Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 11/18/2021

The animal protocol review was considered by the Committee and the following decisions were made:

The protocol was approved.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contact [redacted] to schedule.
☒ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT [redacted] prior to starting surgeries.
☒ Other requirements: IBC approval is required before work with biohazardous materials may begin

Total # of Animals: 8
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 12/1/2021
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

IACUC Use Only	**IACUC Protocol #:** 22-1887R
Date: 10/18/2021	☒ IBC ☐ RSC ☐ Chem

ANIMAL USE PROTOCOL
ARIZONA STATE UNIVERSITY INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
(Revised February 2021)

Read "Instructions for Submitting the ASU Animal Use Protocol" before completing. **Upon approval, this protocol will become a public record so follow instructions carefully.**

PROJECT/PROGRAM TITLE: AAV Trehalose in an NHP model of Alzheimer's Disease

SPECIES REQUESTED: Macaque (*Macaca spp.)*

I. PERSONNEL INFORMATION

A. A single member of the university faculty and/or Principal Investigator (PI) is considered the responsible individual.

NAME: [redacted] TITLE: Director

AFFILIATION: ASU-Banner Neurodegenerative Disease Research Center Office Phone # [redacted]

Cell Phone #: [redacted] E-Mail: [redacted]

B. Additional contact, if any, for IACUC business

NAME: [redacted] TITLE: Primate Lab Supervisor

AFFILIATION: [redacted] Office Phone # [redacted]

Cell Phone #: [redacted] E-Mail: [redacted]

C. Protocol Type

☐ Non-funded research

☐ Internal Funding

Account Number:

☒ External Funding (Grant/Contract)

Granting Agency: [redacted] Deadline:

Co-Investigator(s): N/A

Proposal Title: [redacted]

ASU Proposal or Award #: [redacted]

If, ASU proposal or award number is not provided, attach a copy of the complete proposal or grant document.

☐ Teaching - Course Number and Title:

D. Protocol Status:

☒ New

☐ Renewal—Previous Protocol #:

☐ Revision—Previous Protocol #:

E. Do you plan to use Department of Animal Care & Technologies (DACT) personnel and resources? If yes, describe the support needed? (If this use is new or an expansion of previous use, contact the DACT well in advance of need). Yes, surgical anesthesia and post-op monitoring, as well as standard husbandry and clinical care.

II. PROJECT DESCRIPTION AND PROGRAM REQUIREMENTS

The Institutional Animal Care and Use Committee (IACUC) is composed of both active animal users and lay persons. Regardless of background, each member has a vote, so it is particularly important that the language of the application be understood by all. This applies to all sections of the application, but it is especially important that the goals and justifications of the proposed research be spelled out in the clearest possible terms. NOTE: Upon approval, this protocol will become a public record, so do not disclose proprietary information.

A. Provide a brief (300 words or less) synopsis in **NON-SCIENTIFIC TERMS** of proposed research.

We plan to test the ability of a viral vector to prevent neurodegeneration in a nonhuman primate (NHP) model of Alzheimer's Disease (AD). Animals will first receive an injection into the brain of a viral vector causing them to express a protein, tau, which has been implicated in AD. Then the animals will receive an injection of a second vector causing them to express a naturally occurring sugar that has been shown to prevent the misfolding of tau protein which typically leads to neurodegeneration.

B. PLANNED USE OF ANIMALS. Begin with a clear **statement of purpose** and briefly provide **background** information and **references** to previous work (especially if this is a renewal protocol). Include a clear description of the **experimental design** for all animal experiments planned and explain **why** the experiments must be performed. It is critical that for each procedure you provide a detailed sequence of events that effectively describes what happens to the animals from acquisition to euthanasia (if applicable). As the focus of the IACUC protocol is on animal use, do not simply cut and paste research objective statements from grant proposals. Flow charts, diagrams or tables are strongly recommended for complicated experimental designs. State how the research is expected to benefit the human community, the animal community, and/or society as a whole. **Details regarding surgical procedures, drug treatments, and field techniques are not necessary, as they will be addressed later in the form.**

Background: Trehalose is a naturally occurring sugar that has been shown to be effective in preventing protein misfolding in rodent models of disease (1). It prevents misfolding by activating autophagy pathways. It is currently in clinical trials for other indications. In this study we will create tau misfolding with the AAV-double mutant tau vector which has been demonstrated to cause hippocampal pretangle (AT8) and neurofibrillary tangle (thioflavin S) formation in NHPs (2). We know from previous and ongoing studies that at 3 months following AAV-double mutant tau there are many AT8 positive neurons in the hippocampus but few thioflavin 3 positive cells. There is also no cell loss. By 6 months there are many thioflavin S positive cells in the hippocampus and neuron loss in the CA1, CA3 and subiculum. We hypothesize that injections of AAV-trehalose will reduce the number of hippocampal pretangles and neurofibrillary tangles as well as prevent neuronal loss in the entorhinal cortex (ERC) and hippocampus.

References:

[redacted]

[redacted]

Experimental Design: 8 macaques (M/F, ≥22 years old) will be acquired from commercial vendors. All animals will first have baseline plasma, serum, & cerebrospinal fluid (CSF) collected. We will then deliver injections of AAV-double mutant tau (2 x 20 µL) into the left ERC and AAV-empty vector control into the right ERC (2 x 20 µL). Two months later we will deliver injections of AAV-trehalose or AAV-empty vector control (2 x 20 µL) into the left hippocampus and AAV-empty vector control into the right hippocampus (2 x 20 µL). Three months following the second surgery, all subjects will have plasma, serum, & CSF collected and then they will be sacrificed, the brains removed and punched for neurochemistry, then fixed and sectioned for immunohistochemistry (IHC).

Procedures:
MRI Scanning (two or three times): Stereotaxic intracranial injections are performed under intraoperative MRI guidance. Preop MRIs will be performed on a [redacted] MRI scanner at the [redacted] After transportation to [redacted] animals will be anesthetized with ketamine (3-10 mg/kg, IM) and either dexmedetomidine (0.03 mg/kg, IM) or midazolam (0.05-0.5 mg/kg) and maintained with gas anesthesia (e.g., isoflurane, sevoflurane) or booster injections of ketamine (1.5 mg/kg, IM) and dexmedetomidine (0.015 mg/kg, IM). Animals will be intubated to maintain a stable airway. Cetacaine spray (200 mg, topical) may be applied to the throat to assist with intubation. Animals will be placed in an MRI compatible stereotaxic frame and MRI-opaque fiducial markers will be placed around the skull for neuronavigation registration. T1 and/or T2-weighted images will be obtained. The locations of the fiducial markers will be permanently marked with a tattoo dot on the skin using a commercial tattoo marker [redacted] with sterile needle and ink. The MRI scan time is approximately 20 minutes, sedation is expected to last ≤1 hour. Following the procedure, dexmedetomidine/midazolam may be reversed with atipamezole (0.15-0.3 mg/kg, IM) or flumazenil (0.025 mg/kg, IV), respectively. A new MRI will be performed prior to the second surgery as the skin healing following the first surgery will affect the fiducial locations for neuronavigation registration. As MRI registration is essential for accurate surgical guidance, if in the opinion of the surgeon the MR images prove inadequate (due to animal movement, fiducial placement, or other confounding factors), up to one repeat MRI may be performed.

Euthanasia (once): The animal will be anesthetized with ketamine (10 mg/kg, IM), xylazine (2 mg/kg, IM) or midazolam (0.05-0.5 mg/kg), and either hydromorphone (0.2 mg/kg, IM) or morphine (1 mg/kg, IM), followed by gas anesthesia (e.g., isoflurane, sevoflurane) or additional drug delivered IV [ketamine (≤20 mg/kg, IV), xylazine (≤4 mg/kg, IV), and either hydromorphone (≤0.4 mg/kg, IV) or morphine (≤2 mg/kg, IV)], if needed to achieve a surgical plane of anesthesia. Once a surgical plane of anesthesia is achieved as verified by lack of response to toe/finger pinch, palpebral reflex, and corneal reflex, the thoracic cavity will be opened, heparin (5,000 IU, IC) will be injected into the left ventricle of the heart and the animal will be euthanized via transcardial perfusion of 0.9% saline (1-2 L) followed by 4% buffered formaldehyde (1-2 L). If perfusion cannot be performed for any reason, animals will be anesthetized with ketamine (10 mg/kg, IM) followed by a pentobarbital-containing euthanasia solution (86-120 mg/kg, IV).

C. RATIONALE FOR INVOLVING ANIMALS AND THE APPROPRIATENESS OF THE **SPECIES AND NUMBER** USED. Keeping in mind the principles of the "3 R's" (Refinement, Reduction, and Replacement), answer the following:

1. Why must live vertebrates be used in this study?
Our laboratory's experiments are aimed to help human patients with neurodegenerative diseases. Preclinical research requires a model that can inform about the applicability of diagnostic tools as well as the potential complications of its utilization. To the best of our knowledge, in vitro and computer models are still not able to give us enough information for clinical projection in complex neurodegenerative diseases such as AD.

2. Why are you using the requested species rather than other species?
Macaques were chosen because this model of AD was previously demonstrated in macaques. The brains of NHPs are similar in many respects to humans, enhancing the applicability of the data obtained to human diseases, especially when compared with rodent models.

3. What is the rationale supporting the numbers of animals proposed? Typically, a power analysis should be performed to support the proposed sample sizes. A table depicting the number of animals to be used is required.

This protocol will include an N of 8, which includes 2 groups of 4 animals. We will estimate the number of neurons in the entorhinal cortex and hippocampus by stereological counting of NeuN positive neurons. An n=4/group will give us the ability to detect an effect size of 25% in the number of neurons in the hippocampal subfield ($\beta > 0.8$, $\alpha \leq 0.05$).

Group	First Injection	Second Injection (2 mo later)	N=	Sacrifice
1	AAV-double mutant tau (L. ERC) AAV-empty vector (R. ERC)	AAV-empty vector (L. Hippocampus) AAV-empty vector (R. Hippocampus)	4	3 months post 2nd injection
2	AAV-double mutant tau (L. ERC) AAV-empty vector (R. ERC)	AAV-trehalose (L. Hippocampus) AAV-empty vector (R. Hippocampus)	4	3 months post 2nd injection

4. What refinements, if any, have been made to reduce the number of animals used and the potential detrimental effects on the study animals?

We feel the number of animals requested is the minimum necessary to achieve the aims of this study. Furthermore, we have taken every precaution to avoid pain and discomfort in our animals. The intracranial injections proposed will be conducted under general anesthesia with proper perioperative pain management and postoperative monitoring and care.

III. EMERGENCY CONTACT

A. Who should be contacted in case of an animal emergency? **Note: This information will be redacted if this protocol is requested as a public document.**

Name:
Office Phone #
Home Phone #
Cell Phone #:

IV. DUPLICATION AND ALTERNATIVES PLEASE READ ALL INSTRUCTIONS.

The Animal Welfare Act requires that you document your justifications with data from **two** or more sources. **One source** must **be a set of searches of a relevant database: name the database searched, the keyword and keyword combinations searched, the date the search was performed and the date range searched. The second source can be a set of searches of a second relevant database, or consultation with a laboratory animal science veterinarian, or courses/meetings/consultations with qualified personnel.** Sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge of the availability of alternatives in the specific field of study. Examples of appropriate databases to search include PUBMED, Web of Science, or Animal Welfare Information Center (AWIC – recommended for USDA-covered species https://www.nal.usda.gov/awic/databases).

A. Provide the following details for the most recent literature search used to explore for duplicative research. (The literature search documents that the research will not unnecessarily duplicate previous research). **Teaching protocols do not need to conduct this search.**

Date that search was conducted (*Must be within 60 days of the IACUC review date*): 10/15/2021
Database(s) used: ALTBIB, PUBMED
Publication years covered by the search: 2000 - present
Keyword combinations used: trehalose, tau, nonhuman primate
trehalose, tangles, nonhuman primate

B. Provide the following details for the most recent literature search used to explore for **alternatives to animal use** and **alternatives to painful procedures.** Alternatives should be considered for any aspect of the protocol that

may cause more than momentary or slight pain or distress to the animal. Alternatives to be considered include those that would: 1) refine the procedure to minimize discomfort that the animal(s) may experience; 2) reduce the number of animals used overall; or 3) replace animals with non-animal alternatives (e.g., computer models or tissue culture). **All protocols (research and teaching) MUST conduct this search.**

Date that search was conducted (*Must be within 60 days of the IACUC review date*): 10/15/2021
Database(s) used: ALTBIB, PUBMED
Publication years covered by the search: 2000 - present
Keyword combinations used:
Alzheimer's disease, animal model, nonhuman primate
Nonhuman primate, intracranial injection alternative

C. **Results of literature search for alternatives**: Comment on the application(s) of any identified alternatives (found with your search terms, including how these alternatives may be or may not be incorporated to modify a procedure to either lessen or eliminate potential pain and distress. **All protocols must complete this section and must describe how the literature search results relate to painful procedures and alternatives to animal use.** You must include sufficient information for the IACUC to determine that a reasonable, good faith effort was made to determine the availability of alternatives. If the search identified any alternative methods (ones that could be used to accomplish the goals of the animal use proposal), you must clearly explain and justify why this alternative cannot be used.

For instance, if your search terms retrieved eight publications, summarize how many of those described alternatives to painful procedures and the use of animals.
No studies have been published examining the ability of AAV-trehalose to prevent tangles in an NHP model of AD. While rodents have been used to establish other models of AD, the NHP models better mimic what is seen in the human brain. Additionally, the brains of rodents are less complex than that of NHPs and humans. No alternatives to intracranial injection were found for delivering vector to the ERC and hippocampus.

D. Describe any other procedures (e.g., participation in meetings, review of journals) that are used to explore and evaluate alternatives: The PI, lab manager, post-docs, and graduate students regularly attend national meetings and discuss recent updates in technology and methodology for these experiments with colleagues. Additionally, they remain up to date with the scientific literature on new and alternative procedures.

E. Does this research replicate previous work? **(Your answer will be based in part on the literature search above.)**

☒ No. Proceed to section **VI.**

☐ Yes. Explain why the replication is necessary:

☐ Not applicable. This is a teaching protocol.

V. CATEGORY OF PAIN OR DISTRESS

For non-USDA covered species, answer question A only. For USDA covered species, answer question B only. USDA covered species are all mammals EXCEPT laboratory mice and rats bred for research. All other rodents, including wild mice and rats, are covered.

A. Do the procedures in this protocol have the potential to involve more than slight or momentary pain or distress that will **NOT** be relieved with anesthetics, analgesics, tranquilizer drugs, or other method for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

B. Using the table below, list all USDA covered species to be used in the proposed study and indicate the number of animals to be used under each USDA pain category. For an animal undergoing multiple procedures, include the animal under the highest level of pain/distress expected for that animal.

