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

Animal Protocol Review

ASU Protocol Number: 22-1901R
Protocol Title: Bifunctional intrabody targeting intracellular alpha-synuclein
ASU Principal Investigator: [redacted]
Date of Action: 12/16/2021

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 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 DAC [redacted] prior to starting surgeries.
☒ **Other requirements: PHP.eB-VH14-hPEST and PHP.eB-B8-hPEST need to be added to IBC disclosure**

Total # of Animals: 16
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/16/2021 – 12/15/2024

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

Signature [redacted] Date: 12/23/2021
IACUC Chair or ***Designee***
Cc: IACUC Office, IACUC Chair

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IACUC Use Only	**IACUC Protocol #: 22-1901R**
Date: 11/22/2021	☒ 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: Bifunctional intrabody targeting intracellular alpha-synuclein

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: Laboratory Manager

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

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): [redacted]

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:

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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, anesthesia and post-procedure 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.

Excess levels of the naturally-occurring protein, alpha-synuclein (α-syn), are believed to be one of the causes of Parkinson's disease (PD). Prior research has shown that reducing levels of α-syn in animal models of PD can protect against neurodegeneration. In this study, we seek to test an artificially created nanobody that targets and reduces levels of α-syn in nonhuman primates (NHP). We will test three dose levels of the nanobody delivered into the cerebral spinal fluid to determine how well it is distributed in the central nervous system via this route and to help choose the dosing level for a future study looking at the functional efficacy of this treatment in a NHP model of PD.

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: Many therapies targeting α-syn (e.g. immunotherapies) in PD focus on halting extracellular secretion and subsequent templated propagation of proteopathic seeds. However, these methods do not prevent intracellular accumulation and aggregation, which is more prevalent and, in itself, can be toxic. Furthermore, it is upstream from secretion and spread. A stable, long-term strategy for preventing intracellular α-syn accumulation and aggregation would positively influence both intracellular and extracellular proteotoxicity[1]. Nanobodies are the single domains of the variable region (Fv) of a full-length immunoglobulin. Nanobodies retain many advantages of conventional antibodies, including high specificity and affinity for target epitopes. They can be selected and highly engineered as genes, and, when delivered via viral vectors, become powerful intracellular antibodies[2]. Targeting the α-syn domain integral to driving amyloidogenesis (NAC; non-amyloid component region)[1-3] fused with a proteasomal targeting motif derived from murine ornithine decarboxylase (PEST sequence), we created bifunctional aptamers that both interrupt α-syn aggregation and effectively clear toxic α-syn protein levels in vivo[4]. We have demonstrated that vector delivery of the candidate nanobody, VH14-PEST, provides structural and functional neuroprotection against viral overexpression of α-syn in a rat model of severe synucleinopathy[3]. We then determined the potency of humanized variants of PEST degron fused to VH14 in preventing α-syn pathology in situ, and validated target engagement of lead VH14-hPEST in organoid preparations of dopaminergic cultures derived from human iPS cells. Our successful AAV delivery experiments established the biodistribution and

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transduction efficiency of intracisternal delivery of AAV.PHP.eB-GFP in middle-aged rats and evaluated the structural and functional efficacy of direct (intranigral) and global (intracisternal) delivery of VH14-hPEST in rodent models of synucleinopathy. Here we propose to conduct a biodistribution and dose-ranging study in NHP to obtain additional relevant data for an FDA new drug application for this therapy. This study will not test the efficacy of the treatment, merely the biodistribution of the various nanobody titers via the predicted clinical treatment route (CSF infusion). Nanobody distribution will be measured indirectly via a hemagglutinin (HA) tag which can be visualized histologically once the animals are sacrificed.

Experimental Design: 16 adult macaques (3-15 yrs old, M/F) will comprise this experiment. They will form four groups of four monkeys each. Groups 1-3 will receive low (1 x 10^{10} vg/ml, vector genomes per mL), medium (6 x 10^{11} vg/ml), or high titer (1x10^{13} vg/ml) PHP.eB-VH14-hPEST. Group 4 will receive high titer (1x10^{13} vg/ml) PHP.eB-B8-hPEST for control. All injections will be made into the cisterna magna in 2 mL volumes. As this is not an efficacy study, detailed behavior will not be performed. However, body weights will be taken prior to intracisternal injection (baseline), one month following intracisternal injection, and just prior to necropsy. Animals will also be evaluated on the neurological clinical rating scale prior to and at least once per month following intracisternal injection to test for any unexpected side effects of the treatment. Plasma, serum, and CSF will be collected prior to injection and at sacrifice (during the same session of sedation when possible). Animals will be sacrificed 2 months post-injection and their brains, spinal cords, and peripheral tissues collected for analysis.

