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TITLE: **Brainwide Social Network in Mice Underlying Autism Spectrum Disorder**

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CONTRACTING ORGANIZATION: Hebrew University of Jerusalem

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Ariel Gilad

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14. ABSTRACT:

Social interactions are a crucial part of our human identity and its dysfunction is a core symptom in autism spectrum disorder (ASD). However, the neuronal circuits and their causal underpinnings remain unclear, displaying inconsistent results across studies, partially due to the large heterogeneity of ASD. It is thought that ASD individuals have an underdeveloped Theory of Mind (ToM), unable to grasp the mental states of others, leading to social impairment. Human imaging studies have outlined several key brain areas that are socially-related. Nevertheless, these studies mostly discard individual differences and fail to capture fast network dynamics during real-time and natural social interactions. Alternatively, animal models enable neuronal recordings from freely moving individuals, but usually focus on one or two areas, lacking a brain-wide measurement. Furthermore, similar to human studies, mice are rarely studied at the individual level. To address these limitations, we have developed a multi-fiber method that enables simultaneous recording from dozens of brain areas of freely behaving mice. The proposal studies the personal brain-wide social networks dysfunctions in ASD.

15. SUBJECT TERMS

NONE LISTED

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

Social interactions are a crucial part of our human identity and its dysfunction is a core symptom in autism spectrum disorder (ASD). However, the neuronal circuits and their causal underpinnings remain unclear, displaying inconsistent results across studies, partially due to the large heterogeneity of ASD. It is thought that ASD individuals have an underdeveloped Theory of Mind (ToM), unable to grasp the mental states of others, leading to social impairment. Human imaging studies have outlined several key brain areas that are socially-related. Nevertheless, these studies mostly discard individual differences and fail to capture fast network dynamics during real-time and natural social interactions. Alternatively, animal models enable neuronal recordings from freely moving individuals, but usually focus on one or two areas, lacking a brain-wide measurement. Furthermore, similar to human studies, mice are rarely studied at the individual level. To address these limitations, we have developed a multi-fiber method that enables simultaneous recording from dozens of brain areas of freely behaving mice. The proposal studies the personal brain-wide social networks dysfunctions in ASD.

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

Social interactions, Brain-wide networks, Multi-fiber photometry, SHANK3 mutant mice, Theory of Mind, freely moving mice

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.

Specific Aim 1: To understand the individual personality differences in ASD mice under natural conditions
Major Task 1 Establishment, breeding and behavioral monitoring of ASD (SHANK3 mutation) and wildtype (WT) mice
Major Task 2 Computational framework to map the personality of each mouse

Specific Aim 2: Obtain a brain-wide social network in ASD and WT mice
Major Task 3: Multi-fiber experiments during social interactions

Specific Aim 3: To manipulate the brain-wide network of ASD mice in an attempt to rescue normal social behavior
Major Task 4: Optogenetic experiments.

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.

Major Task 1 We have completed Major task 1.
Subtask 1 – IACUC and ACURO Review and Approval.
Approved by the Animal Care and Use Review Office on March 17th 2023. Completed 100%.

Subtask 2 – Establishing a pipeline that produces groups of 4-6 mice, ASD and WT. We now have a running pipeline between the Amal and Gilad lab.
Specifically, Doron Michaely, a Master student at Amal's lab, is responsible for breeding and genotyping of the ASD mutant lineThe Amal labs has already produced 4 groups of ASD mice (6 mice on average in each group) and we are planned to produce a new group every 4-6 weeks, based on demand. Subtask 2 is completed 100%.

Details:
Group 1 - March 2023. 5 Shank3 mice. 4 underwent successful surgery
Group 2 - June 2023. 6 Shank3 mice. 5 underwent successful surgery
Group 3 - June 2023. 3 Shank3 mice and 3 WT mixed. 5 underwent successful surgery
Group 4 - August 2023. 4 Shank3 mice and 3 WT mixed.
Group 5 - August 2023. 4 Shank3 mice and 3 WT mixed.

Subtask 3 – Each ASD and WT mouse will undergo a set of behavioral tests that are commonly used in the ASD field.
Doron (Master student from the Amal lab) ran the different behavioral tests on all mice that have been transferred to the Gilad lab. Subtask 3 is completed 100%.

