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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The project examines the role of gut dysbiosis in post-traumatic epilepsy (PTE). Using a rat model of PTE - lateral fluid percussion injury (LFPI), the project tests the hypothesis that natural premorbid variations and/or post-LFPI perturbations of gut microbiome contribute to PTE. The goals Year 1 were to (i) obtain administrative approvals (Task 1); (ii) generate rats with LFPI and sham LFPI (iii) collect samples for, and perform longitudinal analysis of microbiome, blood and brain biomarkers of inflammation, biomarkers of intestinal barrier (IB) and blood-brain barrier (BBB) permeability (iv) gather and analyze data of chronic epilepsy after LFPI; (v) collect microbiome samples for subsequent microbiome transfer to recipients for Aim 2/Task3 (ii-v - Task 2). According to plan, by the end of Year 1, Task 2 is to be 66% completed. By the end of the reporting period, generating of experimental subjects and sample collection is on schedule. Sample processing is behind schedule due to the COVID-19 - related research shutdown. Analysis of samples and specimens processed up-to-date shows that after LFPI (i) 1/3 of experimental subjects develop PTE; (ii) there are robust changes in microbiome composition (i.e, dysbiosis); (iii) there is significant increase of plasma inflammatory cytokines, which points to peripheral inflammation; (iv) there are disruptions of intestinal and blood-brain barrier; (v) there is pronounced of microglia activation which points to central inflammation. Overall the results confirm the hypothesis on the dysbiosis-PTE connection.

15. SUBJECT TERMS
Fluid percussion injury; post-traumatic epilepsy; microbiome; dysbiosis; inflammation; intestinal barrier; blood-brain barrier; inflammatory cytokine; seizure; electroencephalogram.

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The project examines the role of perturbations in gut microbiome in post-traumatic epilepsy (PTE). Using a rodent model of PTE following lateral fluid percussion injury (LFPI) in rats, the project tests the hypothesis that gut microbiome, through readily identifiable natural premorbid variations and/or post-LFPI perturbations, modulates the susceptibility to PTE. Two major approaches are used: (i) correlation of composition and function of microbiome obtained at different time points vis-à-vis LFPI with PTE, and with LFPI-induced inflammation, the latter serving as a proposed mechanistic link between dysbiosis and epilepsy following TBI; (ii) examination of effects of fecal microbiome transfer (FMT) from pre- and post-LFPI donors on the susceptibility to, and the trajectory of PTE in recipients.

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

Fluid percussion injury; post-traumatic epilepsy; microbiome; dysbiosis; intestinal barrier; inflammation; blood-brain barrier; inflammatory cytokine; seizure; electroencephalogram.

3. **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.

Major task 1. Obtain animal protocol approvals.
Timeline: This was planned for months 1-4 of the grant.
Status- Completed

Major Task 2. Examine microbiome and inflammation profiles in relation to post-traumatic epilepsy (PTE).
Subtask 3: Generate animals for Aim 1: lateral Fluid Percussion Injury (LFPI), sham LFPI, naïve. n=128 rats, 30 rats per month (for LFPI and sham LFPI; naïve require no intervention)
Subtask 4: Collect and store fecal samples before and at different time points after LFPI. Samples are repeatedly collected between before and 12 months after LFPI, as the animals are generated. Total of 896 samples are scheduled to be collected.
Subtask 5: perform DNA sequencing, and multi-omics of samples collected under sub-task 4. Performed on the ongoing basis.
Subtask 6: Characterize markers inflammation at different time points after TBI in correlation with natural and TBI-induced gut microbiome profiles. Examination of permeability of intestinal and blood-brain barriers, plasma cytokine levels and microglia expression in the brain. Performed on the ongoing basis.
Subtask 7: Electrode implantation, and seizure monitoring. Characterization of PTE: seizure frequency, spikes and high-frequency oscillations (HFOs).

Subtask 8: Kindling in a subset of the animals, which present with no spontaneous seizures as examined under subtask 7.
Subtask 9: Data analysis and integration for Aim 1. Data collected from sub-tasks 5-7 undergo statistical analysis, to correlate microbiome composition and function with PTE, and with markers of inflammation
Milestone(s) Achieved: Identify naturally occurring and/or LFPI-induced specific profiles and perturbations in the gut microbiome which would predict the development of epilepsy after LFPI. Correlate natural and epilepsy-associated microbiome profiles with markers of peripheral and central inflammation.

Timeline: Major task 2 was planned for months 4-19 of the grant.
Status- In progress. Subtasks 3 and 4 are on schedule. Subtasks 5-8: sample collection is on schedule; sample analysis is ongoing, but is behind schedule due to the COVID-19 shutdown and restrictions on non-essential experiments.

