IFOF). To do so, we developed the protocol and tested it on existing data. The protocol and findings from the previous data set were recently published in a study in *Cerebral Cortex* (Scherf et al., 2013) DOH funds were used to support Dr. Scherf's salary when she worked on this project, which is reflected in the acknowledgements of the paper. The protocol that was developed and described in this paper is currently being used to analyze the diffusion tensor imaging (DTI) data that were collected as part of Aim R2.3. In this paper, we established the protocol for defining the regions of interest (ROIs) and for extracting the fiber tracts of interest that is currently being used to analyze the DTI data collected in this project under Aim R2.3 (see Fig 39).

Also, during the course of our award, a new pre-processing stream (TORTOISE) was released from a lab at the National Institutes of Health that is particularly good for processing pediatric DTI data (Pierpaoli et al., 2009). This software produces robust and reliable registration between the DTI and MPRAGE (high resolution anatomical) images by correcting for motion, eddy current distortion, and EPI distortion. It then performs tensor estimation using properly weighted linear and non-linear least squares methods. The resulting diffusion weighted images and tensor qualities can then be exported to several commonly used software packages for fiber tracking. During the course of the award, we have learned to use this pre-processing stream on the DTI data collected under Aim R2.3 and the incorporation of it into our analyses is greatly improving the integrity of the data.

We are currently conducting our analyses of the DTI data using Brain Voyager (BV) (after preprocessing using TORTOISE). The significant benefit of using BV is that all of the functional data are processed in this package, which will expand our capabilities for investigating structure-function relations in our longitudinal analyses. In Fig 40 we have mapped the right ILF on top of face and greeble-related activation at baseline and at follow-up in a single HFA adolescent from Intervention B. We intend to continue developing the full protocol for analyzing the DTI data from both intervention projects and submitting several papers describing potential differences between the HFA and TD adolescents 1) at baseline 2) with respect to long-term developmental changes, and 3) as a result of the intervention within these fasciculi of interest and in structure-function relations.

Future Plans Related to Aim R2 Project

This Aim R2 project has generated a massive and complex set of data. As a result, it will take time for us to fully analyze and understand the existing behavior-brain (structure and function) correspondences among HFA and TD adolescents as well as potential developmental changes and intervention-related changes to them. We anticipate publishing papers describing findings from this longitudinal dataset for the next several years. In so doing, we will be able to train

additional undergraduate and graduate students as well as post-doctoral fellows in the science of autism and developmental neuroimaging. We will also make sure to continue acknowledging the role of the PA Dept of Health and Human Services as the main funding source for supporting this work.

At the same time, Dr. Scherf has forged a new collaboration with Joshua Smyth in Biobehavioral Health at PSU. Dr. Smyth is an expert on designing and evaluating "serious games" to improve health outcomes and treatment compliance in children, adolescents, and adults with various conditions. As part of this collaboration, together with Dr. Elizabeth Whyte, a post-doctoral fellow working with Dr. Scherf, and Dr. Smyth, Dr. Scherf is seeking funding to support the development of a "serious game" to help adolescents with autism learn about and develop social skills, with particular emphasis on learning to use eye gaze information in social contexts. The goal of the game would be to help adolescents with autism develop more natural social skills and generalize their knowledge to real-world social settings, which may also improve their academic and vocational potential. We are waiting to hear about the status of our application to the Penn State Center for Online Innovation in Learning to provide support for the initial development of the game. We have also submitted two post-doctoral fellowship applications to Autism Speaks and one to Autism Science Foundation to support Dr. Whyte so that she can continue training with Dr. Scherf.

Papers in preparation that were supported by the grant:

Whyte, E., Picci, G., and Scherf K.S. Don't forget about us: The need to focus research on understanding adolescence as a unique development period of vulnerability in autism.

Whyte, E., Smyth, J., and Scherf, K. S. Gamification of interventions for individuals with autism: A review of serious games

Picci, G., Behrmann, M., Minshew, N., and Scherf, K. S. It's Not Just Faces: Atypical Neural Activation to Novel Objects in Autism

Scherf, K. S., Whyte, E., Minshew, N., Behrmann, M. Emergence of Holistic Processing of Novel Objects in Autism.

Scherf, K. S., Elbich, D., Minshew, N., Behrmann, M. Neural Plasticity in Face Activation Following Greeble Training in Autism

Whyte, E., Behrmann, M., Minshew, N., Scherf, K. S. Human Faces May Not be Aversive to Individuals with Autism

Haigh, S., Heeger, D., Dinstein, I., Minshew, N. and Behrmann, M. Neural variability in autism disproportionate in visual modality and in right hemisphere, in preparation.

Haigh, S., Heeger, D., Dinstein, I., MInshew, N. and Behrmann, M. Tactile hypersensitivity in autism: behavior and neural correlates, in preparation.

Talks:

Scherf, K. S., Elbich, D.E., Minshew, N., & Behrmann, M. (May, 2013). Core and extended face-processing regions are hypoactive and related to symptom severity in autism. *Annual Meeting of the Vision Sciences Society* Naples, FL.

Whyte, E., Scherf, K. S., Elbich, D., Minshew, N., Behrmann, M. (April, 2013) Human Faces May Not be Aversive to Individuals with Autism. Talk presented at the *Biennial Meeting of the Society for Research in Child Development*, Seattle, WA.

Scherf, K. S., Chair Mini-Symposium: Measuring the Dynamics of Neural Circuits in Awake, Behaving Humans: Functional Connectivity Analyses of fMRI Data. *2012 Annual Meeting of the Society for Neuroscience Meeting* New Orleans, LA.

Scherf, K. S. Face Perception in Autism. (November, 2012) Keynote Address of the *43rd NIPS International Symposium on Face Perception and Recognition, National Institute for Physiological Sciences*, Okasaki, Japan.

Elbich, D. E., Stiffler, J., Hwang, K., & Scherf, K.S. Performance Differences in Face Recognition Behavior is Related to Individual Variation in Size of Right FFA and Real World Social Networks. (November, 2012) *Annual Meeting of the Society for Neuroscience Meeting* New Orleans, LA.

Lerner, Y., Scherf, K.S., Katkov, M., Behrmann, M., & Hasson, U. Developmental Changes in Inter-Subject Correlation of Cortical Activity. (November, 2012) *Annual Meeting of the Society for Neuroscience Meeting* New Orleans, LA.

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Tables & Figures

Table 3. Intervention A Enrollment and Testing Progress at Carnegie Mellon University (CMU)

Group	Enrolled	Baseline Testing	Post-Intervention Testing	1 yr Follow-Up Testing
Autism Intervention	12/10	12/12	11/12^	11/11
Autism Control	10/10	10/10	10/10	10/10
Typical Control	12/10	12/10	11/12*	10/11[#]
Percent Complete:	113%	113%	97%	97%

^One participant was withdrawn from the study because of behavioral and technical problems during intervention.
*One participant withdrew from study because he received braces following the baseline testing.
[#]One participant chose to withdraw from the study.

Table 4. Intervention B Enrollment and Testing Progress at Penn State University (PSU)

Group	Enrolled	Baseline Testing	Post-Intervention Testing	1 yr Follow-Up Testing
Autism Intervention	9/10	9/10	9/10	7/10*
Typical Controls	10/10	10/10		
Percent Complete:	90%	95%	90%	70%

*Two participants were unable to travel to PSU for the final follow-up testing session.

Figure 21. Performance of HFA adolescents during Greeble training. Participants individuated Greebles and categorized Greebles into 1 of 5 Families in separate trials.

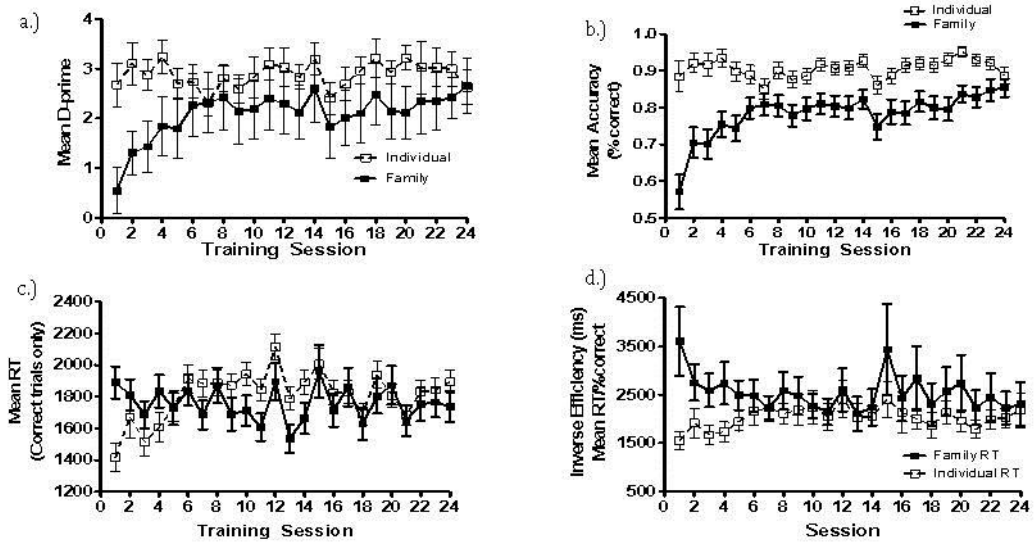


Figure 22 Performance of the group of HFA adolescents during Greeble training plotted as a function of individuation on each of the training Greebles.

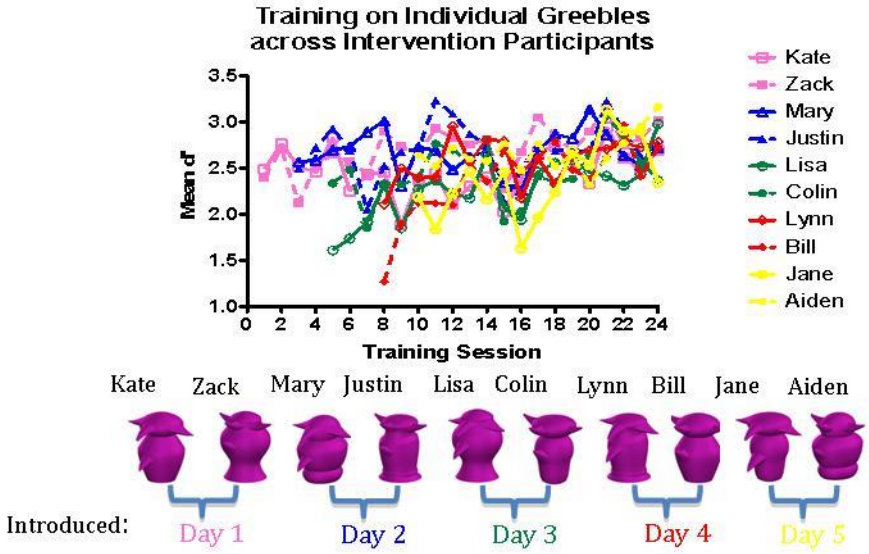


Figure 23. Group level performance on Greeble individuation scaled by the number of Greebles that were required to be individuated on each session.

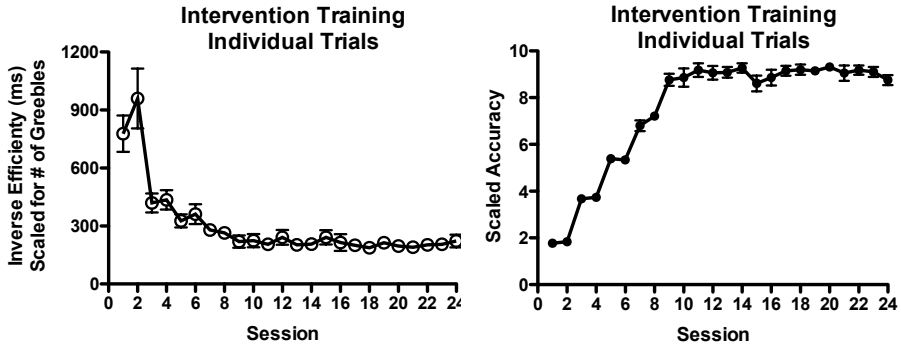


Figure 24. Performance in all groups in the Greeble Sequential Matching task across the three time points.

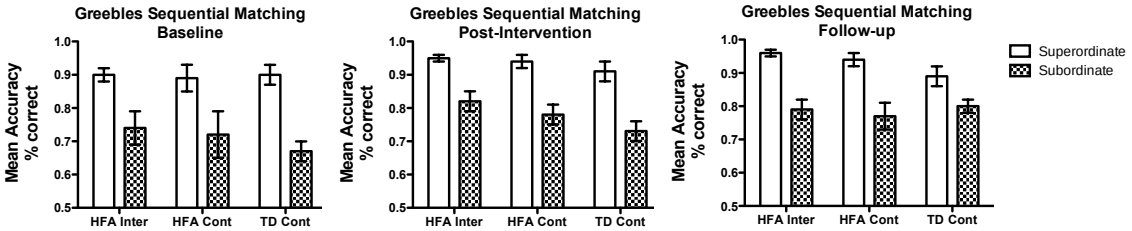


Figure 25. Performance in all groups in the Greeble Part-Whole task across the three time points.

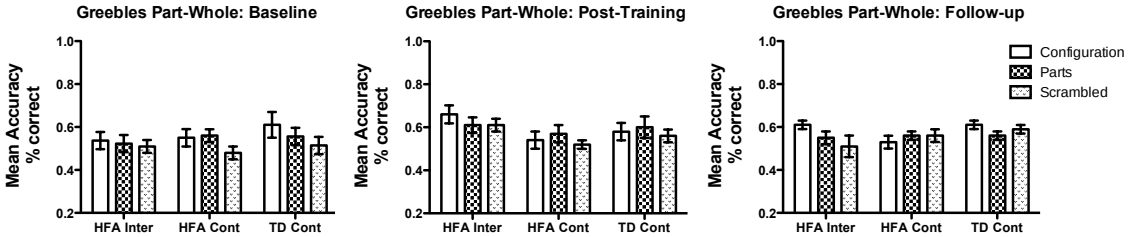


Figure 26. Accuracy in upright (a) and inverted (b) unfamiliar face recognition on the CFMT in each group across 3 testing sessions (pre-intervention, post-intervention, 1-yr follow-up).