Procedures:

Intracisternal Injections (once): 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. 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). The animal will be placed in lateral recumbency. Injection is performed as a sterile procedure. The cervical area of the animal will be shaved and scrubbed alternating with povidone iodine or chlorhexidine solution 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. The head will be held in flexion and a 22G spinal needle will be inserted and stabilized in the cisterna magna, confirmed by back flow of cerebrospinal fluid (CSF). A 36-inch micro-extension line ending in a three-way stock cock will be connected to the spinal needle, and a 1.0-mL sample of CSF will be then obtained using a 3-mL syringe. The syringe will be replaced with a sterile syringe containing 2.0 mL of viral vector infusate. The infusate will be slowly delivered through the spinal needle into the cisterna magna over 20 min (100 µL/min). The needle will then be flushed with 0.1 mL of the patient's CSF at the same rate and removed. The procedure is expected to last ~40 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. Meloxicam may be administered as needed for analgesia.

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Clinical Ratings Scale (at least once per month): Animals will be evaluated with the Clinical Ratings Scale (CRS), a collection of clinical observations, scored and graded in order to determine the severity of an animal's overall impairments (see below). The ratings scale contains scoring in the categories of posture (0-3), gait (0-5), bradykinesia (0-5), balance (0-3), tremor in left and right arms (0-3 for each), gross motor skills in left and right arms (0-4 for each), defense reaction (0-2) and freezing (0-2). The combined scores will determine the impairment level of the animal, a score of 0 indicates a normal animal. Individual scores in each category can be assessed separately as well in order to determine the overall health and well-being of an animal. We do not expect to see an neurological deficits as a result of this treatment, however, if any animals begin to display neurological deficits or other clinical signs that may impact their health and well-being, including a posture score of 3, gait score of 3 or more, bradykinesia score of 3 or more, or gross motor skills score of 3 or more in both arms, they will be referred to the veterinary staff for evaluation and treatment, if necessary.

Posture (0-3)	Gait (0-5)	Bradykinesia (0-5)	Balance (0-3)	Tremor (0-3)	Gross Motor Skills (0-4)	Defense Reaction (0-2)	Freezing (0-2)
0-Normal, upright posture 1-Mildly stooped, neck and shoulders slightly curved 2-Notably stooped 3-Face down, unable to sit	0-Normal, smooth movements 1-Mildly impaired 2-Moderately impaired, some stumbling, abnormal footing (crossover) 3-Severely impaired, stumbling, bradykinetic 4-Severely impaired, loss of balance, freezing 5-Incapable of movement	0-Normal, prompt, brisk, plentiful movements 1-Mild slowness, lesser overall movements than normal 2-Moderate slowness, increasing poverty of movement 3-Moderate slowness with freezing, few and labored movements 4-Severe slowness with freezing, few and labored movements 5-Unable to ambulate	0-Normal 1-Difficulty standing, holding onto cage bars; holds cage bars intermittently but can stand alone 2-Unable to stand without holding onto cage bars 3-Unable to sit without leaning; completely unable to stand	0-Absent 1-Small amplitude and/or infrequent 2-Large amplitude and/or frequent 3-Occurs constantly; interferes with normal behavior	0-Normal 1-Mild impairment, slight clumsiness 2-Moderately impaired, difficulty manipulating small objects, often drops food reward 3-Does not readily use arm to reach for food; can use to ambulate 4-Cannot use arm/hand for feeding or ambulating	0-Normal, aggressive, comes to front of cage, shakes bars, threatens 1-Strong facial threat but does not move 2-Minimal or no response	0-No freezing 1-Notable freezing, freezing readily broken with outside stimulation 2-Large periods of time frozen; interferes with ambulation

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). 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 therapies 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 PD.

2. Why are you using the requested species rather than other species?
Macaques were chosen because their brains closely resemble those of humans, in comparison to a smaller or less complex species. Rodents do not effectively mimic the complex degeneration in nigrostriatal circuitry necessary to appropriately test PD therapies pre-clinically.

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 16 animals, which includes four groups of four animals each. A series of brain regions including frontal, parietal, and temporal cortex, substantia nigra, caudate nucleus, putamen, and

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globus pallidus will be analyzed. We will stereologically estimate the number of HA-immunostained neurons in each region of each animal. We will analyze these stereological estimates using a two-way ANOVA with dose and brain region as the independent. When significant overall effects are found, Tukey's multiple comparison post hoc analyses will be performed. The effect size of data is unable to be measured and the total number of animals proposed has been chosen based on the investigator's prior experience with similar study designs and the expected presentation of data which will guide future research.