USDA Covered Species	Number per USDA Category*				Total number of animals requested
	B	C	D	E	
Macaque spp.			8		8

*USDA PAIN CATEGORIES: (see http://researchintegrity.asu.edu/animals/forms for a more complete description of the below categories)

Classification B: Includes animals that are used solely for breeding or are being acclimatized or held for use in teaching, testing, experiments, research, or surgery but have not yet been used for such purposes.

Classification C: Includes the use of animals in procedures involving no, momentary, or slight pain or distress (e.g., non-invasive parenteral drug delivery, peripheral blood collection, euthanasia, short-term manual or chemical restraint, toe clipping).

Classification D: Includes the use of animals used in procedures that could cause pain or distress but appropriate anesthetics, analgesics, and/or tranquilizing drugs or other methods for relieving pain or distress are used (e.g., surgery, perfusion, administration of irritating chemicals, humane endpoint euthanasia).

Classification E: Includes the use of animals in procedures that have the potential to involve pain or distress that will **not** be relieved with anesthetics, analgesics, tranquilizer drugs, or other method for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia).

VI. ASSURANCE:

The information contained herein is accurate to the best of my knowledge. I have carefully compared the proposed work with the current state of knowledge in this field by reviewing the literature and it is my professional opinion that the proposed work meets high standards of scientific merit. If the study involves pain and distress to the animal, whether or not it is relieved by anesthetics or analgesics, I have (1) reviewed the literature related to this work and have found no significant studies which could make this protocol unnecessarily duplicative, and (2) considered alternatives to animal use and found none available, as described above. Procedures involving animals will be carried out humanely and all procedures will be performed by or under the direction of trained or experienced persons. Any revisions to animal care and use in this project will be promptly forwarded to the Institutional Animal Care and Use Committee for review. Revised protocols will not be used until Committee clearance is received. The use of alternatives to animal models has been considered and found to be unacceptable at this time.

The principal investigator, by signing below, and the IACUC recognize that other medications may be given to the animals for veterinary care purposes. This includes the humane euthanasia of animals in uncontrollable pain or distress as determined by the Attending Veterinarian or the Clinical Veterinarian acting for the Attending Veterinarian. However, the veterinarians will make all efforts to contact and discuss the case with the Principal Investigator or designee prior to making a unilateral decision.

[redacted] 10/15/21

Principal Investigator –Print Date

[redacted] 10/15/21

Principal Investigator Signature Date

NOTE: Principal investigators must submit a current curriculum vitae or biosketch that reflects their most recent pertinent experience.

PERSONNEL CHART

ASU requires that all personnel engaged in animal research or teaching be qualified through training or experience in order to conduct the work humanely. The IACUC requires the following training:

- **Level I Basic** – Required of ALL participants (must be renewed every 4 years)
- **Level II Species-Specific** – Required for each participant that will have direct contact with that species (must be renewed every 4 years)
- **Level III Hands-on Training** – Required to perform specific procedures independently. Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. A Level III Certification form must be submitted to the IACUC office by the person providing the training within 5 days of the training.

You can access the training modules at https://asu.co1.qualtrics.com/jfe/form/SV_b2b2XRXRRs1309f. See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

*** Procedures other than husbandry, handling, or behavioral testing MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel who are not Level III certified are supervised at all times.**

Name	Title	ASURITE name	Role in Protocol: What activities will each person perform on live animals ONLY while under direct supervision?	Role in Protocol: What activities will each person be allowed to perform independently (including appropriate Level 3 certification*) at the time of protocol submission?	Species with which individual will have direct contact ("none, "all", or list species)	FOR IACUC USE ONLY: Training Confirmation
[redacted]	PI	[redacted]	none	Intracranial surgery, blood/CSF collection, MRI, administration of any medications, and necropsy.	All	7/2021 OHSP

For each individual, describe the individual's years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

[redacted] Dr [redacted] has 37 years' experience conducting research with nonhuman primates and is experienced with all procedures in this protocol.

DETAILED USE OF ANIMALS
This section must be completed for each species used.

(additional Detailed Use of Animals forms can be found at https://researchintegrity.asu.edu/animals/forms)

Common Name: Macaque

Scientific Name: *Macaca spp.*

I. ANIMAL INFORMATION

A. Is this a threatened or endangered species?
- ☒ No. Proceed to section I. B.
- ☐ Yes. Describe why this work must be done on this species and why the project will not have a significant negative impact on the species:

B. Maximum # of animals to be used over the 3-year life of the protocol: 8

C. Sex: M/F Age or Weight Range: ≥22 years

D. Source (e.g., commercial, in-house breeding, captured from wild): Commercial

E. List all labs and/or rooms **outside of the ASU centralized vivaria** where you intend to keep or use live animals in connection with the animal use covered under this protocol. This list is for IACUC information to assure each location is inspected semi-annually. **Listing rooms here does not assure approval of this space for use.**

Building	Room #	Max Length of Stay	Method of Transport	Purpose
[redacted]	[redacted]	4 hours	NHP cage inside DACT truck	MR Imaging

F. If you use DEA-controlled substances, list the location where they are stored (building and room number). If you acquire controlled substances from DACT for same day use, state this. The IACUC is required to inspect all controlled substance storage locations semi-annually. Controlled substances will be stored in Dr [redacted] office [redacted]

II. MAJOR CATEGORIES OF USE

A. Will animals be immunized solely for the production and harvesting of antibodies to be used in vitro rather than as a vaccine study?
- ☒ No. Proceed to section II. B.
- ☐ Yes. Complete the following table.

Injection:

Volume of injectate	Adjuvant	Route	Min. Frequency	Max. # of injections

Collection: If terminal, check here ☐ otherwise complete the following.

Route	Max. Volume	Min. Frequency	Max. # of collections

B. Will tissues, blood, or other body fluids be harvested (other than for antibody production)?
- ☐ No. Proceed to section II. C.
- ☒ Yes. Will tissues, blood, or other body fluids be collected post-mortem only?
  - ☐ Yes. Proceed to section II.C.
  - ☒ No. Complete Appendix 1: Antemortem Specimen Collection.

C. Will animals be food restricted (calorically or specific constituents) other than for surgical procedures?
- ☒ No. Proceed to section II. D.

☐ Yes. [note: restriction paradigms exceeding a single 24-hr period must follow the ASU IACUC Standard Institutional Guideline for Food and Water Restriction available at https://researchintegrity.asu.edu/index.php/animals/procedures-library-and-guidelines

1. What are the restriction parameters? Provide scientific justification and include the length of restriction.

2. How will you monitor for negative effects of food restriction (include information on how you will account for animal growth)?

D. Will animals be water restricted?
☒ No. Proceed to section II. E.
☐ Yes. [note: restriction paradigms exceeding a single 24-hr period must follow the ASU IACUC Standard Institutional Guideline for Food and Water Restriction available at https://researchintegrity.asu.edu/index.php/animals/procedures-library-and-guidelines

1. What are the restriction parameters? Provide scientific justification and include the length of restriction.

2. How will you monitor for negative effects of water restriction (include information on how you will account for animal growth)?

E. Will animals be exposed to trauma, injury, burning, freezing, electric shock, UV radiation, magnetic fields, lasers, loud noise, or other physical agents that might cause distress?
☐ No. Proceed to section II. F.
☒ Yes. List and justify each exposure.
Provide scientific justification: **Magnetic Resonance Imaging (MRI):** Due to the variability in NHP neuroanatomy, MR imaging is the best way to accurately target surgical injections within the brain. MRI scans involve strong magnetic fields, and precautions are made to ensure that no incompatible metals are present in the room during the scan. Noise levels inside an MRI machine typically vary from 65 to 95 dB, and intermittent spikes of ~110 dB may be produced. MRI scans are performed under anesthesia, and ear protection using ear plugs or gauze/cotton will be placed in the animal's ears to prevent damage and mitigate distress.

F. Will animals be exposed to environmental stress (e.g., non-natural temperature exposure, prolonged physical restraint, forced exercise)?
☒ No. Proceed to section II. G.
☐ Yes. List and scientifically justify each exposure.

G. Will animals undergo surgery?
☐ No. Proceed to section II. H.
☒ Yes. Complete Appendix 2: Surgical Procedures.

H. Will any animals have a device (e.g., thermocouple, cannula, electrode) that extends chronically through the skin?
☒ No. Proceed to section II. I.
☐ Yes. Describe wound management measures to minimize chances of infection around the device where it penetrates the skin:

I. Will individuals of a social species (e.g., most rodents) need to be housed singly at any time?
☐ No. Proceed to section II. J.
☒ Yes.

1. What would be the maximum duration that an individual would be singly housed? Provide scientific justification for singly housing for this duration:
Animals will be pair housed when possible. However, because of the relatively small number of animals involved, suitable pairing partners may not be available. If necessary, single housing will be determined

in conjunction with the veterinary staff and will continue until a suitable pairing partner becomes available or the experiment concludes.

2. Singly housed animals should receive additional enrichment. Describe what enrichment will be provided or scientifically justify why additional enrichment cannot be provided:
Animals will be housed in a room with other conspecifics and have access to visual, olfactory, and vocal/auditory contact. All animals are also provided a variety of enrichment items including manipulanda and destructibles; these may be increased in number or variety for singly housed animals as determined by the veterinary staff on a case-by-case basis.

J. Will animals need any special husbandry considerations, including but not limited to altering standard cage type, cage change frequencies, housing temperature, or lack of enrichment?
☒ No. Proceed to section II. J.
☐ Yes. Describe special procedures and provide scientific justification:

K. Will animals be transported off campus (e.g., to/from the field, or between institutions) in a vehicle other than one owned by the DACT?
☒ No. Proceed to section II. L.
☐ Yes. Describe details (e.g., vehicle to be used, destinations, and driven by whom), read the IACUC *SIG - Off-campus Transport of Animals by Laboratory Personnel*, and complete and submit with this protocol the *Assurance to Abide by the Requirements for Transporting Live Animals*:

L. Will any work be conducted in the field (this includes field experiments or the capture of animals to be used in laboratory experiments)?
☒ No. Proceed to section II. K.
☐ Yes. Complete Appendix 3: Field Research.

M. Will any animals need to be individually identified?
☐ No. Proceed to section III.
☒ Yes. Describe the marking technique to be used, why that technique was chosen, how it will be performed, and on what age range of animals?
Animals will be tattooed with an identification number on their chest or inner thigh. Animals either have the tattoo upon arrival or are tattooed while under sedation by DACT staff during quarantine. Touch ups may be done while sedated/anesthetized (e.g., for TB testing), and the hair in the region is shaved as needed to maintain visibility of the tattoo. This identification method is the most widely used means of permanently marking macaques.

III. CHEMICALS AND OTHER POTENTIAL HAZARDS

(If you answer yes to any of the following questions, this information may be forwarded to another oversight unit to aid you in assuring safe practices. Approval by these units or additional training may be required prior to using any of these materials)

A. Will drugs or chemicals be used with animals?
☐ No. Proceed to section III. B.
☒ Yes. For each drug or chemical, list the agent, dose, route, purpose, and grade in the table below:

Agent	Dose	Route	Purpose	Frequency	Pharmaceutical grade (Y/N)?	Is this a DEA controlled substance (Y/N)?
Atipamezole	0.15-0.3 mg/kg	IM	Dexmedetomidine reversal	As needed	Y	N
Atropine	0.02-0.05 mg/kg	IM	Reduce respiratory secretions and	As needed	Y	N

			prevent bradycardia			
Betadine	N/A	Topical	Topical disinfectant	As needed	Y	N
Bupivacaine	1-2 mg/kg	SC	Analgesia	Once during closure	Y	N
Buprenorphine Sustained release	0.2 mg/kg	SC	Analgesia	Once post-op	Y	Y
Cefazolin	20-25 mg/kg	IV or IM	Antibiotic	Every 2-4 hours intra-op, as needed	Y	N
Cephalexin	20-30 mg/kg	PO	Antibiotic	Twice daily, as needed	Y	N
Cetacaine Spray (Benzocaine 14%, Butamben 2%, Tetracaine 2%)	200 mg	Topical	Anesthesia	As needed for intubation	Y	N
Chlorhe idine	N/A	Topical	Topical disinfectant	A needed	Y	N
Dexmedetomidine	0.015-0.05 mg/kg	IM	Anesthesia	As needed	Y	N
4% Formaldehyde	1-2 L	IC	Perfusion	Once	N	N
Flumazenil	0.025 mg/kg	IV	Benzodiazepine reversal	As needed	Y	N
Gelfoam	Cut to size	Topical	Hemostasis/Seal surgical holes	As needed	Y	N
Heparin	5,000 IU	IC	Anticoagulant for perfusion	Once	Y	N
Hydromorphone	0.05-0.4 mg/kg	SC, IM, IV	Analgesia	As needed	Y	Y
Isoflurane	0.5-5%	Inhalation	Anesthesia	As needed	Y	N
Isopropyl alcohol	70%	Topical	Topical disinfectant	As needed	Y	N
Ketamine	1.5-20 mg/kg	IM, IV	Anesthesia	As needed	Y	Y
Meloxicam	0.1-0.2 mg/kg	PO, SC	Analgesia	Once daily, as needed	Y	N
Meloxicam Sustained release (10 mg/mL)	0.6 mg/kg	SC	Analgesia	Once post-op	Y	N
Midazolam	0.05-0.5 mg/kg	IM, IV	Sedative, anticonvulsant	As needed	Y	Y
Morphine	1-2 mg/kg	IM, IV	Analgesia	As needed	Y	Y
Ophthalmic ointment	Dab	Topical	Prevent corneal desiccation	As needed	Y	N
Pentobarbital-containing euthanasia solution	86-120 mg/kg	IV	Euthanasia	Once	Y	Y
Propofol	2-5 mg/kg Bolus 0.2-0.6 mg/kg/min CRI	IV	Anesthesia	As needed Continuous, as needed	Y	N

Saline or Lactated Ringer's Solution	5 15 mL/kg/hr	IV	Fluid replacement	Con tant rate infusion	Y	N
Saline	1-2 L	IC	Perfusion	Once	Y	N
Sevoflurane	1-8%	Inhalation	Anesthesia	As needed	Y	N
Sufentanil	0.25-2 µg/kg/hr	IV	Analgesia	Constant-rate infusion	Y	Y
Xylazine	2-4 mg/kg	IM, IV	Anesthesia	As needed	Y	N

1. For each drug or chemical that is not pharmaceutical grade, indicate whether no pharmaceutical grade equivalent exists or provide scientific justification for using the non-pharmaceutical grade product.
   Formaldehyde is not available in a pharmaceutical grade, and is only used once in a terminal procedure.

