

**AWARD NUMBER:** W81XWH-18-1-0539

**TITLE:** The Relationship Between Brain Functioning, Behavior, and Microbiota in Autism Spectrum Disorder

**PRINCIPAL INVESTIGATOR:** Emeran Mayer, MD

**CONTRACTING ORGANIZATION:** University of California, Los Angeles

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Fort Detrick, Maryland 21702-5012

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# REPORT DOCUMENTATION PAGE

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<b>14. ABSTRACT</b>  While social communication deficits are considered the hallmark of autism spectrum disorder (ASD), comorbid gastrointestinal (GI) issues are common and impair quality of life in a large subset of affected individuals. Several studies have linked these GI issues to altered gut microbial composition, or dysbiosis, in ASD. Yet research has not yet clarified how dysbiosis may be related to the core features of ASD or to the symptom heterogeneity of this disorder. A growing number of studies support the concept that gut microbiota can signal to the brain by neural, endocrine, immune, and hormonal pathways. These microbial effects on the brain may modulate outputs in behavior, as well as in autonomic and neuroendocrine function.					
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**1. INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

While social communication deficits are considered the hallmark of autism spectrum disorder (ASD), comorbid gastrointestinal (GI) issues are common and impair quality of life in a large subset of affected individuals. Several studies have linked these GI issues to altered gut microbial composition, or dysbiosis, in ASD. Yet research has not yet clarified how dysbiosis may be related to the core features of ASD or to the symptom heterogeneity of this disorder. A growing number of studies support the concept that gut microbiota can signal to the brain by neural, endocrine, immune, and hormonal pathways. These microbial effects on the brain may modulate outputs in behavior, as well as in autonomic and neuroendocrine function. To our knowledge, no studies have investigated gut-microbiome-brain-behavior interactions in a single population of individuals with ASD to better understand the mechanisms of ASD heterogeneity. By examining these interactions, we aim to test the general hypothesis that alterations in gut microbial composition are correlated with structural and functional brain alterations, as well as with clinical and behavioral features of ASD. The long-term goal of this research is to explain heterogeneity, develop biomarkers, and ultimately build individualized treatments for ASD directed at alterations within the brain-gut-microbiome axis. Accomplishing our specific aims will not only allow us, for the first time, to better understand ASD from a whole body medical perspective, but it will also allow for better understanding of heterogeneity, diagnostic biomarkers, and potentially new forms of treatment for ASD (e.g., fecal microbial transplantation, prebiotics, probiotics, and therapies aimed at specific gut microbes or their products).

**2. KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Autism, Brain Imaging, fMRI, Neural Connectivity, Microbiome

**3. MAJOR GOALS OF THE PROJECT**

**Specific Aim 1: To characterize the composition, metagenome, and metabolome of the gut microbiome in children with ASD**

	Statement of Work Goal Completion Date	Actual Completion Date or Progress	
		Site 1: USC (LAZ)	Site 2: UCLA (EM)
<b>Major Task 1:</b> Prepare regulatory documents and research protocol for IRB Approval			
<i>Milestone # 1: IRB approval received at both USC and UCLA</i>	<b>11/18</b>	<b>9/18</b>	<b>N/A (Exempt)</b>
<i>Milestone #2: Regulatory approval from DoD HRPO</i>	<b>1/19</b>	<b>Determined not necessary till 1/20</b>	<b>N/A</b>
<b>Major Task 2:</b> Hiring and training of study personnel on behavioral	<b>11/18</b>	<b>10/18</b>	<b>N/A</b>

and clinical assessments and MRI/fMRI tasks			
<i>Milestone # 2: Study personnel achieves reliability on the ADOS and ADI-R</i>	<b>11/18</b>	<b>12/18</b>	<b>N/A</b>
<b>Major Task 3:</b> Collect gut microbiome data and brain imaging from typically developing children and children with ASD			
Subtask 1: I. Complete data collection of the 40 ASD and 40 TD participants from ongoing R01 study a. Collect MRI data from these participants (using funds from R01 study) b. Collect microbiota data from these participants (using new funds from current proposal) II. Recruit 20 additional ASD and 20 additional TD participants in each group (using new funds from current proposal)	<b>1/20</b>	<b>83% complete</b>	
	<b>9/20</b>	<b>On Track</b>	
<b>Major Task 4:</b> Conduct metagenomics and metabolomics analysis of the gut microbiome			
Subtask 1: Run shotgun metagenomics	<b>2/21</b>	<b>On Track</b>	<b>On Track</b>
Subtask 2: Run metabolomics analysis	<b>2/21</b>	<b>On Track</b>	<b>On Track</b>
<i>Milestone #3: Present data at conferences and co-author manuscript on metagenomic and metabolic analysis in TD compared to children with ASD</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>

