

Pennsylvania Department of Health Final Performance Summary Report Non-Formula Grants

Overview of the Health Research Project Performance Review Process and Criteria

An applicant that receives a health research grant under Tobacco Settlement Act / Act 77 of 2001, Chapter 9, is subject to a performance review by the Department of Health upon completion of the research project. The performance review is based on requirements specified by Act 77 and criteria developed by the Department in consultation with the Health Research Advisory Committee.

As part of the performance review process, each research project contained in a grant is reviewed by at least three experts who are physicians, scientists or researchers. Reviewers are from the same or similar discipline as the research grant/project under review and are not from Pennsylvania. Reviewers use the applicant's proposed research plan (strategic plan), the annual progress reports, interim review reports, corrective action plan, and final progress report to conduct the review. A grant that receives an unfavorable performance review by the Department may be subject to a reduction in funding or become ineligible for health research funding in the future. The overall grant evaluation rating is based on the ratings for the individual research projects contained in the grant.

This performance review report contains the outcome of the review for the grant as a whole (outstanding, favorable, or unfavorable), strengths and weaknesses of each research project, as well as recommendations for future improvement.

The following criteria were applied to information submitted by research grant recipients:

- **Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?**
 - Did the project meet the stated objectives?
 - Consider these questions about the data and empirical results: Were the data developed sufficiently to answer the research questions posed? Were the data developed in line with the original research protocol?
 - If changes were made to the research protocol, was an explanation given, and, if so, is it reasonable?
 - Consider (only for clinical research grants) the extent of laboratory and clinical activities initiated and completed and the number of subjects relative to the target goal.
 - Were sufficient data and information provided to indicate or support the fact that the project met its objectives or made acceptable progress?
 - Were the data and information provided applicable to the project objectives listed in the strategic plan?

- **Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the dollars budgeted?**
 - Consider any changes in risk factors, services provided, incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of impact and effectiveness of the research being conducted.
 - Consider any major discoveries, new drugs and new approaches for prevention, diagnosis and treatment, which are attributable to the completed research project.
 - What are the future plans for this research project?

- **Criterion 3 - Did the project leverage additional funds or were grant applications submitted?**
 - If leveraging of funds were expected, did these materialize?
 - Are the researchers planning to apply for additional funding in the future to continue or expand the research?

- **Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents, or commercial development opportunities? Were any of these submitted / filed?**
 - If any of the above listed were expected, did these materialize?
 - Are the researchers planning to submit articles to peer-reviewed publications, file for any licenses or patents or begin any commercial development opportunities in the future?
 - Consider the number/quality of each and what was proposed in the original application.

- **Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?**
 - If any improvements in infrastructure were expected, were they made?
 - Were any new investigators added or were any researchers brought into the institution to help carry out this research?
 - Were funds used to pay for research performed by pre- or post-doctoral students?

- **Criterion 6 - Did the project lead to collaboration with research partners outside the institution, or new involvement with the community?**
 - Are the researchers planning to begin any collaborations as a result of the research?
 - For clinical research only: consider the number of hospitals and health care professionals involved and the extent of penetration of the studies throughout the region or the Commonwealth.

Overall Evaluation Rating

An overall evaluation rating is assigned to each research project. The rating reflects the overall progress the project attained in meeting the stated goals and objectives. The rating is based on a scale of 1–3, with 1 being the highest. An average rating is obtained from all the reviews (minimum of 3) of each project and is the basis for the determination of the final overall rating for each project as follows:

1.00 – 1.33 = *Outstanding*

1.34 – 2.66 = *Favorable*

2.67 – 3.00 = *Unfavorable*

The grant level rating is an average rating from all projects as above. The numerical rating appears in parentheses for the grant and each project in the ***Overall Grant Performance Review Rating*** section of the report.

Overall Grant Performance Review Rating

Grant Rating: Favorable (2.00)

Project Rating:

Project	Title	Average Score
08862	Deciphering Altered Brain Connectivity in ASD to Improve Intervention	Favorable (2.00)

Project Number: 08862
Project Title: Deciphering Altered Brain Connectivity in ASD to Improve Intervention
Investigator: Nancy J. Minshew, MD

Section A. Project Evaluation Criteria

Criterion 1 - How well did the project meet its stated objectives? If objectives were not completely met, was reasonable progress made?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Aim R1. Neuropathology & Genetics of Connectivity: Altered Axonal Pathfinding in ASD

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

Strengths: This project was based on the explicit hypothesis that genes related to axonal outgrowth would be preferentially affected in specific facets of brain development and through their dysregulation with the etiology of autism spectrum disorder. In particular, alterations of certain Leucine-rich repeat (LRR) proteins highlighted in the Autism Genome Project would confer risk through alterations of normal axonal growth.

Alternative strategies were developed to examine gene products in tissue sections available through the Autism Tissue Program. Good staining was achieved and LRRN3 showed unusual expression in fetal subplate, a structure that has been implicated in the pathogenesis of ASD. The investigators provided excellent documentation of their immunohistological preparations. Very preliminary studies were carried out in postmortem autistic brain tissue. Findings were too preliminary to draw conclusions. Postmortem staining of interstitial neurons from postnatal/adult brain tissue appeared to demonstrate greater numbers of LRRN3 staining in the subcortical white matter.

Weaknesses: Attempts at determining spatiotemporal patterns of expression of the LRR genes in the fetal brain were limited due to the lack of available postmortem tissue. qRT-PCR and *in situ* hybridization studies were necessarily limited and the investigators were unable to detect statistically significant dynamic changes in expression of their candidate genes.

Despite several manuscripts mentioned in the progress report as in preparation, there are no publications on LRRN3 from these investigators in PubMed.

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

Strengths: Unlike Study 1, this study, carried out in cultured neurons/stem cells, was quite productive. Four LRRs (LINGO1, LRRN1, LRRN3, and LRRTM3) could plausibly be associated with axonal outgrowth and/or guidance. LRRN3 was found in association with extending dendrites and axonal growth cones.

Weaknesses: While the LRRs that were studied may have fundamental effects in the developing nervous system, their potential role in the etiology of autism spectrum disorder was not studied. A survey of leucine rich repeats and autism in PubMed turns up only eight papers. It would appear that this has not proven to be a fruitful hypothesis in the field of autism research.

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

Strengths: The study demonstrated that high functioning adolescents with autism can gain visuo-perceptual expertise for greebles through a computer-based instructional program. The intervention was quite tolerable to the adolescent subjects who were compliant with instructions. Seven to ten adolescent subjects remained within the longitudinal study and participated both in training and in functional imaging.

Based on the Cambridge Face Memory Task, there was some indication that adolescents were better at face recognition following the greeble intervention. However, all groups (including controls) were showing improvement over the course of the study and thus this may simply reflect a practice effect.