B. Does this project involve transgenic, knockout, or knock-in animals?
☒ No. Proceed to section III. C.
☐ Yes. List the strains, any special care needs, and any expected clinical signs that are associated with the strain. Transgenic animals need to be covered by an IBC disclosure.

C. Does this project involve the use of biohazardous agents in animals (microorganisms, microbial toxins, recombinant DNA)?
☐ No. Proceed to section III. D.
☒ Yes. List the agent, as well as concentration, dose, and route if applicable.

Agent	Concentration	Dose	Route	ADMIN. USE ONLY	
				ABSL	IBC # if Req'd
AAV-double mutant tau	1E10^{13}vg/mL	40µL	Intracranial injection into ERC	Pending	SPROTO2021-70
AAV-empty vector	1E10^{13}vg/mL	40 -80µL	Intracranial injection into ERC and hippocampus	Pending	SPROTO2021-70
AAV-trehalose	1E10^{13}vg/mL	40µL	Intracranial injection into hippocampus	Pending	SPROTO2021-70

D. Does this project involve irradiation or the use of radiological material in animals?
☒ No. Proceed to section III. E.
☐ Yes. List the agent, dose, route, and purpose in the table below:

Agent	Dose	Route	Purpose

1. Provide the date of **Radiation Safety Committee** approval:

E. Describe any health hazards to **researchers** and include a description on how the risk is mitigated or managed:
Risk of bites, scratches, or Herpes B (Herpes B virus is not being used in animals but can be transmitted to personnel if there is an NHP bite/exposure). Risks are mitigated with the use of additional PPE as required by University policies (such as, but not limited to, Tyvek sleeves and double gloves), NHP primate certification, annual B Virus training (including Bite/Scratch policy), proof of 2 MMR vaccines or a measles titer, annual TB screening, and ear protection during MRI scans.

F. Describe any health hazards to **animals** and include a description on how the risk is mitigated or managed:
Zoonosis such as TB, measles, and flu are agents of concern that may spread from humans to monkeys. Before working with an NHP, all researchers are required to show proof of 2 MMR vaccines or a measles titer and annual TB screening. All people interacting with the monkeys are also required to wear a surgical mask to prevent the spread of these infections.

IV. DETRIMENTAL SEQUELAE

A. Will animals possibly experience clinical signs intentionally or as a possible side effect of the study?
☐ No. Proceed to section V.
☒ Yes. Complete the following.

Possible Clinical Effect	Probability of Occurrence	Treatment
Intracranial injections may exhibit temporary post-op clinical signs related to the procedure.	Post-op clinical signs occur infrequently following injection and typically resolve after a few days. We expect any clinical signs to be mild and not affect the animals' ability to locomote or eat.	Consult with veterinary staff if clinical signs develop

V. END POINT CRITERIA

A. What clinical signs will be used as a basis for removal of an animal from the study?
If any animals begin to display neurological deficits or other clinical signs that may impact their health and well-being, they will be referred to the veterinary staff for evaluation.
Weight loss in excess of 20% of ideal weight (as determined by veterinary staff based on body weight and body condition score) that does not resolve after two weeks of supportive treatment (as determined and provided in conjunction with the DACT veterinary team).
An animal that becomes laterally recumbent, or has difficulty locomoting or feeding themselves which does not resolve after one week of supportive treatment (as determined and provided in conjunction with the DACT veterinary team), or when determined by the DACT Veterinary Team to have reached an endpoint (veterinary discretion).

VI. EUTHANASIA

A. List the primary method of euthanasia:
Transcardial perfusion under anesthesia. If not perfusing, pentobarbital-containing euthanasia solution.

B. If using a chemical or gas, complete the chart below:
Various combinations of the following drugs may be used in coordination with euthanasia via injection of a euthanasia solution or perfusion.

Agent	Dose	Route	Is this a DEA controlled substance (Y/N)?	Secondary method used to confirm euthanasia
Pentobarbital-containing euthanasia solution	86-120 mg/kg	IV	Y	Removal of brain
Ketamine	10-20 mg/kg	IM, IV	Y	Used in conjunction with perfusion
Xylazine	2-4 mg/kg	IM, IV	N	Used in conjunction with perfusion

Midazolam	0 05 0 5 mg/kg	IM	Y	U ed in conjunction with perfusion
Atropine	0.02-0.05 mg/kg	IM	N	Used in conjunction with perfusion
Morphine	1-2 mg/kg	IM, IV	Y	Used in conjunction with perfu ion
Hydromorphone	0 2 0 4 mg/kg	IM, IV	Y	U ed in conjunction with perfusion
Heparin	5,000 IU	IC	N	Used in conjunction with perfusion
Isoflurane	3-5%	Inhalation	N	Used in conjunction with perfu ion
Sevoflurane	5 8%	Inhalation	N	U ed in conjunction with perfusion
0.9% saline	1-2 L	IC	N	Used in conjunction with perfusion
4% formaldehyde	1-2 L	IC	N	Used in conjunction with perfu ion

C. If euthanasia is being done by a physical means (e.g., decapitation, cervical dislocation) without anesthesia, provide scientific justification:
N/A

APPENDIX 1: ANTEMORTEM SPECIMEN COLLECTION

I. BLOOD COLLECTION

A. Will blood be collected?

☐ No. Proceed to section II.

☒ Yes. Complete the following.

Site	Volume (ml)	% BW	Max. # of collections	Min. Interval
femoral vein	≤10 mL	≤0.5%	Up to 2 planned, 3 max including potential redraws	Typically 5 months; Rarely within 7 days (see below)

B. Will anesthetics, sedatives, or other drugs be used during blood collection?

☐ No. Proceed to section I. C.

☒ Yes. Complete the following.

Drug	Dose	Route	Purpose
Ketamine	3-10 mg/kg	IM	Anesthesia
Dexmedetomidine	0.03 mg/kg	IM	Anesthesia
Midazolam	0.05-0.5 mg/kg	IM	Anesthesia
Atropine	0.02-0.05 mg/kg	IM	Reduce respiratory secretions

C. Describe the methods used to draw the blood including physical restraint, if any.

Animals will be anesthetized with ketamine and either dexmedetomidine or midazolam. Blood samples will be obtained from the femoral vein and separated for plasma and serum collection. Sedation is expected to last 30 minutes.

D. Provide scientific justification for blood collection and justification for the frequency of it.

Plasma and serum will be banked for future analysis. Collections spaced approximately 5 months apart will allow for adequate bodily replacement. In the event that a blood collection attempt does not yield an adequate sample (i.e.: no sample, inadequate volume [<1 mL serum]), the blood collection may be repeated up to one additional time within a 7-day period, well below accepted blood draw volume levels and frequency limits for animals of this size (2 – 10 kg).

II. OTHER TISSUE/BODY FLUID COLLECTION

A. Will other tissues or body fluids be collected prior to death?

☐ No. Appendix 1 is completed.

☒ Yes. Complete the following. Surgical procedures should be described more fully in Appendix 2.

Tissue/Fluid	Site and Method	Amt	# of collections	Min Interval
CSF	lumbar or cisternal puncture	≤0.5 mL	Up to 2 planned, 3 max including potential redraws	Typically 5 months; Rarely within 7 days (see below)

B. Will anesthetics, sedatives, or other drugs be used during tissue/body fluid collection?

☐ No. Proceed to section II. C.

☒ Yes. Complete the following.

Drug	Dose	Route	Purpose
Ketamine	3-10 mg/kg	IM	Anesthesia
Dexmedetomidine	0.03 mg/kg	IM	Anesthesia
Midazolam	0.05-0.5 mg/kg	IM	Anesthesia

Atropine	0 02 0 05 mg/kg	IM	Reduce re piratory secretions
Betadine/Isopropyl alcohol	N/A	Topical	Topical disinfectant

C. Describe the methods used to collect the samples, including physical restraint, if any.
Animals will be anesthetized with ketamine and either dexmedetomidine or midazolam. CSF collection is performed as a sterile procedure. The lumbar or cervical area of the animal will be shaved and scrubbed alternating with povidone iodine and alcohol three times to prepare the collection site. A sterile drape will be placed over the collection site and sterile surgical gloves will be worn for the collection. For lumbar collection, a 22G spinal needle will be advanced into the spinal subarachnoid space until CSF begins to flow spontaneously. For cisternal collection, a 22G spinal needle attached to a 3-6 mL syringe will be advanced into the cisterna magna and CSF will be withdrawn. Sedation is expected to last 30 minutes.

D. Provide scientific justification for the sample collection(s) and justification for the frequency of it
CSF will be banked for future analysis. Collections spaced approximately 5 months apart will allow for adequate bodily replacement. In the event that a CSF collection attempt does not yield an adequate sample (i.e.: no sample, inadequate volume [<0.2 mL], or blood contamination), the CSF collection may be repeated up to one additional time within a 7-day period.

APPENDIX 2: SURGICAL PROCEDURES

I. GENERAL INFORMATION

A. Species
Macaque

B. Surgical Procedure(s)
Intracranial injection

C. Room/location of surgery
[redacted] Surgical Suite

II. PRE-SURGICAL CARE

A. Will the animals undergo pre-surgical fasting?
☐ No. Proceed to section III.
☒ Yes. Provide the details:
The day before a scheduled surgical procedure, animals are offered their full diet allotment in the early afternoon, and any remaining diet is removed at the end of the workday. The animal is then fasted overnight until the scheduled surgery the following morning in order to mitigate the risk of emesis and aspiration during the procedure.

III. SURGICAL PROCEDURE:

☒ Survival ☐ Nonsurvival

***Note:** A surgical checklist is recommended for each survival surgery, and possibly non-survival surgeries. These checklists should be submitted to DACT's Research Support Services [redacted]) for review before implementing procedures.

A. Describe each surgical procedure (e.g., approach, tissue manipulation, closure):
Intracranial Injections:
Anesthesia will be induced with injectable anesthetics, and animals will then be intubated and maintained on gas anesthesia. Morphine or hydromorphone will be administered pre-operatively, as will Cefazolin. Animals will be placed in stereotaxic frames. Surgical targeting will be accomplished using a [redacted] surgical neuronavigation system, which will allow in-op visualization of the surgical instruments within and around the brain. The MRI images will be uploaded to the [redacted] system and coordinates for target areas will be marked. Under sterile conditions, an 8 cm incision will be made along the midsagittal plane of the scalp. Entry points will be identified using the [redacted] system. One entry hole will be drilled in each hemisphere of the skull (10mm x 10mm). During the first surgery, we will deliver injections of AAV-double mutant tau (2 x 20 µL) into the left ERC and AAV-empty vector control into the right ERC (2 x 20 µL). During the second surgery we will deliver injections of AAV-trehalose or AAV-empty vector control (2 x 20 µL) into the left hippocampus and AAV-empty vector control into the right hippocampus (2 x 20 µL). Infusion will be performed with an infusion pump attached to a stereotaxic micromanipulator [redacted] syringes will be lowered to the targets, and the contents infused at a rate of 1 µL/min. After the injection is complete, the needle/syringe will be left in place for an additional 2 minutes to allow infusate to diffuse from the needle tip and prevent backflow prior to retracting the syringe. The entry holes will be filled with Gelfoam. The SC tissues, and skin will then be closed using absorbable suture. Bupivacaine (1-2 mg/kg, SQ) will be administered to the incision site prior to closure. Anesthesia depth will be monitored approximately every 10 minutes through jaw tone, palpebral reflex and vitals measurements (e.g., heart rate, ventilatory rate, oxygen saturation, blood pressure, temperature, CO2 level). Animals will receive Buprenorphine SR and Meloxicam. Surgery is expected to last approximately 2 hours. Should any animal experience adverse effects post-surgery (including signs of infection) as determined by the veterinary staff, they will be evaluated and treated as appropriate.

B. Anesthetic regimen:

Various combinations of the following drugs may be used in the induction and maintenance of anesthesia for surgery.

Drug & concentration (e.g., mg/ml)	Dose (e.g., mg/kg) & maximum volume to be given	Route	Is this a DEA controlled substance (Y/N)?
Ketamine (100 mg/mL)	10-15 mg/kg, 1 mL	IM	Y
Midazolam (5 mg/mL)	0.05-0.5 mg/kg	IM	Y
Dexmedetomidine	0.015-0.05 mg/kg	IM	N
Atropine (0.54 mg/mL)	0.02-0.05 mg/kg	IM	N
Isoflurane	0.5-5%	Inhalation	N
Sevoflurane	1-8%	Inhalation	N
Propofol (10 mg/mL)	2-5 mg/kg, 5 mL (Bolus) 0.2-0.6 mg/kg/min (CRI)	IV	N
Cetacaine Spray (Benzocaine 14%, Butamben 2%, Tetracaine 2%)	200 mg	Topical	N

Please refer to the IACUC approved document "**Macaque Anesthesia/Analgesia/Antibiotics Regimens**"

Note: Use of gas anesthetics requires completion of the EH&S-based Anesthetic Gas Safety training prior to use and refreshed annually.

1. Describe measures used to indicate a surgical plane of anesthesia to keep animals from getting too light or too deep:
   Anesthesia depth will be monitored approximately every 10 minutes through jaw tone, palpebral reflex, and vitals measurements (e.g., ECG, heart rate, ventilatory rate, oxygen saturation, blood pressure, temperature, end tidal gases).

C. Additional pharmacological agents used during surgery (include analgesics, supportive medications, and research drugs):

Drug and concentration	Dose & max volume	Route	Purpose	Frequency	Is this a DEA controlled substance (Y/N)?
Betadine/Chlorhexidine/ Isopropyl alcohol	N/A	Topical	Topical Disinfectant	Once, as needed	N
Bupivacaine (5 mg/mL)	1-2 mg/kg, 2 mL	SC	Analgesia	Once during closure	N
Cefazolin (330 mg/mL)	20-25 mg/kg, 0.76 mL	IV	Antibiotic	Every 2-4 hours, intraoperatively	N
Gelfoam	Cut to size	Topical	Hemostasis/Seal surgical holes	Once, as needed	N
Hydromorphone (2 mg/mL)	0.05-0.2 mg/kg	SC, IM, IV	Analgesia	Once, as needed	Y
Morphine	1-2 mg/kg	IM, IV	Analgesia	Once, as needed	Y
Ophthalmic ointment	Dab	Topical	Prevent corneal desiccation	Once, as needed	N
Saline or Lactated Ringer's Solution	5-15 mL/kg/hr, 300 mL	IV	Fluid replacement	Constant-rate infusion	N
Sufentanil (0.5 µg/mL)	0.25-2 µg/kg/hr, 120 mL	IV	Analgesia	Constant-rate infusion	Y

D. Describe the steps taken to maintain an aseptic surgery:
Trained individuals will perform standard sterile prep of the scalp. The site will be scrubbed alternating with povidone iodine/chlorhexidine and alcohol three times. Sterile drapes, gowns, gloves, and instruments will be used.

