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PRINCIPAL INVESTIGATOR: Dr. Eun Young Choi, PhD

CONTRACTING ORGANIZATION: The Leland Stanford Junior University

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14. ABSTRACT

This grant aims to understand how the circuitry associated with the central lateral thalamic nucleus (CL) plays a role in enhancing the risk for Alzheimer's disease (AD) following a traumatic brain injury (TBI). Animal connectivity work shows that CL has strong connections with the rostral anterior cingulate cortex (rACC), which is heavily involved in both attention and memory. We hypothesize that the downregulation of CL in TBI leads to decreased activity in the rACC, which in turn downregulates the memory system and enhances the risk for AD. This will be tested by quantifying the connectivity strength between CL and rACC in resting-state fMRI and DTI neuroimaging data from Healthy Control versus TBI versus AD subjects from the ADNI and DoD-ADNI databases. We will then examine whether the CL-rACC connectivity strength inversely correlates with markers of AD (performance on the Logical Memory II test, global PET-amyloid burden, and amyloid and tau levels in the cerebrospinal fluid). In Year 2, we have finished the remaining Year 1 task (Major Task 1 Subtask 2) of defining subject-specific CL ROIs. We now have a library of all neuroimaging data and CL, rACC, and parahippocampal cortex (PHC) ROI definitions for all subjects. Major Task 2 Subtask 1 is complete, and Subtask 2 is in progress. The main accomplishment in Year 2 is establishing a working rsfMRI pipeline and generating the CL rsfMRI map for the TBI group. This map shows the expected CL connections with the dorsomedial PFC and striatum. Interestingly, it shows weak correlations with the rACC, consistent with our hypothesis. The next steps will generate CL rsfMRI maps for the Healthy Control and AD groups, and quantitatively and spatially compare them with that of the TBI group.

15. SUBJECT TERMS

Amyloid beta, Alzheimer's disease, central lateral thalamic nucleus, rostral anterior cingulate cortex, diffusion tensor imaging, positron emission tomography, parahippocampal cortex, phosphorylated tau, resting-state functional connectivity MRI, traumatic brain injury

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1. Introduction

The purpose of this grant is to understand the circuitry underlying how sustaining a traumatic brain injury (TBI) leads to enhanced risk for Alzheimer's disease (AD). The central lateral thalamic nucleus (CL) is a key region in the arousal system of the brain that has broad connections throughout the brain. The mesocircuit hypothesis posits that it is through these broad connections that TBIs lead to the common downregulation of CL and subsequent downregulation of connected brain regions. One of CL's strongly connected brain regions is the rostral anterior cingulate cortex (rACC), which is heavily involved in both attention and memory. Animal connectivity work shows that both CL and the parahippocampal cortex (PHC) of the memory system have strong connections with the rACC. Supporting this connectivity, CL stimulation in rodents and a patient showed memory effects: behavioral improvements in memory and increased gene and protein expression changes in the hippocampus. Using our recently developed technology that accurately identifies CL in human anatomical MRI scans, we will map CL's connections to the rACC in TBI subjects, measure its connectivity strengths, and measure its correlation with markers of AD (performance on the Logical Memory II test, global amyloid burden as measured by position emission tomography (PET), and levels of amyloid and tau protein in the cerebrospinal fluid). We hypothesize that the downregulation of CL in TBI leads to decreased connectivity with the rACC, and the enhanced risk of developing AD as assessed by increased levels of AD markers. Results will be compared with those from Healthy Control and AD cohorts: we hypothesize that CL-rACC connectivity will be greater in Healthy Control and AD subjects than TBI subjects, and PHC-rACC connectivity will be weaker in the AD subjects (as part of the AD pathology) than Healthy Control and TBI subjects.

2. Keywords

Abeta—amyloid beta
AD—Alzheimer's disease
CL—central lateral thalamic nucleus
rACC—rostral anterior cingulate cortex
dmPFC—dorsomedial prefrontal cortex
DTI—diffusion tensor imaging
PCC/Rsp—posterior cingulate cortex/retrosplenial cortex
PET—positron emission tomography
PHC—parahippocampal cortex
p-Tau—phosphorylated tau
rsfMRI—resting-state functional connectivity MRI
TBI—traumatic brain injury

3. Accomplishments

What were the major goals of the project?