Details:

We present behavioral results for group 3 which has 3 Shank 3 (KO) and 3 WT mice:

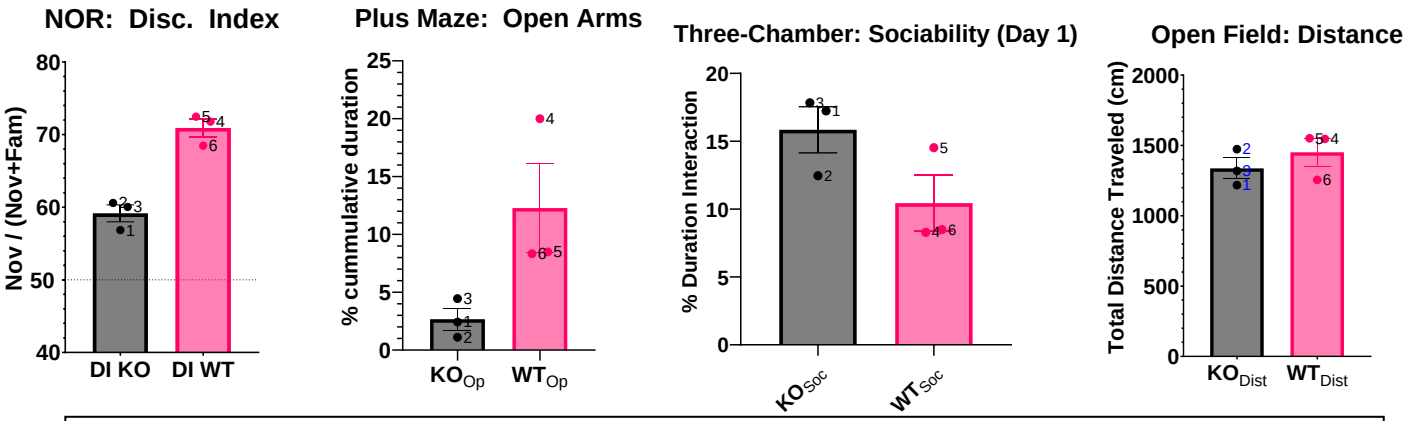

Figure legend: from left to right
- Novel object test. Disc index for behavioral bias to the novel object for Shank 3 (black) and WT (pink) mice
- Plus maze. Percentage of time spent in the open arms
- 3-chamber social test. Duration of interaction with the stranger mouse.
- Open field test. Total distance travelled

We are now analyzing the behavioral results for Groups 4 and 5. Groups 1 and 2 have only Shank3 mice and will be compared to WT later on.

Subtask 4 is completed 100% (for the groups that were transferred to the Gilad lab).
All the groups of mice that were transferred to the Gilad lab underwent monitoring in the social box. Each group was monitored for 5 consecutive days and individual tracking was performed using DeepLabCut. Preliminary results show that tracking of individual mice is reliable and we are able to quantify the position of all mice during a whole recording session.

Details:
We present preliminary monitoring and tracking from the social box for Group 2 and a WT group. In general, we observe that Shank3 mice tend to be less mobile, less social and tend to stay isolated in one corner of the box.

