Response Form for the Final Performance Review Report*

1. Name of Grantee: University of Pittsburgh

2. Year of Grant: 2008 Non-Formula Grant

3. Project Title: Deciphering Altered Brain Connectivity in ASD to Improve Intervention

4. Principal Investigator: Nancy J. Minshew, MD

A. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in <u>Section B</u> of the Final Performance Review Report using the following format.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the Final Performance Review Report the reviewers' comments listed under "Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation in ongoing or future research funded by the Health Research Program*):

Section B. Recommendations

SPECIFIC WEAKNESSES AMD RECOMMENDATIONS

<u>Reviewer 1:</u>

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.

   Response:

   Despite early difficulties, it was possible to examine spatiotemporal patterns of LRR protein expression over the developmental span and identify LRR candidates suitable for follow-up functional studies. Although our time frame was expanded greatly, the consolation is that we have collected formalin-fixed, paraffin-embedded tissue from orbitofrontal cortex, anterior cingulate cortex, dorsolateral prefrontal cortex, frontal operculum, insular cortex, dorsolateral occipital cortex, superior temporal gyrus, hippocampus with lateral geniculate nucleus, striatum, thalamus, cerebellum, and brainstem, as well as key areas representing the ventral visual pathway, i.e. fusiform gyrus, visual cortex, and accessory visual cortex from almost 80 individuals of different ages. While time consuming to acquire, this material is tailored for our planned future studies and is convenient and lower cost. Acquiring samples from individuals aged 5-20 has been a particularly rate limiting step in this process, and is a common deficit in all extant brain banks. However, with supplementation from the Harvard Brain Bank, now that our research has a more narrow focus, we should be able to more fully examine this age span.

* Please note that for grants ending on or after July 1, 2007, grantees' Performance Review Reports, Response Forms, and Final Progress Reports ***will be made publicly available on the CURE Program's Web site.***

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.

   Response:

   If "roll out" is intended to mean dissemination of this intervention to community providers, then this seems premature as we are at least 5 to 8 years from completing our first large controlled trial for which we are submitting an RO-1 shortly. Two such trials will be needed for CET to achieve Medicaid reimbursable status as a treatment for autism; lacking reimbursement status will preclude dissemination to the community. From the beginning, we have been committed to ensuring a compelling evidence base for CET before disseminating clinically. The field is rife with non-evidence based treatments, which are made available to families without evidence of efficacy. We especially wanted to avoid this pitfall. Once we do have evidence of efficacy, we will be committed to large-scale dissemination. In anticipation of a multi-site study, we are encouraging interested groups to identify a graduate student to apply for fellowship funding so that they achieve the required level of CET expertise by the time dissemination is supported by the evidence.

   We have presented the elements of CET at the Northwest Autism Society meeting in Erie, PA last year. This conference was attended by the Pennsylvania Department of Public Welfare Autism Bureau chief, Nina Wall, and by Dennis O'Brien, a Philadelphia city councilman, former state legislator, and longstanding advocate for autism services. These officials who both have family members with autism as well as the other families and professionals attending the meeting were very excited by this new treatment. We expect to have complete data on 40 individuals participating in the RCT of CET and EST by the fall of 2014 and will begin presenting these findings broadly. We are currently scheduled to present these initial findings to the Autism Society of Greater Phoenix meeting of 400 attendees, 60% parents and 40% professionals and we will arrange to receive feedback from the attendees on CET. We have also incorporated many family and adult organizations in our project to provide feedback on treatment experiences, which has included PLEA.

<u>Reviewer 2:</u>

1. Definitive characterization of LRRN3 will require additional work, which I am confident authors are pursuing.

   Response:

   We are resubmitting the R21 "LRRN3, an ASD Candidate, is Involved in Subplate Development" on February 1, 2015. Overall, we had favorable comments for our previous submission but are required to add an experiment involving analysis of behavior of neural stem cell progeny in LRRN3 knock-out mice. We are additionally resubmitting our manuscript "Developmental Brain Expression of LRRN3, an Autism Spectrum Disorder Candidate" to Cerebral Cortex next month after being required to perform additional experiments involving expression of LRRN3 in ASD post-mortem cortex. The Autism Tissue Project has been substantially re-structuring their tissue ordering system but promise to be back on-line this month. We have developed viral vectors to both over-express and knock-down LRRN3 in developing neurons derived from mouse neural stem cells to supplement our siRNA experiments. Our manuscript "LRRN3 is Necessary for Neuritic Outgrowth in Developing Cortical Neurons" will be submitted to Developmental Neuroscience 08/14.