<b>Specific Aim 2: To identify associations between altered brain network connectivity, gut microbial parameters, and behavior.</b>			
<b>Major Task 5:</b> Conduct functional (resting state) and anatomical connectivity (diffusion weighted imaging)			
Subtask 1: Resting state analyses (data preprocessing and QC, seed-to-voxel connectivity, complex network analysis and machine learning approaches)	<b>2/21</b>	<b>On Track</b>	<b>On Track</b>
<i>Milestone #4: Present data at conferences and co-author manuscript on correlations between resting state connectivity, microbiome-related data, and behavioral data</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>
Subtask 2: DTI analyses (deterministic and probabilistic tractography, voxel-based analysis, complex network analysis; machine learning approaches)	<b>2/21</b>	<b>On Track</b>	<b>On Track</b>
<i>Milestone #5: Present data at conferences and co-author manuscript on correlations between DTI analyses, microbiota related data, and behavior</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>
<b>Major Task 6:</b> Conduct correlations brain network analyses, gut microbial parameters, and behavior	<b>12/20</b>	<b>On Track</b>	<b>On Track</b>
Subtask 1: Analyze behavioral data	<b>12/20</b>	<b>On Track</b>	<b>On Track</b>
Subtask 2: Conduct correlations between behavioral data, microbiome-related data, and behavioral data	<b>12/20</b>	<b>On Track</b>	<b>On Track</b>

<i>Milestone #6: Present data at conferences and co-author manuscript on correlations on brain resting state connectivity, microbiota related data, and behavior</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>
<b>Major Task 7:</b> Conduct machine-learning and neural network analyses (classification, ensemble clustering) of the combined microbiota, behavioral, and MRI data to identify microbial features and connectivity patterns that predict clinical diagnosis and heterogeneity within ASD	<b>2/21</b>	<b>On Track</b>	<b>On Track</b>
<i>Milestone #7: Present data at conferences and co-author manuscript based on machine learning analyses combining microbiota, behavioral and structural MRI data that can better understand heterogeneity within ASD and predict clinical diagnosis</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>
<b>Specific Aim 3: To identify associations between altered brain activation patterns and gut microbial parameters</b>			
<b>Major Task 8:</b> Conduct fMRI analyses of emotional face processing in all participants	<b>12/20</b>	<b>On Track</b>	<b>On Track</b>
<b>Major Task 9:</b> Conduct correlations between fMRI analysis results and gut microbial parameters	<b>12/20</b>	<b>On Track</b>	<b>On Track</b>
<i>Milestone #8: Present data at conferences and co-author manuscript on correlations on fMRI data during emotional</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>

<i>face processing and microbiota related data</i>			
<b>Major Task 10:</b> Conduct association network analyses between fMRI data and gut microbial parameters and behavioral data	<b>12/20</b>	<b>On Track</b>	<b>On Track</b>
<i>Milestone #9: Present data at conferences and co-author manuscript on correlations on fMRI data during emotional face processing, microbiota related data, and behavioral data</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>
<b>Major Task 11:</b> Conduct machine-learning analyses (classification, ensemble clustering) of the combined microbiota, behavioral and task based fMRI data to identify microbial features and activation patterns that predict clinical diagnosis and heterogeneity within ASD	<b>12/20</b>	<b>On Track</b>	<b>On Track</b>
<i>Milestone #10: Present data at conferences and co-author manuscript based on machine learning analyses combining microbiota, behavioral and task based fMRI data that can better understand heterogeneity within ASD and predict clinical diagnosis</i>	<b>5/21</b>	<b>On Track</b>	<b>On Track</b>

4. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

**What were the major goals of the project?**

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

### **What was accomplished under these goals?**

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

### **Specific Aim 1: To characterize the composition, metagenome, and metabolome of the gut microbiome in children with ASD**

**Major Task 1:** Prepare regulatory documents and research protocol for IRB Approval

*Milestone # 1: IRB approval received at both USC and UCLA*

*Milestone #2: Regulatory approval from DoD*

As we are currently collecting data from our ongoing NIH R01 study, it was deemed that we do not need new IRB approval till we start collecting data using DoD funding. We also ascertained from the UCLA IRB that since UCLA will use de-identifiable data, they do not need IRB approval. We plan to submit a new IRB using DoD funds by September 2019 and will subsequently send it to DoD for regulatory approval.

