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

PRINCIPAL INVESTIGATORS: Dr. Emeran Mayer, MD

CONTRACTING ORGANIZATION: The Regents of the University of California, Los Angeles

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

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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14. ABSTRACT

Several studies have linked several gastrointestinal symptoms which are common in ASD to altered gut microbial composition (dysbiosis) and function. 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 brain-gut- microbiome interactions and their relationship to behavior 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 USAMRDC RESPONSIBLE PERSON**
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1. INTRODUCTION:

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 interactions and their relationship to behavior 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 and function 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. Such treatments may include fecal microbial transplantation, prebiotics, probiotics, and therapies aimed at specific gut microbes or their products.

2. KEYWORDS:

Autism, Brain Imaging, fMRI, Neural Connectivity, Microbiome

STATEMENT OF WORK – Revised July 2023

3. MAJOR GOALS OF THE PROJECT

STATEMENT OF WORK – 5/25/2023
PROPOSED START DATE July 01, 2018

Site 1:	University of Southern California Los Angeles, CA 90089	Site	University of California, Los Angeles Los Angeles CA 90095
	Initiating PI: Dr. Lisa Aziz-Zadeh		Partnering PI: Dr. Emeran Mayer

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

	Timeline (Months)	**Site 1** Initiating PI	**Site 2** Partnering PI
Major Task 1: Prepare regulatory documents and research protocol for IRB Approval	COMPLETED	Dr. LAZ	Dr. EM
Milestone # 1: IRB approval received at both USC and UCLA	COMPLETED	Dr. LAZ	Dr. EM
Milestone #2: Regulatory approval from DoD HRPO	COMPLETED	Dr. LAZ	Dr. EM
Major Task 2: Hiring and training of study personnel on behavioral and clinical assessments and MRI/fMRI tasks	COMPLETED	Dr. LAZ	
Milestone # 2: Study personnel achieves reliability on the ADOS and ADI-R	COMPLETED	Dr. LAZ	
Major Task 3: 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 stool samples from these participants (using new funds from current proposal)	COMPLETED	Dr. LAZ	

II. Recruit 20 additional ASD and 20 additional TD participants in each group (using new funds from current proposal)	COMPLETED		
Major Task 4: Conduct metagenomic and metabolomics studies of the gut microbiome			
Subtask 1: shot-gun metagenomics analysis	COMPLETED		UCSD under the supervision of Dr. EM (and co-I Dr. RK)
Subtask 2: metabolomics analysis	COMPLETED		Metabolon under supervision of Dr. EM
Milestone #3: Present data at conferences and co-author manuscript on metagenomic and metabolic analysis in TD compared to children with ASD	Dr. LAZ's group has presented metabolite and brain/behavior data at several conferences Manuscripts: 48-60	Dr. LAZ	Dr. EM

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, functional connectivity, complex network analysis)	COMPLETED	Dr. LAZ	Dr. EM
Milestone #4: Present data at conferences and co-author manuscript on correlations between resting state connectivity, microbiota related data, and behavioral data	48-60	Dr. LAZ	Dr. EM
Subtask 2: DTI analyses (deterministic and probabilistic tractography, voxel-based microstructural analysis, complex network analysis)	COMPLETED	Dr. LAZ	Dr. EM
Milestone #5: Present data at conferences and co-author manuscript on correlations between DTI analyses, microbiota related data, and behavior	48-60	Dr. LAZ	Dr. EM
Major Task 6: Conduct correlations between connectivity analyses, gut microbial parameters, and behavior	METABOLITE DATA CORRELATIONS COMPLETED; SHOT-GUN METAGENOMICS	Dr. LAZ	Dr. EM

	CORRELATIONS: 48-60		
Subtask 1: Analyze behavioral data	COMPLETED	Dr. LAZ	Dr. EM
Subtask 2: Conduct correlations between behavioral data, gut microbiome data, and behavioral data	METABOLITE DATA CORRELATIONS COMPLETED; SHOT-GUN METAGENOMICS: 48-60	Dr. LAZ	Dr. EM
Milestone #6: Present data at conferences and co-author manuscript on correlations on brain resting state connectivity, microbiota related data, and behavior	48-60	Dr. LAZ	Dr. EM
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	48-60	Dr. LAZ	Dr. EM

