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Phoenix, Arizona 85006

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**Title and Subtitle**
Cognitive and Neural Correlates of Aging in Autism Spectrum Disorder

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**Sponsoring/monitoring Agency**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**Distribution/Availability Statement**
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This is a collaborative project between SARRC and BNI to produce comprehensive cognitive, behavioral, and neuroimaging data on a set of well-characterized older ASD individuals, which can be used as a reference for clinical diagnosis, therapeutics, and care plans. SARRC is primarily responsible for the identification and phenotyping of each participant (AR140105P1) and BNI conducts additional neurocognitive testing and fMRI scanning protocol (AR140105). Both principal investigators contribute to data analyses and dissemination of findings. We continue to collect new participants to obtain our target enrollment of 70 participants (35 per group). **Results and significance:** We published our first in a series of studies from the initial cross-sectional data analysis in a high impact Autism journal (Autism Research). We found that the older ASD group performed significantly worse in executive functioning and showed decreased white matter connectivity in the frontal and temporal lobes. We also submitted resting state data regarding frontal connectivity group differences to the Society for Neuroscience meeting and Dr. Braden was selected to chair a nanosymposium on Autism: Physiology and Behavior. We have two additional data analyses on other brain-behavior relationships that are being prepared for publication, and we continue to collect data from new recruits and from returning participants.

We also succeeded in sharing our imaging and cognitive data by way of the Autism Brain Imaging Data Exchange 2 (ABIDE-2), thereby providing new data for the greater Autism research community.

15. SUBJECT TERMS
Autism, aging, functional MRI, fMRI, neuroimaging, executive functioning, memory, cognition, cortical thickness, connectivity, white matter, sparse Bayesian networks, machine learning

16. SECURITY CLASSIFICATION OF:

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17. LIMITATION OF ABSTRACT
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18. NUMBER OF PAGES
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19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

As the first diagnosed Autism Spectrum Disorder (ASD) individuals are now reaching old age, it is imperative that we understand the impact of aging on individuals with ASD. Given the striking parallels in ASD of deficits in executive function, subserved by the frontal lobe, and that the frontal lobe is susceptible to normal age-related changes, we combine neuroimaging, cognitive assessments and behavioral measures to examine aging in ASD compared to Typically Developed (TD) adults. We hypothesize that individuals with ASD will have an exacerbation of deficits beyond normal aging, as evidenced by significantly lower scores on tests affected by aging (e.g., executive) along with neuroanatomical markers of dysfunction, and relative preservation of function subserved by more posterior brain regions (memory and local detail processing). Our objective is to produce comprehensive cognitive, behavioral, and neuroimaging data on a group of well-characterized older individuals with ASD who can be used as a reference for clinical diagnosis, therapeutics, and care plans. To achieve this goal, our three-year project involves longitudinal assessment of aging (40–60 y.o.) ASD individuals versus age-matched TD. In addition to commonly used statistical methods, we will use innovative machine learning and sparse Bayesian networks to combine structure, function, cognition, and symptom profiles to specifically address contributions to accelerated aging in ASD.

2. KEYWORDS: Autism, aging, functional MRI, fMRI, neuroimaging, executive functioning, cognition, memory, white matter, cortical thickness, connectivity

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals for the project in the second year of funding were:

A) To continue obtain cognitive and MRI data from ASD and control participants (Major Task 2 and Subtask 3).
B) Analyze and synthesize data to address all specific aims (Major Task 3, Milestones 2).

What was accomplished under these goals?

A) **Obtaining cognitive and MRI data from ASD and control participants:** We continue to collect new participants to obtain our target enrollment of 70 participants (35 per group). At the time of this writing, we have all procedures for Time 1 for 46 of 70 participants. We have also performed the second time point for the initial cohort of participants who reached their 2-year interval time point. We have been able to complete all the procedures for 21 of 24 participants. Two of the ASD participants listed above did not receive MRI scans because they were uncomfortable in the scanner, but we obtained cognitive and other data. The rest of the participants tolerated the scanning procedure well; none of those participants have required any alteration of the MRI or cognitive protocols. In our original Statement of Work, recruitment was scheduled for 3-18 months. Recruitment lags somewhat behind our anticipated schedule, although we continue to receive a small but regular flow of potential recruits. As soon as our new paper is available for viewing (see below), we plan to have increased media exposure, which tends to increase self-referrals.
C) Analyze and synthesize data to address all specific aims (Major Task 3, Milestones 2).