**Major Task 2:** Hiring and training of study personnel on behavioral and clinical assessments and MRI/fMRI tasks

*Milestone # 2: Study personnel achieves reliability on the ADOS and ADI-R*

The USC team has hired a post-doc (Emily Kilroy) to work on this project and an OT doctoral student (Ryann MacMurdo) as well as several undergraduate research assistants. On November 2018 we also finalized a subaward agreement with CHLA for Sharon O'Neil to work as a consultant on this project. An ongoing graduate student, Christiana Butera, whose stipend is paid from an internal USC scholarship, received reliability on the ADI-R by December 2018. Emily Kilroy was already reliable on these measures at the time of hiring.

The UCLA and USC Teams have met on a bimonthly basis to discuss progress of the study, and to identify any potential obstacles to the brain imaging studies and collection and storage of stool samples.

**Major Task 3:** Collect gut microbiome data and brain imaging from typically developing  
Subtask 1:

- I. Complete data collection of the 40 ASD and 40 TD participants from ongoing R01 study
  - a. Collect MRI data from these participants (using funds from R01 study)
  - b. Collect microbiota data from these participants (using new funds from current proposal)

We are 83% complete in collecting reliable data from 40 ASD and 40 TD participants by Dec 31 2019. We currently have reliable data from 36 ASD and 30 TD participants. We prioritized recruiting the ASD group so that we could match the TD participants to them. We are on track to meet the goal of 40 in each group by 12/31/19.

II. Recruit 20 additional ASD and 20 additional TD participants in each group (using new funds from current proposal)

We are on track to meet this goal by September 2020.

**Major Task 4: Conduct metagenomic and metabolomics of the gut microbiome**

Subtask 1: Run shot-gun metagenomics

Subtask 2: Run metabolomics

We are on track to meet this goal by the end of 2/21.

*Milestone #3: Present data at conferences and co-author manuscript on metagenomic and metabolic analysis in TD compared to children with ASD*

We are on track to meet these goals as originally planned for 2/21 for the analyses and 5/21 for the data dissemination.

**Specific Aim 2: To identify associations between altered brain network connectivity, gut microbial parameters, and behavior.**

**Specific Aim 3: To identify associations between altered brain activation patterns and gut microbial parameters**

We will not be able to complete Specific Aim 2 and 3 before we have acquired our full data set and completed the analyses on them. We are on track for completing these aims in the time frame listed above. We note that we can not run preliminary analyses at this time as all genetic data need to be run together once all samples are acquired to avoid batch effects. For this reason, we will leave all data analysis till the final year of the project, as written in our original timeline.

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

*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

This current project has provided several training and professional development opportunities for undergraduate research assistants, graduate students, occupational therapy doctoral residents, and post-graduate trainees who have worked on this project.

*Undergraduate Research Assistant Training:* Several undergraduate research students have been trained in data collection and recruitment protocols as part of this project. Specifically, Jessie Tien and Sharada Krishman, both enrolled in the University of Southern California University (USC) BS/MA Occupational Therapy Program have been receiving ongoing training on this project since September, 2018. Ms. Krishman will be using the data from this study as the foundation independent research the Spring. Both Ms. Tien and Ms. Krishman received training and education in working with those with autism spectrum disorders (ASD), sensory processing deficits, fMRI protocol and Microbiota data acquisition and analysis, in addition to general training in the day-to-day activities associated with scientific research. The trainees were trained in administering and scoring of a variety of behavioral assessments related to the projects aims. Additionally,

the current funding and associated projects have provided both Ms. Tien and Ms. Krishman with training in scientific writing and principles of scientific data dissemination.