Nine of the subjects in intervention A returned for a modified intervention B.

Weaknesses: Analysis of generalization of greeble training to a novel greeble recognition task was complicated due, in part, to "unfortunate experimenter errors of the wrong version of the post-intervention tasks." One conclusion was that any intervention-related boost in performance was short lived.

Given the design of the face processing component of the study, it was not possible to demonstrate that the greeble expertise generalized to better face recognition.

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

Strengths: The investigators carried out baseline measures of functional activity in regions related to face processing and found hypoactivity in many of them. This was particularly true of the fusiform face area.

Weaknesses: It was not clear from the progress report whether the investigators controlled for eye movements when presenting face stimuli. Given that individuals with autism tend to look less at faces and to the eye regions of faces, this could explain, in part, the hypoactivation they observed in face processing areas.

The analyses of the functional MRI data are ongoing and thus there is no answer to the question posed in this component of the project. While the investigators present data for alterations of activity in many face processing regions in the treated individuals with autism over time, there has not yet been an appropriate comparison with the control individuals.

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

Strengths: Baseline MRI processing capacity has been established to define bundles, such as the inferior longitudinal fasciculus, that are involved in face processing.

Weakness: The data analysis component of this project is still underway so there are no conclusions to the question posed in this portion of the project. While it is suggested that the large data set will ultimately lead to multiple publications, none of the intervention-related analyses have reached the publication phase.

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

Strengths: At least nine autism-related CME lectures were prepared and distributed through PittNet. Not clear how many practitioners took advantage of this resource.

Weakness: None

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

Strengths: This appeared to be one of the most successful components of this program of research. While the collaboration with PLEA was judged to be premature given the unproven nature of the intervention, the intervention itself proved to be highly successful. The investigators brought this 18-month neurocognitive and social-cognitive training program to 16 resource poor, verbal and minority individuals with autism.

The program was shown to be feasible and acceptable to adults with autism.

Based on interim progress report and site visit, investigators revised this aim to include a clinical trial of Cognitive Enhancement Therapy (CET) for minority adults with autism.

Pilot data led to the acquisition of additional funding for this project from Autism Speaks and the Department of Defense.

Investigators carried out baseline analysis of cognitive impairments in verbal adults with autism.

The clinical trial is ongoing though initial analyses indicate that CET is promising in developing both cognitive and social enhancements.

Aim HT3. Training 4 Minority Undergraduate & 4 Graduate Students

6 undergraduate students participated in the research - 3 male/3 female - all African-American
4 female graduate students participated in the training program - all African American

Strengths: Strong outreach effort and students are generally pursuing postgraduate medical or graduate education.

Reviewer 2:

Project 08862 (Minshew, PI) was composed of two separate but linked research concentrations. Aim 1 consisted of identification of candidate genetic markers for axonal guidance related to autism risk. Aim 2 consisted of probing for neuroimaging and clinical changes associated with interventions designed to facilitate facial recognition by training on Greebles.

Aim 1/R1:

Aim 1/R1 was revised shortly after the project onset due to unavailability of sufficient developmental brain tissue samples from NICHD and BTB brain banks. Investigators worked around these limitations by recruiting additional samples from their own university autopsy service. Although the lack of developmental materials is unfortunate, and limits interpretation of candidate genetic probes, the researchers were able to identify six candidate leucine-rich repeat proteins that were expressed during second trimester brain samples, of which one (LRRN3) was expressed in fetal subplate with differential results in ASD vs. control tissue samples.

Nevertheless, this success is tempered by a very small tissue pool (3 ASD, 5 TD subjects), which is insufficient to demonstrate that statistically valid differences in ASD and TD individuals are present. This locus did not meet criteria for genomewide significance in the Autism Genome Project. Nevertheless, the study did reveal a candidate locus meriting further characterization.

Aim 2:

The primary research thrust of the project was to demonstrate effects of computer-based greeble training therapy as a strategy for facial recognition, hopefully to recognize benefits in social behavior and holistic perception. While ASD individuals were clearly taught to recognize more greebles with training, no definite generalization was seen for social behavior or facial recognition.

Reviewer 3:

Strengths: The project made reasonable progress toward the stated objectives for Aim 1, which were to narrow down a list of candidate genes from a GWAS study that are likely to impact axonal growth/guidance. The team seemed to deal well with unanticipated difficulties in obtaining tissue samples, and adjusted their approach accordingly. The use of selective spatio-temporal expression (in relevant brain regions and at relevant developmental timepoints) to narrow the candidate genes was a significant strength. They were also responsive to the interim reviewers' suggestion that the genes be examined in tissue from individuals with ASD, which supported the targeting of LRRN3. The role for LRRN3 and other LRR genes in axonal

outgrowth was confirmed in Study 2. The stated objectives for Aim 2 were also achieved in spite of delays associated with a new scanner and the departure of one of the PIs for this Aim. A secondary intervention protocol was also developed to improve on limitations in the design of the initial intervention. Most of the goals for Aim HT2 were met, with promising results for this intervention approach in adults with ASD from economically disadvantaged environments.

Weaknesses: Figure captions or legends, or at least putting the figures in line with the relevant text would have helped with readability of the report. Experimenter error in Aim 2 administration of post-intervention tasks reduced power. The description of Aim 2, Intervention B, Composite Task (p.54) is unclear; the TD control adolescent data is presented first, but then the comment that the result of holistic face processing in the TD group was unexpected, based on holistic face processing deficits in ASD, doesn't make sense.

Reviewer 4:

The project consists of two studies with a central theme of identifying molecular mechanisms underlying ASD that will directly support intervention of the disease.

For the mechanistic study (Neuropathology and Genetics of Connectivity: Altered Axonal Pathfinding in ASD), candidate gene expression of Leucine-rich repeat (LRR) family was to be assayed from postmortem brain to ascertain patterns of temporal and anatomic involvement in ASD. The selection of these axonal pathfinding genes is based on a genome wide association study in ASD families (Autism Genome Project). Those LRR candidate genes showing selective temporospatial brain expression/ localization were to be followed up with *in vitro* functional validations. The original intent was to obtain postmortem brain tissues from 7 brain regions and 3 developmental periods, i.e. midgestation, infancy, and late childhood/early adolescence. Samples were to be obtained from NICHD Brain and Tissue Bank. The projected sample size was 90% of 235, 50 and 55 for each time period (total: ~306). However, due to significant tissue shortage, only 76 samples were collected (all in house), mostly FFPE samples and none from the childhood/early adolescence period. As a result, the scope of the study was significantly compromised (e.g., no western blots, qPCR only on 15 midgestation samples). The main finding of this part of the study is that LRRN3 showed an unusual expression in fetal subplate implicated in the pathogenesis of cortical wiring abnormalities in ASD. As an enhancement, the study also examined 3 brain samples from autistic individuals but the results are considered preliminary given the small sample size. Because of the sample restriction, several key questions originally proposed, such as gene expression at different developmental stages, cannot be examined, which significantly diminished the significance of the study. But such things happen, and it is probably beyond the control of the investigators.