Year	Specific Aim	Major Task	Subtask	Months (per SOW)	% Completed
1	Specific Aim 1: Map the connectivity of CL and PHC in TBI, AD, and Healthy Control groups.	Major Task 1: Obtain all relevant data from databases and define ROIs in structural MRIs in preparation for analyses.	Subtask 1. Goal: Identify TBI and Healthy Controls from the DoD-ADNI database. Identify matched AD patients from the ADNI database. Obtain all the relevant data needed for this grant: structural MRI, rsfMRI, DTI, PET-amyloid imaging, cognitive measures, and CSF biomarker levels. The DoD-ADNI and ADNI databases are publicly available and deidentified of direct identifiers, but retain some indirect identifiers (e.g., birth date, gender, race, ethnicity) that are needed for	1-8	100

			analysis.		
1			Subtask 2. Goal: Define all ROIs for all subjects. All ROIs except CL will be defined by passing the T1 structural images through FreeSurfer. CL will be defined using a modified thalamic segmentation method appropriate for the T1 structural image.	9-10	100
1	PI's Maternity Leave			11-13	100
2		Major Task 2: Map the connections of CL and PHC to the cortex using rsfMRI. Quantify connectivity strength between CL and PHC to rACC.	Subtask 1. Goal: Preprocess all subject rsfMRI data using the CONN toolbox.	14-15	100%
2			Subtask 2. Goal: Run rsfMRI analyses using the CL or PHC ROIs and the cerebral cortex for each subject using the CONN toolbox. Create group correlation maps for each of the TBI, AD, and Healthy Control groups. Quantify the correlation strength between CL or PHC and the rACC for individual subjects and the groups.	16-17	33%
2			Subtask 3. Goal: Conduct a comparative spatial analysis of the maps across TBI, AD, and Healthy Control groups using Matlab. Compare correlation strength between CL or PHC and rACC across groups.	18	0%
2		Major Task 3 Map the connections of CL and PHC to the cortex using DTI. Quantify connectivity strength between CL and PHC to rACC.	Subtask 1. Goal: Preprocess all subjects' DTI data using FSL and NiftyReg.	19-20	0
2			Subtask 2. Goal: Run DTI analyses using the CL or PHC ROIs with the cerebral cortex for each subject using FSL. Create group connectivity maps for each of the TBI, AD, and Healthy Control groups. Quantify the connectivity strength between CL or PHC and the rACC for individual subjects and the groups.	21-22	0
2			Subtask 3. Goal: Conduct a comparative spatial analysis of the maps across TBI, AD, and Healthy Control groups using Matlab. Compare connectivity strength between CL or PHC and rACC	23-24	0

			across groups.		
3			Subtask 4. Goal: Write and submit publication #1 on Specific Aim 1 work.	25-26	0
3	Specific Aim 2: Correlate the connectivity strength between CL or PHC and rACC with AD markers.	Major Task 4 Obtain global PET-amyloid burden measures from all subjects.	Subtask 1. Goal: Preprocess all subject PET-amyloid data using SPM12.	27-28	0
3			Subtask 2. Goal: Obtain global amyloid burden for all subjects using Matlab.	29	0
3		Major Task 5 Test the hypothesis that CL's connections to the rACC are correlated with an increase in markers of AD in TBI.			0
3			Subtask 1. Goal: Examine association of connectivity strength metrics with markers of AD obtained from Major Tasks 1 and 4 (global amyloid burden, memory performance on the Logical Memory II, CSF Abeta42/Abeta40, and CSF p-Tau levels) with a simple linear correlation analysis using SPSS.	30-31	0
3			Subtask 2. Examine association of connectivity strength metrics with markers of AD with multiple linear regression using SPSS.	31-32	0
3			Subtask 3. Goal: Write and submit publication #2 on Specific Aim 2 work.	33-34	0
3			Subtask 4. Goal: Write and submit publication #3 on Specific Aim 2 work.	35-36	0

What was accomplished under these goals?

Per the Statement of Work (revised 10/18/2021), Year 1's tasks are (Major Task 1, Subtask 1) to download all relevant data from the ADNI and DoD-ADNI databases, and (Major Task 1, Subtask 2) to define all regions of interest (ROIs) in all subjects. Year 2's tasks are (Major Task 2, Subtask 1) to preprocess the data for rsfMRI analysis; (Major Task 2, Subtask 2) to conduct the rsfMRI analysis and quantify the correlation strengths between CL-rACC and PHC-rACC for all groups; and (Major Task 2, Subtask 3) to compare these correlation strengths and spatial maps across the TBI, AD, and Healthy Control groups.

