Protocol #: 18-065-CT-19-003

Protocol Title: Optimization & Pre-clinical Testing of Implantable, In-Line High Density 32-Channel Connector

Principal Investigator: [redacted], Ph.D and [redacted], Ph.D

Investigator Assurances

I agree to abide by the policies of the Louis Stokes Cleveland DVA Medical Center Institutional Animal Care and Use Committee (IACUC) and all applicable federal regulations.

I will adhere to the protocol as described and as modified.

I will submit any modifications of the protocol to the IACUC for review and approval before initiating them.

I will notify the IACUC of changes in the location of the animal research.

I will assist the IACUC in verifying compliance with the regulations.

I will notify the IACUC of any unexpected results that affect the welfare of the animals. I will report any unanticipated pain or distress, morbidity, or mortality to the attending veterinarian and the IACUC.

I understand and agree that emergency veterinary care, including euthanasia, will be administered to animals exhibiting unbearable pain distress or illness. Prior to any emergency treatment, the veterinary staff will make every effort to contact my representative or me.

I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. All other personnel involved in animal use in this project have been or will be trained in proper procedures relevant to this protocol, including but not limited to animal handling, administration of anesthetics and analgesics, aseptic technique, postoperative monitoring, and euthanasia. I will notify the IACUC when new employees are hired and will certify when their training to perform the relevant experimental procedures on live animals is complete.

I declare that the information provided in this protocol is accurate. If this project is to be funded, I certify that this protocol accurately describes all procedures in which I intend to involve laboratory animal subjects.

I declare that the studies described here do not unnecessarily duplicate previous work by others or by me.

[redacted] [redacted] May 17. 2019

Signature of Principal Investigator Date

ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)
Main Body
VERSION 4
APPROVED VACO REVISIONS

See Instructions for Completion of the Animal Component of Research Protocol (ACORP Instructions), for help in completing specific items.

A. **ACORP Status.**

1. Full Name of Principal Investigator(s)► [redacted], PhD & [redacted], PhD

2. VA Station Name (City) and 3-Digit Station Number► Cleveland Wade Park, 541

3. Protocol Title► **Optimization & Pre-clinical Testing of Implantable, In-Line High Density 32-Channel Connector**

4. Animal Species covered by this ACORP► Cat

5. Funding Source(s). Check each source that applies:

►(X) Department of Veterans Affairs.
►( ) US Public Health Service (e.g. NIH).
►( ) Private or Charitable Foundation -- Identify the Foundation:
►( ) University Intramural Funds – Identify the University and Funding Component:
►( ) Private Company – Identify the Company:
►( ) Other – Identify Other Source(s):

6. Related Documentation for IACUC reference.

   a. If this protocol applies to a project that has already been submitted to the R&D Committee for review, identify the project:

      (1) Title of project► **Optimization & Pre-clinical Testing of Implantable, In-Line High Density 32-Channel Connector**

      (2) If approved by the R&D Committee, give the date of approval► 02/19/2019

   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:

      (1) Identify the studies described in the previously approved ACORP that have already been completed
      ► Although this study is de novo, it bears some similarity to a recently completed, approved study by Drs. [redacted] in feline subjects at the Cleveland VA, in which

early prototypes of in-line connectors were implanted, that resulted in animals being able to be transferred to an adoption oriented protocol (under VA Adoption guidelines) at the conclusion. In this study, we will test the durability and biocompatibility of new 32 channel connectors that have no internal cavity and are miniaturized so the implanted wires and the connectors are beneath the skin, which avoids the problems of external wires and is expected to allow improved motor and sensory capabilities.

(2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly
► N/A

(3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other Items below.
► In the prior, completed study by Drs. [redacted] of stimulation in feline subjects, it was suggested that it would be best to neuter male cats to reduce aggressive behavior in group housing situations. Depending on each individual cat's behavior and nature, it may or may not be necessary. At the time of the survival surgeries discussed here, the ARF registered veterinary technicians will be consulted to determine whether neutering (or spaying) would be beneficial. This procedure would then take place right before the implantation of the connector(s), and it would be considered part of a given survival surgery. Castration of male cats will decrease inter-cat aggression and decrease the objectionable odor of urine in intact male cats. Castrated and intact cats may not get along well together.

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).

(1) Title of other protocol ► Exploiting selective recruitment to delay fatigue during electrical stimulation
(2) IACUC approval number of other protocol ► [redacted]
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ► Approved by LSCVAMC IACUC

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):

►(X) Research
►( ) Teaching or Training
►( ) Testing
►( ) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of people and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.

► The goal of this study is to improve the safety and inter-operability (functional life) of medical devices, and improve artificial (prosthetic) hands for Veterans and others who have had a hand amputated. Since 2001, over 1,650 U.S. troops have lost hands, arms, legs or feet during the wars in Iraq and Afghanistan. My co-investigator at our VA Medical Center has developed a prosthetic hand that a patient can control with their forearm muscles to make the hand open and close, having pressure sensors in the thumb, index, and middle fingers so that patients can "feel" things, restoring the natural sensation of touch. [redacted] When such prostheses are used, they feel like part of person's body, instead of a separate medical device attached to their own body. In practical use, a prosthetic [redacted] hand also contains multiple components that are implanted within the intact limbs. These include a nerve stimulator, nerve electrodes, and 'in-line' connectors that interconnect these components; the connectors are the subject of this work.