The functional validation study was carried out in primary neural stem cell cultures on 4 candidate LRR genes (LRRN1, LINGO1, LRRN3, LRRTM3). These stem cells were isolated from the cerebral cortex of E10.5 mouse embryos instead of E18 mouse as originally proposed. This improved protocol allows better examination on the effects of LRRs on neural specification as well as earlier neurite out-growth, neuronal maturation and synapse formation.

The intervention study (Inducing Plasticity in Cortical Connectivity Via A Novel Intervention in ASD), was designed to induce visuoperceptual expertise with a novel class of visual objects in

15 HFA adolescents. 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. Despite several setbacks, including the temporary closure of the initial neuroimaging facilities and the transition of Dr. Scherf to a faculty position at Penn State University, the researchers were able to accomplish most of the goals of the study. Using two separate visuoperceptual training intervention methods, the study suggests that HFA adolescents can indeed learn to discriminate and recognize novel perceptually homogenous objects, but not face processing behavior.

Overall, the project was satisfactorily executed. It helped to leverage additional funds, to train numerous investigators and students, to foster additional collaborations, and to enhance the research capacity of the host institutes.

Reviewer 5:

Aim R1 had to pivot on obtaining human brain to rely primarily on a local and not national brain bank. Given this, the number of samples analyzed appeared less than intended. For R1 the project did add assessment of some autism brain samples which strengthens the work. Overall, Aim R1 did make significant progress despite some trouble obtaining brain samples. So, the data obtained and provided did address the project aims. The applicability of the findings to autism specifically remains somewhat in question with only the finding of aberrant LRRN3 showing a potential link between autism and the potential factors in brain development studied. The mouse part of this Aim appeared to be completed without complication. R2 completed a novel pilot study of a computerized training program focused on visual perspective ability in persons with autism. R2 faced several changes including outsourcing some of the work to Pennsylvania State University and some errors in the generalization of intervention A work. The sample of Aim R2 was sufficient for the imaging component which yielded interesting results, but the sample seems small from a pilot clinical trial perspective. It is hard to say from such a small sample that this computer work is feasible long-term. Aim HT2 was interesting and has resulted in essentially all of the additional leverage funding from this project. The cognitive enhancement portion of this project is interesting and holds promise to be adapted widely in the field.

Reviewer 6:

Aim R1. Objectives were not completely met, but reasonable progress was made. A tissue shortage for certain age groups and the absence of non-fetal, non-adult frozen tissue meant several of the planned tissue studies could not be carried out. Primary focus was thus placed on the immunohistochemical studies. These studies were able to identify 4 Leucine-rich repeat (LRR) proteins as potential candidates for ASD risk via the alteration of axonal guidance. An enhancement to Aim 1 repeated these studies in tissue from individuals with ASD and found differences in 1 LRR relative to controls tissue. Results from study 2 of Aim R1 are very preliminary as studies have yet to be completed.

Weaknesses: The inability to conduct more thorough tissue studies due to tissue shortages was disappointing, although this was out of the investigator's control and they were able to

supplement. The absence of quantitative results in support of the immunohistochemistry (the narrative style of the results description for this aim is frustrating to read and belies any quantitative results they may have had).

Aim R2. Objectives were largely met. Enrollment targets were met or nearly met for the Greeble studies, and improvement as a result of training was shown for some of the metrics. The finding that the Greeble training was tolerable during Intervention A was notable. The preliminary results of the fMRI analyses are encouraging, but there is substantial work left to be done.

Weaknesses: The use of Intervention A control participants as subjects for the intervention during Intervention B may introduce familiarity as a potential source of bias; these participants may have exhibited higher scores than non-familiar participants. The use of self-control values from intervention A during intervention B, coupled with the recruitment of new TD controls, introduces another potential source of bias. These adjustments were made due to procedural issues (the moving of the PI) but do hamper the results. The investigators note that the individuation trials included a component in which the difficulty of the trials was increased over time. Speculative inferences are made about this facet of the study, where this factor could have easily been included in the repeated measures ANOVA and more concrete inferences made. Data errors involving 1/3 of the Intervention A participants necessitated the use of missing data methods for the analysis of the generalization of Intervention A learning. Some of the results – for sequential matching and part-whole task – were null in that the intervention group did not show substantial improvement over control groups or any improvement was not sustained over the follow-up period. The finding for the face memory task outcome is weak – a linear trend (which was technically non-significant) over three evaluations only weakly suggests long-term improvement in face recognition, particularly when the marginal average performances of the control group match those of the intervention group. For several results, the investigators quote “trends” for non-significant results. It is not clear that the RM ANOVA model was properly specified for the Intervention B analyses. Subjects receiving the intervention during Intervention B contributed control data to the Intervention B analysis from the results of intervention A testing. It is not clear that the RM ANOVA model was properly specified to account for this source of covariation, nor that their Intervention A data were comparable to their Intervention B data. While this was made necessary by the noted procedural hurdles, this potential source of bias/error should be noted. While statistically significant results were noted for several of the metrics for the Greeble intervention, some measures of effect size should be presented. The graphical evidence makes many of the significant results appear weak. The data from fMRI data from the Greeble intervention study have yet to be fully analyzed. The individual level results (pre-post comparisons of fMRI data) are encouraging but need to be generalized.

Aim HT1: Some objectives were met. Nine lectures on ASD were created and released to pediatric PittNet, 8 of which were accredited for CME.

Weaknesses: No data are presented about the success or efficacy of the lectures. Usage statistics about the web-based lectures would have been helpful to determine if the lectures were being downloaded and viewed. An evaluation of the efficacy of the lectures via a simple comparison of pre- and post-lecture test scores would also have been helpful.

Aim HT2: Objectives were met. The adaptation of cognitive enhancement therapy (CET) to ASD appeared to be successful and tolerated by ASD individuals. Pilot testing of CET was successful and an efficacy analysis showed that CET was beneficial. A notable achievement of this aim was the addition of a revised aim – the conduct of a randomized clinical trial comparing CET in ASD to a comparator therapy (EST). The trial is ongoing and the investigators are attempting to secure funding to expand to a multisite RCT. Preliminary results have demonstrated that cognition is impaired in ASD adults, and that these impairments are similar to impairments seen in schizophrenia, and that CET has shown promise in a preliminary analysis of the first few analyzed subjects. The adapted comparative therapy (EST) also showed promise on some metrics. Neuroimaging studies associated with this aim are in progress.