Group	Treatment	Total N=
1	PHP.eB-VH14-hPEST $1x10^{10}$ vg/ml	4
2	PHP.eB-VH14-hPEST $6x10^{11}$ vg/ml	4
3	PHP.eB-VH14-hPEST $1x10^{13}$ vg/ml	4
4	PHP.eB-B8-hPEST $1x10^{13}$ vg/ml	4

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 intracisternal injections proposed will be conducted under general anesthesia with post-procedure 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 #: [redacted]

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*): 12/4/2021
Database(s) used: PUBMED
Publication years covered by the search: 1980-2021
Keyword combinations used: nonhuman primate, VH14-hPEST

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.**

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Date that search was conducted (*Must be within 60 days of the IACUC review date*): 12/4/2021
Database(s) used: PUBMED
Publication years covered by the search: 1980-2021
Keyword combinations used:
Nonhuman primate, Intracisternal 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 VH14-hPEST in nonhuman primates. While this nanobody has been studied in rodents, NHPs are much closer to humans in size, increasing the applicability of the biodistribution results to human patients and leading the way towards clinical trials of VH14-hPEST therapies. No alternatives to intracisternal injection were found for delivering nanobody to the CSF.

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.

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USDA Covered Species	Number per USDA Category*				Total number of animals requested
	B	C	D	E	
Macaques			16		16

*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).

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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] 11/22/21

Principal Investigator –Print Date

[redacted] 11/22/21

Principal Investigator Signature Date

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

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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]	Intracisternal injection	blood/CSF collection, administration of medications, and necropsy.	All	7/2021 OHSP
[redacted]	Neurosurgeon	N/A	none	Intracisternal injection	All	Visiting surgeon – training and OHSP not required
[redacted]	Laboratory Manager	[redacted]	Intracisternal injection, blood/CSF collection, administration of medications, and necropsy.	None	All	11/2021 OHSP
[redacted]	Laboratory Coordinator	[redacted]	Intracisternal injection, blood/CSF collection, administration of medications, and necropsy.	None	All	11/2021 OHSP
[redacted]	Research Specialist	[redacted]	Intracisternal injection, blood/CSF collection, administration of medications, and necropsy.	None	All	11/2021 OHSP

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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] 37 years' experience in primate research. Experienced with blood/CSF collection, administration of medications, and necropsy. Will be trained in intracisternal injection by Dr. [redacted] a clinical neurosurgeon experienced in this procedure.

[redacted] Dr. [redacted] is Professor of Neurosurgery at th[redacted] He is a clinical neurosurgeon experienced in the intracisternal injection procedure.

[redacted] 17 years' experience in primate research. Experienced with blood/CSF collection, administration of medications, and necropsy. Will be trained in intracisternal injection by D[redacted] a clinical neurosurgeon experienced in this procedure.

[redacted] 2 years' experience in primate research. Experienced with blood/CSF collection, administration of medications, and necropsy. Will be trained in intracisternal injection by D[redacted] a clinical neurosurgeon experienced in this procedure.

[redacted] 2 years' experience in primate research. Experienced with blood/CSF collection, administration of medications, and necropsy. Will be trained in intracisternal injection by Dr[redacted] clinical neurosurgeon experienced in this procedure.

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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: Macaques

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: 16

C. Sex: M/F Age or Weight Range: 3-15 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

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.

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☐ 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:

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:

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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 prevent bradycardia	As needed	Y	N
Betadine	N/A	Topical	Topical disinfectant	As needed	Y	N
Cefazolin	20-25 mg/kg	IV or IM	Antibiotic	Twice daily, as needed	Y	N

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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
Chlorhexidine	N/A	Topical	Topical disinfectant	As needed	Y	N
Dexmedetomidine	0.015-0.05 mg/kg	IM	Anesthesia	As needed	Y	N
Flumazenil	0.025 mg/kg	IV	Benzodiazepine reversal	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	As needed	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	1-2 L	IC	Perfusion	Once	Y	N
Sevoflurane	1-8%	Inhalation	Anesthesia	As needed	Y	N
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.

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)?

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☐ 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
PHP.eB-VH14-hPEST	1E10^{10}vg/mL 6E10^{11}vg/mL 1E10^{13}vg/mL	2mL	Intracisternal injection	tbd	pending
PHP.eB-B8-hPEST	1E10^{13}vg/mL	2mL	Intracisternal injection	tbd	pending

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, and annual TB screening.

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
Intracisternal injections may lead to infection.	Very low, we will perform injections under sterile technique.	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, including a Clinical Ratings Scale posture score of 3, gait score of 3 or more, bradykinesia score of 3 or more, or gross motor skills score of 3 or more in both arms, they will be referred to the veterinary staff for evaluation.