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.

   Response:

   We are very excited to receive the Reviewer's encouragement about publishing the behavioral and neuroimaging findings related to our intervention projects, despite our small sample size. We are in the midst of writing the first manuscript detailing the behavioral findings across the two versions of the intervention. There are consistent findings of successful Greeble learning, the emergence of holistic processing for Greebles, and generalization of learning to novel Greebles. We also can definitely demonstrate that Greeble learning did not interfere with face processing and may have contributed to a slight increase in recognition abilities for unfamiliar faces. We expect to submit this manuscript for publication in the next month.

   We are also in the early stages of determining the set of analyses of the fMRI, sMRI, and DTI data across the three longitudinal time points that illustrate the neural changes (or perhaps lack thereof) across the three participant groups. We expect to begin writing this manuscript near the end of the summer (Sept 2014).

<u>Reviewer 3:</u>

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.

   Response:

   The coherence appears to be limited because each aspect of this work had to have an achievable focus within the time frame of this funding mechanism. A more comprehensive approach would have made the interconnections much more evident but would have been prohibitively expensive and not necessarily feasible with today's methods. Evolution of the technology and science in each of these areas of endeavor is advancing very rapidly and it will be possible to assess all of these aspects in a single cohort in the not too distant future.

   Neural plasticity is the most obvious link between abnormal development of information processing systems in the brain in ASD and response to CET treatment in adults. Cortical systems neuroscience and plasticity are perhaps the two themes that clearly transcend all of our aims.

   The developmental neurobiological studies in this project focused on axonal outgrowth findings because of genetic evidence of their involvement in ASD. However, there is considerable evidence already from imaging research that axonal outgrowth is not normal in ASD. Diffusion tensor imaging and diffusion tensor tractography research studies have already defined abnormalities in major cerebral white matter tracts in 6-7 month old infants later diagnosed with ASD; longitudinal imaging of these infants through 2 to 3 years of age has demonstrated that these white matter tracts continue to develop abnormally. DTI and DTT studies of adolescents and adults with ASD have similarly defined abnormalities in white matter tracts in older aged subjects. Genetic research in autism continues to define

more and more gene loci pertinent to the etiology of autism and RNAomic and proteiomics are defining a highly complex matrix of abnormalities at each of these levels of the pathophysiology to demonstrate a convergence on a small number of processes. In addition to axonal outgrowth, synapse formation and maintenance are also centrally impacted in ASD. We did not investigate the latter in this study. Tying all of these levels of the pathophysiology together depends on further advances in technology to refine the abnormalities and provide a way of assessing abnormalities at each level *in vivo*. Going forward, we continue to obtain blood for DNA from each willing participant and their parents for sequencing studies to provide the potential for future data analyses that link gene findings to behavioral and treatment response variables. In addition, we are accumulating imaging data on each of the CET participants in the CET trial. We are also accumulating a large multi-modal imaging data base of ASD adults from 18 to 65 years and are currently submitting a grant to NIH to develop new analytic methods to apply to this dataset that will enable the type of integration proposed.

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.

   Response:

   This study was motivated by the large number of studies in the existing literature that have attempted (with minimal success) to improve face processing behavior (identity recognition, expression recognition) among individuals with autism. These previous studies have largely shown modest learning during training, and minimal to no generalization of the learning to novel faces. The hypothesis motivating the intervention work in this grant was that the social-affective demands of face processing may fundamentally interfere with the ability to process the visuoperceptual properties of faces in a holistic way. As a result, training on a novel class of stimuli that require similar processing of the stimulus properties might enable the visuoperceptual system to acquire the processing strategies (e.g., holistic processing) in the absence of this interfering information. Prior to this work, it was not clear whether the visuoperceptual systems of individuals with autism are so oriented toward processing features that it compromises the ability to process the stimuli holistically or configurally. Our results provide a proof of concept that this is NOT the case. Individuals with autism can learn to process visual stimuli holistically. The second finding, that holistic processing of one stimulus class does not interfere with or bootstrap holistic processing of other classes of visual stimuli (at least over the course of a single year), will inform current models of the formation of visuoperceptual expertise and the neural basis of such expertise. We have no evidence that the formation of visuoperceptual expertise for one class of objects compromises such expertise for other classes of objects. Unfortunately, the fact that many of our participants with autism exhibited holistic processing of faces at baseline made it difficult to evaluate relative improvements in holistic processing of faces as a result of the intervention.