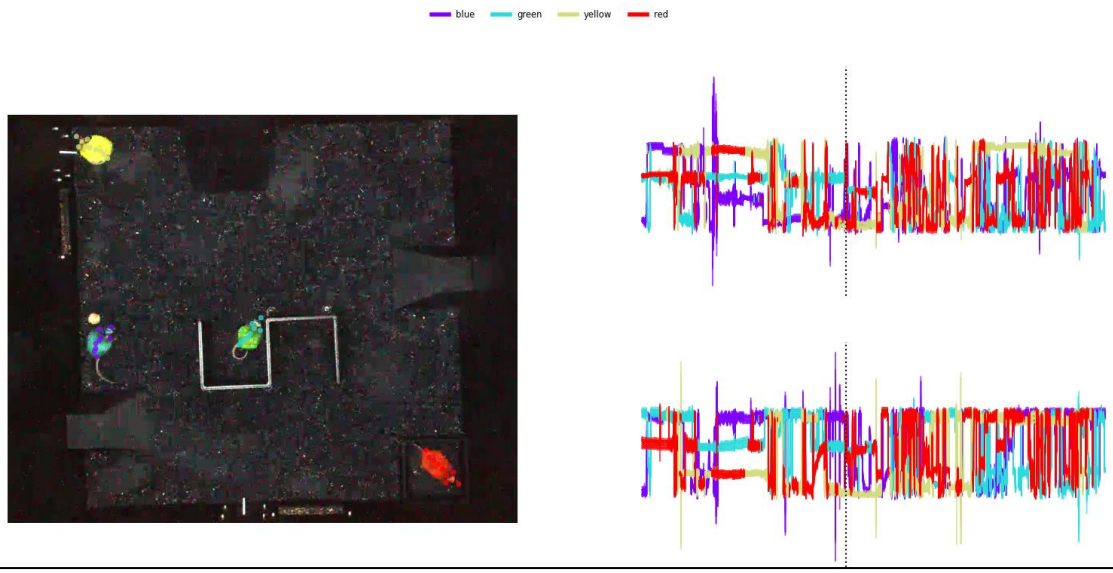

Figure legend. Monitoring and tracking in social box
Left: Snap shot of four Shank3 mice in the social box along with automatic tracking point.
Right: x and y positions of each mouse over the course of 12 hours.

Major task 2 Computational framework to map the personality of each mouse.
Subtask 1 – Automatic tacking of behavioral traits. This task is around 75% complete. We have fully and automatically tracked groups 1 and 2 and we are now tracking group 3. Groups 4 and 5 have not yet been put in the social box. Please see above example for tracking examples in the social box
Subtask 2 – A linear discriminant analysis (LDA) to map personality for each mouse. This is 10% complete. We have started to analyze the track data and extract 60 behavioral traits for each mouse. This task is expected to be complete within the next 6 months.

Major Task 3: Multi-fiber experiments during social interactions
Subtask 1 – Multi-fiber surgeries and experiments.
This task is around 50% complete. We have performed surgeries and recoded brain-wide dynamics from 12 Shank 3 mice and 3 WT mice. We have finished experiments in groups 1 and 2 and we are currently finishing group 3. Groups 4 and 5 will undergo surgeries within the next month. We expect to finish behavioral We have already imaged the first group of ASD mice during social interactions (n=4). Preliminary results show promising behavioral and neuronal differences between ASD and WT mice. We expect to start groups 2 and 3 within the next 2 months. Expected to finish on time.

Details: We show example traces of social interactions between pairs in group 1 - Shank3 mice (n=4).