Major Task 3. Examine effects of fecal microbiome transfer on PTE: allo- and auto- fecal microbiome transfer (FMT).
Subtask 10. Optimize strategy for fecal microbiome transfer studies, based on the outcomes of Aim 1, to decide whether Aim 2.1 OR Aim 2.2 is pursued.
Subtask 11.1: If Aim 2.1 is pursued. Allo-transplantation (epilepsy predisposition factored in). FMT of samples collected from donors with different predisposition to PTE, as obtained under sub-task 4, to recipients 1 week before OR 2 weeks after LFPI. Total of 308 rats, 15-16 rats/week.
Subtask 11.2: If Aim 2.2 is pursued. Allo-transplantation (no epilepsy predisposition is factored in). FMT from donors obtained from sub-task 4 to recipients 2 weeks after LFPI. Total of 132 rats, 15-20 rats/week
Subtasks 11.3-11.7. ONLY if Aim 2.2 is pursued. These sub-tasks are the same as sub-tasks 3-7, with the purpose of increasing of statistical power for sex. A total of 128 rats
Subtask 11.3- 11.7 are same as same as sub-tasks 3-7
Sub-task 12 (for both Aim1 2.1 and 2.2): Auto-transplantation. FMT of samples collected before LFPI and transplanted to the same rats same rats 2 weeks after LFPI. 44 rats. 5-6 rats/week
Subtask 13 (for both Aim1 2.1 and 2.2):: Microbiome sample collection from recipients (both allo- and auto-) generated under sub-tasks 10 and 11. Samples are collected at 5 time points, between 1 and 12 months after LFPI.
Subtask 14 (for both Aim1 2.1 and 2.2): perform DNA sequencing, metabolomics, metatranscriptomics and metagenomics analysis of samples collected under sub-task 12. Performed on the ongoing basis.
Subtask 15 (for both Aim1 2.1 and 2.2): Characterize markers peripheral and central inflammation at different time points after traumatic brain injury in correlation with naturally occurring and brain trauma-induced gut microbiome profiles. Examination of permeability of intestinal and blood-brain barriers, plasma cytokine levels and microglia expression in the brain. Performed on the ongoing basis
Subtask 16 (for both Aim1 2.1 and 2.2): Electrode implantation, and seizure monitoring. Characterization of PTE vis-à-vis seizure frequency, severity, spikes and high-frequency oscillations (HFOs) in allo- and auto-recipients.Subtask 17 (for both Aim 2.1 and 2.2). Kindling in a subset of the animals which present with no spontaneous seizures as identified under subtask 16.

Subtask 18 (for both Aim1 2.1 and 2.2): Data analysis and integration for Aim 2. Data collected from sub-tasks 14-17 undergo statistical analysis and integration, to correlate microbiome composition and function in FMT recipients with PTE, and with markers of inflammation
Milestone(s) Achieved: Determine whether fecal microbiome transfer from animals either predisposed to PTE, or with established PTE, would increase the propensity to and/or severity of post-traumatic epilepsy in recipients, and exacerbate PTE-associated peripheral and central inflammation.
Timeline: This is planned for months 19-36 of the grant.
Status- Not started.

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.

1. Major activities and objectives
1.1. Generating experimental subjects for Aim 1. The progress is indicated in Table 1.

Table 1. Progress in generating animals for Major Task 2 (months 4-19)

Group	*Generated*	*Target (month 19)*
LFPI	68	48
Sham LFPI	25	40
Naive	10	40

Because of higher than expected variability of LFPI severity and long-term morbidity in some subjects (and hence the need for their premature euthanasia), we have generated additional 20 LFPI rats (68 vs. planned 48). Since the project includes longitudinal sample collection, premature animal termination would be highly disruptive to further studies. By generating extra 20 animals, we ensure that by the end of Major Task 2 sufficient number of all samples will be produced. The increased number of LFPI rats does not affect a total number of animals approved for the project, as we were able to curtail mortality, through the optimization of the LFPI technique. During the remaining period of Major Task 2 (months 13-19), we will complete generating sham LFPI and naïve rats.

1.2. Specimens collection for Aim 1 (analysis) and Aim 2 (Fecal Microbiome Transfer, FMT).
Fecal and blood samples are repeatedly collected at different time points vis-a-vis LFPI: before, 1 week, 1,2, 3, 6 and 12 months. Presently, all blood and fecal samples have been collected from all the animals up to 6-month time point, according to schedule. Further, as planned, 25% of animals are being euthanized at 2, 3 and 6 months, and brains processed for the analysis of blood-brain barrier (BBB) permeability (by detecting fluorescein in the brain upon its peripheral administration), and microglia activation (immunohistochemistry- Iba1 expression) in the brain. Additionally, all samples for FMT, which is set to begin on Month 20 (Major Task 3/Aim 2), have been harvested, and are stored.

1.3. Sample processing and data analysis.
The processing of samples has been delayed due to the COVID-related shutdown, when only essential experiments (defined as such by the UCLA Vice-Chancellor for Research) were allowed (for our lab, essential experiments were limited to animal care and data and sample collection).

