

# University of Pittsburgh

## Annual Progress Report: 2008 Nonformula Grant

### Reporting Period

July 1, 2010 – June 30, 2011

### Nonformula Grant Overview

The University of Pittsburgh received \$2,978,656 in nonformula funds for the grant award period June 1, 2009 through May 31, 2013. Accomplishments for the reporting period are described below.

### Research Project: Project Title and Purpose

*Deciphering Altered Brain Connectivity in ASD to Improve Intervention* - Recent scientific research has led to the understanding of autism spectrum disorder (ASD) as resulting from altered information processing by the brain and mind. Research has demonstrated alterations in social understanding, language comprehension, reasoning, emotion, motor movements, sensory processing, and learning related to alterations in brain connections. The goals of this research project are to: (1) develop a new intervention for ASD that enhances thinking capacity or meaningful integration of information and brain circuitry; (2) identify genetic and developmental neurobiologic mechanisms underlying the abnormal development of brain circuitry, as these will provide the basis for long-term definitive intervention; and (3) conduct a pilot study of Cognitive Enhancement Therapy (CET) in minority and resource-poor adults with ASD. CET targets two key underlying mechanisms of a number of signs and symptoms—the slow processing speed and the inability to understand one’s own emotions and the emotions of others. CET is furthermore a comprehensive and long-term program (18 months) with demonstrated efficacy at multiple levels in a similar clinical population with schizophrenia. Adaptions are being made in the pilot study for ASD.

### Anticipated Duration of Project

6/1/2009 - 5/31/2013

### Project Overview

The objectives of this research are to: (1) expand knowledge about the fundamental developmental neurobiologic and genetic mechanisms of autism that will lead to the next generation of discoveries, (2) translate recent scientific advances in ASD into a novel intervention, and (3) pilot a new intervention for adults with ASD that applies the cognitive and neural mechanisms identified as underlying major cognitive and behavioral issues with the aim of improving adaptive function in daily life. In “Neuropathology and Genetics of Connectivity: Altered Axonal Pathfinding in ASD,” developmental neurobiological studies of gene expression

will be conducted in postmortem tissue to ascertain the pattern of temporal and anatomic involvement of brain structures to inform the search for genes in ASD. The selection of these axonal pathfinding genes is based on findings from a genome-wide association study in ASD families. In the study “Inducing Plasticity in Cortical Connectivity via a Novel Intervention in ASD,” a novel-learning paradigm will be used to enhance multi-dimensional information integration and promote development of the supporting neural circuitry with the aim of secondary improvement in related cognitive and affective skills. Phenotypic markers of responders and non-responders will be identified so that the intervention can be refined to address individual variability in initial skill level and rate of response. Pre- and post-functional Magnetic Resonance Imaging (fMRI) will assess intervention effects on neural circuitry. In “CET for Minority and Resource-Poor Adults with ASD,” an established neurocognitive intervention for schizophrenia, head injury, and aging has been demonstrated to increase information processing speed and the capacity for perspective taking. Improvement in these basic skills results in improvement in comprehension, thinking, and social-emotional function in comparable individuals with schizophrenia. Most interestingly, CET not only prevents decline but enhances cortical gray matter volume in individuals with schizophrenia. Health status and access will be addressed through a web-based, archived, and audiotaped continuing medical education (CME)-accredited lecture program on ASD and related medical and behavioral issues created for a large, established, practice-based pediatric research network (Pediatric PittNet) that serves 115,000 families in five counties representing all racial and geographic segments of Western Pennsylvania. Collaboration with Programs for Living, Education, and Advocacy (PLEA), a community organization serving minority and low-income children and adults with ASD, will result in translation of research results to this community to improve intervention. PLEA staff will be trained in the national Autism Treatment Network medical guidelines, research administration of the Autism Diagnostic Observation Schedule (ADOS), and CET program for adults with ASD once pilot studies demonstrate efficacy.