Major Task 1, Subtask 1 was completed in Year 1: data have been identified and downloaded from the ADNI and DoD-ADNI databases onto a workstation for processing and analysis.

Major Task 1, Subtask 2 was partially completed in Year 1, and finished in Year 2. Subject-specific PHC and rACC ROIs have now been defined from the structural MRI scans for all Healthy Control, TBI, and AD subjects using FreeSurfer software (Fig. 1, Fig. 2B-C). Subject-specific CL ROIs were defined using a modified THOMAS thalamic segmentation method that allows for CL to be segmented using the T1-weighted structural MRI scans in the database (Fig. 2A). CL ROIs were further modified to match the spatial resolution of the rsfMRI scans in order to perform rsfMRI analysis.

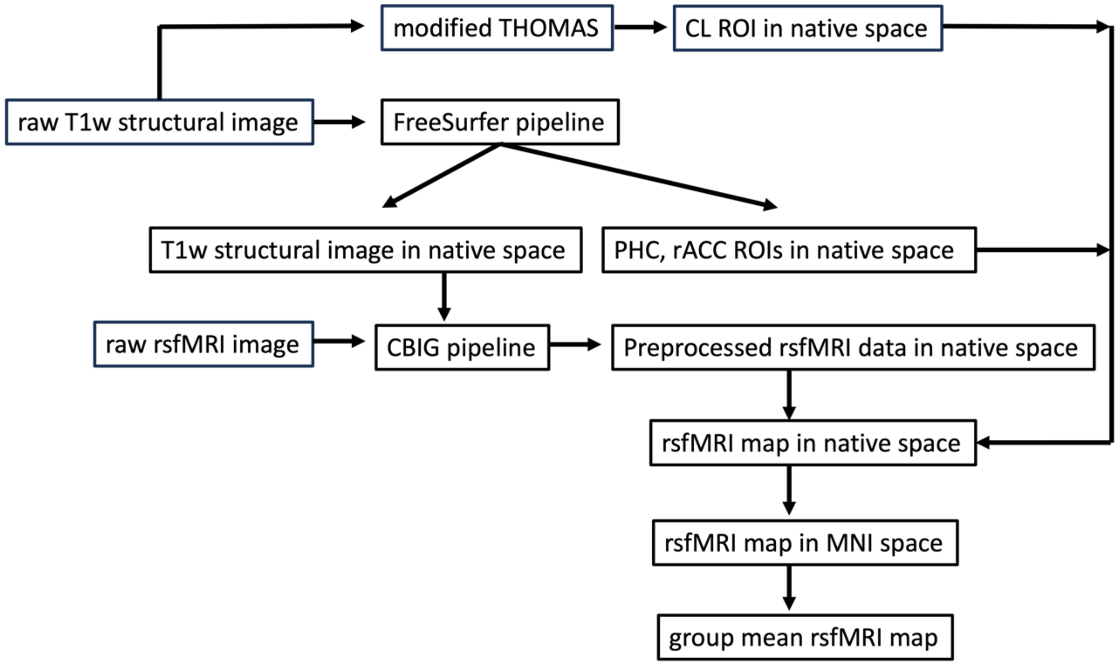

Figure 1. Full processing pipeline.