E. What is the maximum duration of each surgery?
3 hours

F. Will any animals recover from surgery?
☐ No. This involves terminal, or non-survival, procedures; Appendix 2 is complete.
☒ Yes. Complete Section IV.

IV. POST-SURGICAL CARE

A. Is there a potential for post-operative pain or distress?
☐ No. Proceed to section C.
☒ Yes.

B. Will analgesics be used?
(For analgesic options, refer to the IACUC Standard Institutional Guideline on analgesia (https://researchintegrity.asu.edu/animals/procedures-library-and-guidelines) or contact a DACT veterinarian
☐ No. Provide a scientific justification:

☒ Yes. Complete the following.

Drug & concentration	Dose & max. volume	Route	Frequency	Is this a DEA controlled substance (Y/N)?
Buprenorphine Sustained release (1-3 mg/mL)	0.2 mg/kg	SC	Once post-op	Y
Meloxicam Sustained release (10 mg/mL)	0.6 mg/kg	SC	Once post-op	N
Meloxicam (5 mg/mL injection; 1.5 mg/mL oral)	0.1-0.2 mg/kg	SC, PO	SID as needed/ variable duration based on procedure	N

Please refer to the IACUC approved document **"Macaque Anesthesia/Analgesia/Antibiotic Regimens"**

Who will administer these drugs?
Veterinary staff or other trained individuals.

C. Post-operative routine care:

i. What other drugs will be administered, if any (e.g., antibiotics, fluids)?

Drug & concentration	Dose & max. volume	Route	Purpose	Frequency	Is this a DEA controlled substance (Y/N)?
Cefazolin (330 mg/mL)	20-25 mg/kg	IM	Antibiotic	BID as needed/ variable duration based on procedure	N
Cephalexin (50 mg/mL)	20-30 mg/kg	PO	Antibiotic	BID as needed/ variable duration based on procedure	N

Please refer to the IACUC approved document "Macaque Anesthesia/Analgesia/Antibiotic Regimens" (choice of antibiotic and route of administration dictated by patient compliance. We try oral administration first, but default to injectable if NHP is not compliant)

ii. What other post-operative support and monitoring will be provided, how often, for how long, and by whom?
Pain assessment scoring is performed following major surgical procedures and continues until the pain score is 0 as determined by the veterinary or trained research staff. Monitoring is provided by both trained DACT and PI personnel. Should any animal experience adverse effects post-surgery (including signs of cerebral infection, cranial incision complications, or neurologic deficits) as determined by the veterinary staff, they will be evaluated and treated as appropriate by the veterinary staff.

D. Is post-operative intensive care required?
☒ No. Proceed to section E.
☐ Yes.
What special care is required?

Who will provide special care and what are their qualifications?

For how long will special care be needed?

E. Will animals undergo multiple survival surgical procedures?
☐ No. Appendix 2 is complete.
☒ Yes. Describe which surgeries, the sequence (specifying time between surgeries), and frequency. Provide scientific justification:
Two intracranial injection surgeries are necessary to administer the AAV vectors into the target areas of the brain as these vectors cannot cross the blood-brain barrier. A two month period between the two surgeries is necessary to allow time for the animal to express sufficient quantities of tau protein before the administration of the trehalose vector.

IACUC Protocol Trackable Components Checklist

Protocol #: 22-1887R If for amendment, amendment #:

PI [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 10/22/21

☐ No trackable components in this document

Exceptions to the Guide:

☐ Food/Fluid Regulation
- Species:
- What Restricted:
- Parameters:

☐ Prolonged Restraint
- Species:
- Details:

☒ Husbandry Deviation from the Guide
- Species: NHP
- Deviation: Single housing if suitable pairing partners are not available.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)
- Species: NHP
- Surgerie(s): Intracranial injection
- Multiple Major?: ☒ Yes ☐ No

☒ Hazardous Agents
- Biological (list agent and hazard level): AAV constructs
- Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.): 4% Formaldehyde
- Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.): MRI (magnetic fields and up to ~110 dB noise)

☐ Non-Centralized Animal Housing
- Location:
- Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 1
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 12/6/2021

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was administratively approved to add [redacted] and [redacted] as additional personnel to the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contact [redacted] to schedule.
☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT [redacted] prior to starting surgeries.
☒ **Other requirements [redacted] must be added to IBC disclosure before working with biohazardous materials.**

Total # of Animals: 8
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 12/17/2021
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

PERSONNEL MODIFICATION FORM
IACUC and IBC

This form can be used to add or remove participants from both an IACUC protocol and an IBC disclosure. Please submit only one form to Research.Integrity@asu.edu and it will be processed by both committees.

Principal Investigator Name: [redacted]	Phone [redacted]
Dept: ASU-Banner Neurodegenerative Disease Research Center	Email: [redacted]

Participant #1	Add to: ☐ IBC # ☒ IACUC #21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R Delete from: ☐ IBC # ☐ IACUC #	FOR ORIA USE ONLY Training Verification
Name [redacted]	ASURITE: [redacted] Email: [redacted]	
Project Responsibilities in IBC:		
Experience/Training in These Responsibilities:		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Intracranial surgery, intracarotid surgery, intracisternal injection, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified).		11/2021 OHSP
Species: Macaques, Mice **Experience and training with species and procedures:** 17 years' experience in primate research. 14 years' experience in rodent research. Experienced with intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy. Will be trained in intracisternal injection by Dr. [redacted]		

Participant #2	Add to: ☐ IBC # ☒ IACUC #21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R Delete from: ☐ IBC # ☐ IACUC #	FOR ORIA USE ONLY Training Verification
Nam [redacted]	ASURITE [redacted] Emai [redacted]	
Project Responsibilities in IBC:		
Experience/Training in These Responsibilities:		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Intracranial surgery, intracarotid surgery, intracisternal injection, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified).		11/2021 OHSP
Species: Macaques, Mice **Experience and training with species and procedures:** 2 years' experience in primate research. 3 years' experience in rodent research. Experienced with intracranial surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy. Will be trained in intracarotid surgery by [redacted] and [redacted] will be trained in PET scan by [redacted] will be trained in intracisternal injection by Dr. [redacted]		

Assurance

As Principal Investigator, I assure that personnel will receive appropriate training prior to working with animals or biological materials as applicable.

Principal Investigator Signature: [redacted] Date: 12/2/2021

Revised 11/20/12

FOR ORIA USE ONLY	☐ IBC Approved	☒ IACUC Approved 12/6/2021

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 2
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 12/10/2021

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was administratively approved to add [redacted] as additional personnel to the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contac[redacted] to schedule.

☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT[redacted], prior to starting surgeries.

☒ **Other requirements: Kyle must be added to IBC disclosure before working with biohazardous materials.**

Total # of Animals: 8
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 12/17/2021
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

PERSONNEL MODIFICATION FORM
IACUC and IBC

This form can be used to add or remove participants from both an IACUC protocol and an IBC disclosure. Please submit only one form to Research.Integrity@asu.edu and it will be processed by both committees.

Principal Investigator Name [redacted]	Phone [redacted]
Dept: ASU-Banner Neurodegenerative Disease Research Center	Email: [redacted]

Participant #3	Add to: ☐ IBC # ☒ IACUC #21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R Delete from: ☐ IBC # ☐ IACUC #	FOR ORIA USE ONLY Training Verification
Name [redacted]	ASURITE [redacted] Email: [redacted]	
Project Responsibilities in IBC:		
Experience/Training in These Responsibilities:		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Intracranial surgery, intracarotid surgery, intracisternal injection, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified).		11/2021 Basics & NHP 12/2021 Rodent OHSP
Species: Macaques, Mice **Experience and training with species and procedures:** 2 years' experience in primate research. 4 years' experience in rodent research. Experienced with intracranial surgery, MRI, blood/CSF collection, behavioral tests, administrati[redacted] of medications, and necropsy. Will be trained in intracarotid surgery by [redacted] an [redacted] will be trained in PET scan by [redacted] will be trained in intracisternal injection by Dr [redacted]		

Assurance

As Principal Investigator, I assure that personnel will receive appropriate training prior to working with animals or biological materials as applicable.

Principal Investigator Signature: [redacted] Date: 12/2/2021

FOR ORIA USE ONLY	☐ IBC Approved	☒ IACUC Approved 12/10/2021

Revised 11/20/12

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 3
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 4/21/2022

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was approved by Designated Review to add the option for an additional craniotomy during intracranial injection surgeries.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT a[redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contac[redacted] to schedule.
☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT[redacted], prior to starting surgeries.
☐ Other requirements:

Total # of Animals: 8
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 4/22/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

ARIZONA STATE UNIVERSITY

Institutional Animal Care and Use Committee

REQUEST FOR CHANGES TO AN APPROVED PROTOCOL

Protocol No. 21-1867R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1918R
Title: Differential diagnosis of Parkinson's and multiple system atrophy in non-human primate models using a novel a-synuclein retinal contrast agent and AI-assisted analytics
Reprogramming astrocytes to functional dopaminergic neurons in non-human primate brain
Co-Pathologies Drive Neuroinflammation and Progression in PD
Genetic Silencing of Striatal CaV1.3 Calcium Channels as a Potent Antidyskinetic Therapy for PD
AAV Trehalose in an NHP model of Alzheimer's DiseaseAAV-GBA Therapy in an NHP model of PD

Principal Investigator: [redacted] Email Address [redacted]
If not PI, whom should we contact for questions related to this amendment: [redacted] Email Address: [redacted]

☒ Funded ☐ Unfunded

Requested Change (check all that apply):

☐ New procedures to be performed complete Part A, and Appendix 1 and/or 2 as applicable, and sign assurance.
☐ New species and or an increase in the number of animals to be used complete Part A and sign assurance.
☐ New location of housing or procedures complete Part A and sign assurance.
☐ New personnel – complete Part B and sign assurance.
☒ Other (includes changes in dosages, funding, etc.) complete Part A and sign assurance.

A. Description of Requested Changes

For new procedures or additional animals that are USDA-covered species (all mammals EXCEPT mice and rats bred for research), list the **Category of Pain:**

For new procedures or additional animals that are not USDA-covered species, will there be the potential to involve more than slight or momentary pain or distress that will NOT be relieved with anesthetics, analgesics, tranquilizer drugs, or other methods for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

If you are adding a procedure that could create pain or distress, you need to include a **literature search** for alternatives.
If you are adding a new survival surgery, submit a surgical checklist.
If you are requesting an increase in animal numbers, provide justification with supportive statistics.
If you are adding additional funding sources, provide the grant agency, grant title and ASU proposal or award number.

Describe the changes you are requesting. We would like to add the option to make one additional craniotomy to visualize the superior sagittal sinus during intracranial injection surgeries under this protocol. Intraoperative navigation with the [redacted] is generally highly accurate following initial skin registration (i.e., correlation of the MRI scan with the animal's actual position in the stereotaxic frame using fiducial marker locations or tracing the skin surface with a tracked instrument). However, it is occasionally necessary for the surgeon to confirm navigational accuracy after the skin has been retracted with an anatomical landmark that is clearly visible on MRI. The superior sagittal sinus is ideal for this purpose and, prior to the adoption of intraoperative navigation with the [redacted] visualizing the sinus was the primary method of establishing a mediolateral zero point for stereotaxic MRI coordinates in all surgical cases, as described b [redacted]. In cases where visualizing the sinus is deemed necessary, the surgeon will make a small craniotomy (up to 10 x 3 mm) along the mediolateral axis. It is usually not necessary for the craniotomy to fully penetrate the skull and the surgeon will stop once the sinus is visible through the bone. In the very rare occasions when the sinus is inadvertently penetrated, digital pressure with surgical gel foam is sufficient to control bleeding. The craniotomy will be filled with gel foam prior to wound closure, which will be as previously described.

References:

[redacted]

B. Addition of Personnel

All personnel who work with animals are required to have animal care training within the last four years. ASU IACUC training modules can be completed at https://asu.co1.qualtrics.com/jfe/form/SV_b2b2XRXRRs1309f. Personnel are required to have Level III training certification on file with the IACUC office in order to perform procedures independently (without supervision). Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

*** Procedures other than husbandry, handling, or behavioral testing MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel who are not Level III certified are supervised at all times.**

Name	Title	ASURITE name	What activities will each person perform on live animals ONLY while under direct supervision?	What activities will each person be allowed to perform independently (including appropriate Level 3 certification*) at the time of protocol submission?	Species with which individual will have direct contact ("all" or list species) *	IACUC USE ONLY Training (mm/yy)

For each individual, describe the individual's training and years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

Assurance

As Principal Investigator of this protocol, I assure that all procedures will be conducted as described in this request for changes and that personnel will receive appropriate additional training prior to conducting any new procedures that are not listed above.

[redacted] 4/6/2022

Principal Investigator Date

For IACUC use only:

☐ Administratively approved - Approving administrator: Date of approval:

☐ Administratively handled by VCV - Veterinarian providing verification: Date of verification:

Sources used for verification:

☒ Approved by Designated Review – Designated reviewer [redacted] ate of approval: 4/21/2022

☐ Approved by Full Committee Review – Primary reviewer: Date of approval:

From: [redacted]
To:
Cc: [redacted] IACUC@asu.edu
Subject: RE: Action Required: Designated Review fo[redacted] Multiprotocol RFC
Date: Tuesday, April 19, 2022 4:18:09 PM
Attachments: image002.png

Thanks [redacted] I approve the modified amendment as the designated reviewer.

Good luck on the research[redacted]

From: [redacted]
Sent: Tuesday, April 19, 2022 2:51 PM
To [redacted]
Cc: [redacted] IACUC@asu.edu <IACUCasu.edu@mainex1.asu.edu>
Subject: RE: Action Required: Designated Review fo[redacted] Multiprotocol RFC

Great, please see the attached revisions.

[redacted]
Laboratory Manager
ASU-Banner Neurodegenerative Disease Research Center (NDRC)
Arizona State University
[redacted]

From: [redacted]
Sent: Tuesday, April 19, 2022 2:34 PM
To [redacted]
Cc: [redacted] IACUC@asu.edu <IACUCasu.edu@mainex1.asu.edu>
Subject: RE: Action Required: Designated Review for[redacted] Multiprotocol RFC

Hi[redacted] Yes this outlines and justifies the process. This is very helpful. Please add a sentence with the reference to the amendment. Thanks!