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

Major Task 7: Conduct fMRI analyses of emotional face processing in all participants	COMPLETED	Dr. LAZ	
Major Task 8: Conduct correlations between fMRI analyses and gut microbial parameters	METABOLITE DATA CORRELATIONS COMPLETED; SHOT-GUN METAGENOMICS: 48-60	Dr. LAZ	Dr. EM
Milestone #8: Present data at conferences and co-author manuscript on correlations on fMRI data during emotional face processing and microbiota related data	METABOLITE DATA CORRELATIONS COMPLETED; SHOT-GUN METAGENOMICS: 48-60	Dr. LAZ	Dr. EM
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	METABOLITE DATA CORRELATIONS COMPLETED; SHOT-GUN METAGENOMICS: 48-60	Dr. LAZ	Dr. EM
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	48-60	Dr. LAZ	Dr. EM

better understand heterogeneity within ASD and predict clinical diagnosis			

ENROLLMENT SCHEDULE -- COMPLETED

The total sample size is 120. We note that the numbers for each analysis slightly differ due to data quality issues for brain imaging and/or stool samples.

	Year 1				Year 2				Year 3				Year 4			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Target Enrollment This Proposal	**1 ASD 1TD**	**5 ASD 5 TD**	**5 ASD 5 TD**	**5 ASD 5 TD**	**5 ASD 5 TD**	**5 ASD 5 TD**	**Closed due to COVID**	**Closed due to COVID**	**Closed due to COVID**	**1ASD 1 TD**	**2ASD 2 TD**	**4 ASD 4TD**	**3ASD 3TD**	**-**	**-**	**-**

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.

<u>Specific Aim 1</u>: 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*

This milestone has been completed.

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 has been completed.

Major Task 4: Conduct metagenomic and metabolomics of the gut microbiome
Subtask 1: Run shot-gun metagenomics
Subtask 2: Run metabolomics

Subtask 2 is complete. Subtask 1 is complete: We recently received a subcontract for UCSD (Dr. Knight's microbiome lab) to conduct shot-gut metagenomics for us.

- *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 21 different conferences (listed below).

We have published the MRI and behavioral data for this project in 10 pear reviewed research papers (listed below). We further have 2 review papers published and 2 books on the gut microbiome have been published (listed below).

Specific Aim 2 & 3: To identify associations between altered brain network connectivity, gut microbial parameters, and behavior. To identify associations between altered brain activation patterns and gut microbial parameters

Resting state, DTI, functional MRI and behavioral data analysis have been completed. Both shotgun metagenomic and metabolite analyses have been completed. We have also completed correlations between behavioral, brain, and metabolite data. We plan to complete correlations

between shotgun metagenomic data with behavioral and brain data Fall/Winter 2023. We have presented the completed analyses at 6 conferences, and plan to complete manuscripts and more presentations in the year ahead.

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 (UCLA) joined the team as a DIA student in June 2021 and Christine Figueroa (Cal State LA) in June 2022. 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. In November 2021, Ms. Chernikova and Ms. Flores submitted a manuscript entitled "The Brain-Gut-Microbiome System: Pathways and Implications for Autism Spectrum Disorder" which was then published in *Nutrients* in December 2021. Additionally, 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. In June of 2022, Mr. Abrams published a manuscript comparing praxis errors in children with autism and developmental coordination disorder to the

Journal of Autism and Developmental Disorders. Christine Figueroa presented a poster at the 2022 DIA Scholar symposium on alexithymia, personality, and life satisfaction in youth with and without ASD.

In Spring of 2022, Taran Gupta, and Simran Goel, joined as volunteer undergraduate research assistants, followed by Liliana Adzich, Angel Nim, Brooke Barry, and Faiz Ahmed in the summer of 2023. Tarun Seera (Western Washington University) joined in as a research assistant in the summer of 2023 through the USC NEURAL program which focuses on helping students from underprivileged backgrounds gain research experience. All volunteers received general training in the day-to-day activities associated with scientific research.