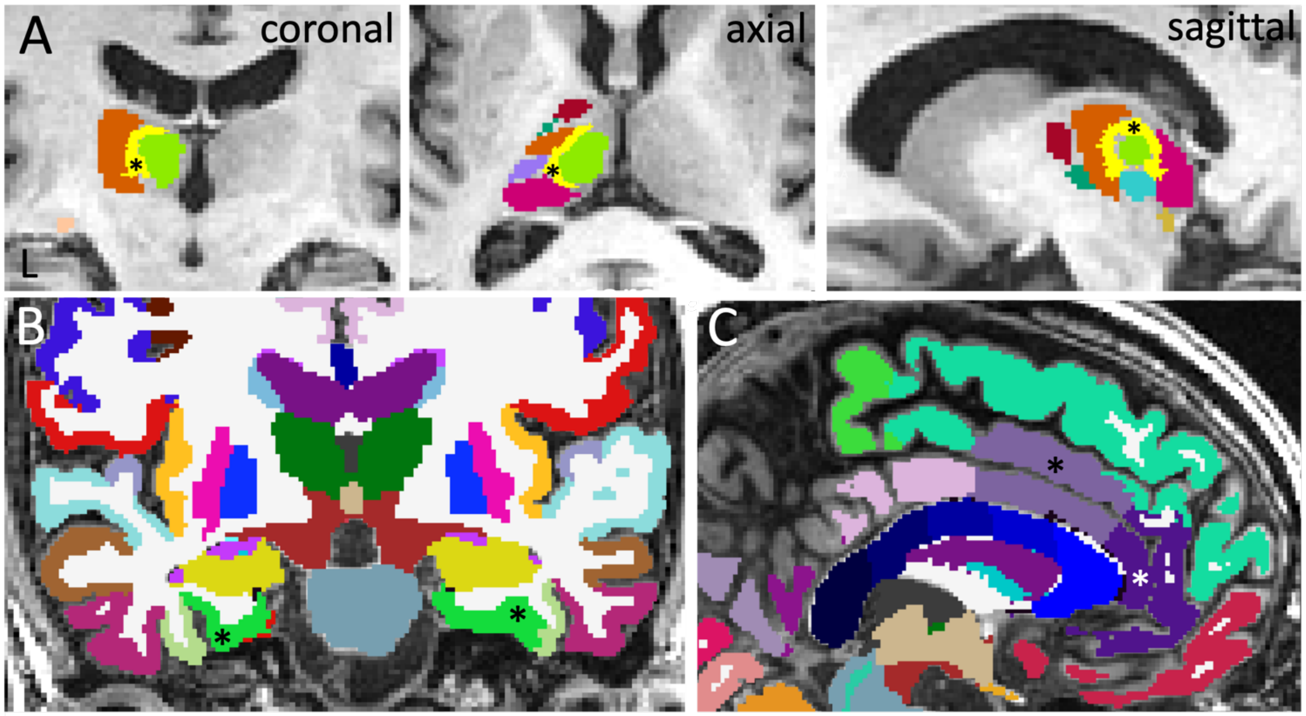

Figure 2. CL, PHC, and rACC ROIs defined in an example TBI subject.
(A) CL was obtained using a modified THOMAS thalamic segmentation method. CL is shown in yellow and denoted with an asterisk. Adjacent thalamic nuclei identified by THOMAS are also shown to demonstrate the accuracy of the THOMAS method. (B) PHC (green, asterisked) and (C) rACC were defined using the Desikan-Killiany atlas in FreeSurfer. Note: rACC is defined as the combination of

segments #1002 (light purple, asterisked) and #1026 (dark purple, asterisked) from the Desikan-Killiany atlas that together covers the rostral half of the ACC.

Major Task 2, Subtask 1 is complete: a CL rsfMRI preprocessing pipeline was built (Fig. 1) (challenges described below), and processing for the TBI, Healthy Control, and AD subjects is complete. Subtask 2 is in progress: CL rsfMRI maps have been created for the TBI group (Fig. 3), but not yet for the Healthy Control and AD groups.