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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	Used 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 perfusion
Hydromorphone	0.2-0.4 mg/kg	IM, IV	Y	Used in conjunction with perfusion
Heparin	5,000 IU	IC	N	Used in conjunction with perfusion
Isoflurane	3-5%	Inhalation	N	Used in conjunction with perfusion
Sevoflurane	5-8%	Inhalation	N	Used in conjunction with perfusion
0.9% saline	1-2 L	IC	N	Used in conjunction with perfusion

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

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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 2 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.

Blood will ideally be collected while animals are sedated for intracisternal injection and euthanasia. Alternatively, animals will be anesthetized with ketamine and either dexmedetomidine or midazolam. Blood samples will be obtained from the femoral vein and separated for serum and plasma collection. If samples are collected independently of intracisternal injection or euthanasia, 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 used for measuring peripheral nanobody concentration. Collections spaced approximately 2 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 plasma/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	0.5-1 mL	Up to 2 planned, 3 max including potential redraws	Typically 2 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

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Atropine	0.02-0.05 mg/kg	IM	Reduce respiratory secretions
Betadine or Chlorhexidine/Isopropyl alcohol	N/A	Topical	Topical disinfectant

C. Describe the methods used to collect the samples, including physical restraint, if any.
CSF will ideally be collected while animals are sedated for intracisternal injection and euthanasia. Alternatively, animals will be anesthetized with ketamine and either dexmedetomidine or midazolam. CSF collection is performed as a sterile procedure except as part of euthanasia. The lumbar or cervical area of the animal will be shaved and scrubbed alternating with povidone iodine or chlorhexidine 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, or CSF will be collected prior to the intracisternal injections as described above. If samples are collected independently of intracisternal injection or euthanasia, 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 used for measuring nanobody concentration. Collections spaced approximately 2 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.

Obtained by Rise for Animals.
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IACUC Protocol Trackable Components Checklist

Protocol #: 22-1901R If for amendment, amendment #:

PI: [redacted]

Species: NHP Highest Category of Pain: D

Completed by [redacted] Date completed: 12/8/21

☐ 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:
Surgerie(s):
Multiple Major?: ☐ Yes ☐ No

☒ Hazardous Agents
Biological (list agent and hazard level): PHP.eB-VH14-hPEST, PHP.eB-B8-hPEST
Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.):
Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.):

☐ Non-Centralized Animal Housing
Location:
Maximum duration:

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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

Animal Protocol Review

ASU Protocol Number: 22-1901R RFC 1
Protocol Title: Bifunctional intrabody targeting intracellular alpha-synuclein
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. 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:

Total # of Animals: 16
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/16/2021 – 12/15/2024

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

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

Cc: IACUC Office
IACUC Chair

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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.		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

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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

[redacted]

Animal Protocol Review

ASU Protocol Number: 22-1901R RFC 2
Protocol Title: Bifunctional intrabody targeting intracellular alpha-synuclein
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] 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: IBC approval of new personnel is required before work with biohazardous materials may begin.**

Total # of Animals: 16
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/16/2021 – 12/15/2024

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

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

Cc: IACUC Office
IACUC Chair

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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

Animal Protocol Review

ASU Protocol Number: 22-1901R RFC 3
Protocol Title: Bifunctional intrabody targeting intracellular alpha-synuclein
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. 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:

Total # of Animals: 16
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/16/2021 – 12/15/2024

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

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

Cc: IACUC Office
IACUC Chair

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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 Addres[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.

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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:

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

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

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 08/15/2023

[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 fo [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
	Dale DeNardo [redacted]	Yes: 8/9/2022 10:10 AM
		Yes: 8/9/2022 10:31 AM
		Yes: 8/10/2022 7:56 AM
	Karen Kibler [redacted]	Yes: 8/9/2022 10:15 AM
		Yes: 8/9/2022 11:17 AM
	[redacted]	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,

Obtained by Rise for Animals.
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IACUC Protocol Trackable Components Checklist

Protocol #: 22-1901R If for amendment, amendment #: 3

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:
- Surgerie(s):
- Multiple Major?: ☐ Yes ☐ No

☒ Hazardous Agents
- Biological (list agent and hazard level): PHP.eB-VH14-hPEST, PHP.eB-B8-hPEST
- Chemical (note category – toxicant, toxin, irritant, carcinogen, etc.):
- Physical (note type - radiation, UV light, lasers, noise, magnetic fields, etc.):

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

☐ Decapitation

☐ USDA-covered Species exempt from USDA reporting

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

Animal Protocol Review

ASU Protocol Number: 22-1901R RFC 4
Protocol Title: Bifunctional intrabody targeting intracellular alpha-synuclein
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 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. 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: 16
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/16/2021 – 12/15/2024

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

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

Cc: IACUC Office
IACUC Chair

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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.		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