At UCLA, undergraduate student Skylar Tanartkit has been receiving training from Dr. Labus (co-I) to help analyze the data.

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. Ms. Nguyen's award was renewed for 2021 – 2022, alongside Gregory Natt and Nandita Raman. Gregory Natt was renewed for 2022-2023, alongside Dhruvina Suru and Miel Krauss. Additionally, Chantal Sotomayor and Tom Williamson, joined the team as volunteers in Fall 2021 followed by Brittany Bynum, Ka Ki Tam, Vanessa Mesa, Stacey Chang, and Tristenne Ocampo in Fall 2022 and Bhargavi Kalanji and Chieh-Yi Chen in Spring 2023. Mr. Williamson has been receiving guidance in scientific writing and principles of scientific data dissemination, focusing on different areas of research with support from PhD student, Sofronia Ringold, and collaborators. Ms. Bynum focused on MRI data pre-processing and analysis with the guidance of PhD candidate, Aditya Jayashankar and together they published a joint first author fMRI paper related to the work completed in *Cortex*. Shahad Almohsen, a master's student from the Occupational Therapy program completed an independent study project with Dr. Aziz-Zadeh. Similarly, Valerie Duarte and Gabrielle Uhrik from the Speech Therapy program also completed independent study projects in the Spring 2023. Students were trained in ASD, microbiota, diet questionnaires, participant recruitment strategies, psychological assessments, behavioral data collection, and/or functional Magnetic Resonance Imaging (fMRI) data collection. They also received training in professional development and scientific writing and data dissemination through mentorship with postdocs and OT residents in the lab.

PhD Graduate Training: Sofronia Ringold began her graduate program in August 2021 and Aditya Jayashankar in August 2018. Sofronia, gained experience with study management and oversight of undergraduate research assistants as well as training in administering behavioral assessments and functional Magnetic Resonance Imaging (fMRI) data collection and analysis, with the support of Aditya. Sofronia presented an abstract at USC's Research Day and the International Society for Research on Emotion (ISRE), and Occupational Therapy Summit of Scholars and Aditya published an abstract in ISRE and the International Society for Autism Research (INSAR). Sofronia published a manuscript related to sensory processing in children with autism to a special issue of *Brain Sciences* in July 2022. Aditya additionally submitted a manuscript entitled "Feeling disgusted: Disgust processing and potential relationships with behaviors in youth with ASD" which is currently under review and a functional brain connectivity paper on the data set which has been accepted for publication in *Cortex.*

Occupational Therapy Doctoral Training: Michelle Canales (2020-2021), Riley McGuire (2021 – 2022), and Nandita Raman (2022 – 2023) were Occupational Therapy Doctoral Residents at USC and 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. Nandita Raman published a manuscript on the Relationships between Affect Recognition, Empathy, Alexithymia, and Co-Occurring Conditions in Autism in July 2023. Nandita Raman was awarded the Margaret S. Rood award for Professional Promise from the USC Chan Division of Occupational Science and Therapy in 2023. In addition, Riley McGuire was awarded the Political action and Advocacy award from the USC Chan Division of Occupational Science and Therapy in 2022.

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 former postdoctoral trainee on the project, and Sofronia Ringold, a PhD Student on the project. As a result of this award, Dr. Aziz-Zadeh, Dr. Kilroy, and Ms. Ringold received 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 (d) mentorship of undergraduate and graduate students, (e) fMRI data analysis (Ringold) and (f) study and personnel management. All the training listed above resulted in increased knowledge and skills pertaining to the project's aims. Additionally, Dr. Aziz-Zadeh and Ms. Ringold have attended conferences and seminars relating to state-of-the-art microbiota analysis and research findings. They 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, Dr. Kilroy and Ms. Ringold have spent one-on-one time working with the PI, Lisa Aziz-Zadeh, furthering their professional development in scientific grant writing and principles of scientific data dissemination. Dr. Kilroy completed her postdoctoral training in December 2021.