### **Principal Investigator**

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### **Other Participating Researchers**

Bernard Devlin, PhD; Shaun M. Eack, PhD; Kathryn A. McFadden, MD; Paula Monaghan-Nichols, PhD - employed by University of Pittsburgh  
Marlene Behrmann, PhD; David C. Plaut, PhD; Kathryn S. Scherf, PhD - employed by Carnegie Mellon University  
Isabel Gauthier, PhD - employed by Vanderbilt University  
Deborah A. Ferraro, BS - employed by Programs for Living, Education, and Advocacy (PLEA)

## Expected Research Outcomes and Benefits

This research project is expected to produce: (1) a model for advancing the discovery of common genes associated with autism and abnormal mechanisms of brain development that can eventually be targeted for corrective action, (2) the first version of the new generation of interventions that will address the core cognitive and social impairments in ASD and enhance neural circuitry, (3) an intervention for adults with ASD that addresses core deficits and adaptive function, (4) publications that establish the three junior researchers as independent investigators qualified to successfully compete for NIH grants on their own, (5) subsequent grants demonstrating refinement and expansion of the interventions and the discovery of genes, (6) minority students who enter a health-related or science field as a result of this and similar research experiences, (7) and minority students who become ambassadors for students with ASD in the community and students who have disabilities of any kind and are of any age.

One of the primary sources of health disparities in ASD is related to the lack of training of providers, especially among those who provide services to the poor and those in rural areas. Those serving minority individuals have challenges related to how parents describe ASD symptoms and its differentiation from other behavior problems. This project will improve health disparities through: (1) a web-based, archived, and transportable lecture series on ASD and related issues for the 106 pediatricians in five counties serving 115,000 children (birth–21 years) and (2) training of staff at a minority and resource-poor serving community organization for ASD on national assessment and medical practice guidelines for ASD and on a new intensive intervention for adults focusing on cognitive-social function.

## Summary of Research Completed

Aim R1. The overall goal for this aim is to assess the plausibility of the hypothesis that alterations in certain leucine-rich repeat (LRR) proteins, implicated by Autism Genome Project linkage, association, and/or copy number variation (CNV) data, confer ASD risk by dysregulating axonal development. The studies are designed to ascertain whether LRR candidate molecules: (1) are expressed in a meaningful temporospatial pattern and/or (2) potentially participate in axonal outgrowth/guidance, at least *in vitro*, and are modeled on previous approaches to assess ASD candidates, such as the MET receptor (a hepatocyte growth factor receptor) and Neurexin 1. We have made significant progress toward completing our specific aims in the reporting period, including acquiring and conducting expression studies on fetal cortical tissue (study 1) and isolating, culturing, and staining mouse cortical neurons (study 2). *Study 1* Despite early difficulties in obtaining fetal cortical tissue, we have now acquired adequate material from the University of Pittsburgh Health Sciences Tissue Bank. Our sample comprises 15 individuals ranging from 16-23 weeks' gestation, with the expectation that we will increase this number to 30 over the next two to three months (ahead of schedule). Tissue samples were homogenized and parallel aliquots taken for ribonucleic acid (RNA) and protein isolation. Levels of gene specific messenger RNAs (mRNAs) were measured by quantitative real-time PCR (qRT-PCR) and quantified as delta cycle threshold (CT) (the gene-specific CT normalized to the CT of the housekeeping gene GAPDH [glyceraldehyde 3-phosphate dehydrogenase]). Figure 1 shows examples of preliminary data for three LRRs—Slit1, Amigo1, and Lrrn1. We have completed qRT-PCR for six candidate LRRs for all 15 specimens. Lrrn1

and Amigo1 appeared to show a general increase in expression during mid-gestation, which agrees with our previous findings, based on immunohistochemical data, that expression of many LRR candidates increased as cortical neurons finished migrating and elaborated neuritic processes. Slit1, while relatively more abundant, does not seem to exhibit this trend. While our initial impressions may change as we analyze more data, there are clearly dynamic changes in LRR expression during mid-gestation, a developmental period marked by significant axonal extension and growth cone migration.

*Study 2* We have made excellent progress establishing mouse cortical neuronal cell cultures and collecting and processing coverslips from the target stages of development (one day *in vitro* (DIV), 2 DIV, 4 DIV, 8 DIV, 14 DIV, 18 DIV, and 20 DIV). Cells were seeded on laminin- and poly-d-lysine-coated coverslips in 24-well plates. Twelve coverslips were collected at each time point and fixed with 4 percent paraformaldehyde, stained, and visualized on a Nikon 400 fluorescent microscope. Figure 2 illustrates the long-term viability of these primary neuronal cultures (18 DIV) and the expression of a normal complement of neuronal maturation markers (synaptotagmin—presynaptic marker; PSD-95 and Homer 1a—postsynaptic markers). Figure 3 shows an example of double-labeled immunohistochemistry for Lrrn1 and synaptotagmin in the same cells under confocal microscopy. Lrrn1 is expressed in the perinuclear cytoplasm and neuritic processes but does not appear to co-localize with synapses. Similar results have been obtained for Lingo1, Slit1, Amigo1, and Lrrn3. We continue to perform immunohistochemistry on cultured neurons at all stages for all LRRs and double labeling with synaptic, postsynaptic, and axonal markers.