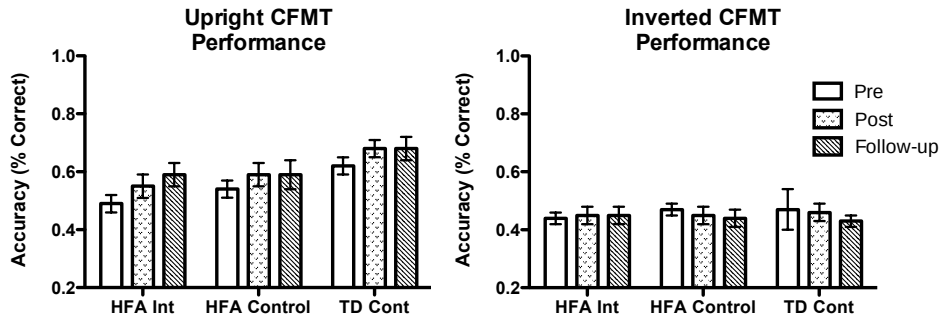


Figure 27. Accuracy in the Faces Sequential Matching task in each of the groups across the three time points.

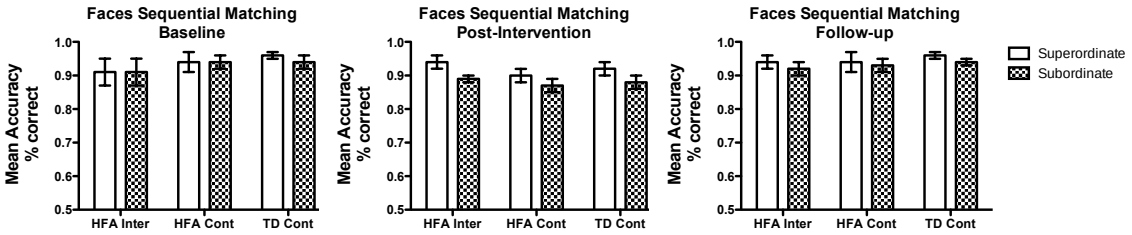


Figure 28. Performance in the Faces Part-Whole task for all three groups at each time point. All three groups evinced holistic processing for faces (config > parts) at baseline and at follow-up.

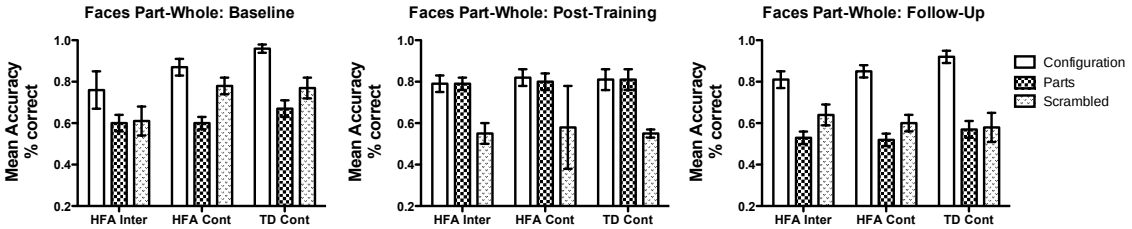


Figure 29. Training performance of HFA adolescents in Intervention B.

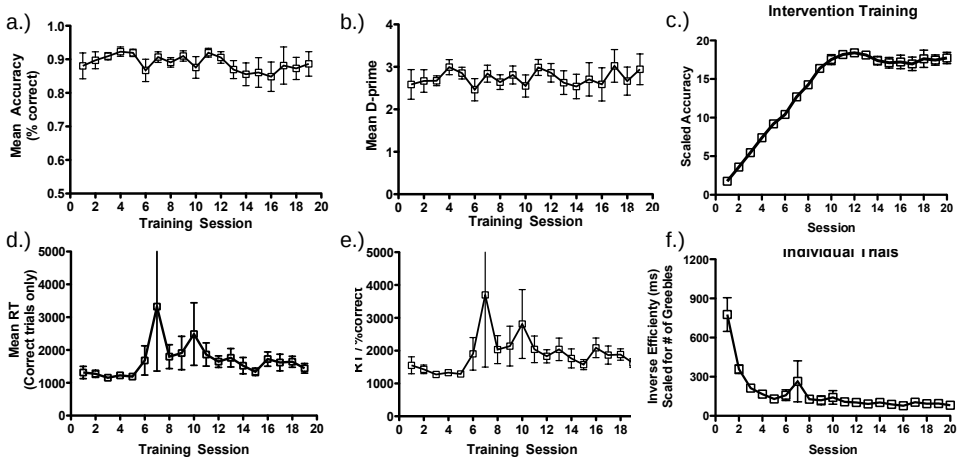


Figure 30. Performance of TD and HFA intervention adolescents in the Greeble Composite Task. TD adolescents did not show holistic processing of Greebles (i.e., aligned < misaligned). HFA participants developed holistic processing of Greebles following training; however, the holistic processing was short lived and was not sustained at follow-up.

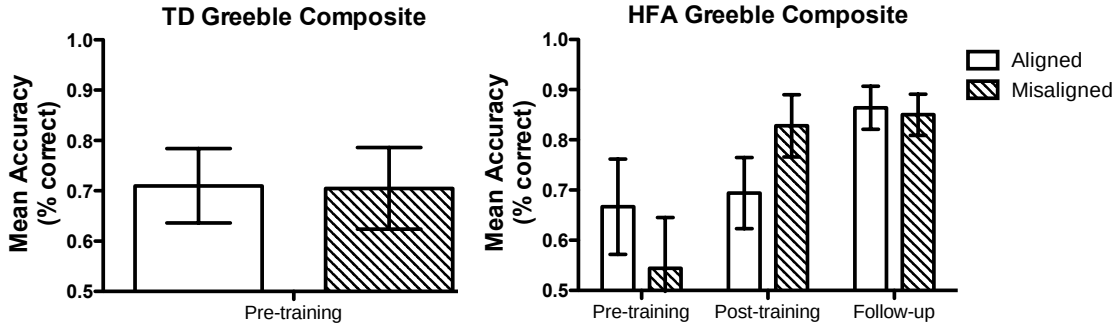


Figure 31. Performance of HFA intervention adolescents in the Greeble and Car Sequential Matching Tasks.

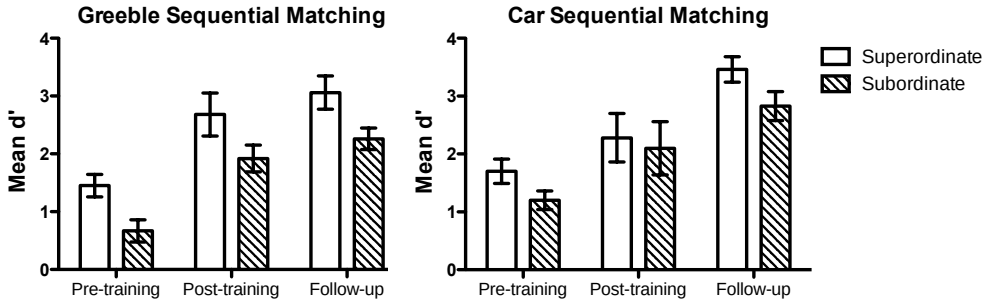


Figure 32. Performance of TD and HFA intervention adolescents in the Face Composite Task. Both TD and HFA adolescents exhibited holistic processing of faces prior to the intervention.

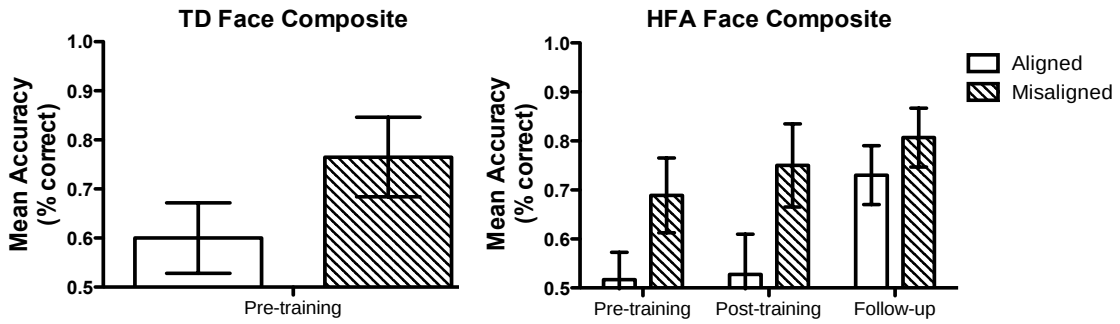


Figure 33. CFMT performance of HFA adolescents across 3 time points in Intervention B as well as TD adolescents.

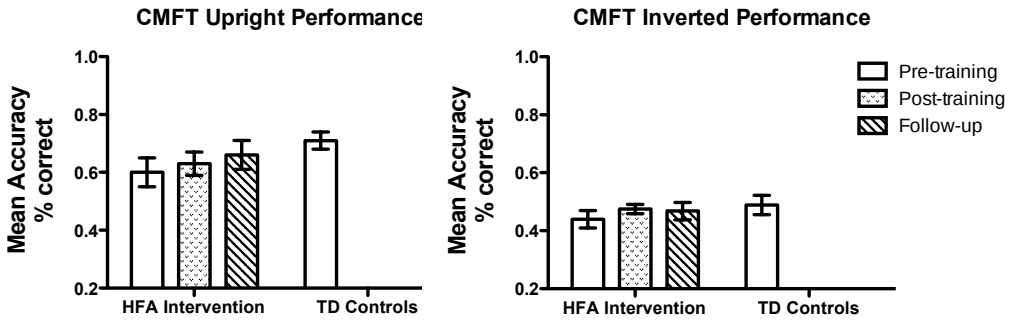


Figure 34. Performance of HFA intervention adolescents in the Face Sequential Matching Task.

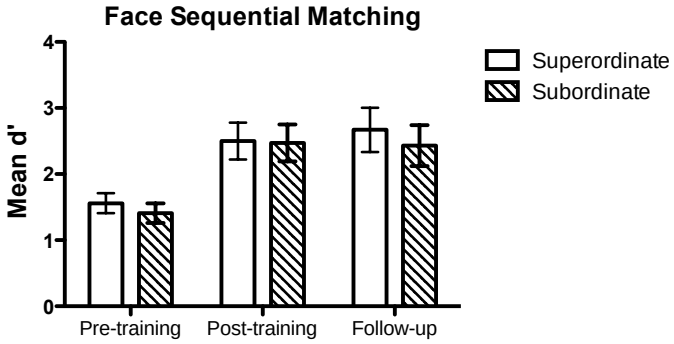


Figure 35. Group differences in category-selective activation for faces (a), common objects (b), and houses (c). Regions in which the HFA adolescents exhibited LESS activation than the TD controls are represented in red, and regions in which they exhibited MORE activation are represented in blue in each map. The maps are all corrected at p < .05.

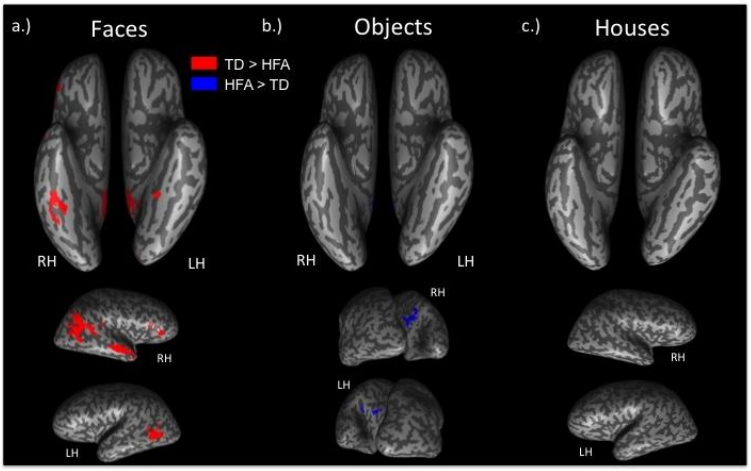


Figure 36. Correlations between face-related activation in the HFA adolescents and symptom severity (a) and adaptive behavior (b).

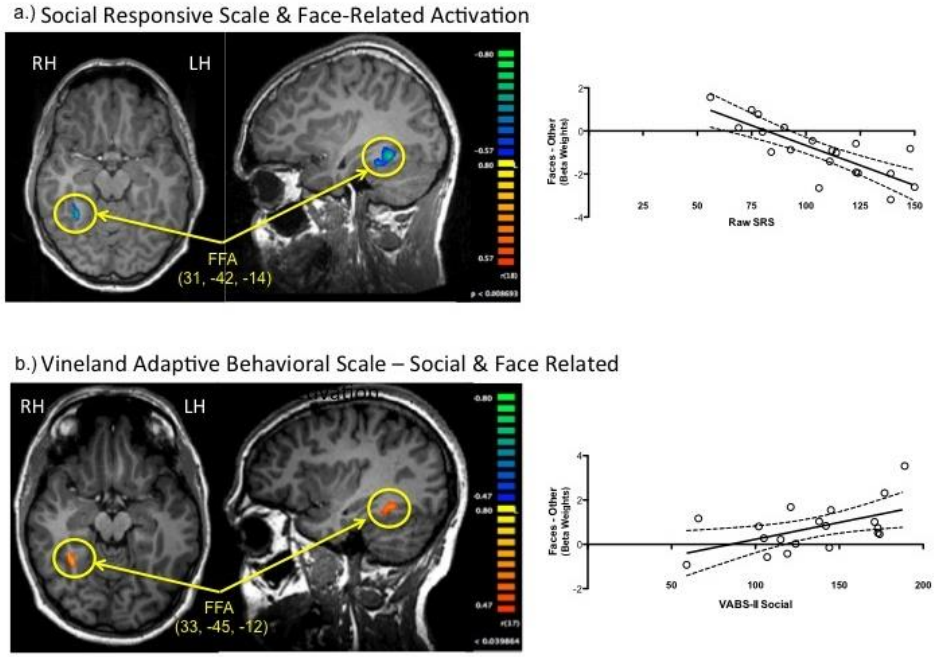


Figure 37. Changes in face-, Greeble-, and object-related activation from baseline to post-intervention and to follow-up in each of the HFA intervention participants from Intervention A. All maps corrected for false positive activation (p<.01).