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

As stated above, we plan to complete the shotgun metagenomic analysis in the next month, which will enable us to running correlations and association network analyses by December 2023 and the writing up of results in publications in early 2024.

5. IMPACT:

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

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". 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. Aziz-Zadeh further discussed the behavioral and brain imaging findings from this study at International Society for Autism Research Annual Meeting in Austin, TX. She presented the current metabolite/brain/behavioral data at the USC and CHLA Autism Interest Group Monthly Meetings in July 2023 and at the FLUX Symposium in September 2023.

Dr. Mayer delivered several talks on gut microbiome brain interactions and clinical implications such as ASD, including the following: International Association for Polyphenol Research Conference, London, 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).

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 2022, 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.

Student presentations at conferences in the past year directly related to this work also include the following:

2023 Ringold, S.M., Kilroy, E., Jayashankar, A., Labus, J., Butera, C., Mahurkar, S., Gupta, A., Mayer, E., **Aziz-Zadeh, L**. Exploring the Relationship Between Tryptophan Metabolites, Neural Activity, and Sensory Processing in Typically Developing and Autistic Children. *Occupational Therapy Summit of Scholars*, Columbus, OH.

2023 Raman, N., Ringold, S., Jayashankar, A., **Aziz-Zadeh, L**. Empathy and Affect Recognition in Children with ASD. *USC Graduate Research Symposium,* Los Angeles, CA.

2023 Raman, N., Ringold, S., Jayashankar, A., **Aziz-Zadeh, L**. Empathy and Affect Recognition in Children with ASD. *USC Herman Ostrow School of Dentistry Research Day,* Los Angeles, CA.

2023 Ringold, S.M. , Gruskin. B., **Aziz-Zadeh, L**., Cogan, A. Scoping Review: Exploring the Evolution and Utility of Neuro-Occupation. *USC Herman Ostrow School of Dentistry Research Day*, Los Angeles, CA.

2023 Jayashankar, A., Bynum, B., Butera, C., Kilroy, E., Harrison, L., **Aziz-Zadeh, L**. Differences in functional connectivity between inferior frontal gyrus and mentalizing brain regions in autism as compared to developmental coordination disorder and neurotypical populations. *Social Affective Neuroscience Society (SANS),* Santa Barbara, CA.

2022 Figueroa, C, Jayashankar, A.*, Ringold, S.*, McGuire, R.*, Aziz-Zadeh, L. Alexithymia, Personality, and Life Satisfaction in Youth with and Without Autism Spectrum Disorder.USC Diversity Inclusion and Access, Los Angeles, CA

2022 Jayashankar, A., Ringold, S., McGuire, R., Butera, C., Kilroy, E., **Aziz-Zadeh, L**. Disgust and life satisfaction outcomes in children with Autism Spectrum Disorder (ASD). *Society for the Study of Occupation (SSO): USA*, San Diego, CA.2022

2022 Jayashankar, A., Ringold, S., McGuire, R., Butera, C., Kilroy, E., **Aziz-Zadeh, L**. Disgust and sensory processing in the anterior insula estimates school performance in children with Autism Spectrum Disorder (ASD). *Flux*, La Sorbonne, Paris.

2022 Ringold, S., Jayashankar, A., Kilroy, E., McGuire, R., Butera, C., **Aziz-Zadeh, L.** Personality and social touch in typically developing and children with ASD. *USC Herman Ostrow School of Dentistry Research Day,* Los Angeles, CA.

2022 Ringold S.**,** Jayashankar, A., Kilroy, E., McGuire, R., Butera, C., **Aziz-Zadeh, L.** Enhanced perception is related to alexithymia differentially in typically developing children and children with autism spectrum disorder (ASD). *International Society for Research on Emotion (ISRE) Conference,* Los Angeles, CA.

2022 Jayashankar, A., Kilroy, E., Butera, C., Harrison, L., Ringold, S., McGuire, R., **Aziz-Zadeh, L.** Anterior insula reactivity is associated with disgust propensity in children with Autism Spectrum disorder (ASD). *International Society for Research on Emotion (ISRE) Conference,* Los Angeles, CA.