Aim R2 We have recruited, enrolled, and tested five additional adolescents for the intervention, as well as five more adolescents with ASD and 10 typically-developing adolescents for the control condition at the first two time points (20 total). We also tested our initial pilot intervention participant at the one-year follow-up session.

*Specific Aim R2.1* Seven adolescents with ASD have completed 22 of 24 sessions of visuo-perceptual expertise training with Greebles. A repeated-measures analysis of variance (ANOVA) with condition (individual, family) and session number (22) as within-participant variables on the proportion of correct trials revealed a main effect of session,  $F(1, 6) = 5.8, p < .001$ , indicating that the participants learned to recognize and categorize the Greebles during the training. There was no main effect of condition or interaction between session and condition.

Despite strong evidence that participants learned the training set, learning has not been generalized to new Greebles or to other classes of visual stimuli by the postintervention testing session. In the sequential matching (SM) task for Greeble recognition, all groups were less accurate on individual compared to subordinate level trials,  $F(1, 13) = 54.0, p < .001$ . There was a similar main effect of condition for faces,  $F(1, 13) = 15.0, p < .001$ ; however, there were no interactions between either session or condition and group for any of the visual classes. The part-whole (PW) task revealed a strange pattern of results that led us to question the validity of this task in its current form. For example, the intervention group exhibited a diminished benefit for configural processing following the training, which is exactly the opposite of the predicted direction of effects. In the Cambridge face memory task, the intervention group exhibited selective increases in upright compared to inverted face processing, which is consistent with our

predictions (see Figure 4). However, this improvement was not selective to the intervention group; all three groups exhibited an improvement in upright face recognition across the testing sessions,  $F(1, 12) = 31.2, p < .001$ , suggesting that this result might be a general developmental effect. Also, accuracy for recognition of upright faces increased across the two testing sessions for all groups, whereas accuracy for the inverted faces did not,  $F(1, 12) = 31.2, p < .001$ . We remain optimistic (based on our pilot participant and neuroimaging data) that the biggest gains in learning will be evident at the one-year follow-up testing session.

*Specific Aim R2.2* Interestingly, even with no obvious changes in behavior following the intervention, our neuroimaging data are very promising. Across both individual participant and group level analyses, selective changes in the right fusiform face area (FFA) for *face* activation are emerging in the intervention group but are not evident in either of the control groups. Figure 5 shows the increase in face-related activation in the localizer task as defined by the group level contrast (post: [faces – (houses + objects)]) – pre: [faces - (houses + objects)]) for each group of participants at the new imaging center (presented at a corrected  $p < .05$  value). Importantly, only the intervention group exhibited a significant increase in face-related activation, specifically in the right FFA. Consistent with these group level analyses, we have also found increased face-related activation in the right FFA in our original pilot participant, who was recently tested one year following the intervention (see Figure 6).

*Specific Aim R2.3:* We are developing the processing stream for these analyses and have conducted the initial preprocessing of all data for all participants across the multiple time points.

*Aim HT2. CET for Underserved Adults with ASD.* The goal of this project aim is to adapt and pilot a novel cognitive rehabilitation approach, CET, for resource-poor and minority adults with ASD. 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.

We have received funding from the National Institute of Mental Health (NIMH) to considerably expand this pilot study. During the previous two years of the project, we conducted an uncontrolled pilot of CET with 16 resource-poor ASD adults. These individuals have received more than half of the 18-month treatment, and 9-month data collection has been completed. Preliminary 9-month results are presented in Figure 7 and already demonstrate highly significant improvements in overall neurocognitive functioning ( $p < .001$ ), speed of processing ( $p = .006$ ), working memory ( $p = .002$ ), and verbal learning and memory ( $p = .007$ ). These mid-treatment effects are larger than expected, and 18-month data are eagerly awaited, which we anticipate to extend to social cognition and adaptive behavior.