Weaknesses: The training of PLEA members was removed as a milestone, as the investigators deemed the procedure too experimental, a justifiable removal. Target enrollment was not met for the pilot study. Information on how enriched supportive therapy (EST) was adapted to ASD individuals was not given. The reported differences in improvement between the CET and EST arms could potentially be due to the amount of effort and care put into the adaptation of CET to ASD. The early results comparing CET to EST are given in terms of effect sizes, but data on the actual marginal effects should be shown (a large effect size could be due to a small actual effect with low variance).

Aim HT3: The program successfully enrolled 6 undergraduate and 4 pre-doctoral underrepresented minority students in a summer research training program.

Reviewer 7:

This set of interrelated projects was based on prior scientific evidence pointing to autism spectrum disorder (ASD) symptoms as resulting from altered information processing – alterations in such parameters as social understanding, language comprehension, reasoning, emotion, motor movements, sensory processing, and learning. The goals of this research project were to: 1) develop a new intervention for ASD that would enhance thinking capacity or meaningful integration of information and brain circuitry; 2) define the brain connections for thinking and emotion that underlie emotion dysregulation, presumed to be the underlying bases for meltdowns, aggression, and withdrawal in ASD, so that more effective and individualized treatment could be developed; and 3) identify genetic and brain development mechanisms underlying this abnormal development of brain circuitry. As part of this project, RT-PCR (Reverse Transcription Polymerase Chain Reaction producing DNA copies of RNA templates, the opposite to the natural order of copying DNA templates to make RNA transcripts, used to make a DNA copy of expressed "exon" RNA sequences without the intervening, non-transcribed "introns.") Primer pairs were designed for LINGO1 (the Leucine rich repeat and Ig domain containing 1 protein from an encoding gene also known as LINGO1), AMIGO1 (Adhesion Molecule with Ig-like domain 1), LRRN1 (Leucine-rich repeat neuronal protein 1), and SLIT1 (the Slit homolog 1 protein) [LINGO1 and SLIT1 were amplified in fetal cortex.] This set of studies, as initially conceived, was a very ambitious overall project.

The objectives of this research were to translate recent scientific advances in ASD research into a novel intervention; to identify the cognitive, neural, and genetic mechanisms underlying major behavioral issues in childhood and adulthood that could directly support improvements in

everyday treatment; and expand knowledge about the fundamental developmental neurobiologic and genetic mechanisms of autism upon which further discoveries and approaches could be based. The project was divided into two main sets of studies. The first, “Inducing Plasticity in Cortical Connectivity via a Novel Intervention in ASD,” in which a paradigm previously utilized for people with schizophrenia was to be adapted 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 for people with ASD. Phenotypic markers of responders and non-responders were to be identified so that the intervention could be refined to address individual variability in initial skill level and rate of response. Before and after functional Magnetic Resonance Imaging (fMRI) was to be utilized to assess intervention effects on neural circuitry. In the second set of studies, “Neuropathology and Genetics of Connectivity: Altered Axonal Pathfinding in ASD,” developmental neurobiological studies of gene expression were to be conducted in postmortem tissue to ascertain the pattern of temporal and anatomic involvement of brain structures to inform the search for important genetic contributions to ASD. The research team ran into some unforeseen complications and delays; for example, the tissue bank originally proposed for use was found to be inadequate to the needs of the study, and the group had to turn to another. A member of the original team was recruited to another university (although he continued to work on the project in his new setting) and a new MRI scanner replaced the one that the team originally had planned to utilize. Nonetheless, the researchers for this project appear to have developed their data in ways which still fulfilled the goals of the original proposal.

Improvement in health status and access was addressed through a web-based, archived, and audiotaped lecture program on ASD and related medical and behavioral issues created for CME delivered to a large, established pediatric research and practice-based network (Pediatric PittNet) serving 115,000 families in 5 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, was to result in translation of this research to the community to improve intervention. PLEA staff was trained in use of the national Autism Treatment Network medical guidelines, and administration of the Autism Diagnostic Observation Schedule. The plan was also to train PLEA staff in the use of the Cognitive Enhancement Treatment (CET), a new comprehensive intervention program that targets cognitive, social, and complex adaptive skills for adults following modification for use with ASD (this technique had previously been studied for schizophrenia); however, it was decided that this technique needed additional standardization before it was ready for this purpose. A total of 16 adults with ASD were to be recruited to constitute pilot groups A-D in the first 6 months of the project by 12/2009, but only 14 adults with ASD were allocated to these pilot groups instead of the 16 originally proposed, the explanation being that groups A-B and C-D were collapsed to constitute larger group sizes in the event of attrition.

The team was able to recruit 14 instead of 16 adult subjects for the clinical portion of the project.

The information provided about the project was sufficient to assess the progress made.

The data and information provided were applicable to the project objectives listed in the strategic plan.

Strengths: The integrated nature of the individual studies incorporated into the project, from biogenetics to useable education for practitioners in the community, especially the minority underserved population. The knowledge of the senior researchers within the field of ASD and its causes and treatments was obvious from study design throughout implementation. The ambitious goals of this project were laudable.

Weaknesses: Minor - The difficulties of obtaining tissue samples may have been foreseeable, and perhaps more preparatory effort could have been done. It is not clear that the proposed connectivity emphasis was maintained throughout the project.

Criterion 2 - What is the likely beneficial impact of this project? If the likely beneficial impact is small, is it judged reasonable in light of the original proposal and the dollars budgeted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

This is a highly varied group of projects ranging from analyses of gene expression during brain development to cognitive enhancement therapies. The beneficial impact will likely be quite variable as well.

Aim R1 has not seemed to lead either to confirmation of the hypotheses of the relation of leucine rich repeat proteins in brain development or in the etiology of autism. A survey of PubMed indicates that this is not a prominent hypothesis in the literature. Perhaps when publications appear related to this work, they will mobilize additional research interest.

Aim R2 may have some impact on the design of future computer-based interventions for autism spectrum disorder. Some of the baseline fMRI and functional connectivity data will also lead to future research. Since it is not clear whether there has been intervention-related plasticity in the brain, it is not clear whether these studies will lead to future efforts to optimize the plasticity for improved intervention.

Aim HT1 produced a valuable resource of clinical information that can be built upon and disseminated further in the future.

Aim HT2 is probably the most beneficial of the projects. Given the increased emphasis of treating adults with autism, it is likely that very close attention will be paid to the outcome of these intervention trials.

Reviewer 2:

Aim 1: LRRN3 remains a potential candidate for an autism risk gene, which will need to be confirmed with future research.

Aim 2: Cognitive Enhancement Therapy appears to be a promising direction for therapeutic intervention, although this was only supported in part through DOH funds (part-time therapist, data management, PI time).

Greeble training demonstrated feasibility for computer-based interventions in autism, and showed that ASD individuals can learn to recognize grebbles, although this knowledge did not appear to generalize to performance gains in facial recognition or social behavior.