2022 Jayashankar, A., Kilroy, E., Ringold, S., McGuire, R., **Aziz-Zadeh, L**. Physical disgust processing in children with autism spectrum disorder (ASD) during observation of rotten foods. *International Society for Autism Research (INSAR) Annual Meeting,* Austin, TX.

2022 Butera, C., Ring, P., Harrison, L., Jayashankar, A., Sideris, J., Kilroy, E , Cermak, S., Chernikova, M., Kaplan, J., **Aziz-Zadeh, L.** Impact of sensory functioning on school performance and social-emotional outcomes in ASD. *International Society for Autism Research (INSAR) Annual Meeting,* Austin, TX.

6. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

As previously noted, we have experienced a year of delays from conducting shotgun metagenomic analysis due to the backlog of analyses experienced over the COVID-19 pandemic. Nevertheless, we hope to acquire a no-cost extension and we anticipate completion of the project goals in the timeline outlined above. We have analyzed all the behavioral and neuroimagiong data and metabolite analyses, as well as correlational analyses for the three datasets, and have submitted all our stool samples for shotgun metagenomic analysis at UCSD. Once the shotgun metagenomics

analysis is complete, the majority of our remaining work is to run statistical analyses on that final component of the data and to write and submit manuscripts.

Changes that had a significant impact on expenditures

We have utilized all our funds and have none to carry over.

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

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:

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

Journal publications.

Review Papers (related to the current project):

1. Jayashankar, A., Aziz-Zadeh, L. Disgust processing and potential relationships with behaviors in autism. *Current Psychiatry Rep* (2023). https://doi.org/10.1007/s11920-023-01445-5
2. Chernikova, M. A., Flores, G. D., Kilroy, E., Labus, J. S., Mayer, E. A., & Aziz-Zadeh, L. (2021). The brain-gut-microbiome system: pathways and implications for autism spectrum disorder. *Nutrients*, *13*(12), 4497.

Published papers (behavioral and brain imaging data from the current study):

1. Raman, N., Ringold, S. M., Jayashankar, A., Butera, C. D., Kilroy, E., Harrison, L., Cermak, S. A., & Aziz-Zadeh, L. (2023). Relationships between Affect Recognition, Empathy, Alexithymia, and Co-Occurring Conditions in Autism. *Brain sciences*, *13*(8), 1161. https://doi.org/10.3390/brainsci13081161
2. Jayashankar, A., Bynum, B., Butera, C., Kilroy, E., Harrison, L., & Aziz-Zadeh, L. (2023). Connectivity differences between inferior frontal gyrus and mentalizing network in autism as compared to developmental coordination disorder and non-autistic youth. *Cortex*, *167*, 115–131. Advance online publication. https://doi.org/10.1016/j.cortex.2023.06.014
3. Butera, C., Kaplan, J.T., Kilroy, E., Harrison, L., Jayashankar, A., Loureiro, F., Aziz-Zadeh, L. (2023) The relationship between alexithymia, interoception, and neural functional connectivity during facial expression processing in autism spectrum disorder. *Neuropsychologia.*
4. Abrams, G., Jayashankar, A., Kilroy, E., Butera, C., Harrison, L., Ring, P., Cermak, S., Aziz-Zadeh, L. (2022) Motor Performance, praxis, and social skills in autism spectrum disorder and developmental coordination disorder. In Review at *Journal of Autism and Developmental Disorders*.
5. Ringold, S., McGuire, R., Jayashankar, A., Kilroy, E., Ring, P., Butera, C., Harrison, L., Aziz-Zadeh, L., Cermak, S. (2022) Sensory sensitivities in developmental coordination disorder as compared to autism. *Brain Sciences*.
6. Kilroy, E., Gerbella, M., Cao, L., Molfese, P., Butera, C., Harrison, L., Jayashankar, A, Rizzolatti, G., Aziz-Zadeh, L. (2022) Specific tractography differences in autism: Comparing autism spectrum disorder and developmental coordination disorder. *Nature: Scientific Reports.*
7. Kilroy, E., Ring, P., Hossain, A., Nalbach, A., Butera, C., Harrison, L., Jayashankar, A., Vigen, C., & Aziz-Zadeh, L. (2022). Motor performance, praxis, and social skills in autism spectrum disorder and developmental coordination disorder. *Autism Research.*
8. Harrison, L. A., Kats, A., Kilroy, E., Butera, C., Jayashankar, A., Keles, U., & Aziz-Zadeh, L. (2021). Motor and sensory features successfully decode autism spectrum disorder and combine with the original RDoC framework to boost diagnostic classification. *Scientific reports*, *11*(1), 1-16.
9. Kilroy, E., Harrison, L., Butera, C., Jayashankar, A., Cermak, S., Kaplan, J., Williams, M., Haranin, E., Bookheimer, S., Dapretto, M. & Aziz-Zadeh, L. (2021). Unique deficit in embodied simulation in autism: An fMRI study comparing autism and developmental coordination disorder. *Human brain mapping*, *42*(5), 1532-1546.
10. Butera, C., Ring, P., Sideris, J., Jayashankar, A., Kilroy, E., Harrison, L., Cermak, S. & Aziz-Zadeh, L. (2020). Impact of sensory processing on school performance outcomes in high functioning individuals with autism spectrum disorder. *Mind, Brain, and Education*, *14*(3), 243-254.