1.3.1. Recurrent seizures (video and EEG recording). Up to date, chronic video and EEG recordings have been completed in a total of 40 LFPI rats, at 3-month and 6-month time points.
1.3.2. Plasma cytokine measurements (enzyme-linked immunosorbent assay, ELISA). Up-to-date, plasma levels of Interleukin-1 beta (IL-1b), Tumor Necrosis Factor Alpha (TNFa), and Interleukin-17 (IL-17), have been analyzed in a total of 19 LFPI rats, at 5 time points vis-à-vis LFPI (baseline, 1 week, 1, 3 and 6 months). Additionally, IL-17 has been analyzed in 8 shLFPI rats. The remaining samples are in storage, and are being analyzed.
1.3.3. Microbiome analysis. UCLA Microbiome core (where microbiome analysis is to be performed) was fully shut down on March 19 2020, and reopened on June 15, 2020. As a result, there is a significant backlog of samples to be analyzed from the laboratories across the campus. Presently, samples from only 10 LFPI rats have been analyzed, at two time points (baseline and 1 week after LFPI). All samples from all rats generated up to date (i.e. LFPI, sham LFPI, naïve, at different time points), were transferred to the UCLA Microbiome core in the mid-July, and the analysis is being performed according to the Core schedule.
1.3.4. Immunohistochemistry of fluorescein (BBB permeability) and Iba1 (microglia activation) has been performed in 32 rats 1 month after LFPI. All brains have been harvested and are being processed.
1.3.5. Intestinal barrier (IB) permeability (measuring plasma FD4 levels upon its oral administration), has been completed in 18 rats at the baseline, 1 week and 1 month after LFPI. Other samples are in storage and are being processed.

2. Major findings
2.1. Chronic epilepsy (video and EEG monitoring).
Spontaneous seizures were documented in 4 rats at 3 months (10%), and in 13 rats at 6 months (33 %). Seizure incidence is in line with the expectations (15% at 3 months and 25% at 6 months). Figure 1 shows an example of electrographic seizure 6 months after LFPI.

Fig. 1. An electrographic seizure 6 months after LFPI (the figure is on the next page). **A.** Arrangement of electrodes for EEG recording. A total of 6 active electrodes were implanted at different spots around the injury. **B-D**. Continuous EEG recording immediately prior to and during the seizure, showing rapid synchronization (B) and spread (C), apparently from channel 7, followed by gradual resolution and post-ictal suppression (D). An episode of post-ictal spikes follows (D). Behaviorally, this was a generalized tonic-clonic seizure with post-seizure depression.

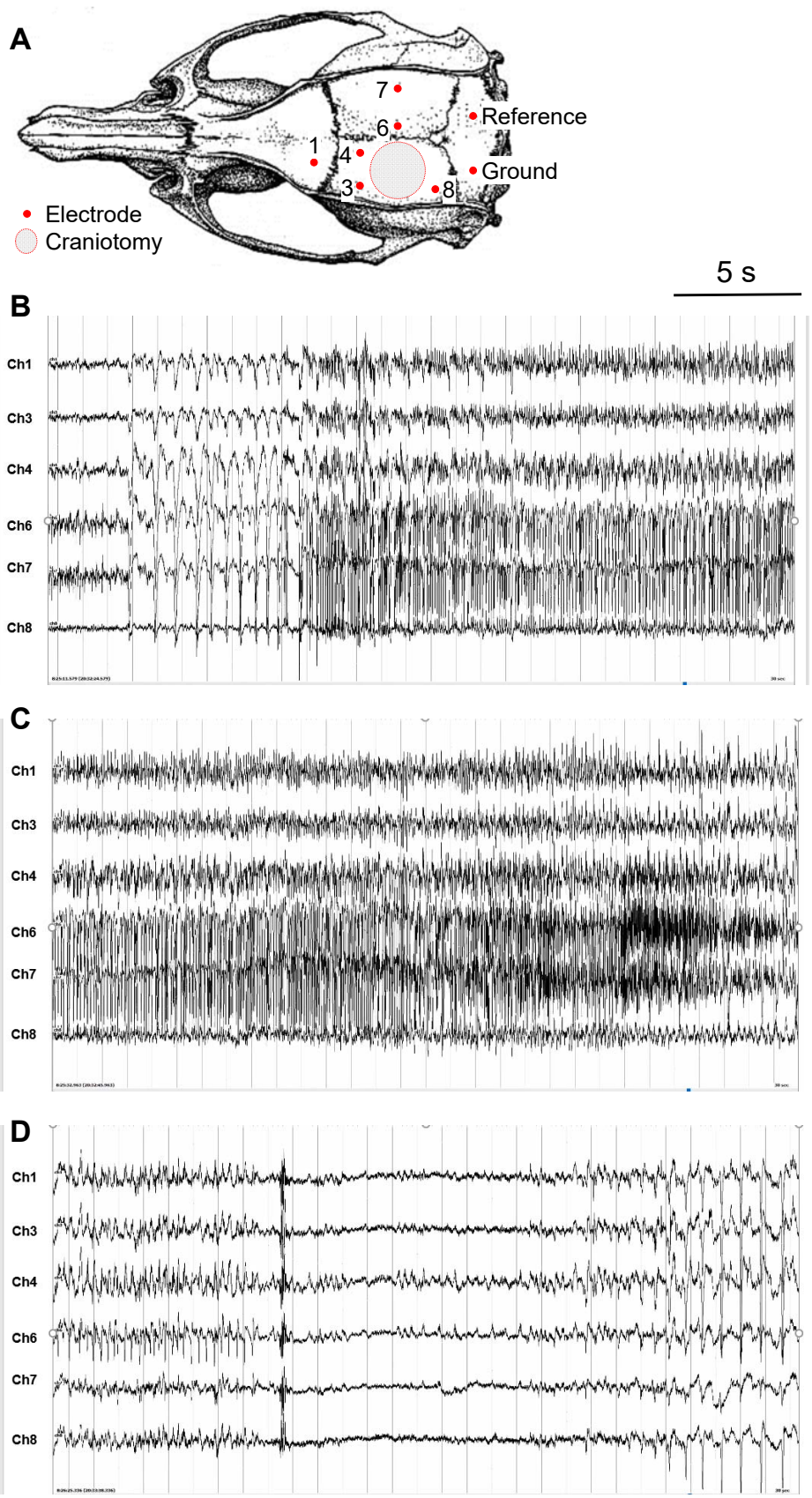
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Fig. 1.