From [redacted]
Sent: Tuesday, April 19, 2022 2:14 PM
To [redacted]
Cc: [redacted] IACUC@asu.edu <IACUCasu.edu@mainex1.asu.edu>
Subject: RE: Action Required: Designated Review fo[redacted] Multiprotocol RFC

H[redacted] he attached paper describes the stereotaxic surgery without the [redacted] neuronavigation, including the exposure of the sagittal sinus for mediolateral zero. Is that what you are looking for?

[redacted]
Laboratory Manager
ASU-Banner Neurodegenerative Disease Research Center (NDRC)
Arizona State University
[redacted]

From [redacted]
Sent: Tuesday, April 19, 2022 10:21 AM
To [redacted]
Subject: FW: Action Required: Designated Review fo[redacted] Multiprotocol RFC

See below

[redacted]
Professor of Life Sciences
College of Liberal Arts and Sciences

Arizona State University
[redacted]

From: [redacted]
Date: Tuesday, April 19, 2022 at 10:15 AM
To [redacted]
Cc: [redacted] "iacuc@asu.edu" <iacuc@asu.edu>
Subject: RE: Action Required: Designated Review fo[redacted] Multiprotocol RFC

H[redacted]

I am the designated reviewer for your amendment. I have no concerns, but can you please add some references to justify your discussion. Thank you!

Best,

[redacted]

Professor of Nutrition
Arizona State University | College of Health Solutions
[redacted]

College of Health Solutions
Arizona State University

From [redacted]
Sent: Tuesday, April 19, 2022 9:47 AM
To: [redacted] Dale DeNard Karen Kibler [redacted]
Cc: IACUC@asu.edu
Subject: Action Required: Designated Review fo[redacted] Multiprotocol RFC
Importance: High

Designated Reviewer: [redacted]
Principal Investigator:
Peer Reviewer:
Protocol Number: **Multiprotocol RFC**

A request for an amendment to the referenced protocols has been submitted. The request for amendment is attached and the various related protoc s are available at the SharePoint site. The Designated Review process allows IACUC members, by voting, to permit the Designated Reviewer to approve or disapprove an amendment request.

Select "**YES**" if you approve the use of the designated review process for this amendment.

Select "**NO**" if you disapprove the use of designated review process regarding this amendment request. The amendment will then be reviewed at the next monthly IACUC meeting.

Select "**Abstain**" if you would like to abstain from the vote for any reason.

Select "**Recuse**" if you have a conflict of interest.

Please indicate your approval or disapproval of the request for designated review by using the YES or NO

[redacted]

To: [redacted]iacuc@asu.edu
Subject: RE: Action Required: Designated Review fo[redacted] Multiprotocol RFC

From [redacted]
Sent: Tuesday, April 19, 2022 10:47 AM
To [redacted] Dale DeNardo Karen Kibler [redacted]
Cc: IACUC@asu.edu
Subject: Action Required: Designated Review fo[redacted] Multiprotocol RFC
Importance: High

Designated Reviewer: [redacted]
Principal Investigator:
Peer Reviewer:
Protocol Number: **Multiprotocol RFC**

Tracking:	**Response**
[redacted]	Yes: 4/19/2022 3:25 PM
Dale DeNardo	Yes: 4/19/2022 4:18 PM
[redacted]	Yes: 4/19/2022 11:56 AM
Karen Kibler	Yes: 4/19/2022 11:02 AM
[redacted]	Yes: 4/19/2022 10:49 AM
	Yes: 4/19/2022 12:20 PM
	Yes: 4/19/2022 4:08 PM
	Yes: 4/19/2022 12:53 PM
	Yes: 4/19/2022 11:51 AM

A request for an amendment to the referenced protocols has been submitted. The request for amendment is attached and the various related protocols are available at the SharePoint site. The Designated Review process allows IACUC members, by voting, to permit the Designated Reviewer to approve or disapprove an amendment request.

Select "**YES**" if you approve the use of the designated review process for this amendment.

Select "**NO**" if you disapprove the use of designated review process regarding this amendment request. The amendment will then be reviewed at the next monthly IACUC meeting.

Select "**Abstain**" if you would like to abstain from the vote for any reason.

Select "**Recuse**" if you have a conflict of interest.

Please indicate your approval or disapproval of the request for designated review by using the YES or NO button in the toolbar at the top of this message. The use of "YES or NO" buttons allows you to submit comments along with your choice. **You may also send your comments to me directly or to the primary reviewer without using the selection buttons. Please copy me on all correspondence and email related to this request.**

Sincerely,

IACUC Protocol Trackable Components Checklist

Protocol #: 22-1887R If for amendment, amendment #: 3

PI [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 4/13/2022

☐ No trackable components in this document

Exceptions to the Guide:

☐ Food/Fluid Regulation
- Species:
- What Restricted:
- Parameters:

☐ Prolonged Restraint
- Species:
- Details:

☒ Husbandry Deviation from the Guide
- Species: NHP
- Deviation: Single housing if suitable pairing partners are not available.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)
- Species: NHP
- Surgerie(s): Intracranial injection
- Multiple Major?: ☒ Yes ☐ No

☒ Hazardous Agents
- Biological (list agent and hazard level): AAV constructs
- Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.): 4% Formaldehyde
- Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.): MRI (magnetic fields and up to ~110 dB noise)

☐ Non-Centralized Animal Housing
- Location:
- Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 4
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
ASU Principal Investigator: [redacted]
Date of Action: 5/31/2022

The animal protocol review was considered by the Committee and the following decisions were made:

Request for changes was approved to modify procedures on the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contac[redacted] to schedule.
☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DAC[redacted] prior to starting surgeries.
☐ Other requirements:

Total # of Animals: 8
Species: NHP **Pain Category: D**

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature [redacted] Date: 6/10/2022
IACUC Chair or *Designee*
Cc: IACUC Office, IACUC Chair

ARIZONA STATE UNIVERSITY

Institutional Animal Care and Use Committee

REQUEST FOR CHANGES TO AN APPROVED PROTOCOL

Protocol No. 22-1887R RFC 4
Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted] Email Address: [redacted]
If not PI, whom should we contact for questions related to this amendment: [redacted] Email Address: [redacted]

☒ Funded ☐ Unfunded

Requested Change (check all that apply):

☐ New procedures to be performed complete Part A, and Appendix 1 and/or 2 as applicable, and sign assurance.
☐ New species and or an increase in the number of animals to be used complete Part A and sign assurance.
☐ New location of housing or procedures complete Part A and sign assurance.
☐ New personnel complete Part B and sign assurance.
☒ Other (includes changes in dosages, funding, etc.) complete Part A and sign assurance.

A. Description of Requested Changes

For new procedures or additional animals that are USDA-covered species (all mammals EXCEPT mice and rats bred for research), list the **Category of Pain:**

For new procedures or additional animals that are not USDA-covered species, will there be the potential to involve more than slight or momentary pain or distress that will NOT be relieved with anesthetics, analgesics, tranquilizer drugs, or other methods for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

If you are adding a procedure that could create pain or distress, you need to include a **literature search** for alternatives.
If you are adding a new survival surgery, submit a surgical checklist.
If you are requesting an increase in animal numbers, provide justification with supportive statistics.
If you are adding additional funding sources, provide the grant agency, grant title and ASU proposal or award number.

Describe the changes you are requesting. After further discussions with the sponsor, in order to better examine the effect of trehalose on tau pathology we would like to modify the study design as follows. Notable changes are moving to bilateral AAV-tau injections in the ERC in both groups in order to have a lesioned and untreated hemisphere against which to compare the lesioned and treated hemisphere. We are also eliminating the second surgery in group 1 and will instead inject the AAV-trehalose in the same surgery as the AAV-tau in order to look at neuroprotection prior to the onset of tau pathology, while group 2 will receive AAV-trehalose two months following the AAV-tau injections, after the onset of early tau pathology. It was decided to eliminate the empty vector control in favor of PBS.

1. PBS is the diluent used in the manufacture of the therapeutic vector and is the solution containing the AAV's, our scientific question in this study is whether the presence or absence of therapeutic AAV product is beneficial in the therapy model
2. The aim of this study is to compare and verify whether the therapy (AAV) has an effect and absence of AAV, i.e. the PBS diluent, is the best comparator currently
3. The FDA and other regulatory agencies are actively debating the role of empty vectors and it will not be a good comparator for the therapy vector (AAV) as the therapy vector is also bound to contain a variable amount of empty vectors
4. There is no truly empty capsids, empty capsids are not truly empty but may contain process- or product-related impurities, cellular debris, or plasmid fragments.
5. Since the same animal will be used as a control and it is well known that the AAV capsids elicit immune response and the current design also calls for multiple dosing using the same AAV9 capsids this can lead to a cascading immune response, adding more aav capsid load to the animals is not the right comparator in the current study design

In conclusion, based on the current understanding of AAV manufacturing technologies, international regulatory agency discussion and guidelines on this topic along with ultimate the goals of this study which follow the 3R's (replacement, reduction, refinement) of primate animal use in research. In our current study we plan to use the same animal as a control (reduction) following the 3R's, thus adding an empty vector only adds and contributes to eliciting an immune response without contributing to our understanding of whether the therapy was beneficial. All these factors together are the reason to use PBS as control.
Finally, we are doubling the volume of the hippocampal injections due to lower than expected production AAV-trehalose viral titer (7.4E12 vg/mL, rather than 3E13 vg/mL, about 4 times lower) in order to maximize the chance of therapeutic efficacy. All animals will receive a total of 80 µL AAV-tau, 80 µL AAV-trehalose, and 80 µL PBS. Due to the increased injection volumes, surgery duration for group 1 is expected to be 5 ½ hours, maximum duration 6 ½ hours. Surgery duration for group 2 first injection is expected to be 2 ½ hours, second injection 4 hours, maximum duration 5 hours.
Finally, we are increasing the study timeline to 6 months following the first surgery in order to maximize tau pathology in our lesions.

Group	First Injection	Second Injection (2 mo later)	N=	Sacrifice
1	AAV-double mutant tau bilaterally in ERC (2 x 20 µL) AAV-trehalose in Left Hippocampus (2 x 40 µL) PBS in Right Hippocampus (2 x 40 µL)	N/A	4	6 mo post 1st injection
2	AAV-double mutant tau bilaterally in ERC (2 x 20 µL)	AAV-trehalose in Left Hippocampus (2 x 40 µL) PBS in Right Hippocampus (2 x 40 µL)	4	4 mo post 2nd injection

B. Addition of Personnel

All personnel who work with animals are required to have animal care training within the last four years. ASU IACUC training modules can be completed at https://asu.co1.qualtrics.com/jfe/form/SV_b2b2XRXRRs1309f. Personnel are required to have Level III training certification on file with the IACUC office in order to perform procedures independently (without supervision). Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

*** Procedures other than husbandry, handling, or behavioral testing MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel who are not Level III certified are supervised at all times.**

Name	Title	ASURITE name	What activities will each person perform on live animals ONLY while under direct supervision?	What activities will each person be allowed to perform independently (including appropriate Level 3 certification*) at the time of protocol submission?	Species with which individual will have direct contact ("all" or list species) *	IACUC USE ONLY Training (mm/yy)

For each individual, describe the individual's training and years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

Assurance

As Principal Investigator of this protocol, I assure that all procedures will be conducted as described in this request for changes and that personnel will receive appropriate additional training prior to conducting any new procedures that are not listed above.

SIG[redacted]

5/20/2022

Principal Investigator Date

For IACUC use only:

- ☐ Administratively approved - Approving administrator: Date of approval:
- ☐ Administratively handled by VCV - Veterinarian providing verification: Date of verification:
  Sources used for verification:
- ☒ Approved by Designated Review – Designated reviewer: Karen K bler Date of approval: 5/31/2022
- ☐ Approved by Full Committee Review – Primary reviewer: Date of approval:

From: [redacted]
To: [redacted] Dale DeNardo [redacted] Karen Kibler [redacted]
Cc: IACUC@asu.edu
Subject: Action Required: Designated Review fo[redacted]22-1887R RFC 4
Date: Wednesday, May 25, 2022 11:14:00 AM
Attachments: [redacted]22-1887R RFC 4 Vet Cleared.docx
image001.png
Importance: High

Designated Reviewer: Karen Kibler
Principal Investigator: [redacted]
Peer Reviewer: TBD by Chair
Protocol Number: 22-1887R RFC 4

A request for an amendment to the referenced protocols has been submitted. The request for amendment is attached and the various related protocols are available at the SharePoint site. The Designated Review process allows IACUC members, by voting, to permit the Designated Reviewer to approve or disapprove an amendment request.

Select "**YES**" if you approve the use of the designated review process for this amendment.

Select "**NO**" if you disapprove the use of designated review process regarding this amendment request. The amendment will then be reviewed at the next monthly IACUC meeting.

Select "**Abstain**" if you would like to abstain from the vote for any reason.

Select "**Recuse**" if you have a conflict of interest.

Please indicate your approval or disapproval of the request for designated review by using the YES or NO button in the toolbar at the top of this message. The use of "YES or NO" buttons allows you to submit comments along with your choice. **You may also send your comments to me directly or to the primary reviewer without using the selection buttons. Please copy me on all correspondence and email related to this request.**

Thank you,

[redacted] | Compliance Coordinator, Office of Research Integrity & Assurance
Arizona State University | Knowledge Enterprise | Operations
[redacted] | http://researchintegrity.asu.edu
How am I doing? Email my[redacted] or send a Sun Award
Chat with me on Teams! (ASU Users Only)

[redacted]22-1887R RFC 4 Designated Review Votes

Name	Vote
[redacted]	Yes: 5/25/2022 1:53 PM
[redacted]	Yes: 5/25/2022 11:31 AM
[redacted]	Yes: 5/25/2022 12:17 PM
Dale DeNardo	Yes: 5/25/2022 11:34 AM
[redacted]	Yes: 5/26/2022 6:59 AM
Karen Kibler	Yes: 5/26/2022 1:36 PM
[redacted]	Yes: 5/25/2022 11:20 AM
[redacted]	Yes: 5/25/2022 11:24 AM
[redacted]	Yes: 5/25/2022 11:16 AM
[redacted]	Yes: 5/26/2022 10:49 AM
[redacted]	

[redacted]

From: Karen Kibler
To: [redacted]
Cc: IACUC@asu.edu
Subject: RE: revised RFC
Date: Thursday, May 26, 2022 1:36:13 PM
Attachments: image001.png

Great – thank you!