We have published the first set of results from our initial cross-sectional analyses in Autism Research, the official journal of the International Society for Autism Research (Impact factor: 3.765; ranked 5/51 for Behavioral Sciences and 9/70 for Psychology Developmental journals). We are currently finishing data analyses and manuscript preparation for two more cross-sectional studies examining other cognitive functions presented below. We have extended our analyses to examine functional connectivity using resting state scans (re-FC) to examine the relationship with symptoms. We have also applied for and received funding to expand our study to include aging women with and without ASD who will be used with the current data to investigate gender differences in brain, cognition and symptoms. Details of significant data results are detailed below.

**Significant results from cross-sectional analyses:**
“Executive Function and Functional and Structural Brain Differences in Middle-Age Adults with Autism Spectrum Disorder” B. Blair Braden, PhD1, Christopher J. Smith, PhD2, Amiee Thompson3, Tyler K. Glaspy, BS3, Emily Wood3, Divya Vatsa3, Angela Abbott3, Samuel C. McGee3, and Leslie C. Baxter, PhD3. Accepted for publication: Autism Research, DOI10.1002/aur.1842. 1Department of Speech and Hearing Science, Arizona State University, 976 S Forest Mall, Tempe, AZ 85281 2Southwest Autism Research & Resource Center, 2225 N 16th Street, Phoenix, AZ 85006 3Department of Neuroimaging Research, Barrow Neurological Institute, St. Joseph’s Hospital and Medical Center, 350 W Thomas Rd, Phoenix, AZ 85013

Our published report included analysis of cognitive and fMRI data from the first 16 ASD and 17 cognitively normal (“neurotypical” or NT) controls. All ASD diagnoses were confirmed by the trained staff at Southwest Autism Resource and Research Center (SARRC) based on the Autism Diagnostic Observation Schedule. All participants were given the Kaufman Brief Intellectual Test to provide an estimate of intelligence to ensure that all participants were above the IQ= 80 cutoff and to assure general equivalence of IQ between groups. Groups were well-matched according to age, IQ, and education. Groups performed similarly on tests of delayed verbal memory (RAVLT) and detailed visual search (Group Embedded Figures Task), but the ASD group made significantly more errors on the executive function task, which requires monitoring, attention, mental flexibility and concept formation (Wisconsin Card Sorting Test (WCST); Table 1). A significant group difference on WCST errors remained after including demographic variables (age, IQ, or education) as covariates (all p<0.05). On fMRI tasks, performance was above 80% on all tasks.

Using independent component analysis (ICA), we evaluated functionally connected neural network activity during the n-back task. The n-back task is a common fMRI task used to interrogate the integrity of networks involved in working memory. The task requires online monitoring and attention to a series of letters. The ASD participants’ reaction time was slower on the 2-back condition. We demonstrated older adults with ASD have weaknesses on an executive function task emphasizing flexibility, working memory, and inhibition (WCST) and are less likely to engage a CSTC network when switching between low-load and high-load working memory conditions. The beta weights were more positive for the 2-back condition [F(1,28) = 11.182; p = 0.002] and more negative for the 0-back condition [F(1,28) = 17.471; p < 0.001] for the NT group. Striato-thalamo-cortical networks are known to be involved in working memory, impulse control and other aspects of executive functioning (Miller and Cohen, 2001). Recently, Gordon et al. (2015) demonstrated that differences in functional connectivity between regions of this subcortical-frontal network were associated...
with variability in normal controls. Specifically, lack of engagement of this network resulted in weak performance on the n-back task and increased impulsivity scores within a group of healthy controls. The findings from our group differences in engagement of this network along with the greater number of errors produced by the ASD group on the WCST, which requires error monitoring, updating and flexible thinking, suggests that this is a relative weakness within our ASD group, detectible within a small group. We will begin collecting our second longitudinal data point for those already enrolled in the study to determine whether aging exacerbates these differences.

**White matter:**

The ASD group showed reduced white matter integrity (voxel-wise differences in Fractional Anisotropy; FA) in the genu of the corpus callosum and bilaterally in the fimbria of the hippocampi. There were no areas of greater FA in the ASD cohort. No group differences in Mean Diffusivity, another measure of white matter integrity survived correction for multiple comparisons, but consistent with the spatial location of FA.
differences, two clusters in the bilateral anterior corona radiata (projections from of the genu of the corpus callosum) showed increased MD in the ASD group at \( p=0.001 \) uncorrected (not shown).