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

Animal Protocol Review

ASU Protocol Number: 22-1901R RFC 5
Protocol Title: Bifunctional intrabody targeting intracellular alpha-synuclein
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] and [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. 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: 16
Species: NHP **Pain Category:** D

Protocol Approval Period: 12/16/2021 – 12/15/2024

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

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

Cc: IACUC Office
IACUC Chair

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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**

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	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

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Revised 11/20/12

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

Animal Protocol Review

ASU Protocol Number: 22-1903R
Protocol Title: Primate Holding, Assessment, and Training
Principal Investigator: [redacted]
Date of Action: 1/27/2022

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:

Total # of Animals: 150
Species: NHP **Pain Category: D**

Protocol Approval Period: 1/27/2022 – 1/26/2025

Sponsor: N/A
ASU Proposal/Award #: N/A
Title: N/A

Signature: [redacted] Date: 1/31/2022
IACUC Chair or Designee

Cc: IACUC Office
IACUC Chair

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IACUC Use Only	**IACUC Protocol #:** 22-1903R
Date: 12/29/2021	☐ 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: Primate Holding, Assessment, and Training

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: Laboratory Manager

AFFILIATION: ASU-Banner Neurodegenerative Disease Research Center 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: Deadline:

Co-Investigator(s):

Proposal Title:

ASU Proposal or Award #:

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

☐ Teaching - Course Number and Title:

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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, 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.

The goal of this protocol is to obtain nonhuman primates (NHPs) for the P.I.'s inventory based on current animal availability and the expected needs of our research. The availability of NHPs for research use is extremely variable over time, especially with rare populations such as aged (≥22 yrs old) NHPs, which are invaluable for studying neurodegeneration. In order to take advantage of these resources as they become available, we may need to procure animals prior to finalization and approval of the eventual study protocol on which they will be used. This will also allow us to evaluate animals for their capability to perform motor and/or cognitive tasks and perform baseline behavioral assessments before assigning them to a particular project.

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.**

Experimental Design: Up to 150 macaques (M/F) (2-30 yrs old) will be acquired from commercial vendors based on availability and planned study needs. Animals may have plasma, serum, & cerebrospinal fluid (CSF) collected for baseline or future untreated control use (under the same sedation when possible). Animals may be trained on a hand reach task and have baseline data recorded. Animals may also have baseline general activity recorded and be rated on a clinical rating scale. Animals may be kept on this protocol up to three months before being transferred to the appropriate approved study protocol. All animals will eventually be transferred to a study protocol; in the rare case that an animal cannot be used, the veterinarians will be consulted, and the animals will be transferred to another investigator at ASU or another primate facility. If animals reach an end point criterion due to health reasons, they may be euthanized upon consultation with DACT veterinary staff and their tissues may or may not be collected for post-mortem diagnosis or for research control uses.

Procedures:

Activity Monitoring (One week per month)

Activity monitoring may be performed to assess general motor function. Animals will be fitted with an appropriate sized collar containing an activity monitor which senses and records any excessive acceleration. Animals will be sedated with ketamine alone (10 mg/kg) or ketamine (3-10 mg/kg, IM) and either dexmedetomidine (0.03 mg/kg, IM) or midazolam (0.05-0.5 mg/kg) in order to fit them with collars [redacted] that

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will hold Actical activity monitors [redacted] 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. The monitor senses acceleration that exceeds 0.05 G, recorded up to 32 times per second. The number of acceleration pulses within a pre-selected time period are then recorded. Animals will be acclimated to the collars for at least 2 days prior to any data collection. Recording will take place for 5 days per collection interval, after which time the animals will be sedated as above and the collars will be removed. Following collar removal, animals will be examined for any chafing or irritation and the veterinarian will be notified if any skin abrasions are present.

Fine Motor Skills Test (Hand Reach Task) (three-five times per week): Animals may be tested for fine motor performance in both upper limbs using a hand reach task (HRT). Animals will be transported to a modified testing cage from their home cage and presented with a 3x3 well matrix plexiglass testing board. Six pieces of food will be placed within the wells for each trial, and time will be recorded for how long it takes the animal to retrieve them. The board is configured so that only one limb will be tested at a time. Animals will undergo 10 trials per limb, with each trial being alternated between the left and right limbs. Each animal will be tested by the same investigator 3-5 times per week. Investigators will be blinded to the animal's treatment groups. If necessary, food restriction will be used to encourage animal participation in this activity.

