

AWARD NUMBER: W81XWH-18-1-0538

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

PRINCIPAL INVESTIGATOR: Lisa Aziz-Zadeh, PhD

CONTRACTING ORGANIZATION: University of Southern California

REPORT DATE: SEPTEMBER 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE SEPTEMBER 2021		2. REPORT TYPE Annual		3. DATES COVERED 09/01/2020 – 08/31/2021	
4. TITLE AND SUBTITLE The Relationship Between Brain Functioning, Behavior, and Microbiota in Autism Spectrum Disorder				5a. CONTRACT NUMBER W81XWH-18-1-0538	
				5b. GRANT NUMBER AR170062	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Lisa Aziz-Zadeh, PhD (lazizzad@usc.edu), Emeran Mayer, MD, (emayer@ucla.edu) E-Mail:				5d. PROJECT NUMBER 0011187525	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) UNIVERSITY OF SOUTHERN CALIFORNIA U S C 3720 S FLOWER ST FL 3 LOS ANGELES CA 90007-4318				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT 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. 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. 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. The long-term goal of this research is to explain heterogeneity, develop biomarkers, and ultimately build individualized treatments for ASD directed at brain-gut-microbiome pathways.					
15. SUBJECT TERMS Autism, Brain Imaging, fMRI, Neural Connectivity, Microbiome					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)
Unclassified	Unclassified	Unclassified	Unclassified	25	USAMRMC

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	2
2. Keywords	3
3. Major Goals of the Project	3
4. Accomplishments	9
5. Impact	14
6. Changes/Problems	15
7. Products	16
8. Participants & Other Collaborating Organizations	19
9. Special Reporting Requirements	23
10. Appendices	24

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

STATEMENT OF WORK – 9/29/2017 (REV 04/19/2021)

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)
Major Task 1: Prepare regulatory documents and research protocol for IRB Approval			
<i>Milestone # 1: IRB approval received at both USC and UCLA</i>	11/18	9/18	N/A (Exempt)
<i>Milestone #2: Regulatory approval from DoD HRPO</i>	1/19	Approved 9/27/2019 1/27/2021 (COVID related changes)	N/A (Exempt)
Major Task 2: Hiring and training of study personnel on behavioral and clinical	11/18	10/18	N/A

	Statement of Work Goal Completion Date	Actual Completion Date or Progress	
		Site 1: USC (LAZ)	Site 2: UCLA (EM)
metabolomics analysis of the gut microbiome			
Subtask 1: Run shot-gun metagenomics	2/21		Delayed due to COVID-19 Planning to complete by Feb 2022
Subtask 2: Run metabolomics analysis	2/21		Delayed due to COVID-19 Planning to complete by Feb 2022
<i>Milestone #3: Present data at conferences and co-author manuscript on metagenomic and metabolic analysis in TD compared to children with ASD</i>	5/21	Delayed due to COVID-19 Submitting a comprehensive review article by Aug 1, 2021 Planning to submit data papers mid-way through 2022	Delayed due to COVID-19 Submitting a comprehensive review article by Aug 1, 2021 Planning to submit data papers mid-way through 2022
Specific Aim 2: To identify associations between altered brain network connectivity, gut microbial parameters, and behavior.			
Major Task 5: 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)	2/21	Preprocessing initiated, rest of analysis will be completed early 2022 after all data collected	Preprocessing initiated, rest of analysis will be completed early 2022 after all data collected

	Statement of Work Goal Completion Date	Actual Completion Date or Progress	
		Site 1: USC (LAZ)	Site 2: UCLA (EM)
<i>Milestone #4: Present data at conferences and co-author manuscript on correlations between resting state connectivity, microbiome- related data, and behavioral data</i>	5/21	Delayed due to COVID-19; Expected to complete in spring/summer of 2022	Delayed due to COVID-19 Expected to complete in spring/summer of 2022
Subtask 2: DTI analyses (deterministic and probabilistic tractography, voxel-based analysis, complex network analysis; machine learning approaches)	2/21	Delayed due to COVID-19 Expected to complete in early 2022	Delayed due to COVID-19 Expected to complete in early 2022
<i>Milestone #5: Present data at conferences and co-author manuscript on correlations between DTI analyses, microbiota related data, and behavior</i>	5/21	Delayed due to COVID-19 Expected to complete in spring/summer of 2022	Delayed due to COVID-19 Expected to complete in spring/summer of 2022
Major Task 6: Conduct correlations brain network analyses, gut microbial parameters, and behavior	12/20	Delayed due to COVID-19	Delayed due to COVID-19
Subtask 1: Analyze behavioral data	12/20	Current data has been scored. Group analysis will occur after all data acquired, January 2022	
Subtask 2: Conduct correlations between	12/20	Delayed due to COVID-19	Delayed due to COVID-19