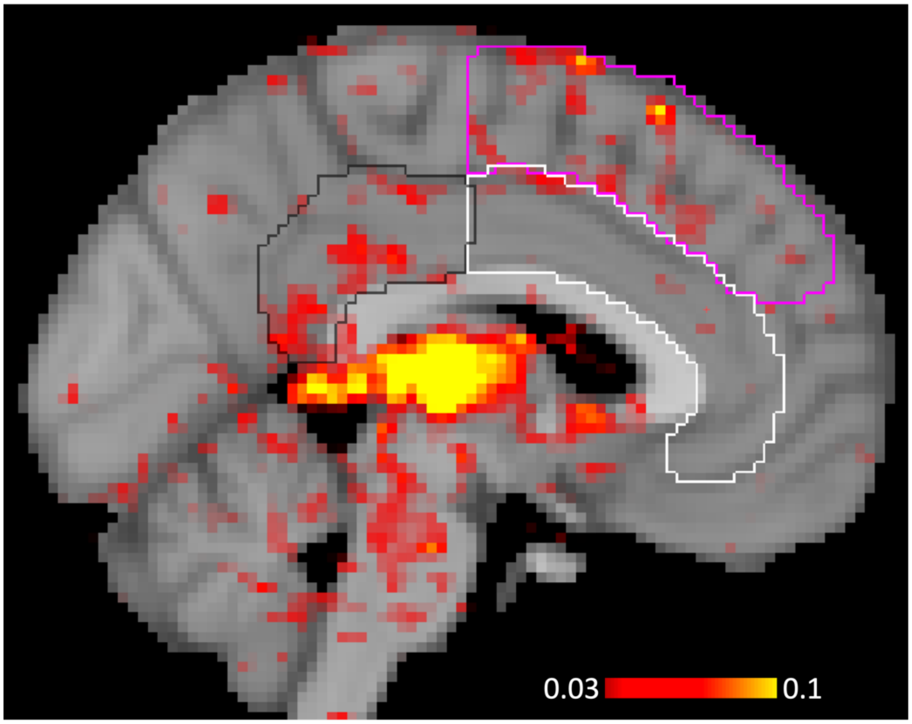

Figure 3. CL rsfMRI map for the TBI group (N=23) overlaid on an average MNI atlas brain. Cortical regions are outlined in the following colors: rACC (white), dmPFC (pink), and PCC/Rsp (black). Note: the hotspot of strong correlation in the thalamus is the typically observed strong correlations between the seed region and adjacent voxels, which is due to partial volume effects and/or intrinsic connectivity between the seed region and adjacent regions; these correlations are not interpreted here.

The CL rsfMRI map for the TBI group shows the expected correlations between CL and dorsomedial prefrontal cortex (dmPFC) (Fig. 2, pink outline) and striatum (not shown) based on monkey anatomical tract-tracing. Correlations were also observed in the posterior cingulate cortex and retrosplenial cortex (PCC/Rsp) (Fig. 2, black outline), also consistent with monkey anatomical tract-tracing. Notably, there were weak or no correlations between CL and the rACC (Fig. 2, white outline), which is consistent with our hypothesis that CL-rACC connectivity is decreased in TBI subjects.

The main problems in Year 2 have been technical challenges in building a full pipeline to perform rsfMRI analysis of small nucleus such as CL, which required additional time to try different tools and find solutions. The major challenges and solutions are detailed as follows.

(1) The T1-weighted structural images from which the CL ROI is derived (as output from the THOMAS algorithm), and therefore also the CL ROI, is at a smaller voxel resolution than the rsfMRI images. Multiple approaches were tried to solve this problem, including transforming the CL ROI to rsfMRI space or upsampling the rsfMRI map to the CL ROI resolution. The final and best solution was to spatially enlarge the CL ROI by one voxel, nonlinearly warp the ROI to rsfMRI space, and downsample the ROI to the rsfMRI voxel resolution.

(2) The CONN toolbox was proposed and initially used to perform the rsfMRI preprocessing and analysis. However, since it is a package with a graphical user interface (GUI), it was difficult to make modifications to the pipeline to tailor processing steps, such as altering the

structural to functional registration method, altering the white matter nuisance regression mask, and transforming the CL ROI to the subject or template MNI atlas space. After trying multiple ways to circumvent these issues, Dr. Choi decided to try another toolbox, the CBIG toolbox that was based on code that Dr. Choi had used in graduate school and has since been updated to include improved denoising steps. The CBIG toolbox is also command-line based, which made it easier to make modifications to steps within the scripts. All rsfMRI data were preprocessed with the CBIG toolbox.

(3) The CBIG toolbox is focused on rsfMRI of the cerebral cortex (modeled as a surface using FreeSurfer). It did not have a script for a ROI-based volumetric rsfMRI analysis. Dr. Choi wrote and tested a new script to do this.

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

Training: During Year 1, Dr. Choi has received one-on-one weekly training with Dr. Nicholas Schiff (Mentor) to enhance Dr. Choi's knowledge of CL's connectivity. Dr. Choi has also received one-on-one weekly training with Dr. Brian Rutt (Collaborator) to utilize the modified THOMAS thalamic segmentation method to define CL, as well as to build a rsfMRI preprocessing and analysis pipeline that is adapted to the technical challenges of conducting rsfMRI analysis with a small thalamic nucleus.