2.2. Microbiome composition (16S ribosomal RNA gene sequencing).
Summary of changes in microbiota diversity and composition one week after LFPI are shown in Figures 2-5. We observed identifiable changes in gut microbiome diversity and composition 1 week after LFPI.

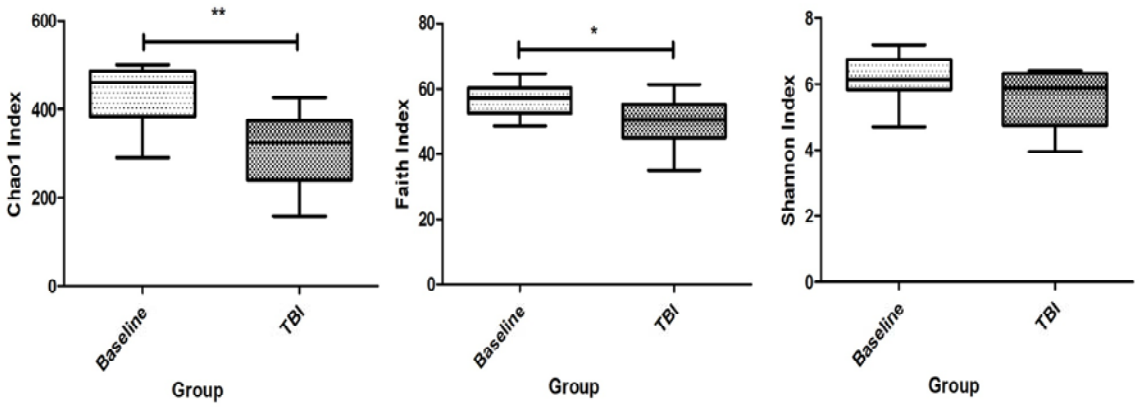

Fig 2. Alpha-diversity (variation of microbes within a single animal). There was significant decrease in the alpha-diversity of gut microbiota one week after LFPI as compared to pre-LFPI diversity. *p<0.05; **p<0.01 significant differences as analyzed by paired T-Test.

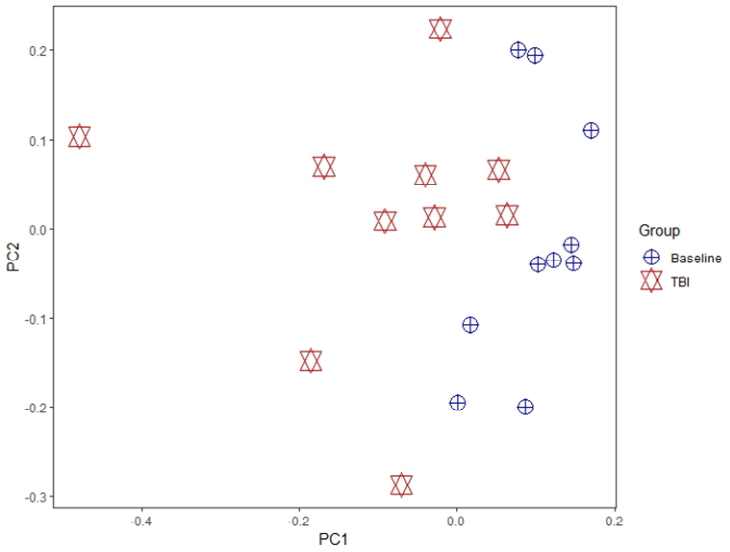

Fig. 3. Beta-diversity (variations of microbial communities among the animals). There was significant decrease in beta diversity of microbiota 1 week after LFPI as compared with pre-LFPI diversity (p<0.01, Adonis test)