Clinical Ratings Scale (Once per month): Animals may be evaluated with the Clinical Ratings Scale (CRS), a collection of clinical observations, scored and graded in order to determine the severity of an animal's overall impairments (see below). The ratings scale contains scoring in the categories of posture (0-3), gait (0-5), bradykinesia (0-5), balance (0-3), tremor in left and right arms (0-3 for each), gross motor skills in left and right arms (0-4 for each), defense reaction (0-2) and freezing (0-2). The combined scores will determine the impairment level of the animal, a score of 0 indicates a normal animal, and a max score of 34 indicates severely impaired. Young, healthy animals typically score 0 and aged, healthy animals typically score 0-3. Individual scores in each category can be assessed separately as well in order to determine the overall health and well-being of an animal.

Posture (0-3)	Gait (0-5)	Bradykinesia (0 5)	Balance (0-3)	Tremor (0-3)	Gross Motor Skills (0-4)	Defense Reaction (0 2)	Freezing (0-2)
0-Normal, upright posture 1-Mildly stooped, neck and shoulders slightly curved 2-Notably stooped 3-Face down, unable to sit	0-Normal, smooth movements 1-Mildly impaired 2-Moderately impaired, some stumbling, abnormal footing (crossover) 3-Severely impaired, stumbling, bradykinetic 4-Severely impaired, loss of balance, freezing 5-Incapable of movement	0-Normal, prompt, brisk, plentiful movements 1-Mild slowness, lesser overall movements than normal 2-Moderate slowness, increasing poverty of movement 3-Moderate slowness with freezing, few and labored movements 4-Severe slowness with freezing, few and labored movements 5-Unable to ambulate	0-Normal 1-Difficulty standing, holding onto cage bars; holds cage bars intermittently but can stand alone 2-Unable to stand without holding onto cage bars 3-Unable to sit without leaning; completely unable to stand	0-Absent 1-Small amplitude and/or infrequent 2-Large amplitude and/or frequent 3-Occurs constantly; interferes with normal behavior	0-Normal 1-Mild impairment, slight clumsiness 2-Moderately impaired, difficulty manipulating small objects, often drops food reward 3-Does not readily use arm to reach for food; can use to ambulate 4-Cannot use arm/hand for feeding or ambulating	0-Normal, aggressive, comes to front of cage, shakes bars, threatens 1-Strong facial threat but does not move 2-Minimal or no response	0-No freezing 1-Notable freezing; freezing readily broken with outside stimulation 2-Large periods of time frozen; interferes with ambulation

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) and may be followed by 4% buffered formaldehyde (1-2 L) for fixation if needed. If perfusion cannot be performed for any reason or is not deemed necessary, 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:

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1. Why must live vertebrates be used in this study?
Our laboratory's experiments are aimed at helping human patients with neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (PD). 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 these.

2. Why are you using the requested species rather than other species?
Macaques were chosen because many models of neurodegenerative diseases such as PD and AD have been established in this species. 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. NHP models are considered the gold standard for preclinical testing of potential therapies for neurodegenerative diseases.

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.
Up to 150 macaques will be acquired over three years based on availability and planned study needs. Our lab's typical NHP study protocols include between 8 and 50 animals each and last from 1 to 18 months. Based on our experience, this number of animals will be sufficient to fill our study needs over this time period.

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 our lab's aims.

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*): 12/29/2021
Database(s) used: ALTBIB, PUBMED
Publication years covered by the search: 1980 - 2021
Keyword combinations used: Parkinson's disease, animal model, nonhuman primate
Alzheimer's disease, animal model, nonhuman primate

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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*): 12/29/2021
Database(s) used: ALTBIB, PUBMED
Publication years covered by the search: 1980 - 2021
Keyword combinations used: Parkinson's disease, animal model, nonhuman primate
Alzheimer's disease, animal model, nonhuman primate

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.
While rodents have been used to establish other models of PD and 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. Non-animal models are still not able to give us enough information for clinical projection in complex neurodegenerative diseases such as these. The literature search yielded no duplications or viable alternatives to stated procedures.

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

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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	
Macaques			150		150

*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).

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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] 12/29/21

Principal Investigator –Print Date

[redacted] 12/29/21

Principal Investigator Signature Date

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

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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	blood/CSF collection, administration of any medications, and necropsy.	All	7/2021 OHSP
[redacted]	Laboratory Manager	[redacted]	blood/CSF collection, administration of any medications, and necropsy.	None; May perform all procedures independently once Level 3 certified	All	11/2021 OHSP
[redacted]	Laboratory Coordinator	[redacted]	blood/CSF collection, administration of any medications, and necropsy.	None; May perform all procedures independently once Level 3 certified	All	11/2021 OHSP
[redacted]	Research Specialist	[redacted]	blood/CSF collection, administration of any medications, and necropsy.	None; May perform all procedures independently once Level 3 certified	All	11/2021 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] 37 years' experience in primate research. Experienced with all procedures in this protocol.