Professional development: Dr. Choi did not participate in formal professional development activities. However, she received one-on-one professional development guidance from Drs. Schiff and Rutt in their weekly meetings. Dr. Choi has received guidance on her next grant applications and potential faculty job applications, as well as networked with other faculty to gain further knowledge and techniques related to the project and her professional development.

How were the results disseminated to communities of interest?

Nothing to Report.

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

Dr. Choi will complete the remainder of the tasks from Year 2: generate CL and PHC rsfMRI maps for all three groups, and quantitatively and spatially compare these maps across the three groups. Given that the pipeline has been built, we anticipate that this will take 1 or 2 months. Dr. Choi will then work on the Year 3 tasks (Major Task 3): build a CL and PHC DTI pipeline, generate CL and PHC DTI maps for the three groups, and analyze these maps across the three groups. When these tasks are completed, she will commence work on the remaining Major Tasks 4 and 5.

Dr. Choi will also continue to receive scientific and professional training from Drs. Nicholas Schiff, Brian Rutt, and her mentoring team, and participate in training and professional development opportunities as they arise. This will include planning for Dr. Choi's next papers and grants based on this work, and presenting this work at conferences.

4. Impact

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

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. Changes/Problems

Changes in approach and reasons for change

There have not been any changes in approach.

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

Firstly, as processing progressed, we found that not all the subjects in each group had T1w structural and rsfMRI images, or the existing images were not of sufficient quality for the pipelines to work. For the TBI group, we started with 51 subjects downloaded from the DoD-ADNI database and ended with 23 subjects whose data contributed to the group rsfMRI map. After generating the rsfMRI maps for the Control and AD groups, we will assess whether the differences between the groups are statistically significant or whether we need to search for additional subjects (i.e., the FITBIR database).

Secondly, CL is a thin (~2mm width) nucleus, which is smaller than the ~3mm isotropic voxel size of the rsfMRI images. This leads to partial volume effects in which the signal from adjacent structures is mixed with that of CL within a CL-defined voxel. This problem was mitigated by removing the typical preprocessing step of Gaussian smoothing (which increases the signal-to-noise ratio, but reduces the spatial resolution). This is the method used to generate the CL rsfMRI map in Figure 2. Depending on the results of the CL rsfMRI maps of the Healthy Control and AD subjects (i.e., if these maps do not look significantly different from the TBI map), a second solution that Dr. Choi may try is to regress out the signal of adjacent nuclei from that of CL that may be masking the CL signal. A third solution that may be explored is to examine higher spatial resolution rsfMRI data. There are publicly available 7T rsfMRI data from healthy young adult subjects from the Human Connectome Project database. While these are not part of the three cohorts of this proposal, these data with higher spatial resolution (1.6mm isotropic voxels) and many more subjects (N=184), could provide a high quality healthy adult reference map of CL connectivity to help interpret the maps generated in this proposal.

Thirdly, Dr. Choi has been performing these analyses on her workstation computer, which is powerful but nonetheless limited in computing power. She will set up her analyses on the Stanford computing cluster to significantly reduce processing times.

Changes that had a significant impact on expenditures

Previously, we had planned for a Data Analyst to assist with work in Year 1, with the budget including the salary of a Data Analyst. However, there have been significant methods development and prototyping challenges that were better suited for Dr. Choi to address than a Data Analyst. Thus, those funds have not yet been used and may be used in the future for the salary of Dr. Choi or a Data Analyst.

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

N/A.

Significant changes in use or care of human subjects

N/A.

Significant changes in use or care of vertebrate animals.

N/A.

Significant changes in use of biohazards and/or select agents

N/A.

6. Products

Publications, conference papers, and presentations

Journal publications

Nothing to Report.

Books or other non-periodical, one-time publications

Nothing to Report.

Other publications, conference papers, and presentations

Nothing to Report.

Website(s) or other Internet site(s)
Nothing to Report.

Technologies or techniques
Nothing to Report.

Inventions, patent applications, and/or licenses
Nothing to Report.

Other Products
Nothing to Report.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	Eun Young Choi
Project Role:	PI
Research Identifier (e.g. ORCID ID):	0000-0003-3226-1486
Nearest person month worked:	15
Contribution to Project:	Dr. Choi has performed all work thus far.
Funding Support:	NIH BRAIN

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report.

8. Special Reporting Requirements

N/A.

9. Appendices

None.