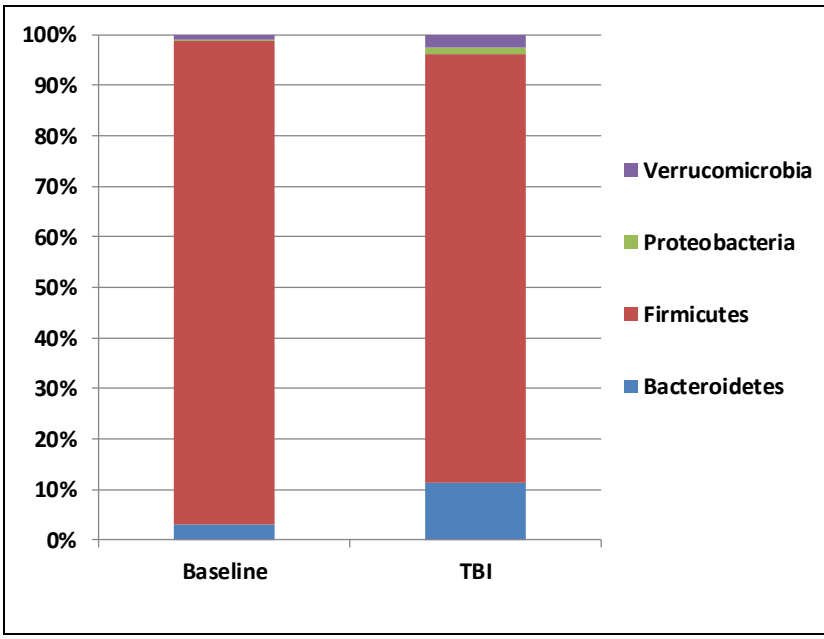

Fig. 4. Microbiota composition, phylum level. The graph compares microbiota composition between the baseline and 1 week post-LFPI time points. There was an increase in Bacteroidetes and reciprocal decrease in Firmucutes.

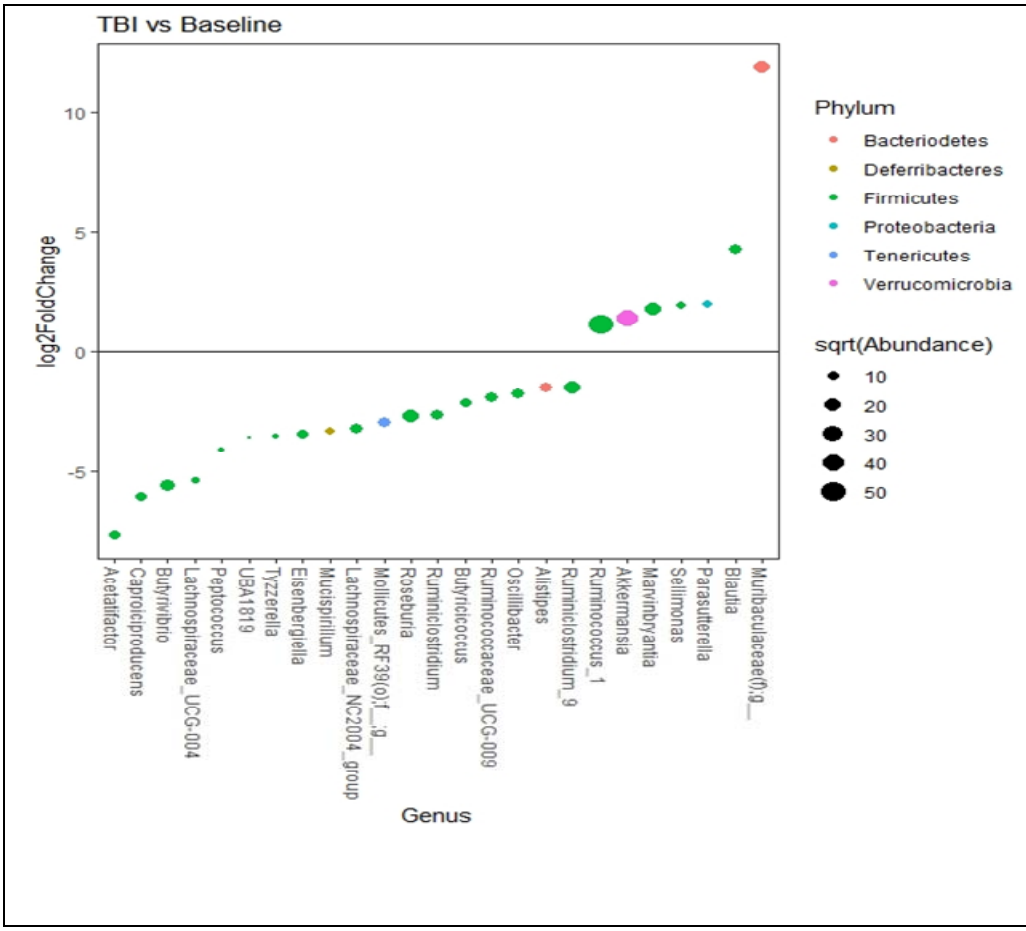

Fig. 5. Microbial composition, genus level. Only those microbes whose content changed significantly (either decreased or increased) are shown. There is specific decrease and increase in certain types of microbiota 1 week after LFPI, as compared with pre-LFPI levels, which are shown as 0 line.