Karen

From: [redacted]
Sent: Thursday, May 26, 2022 1:33 PM
To: Karen Kible[redacted]
Cc: IACUC@asu.edu
Subject: RE: revised RFC

Hi Karen,

The 2 business day time frame is not over yet, and we do not have all members approving the use of the DR process (but we do have a quorum). So we can change the approved version no problem

Thank you,

[redacted] | Compliance Coordinator, Office of Research Integrity & Assurance
Arizona State University | Knowledge Enterprise | Operations
[redacted] | http://researchintegrity.asu.edu
How am I doing? Email m[redacted] or send a Sun Award
Chat with me on Teams! (ASU Users Only)

From: Karen Kible[redacted]
Sent: Thursday, May 26, 2022 1:30 PM
To: [redacted]
Cc: IACUC@asu.edu
Subject: revised RFC

Hi [redacted]an [redacted]

After I heard from the peer reviewer and from [redacted] I approved as writter[redacted] sent this version. Can we make this one the official?

Thanks,
Karen

IACUC Protocol Trackable Components Checklist

Protocol #: 22-1887R If for amendment, amendment #:

PI: [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 5/23/22

☐ No trackable components in this document

Exceptions to the Guide:

☐ Food/Fluid Regulation
- Species:
- What Restricted:
- Parameters:

☐ Prolonged Restraint
- Species:
- Details:

☒ Husbandry Deviation from the Guide
- Species: NHP
- Deviation: Single housing if suitable pairing partners are not available.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)
- Species: NHP
- Surgerie(s): Intracranial injection
- Multiple Major?: ☒ Yes ☐ No

☒ Hazardous Agents
- Biological (list agent and hazard level): AAV constructs
- Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.): 4% Formaldehyde
- Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.): MRI (magnetic fields and up to ~110 dB noise)

☐ Non-Centralized Animal Housing
- Location:
- Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 5
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 7/6/2022

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was administratively approved to add [redacted] as additional personnel to the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contac[redacted]to schedule.
☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT [redacted] prior to starting surgeries.
☐ Other requirements:

Total # of Animals: 8
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 7/7/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

PERSONNEL MODIFICATION FORM
IACUC and IBC

This form can be used to add or remove participants from both an IACUC protocol and an IBC disclosure. Please submit only one form to Research.Integrity@asu.edu and it will be processed by both committees.

Principal Investigator Name: [redacted]	Phone [redacted]
Dept: ASU-Banner Neurodegenerative Disease Research Center	Email: [redacted]

Participant #1	**Add to:** ☒ **IBC #**SPROTO202100000070 ☒ **IACUC #** 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R **Delete from:** ☐ **IBC #** ☐ **IACUC #**	**FOR ORIA USE ONLY Training Verification**
Name [redacted]	**ASURIT** [redacted] **Email:** [redacted]	
Project Responsibilities in IBC: Will handle AAV viral vectors, alpha-synuclein preformed fibrils, Human Lewy Body extracts, and mouse/rat/nonhuman primate blood/CSF/brain tissue.		Added in ERA
Experience/Training in These Responsibilities: 7 years' experience in rodent and macaque research with ASU DACT.		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Macaques: Intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified). Rats: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified). Mice: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified).		5/2019 OHSP
Species: Macaques, Rats, Mice **Experience and training with species and procedures:** 7 years' experience in rodent and macaque research with ASU DACT.		

Assurance

As Principal Investigator, I assure that personnel will receive appropriate training prior to working with animals or biological materials as applicable.

Principal Investigator Signature: [redacted] Date: 7/1/22

FOR ORIA USE ONLY	☐ **IBC Approved**	☒ **IACUC Approved 7/6/2022**

Revised 11/20/12

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 6
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 7/15/2022

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was approved by Designated Review to alter previously approved procedures.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contact [redacted] to schedule.
☐ This protocol indicates that there are surgical procedures. A surgical [redacted] hecklist may be required to be submitted to Research Support Services within DAC [redacted] prior to starting surgeries.
☐ Other requirements:

Total # of Animals: 8
Species: NHP **Pain Category: D**

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature [redacted] Date: 7/15/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

ARIZONA STATE UNIVERSITY

Institutional Animal Care and Use Committee

REQUEST FOR CHANGES TO AN APPROVED PROTOCOL

Protocol No. 22-1887R RFC 6
Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted] Email Address: [redacted]
If not PI, whom should we contact for questions related to this amendment [redacted] Email Address: [redacted]

☒ Funded ☐ Unfunded

Requested Change (check all that apply):

☐ New procedures to be performed complete Part A, and Appendix 1 and/or 2 as applicable, and sign assurance.
☐ New species and or an increase in the number of animals to be used complete Part A and sign assurance.
☐ New location of housing or procedures complete Part A and sign assurance.
☐ New personnel complete Part B and sign assurance.
☒ Other (includes changes in dosages, funding, etc.) complete Part A and sign assurance.

A. Description of Requested Changes

For new procedures or additional animals that are USDA-covered species (all mammals EXCEPT mice and rats bred for research), list the **Category of Pain:**

For new procedures or additional animals that are not USDA-covered species, will there be the potential to involve more than slight or momentary pain or distress that will NOT be relieved with anesthetics, analgesics, tranquilizer drugs, or other methods for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

If you are adding a procedure that could create pain or distress, you need to include a **literature search** for alternatives.
If you are adding a new survival surgery, submit a surgical checklist.
If you are requesting an increase in animal numbers, provide justification with supportive statistics.
If you are adding additional funding sources, provide the grant agency, grant title and ASU proposal or award number.

Describe the changes you are requesting. The sponsor has conducted an internal study with multiple different AAV9-test vectors and PBS control, and as expected the AAV vector injected hemispheres in the animals had a greater response compared to PBS only injected hemispheres in the same animals. This led to the concern that using only PBS injections in the control hemisphere will lead to an unfair comparison as a control and not serve its purpose. For this reason, we have made a new control AAV-scramble vector with the same sequences as the test vector, except the region coding for the trehalose enzymes has been replaced with a scramble sequence which is a superior control to using either an empty capsid or PBS only injection. To ensure both hemispheres receive the same AAV9 capsid load, we will use the same vector titer and volume for the AAV-double mutant tau, AAV-trehalose, and AAV-scramble vectors in each hemisphere. It was also decided to eliminate the second surgery in group 2 to avoid the risk that the immune response to the AAV9 capsid following the first vector injection would prime the immune system to target the test AAV-trehalose vector during the second injection. Instead, we will focus solely on achieving neuroprotection prior to the onset of tau pathology using two dose levels of the AAV-trehalose vector, a high dose (7.4×10^{12} vg/mL) and a low dose (3.7×10^{12} vg/mL). As in RFC 4, surgery duration is expected to be 5 ½ hours, maximum duration 6 ½ hours. All animals will be sacrificed 6 months following injection.

Group	Treatment	N=	Sacrifice
1 [redacted]	AAV-double mutant tau bilaterally in ERC (2 x 20 µL) (1×10^{13} vg/mL) AAV-trehalose in Left Hippocampus (2 x 40 µL) pBK1802 [redacted] AAV-scramble in Right Hippocampus (2 x 40 µL) pBK180 [redacted]	4	[redacted]

2 [redacted]	AAV-double mutant tau bilaterally in ERC (2 x 20 µL) (1 x 10^{13} vg/mL) AAV-trehalose in Left Hippocampus (2 x 40 µL) pBK180 2 [redacted] AAV-scramble in Right Hippocampus (2 x 40 µL) pBK180 3	4	[redacted]

B. Addition of Personnel

All personnel who work with animals are required to have animal care training within the last four years. ASU IACUC training modules can be completed at https://asu.co1.qualtrics.com/ife/form/SV_b2b2XRXRRs1309f. Personnel are required to have Level III training certification on file with the IACUC office in order to perform procedures independently (without supervision). Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

*** Procedures other than husbandry, handling, or behavioral testing MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel who are not Level III certified are supervised at all times.**

Name	Title	ASURITE name	What activities will each person perform on live animals ONLY while under direct supervision?	What activities will each person be allowed to perform independently (including appropriate Level 3 certification*) at the time of protocol submission?	Species with which individual will have direct contact ("all" or list species) *	IACUC USE ONLY Training (mm/yy)

For each individual, describe the individual's training and years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

Assurance

As Principal Investigator of this protocol, I assure that all procedures will be conducted as described in this request for changes and that personnel will receive appropriate additional training prior to conducting any new procedures that are not listed above.

SIGNED: [redacted]

Principal Investigator

7/6/22
Date

For IACUC use only:

☐ Administratively approved - Approving administrator: Date of approval:
☐ Administratively handled by VCV - Veterinarian providing verification: Date of verification:
Sources used for verification:
☒ Approved by Designated Review – Designated reviewer: Karen K bler Date of approval: 7/15/2022
☐ Approved by Full Committee Review – Primary reviewer: Date of approval:

From: Karen Kibler
To: [redacted]
Cc: IACUC@asu.edu
Subject: RE: [redacted]2-1887R RFC 6 - Ready for Assignment
Date: Friday, July 15, 2022 1:30:16 PM
Attachments: image001.png

Hello, [redacted]

The peer reviewer approved, and I have no questions.

Thanks,
Karen

From: [redacted]
Sent: Friday, July 15, 2022 12:02 PM
To: Karen Kible[redacted]
Cc: IACUC@asu.edu
Subject: RE [redacted]22-1887R RFC 6 - Ready for Assignment

Hi Karen,

I am just following up on the emails below and the Designated Review for [redacted]22-1887R RFC 6 (attached).

Please let us know if, as DR, you approve of the RFC as is or if you are still working with the PI, and if the peer review is done.

Thanks!

[redacted] Compliance Coordinator, Office of Research Integrity & Assurance
Arizona State University | Knowledge Enterprise | Operations
[redacted] http://researchintegrity.asu.edu
How am I doing? Email m[redacted]or send a Sun Award
Chat with me on Teams! (ASU Users Only)

From: Karen Kible[redacted]
Sent: Monday, July 11, 2022 9:49:27 PM (UTC+00:00) Monrovia, Reykjavik
To: [redacted]
Cc: IACUC@asu.edu <iacuc@asu.edu>

[redacted]

To: IACUC@asu.edu
Subject: RE: Action Required: Designated Review fo[redacted]22 1887R RFC 6

From [redacted]
Sent: Monday, July 11, 2022 8:22:05 PM (UTC+00:00) Monrovia, Reykjavik
To: [redacted] Dale DeNardo [redacted] Karen Kibler [redacted]
Cc: IACUC@asu.edu <iacuc@asu.edu>
Subject: Action Required: Designated Review for [redacted] 22-1887R RFC 6

Designated Reviewer: Karen Kibler
Principal Investigator: [redacted]
Peer Reviewer: TBD by Chair
Protocol Number: 22-1887R RFC 6

Tracking:	Recipient	Response
	[redacted]	Yes: 7/11/2022 3:35 PM
		Yes: 7/11/2022 5:48 PM
		Yes: 7/11/2022 4:22 PM
	Dale DeNardo	
	[redacted]	Yes: 7/12/2022 6:13 AM
	Karen Kibler	Yes: 7/11/2022 2:28 PM
	[redacted]	Yes: 7/11/2022 5:04 PM
		Yes: 7/11/2022 2:23 PM
		Yes: 7/11/2022 2:29 PM
		Yes: 7/11/2022 2:47 PM

A request for an amendment to the referenced protocols has been submitted. The request for amendment is attached and the various related protocols are available at the SharePoint site. The Designated Review process allows IACUC members, by voting, to permit the Designated Reviewer to approve or disapprove an amendment request.

Select "**YES**" if you approve the use of the designated review process for this amendment.

Select "**NO**" if you disapprove the use of designated review process regarding this amendment request. The amendment will then be reviewed at the next monthly IACUC meeting.

Select "**Abstain**" if you would like to abstain from the vote for any reason.

Select "**Recuse**" if you have a conflict of interest.

Please indicate your approval or disapproval of the request for designated review by using the YES or NO button in the toolbar at the top of this message. The use of "YES or NO" buttons allows you to submit comments along with your choice. **You may also send your comments to me directly or to the primary reviewer without using the selection buttons. Please copy me on all correspondence and email related to this request.**

Thank you,

[redacted] Compliance Coordinator, Office of Research Integrity & Assurance

IACUC Protocol Trackable Components Checklist

Protocol #: 22-1887R If for amendment, amendment #: 6

P[redacted]

Species: NHP Highest Category of Pain: D

Completed by: [redacted] Date completed: 7/7/22

☐ No trackable components in this document

Exceptions to the Guide:

☐ Food/Fluid Regulation
- Species:
- What Restricted:
- Parameters:

☐ Prolonged Restraint
- Species:
- Details:

☒ Husbandry Deviation from the Guide
- Species: NHP
- Deviation: Single housing if suitable pairing partners are not available.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)
- Species: NHP
- Surgerie(s): Intracranial injection
- Multiple Major?: ☒ Yes ☐ No

☒ Hazardous Agents
- Biological (list agent and hazard level): AAV constructs
- Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.): 4% Formaldehyde
- Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.): MRI (magnetic fields and up to ~110 dB noise)

☐ Non-Centralized Animal Housing
- Location:
- Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University
[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 7
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 8/2/2022

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was administratively approved to add [redacted] an [redacted] as additional personnel to the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contact [redacted] to schedule.

☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT [redacted] prior to starting surgeries.

☒ **Other requirements: IBC approval of new personnel is required before work with biohazardous materials may begin.**

Total # of Animals: 8
Species: NHP **Pain Category: D**

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 8/2/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

PERSONNEL MODIFICATION FORM
IACUC and IBC

This form can be used to add or remove participants from both an IACUC protocol and an IBC disclosure. Please submit only one form to Research.Integrity@asu.edu and it will be processed by both committees.

Principal Investigator Name [redacted]	Phone [redacted]
Dept: ASU-Banner Neurodegenerative Disease Research Center	Email: [redacted]

Participant #1	**Add to:** ☒ **IBC #**SPROTO202100000070 ☒ **IACUC #** 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R **Delete from:** ☐ **IBC #** ☐ **IACUC #**	**FOR ORIA USE ONLY Training Verification**
Name [redacted] **ASURITE** [redacted] **Email:** [redacted]		
Project Responsibilities in IBC: Will handle AAV viral vectors, alpha-synuclein preformed fibrils, Human Lewy Body extracts, and mouse/rat/nonhuman primate blood/CSF/brain tissue.		
Experience/Training in These Responsibilities: No previous experience		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Macaques: Intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified). Rats: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified). Mice: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified).		7/2022 OHSP
Species: Macaques, Rats, Mice **Experience and training with species and procedures:** No previous experience		
Participant #2	**Add to:** ☒ **IBC #**SPROTO202100000070 ☒ **IACUC #** 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R **Delete from:** ☐ **IBC #** ☐ **IACUC #**	**FOR ORIA USE ONLY Training Verification**
Name [redacted] **ASURITE** [redacted] **Email** [redacted]		
Project Responsibilities in IBC: Will handle AAV viral vectors, alpha-synuclein preformed fibrils, Human Lewy Body extracts, and mouse/rat/nonhuman primate blood/CSF/brain tissue.		
Experience/Training in These Responsibilities: No previous experience		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Macaques: Intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified). Rats: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified). Mice: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified).		7/2022 OHSP
Species: Macaques, Rats, Mice **Experience and training with species and procedures:** No previous experience		

Assurance

As Principal Investigator, I assure that personnel will receive appropriate training prior to working with animals or biological materials as applicable.