	Statement of Work Goal Completion Date	Actual Completion Date or Progress	
		Site 1: USC (LAZ)	Site 2: UCLA (EM)
behavioral data, microbiome-related data, and behavioral data		Expected to complete in spring/summer of 2022	Expected to complete in spring/summer of 2022
<i>Milestone #6: Present data at conferences and co- author manuscript on correlations on brain resting state connectivity, microbiota related data, and behavior</i>	5/21	Delayed due to COVID-19 Expected to complete in spring/summer of 2022	Delayed due to COVID-19 Expected to complete in spring/summer of 2022
Major Task 7: 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	REMOVED FROM SOW DUE TO COVID-19	N/A	N/A
<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>	REMOVED FROM SOW DUE TO COVID 19	N/A	N/A
Specific Aim 3: To identify associations between altered brain activation patterns and gut microbial parameters			

	Statement of Work Goal Completion Date	Actual Completion Date or Progress	
		Site 1: USC (LAZ)	Site 2: UCLA (EM)
Major Task 8: Conduct fMRI analyses of emotional face processing in all participants	12/20	Individual subject data has been preprocessed. Group analysis will occur after completion of data acquisition in early 2022	Individual subject data has been preprocessed. Group analysis will occur after completion of data acquisition in early 2022
Major Task 9: Conduct correlations between fMRI analysis results and gut microbial parameters	12/20	Delayed due to COVID-19 Expected completion by spring 2022	Delayed due to COVID-19 Expected completion by spring 2022
<i>Milestone #8: Present data at conferences and co-author manuscript on correlations on fMRI data during emotional face processing and microbiota related data</i>	5/21	Delayed due to COVID-19 Expected completion by summer 2022	Delayed due to COVID-19 Expected completion by summer 2022
Major Task 10: Conduct association network analyses between fMRI data and gut microbial parameters and behavioral data	12/20	Delayed due to COVID-19 Expected completion by spring 2022	Delayed due to COVID-19 Expected completion by spring 2022
<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>	5/21	Delayed due to COVID-19 Expected completion by summer 2022	Delayed due to COVID-19 Expected completion by summer 2022

	Statement of Work Goal Completion Date	Actual Completion Date or Progress	
		Site 1: USC (LAZ)	Site 2: UCLA (EM)
Major Task 11: 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	REMOVED FROM SOW DUE TO COVID-19	N/A	N/A
<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>	REMOVED FROM SOW DUE TO COVID-19	N/A	N/A

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

IRB has been approved by USC and DoD.

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

Milestone # 1: Study personnel achieves reliability on the ADOS and ADI-R

In August 2020, the USC team hired one new OT doctoral student (Michelle Canales) as well as several undergraduate research assistants. All staff were trained on the protocol and assisted with subject recruitment, data acquisition, and data entry. The project's post-doc (Emily Kilroy), graduate student (Christiana Butera) have all remained on the project with the addition of a new graduate student (Aditya Jayashankar).

The UCLA and USC Teams have communicated regularly 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: Complete data collection of the 40 ASD and 40 TD participants from ongoing R01 study.

Milestone #1: Collect MRI data from these participants (using funds from R01 study)

This milestone has been completed.

Subtask 2: Recruit 20 additional ASD and 20 additional TD participants in each group (using new funds from current proposal).

Milestone #2: Collect microbiota data from these participants (using new funds from current proposal)

This milestone is 80% completed, due to delays from COVID-19. We expect all data acquisition to be completed by Dec 31, 2021.

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

Subtask 1: Run shot-gun metagenomics

Subtask 2: Run metabolomics

Our plans to meet these goals as originally planned for 2/21 for the analyses have been delayed due to COVID-19. We now plan to complete this goal in January 2022.

Milestone #1: Present data at conferences and co-author manuscript on metagenomic and metabolic analysis in TD compared to children with ASD

Preliminary data from this project and theory papers related to this project were delivered at 5 different conferences (listed below). Dr. Aziz-Zadeh presented preliminary data from this project at 2 conferences, Dr. Mayer at 2 separate conferences, and an undergraduate volunteer in the lab at 1 conference.

We have written a review article about brain gut microbiome interactions in ASD, which will be submitted in August 2021. The co-authoring of data-based manuscripts based on the full data set have been delayed due to COVID-19.