Reviewer 3:

Strengths: The potential impact of Aim 1 includes a candidate gene target but will require considerable additional evidence in larger samples of ASD tissue, as well as further gene expression studies. The creativity in the design of the intervention protocol in Aim 2 may inform future interventions, as the training itself was very successful in spite of limited impact (see below). The CET intervention for underserved adults with ASD was very successful and likely to translate to a large scale RCT to examine its potential to improve outcomes in adults with ASD. Intervention approaches that are targeted to adults are difficult to find, thus if the preliminary data generated by this study bear out in a larger study, it will represent a significant contribution.

Weaknesses: The impact of Aim 2 is limited. Specific weaknesses: 1) It is unclear why the researchers implemented an expertise training protocol using Grebbles, expecting perceptual improvement to generalize to face processing. Why not design a face processing intervention directly? We already know that individuals with ASD can develop considerable perceptual expertise in non-face stimuli (e.g., objects or even animated characters related to restricted interests), and to my knowledge this is not limited to local processing. 2) Although the intervention group did show improvement in Greeble recognition, it was not significantly different from the improvement seen in controls groups that did not receive the intervention. Some limited gains in the part-whole task could be attributed to the intervention (i.e., intervention ASD group performed better at time 2 than non-intervention ASD group, but not better than TD controls who did not receive the intervention). The effects of the intervention on face processing are dubious, with no group*session interaction, and a puzzling main effect of group suggesting that the ASD intervention group was less accurate than TD controls, but the ASD control group was not, and the two ASD groups did not differ from each other. The lack of group*session interaction would typically dictate that within-group effects of session should not technically be further examined, but they showed a trend for linear improvement in the intervention group only. In my opinion, the conclusion that “the intervention may have helped to improve unfamiliar face recognition abilities in HFA adolescents” is not supported by the data. Despite improvements in the design, Intervention B had an even less convincing effect on face recognition ability. 3) The imaging data are not completely analyzed at this point, and show time-related effects in the intervention group (based on individual level data), but no comparison with the other groups. These analyses will surely be forthcoming, but it is unfortunate that there is no way to assess training-specific effects on the brain of the intervention. Additionally, although it is stated that effective connectivity protocols are under development, the reported imaging analysis approach does not really capture anything about connectivity, which is the theme of the grant and presumably the reason for its juxtaposition with axonal growth genetic

studies. A DTI protocol was developed using the funds and used on previously collected data, with plans to use it on the data from the current study in the near future.

Reviewer 4:

The first study is considered a mechanistic study. The main finding is that LRRN3 seems to play a role in ASD etiology. The impact of this finding is moderate and whether a drug/treatment can be developed to modulate LRRN3 remains to be seen. Nevertheless, this type of research is important in clarifying ASD etiology because the pathogenesis of ASD is largely unknown. For the intervention study, the researchers accomplished a lot in conducting longitudinal studies of developing changes in the behavioral and neural foundation of face and object processing in both HFA and TD adolescents. Results from the study help delineate between two competing theories for explaining face-processing deficits in autism, which may guide future strategy to improve face processing, and social information processing.

Reviewer 5:

The CET portions of this project with associated imaging work holds great promise to improve treatment in autism. The first aim of this project seems detached from the other aims and appears less promising regarding impact in the field long-term. The CET work is clearly moving forward with additional funding. The genetic portions of this grant and the Greebles work appear to not have significant impact.

Reviewer 6:

Cognitive Enhancement Therapy (CET) shows promise as a therapeutic strategy for adults with ASD. If the investigators continue to experience success with CET, it could become a standard of care and an insurance-coverable mode of treatment. There were promising results for Greeble training, particularly in terms of facial recognition and potential brain alteration. The results from Aim 1 identified novel neurobiological mechanisms and genes as potential causative factors for ASD. Future research will include further genetic and neurobiological testing, closer inspection of functional neural imaging data from Aim R2, and explanation of the RCT of CET.

Weaknesses: Several components of Aims R1 and R2 have yet to be completed and are pending data analysis. Some of these components appear to be the most substantial portions of these aims. Many of the positive impacts of Greeble training, although statistically significant, appeared to be small in magnitude and potentially clinically irrelevant, although no commentary on clinical relevance is provided.

Reviewer 7:

The benefits of this project to the larger field of ASD research include a better understanding of the candidate genes for the neurological underpinnings of ASD and how their effects occur. This impact alone should justify the work done as part of this grant. The recruitment of minority candidates into the labs and clinics at the University of Pittsburgh and the education of clinicians who care for so many families in Western Pennsylvania are also very useful results.

In terms of the specific treatment resources available in Pennsylvania, the educational components and the efforts to recruit more minority candidates to take an active interest in ASD research and treatment ought to be helpful to the diagnosis and treatment of ASD - but it should

be noted that the relative numbers were low. There is no reason why the educational programming developed cannot be utilized elsewhere in the state, as well as exported to other states.

The following listing of topics was supplied:

- 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, M.D.
- The Science of Autism: Transformative Advances in the Making (Part 2)* - Nancy Minshew, M.D.
- The Verbal Individual with Autism: Have you seen this Patient?* - Nancy Minshew, M.D, and Diane Williams, Ph.D.

(CME accreditation was in process for all topics at the time of the final report.)

With disorders as complex as ASD, the progress tends to be incremental. In terms of diagnosis, work to educate PLEA staff in the use of the national Autism Treatment Network medical guidelines and use of the Autism Diagnostic Observational Schedule should be helpful. The project also provided an opportunity for some study of a new ASD treatment program, CET. In addition, the team was able to develop a web-based educational program for pediatricians and there is no reason why it could not be utilized beyond the Pediatric PittNet service area. The PI lists the major developments as follows:

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 were also demonstrated.
2. Enriched Support Therapy was also adapted for individuals with ASD, demonstrated to be acceptable to them and initial efficacy data provided.
3. The “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; and this cognitive training approach also showed alterations in the brain as a basis for assessing the improvement generated -- but no improvement in facial recognition was demonstrated.

The project was able to benefit the development of the careers of three early-career investigators (Drs. Eack, McFadden, and Scherf). They reportedly plan additional NIH grant applications – Kathryn McFadden, M.D., submitted a grant to NIH to extend and expand on the research of Aim R1, and once the papers are published reporting the original data from this project, she plans to submit a larger grant application to the NIH. K. Suzanne Scherf, Ph.D., has submitted three post-doctoral fellowship grant applications to Autism Speaks and to the Autism Science Foundation to support fellows to continue the publication work of Aim R2. Once the original

data from this aim has been published, Dr. Scherf then reportedly plans to submit an R01 grant to the NIH to extend and expand on Aim R2 work. For Aim HT2, Shaun Eack, Ph.D., and Dr. Minshew plan to submit an R01 proposing a multisite controlled trial of CET for adults with ASD to expand the evidence for efficacy of this treatment and to try to demonstrate reliable dissemination of this treatment across sites with good fidelity.