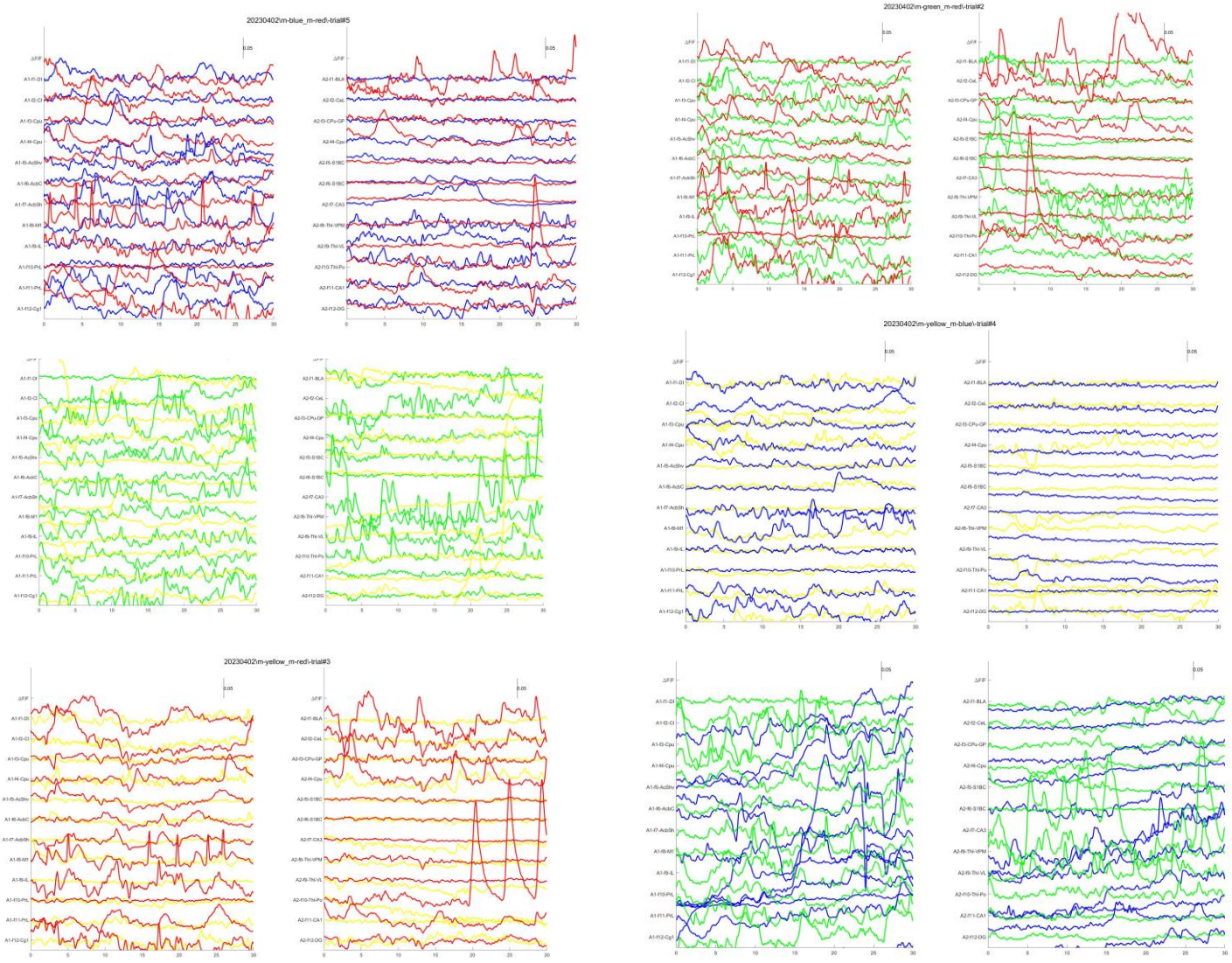

Figure legend. Example traces in 24 brain areas from different pairs of Shank3 mice within group 1. Each color is a different mouse (n=4)

Subtask 2 – Calculate a brain-wide social network for each mouse and each type of social interaction.

This task is 10% complete. We have started developing a network analysis to outline important brain areas within and between mice underlying social interactions. We have already analyzed a group of WT mice and are currently implementing the network analysis to the Shank 3 mice. We expect to finish the network analysis within the next 10 months.

Details:
We present correlation network analysis between and within WT mice during social interactions

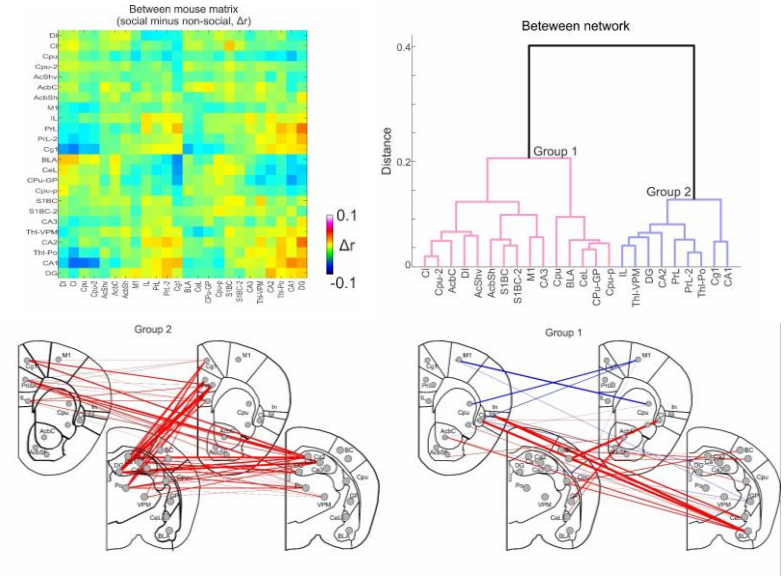

Figure legend. Network analysis between WT mice.
Left up: Average correlation matrix between two mice during social interaction (normalized to non-contact; n=15 pairs).
Right up: Dendrogram of the correlation matrix divides into two subnetworks (groups)
Bottom: The two subnetworks superimposed on the two brains.