   At Penn State University, Dr. Scherf has also begun a new collaboration in which she has received pilot funding from the Center for Innovation in Online Learning to design a “serious game” to improve sensitivity to and use of eye gaze information in adolescents with autism. This is a core symptom of autism that appears to directly interfere with social interactions for individuals with autism. The studies funded by the PA Department of Health and Human Services were critical in providing Dr. Scherf with the expertise to explore this new direction in intervention.

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.

   Response:

   We appreciate the concern that all of the neuroimaging analyses were not finished at the time of the submission of the final report. We are still in the process of developing a protocol for analyzing longitudinal changes in the distributed patterns of activation for faces and other stimuli, the functional connectivity between neural regions in the face- and object-processing systems, as well as for the micro- and macro-structural properties of the white matter tracts. This is an ongoing effort that is the basis of several graduate student thesis projects at Penn State University. We expect to prepare several manuscripts describing the results from these protocols in the course of the next several months.

   We will definitely complete our original aims related to connectivity and network analyses as originally proposed. Dr. Marcel Just and Dr. Tim Keller in our autism research group are also working on developing new methods to analyze behavioral and multi-modal imaging data of this type, as is the NIH and Canadian funded US-Canadian child imaging research collaboration. These local, national and international efforts will result in the needed advances in image analysis methods in the very near future that can be applied to our data sets.

4. Following up on the autism tissue aspect of Aim I with greater numbers will be important for interpretation.

   Response:

   We are resubmitting the R21 "LRRN3, an ASD Candidate, is Involved in Subplate Development" on February 1, 2015. Overall, we had favorable comments for our previous submission but are required to add an experiment involving analysis of behavior of neural stem cell progeny in LRRN3 knock-out mice. We are additionally resubmitting our manuscript "Developmental Brain Expression of LRRN3, an Autism Spectrum Disorder Candidate" to Cerebral Cortex next month after being required to perform additional experiments involving expression of LRRN3 in ASD post-mortem cortex. The Autism Tissue Project has been substantially re-structuring their tissue ordering system but promises to be back on-line this month. We have developed viral vectors to both over-express and knock-down LRRN3 in developing neurons derived from mouse neural stem cells to supplement our siRNA experiments. Our manuscript "LRRN3 is Necessary for Neuritic Outgrowth in Developing Cortical Neurons" will be submitted to Developmental Neuroscience 08/14.

<u>Reviewer 4:</u>

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.

Response:

We have made progress on this goal. We have collected key areas representing the ventral visual pathway, i.e. fusiform gyrus, visual cortex, accessory visual cortex, and are analyzing these with LRRN3 antibody. So far, LRRN3-positive subcortical white matter neurons appear to be particularly densely distributed in the visual cortex and are more frequently observed to be connected with each other. If we need to supplement our sample, we can make a more targeted request to the Harvard Brain Bank as these brain regions are not in as high demand. However, this will be contingent on funding.

Reviewer 5:

The grantee should expand future work in Aim R1 into autism with a 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.

Response:

We also hope to pursue these next logical steps and are building the infrastructure in papers and data to obtain grant funding to do this. We are resubmitting the R21 "LRRN3, an ASD Candidate, is Involved in Subplate Development" on February 1, 2015. Overall, we had favorable comments for our previous submission but are required to add an experiment involving analysis of behavior of neural stem cell progeny in LRRN3 knock-out mice. We are additionally resubmitting our manuscript "Developmental Brain Expression of LRRN3, an Autism Spectrum Disorder Candidate" to Cerebral Cortex next month after being required to perform additional experiments involving expression of LRRN3 in ASD post-mortem cortex. The Autism Tissue Project has been substantially re-structuring their tissue ordering system but promise to be back on-line this month. We have developed viral vectors to both over-express and knock-down LRRN3 in developing neurons derived from mouse neural stem cells to supplement our siRNA experiments. Our manuscript "LRRN3 is Necessary for Neuritic Outgrowth in Developing Cortical Neurons" will be submitted to Developmental Neuroscience 08/14.

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.

   Response:

   We are resubmitting the R21 "LRRN3, an ASD Candidate, is Involved in Subplate Development" on February 1, 2015. Overall, we had favorable comments for our previous submission but are required to add an experiment involving analysis of behavior of neural stem cell progeny in LRRN3 knock-out mice. We are additionally resubmitting our manuscript "Developmental Brain Expression of LRRN3, an Autism Spectrum Disorder Candidate" to Cerebral Cortex next month after being required to perform additional experiments involving expression of LRRN3 in ASD post-mortem cortex. The Autism Tissue Project has been substantially re-structuring their tissue ordering system but promise to be back on-line this month. We have developed viral vectors to both over-express and knock-down LRRN3 in developing neurons derived from mouse neural stem cells to supplement our siRNA experiments. Our manuscript "LRRN3 is Necessary for Neuritic

Outgrowth in Developing Cortical Neurons" will be submitted to Developmental Neuroscience 08/14.