Strengths: The immediate plans for additional grant projects to build upon the work done under this grant are commendable. The modification and standardization of CET and a plan for a multi-center controlled trial of this treatment program are also specific strengths.

Weaknesses: Minor - The lack of standardization of the CET program to the ASD population ahead of time was a foreseeable issue, but the subsequent work done was admirable. Connectivity did not appear to be as much of a focus as was originally intended.

Criterion 3 - Did the project leverage additional funds or were additional project applications submitted?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Aim HT2 was the most successful in leveraging additional funds. The following grants were received to support this:

1. Evidence-Based Cognitive Rehabilitation to Improve Functional Outcomes for Young Adults with Autism-Spectrum Disorders (Autism Speaks) \$300,000
2. A Randomized Clinical Trial of Cognitive Enhancement Therapy for Adults with Autism Spectrum Disorders (NIH) \$1,412,388
3. Adapting Cognitive Enhancement Therapy for ASD (NIH) \$644,278
4. Also for HT2, Shaun Eack, Ph.D., 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.
5. Kathryn McFadden, M.D., has a grant under review at the NIH to extend and expand on the research of Aim R1.
6. K. Suzanne Scherf, Ph.D. 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.

Reviewer 2:

Aim 1: The research has sponsored an additional R21 NIH grant submission, listed as under review in the final report.

Aim 2: Numerous additional grants were obtained to pursue validation of Cognitive Enhancement Therapy.

Reviewer 3:

Strengths: The team applied for and received three additional grants (two federal, one foundation) to extend the CET intervention study.

Weaknesses: None noted.

Reviewer 4:

A grant to Autism Speak was funded. The DOH support contributed significantly to the positive decision by Autism Speaks to contribute funding for this effort. Several others, to DOD and NIH, have been submitted, but it is unclear whether any of them received the funding. Given the amount of data that was generated from this project, it is believed that some of them will materialize in the future.

Reviewer 5:

Additional leveraged funding appears to be all related to Aim R2. Nothing appears to have been leveraged related to the preclinical work. Given this, Aim R2 appears to have leveraged \$2 million in Department of Defense Funding and \$300,000k of foundation funding to support research into Cognitive Enhancement therapy in autism.

Reviewer 6:

One existing grant through Autism Speaks was used to support the adaptation of cognitive enhancement to those with autism. An NIH grant was awarded during the study period for the same goal. A large DOD grant was used to support the conduct of a randomized clinical trial comparing cognitive enhancement therapy to a control therapy (EST). Several additional grants are planned after additional publications are drafted based on the results of the current study, including an NIH grant to expand the cognitive enhancement therapy RCT to a multisite study.

No weaknesses were noted.

Reviewer 7:

The project scientists obtained three additional grants to enhance this project: one from Autism Speaks (\$300,000), another from the Department of Defense (\$1,412,388), and the third from the NIH (\$644,278). In addition, an NIH/NIMH K23 grant with Dr. Eack as PI was obtained for the period 09/01/2012 - 05/31/2016 in the amount of \$663,508 to provide additional investigator effort at no cost to DOH, and additional funding was obtained to provide junior scholar training utilizing the resources from this project for the period 08/01/2013 – 07/31/2015 in the amount of \$59,000 through the Autism Speaks Weatherstone Fellowship on the topic of: “Stress and Social Disability in Adults with Autism Spectrum Disorders.”

It would appear that the PIs knew that they would need to apply for these funds from the beginning, and did so in order to enhance and complete the DOH project, and were successful.

Kathryn McFadden, M.D, K. Suzanne Scherf, Ph.D., and Shaun Eack, Ph.D, (in collaboration with Dr. Minsheu) all plan to submit additional grants.

Strength: The leveraging of an aggregate \$2,356,666 is certainly laudable, and the plans for further grant submissions (some of which may have already borne fruit by now) certainly show excellent follow-through.

Weaknesses: None.

Criterion 4 - Did the project result in any peer-reviewed publications, licenses, patents or commercial development opportunities? Were any of these submitted/filed?

STRENGTHS AND WEAKNESSES

Reviewer 1:

Ten papers related to the research in this project have been published and provided. No patents or commercial development were undertaken.

Reviewer 2:

Aim 1:

Only a single abstract and 1 review article (Frontiers in Human Neuroscience) are listed in the final report for this aim. Authors describe 4 potential manuscripts, 2 of which were fully funded by the Pennsylvania Department of Health (DOH) and 2 were partially funded by DOH. The impact of these manuscripts is not clear at the present time.

Aim 2:

DOH Support:

Scherf et al. Brain 2014
Scherf et al. Cerebral Cortex 2013
Scherf et al. Hormones and Behavior 2013
Dinstein et al. Neuron 2012
Scherf et al. Dev. Cog. Neurosci. 2012

Partial DOH Support (CET also had dedicated support from NIH R33, K23, DOD, Autism Speaks):

Cognitive Enhancement Therapy description: 2 papers published in Journal of Aut. & Devel. Disorders (Eack 2013, Eack 2013).

Review paper on psychosocial interventions (Eack JADD, 2013)

Comparison of social impairments in adults with ASD and Schizophrenia, published in Schizophrenia Research (Eack 2013)

Several additional papers are listed as in preparation, and the impact of project 2 will likely continue to grow.

Reviewer 3:

Strengths: Ten papers are accepted or published in a variety of clinical and neuroscience journals.

Weaknesses: Many of these are review articles, rather than primary literature. Very little is published on the first two aims of the project at this point, although several “in preparation” manuscripts are listed.

Reviewer 4:

The project resulted in 10 publications (9 published and 1 accepted), which is an excellent achievement overall. However, because of the delay in sample accrual, Project 1 (led by Drs. McFadden and Devlin) did not seem to produce any research papers. One paper by Drs. Minschew and McFadden (published in *Frontiers in Neuroscience*) is a review article. Investigators are encouraged to publish their study results in the near future.

Reviewer 5:

No commercial or IP development was noted. Peer reviewed publications have resulted and many appear in preparation. The participants appear to be doing a good job of getting manuscripts in print as final data is available. Both the CET and Greebles approaches appear unique in autism work and would hold potential for some commercialization, but this direction does not appear to be something being pursued.

Reviewer 6:

No licenses, patents, or inventions resulted from this project. 10 peer-reviewed publications resulted from this project including four papers about cognitive enhancement therapy (Aim HT2 – review, relation to schizophrenia, CET feasibility, and early trial report), five publications regarding neural connectivity (Aim R1), and one regarding face recognition and brain development (Aim R2). Investigators are planning several additional years’ worth of publications, based particularly on extensions of Aim R1, results from functional neural imaging data of Aim R2, and the results from the RCT of cognitive enhancement therapy (Aim HT2).