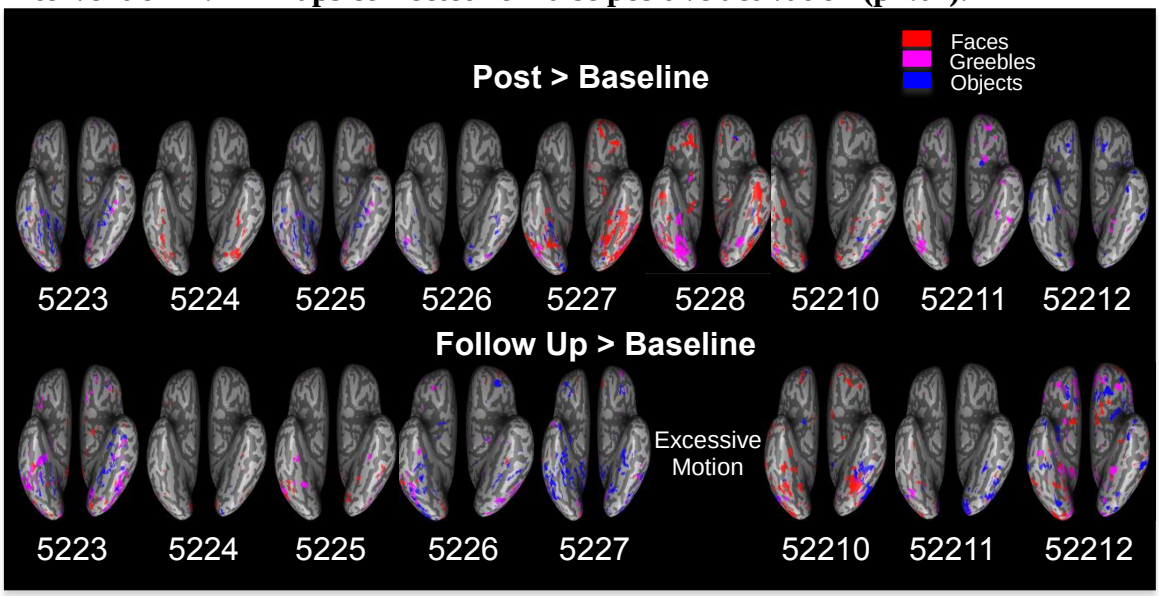


Figure 38. Changes in face-, Greeble-, and object-related activation from baseline to post-intervention and to follow-up in each of the HFA intervention participants from Intervention B. All maps corrected for false positive activation (p<.01).

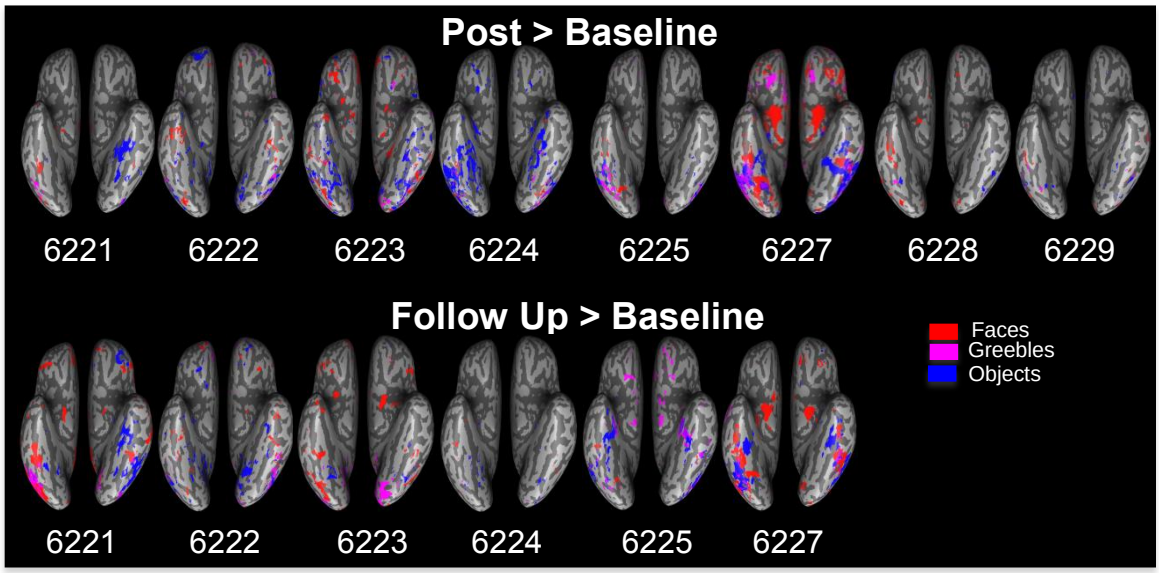


Figure 39. Procedure for defining structural regions of interest to extract the ILF, IFOF, and control tracts (Figure 3 in Scherf et al., 2013).

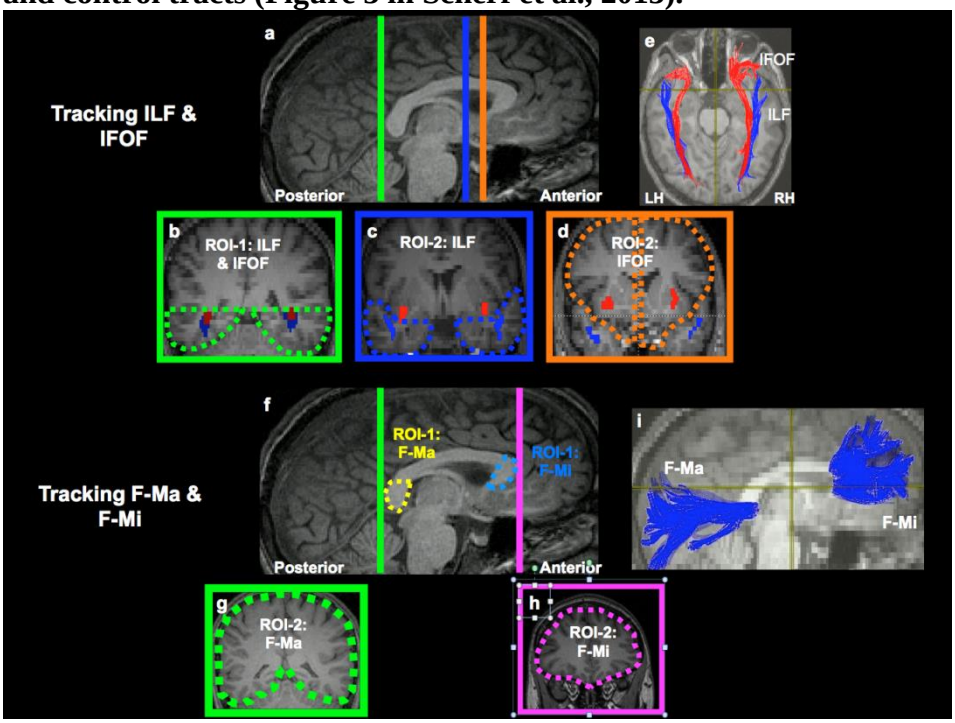
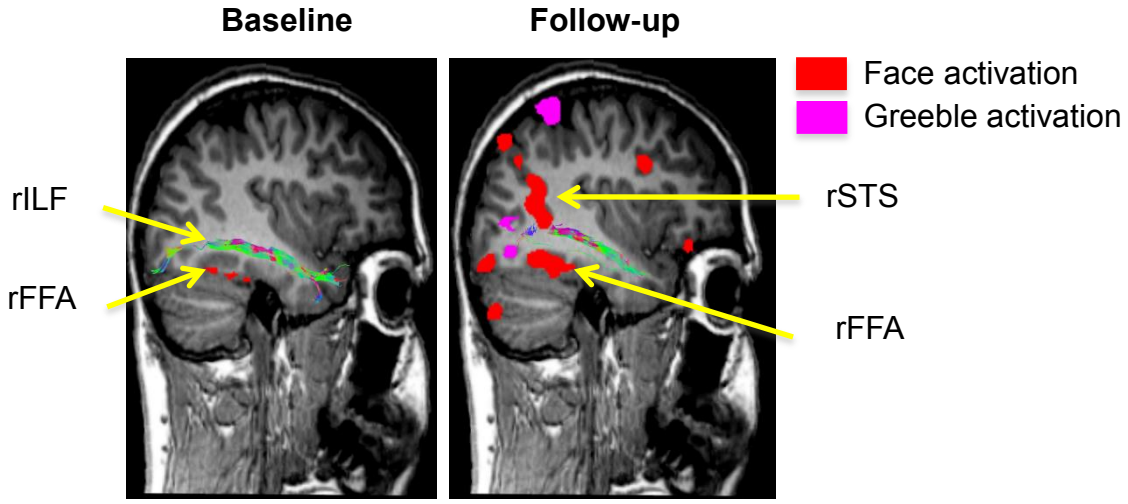


Figure 40. Face- and Greeble-related activation as well as delineation of the ILF in the right hemisphere at baseline and follow-up in a single HFA adolescent from Intervention B.



Aim HT1: Addressing Health Disparities in Practices Through An Educational Program for PittNet, A Practice-Based Pediatric Research Network of 106 Pediatricians, 5 Counties, and 115,000 Children

Formative and Summative Evaluation Process.
We proposed to improve health status and access through a web-based, archived CME accredited lecture program on ASD and related medical and behavioral issues created for a large established pediatric research network (PittNet) that serves 115,000 families in 5 counties representing all racial and geographic segments of Western PA. Dr. Minshew attended an initial meeting with the PittNet executive committee to get ideas for presentation topics. Dr. Minshew identified presenters for different lecture topics. PittNet sent out email blasts when new lectures were available for viewing and included information about CMEs that could be earned by taking part in the lectures and pre and post-tests.

Summative Evaluation.
The lectures were broken into two 30 minute lectures with a pre and post-test.

Formative Evaluation.
We provide email contact information so that participants could send in questions to the presenter and provide feedback about the presentations.

The following CME accredited lectures have been provided to PittNet for their pediatricians to access:

- Early Identification of Autism-Susan Campbell Ph.D.
- Autism and My Sensory Based World-Temple Grandin Ph.D.
- Different Kinds of Minds-Temple Grandin Ph.D.
- Normative Patterns, Individual Differences and Signs of Risk in Infant Motor Development-Jana Iverson Ph.D.
- Late to Talk: Sign of a Developmental Problem or a Developmental Difference-Diane Williams Ph. D.
- Understanding the Differences in ASD-Holly Gastgeb Ph.D.
- The Science of Autism: Transformative Advances in the Making (Part 1)-Nancy Minshew MD.
- The Science of Autism: Transformative Advances in the Making (Part 2)-Nancy Minshew MD.
- The Verbal Individual with Autism: Have you seen this Patient?-Nancy Minshew MD and Diane Williams Ph.D.
    - CME accreditation in process

Plans:
Once all lectures have been accredited and released to the Pediatric PittNet practices, we will seek approval to make them widely available to anyone in the region and state who is interested. We will also provide them to the Pennsylvania Department of Public Welfare Autism Bureau for distribution.

Aim HT2 (original). Addressing Health Disparities Through Collaboration With A Minority Serving Community Organization- PLEA, Programs for Living, Education and Advocacy: Training & Adult Intervention

The goal of Aim HT2 (original) was to adapt and pilot a novel cognitive rehabilitation approach, *Cognitive Enhancement Therapy (CET),* in 16 resource poor and/or minority verbal adults with ASD in collaboration with a community partner, PLEA. This intervention consists of a comprehensive 18-month neurocognitive and social-cognitive rehabilitation program that, for the first time, targets the core cognitive deficits of ASD, and is expected to improve the cognitive and adaptive skills necessary for employment, independent living, and interpersonal success.

Overview of Cognitive Enhancement Therapy

Cognitive Enhancement Therapy is an evidence-based cognitive rehabilitation intervention that was originally designed for individuals with schizophrenia. The focus of this DOH study aim was to adapt CET to verbal adults with ASD because they experience similar social and cognitive challenges to those in schizophrenia. CET integrates 60 hours of neurocognitive training with 45 one and one-half hours of weekly social-cognitive group sessions over the course of 18 months. Following baseline assessments, CET begins by pairing two participants with ASD to work together with a therapist-coach on computer-assisted neurocognitive training exercises designed to enhance attention, memory, and problem-solving abilities. These weekly training sessions not only focus on enhancing cognition within these domains, but also facilitate secondary socialization by encouraging participants to actively provide support to each other during the sessions and to respond to Socratic coaching questions, prompts, and cues to enhance performance. CET "coaches" actively integrate key CET concepts during the computer sessions (e.g., managing emotions, working memory, cognitive flexibility, and giving support).

After 2-3 months of computer training in the Attention module of the program, three participant pairs (6 people) join to form a weekly CET social-cognitive group. The neurocognitive training continues concurrently, progressing through the completion of the attention, memory and problem-solving modules. The focus of the weekly social-cognitive groups is to enhance social cognition in an effort to improve socialization, interpersonal effectiveness, and overall social adjustment. By design, there is a structured format for each group session. During the social-cognitive groups, a recovery plan is developed collaboratively with each participant. Recovery plans personalize the experience, help individuals focus on a problem area that is important for them to work on in the group, and provide an individualized way of measuring progress over time. Recovery plans are developed at three points during the group experience (beginning, approximately mid-treatment, and towards the end of the group sessions). In total, there are three modules in the CET social-cognitive curriculum that progress from basic concepts, to core components of social cognition, and application. Each module contains relevant psychoeducational lectures, homework, and in-group exercises.

Rationale for CET in Autism Spectrum Disorders

While important differences exist between autism and schizophrenia (e.g., age of onset, psychosis, restricted repetitive behavior), convergence in the cognitive manifestations of these conditions is becoming increasingly recognized as a result of the growing number of individuals with ASD without intellectual disability has increased and aged into adulthood. Both autism and schizophrenia are well-known to be characterized by significant impairments in neurocognitive and social-cognitive functioning (Penn, Corrigan, Bentall, Racenstein, & Newman, 1997; Volkmar, Lord, Bailey, Schultz, & Klin, 2004). Indeed, numerous direct comparisons of the two conditions have found similar degrees of impairment in social and non-social cognitive domains, including theory of mind (Pilowsky, Yirmiya, Arbelle, & Mozes, 2000), gaze orientation (Sasson et al., 2007), emotion perception (Couture et al., 2010), speed of processing (Goldstein, Minshew, Allen, & Seaton, 2002; Schneider & Asarnow, 1987), and executive functioning (Schneider & Asarnow, 1987). CET is one of the only cognitive rehabilitation interventions that systematically targets both social and non-social cognitive impairments. These deficits in social and non-social cognition are known to be related (and perhaps dependent), such that challenges in a non-social domain (e.g., slow speed of processing) can negatively affect performance in a social domain (e.g., identifying social cues) (e.g., Sergi, Rassovsky, Nuechterlein, & Green, 2006). Given the interrelationships between these areas and challenges that verbal adults with ASD have in both of these domains, the comprehensive nature of CET may afford the greatest opportunity to these individuals for cognitive improvement that results in meaningful gains in functional outcome. Further, many of the specific targets of CET (processing speed, perspective-taking, social context appraisal, emotion perception, emotion management) are among the most common and challenging areas for adults with autism, suggesting a congruence between the targets of the approach and the areas of greatest need for treatment in the ASD population.