Principal Investigator Signature: [redacted] Date: 8/1/22

FOR ORIA USE ONLY	☐ IBC Approved	☒ IACUC Approved 8/2/2022

Revised 11/20/12

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 9
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 8/11/2022

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was approved by Designated Review to update the possible detrimental sequelae on the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

- ☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contac[redacted]to schedule.
- ☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DAC[redacted] prior to starting surgeries.
- ☐ Other requirements:

Total # of Animals: 8
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 8/11/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

ARIZONA STATE UNIVERSITY

Institutional Animal Care and Use Committee

REQUEST FOR CHANGES TO AN APPROVED PROTOCOL

Protocol No. 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R

Title: Differential diagnosis of Parkinson's and multiple system atrophy in non-human primate models using a novel a-synuclein retinal contrast agent and AI-assisted analytics
Kinase activation in multiple system atrophy
Reprogramming astrocytes to functional dopaminergic neurons in non-human primate brain
Co-Pathologies Drive Neuroinflammation and Progression in PD
Genetic Silencing of Striatal CaV1.3 Calcium Channels as a Potent Antidyskinetic Therapy for PD
AAV Trehalose in an NHP model of Alzheimer's Disease
BAG3 in Rodent Models of Neurodegenerative Disease
Bifunctional intrabody targeting intracellular alpha-synuclein
Primate Holding, Assessment, and Training
AAV-GBA Therapy in an NHP model of PD

Principal Investigator: [redacted] Email Address: [redacted]

If not PI, whom should we contact for questions related to this amendment [redacted] Email Address: [redacted]

☒ Funded ☐ Unfunded

Requested Change (check all that apply):

☐ New procedures to be performed – complete Part A, and Appendix 1 and/or 2 as applicable, and sign assurance.
☐ New species and or an increase in the number of animals to be used – complete Part A and sign assurance.
☐ New location of housing or procedures – complete Part A and sign assurance.
☐ New personnel – complete Part B and sign assurance.
☒ Other (includes changes in dosages, funding, etc.) – complete Part A and sign assurance.

A. Description of Requested Changes

For new procedures or additional animals that are USDA-covered species (all mammals EXCEPT mice and rats bred for research), list the **Category of Pain:**

For new procedures or additional animals that are not USDA-covered species, will there be the potential to involve more than slight or momentary pain or distress that will NOT be relieved with anesthetics, analgesics, tranquilizer drugs, or other methods for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

If you are adding a procedure that could create pain or distress, you need to include a **literature search** for alternatives.
If you are adding a new survival surgery, submit a surgical checklist.
If you are requesting an increase in animal numbers, provide justification with supportive statistics.
If you are adding additional funding sources, provide the grant agency, grant title and ASU proposal or award number.

Describe the changes you are requesting. We would like to add additional possible detrimental sequelae.

Possible Clinical Effect	Probability of Occurrence	Treatment
Surgical and other procedures performed under anesthesia may rarely result in death or permanent disability due to hemorrhage, edema, thrombosis, infection, toxicity, or complications due to anesthesia.	Rare	Consult with veterinary staff if clinical signs develop. Euthanasia may be considered.

B. Addition of Personnel

All personnel who work with animals are required to have animal care training within the last four years. ASU IACUC training modules can be completed at https://asu.co1.qualtrics.com/jfe/form/SV_b2b2XRXRRs1309f. Personnel are required to have Level III training certification on file with the IACUC office in order to perform procedures independently (without supervision). Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

*** Procedures other than husbandry, handling, or behavioral testing MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel who are not Level III certified are supervised at all times.**

Name	Title	ASURITE name	What activities will each person perform on live animals ONLY while under direct supervision?	What activities will each person be allowed to perform independently (including appropriate Level 3 certification*) at the time of protocol submission?	Species with which individual will have direct contact ("all" or list species) *	IACUC USE ONLY Training (mm/yy)

For each individual, describe the individual's training and years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

Assurance

As Principal Investigator of this protocol, I assure that all procedures will be conducted as described in this request for changes and that personnel will receive appropriate additional training prior to conducting any new procedures that are not listed above.

SIGNED: [redacted] 8/4/2022

Principal Investigator Date

For IACUC use only:

- ☐ Administratively approved - Approving administrator: Date of approval:
- ☐ Administratively handled by VCV - Veterinarian providing verification: Date of verification:
  Sources used for verification:
- ☒ Approved by Designated Review – Designated reviewer: Karen K bler Date of approval: 8/11/2022
- ☐ Approved by Full Committee Review – Primary reviewer: Date of approval:

From: Karen Kibler
To: [redacted]
Cc: IACUC@asu.edu
Subject: Multiprotocol RFC
Date: Tuesday, August 9, 2022 10:46:50 AM
Attachments: [redacted]Multiprotocol RFC 8.5.2022 Final.docx

Hello [redacted]

The attached version is DR approved.

Thanks,
Karen

[redacted]

To: [redacted]iacuc@asu.edu
Subject: RE: Action Required: Designated Review fo[redacted]Multiprotocol RFC 8.5.2022

From[redacted]
Sent: Tuesday, August 9, 2022 9:47 AM
To[redacted]Dale DeNardo[redacted]Karen Kibler[redacted]
Cc: IACUC@asu.edu
Subject: Action Required: Designated Review for[redacted]Multiprotocol RFC 8.5.2022
Importance: High

Designated Reviewer: Karen Kibler
Principal Investigator: [redacted]
Peer Reviewer: N/A
Protocol Number: [redacted]**Multiprotocol RFC 8.5.2022**

Tracking:	Recipient	Response
	[redacted]	Yes: 8/9/2022 10:15 AM
		Yes: 8/9/2022 10:10 AM
	Dale DeNardo	Yes: 8/9/2022 10:31 AM
	[redacted]	Yes: 8/10/2022 7:56 AM
	Karen Kibler	Yes: 8/9/2022 10:15 AM
	[redacted]	Yes: 8/9/2022 11:17 AM
		Yes: 8/9/2022 3:29 PM
		Yes: 8/9/2022 10:13 AM
		Yes: 8/10/2022 5:06 PM

A request for an amendment to the referenced protocols has been submitted. The request for amendment is attached and the various related protocols are available at the SharePoint site. The Designated Review process allows IACUC members, by voting, to permit the Designated Reviewer to approve or disapprove an amendment request.

Select "**YES**" if you approve the use of the designated review process for this amendment.

Select "**NO**" if you disapprove the use of designated review process regarding this amendment request. The amendment will then be reviewed at the next monthly IACUC meeting.

Select "**Abstain**" if you would like to abstain from the vote for any reason.

Select "**Recuse**" if you have a conflict of interest.

Please indicate your approval or disapproval of the request for designated review by using the YES or NO button in the toolbar at the top of this message. The use of "YES or NO" buttons allows you to submit comments along with your choice. **You may also send your comments to me directly or to the primary reviewer without using the selection buttons. Please copy me on all correspondence and email related to this request.**

Sincerely,

IACUC Protocol Trackable Components Checklist

Protocol #: 22-1887R If for amendment, amendment #: 9

PI [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 8/8/22

☐ No trackable components in this document

Exceptions to the Guide:

☐ Food/Fluid Regulation
- Species:
- What Restricted:
- Parameters:

☐ Prolonged Restraint
- Species:
- Details:

☒ Husbandry Deviation from the Guide
- Species: NHP
- Deviation: Single housing if suitable pairing partners are not available.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)
- Species: NHP
- Surgerie(s): Intracranial injection
- Multiple Major?: ☒ Yes ☐ No

☒ Hazardous Agents
- Biological (list agent and hazard level): AAV constructs
- Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.): 4% Formaldehyde
- Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.): MRI (magnetic fields and up to ~110 dB noise)

☐ Non-Centralized Animal Housing
- Location:
- Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 8
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
ASU Principal Investigator: [redacted]
Date of Action: 8/16/2022

The animal protocol review was considered by the Committee and the following decisions were made:

Request for changes was approved to add additional procedures and 12 animals to the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contac[redacted]to schedule.
☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT[redacted] prior to starting surgeries.
☐ Other requirements:

Total # of Animals: 20
Species: NHP **Pain Category: D**

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 8/19/2022
IACUC Chair or ***Designee***
Cc: IACUC Office, IACUC Chair

ARIZONA STATE UNIVERSITY

Institutional Animal Care and Use Committee

REQUEST FOR CHANGES TO AN APPROVED PROTOCOL

Protocol No. 22-1887R RFC 8
Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted] Email Address: [redacted]
If not PI, whom should we contact for questions related to this amendment: [redacted] Email Address: [redacted]

☒ Funded | | Unfunded

Requested Change (check all that apply):

☒ New procedures to be performed complete Part A, and Appendix 1 and/or 2 as applicable, and sign assurance.
☒ New species and or an increase in the number of animals to be used complete Part A and sign assurance.
☐ New location of housing or procedures complete Part A and sign assurance.
☐ New personnel complete Part B and sign assurance.
☒ Other (includes changes in dosages, funding, etc.) complete Part A and sign assurance.

A. Description of Requested Changes

For new procedures or additional animals that are USDA-covered species (all mammals EXCEPT mice and rats bred for research), list the **Category of Pain:** D

For new procedures or additional animals that are not USDA-covered species, will there be the potential to involve more than slight or momentary pain or distress that will NOT be relieved with anesthetics, analgesics, tranquilizer drugs, or other methods for relieving pain or distress (e.g., negative conditioning, unrelieved post-surgical pain, death without euthanasia)? ☐ No ☐ Yes

If yes, describe and justify:

If you are adding a procedure that could create pain or distress, you need to include a **literature search** for alternatives.
If you are adding a new survival surgery, submit a surgical checklist.
If you are requesting an increase in animal numbers, provide justification with supportive statistics.
If you are adding additional funding sources, provide the grant agency, grant title and ASU proposal or award number.

Describe the changes you are requesting. The sponsor is currently conducting multiple phase II and III clinical trials of intravenous trehalose dosing for the treatment of neurodegenerative diseases (Alzheimer's Disease, Spinocerebellar Ataxia, and Amyotrophic Lateral Sclerosis). In order to better match the intended clinical dosing route, we have elected to give trehalose intravenously in our NHPs rather than through an AAV-trehalose vector. We have also elected to add a third group of four additional animals to receive a new Poly(trehalose) formulation to compare its efficacy vs the trehalose-dihydrate formulation being used in the current clinical trials. In addition to testing a new trehalose dosing formulation, this experiment is not duplicative of the upcoming clinical trial for AD patients because we will be able to collect histopathological and molecular (via tissue punches) data on the effects of this trehalose dose on early tau pre-tangle development in the NHP model that we would be unable to obtain from the clinical trial participants, who are already showing clinical signs of dementia and whose brain tissues will not be collected as part of the trial. Poly(trehalose) and trehalose-dihydrate solutions used will be pharmaceutical grade and are not DEA-controlled. All animals will receive bilateral AAV-tau injections in the ERC as previously described. One month following surgery, four animals will receive trehalose-dihydrate (750 mg/kg, 8.3 mL/kg, IV) once per week until sacrifice, four animals will receive Poly(trehalose) (100 mg/kg, 8.3 mL/kg, IV) once per week until sacrifice, and four animals will receive saline control (8.3 mL/kg, IV) once per week until sacrifice. All animals will be sacrificed 6 months post-AAV as previously described. This experimental design will include an N of 12, which includes 3 groups of 4 animals. We will estimate the number of neurons in the entorhinal cortex and hippocampus by stereological counting of NeuN positive neurons. An n=4/group will give us the ability to detect an effect size of 25% in the number of neurons in the hippocampal subfield ($\beta > 0.8$, $\alpha \leq 0.05$). We feel the number of animals requested is the minimum necessary to achieve the aims of this study.

Group	Surgery	Treatment	N=	Sacrifice

1	AAV-double mutant tau bilaterally in ERC (2 x 20 µL) (1 x 10^{13} vg/mL)	Trehalose-dihydrate (750 mg/kg, 8.3 mL/kg, IV)	4	6 months post-AAV
2	AAV-double mutant tau bilaterally in ERC (2 x 20 µL) (1 x 10^{13} vg/mL)	Poly(trehalose) (100 mg/kg, 8.3 mL/kg, IV)	4	6 months post-AAV
3	AAV-double mutant tau bilaterally in ERC (2 x 20 µL) (1 x 10^{13} vg/mL)	Saline control (8.3 mL/kg, IV)	4	6 months post-AAV

New Procedures:

Trehalose Dosing: (once per week for five months): Animals will be anesthetized with ketamine (3-10 mg/kg, IM) and either dexmedetomidine (0.02-0.03 mg/kg, IM) or midazolam (0.05-0.5 mg/kg) and maintained with booster injections of ketamine (1.5 mg/kg, IM) and dexmedetomidine (0.015 mg/kg, IM). Trehalose or saline control (8.3 mL/kg, 41.5 mL for a 5.0 kg animal) will be administered via IV injection in the cephalic or saphenous vein with a syringe pump or infusion pump over approximately 3 minutes. Veins will be rotated every week. Sedation is expected to last ≤30 minutes. Following the procedure, dexmedetomidine/midazolam may be reversed with atipamezole (0.15-0.3 mg/kg, IM) or flumazenil (0.025 mg/kg, IV), respectively.

A. Provide the following details for the most recent literature search used to explore for duplicative research. (The literature search documents that the research will not unnecessarily duplicate previous research). **Teaching protocols do not need to conduct this search.**

Date that search was conducted (*Must be within 60 days of the IACUC review date*): 08/02/2021
Database(s) used: ALTBIB, PUBMED
Publication years covered by the search: 2000 - present
Keyword combinations used: trehalose, tau, nonhuman primate
trehalose, tangles, nonhuman primate

B. Provide the following details for the most recent literature search used to explore for **alternatives to animal use** and **alternatives to painful procedures.** Alternatives should be considered for any aspect of the protocol that may cause more than momentary or slight pain or distress to the animal. Alternatives to be considered include those that would: 1) refine the procedure to minimize discomfort that the animal(s) may experience; 2) reduce the number of animals used overall; or 3) replace animals with non-animal alternatives (e.g., computer models or tissue culture). **All protocols (research and teaching) MUST conduct this search.**

Date that search was conducted (*Must be within 60 days of the IACUC review date*): 08/02/2022
Database(s) used: ALTBIB, PUBMED
Publication years covered by the search: 2000 - present
Keyword combinations used:
Alzheimer's disease, animal model, nonhuman primate
Nonhuman primate, intracranial injection alternative

C. **Results of literature search for alternatives**: Comment on the application(s) of any identified alternatives (found with your search terms, including how these alternatives may be or may not be incorporated to modify a procedure to either lessen or eliminate potential pain and distress. **All protocols must complete this section and must describe how the literature search results relate to painful procedures and alternatives to animal use.** You must include sufficient information for the IACUC to determine that a reasonable, good faith effort was made to determine the availability of alternatives. If the search identified any alternative methods (ones that could be used to accomplish the goals of the animal use proposal), you must clearly explain and justify why this alternative cannot be used.