“Autism and age-related network differences in verbal fluency in adult autism”
Baxter LC\(^1\), Wood E\(^1\), Singh K\(^1\), Smith CJ\(^2\), Braden BB\(^3\).
\(^1\)Department of Neuroimaging Research, Barrow Neurological Institute, Phoenix, AZ
\(^2\)Southwest Autism Research & Resource Center, Phoenix, AZ
\(^3\)Speech and Hearing Sciences, Arizona State University, Tempe, AZ

Language functioning is variable in autism, ranging from nonverbalism in more severely affected individuals, to pragmatic aspects of language comprehension and mild problems with language processing in high functioning individuals. Using a commonly used fMRI fluency task, we determined the effects of ASD and aging on language networks.

Fluency tasks are complex tasks that combine language and executive functions since they require the individual to engage associative processes and strategies to search and produce words according to a given demand (e.g., words with the same first letter). In our study, participants perform a “fluency” fMRI task, which is based on the Controlled Oral Word Association Test (COWAT), a task often used to assess expressive language abilities. This is a commonly-used fluency paradigm, requiring the participant to silently generate as many words they can to a designated first letter.

The task has blocks of 16 seconds, during which the participant thinks of words to two letters (i.e., 8 seconds per letter) followed by a baseline block during which the word “Relax” is presented and the participant is instructed to not talk to themselves, and encouraged to “think of a blank blackboard”. The participant presses a button with their right forefinger for each word they generate to estimate the rate of word generation. We analyzed cross-sectional data from middle age (40-60) and young adult (18-25) high-functioning ASD and age-match TDs. There were no significant group differences for fMRI task performance, as measured by mean number of words produced on the task (about 50 words, \( p = .66 \)). We first used ICA (described above) for each of the 4 groups to visualize any network differences across groups. We rejected any components indicating movement or the motor network associated with finger movement. We found several components that significantly correlated with the task. All groups produced a network involving left inferior frontal cortex (LIFC), in the general area often termed “Broca’s area” that is critical for expressive language. Other networks were found that included the right cerebellum, anterior cingulate, left hippocampus, and the precuneus/posterior cingulate region reflecting engagement of this network during the baseline condition.
Using SPM, we extracted values from any nodes found from networks significantly engaged during any of each of the 4 group ICA analyses in order to test for group, age, and group-by-age interactions. Our results indicated that not all of the groups utilized these regions to the same extent when performing the fluency task, despite all groups having very similar task performance. Specifically, some regions contributing to this fluency task showed clear aging effects, while others showed differences between the ASD group and the TD groups (Figure 2). For example, the left hippocampus was far less engaged in the fluency task for both older ASD and TD individuals; in contrast, both older and younger adult ASD groups showed less cerebellar activation compared to the TD groups. Interestingly, the older ASD group shows a level of engagement that is similar to young controls, possibly suggesting a compensatory mechanism.

Figure 3. Regional BOLD activity during a fluency task. Both ASD groups show decreased right cerebellar activity while both older groups engage the hippocampus to a much lesser extent than their younger cohorts. The ASD groups show less engagement of the precuneus region that is part of the Default Mode Network during the baseline rest condition. The older ASD group shows a relatively high degree of engagement of Broca’s area, possibly as a compensation for weaknesses in other regions. All y axes are contrast estimates obtained from whole brain analysis in SPM(12).

“Motor system integrity in older adults with autism spectrum disorder”

Deatherage BR\(^1\), Braden BB\(^2\), Smith CJ\(^3\), McBeath MM\(^4\), Singh K\(^1\), Thompson AM\(^1\), Wood EG\(^1\), Vasta D\(^1\), McGee S\(^1\), Baxter LC\(^1\)

\(^1\)Department of Neuroimaging Research, Barrow Neurological Institute, Phoenix, AZ  
\(^2\)Speech and Hearing Sciences, Arizona State University, Tempe, AZ  
\(^3\)Southwest Autism Research & Resource Center, Phoenix, AZ  
\(^4\)Department of Psychology, Arizona State University, Tempe, AZ

We are finalizing a manuscript that in part includes an honor’s project by ASU undergraduate student Brandon Deatherage. Gait disturbance, clumsiness, and other mild movement problems are often observed in children with autism spectrum disorder (ASD) (Maurer, 1982). As the brain ages, these symptoms may persist into late adulthood in those diagnosed with ASD. This study focused on middle-age adults with ASD to study motor behavior and underlying brain integrity in older individuals. Using a finger tapping task, motor performance was measured in a cross-sectional study comparing middle-aged adults with ASD and age-, education- and IQ-matched typically developing (TD) controls. We hypothesized that older adults with ASD
would show poorer motor performance (slower finger tapping speed). We also hypothesized that underlying brain differences, measured using MRI, in regions associated with motor function including the primary motor cortex, basal ganglia, and cerebellum, as well as the white matter connecting tracts would exist between groups and may explain the proposed disparity in motor performance.