*Master's Level Graduate Training:* Michelle Canales, a master's student in the USC Occupational Therapy program, was awarded the Centennial Vision Scholarship for the 2018-2019 academic year to work with Dr. Aziz-Zadeh's lab on this project. She was trained in ASD, microbiota, diet questionnaires, psychological assessments and functional Magnetic Resonance Imaging (fMRI) data collection and analysis. She also received training in professional development through mentorship with postdocs in the lab and by attending team meetings with UCLA collaborators.

*PhD Graduate Training:* Christiana Butera, a graduate student under Dr. Aziz-Zadeh at USC, became research certified on the ADI-R (an autism diagnostic measure) in 12/18. All graduate student members of the lab also received biohazard training. She furthermore gained experience with study management and oversight of undergraduate research assistants as well as furthered her training in administering behavioral assessments and functional Magnetic Resonance Imaging (fMRI) data collection and analysis. Furthermore, she attended the Organization for Brain Mapping Annual Conference where presented some previous findings on autism and brain imaging, expanded her knowledge in brain imaging and made new professional contacts.

*Occupational Therapy Doctoral Training:* Ryann MacMurdo, an Occupational Therapy Doctoral Resident at USC gained training and experience in lab protocols specific to this project, as well as educational and professional development. She received training on ASD, microbiota data collection, clinical assessments, ASD participant recruitment, as well as, general training in the day-to-day activities associated with scientific research including internal review board submissions and maintenance, database software (RedCap), and research lab management. Furthermore she attended the annual AOTA conference to expand her professional experience.

*Professional Development and Post-Graduate Training:* Additional training and professional development has been provided to Dr. Aziz-Zadeh, PI of this project and Dr. Emily Kilroy, a postdoctoral trainee on the project. As a result of this award, Dr. Aziz-Zadeh and Dr. Kilroy have received training in (a) sensory processing assessments in young children with ASD, (b) microbiota research through online courses and through meetings with UCLA collaborators (c) microbiota data acquisition and analysis (c) mentorship of undergraduate and graduate students, and (d) study and personnel management. All of the training listed above resulted in increased knowledge and skills pertaining to the projects aims. Additionally, Dr. Aziz-Zadeh has attended conferences (The Annual Gut Microbiome National Conference) and seminars relating to state of the art microbiota analysis and research findings. Dr. Kilroy attended the Organization for Human Brain Mapping Annual conference where she presented work from a previous study on autism and brain imaging, made contacts, and furthered her knowledge on brain imaging and autism. She additionally received lab safety training become certified in biohazard shipping and transportation in preparation for maintenance and preservation of microbiota data collection. Furthermore, she has spent one-on-one time working with the PI, Lisa Aziz-Zadeh, furthering her professional development in scientific grant writing and principles of scientific data dissemination.

The UCLA Team has ongoing training and mentoring activities involving a large number of undergraduate students, and postdoctoral fellows. As the major analyses involving brain imaging and gut microbiome data for this study will not start before the enrollment has been completed, no direct training on the study's specific data set has begun.

### **How were the results disseminated to communities of interest?**

*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of*

*these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

Nothing to report at this time.

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

*If this is the final report, state “Nothing to Report.”*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

As stated in our timeline, we plan to complete data acquisition by the end of September 2020. Thus in the next reporting period (Aug 1, 2020), we will plan to have data from 58 ASD participants and 58 TD participants. This will leave us with just 4 more participants to acquire data from by September 2020. We will accomplish these goals by recruiting via posting on relevant list serves and social media groups, reaching out to schools, clinics, and community centers, and attending relevant events for recruitment. These recruitment practices have proved successful in the past, and we plan to continue them in the coming year.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

There have been two news articles on our project in the last year, one on the UCLA news website (<https://dailybruin.com/2019/05/20/ucla-usc-researchers-study-role-of-gut-microbiome-in-development-of-autism>) and one on the USC news website (<https://hscnews.usc.edu/more-than-a-gut-feeling-study-looks-at-link-between-microbiome-autism>). This has brought more attention to the ideas we are working on in this project, namely trying to understand autism from a whole body medical perspective by bringing in data from behavior, brain imaging, and the gut microbiome. Dr. Mayer and Dr. Aziz-Zadeh were contacted by ASD patient organization and ASD researchers showing interest in the project once the new articles came out. It is our hope that once the data comes out, we will have stronger more concrete impact in our fields and beyond as well.