The pathophysiology of autism and schizophrenia has also begun to show considerable overlap, as studies have reported similar neurobiologic and genetic pathways affected in both conditions. Shared genetic abnormalities in regions of the genome coding for synaptic formation and neurotransmission have been found in both disorders (e.g., Guilmatre et al., 2009), and studies have noted similar functional abnormalities in affected brain regions (Pinkham, Hopfinger, Pelphrey, Piven, & Penn, 2007; Sugranyes, Kyriakopoulos, Corrigall, Taylor, & Frangou, 2011), particularly in those areas associated with social cognition. Finally, recent neuroimaging findings from a CET trial in early course schizophrenia have shown that at least some of the beneficial effects of the treatment are due to a neuroprotective effect of CET on brain structures (e.g., amygdala, fusiform gyrus) commonly implicated in social-cognitive impairment in both schizophrenia and autism (Eack et al., 2010b), thus providing initial evidence that the approach may target underlying neural pathways that are shared between these disorders. Collectively these observations suggest that a treatment that effectively addresses the neural basis of information processing deficits in schizophrenia is likely to have promise for treating similar impairments in autism.

Original Milestones and Accomplishments
The milestones and timeline for this aim originally outlined in the application are below:

Period	Milestones
6/1/09-6/30/09	• Hire clinicians and prepare clinical infrastructure
7/1/09-6/30/10	• Adapt CET for ASD, recruit 4 patients for CET treatment, start CET pilot group A
7/1/10-6/30/11	• Start CET pilot group B, begin training PLEA clinicians, recruit 4 additional patients
7/1/11-6/30/12	• Start CET pilot group C, transition program operation completely to PLEA, supervise PLEA clinicians
7/1/12-5/31/13	• Start CET pilot group D, recruit 4 additional patients, begin data analysis of treatment effects on cognition and functioning

The hiring of initial clinicians and preparation of clinical infrastructure for adapting and piloting CET for adults with ASD was completed in 6/2009. Due to faster than expected recruitment, as well as additional funding from NIMH, a total of 14 adults with ASD were recruited to constitute pilot groups A-D in the first 6 months of the project by 12/2009. Only 14 adults with ASD were allocated to these pilot groups instead of the 16 originally proposed, and groups A-B and C-D were collapsed to constitute larger group sizes in the event of attrition. DOH funds supported 1 part-time clinician and partial investigator effort to complete the project, as originally outlined in the proposal. As reported in our 2010 progress report, on 1/2010 the milestone of training PLEA clinicians in CET was not pursued in favor of building a stronger evidence base for CET in autism before dissemination (primarily through completing the pilot and subsequent randomized controlled trial). Given that there was no evidence base for CET in autism, it was viewed as too experimental to proceed with dissemination. Nonetheless, we continued to collaborate with PLEA to educate their staff on autism and receive minority and resource poor referrals for the CET groups.

Original Milestones Met:

1. Adapt CET for Adults with ASD – completed 1/2010
2. Pilot CET in 14 adults with ASD – completed 10/2011
3. Coordinate with PLEA to include underserved minority and/or resource poor adults with ASD – ongoing throughout 6/2009 – 5/2013
4. Conduct data analysis of CET effect on cognition and functioning – completed 2/2013
5. Publish results from pilot groups A-D – published 4/2013

Original Milestones Not Met:

1. Train PLEA in CET
2. Pilot Cohorts A-D included 14 instead of 16 adults with ASD

Study Results for Original Aim HT2

Finding 1. Cognitive Enhancement Therapy is Feasible and Acceptable in Adults With ASD

This finding was supported in part by DOH funds, and the results have been published in the following paper with acknowledgment of DOH funding:

Eack, S. M., Greenwald, D. P., Hogarty, S. S., Bahorik, A. L., Litschge, M. Y., Mazefsky, C. A., & Minshew, N. J. (2013). Cognitive Enhancement Therapy for adults with autism spectrum disorder: Results of an 18-month feasibility study. Journal of Autism and Developmental Disorders, 43(12), 2866-2877.

The primary focus of this DOH study was to adapt CET to be a feasible and acceptable intervention that would meet the treatment needs of verbal adults with ASD. Data from this pilot, which was the focus of the **Original Aim HT2** have now been collected, cleaned, fully analyzed and published. Participants included 14 verbal adults enrolled in a feasibility study of CET for ASD. Individuals were included if they met expert clinical opinion and research criteria for autistic disorder or autism spectrum disorder using the Autism Diagnostic Observation Schedule (Lord et al., 2000), met autism cutoffs on the Autism Diagnostic Interview-R (Lord, Rutter, & Couteur, 1994), were age 18-45 years, had an IQ $\geq$ 80 as assessed by the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999), had not abused substances in the 3 months prior to enrollment, did not exhibit behavioral problems that would negatively impact other participants in the program, and demonstrated cognitive and social disability on the Cognitive Style and Social Cognition Eligibility Interview (Hogarty et al., 2004). This semi-structured interview has been validated in previous studies of CET for patients with schizophrenia, and is used to provide a clinical assessment of cognitive dysfunction and social impairment indicative of the need for treatment.

<u>Enrolled participants</u> were mostly young adults, with an average age of 25.29 (*SD* = 5.72) years, predominantly male (*n* = 12), and all Caucasian. Over half (*n* = 8) of the participants met criteria for autism, with the remaining meeting criteria for ASD. Psychiatric, learning, and other developmental comorbidities were common (*n* = 7) and included anxiety disorders (*n* = 4), depressive disorders (*n* = 4), personality disorders (*n* = 1), developmental dysgraphia (*n* = 1), learning disorder NOS (*n* = 1), mathematics disorder (*n* = 1), and motor coordination disorder (*n* = 1); four participants had more than one significant comorbidity. Although the majority (*n* = 12) of individuals had attended some college and the average full scale IQ for the sample was above average (*M* = 117.70, *SD* = 16.77, range = 92 to 157), only half (*n* = 7) of the participants were employed, and all participants, except for one, were living with their family. Of those individuals employed, all were in jobs below levels commensurate with their education and intellectual level; every employed participant had received at least some college education and had higher levels of intellectual functioning (range of IQ = 107 to 157), yet none were employed in positions greater than clerical work.

Several adaptations to CET were made to ensure the applicability of the treatment to the unique needs of adults with ASD. The largest adaptations occurred with regard to the early components of the social-cognitive group curriculum, which originally focused on psychoeducation about schizophrenia. Such content was removed and replaced with the latest knowledge and understanding of ASD and its impact upon cognition, information processing, social cognition, sensory perception, and emotion management. In addition, some of the computer exercises in the neurocognitive training produced sounds that were uncomfortable to some participants with ASD, and these exercises were altered to mute such sounds. Coaches also had to alter their approach in working with participants with ASD, who unlike individuals with schizophrenia, often do not ask for help when they needed it and commonly needed greater clinical outreach and engagement (more structured support) in order to participate. A more guided, repetitive, and elaborated approach was also employed in the training of some advanced abilities (e.g., providing support, perspective-taking) in the social-cognitive groups, as the impairments in social cognition experienced by individuals with ASD who have not had normal early periods of social development were at times considerably greater than those observed in schizophrenia. Overall, however, we found the need for adaptations to be surprisingly minimal compared to initial expectations, as the majority of the content in CET was perceived as highly applicable by both ASD participants and clinicians. These adaptations are being collated in a supplement to the existing CET treatment manual.

Measures of feasibility and acceptability included session attendance, treatment dropout, and standardized assessments of satisfaction administered by non-treating research staff. In addition, a battery of neurocognitive and social-cognitive assessments were administered prior to treatment, and at 9 (mid-treatment) and 18 (treatment completion) months. Of the 14 individuals who enrolled in the study, 11 (79%) completed the entire 18 months of treatment. One participant withdrew at 9 months due to increased hours of employment; 1 was administratively terminated at 9 months due to personality disorder instability; and 1 completed the entire 18 months of the study, but could not attend the social-cognitive groups due to persistent family and transportation problems, and thus was not considered to have completed treatment. Treatment adherence was high across both neurocognitive training (89%) and social-cognitive group (85%) sessions, with an 87% average overall attendance rate at treatment sessions. In addition, treatment satisfaction among all participants (treatment completion ratings for completing participants and interim ratings for partial completers) was also high with overall satisfaction scores for the program of 3.57 (SD = .51) out of 4.00. These ratings indicate that individuals were "mostly satisfied" to "very satisfied" with CET (see *Table 5*).

Although the emphasis of this study was to assess the feasibility of adapting and applying CET to verbal adults with ASD, preliminary cognitive and behavioral outcome data were examined to provide an initial assessment of treatment efficacy. As can be seen in *Figure 41*, highly significant (all $p < .001$) and large (d = 1.40 to 2.29) levels of improvement were observed across composite domains of neurocognition, cognitive style, social cognition, and social adjustment. Neurocognitive improvement was particularly large in the domain of processing speed, which was also the greatest area of non-social cognitive impairment in the sample prior to treatment. Significant levels of improvement were observed in all neurocognitive domains, with the

exception of attention/vigilance (see *Table 6*). In addition, all clinician-rated aspects of dysfunctional cognitive style showed significant levels of improvement.

Social cognition and social functioning proved to be the largest domains of improvement in this study (see *Figure 41*). Social cognition was significantly improved across both clinician-rated and performance-based measures, particularly with regard to emotion understanding and management. A trend-level (p = .055) effect was observed for improvements in emotion perception, which was primarily due to an improvement in accuracy in the perception of sad faces, $t(25) = 2.43$, $p = .023$, $d = .61$. Importantly, these social-cognitive gains generalized to broader improvements in adaptive function and social adjustment, as large and highly significant levels of improvement were observed in vocational effectiveness, interpersonal effectiveness, and participants' ability to adjust to their condition, as measured by the Cognitive Style and Social Cognition Eligibility Interview (see *Table 6*). Taken together, such findings suggest that CET is a feasible, acceptable, and potentially effective approach to the treatment of cognitive impairments in adults with ASD that can confer substantial benefits to social and adaptive function in these individuals.

Conclusions. In summary, this research provides the first evidence of the feasibility of CET, a comprehensive neurocognitive and social-cognitive remediation approach, in verbal adults with ASD. Such cognitive rehabilitation interventions have been available and highly successful with individuals with other neurological disorders, and although these results are limited by a modest sample size and the absence of a treatment control condition, findings suggest that CET is an acceptable and satisfying treatment for verbal individuals with ASD that may have substantial benefits for cognitive and functional outcomes in this population.

Table 5
Acceptability and Adherence of Cognitive Enhancement Therapy in Adults with Autism Spectrum Disorder (N = 14).

Measure	*N*	%
Adherence		
Number completing first 9 months of CET	14	100
Number completing entire 18 months of CET	11	79
Average percent of neurocognitive training sessions attended (*M* / *SD*)	89	15
Average percent of social-cognitive group sessions attended (*M* / *SD*)	85	14
Acceptability		
Average overall satisfaction with CET (*M* / *SD*)[a]	3.57	.51
Average CSQ-8 total satisfaction score (*M* / *SD*)[a]	3.27	.46
Number "mostly satisfied" with CET	14	100

Note. CET = Cognitive Enhancement Therapy; CSQ-8 = Client Satisfaction Questionnaire 8
[a]Rated on a 1 to 4 scale, with higher scores indicating greater satisfaction

Figure 41. Effects of Cognitive Enhancement Therapy on Composite Indices of Cognition and Behavior in Adults with Autism Spectrum Disorder (*N* = 14).

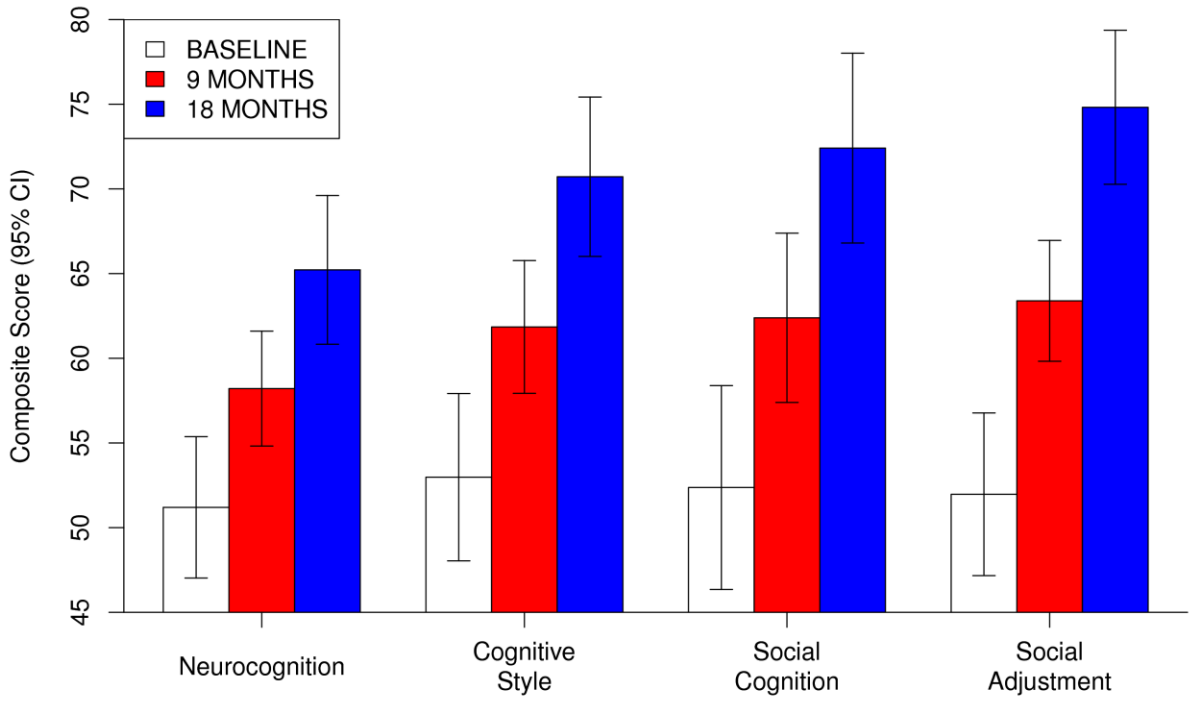


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Table 6
Univariate Effects of Cognitive Enhancement Therapy on Cognition and Behavior in Adults with Autism Spectrum Disorder (N = 14).