Summary. There are at least early changes in microbiome composition after LFPI. Pathophysiological significance of these changes is not clear at this point. The interpretation

requires bioinformatics, to integrate data from other assays (seizures, markers of inflammation etc.), and will be done once all samples from all time points are analyzed.

2.3. Plasma cytokine levels (ELISA). Peripheral cytokines are markers of systemic inflammation, which, according ti my hypothesis, serves as a link between dysbiosis and PTE. Fig. 7 summarizes plasma levels on the three cytokines of interest, as analyzed up-to-date. (A) We observed significant decrease of IL-b 6 months after LFPI. This was an unexpected result, contrary to the expectations. (B) We observed significant increase in TNFa 6 months after LFPI, which is in line with the expectations, although I expected to see the change earlier. (C). We observed significant increase of plasma IL-17 at 1 week (as hypothesized), followed by the decrease (uncertain). IL-17 effects are complex and may be both damaging and protective. In either way, as IL-17 levels are strongly regulated by gut bacteria, changes in this cytokine confirm that dysbiosis is indeed triggered by brain trauma. Once all the data are collected and processed, they will be integrated in biostatistical analysis, to provide a conclusive interpretation of these changes.

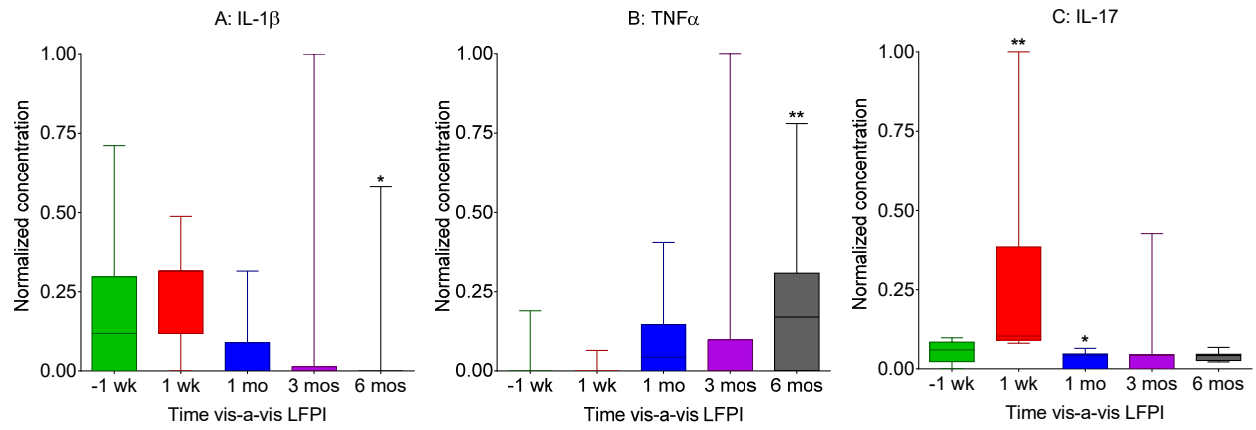

Fig. 6. Time-course of plasma cytokine levels vis-à-vis LFPI. Data are shown as box (Median/First quartile/Third quartile) and whiskers (Minimal to Maximal) for 19 LFPI rats at different time points vis-à-vis LFPI. Data normalized against the lowest cytokine level. *- $p<0.05$, **- $p<0.01$ in comparison with "-1 wk" (i.e. baseline; Friedman test with Dunn's *post hoc*).

We also compared IL-17 levels between 19 LFPI and 8 sham LFPI rats at 6-month time point, and found that in sham LFPI rats the level was lower than in 6-month LFPI subjects, and comparable to the baseline level in the latter (data not shown). Therefore, the changes observed in Fig. 6C appear to be a consequence of trauma, not of ageing.

2.4. Microglia activation (Iba1 immunochemistry). We observed significant over-expression of Iba1 1 month after LFPI in the area of the injury (Fig. 7, A, B), which is in line with our hypothesis on the involvement of central inflammation in PTE.

2.5. BBB permeability (fluorescein staining). BBB permeability may be assessed by analyzing fluorescein staining in the brain upon its i.v. administration, and is based on the fact that fluorescein does not penetrate intact BBB. Hence, fluorescein appearance in the brain after peripheral injection is a sign of BBB disruption. One month after LFPI, we detected fluorescein staining in the area of

the injury (Fig. 7D), as well as in the hippocampal alveus (Fig. 7C), which points to extensive BBB disruption, and is along the line of out hypothesis that BBB disruption may contribute to PTE. Microglia and BBB integrity analysis is being performed at other time points, as well as in control rats, and will be integrated into bioinformatics when completed. This will allow to connect these impairments to dysbiosis and to PTE.