**What was the impact on other disciplines?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Nothing to report at this time.

**What was the impact on technology transfer?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

**What was the impact on society beyond science and technology?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

As stated above, two news articles on our project came out in the last year. These articles improved public knowledge on the potential role of the microbiome in autism, and its interactions with the brain and behavior. The public seemed very much interested in this topic, especially families with individuals with autism, as the PIs received multiple emails following the news article from the public.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

**Changes in approach and reasons for change**

*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

Nothing to report.

**Actual or anticipated problems or delays and actions or plans to resolve them**

*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

Nothing to report.

**Changes that had a significant impact on expenditures**

*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Nothing to report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

Nothing to report.

**Significant changes in use or care of vertebrate animals**

Nothing to report

**Significant changes in use of biohazards and/or select agents**

Nothing to report.

**6. 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.”*

- **Publications, conference papers, and presentations**

*Report only the major publication(s) resulting from the work under this award.*

**Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

**Other publications, conference papers and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (\*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**

*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. It is not necessary to include the publications already specified above in this section.*

<https://dailybruin.com/2019/05/20/ucla-usc-researchers-study-role-of-gut-microbiome-in-development-of-autism>

<https://hscnews.usc.edu/more-than-a-gut-feeling-study-looks-at-link-between-microbiome-autism>

- **Technologies or techniques**

*Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

Nothing to report

- **Inventions, patent applications, and/or licenses**

*Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

Nothing to report

- **Other Products**

*Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Database of MRI, behavioral data, and microbiome data for children with autism and typically developing children. The MRI and behavioral data have been uploaded onto NDAR.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### **What individuals have worked on the project?**

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.*

Name: Lisa Aziz-Zadeh, PhD

Project Role: Principal Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: oversees all aspects of proposed study, including overall recruitment strategy, behavior assessments, research design, data acquisition, oversight of the NDAR data sharing protocol, data analysis, and manuscript preparations.

Name: Emeran Mayer, MD

Project Role: Partnering Principal Investigator (UCLA)

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: oversee the bioinformatics and multiomics integration of behavioral, brain and microbiome data. He works closely with Drs. Jacobs and Labus in the planning and interpretation of these analyses, and will be involved in manuscripts resulting from this study.

Name: Jennifer Labus PhD

Project Role: Co-Investigator (UCLA)

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contributions to Project responsible for cross-correlating the neuroimaging data with microbiome-related data, machine learning analyses, and performing brain-gut-microbiome network analysis in conjunction with Dr. Jacobs. She will also participate in the preparation of manuscripts.

Name: Jonathan Jacobs, MD PhD

Project Role: Co-Investigator (UCLA)

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contributions to Project: expertise in the intestinal microbiome and immunology, processing samples for microbiome characterization by 16S ribosomal RNA and shotgun metagenomics sequencing and preparing samples for untargeted metabolomics by Metabolon, Inc. He will also contribute to the preparation of manuscripts.

Name: Mirella Dapretto, PhD

Project Role: Co-Investigator (UCLA)

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contributions to Project: provide clinical and scientific expertise in ASD, brain-imaging studies, and the interpretation of study results.

Name: Sharon O’Neil, PhD

Project Role: Consulting Psychologist

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Training in the administration of ADOS and ADI-R and verification of autism diagnosis.

Name: Emily Kilroy, PhD

Project Role: Post-Doctoral Research Associate

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 12

Contribution to Project: Involved in all aspects of the study such as subject recruitment; data acquisition (e.g. behavioral, stool, and brain imaging); data coordination for National Database for Autism Research (NDAR)); administering behavioral assessments and scoring them, stool collection and storage, and cross data analysis (behavioral and microbiota).

Funding: 2.4 calendar months (of 12) supported by institutional resources.

Name: Christiana Butera,

Project Role: Graduate student

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 12

Contribution to Project: Subject recruitment; data acquisition (e.g. behavioral, stool, and brain imaging); administering behavioral assessments and scoring them, stool collection and storage, and cross data analysis (behavioral and microbiota).

Funding: Academic scholarship supported by institutional resources.

Name: Ryann MacMurdo, MA, OTR/L

Project Role: Occupational Therapy Doctoral Resident

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 9

Contribution to Project: responsible for administering some of the behavioral assessments, assist with screening participants for participation in the study.