The goals for this reporting period were to: (1) recruit four additional adults with ASD, (2) continue piloting CET in participants already enrolled, and (3) begin training community clinicians in CET. In response to feedback from an interim performance review for this project, we have greatly expanded the first goal to conduct a randomized-controlled trial of CET versus an active supportive therapy control. To achieve this goal, we have hired and trained an additional study therapist and independent, blind rater and have begun enrolling adults with ASD

into this trial. We have screened 81 adults, 31 of whom are potentially eligible. To date, 12 adults (seven resource-poor, one minority, 10 males) (mean age = 24.08, mean IQ = 107.80, 60 percent unemployed) with ASD have been randomized to CET or supportive therapy, baseline assessments of cognitive/functional outcome have been completed, and these individuals have begun their treatment condition. The remaining 19 adults are in the process of enrollment/eligibility testing. The expansion has also been supported by new awards from Autism Speaks, NIMH, and the U.S. Department of Defense (DoD).

We have continued to treat 16 adults with ASD in an uncontrolled pilot and will be completing 18-month data collection with these individuals. With the expanded federal grant support, we also plan to assess this original pilot cohort again at one-year post-treatment to begin to gauge treatment durability. The DoD funding will also allow us to conduct neuroimaging assessments on incoming participants enrolling in the randomized trial.

Finally, we have found the need to modify our initial plans to disseminate CET to PLEA and other community clinicians at this stage so that we may focus on collecting more efficacy data. Currently, the evidence base for CET in adults with ASD is limited to this first pilot cohort, which is approaching treatment completion. While the preliminary evidence of the effects of CET is exciting, results from a controlled trial need to verify these results before agencies expend the time and resources needed to learn and implement CET. As such, we have not begun training PLEA clinicians in CET but have continued to collaborate with them to educate their clinicians about ASD and receive their advice about how to structure the treatment program to support implementation.

#### *Specific Aim 5 Minority Training:*

The two programmatic endeavors launched under this specific aim continue to flourish. One training component of the project is a seven-week research program, the goal of which is to expose underrepresented and disadvantaged students to biomedical research in autism. The second is a nine-month postbaccalaureate research program for students interested in progressing to graduate training.

#### Summer Research

##### *Admission Procedures*

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. For summer 2010, 271 students submitted the basic application, and 161 completed the full application with all required documentation. In 2011, 299 submitted basic applications, while 178 submitted the complete application.

##### *Program Structure*

The comprehensive training curriculum requires each student to spend 4.5 days each week performing mentored laboratory research and .5 days each week on enrichment activities, such as attending application skills seminars and talks by underrepresented role model physicians and

scientists, shadowing physicians and scientists, and completing a mock interview. Students spend several Saturday mornings learning about test-taking skills for the Medical College Admission Test (MCAT). Finally, each student must present 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 serve as the faculty for the admissions skills workshops. A former program participant serves as the dormitory monitor/student coordinator. Richard Levitt, MA, academic development coordinator for UPSOM, provides the study and reading skills assessment and meets individually with each student to discuss his/her approach to learning as it relates to his/her future goals.

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). The trainees during this reporting period were:

<b>Student</b>	<b>Home Institution</b>	<b>Preceptor</b>
<b>2009</b>		
Derrick Johnson	University of Pittsburgh	Kate McFadden, MD
Kene Ukeje	University of Pittsburgh	Paula Monahan-Nichols, PhD
<b>2010</b>		
Derrick Johnson	University of Pittsburgh	Kate McFadden, MD
Olivia Beaubrun	University of Pittsburgh	Kate McFadden, MD
<b>2011</b>		
KaHill Liddell	University of Pittsburgh	Shaun Eack, PhD

Mr. Kene Ukeje will enter UPSOM in August 2011. Mr. Derrick Brooks is in the final stages of choosing an academic postbaccalaureate program, which he will complete before applying to medical or graduate school. Ms. Olivia Beaubrun is in the process of applying to medical school for 2012 entry. Mr. KaHill Liddell is a rising junior with aspirations in rehabilitation medicine.