Weaknesses: The publications from Aim HT2 – the randomized trial of CET – seem incremental and minimal in scope, as do the planned publications. The results of Aim R2 are unpublished, where results appear to be ready. The plans for these publications also appear to be incremental in scope.

Reviewer 7:

Ten papers have been listed in the final report from the project, and PDFs are available.

Additional publications are anticipated.

Three of the ten publications listed are in the *Journal of Autism and Developmental Disorders* and one each in *Schizophrenia Research*, *Frontiers of Neuroscience*, *Brain*, *Cerebral Cortex*, *Hormones and Behavior*, *Neuron*, and *Developmental Cognitive Neuroscience* – all high quality, well known journals and very relevant to the field.

Strengths: Ten articles were listed -- but they were not focused across the board of all aims, however. There were no patents listed.

Weaknesses: Minor - It would be highly desirable to see additional articles covering more areas of the work submitted for publication.

Criterion 5 - Did the project enhance the quality and capacity for research at the grantee's institution?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The PI and investigators indicate that the funding provided to this program of research has increased the capacity of several Pennsylvania institutions to carry out autism-related research. For example, Dr. Scherf (Aim R2) was recruited to Penn State University, creating a new research effort in autism in the middle region of the Commonwealth.

New faculty members involved in autism research have been recruited to the parent institution.

Reviewer 2:

The project clearly demonstrated enhanced capacity for research both at Carnegie Mellon and Pittsburgh, with admirable record of student and fellow training and development of new funded research projects.

Reviewer 3:

Strengths: The investigators report that the funds expanded and enhanced their capacity to pursue three new, innovative research efforts, as well as to launch three new independent investigators, in the field of ASD research.

Weaknesses: None noted.

Reviewer 4:

The host institution seems to have benefited from the award. There are a couple of NIH and other grants under development. Three post-doctoral fellowship grant applications have been submitted. The award also helped to train 4 doctoral and 6 undergraduate students. Importantly, this project greatly expanded and enhanced the research capacity for ASD in the host institution by adding three new researchers in the field and establishing infrastructure to support the research. A new research effort in autism in the middle region of the Commonwealth was created with the relocation of one of the researchers on the team, Dr. Scherf. The award also resulted in many new collaborations between the research team and other faculty members in the institution.

Reviewer 5:

It appears that this project led to some additional collaborations and leveraged additional funding that enhanced the research capacity for this autism program. This is more apparent on the clinical aims as opposed to the preclinical aim. It is unclear how much this project meant to the development of the preclinical work at this institution.

Reviewer 6:

Dr. Scherf was added to the faculty of the host institution. Three research lines (and three researchers) were brought together under the same funding umbrella. Six undergraduate and 4 pre-doctoral students were recruited to participate in research through the minority training program. The current grant has served as a springboard for two additional funded studies and several forthcoming applications.

Weaknesses: It's not entirely clear that there was any formal broad impact on the research infrastructure at the host institution, other than linking three disparate lines of research under a single funding mechanism.

Reviewer 7:

The project expanded and enhanced the University of Pittsburgh's capacity to pursue new, innovative research efforts in ASD. The team reports that none of this research would have been possible without this award. In addition, support for these faculty members enabled them to expand their autism research efforts beyond the Pennsylvania Department of Health (DOH) protocols and to pursue ancillary projects. An example of this multiplicative effect is that participants recruited for the DOH-sponsored treatment studies also participated in other ASD research, allowing this group to further refine the definition of the cognitive and neural mechanisms underlying ASD manifestations. These additional studies demonstrated that neither attention abnormalities nor abnormalities in the brain's exhibition-inhibition balance were responsible for ASD manifestations, although both of these deficits had been widely hypothesized to be involved in ASD. In addition, studies of sensory perception by Marlene Behrmann, Ph.D., 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, the team 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 and funding was obtained from the state for this project. Dr. Eack was also 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 although the treatments used are different. Publications from this group are reflective of the multiplicative effect of this DOH grant.

There was no new equipment reported as being directly funded through this DOH grant. The project was intended to bring three investigators to the next level of recognition, and this seems to have been accomplished, although one of these faculty members was recruited by another university (fortunately also located in Pennsylvania, however).

This health research project enabled the institution to bring three early-career researchers to this field to the next level - Drs. McFadden, Scherf, and Eack. The grant also reportedly furthered three new lines of research at the institution. According to Dr. Minshew, each of these three faculty member has made progress during this project that will lead to major advances in research and diagnosis in ASD and will help them further establish their careers.

Pre-doctoral minority students were given educational enhancements to develop their interest in research in general and in the field of developmental disorders in particular.

Strengths: Two faculty members were able to move into the next level of research expertise within the area of ASD, and another to move to a faculty position at another university.

Weaknesses: No major weaknesses.

Criterion 6 - Did the project lead to collaboration with research partners outside of the institution, or new involvement with the community?

STRENGTHS AND WEAKNESSES

Reviewer 1:

The project did not lead to new collaborations with research partners outside of the institution. While the initial version of the project description had included partnership with community groups for dissemination of the Cognitive Enhancement Therapy component of HT2, it was deemed premature to pursue this given the initial uncertain effectiveness of the treatment.

Reviewer 2:

Additional collaborations were formed, for example with Dr. Scherf and Smyth. Support from the Pennsylvania Department of Health will also likely be contributory to integration of therapeutic innovations in cognitive enhancement therapy into standard of care.

Reviewer 3:

Strengths: The investigators state that the departure of Dr. Scherf to Pennsylvania State University for a new position has strengthened collaborations between the institutions, and that the team has developed new collaborations with researchers at Carnegie Mellon University. The investigators also report stronger collaboration with community/parent/advocacy groups in their community.

Weaknesses: The links between the new collaborations with Carnegie Mellon University (rTMS and mindfulness meditation) to the current project are unclear. It is surprising that the investigators do not mention the community partner from which their adults were recruited (and for which one of the unmet goals of Aim HT2 was staff training in CET, although the rationale is given that the investigators wanted to build a stronger evidence base before taking this step)—the Program for Living, Education, and Advocacy (PLEA).

Reviewer 4:

The project led to collaboration with research partners at Carnegie Mellon University.

Reviewer 5:

This project expanded collaborations within a limited scope to Pennsylvania State University and to Carnegie Mellon University. Broader expansion of collaborations was not clearly documented. The Pennsylvania State University collaboration occurred because a key member of the original study team changed institutions. It would appear that involvement beyond the

collaborators noted would be helpful in future planning of potential multi-site Greebles or CET trials.