Funding: Academic scholarship supported by institutional resources.

Name: Priten Vora

Project Role: Programmer/Analyst (UCLA)

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: perform quality control of all neuroimaging data and will prepare the neuroimaging datasets for analyses.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Nothing to report

**What other organizations were involved as partners?**

*If there is nothing significant to report during this reporting period, state “Nothing to Report.”*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*

*Organization Name:*

*Location of Organization: (if foreign location list country)*

*Partner’s contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Nothing to report.

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

# The Relationship Between Brain Functioning, Behavior, and Microbiota in Autism Spectrum Disorder

Insert ERMS/Log Number and Task Title Here

W81XWH1810538

PI: Lisa Aziz-Zadeh and Emeran Mayer **Org:** USC, UCLA

**Award Amount: \$300,954**



## Study/Product Aim(s)

**Aim 1.** To characterize the composition, metagenome, and metabolome of the gut microbiome in children with ASD.

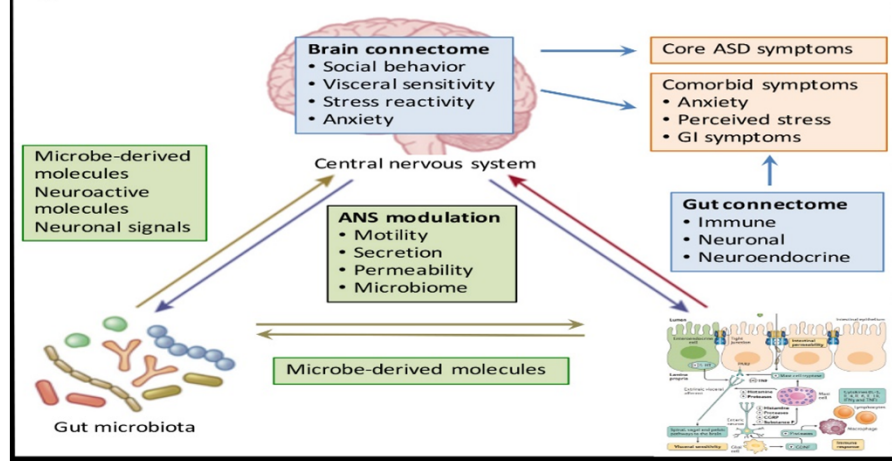
**Aim 2.** To identify associations between altered brain network connectivity and gut microbial parameters.

**Aim 3.** To identify associations between altered brain activation patterns and gut microbial parameters.

## Approach

Building on the brain imaging and behavioral data generated by the Co-PI's R01 grant, we will acquire stool samples from all participants and characterize the composition, metagenome, and metabolome of their gut microbiome. We will relate individual differences across three continuums of symptoms (GI issues, social deficits, and sensory deficits) to activity in selected brain networks and microbiome-related data. We will conduct group analyses as well. By applying machine-learning analyses to the combined microbiota, brain imaging, and behavioral data we will determine if distinct biological signatures are associated with symptom heterogeneity.

Figure 1. Putative Bidirectional Brain-Gut-Microbiome Interactions in ASD



Accomplishment: 1) Approved IRB at institution level; 2) Hired Personnel; 3) Collected 83% of target number for Dec 2019 and 55% of total project data.

## Timeline and Cost

Activities	CY	18	19	20
Hiring & IRB & Design		X		
Data Acquisition		X	X	
Data Analysis			X	X
Data Dissemination				X
<b>Estimated Budget (\$K)</b>		\$100,208	\$100,055	\$100,692

Updated: August 2019

## Goals/Milestones

**CY18 Goal** – Hiring, IRB, Study Design, Data Acquisition

- Hiring of Personnel
- IRB approved at institutions
- Study Design in place and validated
- 83% of target number for Dec 2019 and 55% of total project collected

**CY19 Goals** – Data Acquisition Completed

- Data Acquisition Completed

**CY20 Goal** – Data Analysis and Data Dissemination

- Brain imaging data analysis
- Microbiota data analysis
- Correlations between imaging and microbiome and behavior
- Machine learning analyses
- Publications
- Conference Presentations

**Comments/Challenges/Issues/Concerns:** N/A

**Budget Expenditure to Date**

Projected Expenditure: \$300,954

Actual Expenditure: \$50,368