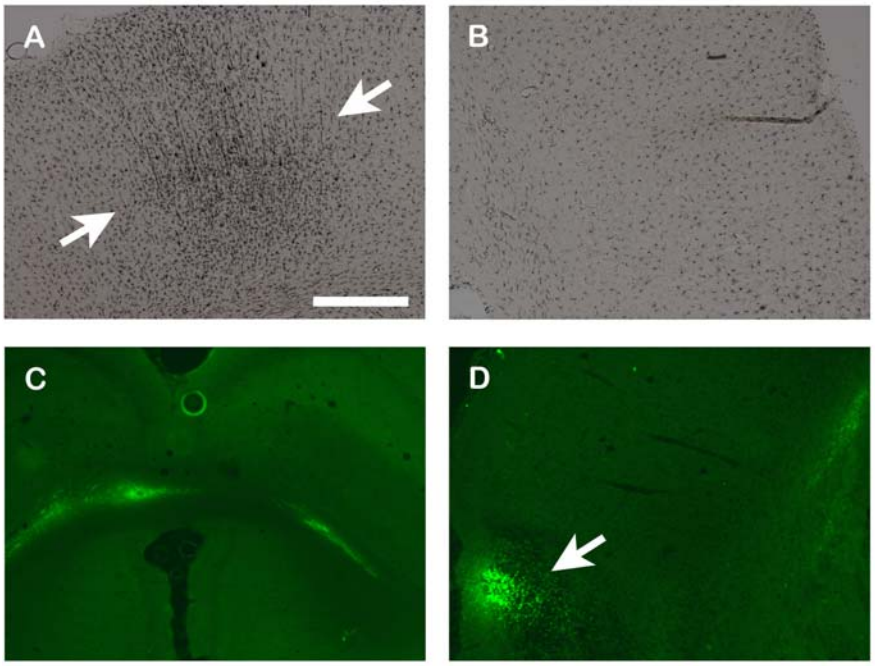

Fig. 7. Histology 1 month after LFPI. A, B- microglia staining (Iba1 antibody). A. In the area of the LFPI, there is the increased Iba1 expression (area between the arrows). B. Area under craniotomy from sham-LFPI (control) shows no such Iba1 over-expression. C,D.- Blood-brain barrier (BBB) permeability as measured by fluorescein accumulation in the brain after its systemic injection. C. – Bilateral fluorescein accumulation in the area of alveus hippocampi suggests BBB disruption distant from LFPI. D. Area of LFPI proper shows massive BBB breakdown. Scale bar is 500microns for A,B,D and 1100microns for C.

2.6. IB permeability.

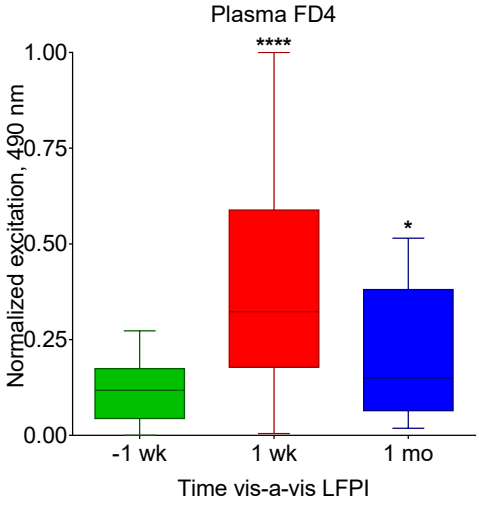

Fig. 8. Intestinal barrier (IB) permeability. IB permeability was assessed by measuring plasma levels of FD4 after its central administration. Data are shown as box (Median/First quartile/Third quartile) and whiskers (Minimal to Maximal). There was pronounced appearance of FD4 in plasma at both 1 week and 1 month after LFPI, which [points to the leaky gut. The excitation at 490 nm is normalized against the lowest values. *- $p<0.05$; ***- $p<0.0001$ as compared with the baseline ("- 1 wk"). Friedman test with Dunn's *post hoc*.

The integrity of IB can be assessed by measuring plasma concentration of fluorescein isothiocyanate-labelled dextran (FD4, MW=4 kDA) after its oral administration and is based on the fact that intact IB is impermeable to FD4. Hence, the appearance of FD4 in plasma after its oral injection points to the IB disruption. WE observed appearance of FD4 in plasma both at 1 week and at 1 month after LFPI (Fig. 8), which is the evidence of early and persistent IB disruption. This confirms our hypothesis that connects dysbiosis to peripheral inflammation via the disruption of IB (a.k.a "leaky gut").

Conclusions. So far, major hypotheses put forward in the proposal are being confirmed. Specifically, we observe perturbations in the gut microbiome composition after LFPI; signs of peripheral inflammation after LFPI (increased plasma TNFa and IL17 levels); disruption of tissue barriers (IB and BBB), and central inflammation (microglia activation). The incidence of PTE is within the expectations.

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.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

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.
Major task 2, which includes generating all animals of all groups, and analyzing all samples under Aim 1 is to be completed by month 19 of the project. During the next two quarters, we will be

completing generating all the experimental subjects of all groups, to meet Major Task 2 goals, collecting samples and analyzing the data. All these activities are prerequisites for moving into the Major Task 3 (starting from month 20). In addition, during the next quarter, we are planning to produce limited number of animals (10-12) for Major task 3. These will be recipients, which will receive FMT from donors (generated under Major Task 2) 1 month after LFPI. This would allow us to detect any possible technical problems and take steps towards their resolution and optimization.