	Baseline		9 Months		18 Months		Analysis		
Variable	*M*	*SE*	*M*	*SE*	*M*	*SE*	*t*	*p*	*d*
Neurocognition[a]	51.20	2.13	58.21	1.73	65.22	2.24	5.23	.000	1.40
Processing speed[b]	38.69	8.26	57.33	6.96	75.98	8.30	4.16	.000	1.22
Attention/vigilance[b]	49.28	8.90	50.97	8.08	52.66	8.93	.45	.657	.12
Working memory[b]	55.59	8.32	71.66	6.84	87.72	7.57	3.96	.001	.81
Visual learning[b]	53.20	8.10	60.12	7.55	67.03	8.23	2.24	.034	.43
Verbal learning[b]	41.03	6.28	55.47	4.26	69.92	6.15	3.19	.004	1.13
Problem-solving[b]	45.82	8.93	56.85	8.18	67.88	7.99	5.00	.000	.63
Cognitive Flexibility									
WCST: Perseverative errors (log)	2.23	.16	1.78	.08	1.32	.19	-2.80	.010	-1.33
WCST: Non-perseverative errors (log)	2.10	.19	1.69	.10	1.27	.19	-2.54	.018	-.93
Cognitive Style[a]	52.98	2.52	61.85	2.00	70.72	2.40	6.15	.000	1.77
Impoverished style[c]	9.54	.44	8.34	.37	7.14	.42	-5.27	.000	-1.01
Disorganized style[c]	8.71	.57	7.68	.50	6.65	.55	-4.22	.000	-.95
Rigid style[c]	10.71	.52	9.49	.44	8.26	.46	-5.81	.000	-1.42
Total impairment, disability, and social handicap[d]	28.77	.99	25.32	.76	21.87	.94	-5.79	.000	-1.69
Highest cognitive style score[c]	11.59	.46	10.13	.40	8.67	.45	-6.28	.000	-1.70
Social Cognition[a]	52.37	3.07	62.39	2.55	72.41	2.86	6.60	.000	2.00
Social Cognition Profile[e]									
Tolerant factor	3.35	.11	3.73	.09	4.11	.10	8.02	.000	1.75
Supportive factor	2.45	.14	3.05	.13	3.65	.14	9.79	.000	2.39
Perceptive factor	2.58	.13	3.15	.08	3.72	.11	7.09	.000	2.04

Confident factor	2.62	.13	3.10	.09	3.58	.10	6.38	.000	1.36
MSCEIT									
Emotion facilitation (*z*)	.21	.28	.16	.23	.11	.23	-.44	.661	-.10
Emotion understanding (*z*)	-.11	.31	.26	.25	.63	.28	2.35	.027	.73
Emotion management (*z*)	-.02	.27	.30	.20	.61	.21	2.19	.038	.62
Penn Emotion Recognition Test-40[f]	30.80	1.09	31.32	1.08	31.85	1.12	2.01	.055	.24
Social Adjustment[a]	51.97	2.45	63.39	1.82	74.82	2.32	7.43	.000	2.29
Cognitive Style and Social Cognition Eligibility Interview									
Vocational ineffectiveness[g]	3.77	.16	3.24	.13	2.71	.17	-5.53	.000	-1.52
Interpersonal ineffectiveness[g]	4.03	.13	3.43	.12	2.82	.16	-7.48	.000	-2.54
Adjustment to disability[g]	3.12	.15	2.52	.09	1.92	.08	-7.22	.000	-1.82

Note. Means and standard errors are adjusted from linear mixed-effects intent-to-treat models. Effect sizes, *t*-tests, and *p*-values reflect the results of these mixed-effects models evaluating the size and significance of change over the entire 18-months of treatment in reference to the null hypothesis of no change during this time period.

[a]Composite score scaled with a mean (*SD*) of 50 (10), with higher scores indicating better cognitive and behavioral functioning

[b]Percentile score

[c]Scores range from 3 to 15, with higher scores indicating greater cognitive dysfunction

[d]Scores range from 9 to 45, with higher scores indicating greater impairment from cognitive dysfunction

[e]Scores range from 1 to 5, with higher scores indicating better social-cognitive functioning

[f]Scores range from 0 to 40, with higher scores indicating better social-cognitive functioning

[g]Scores range from 1 to 5, with higher scores indicating worse social adjustment

MSCEIT = Mayer-Salovey-Caruso Emotional Intelligence Test, WCST = Wisconsin Card Sorting Test

Revised Aim HT2: Clinical Trial of Cognitive Enhancement Therapy for Underserved Adults with ASD

In response to our site visit and first progress report, which indicated that a randomized-controlled trial of CET would generate the strongest evidence for its efficacy in adults with ASD, we made revisions to Aim HT2 on 1/2010 by introducing the following new sub-aims:

1. Develop infrastructure to conduct a randomized-controlled trial of CET in verbal adults with ASD
2. Assess the level of cognitive impairment in verbal adults with ASD to gauge the need for cognitive rehabilitation
3. Compare cognitive performance deficits in verbal adults with ASD to adults with schizophrenia, for whom CET was originally developed
4. Initiate a randomized-controlled trial of CET in verbal adults with ASD
5. Conduct preliminary analyses of the randomized-controlled trial of CET for verbal adults with ASD on measures of cognition and adaptive function
6. Collect preliminary pre-post neuroimaging in the randomized-controlled trial of CET for verbal adults with ASD to assess neural mechanisms of treatment effects

DOH funds partially supported this considerable expansion of our original Aim HT2 by continuing to support a part-time therapist, investigator effort, and data management. In addition, new funds were attracted based on preliminary data collected in original Aim HT2 from Autism Speaks (obtained 6/2010), NIMH (obtained 5/2011), and the Department of Defense (obtained 9/2011). It is important to recognize that these new sub-aims required significantly more resources than provided by the DOH, given the high costs of randomized trials and expansion of participants. *None of these new sub-aims affected the completion of the original milestones for Aim HT2. Rather, these new sub-aims reflect the success of original Aim HT2 that was completed earlier than expected and quickly attracted new funders to partner with our DOH project to provide a more rigorous evaluation of CET in verbal adults with ASD.*

Revised Aim HT2 Sub-Aims Completed:

1. Develop infrastructure to conduct a randomized-controlled trial of CET in verbal adults with ASD – completed 8/2010
2. Assess the level of cognitive impairment in verbal adults with ASD to gauge the need for cognitive rehabilitation – completed and published 9/2013
3. Compare cognitive performance deficits in verbal adults with ASD to adults with schizophrenia, for whom CET was originally developed – completed and published 8/2013
4. Initiate a randomized-controlled trial of CET in verbal adults with ASD – completed 8/2010
5. Conduct preliminary analyses of the randomized-controlled trial of CET for verbal adults with ASD on measures of cognition and adaptive function – completed 6/2013

6. Collect preliminary pre-post neuroimaging in the randomized-controlled trial of CET for verbal adults with ASD to assess neural mechanisms of treatment effects – completed 6/2013

Revised Aim HT2 Sub-Aims Not Completed:

None

Study Results for Revised Aim HT2

Finding 1 (Sub-Aim #2, completed and published 9/2013): Cognition is Highly Impaired in Verbal Adults with ASD

This finding was supported in part by DOH funds, and the results have been published in the following paper with acknowledgment of DOH funding:

Eack, S. M., Bahorik, A. L., Hogarty, S. S., Greenwald, D. P., Litschge, M. Y., Mazefsky, C. A., & Minshew, N. J. (2013). Is cognitive rehabilitation needed in verbal adults with autism? Insights from initial enrollment in a trial of Cognitive Enhancement Therapy. Journal of Autism and Developmental Disorders, 43(9), 2233-2237.

Pre-treatment data from participants enrolled in our DOH-funded pilot (original Aim HT2) and randomized-controlled trial (revised Aim HT2) of CET were used to assess the degree to which the primary targets of CET, social and non-social cognition, were impaired in verbal adults with ASD. Participants consisted of 40 verbal adults with ASD recruited for a pilot trial of Cognitive Enhancement Therapy (Hogarty & Greenwald, 2006). Eligibility criteria for the study included a diagnosis of autism or ASD using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000), age 16 to 45, IQ $\geq$ 80 assessed by the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999), ability to speak and read English, the presence of significant social and cognitive disability based on the Cognitive Styles and Social Cognition Eligibility Interview (Hogarty et al., 2004), and the absence of significant substance use problems within the past 3 months. The Cognitive Styles and Social Cognition Eligibility Interview is a structured interview developed specifically for trials of CET (Hogarty et al., 2004) and designed to elicit information from participants on the degree to which they experience social and cognitive disability that could represent meaningful targets for treatment. Of the over 100 participants screened, none were excluded because they failed to demonstrate significant cognitive and social disability during this interview. Participants were excluded primarily due to lack of willingness to enroll in an experimental treatment trial (39%), IQ < 80 (13%), and the absence of a research diagnosis of ASD by the ADOS (12%). Participants were young adults, most were male, and the sample was predominantly Caucasian. Over half of the participants met ADOS criteria for autism, with the remaining meeting criteria for ASD. While most participants had attended some college, less than half were employed and only 6 (15%) were living independent of family.

A comprehensive battery of neuropsychological tests and performance-based assessments of social cognition was used to characterize the degree of cognitive disability experienced by verbal adults with ASD. Neurocognitive assessments included the NIMH-recommended MATRICS Consensus Cognitive Battery, which was designed to provide a broad assessment of cognitive function for use in clinical trials of cognitive enhancement interventions in patients with schizophrenia (Green et al., 2004). The Wisconsin Card Sorting Test was utilized to assess cognitive flexibility (Heaton, Chelune, Talley, Kay, & Curtiss, 1993). Social cognition was assessed with the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT; Mayer, Salovey, Caruso, & Sitarenios, 2003) and the Penn Emotion Recognition Test-40 (Kohler et al., 2003).

Clinical and cognitive characteristics of the ASD participants are presented in *Table 7*. Full scale IQ scores were within or above normative ranges (range = 80 to 157) for the sample. Despite mean intelligence scores for this sample in the above-average range, performance on tests of neurocognition and social cognition were substantially below average. Overall neurocognitive performance was below the 35th percentile, and ranged from a low of 0% to a high of 81.60%, with considerable heterogeneity in ability across domains. Nearly half (45%) of the sample performed below the 25th percentile for neurocognitive functioning, with the most marked impairments observed in speed of processing, working memory, and visual learning. All but four participants demonstrated moderate ($\geq$ .50 *SD*) or greater deficits in at least one neurocognitive domain on the MATRICS battery, and most (75%) displayed impairments in multiple domains. With regard to social cognition, participants also displayed substantial impairments in overall emotional intelligence, particularly emotion understanding and management. In addition, significant impairments in facial emotion perception were observed. On average, full scale IQ scores accounted for only 14.9% (range = 0% to 51%) of the variance in performance on these cognitive domains, and no significant differences in level of impairment were observed in any domain between those meeting ADOS criteria for autism compared to those meeting criteria for ASD (all $p > .172$). Taken together, these findings indicate significant cognitive disability among verbal adults with autism and ASD and a great need for cognitive rehabilitation treatment.

Table 7
Demographic, Cognitive, and Clinical Characteristics of Verbal Adults with Autism Spectrum Disorders (N = 40).

			Range			Normative Difference
	M / *N*	*SD* / %	Low	High	*V*	*d*[a]
Demographic						
Age	25.20	5.82	16.00	44.00	.23	-
Male	36	90%	-	-	-	-
White	34	85%	-	-	-	-
Attended Some College	28	70%	-	-	-	-
Employed	16	40%	-	-	-	-
Clinical						
ADOS Diagnosis						
Autism	23	57%	-	-	-	-
Autism Spectrum	17	42%	-	-	-	-
IQ	113.22	15.47	80.00	157.00	.14	.87
Verbal IQ	112.88	13.39	82.00	138.00	.12	.91
Performance IQ	108.65	14.61	76.00	137.00	.13	.58
Cognitive						
Neurocognition (percentile)						
Overall Composite	34.79	26.76	.00	81.60	.77	-.60
Processing Speed	38.58	31.89	.00	97.10	.83	-.46
Vigilance	46.79	31.24	.30	95.60	.67	-.16
Working Memory	38.02	31.08	.00	99.90	.82	-.37
Visual Learning	46.76	29.71	1.10	94.50	.64	-.12
Verbal Learning	37.57	28.23	1.10	90.30	.75	-.45
Problem Solving	45.71	30.71	1.40	93.30	.67	-.16
Cognitive Flexibility						
WCST - Perseverative Errors	14.90	9.49	4.00	41.00	.64	-.54
WCST - Non-Perseverative Errors	14.50	9.67	2.00	37.00	.67	-.46
Social Cognition						
Emotional Intelligence[b]	93.44	19.06	9.55	116.57	.20	-.38
Emotion Facilitation	94.12	20.65	1.46	124.28	.22	-.33
Emotion Understanding	92.60	16.33	24.04	117.96	.18	-.47
Emotion Management	89.78	12.73	41.55	110.14	.14	-.73
Facial Emotion Perception[c]	30.82	4.19	19.00	37.00	.14	-.75
Vocational Impairment[d]	3.90	.67	3.00	5.00	.17	-

Note. ADOS = Autism Diagnostic Observation Schedule, WCST = Wisconsin Card Sorting Test
[a]Effect sizes are based upon comparisons with normative test values
[b]Scores are standardized with a mean (*SD*) of 100 (15)
[c]Emotion perception accuracy scores range from 0 to 40
[d]Impairment was rated on a 5-point scale (1 = rare, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe) using the Cognitive Styles and Social Cognition Eligibility Interview

Finding 2 (Sub-Aim #3, completed and published 8/2013). Cognitive Impairments in Autism and Schizophrenia are Strikingly Similar

This finding was supported in part by DOH funds, and the results have been published in the following paper with acknowledgment of DOH funding:

Eack, S. M., Bahorik, A. L., McKnight, S. A. F., Hogarty, S. S., Greenwald, D. P., Newhill, C. E., Phillips, M. L., Keshavan, M. S., & Minshew, N. J. (2013). Commonalities in social and non-social cognitive impairments in adults with autism spectrum disorder and schizophrenia. Schizophrenia Research, 148(1-3), 24-28.