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 delayed for completing these aims in the timeframe listed above due to COVID-19. We note that we cannot 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 all the data is acquired, as written in our original timeline. However, we note that all behavioral data has been entered and all MRI data (structural, functional, and resting state) has been preprocessed at the individual subject level.

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 at USC. Specifically, Ariel Pruyser (Loyola Marymount University), Genesis Flores (California State Polytechnic University, Pomona) and Michelle Chernikova (Loyola Marymount University), all enrolled through the University of Southern California (USC) Diversity, Inclusion & Access (DIA) Jumpstart fellowship, have been receiving ongoing training in this project since June 2020. Additionally, Samantha Antekeier joined the team as a DIA student in June 2021. All DIA Jumpstart fellows received training and education in working with those with autism spectrum disorders (ASD), 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 designing and developing parent surveys related to the project's aims. Additionally, the current funding and associated projects have provided Ms. Pruyser, Ms. Flores, Ms. Antekeier and Ms. Chernikova with training in scientific writing and principles of scientific data dissemination. Ms. Pruyser, Ms. Flores, and Ms. Chernikova presented posters at the 2020 DIA Scholar symposium at USC. Both Ms. Chernikova and Ms. Antekeier completed posters presented at the 2021 DIA Scholar symposium at USC. Ms. Pruyser is a McNair scholar and used data from the lab for her presentation on how embodied communication is reduced during emotion experience description in children with ASD at the 2020 UCLA Virtual National McNair conference. Ms. Flores used data collected as part of the project for her final presentation on the preliminary results of microbiome-gut-brain axis influence on emotion processing in typically developing children. Ms. Chernikova, Ms. Pruyser, and Ms. Flores also completed a poster presentation titled Caregiver and Youth Inter-Rater Assessment Agreement in Autism Spectrum Disorder and Developmental Coordination Disorder at the Association for Psychological Science (APS) 2021 Virtual Convention. Finally, another undergraduate researcher at the USC laboratory, Gabriel Abrams, used data collected on this project to complete his undergraduate thesis in 2021 with help from graduate students and postdocs.

Master's Level Graduate Training: Lamoni Lucas, Trinh Nguyen, and Corinne Archer, master's students in the USC Occupational Therapy program, were awarded research assistant placements to work with Dr. Aziz-Zadeh's lab on this project for the academic years 2019-2021. They were trained in ASD, microbiota, diet questionnaires, participant recruitment strategies, psychological assessments and functional Magnetic Resonance Imaging (fMRI) data collection. They also received training in professional development through mentorship with postdocs and OT residents in the lab.

PhD Graduate Training: Christiana Butera and Aditya Jayashankar, graduate students in Dr. Aziz-Zadeh's lab at USC, continued to gain 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, Christiana and Aditya had posters accepted to the American Occupational Therapy Annual Conference: one exploring sensory differences, alexithymia, and neural activity in autism spectrum disorder (Christiana) and one exploring how anxiety and physical hyperarousal affect competence in Autism Spectrum Disorder (Aditya). Aditya had another poster accepted to the Organization of Human Brain Mapping comparing the efficacy of two automated motion correction strategies in correcting multiband-acquired images. Christiana also published an article in *Mind, Brain and Education* entitled "Impact of Sensory Processing on School Performance

Outcomes in High Functioning Individuals with Autism Spectrum Disorder.” Christiana successfully defended her dissertation in June 2021 and will be moving to a post-doctoral position at USC in August 2021. Aditya successfully passed his candidacy examination in December 2020 and will be defending his dissertation proposal in August 2021.

Occupational Therapy Doctoral Training: Anusha Hossain (2019-2020), Alexis Nalbach (2019-2020), and Michelle Canales (2020-2021), Occupational Therapy Doctoral Residents at USC gained training and experience in lab protocols specific to this project, as well as educational and professional development. They 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.

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 continued to receive training in (a) sensory processing assessments in young children with ASD, (b) microbiota research through online courses, conferences, 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 project’s 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 planned on attending the Social and Affective Neuroscience Conference and the American Occupational Therapy Association conference to present work on autism before they were canceled due to COVID-19 pandemic. She additionally mentored four undergraduate Diversity, Inclusion, and Access Jumpstart Scholar Interns over the past two summers and worked with them specifically on projects relating to the current project (i.e., refining assessment protocols and writing a review manuscript of current ASD and microbiota literature). 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 many undergraduate students, and postdoctoral fellows. As the major multiomics analyses involving brain imaging, gut microbiome and behavioral data for this study will not start before the enrollment has been completed, no training on the study’s specific data set has begun.