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.

   Response:

   We will consult with a statistician at Penn State University to gather additional expertise to complete these analyses.

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).

   Response:

   We are continuing our randomized-controlled trial with support from the Department of Defense in order to accumulate efficacy data on CET in adults with ASD. In addition, based on our promising preliminary data from this trial, we have recently (June 1, 2014) submitted a large-scale efficacy R01 application to NIMH to continue and extend this work to 100 additional adults with ASD. Together, this will provide the efficacy data needed to evaluate the potential benefits of CET in this population, and if supported, ultimately provide the evidence needed for third-party reimbursement and implementation. We hope to develop and acquire funding for training fellowships so that we will produce CET competent clinicians as we conduct this next RCT to support generalization to other sites once the replication is complete.

4. Some data about the online lectures – usage statistics, performance on pre- and post-tests – should be presented so efficacy can be determined.

   Response:

   The online lectures were distributed by a different department within the university, so our access to these data is limited at present. From the data given to us, we found that test scores increased from the pre-test to the post-test. In addition, the majority of people who viewed the videos indicated on a survey that the information enhanced their knowledge of autism and was relevant to their work setting. We will make these lectures available more broadly in the upcoming year and require specific feedback on utilization and impact. We apologize that we are not able to provide more specifics for our experience to date. We will continue to use this mechanism to disseminate research findings that can be used to improve practice.

<u>Reviewer 7:</u>

1. Prior due diligence on tissue availability should be seen as highly important as part of the grant submission in such projects.

   Response:

Despite early difficulties, it was possible to examine spatiotemporal patterns of LRR protein expression over the developmental span and identify LRR candidates suitable for follow-up functional studies. Although our time frame was expanded greatly, the consolation is that we have collected formalin-fixed, paraffin-embedded tissue from orbitofrontal cortex, anterior cingulate cortex, dorsolateral prefrontal cortex, frontal operculum, insular cortex, dorsolateral occipital cortex, superior temporal gyrus, hippocampus with lateral geniculate nucleus, striatum, thalamus, cerebellum, and brainstem, as well as key areas representing the ventral visual pathway, i.e. fusiform gyrus, visual cortex, and accessory visual cortex from almost 80 individuals of different ages. While time consuming to acquire, this material is tailored for our planned future studies and is convenient and lower cost. Acquiring samples from individuals aged 5-20 has been a particularly rate limiting step in this process, and is a common deficit in all extant brain banks. However, with supplementation from the Harvard Brain Bank, now that our research has a more narrow focus, we should be able to more fully examine this age span.

2. Forging better bonds among the research labs and projects would seem very useful in furthering the research collaborations started under this grant.

   Response:

   Dr. Eack and the CET research team are an integral part of the main clinical research site. We continue to work closely with Dr. Just to provide input on imaging paradigms for this study. The shared focus on adults with ASD will continue to produce overlapping data sets. Dr. Just and Dr. Minshew have been close collaborators since 2007 and this is continuing to be active. Likewise, Dr. Minshew and Drs. Behrman and Scherf continue to be close collaborators supporting new research. Dr. Scherf has relocated to Penn State University in State College, PA. Nonetheless, we continue to recruit participants for her ongoing research and Dr. Scherf and Dr. Behrmann continue to be close collaborators.

   Dr. McFadden continues to work on completing her studies for publication with the support and assistance of Dr. Peter Strick. This grant played a central role in enabling all of this work to move forward. Lack of an autism institute and further center funding has imposed limits on the capacity to integrate this work. Nonetheless, we continue to move ahead though in a somewhat fragmented manner.

3. Several plans for future publications are mentioned and those must be pursued for this project to have the future impact that it should.

   Response:

   We are absolutely committed to the publication of all data from this study as analyses are finalized.

B. If the grant received a rating of "unfavorable," please indicate the steps that you intend to take to address the criteria that the project failed to meet, and to modify the grant oversight process so that future projects will not receive "unfavorable" ratings.

Response: This project received a "Favorable" rating.

C. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: We greatly appreciate the support of this grant funding that allowed us to develop several new aspects of research. Each aspect has achieved a "life of its own" and will continue to contribute new advances to the fundamental understanding of the etiology of ASD and new treatments for ASD that will improve lives in the community. We would like to thank everyone who has made this funding possible including all program officers and staff and all reviewers.