Having documented the considerable cognitive impairments that verbal adults with ASD experience, despite normal or above-average IQ levels, we next proceeded to examine the degree to which these impairments were similar to those with schizophrenia for whom CET was originally developed. Any cognitive differences between these two samples could indicate important adaptations that need to be made to CET for the autism population. Participants included 43 verbal adults with ASD who were enrolled in the CET pilot/clinical trial, 47 outpatients with schizophrenia, and 24 healthy control individuals. Inclusion criteria for the ASD sample were the same as those described above for the pilot/trial. Schizophrenia patients were included if they: (1) were between the ages of 18 and 60; (2) were diagnosed with schizophrenia or schizoaffective disorder using the Structured Clinical Interview for DSM-IV (SCID; First, Spitzer, Gibbon, & Williams, 2002); (3) were receiving and adherent to antipsychotic medication; and (4) had an IQ $\geq$ 80. Healthy volunteers were age- and gender-matched to the ASD sample. The average age for the overall sample was 29.32 (SD = 10.23) years, participants were predominantly male and mostly Caucasian. All individuals with schizophrenia were receiving antipsychotic treatment, and none of the adults with autism were taking antipsychotic medication. Patients in the schizophrenia sample were significantly older, had lower IQ scores, and were less likely to be Caucasian than the autism and healthy control groups. In addition, as is common in the disorder, 28 of the patients with schizophrenia also met SCID criteria for substance abuse or dependence. Patients with schizophrenia were significantly less likely to be employed than adults with ASD, who were less likely to be employed than healthy individuals.

Investigation of overall performance differences on composite measures of neurocognition and social cognition between adults with ASD, schizophrenia, and healthy volunteers, after adjusting for demographic and IQ characteristics, indicated that significant differences were observed in neurocognitive, $F(2, 107) = 26.75$, $p < .001$, and emotion processing social-cognitive function, $F(2, 107) = 7.51$, $p < .001$, between the study groups. Planned follow-up pairwise comparisons indicated that neurocognitive performance was greatly impaired in adult autism ($d = -1.71$, $p < .001$) and schizophrenia ($d = -1.48$, $p < .001$) participants, compared to healthy controls, but that the magnitude of neurocognitive impairment was not significantly different between those with ASD and schizophrenia ($p = .710$). Similarly, both autism ($d = -.97$, $p < .001$) and schizophrenia ($d = -.65$, $p = .016$) participants also demonstrated significant impairments in emotion processing

compared to healthy controls, but again no significant differences were observed in the magnitude of these impairments between the two patient groups (p = .331) (see *Figure 42*).

As can be seen in *Table 8*, both patient groups demonstrated significant impairments relative to healthy individuals on nearly every neurocognitive domain assessed. The largest domain of neurocognitive impairment for both groups was speed of processing. No significant differences were found in the degree of neurocognitive deficit between autism and schizophrenia participants. When examining the social-cognitive domain of emotion processing, areas affected were less uniform than basic cognitive processes. Overall emotional intelligence and emotional understanding as measured by the MSCEIT was significantly impaired in both adults with ASD and schizophrenia. Conversely, emotion recognition performance was only significantly impaired relative to healthy controls in the autism (p = .004), but not in the schizophrenia group (p = .126), although the average performance advantage for those with autism versus schizophrenia was small (d = .33) and the two groups did not significantly differ in emotion recognition ability before (p = .126) or after correction for multiple inference testing (p = .126). The greatest domain of emotion processing impairment was emotional understanding in both autism and schizophrenia, and no significant differences in degree of impairment were found between adults with ASD and schizophrenia on any social-cognitive domain of emotion processing studied. Taken together, these findings indicate considerable similarity in social and non-social cognitive impairment in verbal adults with ASD and outpatients with schizophrenia, with particularly marked deficits in speed of processing and emotion understanding observed in both of these conditions. Such findings indicate that intervention approaches such as CET that transcend diagnostic boundaries and integrate neurocognitive and social-cognitive remediation are needed to improve adaptive function and quality of life in these adults with serious neuropsychiatric disorders and poor functional outcomes.

Figure 42
Performance on Composite Indexes of Neurocognition and Social Cognition Among Adults with Autism (ASD), Schizophrenia (SZ), and Healthy Individuals (HC).

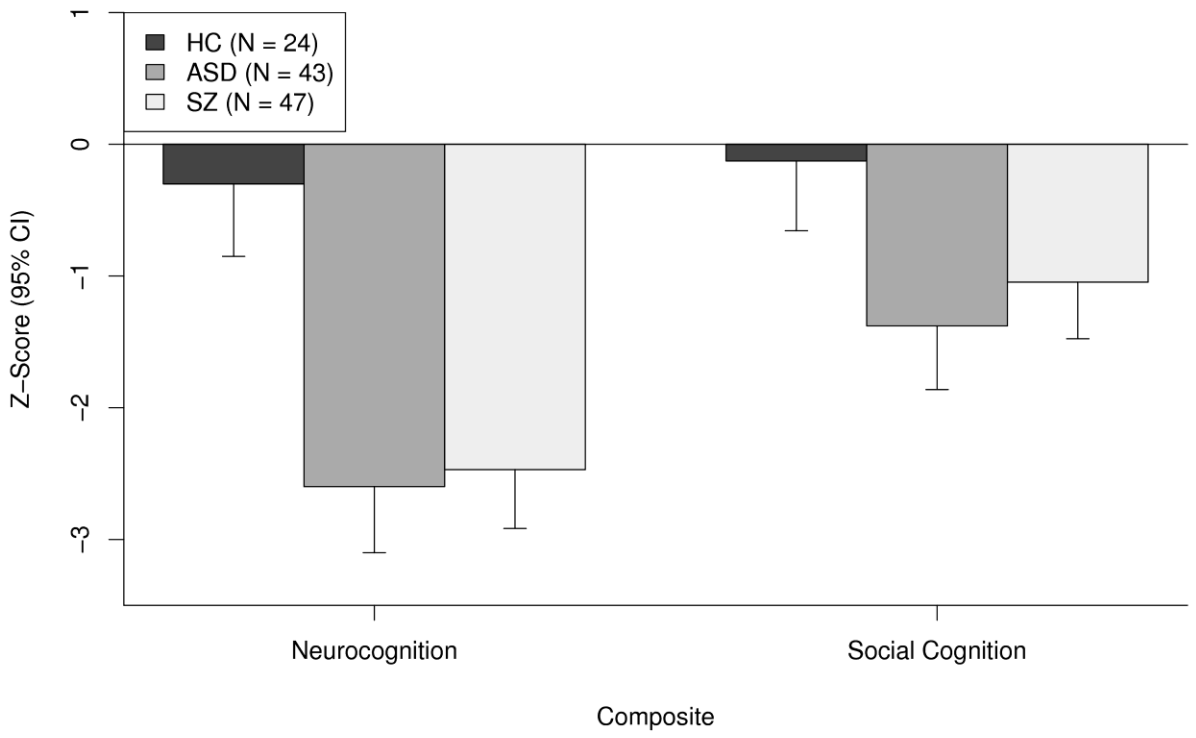


Table 8
Neurocognitive and Social-Cognitive Performance Across Adults with Autism, Schizophrenia, and Healthy Individuals.

	Healthy (HC; *N* = 24)		Autism (ASD; *N* = 43)		Schizophrenia (SZ; *N* = 47)		Analysis[a]				
Variable	*M*	*SD*	*M*	*SD*	*M*	*SD*	d_{ASD}	d_{SZ}	$d_{ASD\ v\ SZ}$	*p*	Direction
Neurocognition											
MCCB											
Speed of processing	79.04	19.15	34.92	31.63	32.80	28.35	-1.53	-1.48	-.06	< .001	HC > ASD, SZ
Attention/vigilance	52.29	27.89	36.01	32.06	27.03	24.14	-.57	-.81	-.25	.006	HC > ASD, SZ
Working memory	56.00	26.01	22.17	30.72	31.61	27.44	-1.29	-.86	.29	< .001	HC > ASD, SZ
Visual learning	62.83	23.93	40.10	29.44	38.56	30.67	-.85	-.83	-.05	.001	HC > ASD, SZ
Verbal learning	50.25	25.28	31.06	28.71	32.27	26.90	-.72	-.62	.04	.010	HC > ASD, SZ
Problem-solving	59.02	24.08	29.86	30.69	42.03	30.58	-1.05	-.56	.35	< .001	HC > ASD, SZ
WCST											
Perseverative errors	8.17	3.75	17.86	9.27	17.47	15.55	.84	.74	-.03	.002	HC < ASD, SZ
Non-perseverative errors	7.92	4.56	18.35	9.63	18.00	13.14	1.01	.89	-.03	< .001	HC < ASD, SZ
Social Cognition											
MSCEIT	101.50	12.98	91.79	14.44	92.86	14.86	-.70	-.57	.06	.017	HC > ASD, SZ
Perceiving emotions	103.54	15.92	100.56	19.58	98.56	21.05	-.15	-.24	-.08	.635	-
Facilitating emotions	102.48	13.32	94.96	14.74	98.72	15.51	-.49	-.23	.20	.157	-
Understanding emotions	102.54	9.88	88.86	13.31	90.12	12.43	-1.17	-.98	.09	< .001	HC > ASD, SZ
Managing emotions	95.25	11.60	88.98	12.54	91.22	12.86	-.49	-.29	.14	.153	-
Penn Emotion Recognition Test											
Total correct	34.25	2.40	30.77	4.14	32.44	4.34	-.84	-.40	.33	.005	HC > ASD
Reaction time (log)	7.55	.15	7.74	.22	7.67	.37	.73	.43	-.21	.019	HC < ASD

MCCB = MATRICS Consensus Cognitive Battery; MSCEIT = Mayer-Salovey Caruso Emotional Intelligence Test; WCST = Wisconsin Card Sorting Test
[a]Omnibus *F*-test results for group differences from general linear models, two-tailed, adjusting for age, gender, race, and IQ

Finding 3 (Sub-Aims #4-5, completed 6/2013). Results From an Initial Randomized Controlled Trial of CET Are Promising

This finding was supported in part by DOH funds through the funding of a part-time therapist, data management, and partial investigator effort. There are no current manuscripts or published papers on this finding, as the controlled trial remains ongoing.

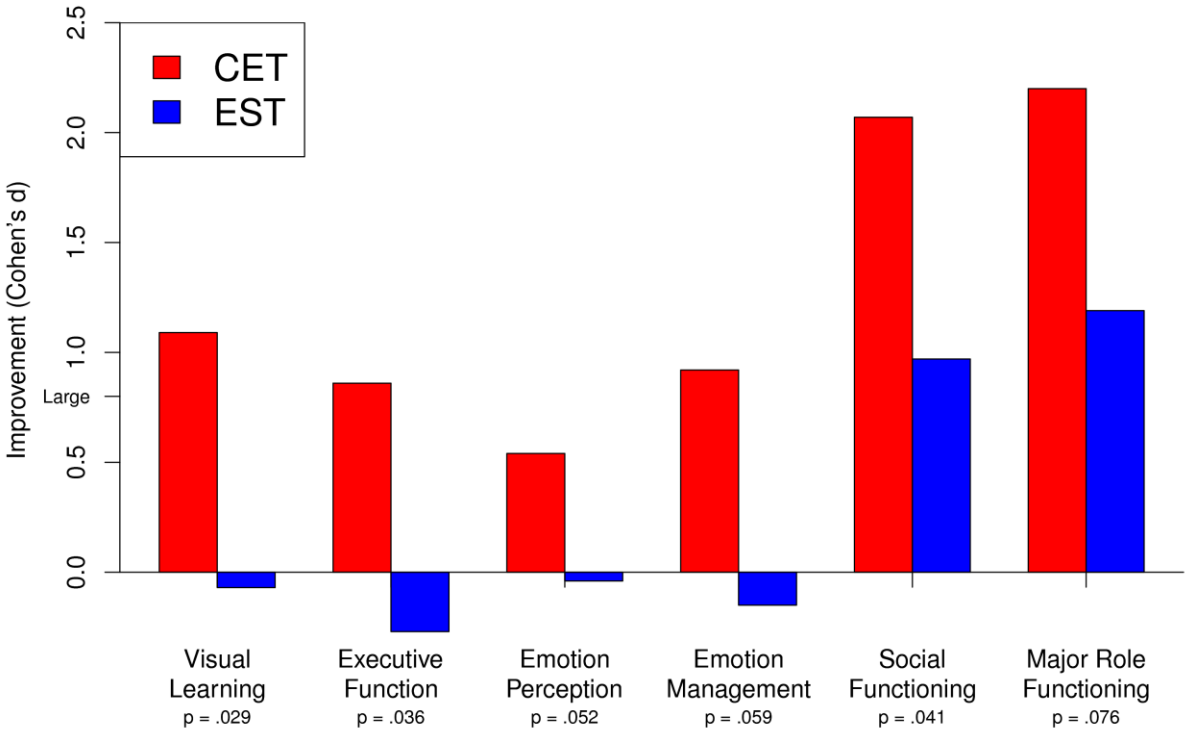
Demonstrating the initial feasibility and efficacy of CET through **Original Aim HT2** enabled us to attract additional funds from Autism Speaks, NIMH and the Department of Defense to conduct a full randomized-controlled trial of CET in verbal adults with ASD. For this trial, 164 adults have been screened, 64 have been recruited, and 38 have been randomized to either CET (n = 20) or an Enriched Supportive Therapy (EST) control (n = 18). The remaining 26 individuals who have been enrolled but not randomized are currently being screened or are on a waiting list for study enrollment. Individuals not admitted to the trial have been excluded primarily due to failure to meet inclusion criteria (39%), lack of interest on the part of the participant (37%) usually from contacts initiated by interested family, or distance (9%). The trial was to be initiated with a treatment as usual comparison group, but we quickly realized that usual care for adults with ASD does not exist. Therefore, we adapted a second intervention, Enriched Supportive Therapy or EST, focused on stress and emotion management to serve as a comparison condition for this trial. EST has been used as an active comparison condition in our previous trials with schizophrenia because it offers an effective intervention to everyone in the study and controls for the non-specific effects of CET (e.g., provision of a skilled and empathic study therapist, psychoeducation on autism, coping skills training).