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 originally planned to complete data acquisition by the end of September 2020. However, we were unable to collect data from any participants from March 2020 – January 2021 due to COVID-19, and thus we are behind in reaching this goal. We believe that we will be able to complete the remaining participants by December 2021. We continue to recruit via posting on relevant listservs and social media groups, reaching out to schools, clinics, and community centers, and attending relevant online events for recruitment. These recruitment

practices have proved successful in the past, and we plan to continue them as soon as we are able to collect data again.

As soon as we complete data collection, we will complete data analyses, including multiomics integration of brain, microbiome and behavioral data, and subsequently work on manuscript write-up and dissemination. We have already completed scoring of behavioral data as well as preprocessing of individual brain imaging data and individual level fMRI analysis.

5. 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).

Nothing to report at this time.

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 at this time.

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.*

Dr. Aziz-Zadeh delivered a talk at the Help Group Annual Symposium (2019) which includes scientists, practitioners, clinicians, and individuals and their families with autism. The title of the talk was “The brain, the microbiome, and behavior in autism”, and it was well received, with the public very much interested in this topic, especially families with individuals with autism, as the PIs received multiple emails following the symposium from audience members. Dr. Aziz-Zadeh also delivered a keynote talk at the Annual Meeting of Neuroscience National Research Council in Italy (2020) which included discussion on and preliminary data from this project. She also delivered a talk at the STAR Institute Summit which is a Summit attended by families with autism, practitioners, and researchers.

Dr. Mayer delivered several talks on gut microbiome brain interactions including the following: Microbiome for Mars Virtual Conference, July 13, 2020 (Title: Gut Brain Connections to Behaviors in Humans); MENLA Virtual Symposium “Weeding, Feeding and Seeding”, Jan 21, 2021 (Title: The Gut Immune Connection); Columbia University Digestive, Liver Disorders Research Center Seminar on June 2, 2021 (Title: The Brain Gut Microbiome System – Implications for GI Disorders); NuRhoPsi Neuroscience Symposium, Atlanta, April 9, 2021 (Title: The Gut Immune Connection and how it affects the brain); Oregon Society for Neuroscience Chapter, April 4, 2021 (Title: the Gut Immune Connection and how it affects the brain).

In order to reach clinicians and practitioners, PhD student, Christiana Butera, had a poster accepted to the 2020 American Occupational Therapy Association Annual Conference to discuss work entitled “*Sensory differences, alexithymia, and neural activity during emotion observation in youth with Autism Spectrum Disorder,*” however, this conference was cancelled due to COVID-19. Additionally, in Spring 2021, Christiana gave an invited lecture entitled “Sensorimotor and socio-emotional functioning in autism spectrum disorder” to both the biokinesiology and physical therapy department at the University of Southern California, and to the Center for Interdisciplinary Brain Sciences at Stanford University.

Finally, undergraduate student Genesis Flores, who spent the summer of 2020 in Dr. Aziz-Zadeh’s lab as part of the Diversity, Inclusion, and Access Program at USC and continued in the lab till June 2021, gave a virtual seminar on potential involvement of the gut-microbiome brain axis on emotional expressions in autism. All the talks were well received and were followed by ample discussion and interest.

6. 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.

As previously stated, we have been greatly delayed by the COVID-19 bar on testing participants in person for much of last year. Nevertheless, with a no-cost extension we anticipate completion of the project goals in the timeline outlined above.

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.

Due to COVID-19, our participant expenditure (fMRI costs, etc.) were less than expected of the last year.

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.

7. 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).*

1. Frith, J., Grangwisch, JE., Borisini, A. Wootton RE., Mayer. EA. (2020) Food and mood: How do diet and nutrition affect mental well being? BMJ. PMID: 32601102.
2. Osadchiy, V., Martin, CR, Mayer EA. (2019) Gut microbiome and modulation of CNS function. Comp Physiology. PMID: 31853944
3. Bhatt, RR., Gupta, A., Mayer, EA, Zeltzer LK. (2019) Chronic pain in children: Structural and resting state functional brain imaging within a developmental perspective. Pediatric Research. PMID: 31791045.
4. LaPelusa M, Donoviel D, Branzini SE, Carlson PE Jr, Culler S, Cheema AK, Kaddurah-Daouk R, Kelly D, de Cremoux I, Knight R, Krajmalnik-Brown R, Mayo SL, Mazmanian SK, Mayer EA, Petrosino JF, Garrison K. (2021) Microbiome for Mars: surveying microbiome connections to healthcare with Implications for long-duration human spaceflight, virtual workshop. Microbiome. PMID: 33397500
5. Bonaz B, Lane RD, Oshinsky ML, Kenny PJ, Sinha R, Mayer EA, Critchley HD. (2020) Diseases, Disorders, and Comorbidities of Interoception, Trends Neurosci. PMID: 33378656
6. Kilroy, E. Chernikova, Flores, G., Pruyser, A., Canales, M., Labus, J., Mayer, E., Aziz-Zadeh, L. (in preparation). Potential role of the gut microbiome in autism spectrum disorder. Expected submission: date Aug 1, 2021.