Data from the study show that older ASD participants have greater variability in right hand finger tapping than the TD group, although there was not a significant difference in mean group values. On a brain level, the left motor cortical region was the only gray matter region showing a significant effect, with thinning occurring in the ASD group. In contrast, group differences were found on several measures of white matter including the cortical spinal tract, posterior limb of the internal capsule, retroventricular internal capsule and middle cerebellar peduncle. Brain-behavior correlations showed that dominant finger tapping speed, which showed greater variability among the ASD group, correlated with left hemisphere white matter integrity measures of the left corticospinal tract and posterior limb and retroventricular segment of the internal capsule and cerebellar white matter.

No significant differences were observed between groups in finger tapping speed but the dominant, right-hand tapping showed greater variability in the ASD cohort. Decreased white matter integrity was observed in the ASD group for many regions associated with motor functioning. Given that white matter integrity, not gray matter thickness, correlated with right hand tapping in the ASD suggests that white matter differences may account for the heterogeneity and intermittent findings of motor dysfunction in ASD groups.

**Other related accomplishments:**

**Received funding to include a cohort of older women for longitudinal assessment:**

We received a 3-year, $225,000(DC) grant from the Arizona Biomedical Research Commission (ABRC) to investigate aging effects in women with ASD. The grant was awarded to Dr. Blair Braden under the New Investigator mechanism. As with the DoD grant, this funding continues the collaboration between Barrow, SARRC, and ASU. The new funding will allow us to include a group of 20 older ASD women and their age-matched TDs. The procedures will mirror our DoD-funded study in order to allow us to directly test gender differences in symptoms, cognition and brain functioning as they age. The funding also allows for longitudinal assessment over 2 time points.

a. **What opportunities for training and professional development has the project provided?**

As noted in the year 1 annual report, this project provided the environment to train and promote Dr. Baxter’s post-doctoral resident, who is one of the key personnel in this study. B. Blair Braden, PhD has been active in all of our projects on ASD and aging. She came to the lab after graduate training in neuroscience, studying hormone and aging in animal models specifically to help develop our cognitive and imaging studies in aging and Autism. She has successfully transitioned to a tenure-track Assistant Professor position at Arizona State University and established a lab in Autism and Aging. Prior to this move when she was still at Barrow Neurological Institute, Dr. Braden applied for a New Investigator Award from the Arizona Biomedical Research Commission. She received her award after she transitioned to her ASU position. Dr. Braden has 1 graduate student and several undergraduate students dedicated to this project. The Barrow lab has one to two Barrett honors students working on this project per year. Through a high school program, the BNI lab had 4 high school students. At the end of the last year, two high school students received 2 INTEL awards for analyses related to this project. These two students are matriculating and attending Harvard University and Washington University in the fall. One of the new students will submit for the Arizona Science Fair this fall.
b. How were the results disseminated to communities of interest?

The following presentations were made or are scheduled:

1) “Motor System Integrity in Older Adults with Autism Spectrum Disorder.”
   Brandon R. Deatherage*, B. Blair Braden, Christopher J. Smith, Michael K. McBeath, Samuel C. McGee,
   Emily G. Wood, Krishna Sinha, Leslie C. Baxter *Mentored Honors Undergraduate Student (Baxter)

   Cognitive and Brain Aging in Autism Spectrum Disorder: Executive Functioning and Frontal and Temporal
   Lobe Differences. Presented at the International Meeting for Autism Research, San Francisco, CA May 2017,

3) Braden, B.B., Riecken, C. Age-Related Cortical Thickness Differences in Adults with Autism Spectrum
   Adventures in Learning, Chandler-Gilbert Community College, AZ, July 13, 2017 Society for Neuroscience
   Annual Meeting, Washington D.C., Nov 2017

4) Stoeckmann, M, Baxter, L, Smith, CS, Braden, B*. Large-scale brain networks in middle-aged adults autism
   spectrum disorder: Functional connectivity differences and relationships with symptoms. Society for

Autism Brain Imaging Data Exchange: The Autism Brain Imaging Data Exchange II is large-scale data
repository of ASD and TD controls from 17 sites. Our contribution of 58 samples (which includes participants
that are part of the DoD study) include the oldest sample in the group (aged 64 years old) and represents the
first substantial set of older adults in the exchange. Our contribution to this high-profile, international group
of researchers helps us to disseminate our study to the ASD research community.