Our initial treatment experiences in this trial have been very positive. Currently, the 20 individuals being treated with CET are at various phases of the treatment. Those in the initial phase are focusing on neurocognitive computer-based training to improve attention and processing speed, and building interpersonal cooperative skills. Those in the later phase are participating in the CET social-cognitive groups and continuing concurrently with computer-based neurocognitive training in higher-order memory and executive function. Individuals are engaging well in the treatment, which is reflected in a 80% retention rate in the CET group (1 individual withdrew from CET at 9 months due to residential instability, 1 withdrew prior to 9 months due to increased employment, 1 withdrew prior to 9 months due to an unmanaged anxiety disorder, and 1 withdrew from CET at study baseline due to interest only in EST). The 18 individuals being treated with EST are participating in individual sessions with their study clinician and learning about their condition and how to identify and manage stress. Treatment engagement in EST has also been successful, with 89% of participants attending EST sessions (1 withdrew from EST at study baseline due to a lack of interest, 1 withdrew from EST at baseline due to interest only in CET).

While many participants are still completing their respective study treatment condition in either CET or EST, sufficient post-treatment (9- or 18-month, depending on length of enrollment) data have been cleaned for 20 of the randomized participants (9 in CET; 11 in EST) to facilitate an initial examination of the effects of these two interventions on cognition and behavior. As can be seen in *Figure 43***,** CET is already demonstrating a consistent medium-to-large benefit on multiple aspects of social cognition, including emotion perception and management, which are

approaching statistical significance using one-tailed tests. Most critically, these gains are generalizing to large improvements on blinded measures of functional outcome, including social functioning and major role functioning. Basic improvement in neurocognition has also shown promising effects favoring CET on higher-order domains of complex visual learning and executive functioning (see *Figure 43*). While these findings suggest that CET is having a key advantage over EST in multiple cognitive domains and in terms of adaptive function, EST also demonstrates significant benefits. EST participants are also demonstrating medium-to-large levels of improvement in social adjustment, indicating that this intervention is also helping participants. Taken together, these preliminary findings from a rigorous controlled trial tentatively suggest that the benefits of CET for core social-cognitive and neurocognitive processes in verbal adults with ASD are translating into large and meaningful gains in adaptive behavior. These results confirm the findings of our initial uncontrolled feasibility study. In addition, the results for EST indicate that this less specialized treatment could produce substantial clinical improvement in a large number of adults with ASD in the community with a modest clinician training effort. The above results should be interpreted with caution as they represent interim treatment results.

Figure 43
Preliminary Effects of a Randomized Trial of CET Versus EST for Verbal Adults with ASD (*N* = 20).



Finding 4. Effects of CET on Underlying Brain Function in Adults with ASD

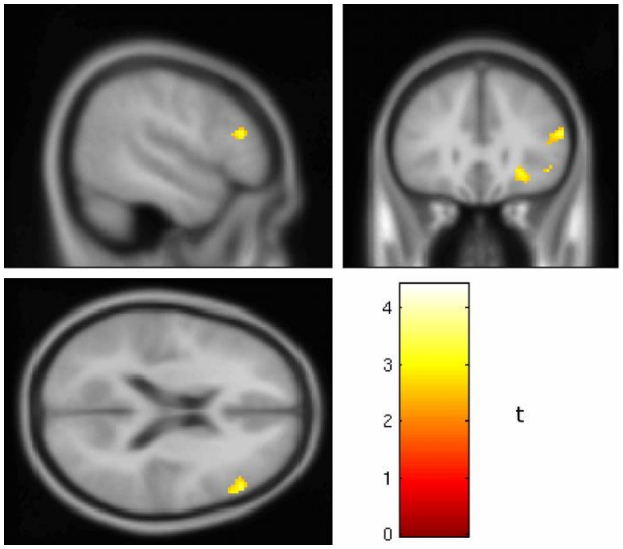
This finding was supported in part by DOH funds through the funding of a part-time therapist, data management, and partial investigator effort. There are no current manuscripts or published papers on this finding, as the controlled trial remains ongoing.

An important advance to this program of research that was added to this clinical trial with funding from the NIMH and DoD is the use of pre-/post-treatment neuroimaging scans to assess the impact of CET on brain structure, function, and connectivity in verbal adults with ASD. Our previous research in schizophrenia showed that the cognitive improvements observed in schizophrenia patients who received CET were reflected in a neuroprotective effect against brain loss (Eack et al., 2010b), and we hypothesized that similar neuroplastic changes would be observed in the brain in participants with autism who received this treatment.

Preliminary post-treatment (9- or 18-month, depending on length of enrollment) analyses of fMRI tasks involving speed of processing and perspective-taking/theory of mind (two key putative mechanisms of CET effects) have now been completed in 11 participants (6 in CET; 5 in EST). As can be seen in *Figure 44*, those treated with CET are demonstrating significant differential increases in brain activity in the right dorsolateral prefrontal and orbitofrontal cortices (all uncorrected $p < .005$) during processing speed/load compared to those treated with EST. This suggests that CET may be associated with increased recruitment of executive and emotional regulatory regions that enables a more rapid processing of information. Participants treated with CET are also showing differential increases in bilateral activation of the orbitofrontal cortex during mentalizing conditions of our theory of mind task (all uncorrected $p < .004$), suggesting a strengthening of the perspective-taking/theory of mind network in those treated with CET versus EST (see *Figure 44*). While preliminary, these exciting findings support the feasibility of elucidating and characterizing the neural mechanisms of response to cognitive rehabilitation in adults with ASD, and indicate the potential of neuroplasticity well into adulthood in ASD.

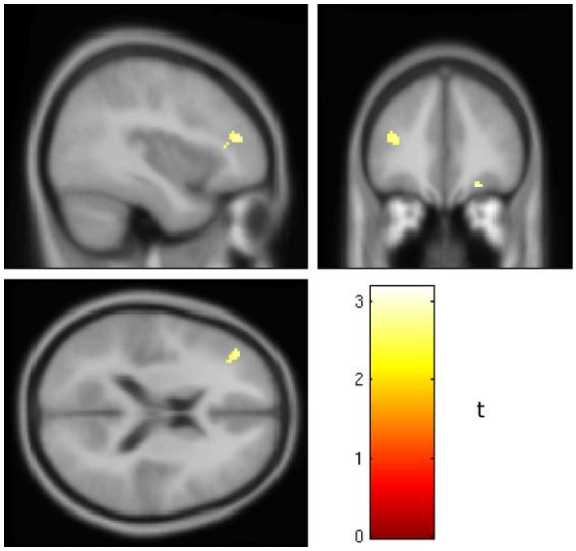
Figure 44
Preliminary Effects of CET versus EST on Brain Function During Speed of Processing and Theory of Mind Tasks (N = 11).

Processing Speed



CET > EST

Theory of Mind



CET > EST

Additional Funding Obtained That Supported This Research

The following additional funding sources were attracted to this DOH project to provide supplementary resources to support its expansion to a randomized-controlled trial, and were specifically used to supplement DOH funds to support sub-aims #1-6 of Revised Aim HT2.

R33 MH-85851 *Minshew & Eack, PIs 05/01/2011 – 04/30/2014*
NIH/NIMH $644,278
Adapting Cognitive Enhancement Therapy for ASD
The purpose of this grant is to adapt, pilot and preliminarily test of the effects of Cognitive Enhancement Therapy (CET) for young transitional age high functioning adults with autism spectrum disorders to improve adaptive functioning and adult life achievement in this population.
Role: Co-PI

AR100344 *Minshew & Eack, PIs 09/30/2011 – 09/29/2015*
Department of Defense $1,412,388
A Randomized Clinical Trial of Cognitive Enhancement Therapy for Adults with Autism Spectrum Disorders
The purpose of this project is to conduct a randomized-controlled trial of Cognitive Enhancement Therapy (CET) in adults with high function autism spectrum disorders. Specific aims are to evaluate the efficacy of CET for improving cognitive and behavioral outcomes in autism spectrum disorders, to examine the 6-month post-treatment durability of CET effects in adults with autism, and to examine the impact of CET on neurobiologic processes and brain connectivity in these disorders.

Minshew & Eack, PIs *06/01/2010 – 05/31/2013*
Autism Speaks $300,000
Evidence-Based Cognitive Rehabilitation to Improve Functional Outcomes for Young Adults with Autism-Spectrum Disorders
The purpose of this grant is to conduct a randomized-controlled trial of Cognitive Enhancement Therapy (CET) in young adults with high function autism spectrum disorders to improve adaptive functioning and adult life achievement in this population.
Role: Co-PI

The following additional funding sources were obtained to provide additional investigator effort at no cost to the DOH on this project.

K23 MH-95783 *Eack (PI)* *09/01/2012 - 05/31/2016*
NIH/NIMH $663,508
Social-Cognitive Rehabilitation and Brain Function in Early Schizophrenia
The purpose of this career development award is to provide the applicant with training in social-cognitive and affective neuroscience and functional magnetic resonance imagining methods to advance a translational program of research designed to elucidate the effects of psychosocial interventions on the brain in schizophrenia. This training plan will be carried out in the context

of a project designed to examine the impact of Cognitive Enhancement Therapy on social-cognitive brain function and connectivity in schizophrenia using novel fMRI paradigms, which is expected to lead to new insights regarding the plasticity of the social brain in the disorder.

The following additional funding sources were obtained to provide junior scholar training utilizing the resources from this project.

Fitzpatrick, PI *Eack & Minshew, Mentors* *08/01/2013 – 07/31/2015*
Autism Speaks Weatherstone Fellowship $59,000
Stress and Social Disability in Adults with Autism Spectrum Disorders
Stress as a Predictor of Outcomes for Adults with Autism Spectrum Disorders The purpose of this grant is to examine the differences in stress response between adults with ASD and adults who do not have ASD, examine the relationship between stress and meaningful adult outcomes for people with ASD, and use advanced statistical procedures to identify meaningful subgroups in terms of stress response for adults with ASD. This will contribute to the understanding of differing stress responses among a growing and diverse population of adults with ASD, and their impact on outcome.

Talks:

Fitzpatrick, L. B., Minshew, N. J., & Eack, S. M. (2012, May). A systematic review of psychosocial interventions for adults with autism spectrum disorders. Poster presented at the International Meeting for Autism Research, Toronto, Ontario, Canada.

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Aim HT3. Training 4 Minority Undergraduate & 4 Graduate Students

Two programmatic endeavors were launched under this specific aim: a seven-week summer research program (the goal of which was to expose underrepresented and disadvantaged students to biomedical research in autism) and a nine-month postbaccalaureate research program for students interested in progressing to graduate training.

Summer Research

1. Admission Procedures and Program Structure

To support this programmatic aim, students were recruited from the applicant pool of the University of Pittsburgh School of Medicine (UPSOM) Summer Premedical Academic Enrichment Program (SPAEP), Level II. Students were selected according to CURE requirements that they belong to an underrepresented group, paying special attention to students attending Pennsylvania's historically black colleges and universities (HBCUs). In 2012, 311 submitted basic applications and a record 242 completed their applications. The Summer Research component went beyond its initial goal of training four (4) students, providing seven (7) research placements to six (6) students across the life of the grant.

The comprehensive training curriculum required each student to spend 4.5 days each week performing mentored laboratory research and .5 days each week on enrichment activities (graduate school application skills seminars and talks by underrepresented role model physicians and scientists, shadowing physicians and scientists, and completing a mock interview). Students spent several Saturday mornings learning about test-taking skills for standardized admission tests. Finally, each student presented her/his research to an audience of peers and mentors, producing a formal research poster and a PowerPoint presentation.

The training coordinator, Paula K. Davis, MA, assistant vice chancellor for health sciences diversity, and staff in UPSOM's Offices of Admissions and Financial Aid and Student Affairs/Diversity Programs served as the faculty for the enrichment core. Richard Levitt, MA, academic development coordinator for UPSOM, provided the study and reading skills assessments and met individually with each student to discuss his/her approach to learning as it relates to future goals. (See *Table 9.* Trainees and Current Status and *Table 10.* Student's Completed Research Projects/Presentations)

To meet the goals of the post-baccalaureate training aim, we created the University of Pittsburgh Intramural Research Training Award (UPIRTA) program (http://www.healthdiversity.pitt.edu/programs/upirta.php), a nine-month to one-year post-baccalaureate experience for underrepresented students who are interested in biomedical graduate training (See *Table 11* Post-Baccalaureate Training). Students were placed with research preceptors (under the umbrella of Dr. Minshew's lab) according to their interests and

abilities. Experiences began in the fall of each award year. As with the summer research program, all students were eligible to apply; however, preference in admission was given to students from Lincoln and Cheyney Universities (Pennsylvania's two HBCUs), University of Pittsburgh students, and Pittsburgh-area and Pennsylvania natives.

Students were recruited at minority pre-health and graduate opportunity fairs (such as the Annual Biomedical Research Conference for Minority Students, the Society for the Advancement of Chicanos and Native Americans in Science, and the eastern-PA-based National Conference on Higher Education), through the honors and pre-health programs of Lincoln and Cheyney Universities, and through the National Association for Advisors of the Health Professions. Applicants were vetted by Ms. Davis and John P. Horn, PhD, associate dean for graduate studies in the School of Medicine. Ms. Davis and Dr. Horn screened applications for each student's interest and potential for success (as judged by grade point average [GPA] and prior research activity). In addition to our general recruitment process, we engaged in focused marketing nationally to pre-health advisors and to the Society for Teachers of Neuroscience.

In addition to their research, the students engaged in a comprehensive schedule of enrichment activities, including lab meetings and progress meetings. In addition, they participated in the University of Pittsburgh's "Survival Skills and Ethics" program (until it was shuttered in 2012) or CEED, the Career Education and Enhancement for Health Care Research Diversity Program (http://www.icre.pitt.edu/ceed), both programs were designed to teach early career researchers key professional "survival" skills such as making effective presentations, navigating career paths, and obtaining grant funding. CEED exists specifically to support underrepresented individuals, so UPIRTA students had an opportunity, through this experience, to network with resident, fellow and junior faculty underrepresented role models. (See *Table 12.* Post-Baccalaureate Project Presentations)

2. Outcomes

The two programmatic efforts were successful in exposing underrepresented students to both clinical and basic research into the causes and treatments of autism. Outcomes were mixed among the two groups, however, with the summer students largely moving along clinical career paths. However, LaToya Riley sought a post-baccalaureate research experience. Had funding persisted, she would have been a very appropriate candidate for the post-baccalaureate experience, enabling her further preparation for graduate study.

In the post-baccalaureate group, 50% (2 of 4) are proceeding to graduate school, while 25% (1) is headed for a clinical career in physician assistant studies. Chizelle Rush, who had the opportunity to present at an international autism research conference while in the program, intends to pursue medicine with research as a strong part of her career.