[redacted] 7 years' experience in primate research. Experienced with all procedures in this protocol.

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[redacted] 2 years' experience in primate research. Experienced with all procedures in this protocol.

[redacted] 2 years' experience in primate research. Experienced with all procedures in this protocol.

Level 3 certification training will be performed by an authorized trainer or the RSS team.

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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: 150

C. Sex: M/F Age or Weight Range: 2-30 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

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.

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☒ 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.
Some animals are initially resistant to performing the hand reach task (HRT). In order to provide increased incentive for these animals, we will offer different food treats (cereal, marshmallows, raisins, peanuts, etc.) to discover the animals' preferences (stage 1). If that is unsuccessful after one week, we will postpone daily feeding of the normal food allotment until after the animal has been tested (stage 2). If that is unsuccessful after one week, we would like to temporarily restrict the number of food biscuits fed to the animals (stage 3). Adult cynomolgus monkeys normally receive 6-12 biscuits per day and a half a fruit or vegetable, food restricted animals will receive a minimum of half their normal allotment of biscuits based on veterinarian recommendation (primate veterinarian at the University of Illinois), along with a half a fruit or vegetable. Food restriction during the initial HRT training period will last a maximum of one month. Previous animals that have been placed on similar food restriction have not become combative in our experience, but food restricted animals will be monitored and will be temporarily separated from their partners during feeding if necessary. This incentive program will apply to all animals participating in the HRT initial training and baseline. A log of the HRT incentive program will be maintained.

2. How will you monitor for negative effects of food restriction (include information on how you will account for animal growth)?
Prior to the initiation of food restriction, the veterinarian will be consulted on the animal's ideal body weight and current body condition score (BCS) on a 9-point scale (see below). Animals with a BCS of 3.5/9 or less will not be placed on food restriction. During food restriction, the animal's body weight will be monitored every week or twice per week if body mass loss exceeds 8% of starting mass. An animal that loses 10% or more of its body weight from the start of food restriction or whose BCS drops to 3.5/9 or below will be removed from food restriction and will return to stage 2 (postponement of feeding full daily ration until after testing). Food restriction will last up to a maximum of one month during the initial HRT training period.

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:

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.

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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 or group 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. Animals that will undergo HRT will be permanently exempt from the establishment of new social housing pairs (contact or mesh) beginning with baseline behavioral data collection. An injury to the arm, hand, or fingers can severely affect the HRT results and the establishment of new social housing pairs is a common cause of injuries. Animals in established pairs will be allowed to remain partnered. However, if injurious fights occur between established pairs, the animals may be permanently separated based on consultation with the veterinary staff.

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

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(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 rever al	As needed	Y	N
Atropine	0 02 0 05 mg/kg	IM	Reduce respiratory secretions and prevent bradycardia	A needed	Y	N
Betadine	N/A	Topical	Topical disinfectant	As needed	Y	N
Dexmedetomidine	0.03 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 rever al	As needed	Y	N
Heparin	5,000 IU	IC	Anticoagulant for perfusion	Once	Y	N
Hydromorphone	0.2-0.4 mg/kg	IM, IV	Analgesia	As needed	Y	Y
Isoflurane	3-5%	Inhalation	Anesthesia	As needed	Y	N
Isopropyl alcohol	70%	Topical	Topical disinfectant	As needed	Y	N
Ketamine	3-20 mg/kg	IM, IV	Anesthesia	As needed	Y	Y
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
Pentobarbital-containing euthanasia solution	86-120 mg/kg	IV	Euthanasia	Once	Y	Y
Saline	1-2 L	IC	Perfusion	Once	Y	N
Sevoflurane	5-8%	Inhalation	Anesthesia	As needed	Y	N
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)?

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☒ 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

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

V. END POINT CRITERIA

A. What clinical signs will be used as a basis for removal of an animal from the study?
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 return to ≥80% of ideal weight after two weeks of supportive treatment (as determined and provided in conjunction with the DACT veterinary team) or with weight loss in excess of 30% of ideal weight at any time.

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

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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	Used 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 perfusion
Hydromorphone	0.2-0.4 mg/kg	IM, IV	Y	Used in conjunction with perfusion
Heparin	5,000 IU	IC	N	Used in conjunction with perfusion
Isoflurane	3-5%	Inhalation	N	Used in conjunction with perfusion
Sevoflurane	5-8%	Inhalation	N	Used 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 perfusion

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

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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 3 planned, 4 max including potential redraws	Typically 1 month; 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 may be collected for baseline analysis for later study use or for use as a normal control sample. Collections spaced approximately 1 month 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 plasma/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 3 planned, 4 max including potential redraws	Typically 1 month; 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 respiratory secretions

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Betadine/I opropyl alcohol	N/A	Topical	Topical di infectant

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 may be collected for baseline analysis for later study use or for use as a normal control sample. Collections spaced approximately 1 month 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.