Reviewer 6:

Collaborations with investigators at Carnegie Mellon University were developed. Investigators have begun working in conjunction with parent groups in Pittsburgh (Autism Connections and the Autism Society of America, Pittsburgh chapter) and an adult group (Cranberry Cares). The proposed extension of the RCT of CET will make it a multisite study, presumably extending the collaborative sphere of this project.

No weaknesses.

Reviewer 7:

The project led to collaboration with research partners outside of the institution, and to new involvement with the community.

There will be more work on ASD done at Dr. Scherf's new institution, Penn State University, and Drs Eack and Minshew have reportedly developed new collaborations with Robert Mason, Ph.D., (Carnegie Mellon University), who does research in repetitive Transcranial Magnetic Stimulation (rTMS) and J. David Cresswell, Ph.D., (Carnegie Mellon University) who does research on mindfulness meditation and its potential for changing cognition and related brain circuitry. They anticipate developing new treatments for ASD that combine rTMS with a neurocognitive approach. New community partner relationships were developed at the University of Pittsburgh – Autism Connections of Pennsylvania and the Pittsburgh chapter of the Autism Society of America. The investigators are also now 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 William Rock, an artist with a track record of promoting the growth of young adults with ASD using art as a nonverbal medium for self-expression and building self-esteem. Mr. Rock is also reportedly planning to begin working with Drs. Cresswell and Minshew in the near future to develop a mindfulness meditation program for adults with ASD.

This was a large project, part of which was clinical in nature and part of which was more basic science. The studies involved faculty from the University of Pittsburgh, Carnegie-Mellon University, and Penn State University, and a consultant from Vanderbilt University. There were a number of healthcare professionals and trainees involved – the project also involved medical education for 20 PittNet group practices encompassing 106 pediatricians serving approximately 115,000 families living in five Western Pennsylvania counties.

Strengths: The project involved collaborations with multiple research teams and community groups. Several new alliances were formed in both arenas. Some of the direct relationship of this grant to the new community connections was not made clear in the reports.

Weaknesses: No major weaknesses were noted, but more work on community and professional collaborative work seems indicated.

Section B. Recommendations

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. Aim R1 made the untenable assumption that fetal postmortem tissue would be available for their studies and thus the overarching goal of that project, which was to study spatiotemporal distribution of LRR gene expression, was not possible. While these types of studies are incredibly valuable, it is essential that the investigators establish a source of tissue prior to defining the scope of the work.
2. Investigators should make a greater effort to approach the community to get input on the roll out of CET. It was regrettable that PLEA was not incorporated in the project.

Reviewer 2:

1. Definitive characterization of LRRN3 will require additional work, which I am confident authors are pursuing.
2. Manuscripts detailing greeble training results should be submitted for publication. It is common for intervention trials to have small n, and very few imaging intervention trials have been published. Early experience like this really can be informative even when not entirely successful.

Reviewer 3:

1. The coherence of the three aims of the project is limited; it is difficult to link the processes of early axonal outgrowth and neuroimaging/intervention in adolescence and adulthood. While interventions targeted to adults are clearly needed and the success of the CET was a significant strength, the investigators should look for ways to tie the three aims together better moving forward.
2. It seemed unrealistic to expect Greeble training to generalize to face processing skills. Future intervention studies should target the behavior of interest more directly.
3. The imaging analyses are incomplete and this makes it very difficult to evaluate the effects of training on brain function in a meaningful way. The emphasis on connectivity and networks in the original application should be honored as the analyses progress.
4. Following up on the autism tissue aspect of Aim I with greater numbers will be important for interpretation.

Reviewer 4:

The major weakness of the project is the failure to obtain the brain tissues as originally proposed, which significantly compromises the ability for the researchers to pinpoint when developmentally the functional connectivity in the ventral visual pathway that supports both face and object processing become disrupted in autism. Efforts should be made to search other tissue banks so this important question can be answered.

Reviewer 5:

The grantee should expand future work in Aim R1 into autism with larger autism brain study and potentially into mouse models of autism. Right now, the translational linkage between what has been done in Aim R1 is limited and could be expanded. The hope is the NIH grant related to Aim R1 is funded to provide this needed expansion.

Reviewer 6:

1. Analysis of data from Aim R1 needs to proceed and papers published. Further investigation on additional tissue samples representing the targeted diversity in age should be pursued. Further analysis of tissue samples from ASD individuals should be pursued.
2. Additional analyses with proper specification of factor effects for the data from Aim R2 should be conducted and results updated if necessary. Use of generalized estimating equations and/or nonlinear mixed effects models may be justified. The analysis of neuroimaging data should proceed and population level analyses pursued.
3. Continued conduct of the RCT for CET is encouraged, and the investigators have additional resources to do so (including potential funding to expand to a multisite study).
4. Some data about the online lectures – usage statistics, performance on pre- and post-tests – should be presented so efficacy can be determined.

Reviewer 7:

1. Prior due diligence on tissue availability should be seen as highly important as part of the grant submission in such projects.
2. Forging better bonds among the research labs and projects would seem very useful in furthering the research collaborations started under this grant.
3. Several plans for future publications are mentioned and those must be pursued for this project to have the future impact that it should.

Generic Recommendations for the University of Pittsburgh

Reviewer 7:

This was a well-conceived and reasonably well-executed set of interrelated studies regarding ASD. But some changes in initial plans had to be made due to circumstances that had the

potential to be seen as likely ahead of time. The scientists involved took steps to rectify these situations but might have had greater success with the studies with a bit more preparation. The most obvious example of this would be in the case of the tissue acquisition. The research team members were able to leverage additional funding in order to complete and enhance the proposed studies and have laid the groundwork for translation of some of their work into what are likely to become useful clinical strategies and educational programming.

ADDITIONAL COMMENTS

Reviewer 2:

- 1) Sample size for ASD vs. TD tissue comparison for LRRN3 remains too small to demonstrate ASD specific alterations, which will require future work.
- 2) Greeble training manuscripts should be publishable, and would be informative to the research community if not already submitted.

Reviewer 5:

The project would also be well served to take steps to enhance the translational potential of the preclinical Aim to attract future funding. Expansion of the CET work to multiple sites will be helpful. Additional ASD sample analysis will be needed. Also the work performed on Aim 1 needs to be published.

Reviewer 6:

Further publications derived from all aims need to be pursued. Tissue analyses to fill in the under-sampled age groups in Aim R1 should be pursued, although this would likely need to occur under some other funding mechanism. Reanalysis of some of the results from Aim R2 under proper specification of factor effects (potentially using GEE or mixed effects models) would strengthen the results of the study. Some measure of analysis of data (usage statistics, pre- vs. post-lecture performance) from Aim HT1 should be provided, if data are available. If not, those data should be collected.

Reviewer 7:

None of the weaknesses came to the level of an overall unfavorable rating.