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).

1. Labus JS, Tun G, Kilpatrick, LA, Rao, SC, Mayer, EA, Tillisch, K. Neuroimaging and Biomarkers in functional gastrointestinal disorders: What scientists and clinicians need to know about basic neuroimaging, biomarkers, microbiome, gut, and brain interactions. In: Rao, SC, Lee YY, Ghosal, UC (eds.) *Clinical and Basic Neurogastroenterology and Motility* (Ch. 3. Pp. 31-53) Academic Press, Elsevier Inc, 2020

2. Mayer, EA. *The Gut-Immune Connection: The Invisible link between the food we eat and the microbes within us -- How we can take back our health.* Harper & Collins, June 2021

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.*

1. Aziz-Zadeh, L. (2020) Understanding the neural basis of social, motor, and microbial deficits in autism. Keynote Speaker, *Annual Meeting of Neuroscience National Research Council, Italy* [online live seminar due to COVID-19]
2. Aziz-Zadeh, L. (2020) Toward A Better Understanding of Social & Motor Deficits in Children with Autism Speaker at STAR Institute Virtual Summit: Sensory Processing in Autism
3. Aziz-Zadeh, L. (2020). Sensorimotor and gut differences in ASD. Speaker at Occupational Therapy Summit (Canceled due to COVID-19).
4. Aziz-Zadeh, L. (2019) The brain, the microbiome, and behavior in autism. Help Group Annual Symposium. Los Angeles.
5. Mayer, M. (2019) Brain gut microbiome interactions & brain health. NeoTriton Conference. Calgary.
6. Mayer, E. (2020) Gut-Brain connections to behaviors in humans. Microbiome for mars virtual workshop. Online
7. G. Flores, E. Kilroy, A. Jayashankar, C. Butera, L. Harrison, A. Hossain, A. Nalbach, L. Aziz-Zadeh (2020). Emotion processing in autism spectrum disorder: Potential influence of the gut-brain axis. Diversity, Inclusion & Access (DIA) Jumpstart Research Presentation. Online presentation.
8. Noor, S., Jayashankar, A., & Harrison, L. (2021). Virtual Reality Adaptation of Cyberball paradigm increases Sense of Social Presence and Ostracism. Society of Affective Science (SAS) Annual Conference. (Virtual conference)
9. Jayashankar, A., Harrison, L., Butera, C., Kilroy, E., Kaplan, J., Hossain, A., Nalbach, A., & Aziz-Zadeh, L. (2020). Comparison of AROMA and FIX in motion correction for multiband pediatric clinical neuroimaging. 26th Annual Meeting of the Organization of Human Brain Mapping (OHBM). (Virtual conference)

· **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.

· **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.

8. 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: Aditya Jayashankar
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: Anusha Hossain, OTD, OTR/L
Project Role: Occupational Therapy Doctoral Resident
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
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: Michelle Canales, OTD, 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.

New funding for Dr. Mayer:

1. Social Isolation and Discrimination as Stressors Influencing Brain-Gut Microbiome Alterations Among Filipino and Mexican Americans 05/01/21-12/30/2026

0.30 CM

This proposal aims to examine how the stressors of social isolation and discrimination are related to eating behaviors and dietary patterns, and further, how these behaviors affect the brain-gut-microbiome axis in 300 Mexican and Filipina women because research shows that they encounter a high burden of obesity as well as exposure to social stressors.

Role: Co-I

2. Danone (PI: Gupta)

12/16/2020-03/01/2022

0.60 CM

Pilot study aimed to determine the optimal design for the UCLA/Danone probiotic intervention study related to stress

The goal of this pilot study is to identify the optimal population and methodology for the planned probiotic intervention study in Phase II of the collaboration.

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.

9. 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.*

10. 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.*