Table 9. Trainees and Current Status			
Student (Race/Ethnicity)	**Home Institution**	**Preceptor**	**Current Status**

2009			
Derrick Brooks (AfAm)	University of Pittsburgh	Kate McFadden, MD	UPSOM MS1
Kene Ukeje (AfAm)	University of Pittsburgh	Paula Monahan-Nichols, PhD	UPSOM MS3
2010			
Derrick Brooks (AfAm)	University of Pittsburgh	Kate McFadden, MD	UPSOM MS1
Olivia Beaubrun (AfAm)	University of Pittsburgh	Kate McFadden, MD	George Washington SOM, Fall 2013
2011			
KaHill Liddell (AfAm)	University of Pittsburgh	Shaun Eack, PhD	April 2013 grad
2012			
Kelsey Stegall (AfAm)	Clemson University	Paula Monahan-Nichols, PhD	May 2013 grad - Joining Teach for America
LaToya Riley (AfAm)	Cheyney University	Paula Monahan-Nichols, PhD	May 2013 grad

Table 10. Student's Completed Research Projects/Presentations

Student	Home Institution	Research Project/Presentation
2009		
Derrick Brooks	University of Pittsburgh	"Developmental Brain Expression of Lingo-2 and Amigo2: Leucine-rich Repeat Proteins Implicated in Autism"
Kene Ukeje	University of Pittsburgh	"Developmental Brain Expression of Lingo-1: A Leucine-rich Repeat Protein Implicated in Autism"
2010		
Derrick Brooks	University of Pittsburgh	"Developmental Brain Expression of Lingo2 and Amigo2: Leucine-rich Repeat Proteins Implicated in Autism" *(project continued from summer 2009)*
Olivia Beaubrun	University of Pittsburgh	"Effect of the Absence of Sall4 in the Dorsal Cortex"

2011		
KaHill Liddell	University of Pittsburgh	"Increased Attention and Working Memory Among Individuals with Autism Spectrum Disorder Not Living with Family"
2012		
Kelsey Stegall	Clemson University	"Protein expression of LRRTM3: A Possible Autism Susceptibility Gene"
LaToya Riley	Cheyney University	"Developmental Brain Expression of LRRN1 A Leucine-rich Repeat Protein Implicated in Autism"

Table 11. Post-Baccalaureate Training

Student	**Undergraduate Institution**	**Preceptor**	**Current Status**
2010-11			
Chizelle Rush	Northwestern University	Jana Iverson, PhD	Postbacc pre-med at Wash U SOM
2012-13			
Candace Cooper	Norfolk State University	Paula Monahan-Nichols, PhD	Awaiting news of grad school admission at UT
Natalie Kendall	Cheyney University	Paula Monahan-Nichols, PhD	Entering neuroscience graduate program Fall 2013 - Delaware State U
Bianca Montalmont	Lincoln University	Carla Mazefsky, PhD	Will begin masters in Physician Assistant studies at Drexel in Fall 2013

Table 12. Post-Baccalaureate Project Presentations		
Student	**Preceptor**	**Presentation**
Chizelle Rush	Jana Iverson, PhD	“Development of Language and Communicative Gestures in Children at High Risk for Autism Spectrum Disorders”
Candace Cooper	Paula Monahan-Nichols, PhD	“LRRN3: An Autism Candidate Gene”
Natalie Kendall	Paula Monahan-Nichols, PhD	"The effects of synthetic glucocorticoids on neural development and cognition"
Bianca Montalmont	Carla Mazefsky, PhD	”Clinical Investigations of Autism”

Book Chapters resulting from the project:

Title of Book Chapter:	Authors:	Name of Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Cognitive Enhancement Therapy	Eack, S. M.	*Encyclopedia of Autism Spectrum Disorders*	2012	☐Submitted ☐Accepted ☒Published
2. Neuroanatomy	McFadden, K.	*Encyclopedia of Autism Spectrum Disorders*	2013	☐Submitted ☐Accepted ☒Published
3. Neuropathology	McFadden, K.	*Autism Spectrum Disorders*	2013	☐Submitted ☐Accepted ☒Published
4. Neurobiology of autism spectrum disorder.	McFadden, K., Minshew, N.J. & Scherf, K.S.	*Autism spectrum disorder: Pittsburgh Pocket Psychiatry.*	2011	☐Submitted ☐Accepted ☒Published
5. Information processing, neural connectivity, and neuronal organization	Minshew, N.J., Williams, D.L. & McFadden, K.	*Autism: Current theories and evidence*	2009	☐Submitted ☐Accepted ☒Published

Publications resulting from the project that did not include acknowledgement of the Pennsylvania Department of Health as a funding source:

Title of Journal Article:	Authors:	Name of Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Distributed circuits, not circumscribed centers, mediate visual recognition.	Behrmann, M, Plaut, D.C.	*Trends in Cognitive Science*	May, 2013 PMID: 23608364	☐Submitted ☐Accepted ☒Published
2. Practice makes improvement: How autistic adults out-perform others in luggage screening	Gonzalez, C., Martin, J., Behrmann, M., Minshew, NJ	*Journal of Autism and Developmental Disorders*	Feb, 2013 PMID: 23381483	☐Submitted ☐Accepted ☒Published
3. Normal Binocular Rivalry in Autism: Implications for the Excitation/Inhibition Imbalance Hypothesis.	Said, C., Heeger, D., Egan, R., Minshew, N. Behrmann, M.	*Vision Research*	Jan, 2012 PMID: 23200868	☐Submitted ☐Accepted ☒Published
4. Connecting Developmental Trajectories: Biases in Face Processing from Infancy to Adulthood.	Scherf, K.S., & Scott, L.S.	*Developmental Psychobiology*	Sep, 2012 PMID: 22711622	☐Submitted ☐Accepted ☒Published
5. Exogenous spatial attention is intact in autism spectrum disorders: evidence from contrast sensitivity, crowding, and visual search	Grubb, M. A., Behrmann, M., Egan, R., Minshew, N, J., Heeger, D. and Carrasco, M.	*Journal of Vision*	Dec. 2013 PMID: 24326863 PMCID: PMC3859 176	Submitted ☐Accepted ☒Published

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

___X_Yes

_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

___X_Yes
_____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

__9_ Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

___76_Number of subjects originally targeted to be included in the study
__107_Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
__100_Males
____7_Females
_____Unknown

Ethnicity:
___2__Latinos or Hispanics
__105_Not Latinos or Hispanics
_____Unknown

Race:
__1___American Indian or Alaska Native
__4___Asian
__6___Blacks or African American
__0___Native Hawaiian or Other Pacific Islander
_95___White

__1___Other, specify:_____biracial_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

Allegheny County
Center County

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase

Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

PDF's have been provided for the 10 published or accepted papers.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Cognitive Enhancement Therapy for adults with autism spectrum disorder: Results of an 18-month feasibility study	Eack, S. M., Greenwald, D. P., Hogarty, S. S., Bahorik, A. L., Litschge, M. Y., Mazefsky, C. A., & Minshew, N. J.	*Journal of Autism and Developmental Disorders*	Apr, 2013. [Epub ahead of print] PMID: 23619953	☐Submitted ☐Accepted ☒Published
2. Is cognitive rehabilitation needed in verbal adults with autism? Insights from initial enrollment in a trial of Cognitive Enhancement Therapy	Eack, S. M., Bahorik, A. L., Hogarty, S. S., Greenwald, D. P., Litschge, M. Y., Mazefsky, C. A., & Minshew, N. J.	*Journal of Autism and Developmental Disorders*	Feb, 2013. [Epub ahead of print] PMID: 23381484 PMCID: PMC3686872	☐Submitted ☐Accepted ☒Published
3. A systematic review of psychosocial interventions for adults with autism spectrum disorders	Fitzpatrick, L. B., Minshew, N. J., & Eack, S. M.	*Journal of Autism and Developmental Disorders*	Mar, 2013 PMID: 22825929	☐Submitted ☐Accepted ☒Published
4. Commonalities in social and non-social cognitive impairments in adults with autism spectrum disorder	Eack, S. M., Bahorik, A. L., McKnight, S. A. F., Hogarty, S. S., Greenwald, D. P., Newhill, C. E.,	*Schizophrenia Research*	June, 2013 [Epub ahead of print] PMID: 23768814	☐Submitted ☐Accepted ☒Published

and schizophrenia	Phillips, M. L., Keshavan, M. S., & Minshew, N. J.			
5. Evidence for Dysregulation of Axonal Growth and Guidance in the Etiology of ASD	McFadden, K., & Minshew, N.J.	*Frontiers in Neuroscience*	October, 2013 PMID: 24155705 PMCID: PMC3804918	☐Submitted ☐Accepted ☒Published
6. Vulnerability of The Social Brain During Adolescence in Autism.	Scherf, K.S., Elbich, D., Minshew, N., Behrmann, M.	Brain	2014 (in press)	☐Submitted ☒Accepted ☐Published
7. Emerging structure-function relations in the developing face processing system	Scherf, K.S., Thomas, C., Doyle, J., & Behrmann, M.	*Cerebral Cortex*	June, 2013 PMID: 23765156	☐Submitted ☐Accepted ☒Published
8. The Amygdala: An Agent of Change in Adolescent Neural Networks.	Scherf, K.S., Smyth, J., & Delgado, M.	*Hormones and Behavior*	July, 2013 PMID: 23756154 PMCID: PMC3781589	☐Submitted ☐Accepted ☒Published
9. Unreliable evoked responses in autism	Dinstein, I., Heeger, D. J., Lorenzi, L., Minshew, N. J., Malach, R., Behrmann, M.	*Neuron*	Sep, 2012 PMID: 22998867	☐Submitted ☐Accepted ☒Published
10. Facing changes and changing faces in adolescence: A new model for investigating adolescent-specific interactions between pubertal, brain and behavioral development.	Scherf, K.S., Behrmann, M. & Dahl, R.	*Developmental Cognitive Neuroscience*	Apr, 2012 PMID: 22483070	☐Submitted ☐Accepted ☒Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

These plans are described in the report for each aim. Numerous additional publications are planned, in preparation or awaiting completion of data processing or analysis for each aim.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Aim R1 generated evidence implicating a previously not considered disturbance in brain development as contributing to the cause of autism, e.g., formation of the subplate region of the cerebral cortex. Disturbances in this mechanism may explain how the gray matter-white matter junction is less distinct in the ASD brain. This study also provided evidence for disturbances in axonal outgrowth and guidance mechanisms in the cause of ASD. Most theories have focused on alterations in synapse formation and maintenance but the increase in brain growth in early life in ASD would suggest that axonal outgrowth is commonly impacted. Functional Magnetic Resonance Imaging (FMRI) and Diffusion Tensor Tractography (DTT) studies in ASD demonstrating that the trajectory and endpoint of major white matter tracts are displaced compared to the typical brain are consistent with alterations in axonal outgrowth and pathfinding. Aim R1 also provides evidence of new genes that are involved in causation of autism that may improve genetic diagnosis and become targets for new drugs that alter gene expression or the products of gene expression.

Aim R2 demonstrated that impaired multi-dimensional integration of information in ASD can be addressed through computer based learning tasks that promote the emergence of category level knowledge that is independent of any specific detail and is an emergent, higher level derivative. This study also demonstrated that this intervention impacted the underlying brain systems involved in integration of information. This deficit in global processing/multi-dimensional integration of information likely underlies many of the manifestations of autism and may be treatable if it is addressed at the cognitive level rather than at the behavioral level. This approach can be readily converted to a gaming format and distributed widely in an appealing format. Individuals with ASD who are typically rule based in their thinking and fail to comprehend concepts may well benefit from this intervention. I would also expect that non-ASD individuals who have difficulties with concept formation/common sense may also benefit, e.g., that approaches developed for specific cognitive deficits will generalize to other disorders/behavioral challenges that also affect these same cognitive abilities and neural systems.

HT1 involved several efforts that were designed to improve community function and practices in relation to ASD. This effort involved training of minority students in ASD

health research, development of a lecture series to improve ASD care in pediatric practices locally, and piloting of an entirely new intervention for the treatment of verbal adults with ASD. In Aim HT1, 10 minority students (three more than the seven we proposed to train in the original application) had ASD research experiences and training, and all have now embarked on science/medicine/research careers including several who have entered medical school. In addition, I expect that these students will become community advocates for individuals with ASD and other cognitive disabilities as a result of their experiences in this training program. Aim HT1b involved creating eight web based CME credit presentations that would improve the early detection and diagnosis of ASD by community pediatricians. These presentations were distributed to pediatric research practices that serve approximately 120,000 children in this area. These presentations will be made broadly available throughout Pennsylvania in the fall. Aim HT2 began the adaptation of Cognitive Enhancement Therapy for verbal adults with ASD. CET is the first treatment approach for adults with ASD that targets the core deficits. CET was originally developed at the University of Pittsburgh for the treatment of adults with schizophrenia. Several NIH funded research studies have demonstrated its effectiveness in improving adaptive function in adults with schizophrenia, resulting in the acceptance of CET as a reimbursable treatment for those with schizophrenia. This DOH funding enabled us to adapt CET for use in autism and to gain funding from several sources to support the first controlled trial of CET which we compared to Enhanced Supportive Therapy, another treatment that we introduced since there is no standard of care model for adults with ASD in the community. We know have preliminary evidence of the efficacy of both CET and EST, with CET leading to larger and broader scale improvements than EST. Nonetheless EST is also effective and could be relatively quickly disseminated to community practice. As a result, we know have evidence of the effectiveness of two new treatments for verbal adults with ASD that target core symptoms rather than behavior. Our goal is to establish evidence of efficacy that will meet Medicare standards and enable us to disseminate these treatments throughout the state. Because of the involvement of Dr. Eack in ASD work, we were able to move another cognitive based intervention (ICONz method) into a community trial that will start in area high schools this fall.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert “None”; do not use “Not applicable.” Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

New Discoveries:

1. New developmental neurobiologic mechanism involving subplate neurons was implicated in the cause of ASD.
2. A new marker for subplate neurons in human cortex was developed.
3. New genes were implicated in the cause of ASD.

New Approaches for Treatment of ASD:

1. Cognitive Enhancement Therapy was adapted for individuals with ASD, demonstrated to be acceptable to them and initial efficacy data provided. Improvements in adaptive function also demonstrated.
2. Enriched Support Therapy was also adapted for individuals with ASD, demonstrated to be acceptable to them and initial efficacy data provided. Improvements in adaptive function also demonstrated.
3. Greeble (a created object that eliminates the variable influence of prior experience) training was demonstrated to improve multi-dimensional integration of information in adolescents with ASD that generalized; this cognitive training approach also produced alterations in the brain, e.g., a brain basis for improvement.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*