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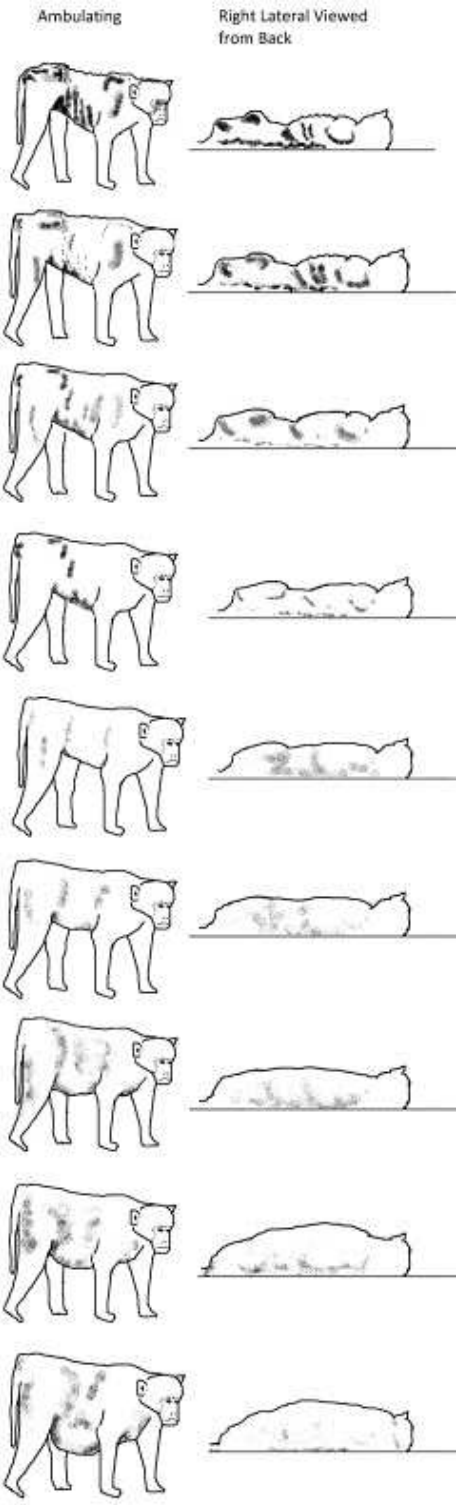
9-point Body Condition Score

1	**EMACIATED** – Very prominent hip bones (easily palpable and likely visible), prominent facial bones, spinous processes and ribs. Minimal to no muscle mass is palpable over ileum or ischium. Anus may be recessed between ischial callosities. Body is very angular, no subcutaneous fat layer to smooth out prominences.
2	**VERY THIN** – Hips, spinous processes, and ribs are prominent. Facial bones may be prominent. There is very little muscle present over the hips and back. Anus may be recessed between ischial callosities. Body is angular, no subcutaneous fat to smooth out prominences
3	**THIN** – Very minimal fat reserves, prominent hip bones and spinous processes. Hips, spinous processes and ribs are easily palpable with only a small amount of muscle mass over hips and lumbar region.
4	**LEAN** – Overlying muscle gives hips and spine a more firm feel. Hip bones and spinous processes are readily palpable, but not prominent. Body is less angular because there is a thin layer of subcutaneous fat.
5	**OPTIMUM** –Hip bones, ribs and spinous processes are palpable with gentle pressure but generally not visible. Well developed muscle mass and subcutaneous fat layer gives spine and hips smooth but firm feel. No abdominal, axillary or inguinal fat pads.
6	**SLIGHTLY OVERWEIGHT** – Hip bones and spinous processes palpable with firm pressure but are not visible. Bony prominences smooth. Rib contours are smooth and only palpable with firm pressure. Small abdominal fat pad may be present.
7	**HEAVY** – Bony contours are smooth and less well defined. Hip bones, spinous processes and ribs may be difficult to palpate due to more abundant subcutaneous fat layer. May have fat deposits starting to accumulate in the axillary, inguinal or abdominal areas.
8	**OBESE** – This animal will often have prominent fat pads in the inguinal, axillary or abdominal region. Abdomen will be pendulous when animal sitting or ambulating. Hip bones and spinous processes difficult to palpate. Bony contours smooth and poorly defined.
9	**GROSSLY OBESE** –Obvious, large fat deposits in the abdominal, inguinal and axillary regions. Abdominal palpation is very difficult due to large amount of mesenteric fat. Pronounced fat deposits may alter posture/ambulation. Hip bones, rib contours and spinous processes only palpable with deep palpation.



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