Research projects/presentations completed:

<b>Student</b>	<b>Home Institution</b>	<b>Research Project/Presentation</b>
<b>2009</b>		
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"
<b>2010</b>		
Derrick Brooks	University of Pittsburgh	"Developmental Brain Expression of Lingo2 and Amigo2: Leucine-rich Repeat Proteins Implicated in Autism" ( <i>project continued from summer 2009</i> )

Olivia Beaubrun	University of Pittsburgh	“Effect of the Absence of Sall4 in the Dorsal Cortex”
<b>2011</b>		
KaHill Liddell	University of Pittsburgh	“Increased Attention and Working Memory Among Individuals with Autism Spectrum Disorder Not Living with Family”

To meet the goals of the postbaccalaureate 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 postbaccalaureate experience for underrepresented students who are interested in biomedical graduate training. Students are placed with research preceptors (under the umbrella of Dr. Minshew’s lab) according to their interests and abilities. Experiences begin in the fall of each award year.

As with the summer research program, all students are eligible to apply; however, preference in admission is given to students from Lincoln and Cheyney Universities (Pennsylvania’s two HBCUs), University of Pittsburgh students, and Pittsburgh-area and Pennsylvania natives.

Students are 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 Chaka Fattah Graduate Opportunities Conference), through the honors programs of Lincoln and Cheyney Universities, and through the National Association for Advisors of the Health Professions. Applicants are vetted by Ms. Davis and John P. Horn, PhD, associate dean for graduate studies in the School of Medicine. Ms. Davis and Dr. Horn screen applications for each student’s interest and potential for success (as judged by grade point average [GPA] and prior research activity).

It has been particularly challenging to attract underrepresented students to the autism training core. In addition to our general recruitment process, we have engaged in focused marketing nationally to pre-health advisors and to the Society for Teachers of Neuroscience. We have had one program participant to date.

<b>Student</b>	<b>Undergraduate Institution</b>	<b>Preceptor</b>
<b>2010-11</b>		
Chizelle Rush	Northwestern University	Jana Iverson, PhD

In addition to their research, the students engage in a comprehensive schedule of enrichment activities, including lab meetings and progress meetings. In addition, they participate in the University of Pittsburgh’s “Survival Skills and Ethics” program, designed to teach graduate students and early career researchers key professional “survival” skills such as making effective presentations, navigating career paths, and obtaining grant funding. Ms. Rush spent her non-lab time with the 2010 participants of the infectious disease training core postbaccalaureate program.

The 2011 project presented was:

Student	Preceptor	Presentation
Chizelle Rush	Jana Iverson, PhD	“Development of Language and Communicative Gestures in Children at High Risk for Autism Spectrum Disorders”