Aging in Autism Special Interest Group, International Society for Autism Research (INSAR). Drs. Smith,
Baxter, and Braden actively participated in the second meeting of the INSAR group of Autism researchers
dedicated to the study of older individuals with Autism. The group was formed by Hilde Geurts, PhD of the
University of Amsterdam to work towards common goals for studying older adults with ASD. Both Drs. Baxter
and Braden are contributing to the establishment of a core set of cognitive tests and other data that can be
collated across studies from around the world.

c. What do you plan to do during the next reporting period to accomplish the goals?

d. Continue with recruitment of ASD and age-matched TDs to reach our target sample of 35/group.

e. Continue to acquire second time point evaluations of those participants who are two years from their
   original study.

f. Begin machine learning data analysis with collaborator Dr. Jieping Ye (University of Michigan) to
   investigate multimodal influences of aging on ASD.

B) IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

   We published a publication that is one of the first to focus on both behavior and brain imaging in
   older adults. The reviewers’ utilized by the Autism Research editors reflect the importance of our
   study: “This is an important topic in need of further investigation, and this is a contribution in this
   direction” and “this work is both timely and important”. We have significantly increased the
   number of older adults that are freely available to others via the national data exchange, “ABIDE
II”. Our study is complementing the established cognitive studies in older individuals with Autism to emphasize the importance of developing a greater understanding of aging in Autism to inform treatment.


What was the impact on other disciplines?

Nothing to Report.

b. What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

i. As reported in the Year 1 summary, an intent of our study is to develop a plan of action to help keep older adults with ASD as independent as possible for as long as possible. We foresee that the results of our study, which is one of the first of its kind, will be able to inform state agencies and community aging programs to develop interventions that will help keep older ASDs independent. We plan on continuing to publish our results, and becoming a voice for the older ASD population, to help form effective and meaningful supports and treatments for this group.

C) CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

a. Changes in approach and reasons for change

Nothing to Report

b. Actual or anticipated problems or delays and actions or plans to resolve them

Obtaining institutional and HRPO approval for the study took longer than expected, so recruitment of our cohort is somewhat behind schedule. Our goals are to use all available resources to recruit our target number as soon as possible.

c. Changes that had a significant impact on expenditures

Our expenditures for MRI scans and patient reimbursement is less than expected, as our recruitment has slowed.
d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

i. We changed our local consent and HIPAA forms to allow us to share anonymized data with the Autism Brain Imaging Date Exchange (detailed above). This was approved by our institution. We informed the HRPO, who also approved this. Current approval dates are: Informed Consent: 4/20/16; HIPAA: 3/02/16

e. Significant changes in use or care of human subjects:  Nothing to Report

f. Significant changes in use or care of vertebrate animals. N/A

g. Significant changes in use of biohazards and/or select agents N/A

D) PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

a. Publications, conference papers, and presentations
   Nothing to Report

   i. Journal publications.  Nothing to Report

   ii. Books or other non-periodical, one-time publications.  Nothing to Report

b. Other publications, conference papers, and presentations.  Nothing to Report

c. Website(s) or other Internet site(s)

d. List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. Nothing to report

e. Technologies or techniques
   None to report

f. Inventions, patent applications, and/or licenses
   None to report

g. Other Products
   None to report
4. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- What individuals have worked on the project?

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<tr>
<th>Name:</th>
<th>Christopher J. Smith, Ph.D.</th>
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<td>PI</td>
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<tr>
<td>Contribution to Project:</td>
<td>Dr. Smith oversaw regulatory approvals, recruitment strategies, data collection, and participated in analysis and interpretation of results for presentation</td>
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<td>Funding Support:</td>
<td>1R01 MH104446, NIH 08/25/14-06/30/19 Detection of ASD at the 1st birthday as a standard of care: The Get SET Early Model 1R44EB020565-01, NIH 9/1/2015 – 8/31/2018 Increasing Access to Earlier Diagnostic Assessment for Autism in Rural Idaho SARRC internal funds</td>
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<tr>
<th>Name:</th>
<th>Andrew Mason, BA</th>
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<td>Project Role:</td>
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<td>Researcher Identifier (e.g. ORCID ID):</td>
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<td>Nearest person month worked:</td>
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<tr>
<td>Contribution to Project:</td>
<td>Mr. Mason presented the study at numerous support meetings and conferences for recruitment, contacted and recruited participants, and coordinated phenotyping.</td>
</tr>
<tr>
<td>Funding Support:</td>
<td>Roche Pediatric Clinical Trial</td>
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- Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
  - Nothing to Report during Year 2.

- What other organizations were involved as partners?
  - Organization Name: St. Joseph’s Hospital (Barrow Neurological Institute)
  - Location of Organization: 350 West Thomas Road Phoenix, AZ 85013
  - Partner's contribution to the project Partnering PI

- Collaboration Further testing and scanning of participants, collaboration with data interpretation and manuscript preparation