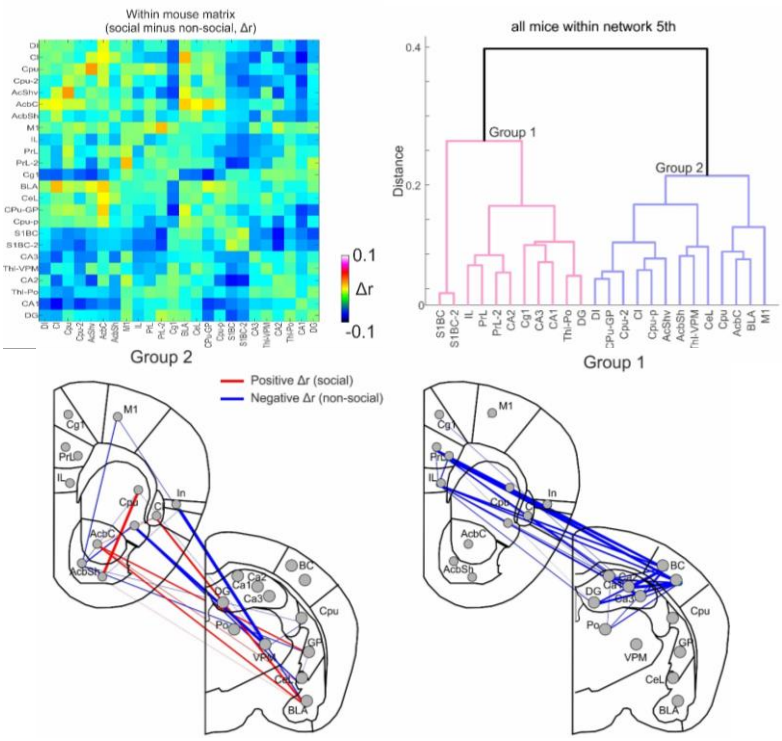

Figure legend. Network analysis within WT mice.
Left up: Average correlation matrix within each mouse during social interaction (normalized to non-contact; n=9 individuals).
Right up: Dendrogram of the correlation matrix divides into two subnetworks (groups)
Bottom: The two subnetworks superimposed on the brain.

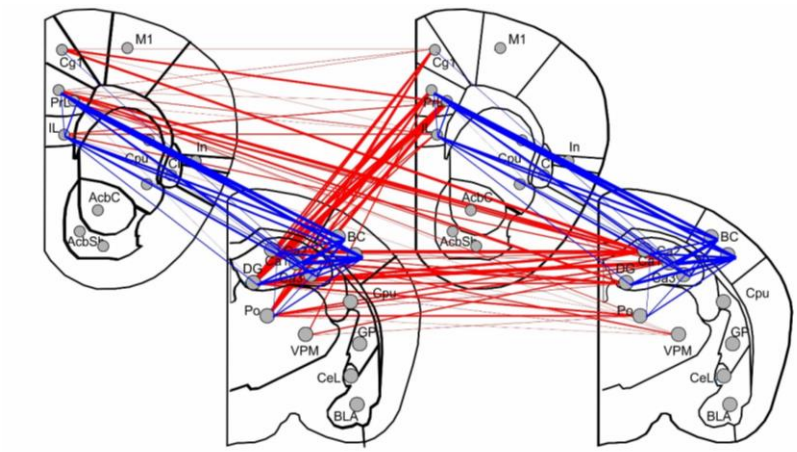

Figure legend: Total network during social interactions.
Superposition of two subgroups from the within and between networks from the above figure. Red and blue colors indicate an increase and decrease in correlation during social interaction respectively.

Major Task 4: Optogenetic experiments. This part is 0% complete. We plan to start with this section by the end of 2023.

Subtask 1 – Perform optogenetic experiment in each ASD mouse**.** 0% complete
Subtask 2 – Mice will be imaged and manipulated during the standard behavioral tests described in major task. 0% complete

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.

The Gilad lab has managed to recruit a Master student, Renana Terner, to work on the project along with Dr. Odeya Marmor. Renana has taken MATLAB a courses and also relevant neuroscience courses as part of her training.

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

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

We are now working on major task 2, subtask 2, aiming to finish within the next 6 months. We aim to continue on Major task 3, which is to continue with social experiments with multi-fiber implants. We aim to obtain a full dataset by the end of the upcoming reporting period. We also aim to start with Major task 4 (optogenetics experiments) during the beginning of 2024. We will continue with data analysis to further consolidate the results. We also plan to present this project at the upcoming SFN meeting in the ISFN, during 14-16 of January in Israel.

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

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

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

Nothing to report

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.

No anticipated delays or problems at this time

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

Nothing to report

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

Nothing to report

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

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

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

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

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

Nothing to report

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

Nothing to report

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

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	*Odeya Marmor*
Project Role:	*Research Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Preforming surgeries, social experiments and data analysis*
Funding Support:	

Name:	*Renana Terner*
Project Role:	*Master student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Preforming surgeries, social box and behavioral experiments, social experiments*
Funding Support:	*Excellence scholarship for Master students*

Name:	*Doron Michaeli*
Project Role:	*Master student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Preforming genotyping and behavioral experiments in the Amal lab.*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Nothing to report

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

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

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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: N/A**

   COLLABORATIVE AWARDS: *N/A*

   QUAD CHARTS: *N/A*

9. **APPENDICES:** *N/A*