Figure 1: Delta CT

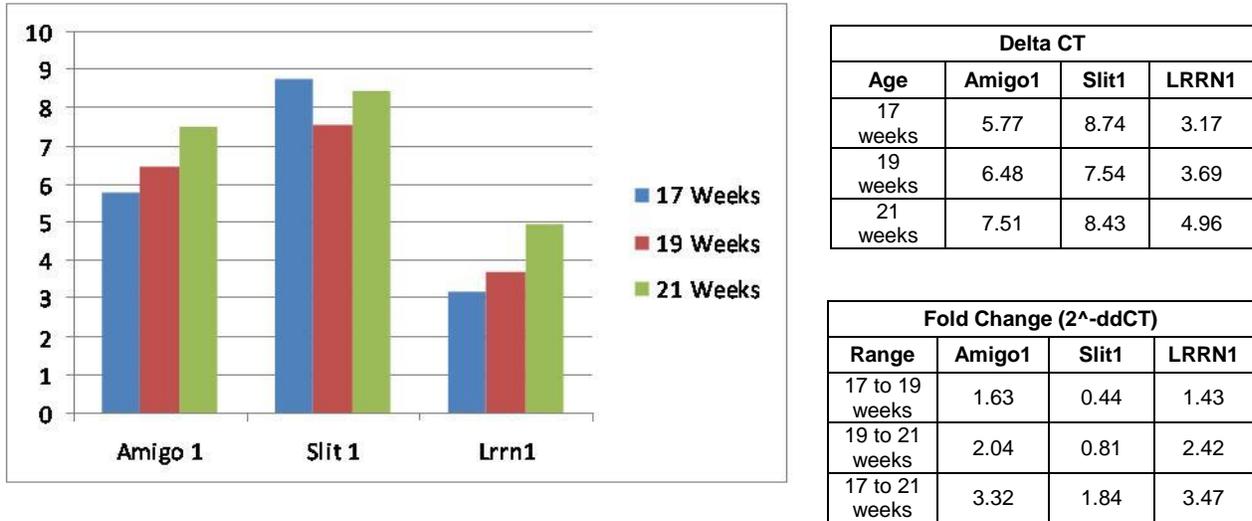


Figure 2: Maturation of 18 DIV Mouse Cortical Neurons

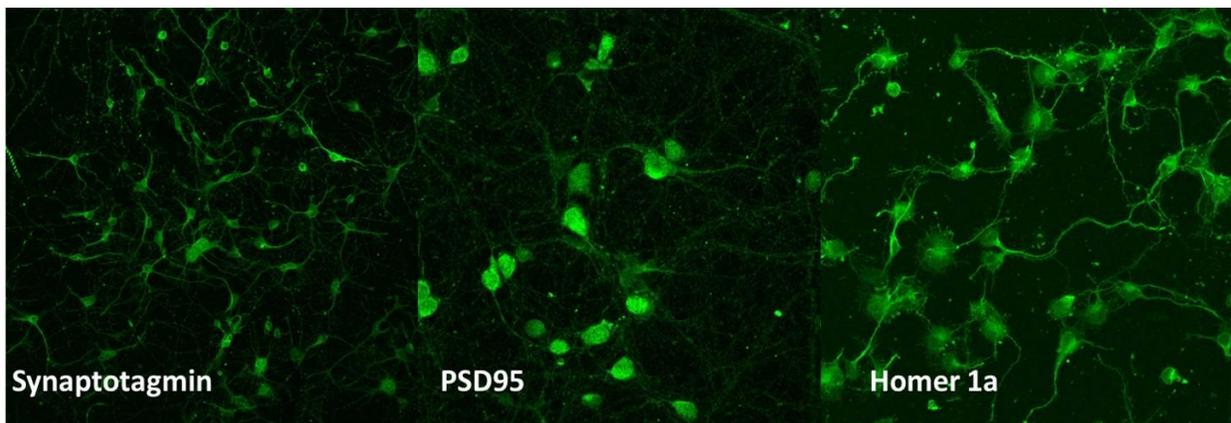


Figure 3: Double Fluorescence Lrrn1/Synaptotagmin

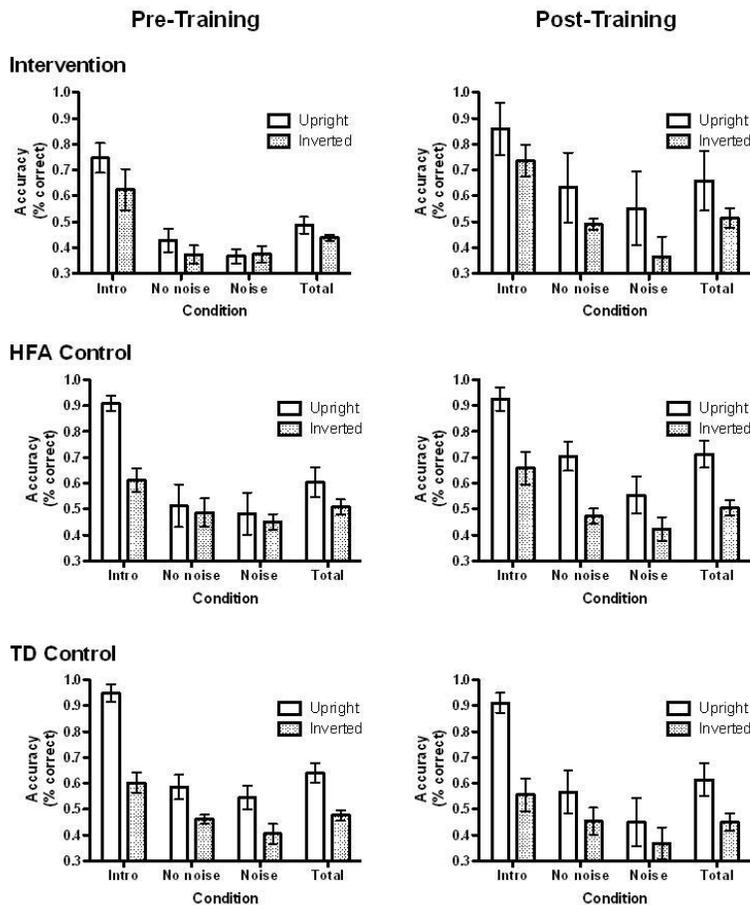
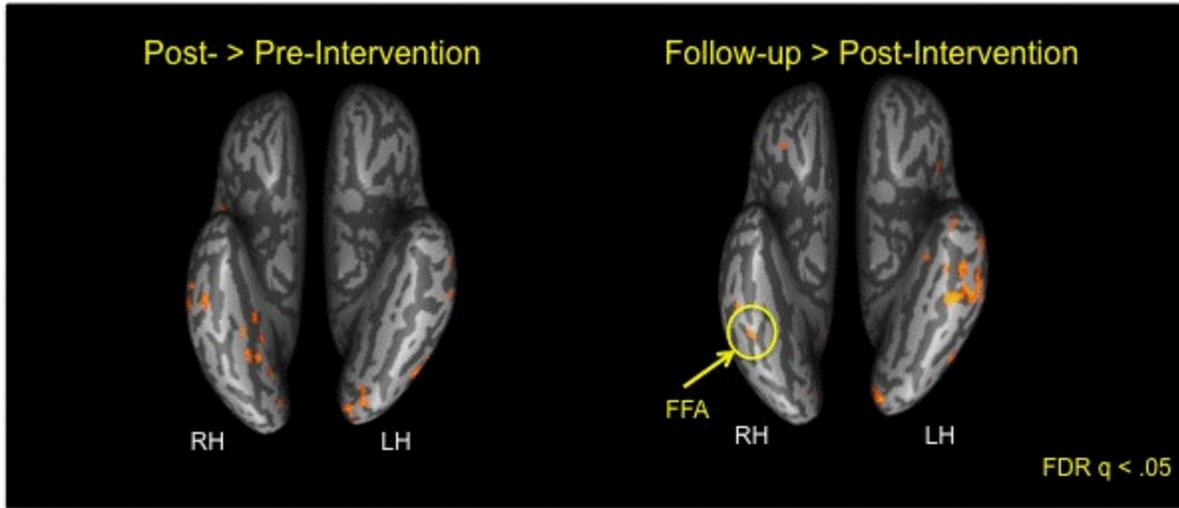