Published papers related to the gut-microbiome (though not from the current data set):

1. LaPelusa, M., Donoviel, D., Branzini, S.E., Carlson, P.E., Jr, Culler, S., Cheema, A.K., Kaddurah-Daouk, R., Kelly, D., de Cremoux, I., Knight, R., Krajmalnik-Brown, R., Mayo, S.L., Mazmanian, S.K., Mayer, E.A., Petrosino, J.F, Garrison, K. (2021) Microbiome for Mars: surveying microbiome connections to healthcare with implications for long-duration human spaceflight, virtual workshop. *Microbiome*. PMID: 33397500
2. Frith, J., Grangwisch, J.E., Borisini, A. Wootton, R.E., Mayer, E.A. (2020) Food and mood: How do diet and nutrition affect mental well being? BMJ. PMID: 32601102.
3. Bonaz, B., Lane, R.D., Oshinsky, M.L., Kenny, P.J., Sinha, R., Mayer, E.A., Critchley, H.D. (2020) Diseases, Disorders, and Comorbidities of Interoception. *Trends Neuroscience*. PMID: 33378656
4. Bonaz B, Lane RD, Oshinsky ML, Kenny PJ, Sinha R, Mayer EA, Critchley HD. (2020) Diseases, Disorders, and Comorbidities of Interoception. *Trends Neuroscience*. PMID: 33378656
5. Osadchiy, V., Martin, C.R., Mayer, E.A. (2019) Gut microbiome and modulation of CNS function. *Comp Physiology*. PMID: 31853944
6. Bhatt, R.R., Gupta, A., Mayer, E.A., Zeltzer L.K. (2019) Chronic pain in children: Structural and resting state functional brain imaging within a developmental perspective. *Pediatric Research*. PMID: 31791045.

Books or other non-periodical, one-time publications.