For instance, if your search terms retrieved eight publications, summarize how many of those described alternatives to painful procedures and the use of animals.
No studies have been published examining the ability of IV trehalose to prevent tangles in an NHP model of AD. While rodents have been used to establish other models of AD, the NHP models better mimic what is seen in the human brain. Additionally, the brains of rodents are less complex than that of

NHPs and humans. No alternatives to intracranial injection were found for delivering AAV-tau vector to the ERC.

D. Describe any other procedures (e.g., participation in meetings, review of journals) that are used to explore and evaluate alternatives: The PI, lab manager, post-docs, and graduate students regularly attend national meetings and discuss recent updates in technology and methodology for these experiments with colleagues. Additionally, they remain up to date with the scientific literature on new and alternative procedures.

B. Addition of Personnel

All personnel who work with animals are required to have animal care training within the last four years. ASU IACUC training modules can be completed at https://asu.co1.qualtrics.com/jfe/form/SV_b2b2XRXRRs1309f. Personnel are required to have Level III training certification on file with the IACUC office in order to perform procedures independently (without supervision). Training can be received from DACT staff or PIs can have a member of their lab approved by DACT to be a Certified Trainer who can then train others. Simply being Level III certified does not allow the training of others. See the IACUC web site (https://researchintegrity.asu.edu/animals/training) for more information on training and Level III forms.

*** Procedures other than husbandry, handling, or behavioral testing MUST be performed under supervision unless the person is Level III certified to conduct the procedure independently. Personnel are not Level III certified until the IACUC has reviewed and approved the Level III training documentation. The PI is responsible for ensuring that personnel who are not Level III certified are supervised at all times.**

Name	T t e	ASURITE name	What activit es will each person perform on live animals ONLY while under direct supervision?	What activities will each person be allowed to perform ndependent y (including appropriate Level 3 certification*) at the time of protocol submission?	Spec es w th wh ch nd v dua w have d rect contact ("al " or st spec es) *	IACUC USE ONLY Tra n ng (mm/yy)

For each individual, describe the individual's training and years of experience with all listed species and procedures they will be conducting under this protocol. For procedures for which they are not yet trained, but will likely be trained to do during the activity period of this protocol, provide a description of who will provide such training:

Assurance

As Principal Investigator of this protocol, I assure that all procedures will be conducted as described in this request for changes and that personnel will receive appropriate additional training prior to conducting any new procedures that are not listed above.

SIG[redacted]

Principal Investigator

8/4/22
Date

For IACUC use only:

☐ Administratively approved - Approving administrator: Date of approval:
☐ Administratively handled by VCV - Veterinarian providing verification: Date of verification:
Sources used for verification:
☒ Approved by Designated Review – Designated reviewer: Karen K bler Date of approval: 8/16/2022
☐ Approved by Full Committee Review – Primary reviewer: Date of approval:

From: IACUC@asu.edu
To: [redacted]
Subject: FW: Action Required: Designated Review fo[redacted]22-1887R RFC 8
Date: Thursday, August 11, 2022 12:10:18 PM
Attachments: image001.png
[redacted]22-1887R RFC 8 Vet Cleared_SJM.docx
Importance: High

From: [redacted]
Sent: Thursday, August 11, 2022 7:08:46 PM (UTC+00:00) Monrovia, Reykjavik
To: [redacted]
Dale DeNardo
Karen Kibler
[redacted]
Cc: IACUC@asu.edu <iacuc@asu.edu>
Subject: Action Required: Designated Review fo[redacted]22-1887R RFC 8

Designated Reviewer: Karen Kibler
Principal Investigator: [redacted]
Peer Reviewer: N/A
Protocol Number: [redacted]22-1887R RFC 8

A request for an amendment to the referenced protocols has been submitted. The request for amendment is attached and the various related protocols are available at the SharePoint site. The Designated Review process allows IACUC members, by voting, to permit the Designated Reviewer to approve or disapprove an amendment request.

Select "**YES**" if you approve the use of the designated review process for this amendment.

Select "**NO**" if you disapprove the use of designated review process regarding this amendment request. The amendment will then be reviewed at the next monthly IACUC meeting.

Select "**Abstain**" if you would like to abstain from the vote for any reason.

Select "**Recuse**" if you have a conflict of interest.

Please indicate your approval or disapproval of the request for designated review by using the YES or NO button in the toolbar at the top of this message. The use of "YES or NO" buttons allows you to submit comments along with your choice. **You may also send your comments to me directly or to the primary reviewer without using the selection buttons. Please copy me on all correspondence and email related to this request.**

Sincerely,

IMPORTANT *- All ERA applications (including the Conflict of Interest, Institutional Review Board, and Institutional Biosafety Committee modules) will be unavailable during an ERA upgrade outage* ***Friday, August 12 at 5pm until Monday, August 15 in the afternoon.*** *If you have submissions to these compliance modules that are due August 12, August 15, or August 16, please submit them no later than noon AZ time on August 10 to give the ORIA team an opportunity to get them in the review cycle prior to the outage.*

[redacted] Compliance Coordinator, Office of Research Integrity & Assurance

Arizona State University | Knowledge Enterprise | Operations
[redacted] | http://researchintegrity.asu.edu
How am I doing? Email my [redacted] or send a Sun Award
Chat with me on Teams! (ASU Users Only)

From: Karen Kibler
To: [redacted]
Cc: IACUC@asu.edu
Subject: DR-approved
Date: Thursday, August 11, 2022 3:08:52 PM
Attachments: [redacted]2-1887R RFC 8 Vet Cleared_DR cleared.docx

---

Hi [redacted]

If this RFC is approved for the DR process, the attached version has cleared DR.

Thanks,
Karen

[redacted]22-1887R RFC 8 Designated Review Votes

Name	Vote
[redacted]	Yes: 8/11/2022 12:30 PM
[redacted]	Yes: 8/11/2022 2:54 PM
[redacted]	Yes: 8/11/2022 2:32 PM
Dale DeNardo	Yes: 8/11/2022 12:10 PM
[redacted]	Yes: 8/12/2022 6:20 AM
Karen Kibler	Yes: 8/11/2022 3:08 PM
[redacted]	Yes: 8/11/2022 12:42 PM
[redacted]	
[redacted]	Yes: 8/11/2022 12:41 PM
[redacted]	Yes: 8/11/2022 1:04 PM
[redacted]	Yes: 8/11/2022 4:42 PM

[redacted]

IACUC Protocol Trackable Components Checklist

Protocol #: 22-1887R If for amendment, amendment #: 8

PI: [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 8/5/22

☐ No trackable components in this document

Exceptions to the Guide:

☐ Food/Fluid Regulation
- Species:
- What Restricted:
- Parameters:

☐ Prolonged Restraint
- Species:
- Details:

☒ Husbandry Deviation from the Guide
- Species: NHP
- Deviation: Single housing if suitable pairing partners are not available.

☐ Other:

Other Trackable Components:

☒ Survival Surgerie(s)
- Species: NHP
- Surgerie(s): Intracranial injection
- Multiple Major?: ☐ Yes ☒ No (Converted to no multiple major surgeries in RFC 8)

☒ Hazardous Agents
- Biological (list agent and hazard level): AAV constructs
- Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.): 4% Formaldehyde
- Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.): MRI (magnetic fields and up to ~110 dB noise)

☐ Non-Centralized Animal Housing
- Location:
- Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 10
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 9/2/2022

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was administratively approved to ad [redacted] as additional personnel to the protocol.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT a[redacted]

Additional requirements:

- ☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contact [redacted] to schedule.
- ☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT [redacted] prior to starting surgeries.
- ☒ Other requirements: IBC approval of new personnel is required before work with biohazardous materials may begin.

Total # of Animals: 20
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 9/7/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

PERSONNEL MODIFICATION FORM
IACUC and IBC

This form can be used to add or remove participants from both an IACUC protocol and an IBC disclosure. Please submit only one form to Research.Integrity@asu.edu and it will be processed by both committees.

Principal Investigator Name [redacted]	Phon[redacted]
Dept: ASU-Banner Neurodegenerative Disease Research Center	Email: [redacted]

Participant #1	**Add to:** ☒ **IBC #**SPROTO202100000070 ☒ **IACUC #** 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R **Delete from:** ☐ **IBC #** ☐ **IACUC #**	**FOR ORIA USE ONLY Training Verification**
Name: [redacted]	**ASURITE:** [redacted] **Email:** [redacted]	
Project Responsibilities in IBC: Will handle AAV viral vectors, alpha-synuclein preformed fibrils, Human Lewy Body extracts, and mouse/rat/nonhuman primate blood/CSF/brain tissue.		Being added in ERA
Experience/Training in These Responsibilities: No previous experience		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Macaques: Intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified). Rats: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified). Mice: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified).		8/2022 OHSP
Species: Macaques, Rats, Mice **Experience and training with species and procedures:** 1 year experience working with rats in research		

Assurance

As Principal Investigator, I assure that personnel will receive appropriate training prior to working with animals or biological materials as applicable.

Principal Investigator Signature: [redacted] Date: 9/1/22

FOR ORIA USE ONLY	☐ **IBC Approved**	☒ **IACUC Approved 9/2/2022**

Revised 11/20/12

Institutional Animal Care and Use Committee (IACUC)
Office of Research Integrity and Assurance
Arizona State University

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1887R RFC 11
Protocol Title: AAV Trehalose in an NHP model of Alzheimer's Disease
Principal Investigator: [redacted]
Date of Action: 9/23/2022

The animal protocol review was considered by the Committee and the following decisions were made:

The request for changes was administratively approved to add [redacted] as additional personnel.

NOTE: If you have not already done so, documentation of Level III Training (i.e., procedure-specific training) will need to be provided to the IACUC office before participants can perform procedures without supervision. For more information on Level III requirements see https://researchintegrity.asu.edu/animals/training, or contact Research Support Services within DACT at [redacted]

Additional requirements:

☐ This protocol requires that Research Support Services group within DACT provide supervision for the first time a procedure is conducted. Contac[redacted] to schedule.

☐ This protocol indicates that there are surgical procedures. A surgical checklist may be required to be submitted to Research Support Services within DACT[redacted] prior to starting surgeries.

☒ **Other requirements: IBC approval of new personnel is required before work with biohazardous materials may begin.**

Total # of Animals: 20
Species: NHP **Pain Category:** D

Protocol Approval Period: 11/18/2021 – 11/17/2024

Sponsor: [redacted]
ASU Proposal/Award #:
Title:

Signature: [redacted] Date: 9/27/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

PERSONNEL MODIFICATION FORM
IACUC and IBC

This form can be used to add or remove participants from both an IACUC protocol and an IBC disclosure. Please submit only one form to Research.Integrity@asu.edu and it will be processed by both committees.

Principal Investigator Name: [redacted]	Phone: [redacted]
Dept: ASU-Banner Neurodegenerative Disease Research Center	Email: [redacted]

Participant #1	**Add to:** ☒ **IBC #**SPROTO202100000070 ☒ **IACUC #** 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R **Delete from:** ☐ **IBC #** ☐ **IACUC #**	**FOR ORIA USE ONLY** **Training Verification**
Name [redacted]	**ASURITE** [redacted]	**Email:** [redacted]
Project Responsibilities in IBC: Will handle AAV viral vectors, alpha-synuclein preformed fibrils, Human Lewy Body extracts, and mouse/rat/nonhuman primate blood/CSF/brain tissue.		Already on IBC in ERA
Experience/Training in These Responsibilities: No previous experience		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Macaques: Intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified). Rats: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified). Mice: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified).		8/2022 OHSP
Species: Macaques, Rats, Mice **Experience and training with species and procedures:** No previous experience		
Participant #2	**Add to:** ☒ **IBC #**SPROTO202100000070 ☒ **IACUC #** 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R **Delete from:** ☐ **IBC #** ☐ **IACUC #**	**FOR ORIA USE ONLY** **Training Verification**
Name [redacted]	**ASURITE:** [redacted]	**Email:** [redacted]
Project Responsibilities in IBC: Will handle AAV viral vectors, alpha-synuclein preformed fibrils, Human Lewy Body extracts, and mouse/rat/nonhuman primate blood/CSF/brain tissue.		Being added in ERA
Experience/Training in These Responsibilities: No previous experience		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Macaques: Intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified). Rats: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified). Mice: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified).		10/2018 9/2022 NHP OHSP
Species: Macaques, Rats, Mice **Experience and training with species and procedures:** 3 years experience working with mice in research		
Participant #3	**Add to:** ☒ **IBC #**SPROTO202100000070 ☒ **IACUC #** 21-1867R, 22-1872R, 22-1873R, 22-1880R, 22-1886R, 22-1887R, 22-1898R, 22-1901R, 22-1903R, 22-1918R	**FOR ORIA USE ONLY** **Training Verification**

	Delete from: ☐ IBC # ☐ IACUC #	
Name [redacted]	ASURITE [redacted] Email: [redacted]	
Project Responsibilities in IBC: Will handle AAV viral vectors, alpha-synuclein preformed fibrils, Human Lewy Body extracts, and mouse/rat/nonhuman primate blood/CSF/brain tissue.		Need to add in ERA
Experience/Training in These Responsibilities: No previous experience		
What procedures are they responsible for on the IACUC protocol (please note which procedures are being done independently and which are done under supervision: Macaques: Intracranial surgery, intracarotid surgery, MRI, PET scan, blood/CSF collection, behavioral tests, administration of medications, and necropsy (all under direct supervision until certified). Rats: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified). Mice: Intracranial surgery, blood/CSF collection, behavioral tests, administration of any medications, and necropsy (all under direct supervision until certified).		9/2022 OHSP
Species: Macaques, Rats, Mice **Experience and training with species and procedures:** No previous experience		

Assurance

As Principal Investigator, I assure that personnel will receive appropriate training prior to working with animals or biological materials as applicable.

Principal Investigator Signature: [redacted] Date: 8/25/22

FOR ORIA USE ONLY	☐ **IBC Approved**	☒ **IACUC Approved 9/23/2022**

Revised 11/20/12