Figure 4. Performance in the Cambridge Face Memory Task across Sessions and Group

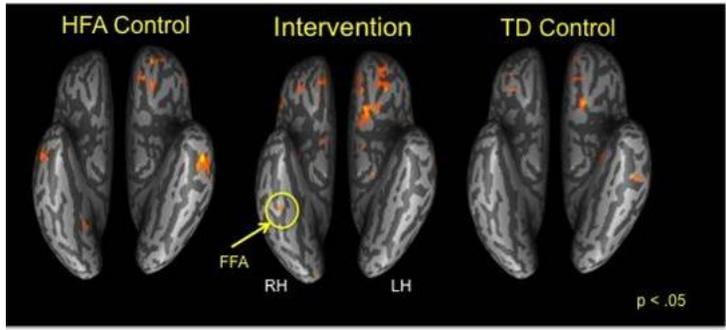


Figure 5. Increase in Face-Related Activation in Each Group from Pre- to Post-Intervention

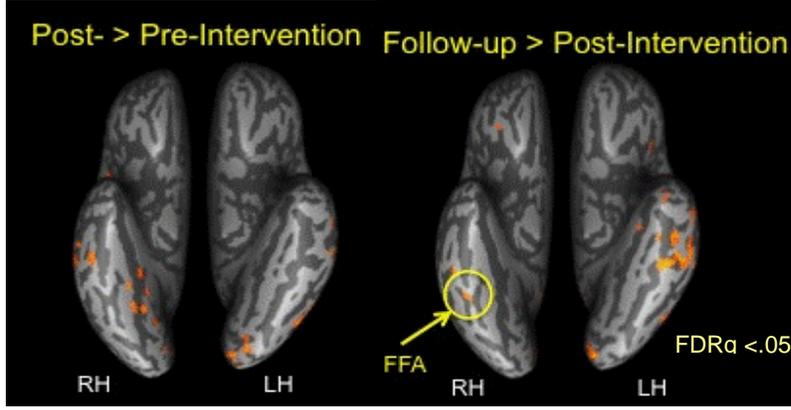


Figure 6. Increase in Face-Related Activation in First Participant to Complete 1-Year Follow-Up

Figure 7. Nine-Month Effects of Cognitive Enhancement Therapy in Adults with ASD