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

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

While the data obtained up to date are promising, they are not complete to the extent, which would allow to judge on their potential impact on the principal discipline. Potential impact is in furthering understanding of mechanisms that underlie epilepsy following traumatic brain injury (TBI) with further prospects of new diagnostic and therapeutic tools. The latter is especially relevant to the Major Task 2 goals. The ability to use microbiome for early detection and risk stratification purposes could be of enormous benefit, especially considering high accessibility of microbiome samples and availability of well-developed techniques for its analysis. Our findings may ultimately lead to a development of a simple, quick, non-invasive early detection/risk stratification PTE assay based on the identified microbiome profiles. The assay could be performed at a critical time after TBI (or, possibly even before the exposure to conditions with increased risk to TBI, such as war zone) to promptly and cost-effectively identify patients at high PTE risk.

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.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

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

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.
Problems. While generating of experimental subjects and sample collection have been on schedule, due to the COVID-19 pandemic, we have encountered significant delays in sample assays and subsequent analysis. Between March 19 and June 8, only essential experiments were allowed, and this did not include running *ex vivo* assays. Effective June 15, these activities resumed, but there are still delays due to the personnel and equipment capacities and limitations both in the PI's lab and in core facilities, specifically the Microbiome Core, where microbiome samples are analyzed. Furthermore, these delays also hinder data integration via bioinformatics, whereby all the data should be loaded into a single database, and connections between various outcome measures should be established.
Another problem is that we were not able to present research-ready data at scientific meetings, which were cancelled due to the pandemic (this was planned for the Society for Neuroscience in November and American Epilepsy Society in December).
Actions. With regard to out laboratory, we have been working overtime to run the assays of existing samples (ELISA, immunohistochemistry), to implant animals for and to record EEGs. With regard to the Microbiome Core, their personnel is working on the backlog of sample. We have 7 more

months to complete Major task 2, and since generating animals and sample collection is on schedule, we are hoping that we will be able complete Major Task 2 (month 19) with either no, or minimal delays. Furthermore, some (although not all) experiments under Major Task 3/Aim 2 can proceed without completing Task 2. Therefore, if necessary, we should be able to start Major Task 3 studies without any delays, and to complete data analysis and bioinformatics under Major Task 2 while Major Task 3 is already under way.

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.

None

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
Not applicable

Significant changes in use or care of vertebrate animals
None

Significant changes in use of biohazards and/or select agents
None

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

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

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

  Nothing to report

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

Nothing to report

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

Nothing to report. We were planning on submitting abstracts and to present at the annual meeting of the Society for Neuroscience (November 2020) and American Epilepsy Society (December 2020), but both meetings were cancelled due to the pandemic.

- **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.

  Nothing to report

- **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.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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

Name	Andrey Mazarati
Project role	Principal Investigator
ORCID ID	0000-0001-9693-5297
Nearest person months worked	1.2
Contribution to the project	Project oversight. Interaction with collaborators. EEG monitoring and analysis. LFPI surgery. Care of animals under seizure monitoring. Seizure, immunochemistry and ELISA analysis. Coordination of activities of the personnel, data integration and quality control.
Funding support	DoD ERP Grant W81XWH-18-1-0612 (Galanopoulou, PI)- 1.8 months. Harinarayan Epilepsy Research Fund, Dept. of Pediatrics, UCLA- 9 months.

Name	Jesus-Servando Medel-Matus
Project role	Project Scientist
ORCID ID	0000-0003-1103-468X
Nearest person months worked	6.0

Contribution to the project	LFPI surgery. Microbiome and blood sample collection. Animal surgery for EEG seizure monitoring. Fluorescein and FD4 injections. Brain processing for immunohistochemistry. Animal care in the study area.
Funding support	Harinarayan Epilepsy Research Fund, Dept. of Pediatrics, UCLA- 6 months.

Name	Don Shin
Project role	Staff Research Associate
ORCID ID	n/a
Nearest person months worked	3.5
Contribution to the project	Animal for immunohistochemistry (perfusion, brain harvesting, cutting, staining). ELISA of plasma cytokines. Blood and microbiome sample collection. Immunohistochemistry (Iba1, fluorescein). Animal care in the study area. Supply orders. Compliance with UCLA rules and regulatiosn.
Funding support	DoD ERP Grant W81XWH-18-1-0612 (Galanopoulou, PI)- 3.84 months. Harinarayan Epilepsy Research Fund, Dept. of Pediatrics, UCLA- 4.66 months.

Name	Venu Lagishetty
Project role	Staff Research Associate
ORCID ID	0000-0001-6500-8255
Nearest person months worked	1.2
Contribution to the project	Microbiome assay and analysis.
Funding support	Crohn's and Colitis Foundation Research Initiatives Award 563138 (PI: Mayer), 1.2 months. VA CDAII IK2CX001717 (PI: Jacobs), 3 months.

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.

No changes

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

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

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

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

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

Not applicable

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

None