1. Labus, J.S., Tun, G., Kilpatrick, L.A., Rao, S.C., Mayer, E.A., 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.) Clincial and Basic Neurogastroenterology and Motility (Ch. 3. Pp. 31-53) Academic Press, Elsevier Inc., 2020.
2. Mayer EA. The Mind Gut Connection. How the hidden conversation within our bodies impacts our mood, our choices and our overall health. Harper&Collins 2016
3. Mayer, E.A. 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., Jayshankar, A., Ringold, S., Kilroy, E., Butera, C., Mahurkar-Joshi, S., Dapretto, M., Gupta, A., Labus, J., Mayer, E. (2023) Relationships between gut metabolites, socio-emotional brain processing, and behavior in youth with autism. FLUX Congress, Santa Rosa, CA.
2. Aziz-Zadeh, L. Unique Motor Differences in ASD and their Structural and Functional Neurological Basis & Potential Correlations with Gut Metabolites. (2023) USC & CHLA Autism Interest Group Online Meeting.
3. Ringold, S., Jayashankar, A., Kilroy, E., McGuire, R., Butera, C., Aziz-Zadeh, L. Personality and Social Touch in Typically Developing and Children with ASD. (2022) USC Herman Ostrow School of Dentistry Research Day. Los Angeles, CA.
4. Ringold S., Jayashankar, A., Kilroy, E., McGuire, R., Butera, C., Aziz-Zadeh, L. Enhanced Perception is Related to Alexithymia Differentially in Typically Developing Children and Children with Autism Spectrum Disorder (ASD). (2022) International Society for Research on Emotion (ISRE) Conference. Los Angeles, CA, United States.
5. Jayashankar, A., Kilroy, E., Butera, C., Harrison, L., Ringold, S., McGuire, R., Aziz-Zadeh, L. Anterior insula reactivity is associated with disgust propensity in children with Autism Spectrum disorder (ASD). (2022) International Society for Research on Emotion (ISRE) Conference. Los Angeles, CA, United States.
6. Jayashankar, A., Kilroy, E., Ringold, S., McGuire, R., Aziz-Zadeh, L. Physical disgust processing in children with Autism Spectrum Disorder (ASD) during Observation of Rotten Foods. (2022) International Society for Autism Research (INSAR) Conference. Austin, TX, United States.
7. Butera, C., Ring, P., Harrison, L., Kilroy, E. , Jayashankar, A., Sideris, J., Cermak, S., Chernikova, M., Kaplan, J., Aziz-Zadeh, L. Impact of Sensory Functioning on School Performance and Social-Emotional Outcomes in ASD. (2022) International Society for Autism Research Annual Meeting. Austin, Texas.
8. Aziz-Zadeh, L. (2022) Understanding dissimilar others. NeuroLeadership Annual Summit, Virtual.
9. Aziz-Zadeh, L. (2022) Motor processing in autism (behavioral and neural) and the relationship between motor and social processing. International Ayres Sensory Integration® Annual Congress, Redondo Beach, CA.
10. 2022 Aziz-Zadeh, L. (2022) Brain-gut-microbiome system: Pathways and implications for autism spectrum disorder. Microbiome First: Pathway to Sustainable Healthcare Summit, Virtual.
11. 2021 Aziz-Zadeh, L., (2021) Rock, David. Managing humans: The neuroscience of empathy. NeuroLeadership Institute: Your Brain at Work Live, Virtual.
12. 2022 Aziz-Zadeh, L. Unique motor differences in ASD and their structural and functional neurological basis. International Society for Autism Research Annual Meeting, Austin, TX
13. 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]
14. 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
15. Aziz-Zadeh, L. (2020). Sensorimotor and gut differences in ASD. Speaker at Occupational Therapy Summit (Canceled due to COVID-19).
16. Aziz-Zadeh, L. (2019) The brain, the microbiome, and behavior in autism. Help Group Annual Symposium. Los Angeles.
17. Mayer, E.M. (2019) Brain gut microbiome interactions & brain health. NeoTrition Conference. Calgary.

18. Mayer, E. (2020) Gut-Brain connections to behaviors in humans. Microbiome for Mars virtual workshop. Online.
19. 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.
20. 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)
21. 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.

  https://dornsife.usc.edu/cenec/

- **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: ORCID ID: 0000-0003-3923-3349
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. 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: 4
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: 1 calendar month (of 4) 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: Sofronia Ringold
Project Role: Graduate student
Researcher Identifier (e.g. ORCID ID): 0000-0002-8020-7735
Nearest person month worked: 3
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: Nandita Raman, OTR/L
Project Role: Occupational Therapy Doctoral Resident
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 20
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 and fulfillment of doctoral immersion experience

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?

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 (PIs: Gupta, Mayer) 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.

New Funding for Dr. Aziz-Zadeh:

1. Google Faculty Grant. (PI: Aziz-Zadeh)
   Language Signals for Belonging Bridging, and Othering
   08/24/22 - 08/23/23

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: *N/A*

QUAD CHARTS: *N/A*

10. APPENDICES: *N/A*