Unfortunately, the highest-density in-line implantable connector currently available to interconnect medical device parts in the body, such as the prosthetic hand sensors above, supports only 8 channels. In this sense, a channel represents each electrical signal path leading from a specific sensor (feeling of touch or pressure) or actuator (movement), to a nerve, and from there, to or from the brain. Each sensor needs a separate 'channel', and the more sophisticated the prosthetic, the more channels are needed – and thus, more in-line connectors. For example, the prosthetic hand system will require between 32 and 64 channels, and the large size and design of currently available connectors limits how many sensors can be put in an artificial hand, and what movements it could enable. Furthermore, a large number of existing connectors would be required to attain the total channel number needed to improve touch sensation and hand function, which would be extremely bulky, possibly not even fitting within a person's arm. While it is possible to avoid implanted connectors by using wire leads that exit the skin of the patient, the place where the wires come out of the patient's arm or leg can become infected, and wires can be accidentally damaged or pulled out (e.g. by an external wire becoming caught on a protruding object, such as a cabinet knob).

With support from the VA, we have developed and performed preliminary testing on a new connector with 32 channels that is miniaturized so that implanted wires and the connectors can be run under the skin, avoiding all the problems of external wires. Furthermore, this new connector allows the wires to be detached and reconnected with improved or repaired hardware at a later time, so that future sensor upgrades, for example, could be put into the artificial hand and re-attached through the connectors. Our new VA High Density (HD) connector will allow many more sensors to be added to the prosthetic hand, such as sensors on the other fingers, palm, and wrist, and restore the ability to sense for heat, cold, and pressure/touch. With these HD connectors, more input from the forearm muscles is possible so that more hand movements could be performed. Eventually, this same technology could be used for arm, leg, or foot amputees, and for other problems having to do with movement and sensation.

Since this new VA HD Connector will be implanted under the skin, it must be "bio-compatible", which means it must not cause tissue inflammation or injury and be durable. Durable means the connector will withstand normal body movements (including running and jumping), and will not break or allow body fluids to leak into the connector, possibly 'shorting out' the connections. We have done initial lab bench tests, by having the connectors sit in a solution similar to body fluids, and we checked them for leakage and corrosion (there was none). However, the FDA requires that the connectors be tested in living animals before implanting them in human patients.

Cats are ideal for testing the new HD connectors because of their size; the torso is approximately the girth of a human limb, and they are also natural athletes (running, jumping, etc.). The connector will be implanted using standard veterinary surgery, anesthesia, and analgesia techniques. The cats will recover quickly from surgery; the implanted connector merely "sits" in the cat's body for up to six months, and no nerve stimulation will occur. The cats will be encouraged to play and jump using the laser pointer and other toys to test the connector's durability. At the end of the study, the connectors will be removed for examination, and the cats will be offered for adoption according to VA adoption guidelines. The cats will experience very minor distress (similar to being spayed), and the benefits of this work for Veteran amputees far outweighs any distress in the cats.

C. **Experimental Design.**

1. **Lay Summary**. Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.

► As opposed to connectors in current use, the new connectors are quite small and have a clamshell design with no internal cavity, which makes them less vulnerable to corrosion by body fluids and more durable. These new connectors will be implanted in seven cats using standard veterinary surgical techniques, anesthesia, and pain relief (analgesia). Although, the cats recover from surgery within a few days, we will wait three to four weeks before testing the connector. The connector itself will not stimulate the cat – it will just sit there within the body. Each cat will be regularly encouraged to run and jump using the laser pointer and other toys in order to challenge the connectors' durability and to provide enrichment for the animals. As often as weekly after the recovery period, we will anesthetize the cats and check the connectors to make sure they can still carry signals well. This testing procedure is noninvasive, causes no pain, and should take less than 15 minutes to complete. After six months, the connectors will be surgically removed, and the cats will be checked for any signs of inflammation or tissue damage from the connector. The connectors will also be examined to make sure that no body fluids leaked in, that they still function properly, and that there were no abnormal tissue responses to the connectors' presence. After the cats have recovered from the connector removal surgery, they will be put up for adoption according to VA adoption guidelines.

2. **Complete description of the proposed use of animals.** Use the following outline to detail the proposed use of animals.

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.

► We are proposing to take implantable connector density to new levels using improved, cavity-free clamshell designs and compressible, laser-machined spring pins transverse to the line of the incoming leads. In this way, we do not have internal voids that over time are vulnerable to the corrosive effect of salts found in body fluids. Overall, in-line connectors are an important component of systems to ultimately be implanted in humans. As nerve interfaces increase in number of contacts, it is necessary to reduce and organize the number of wires implanted. The addition of an inline

connector makes a system modular, meaning it would be possible to switch out either an electrode or a stimulator in case of malfunction or in the event of future improvements to medical device technology without disturbing the nerve as is the case with hard-wired prosthetic devices. This style of connector is already in use in humans; but, it is necessary to test the biocompatibility and robustness of these new HD components. The data gathered in these preliminary tests will be used to justify further testing and independent studies of the connectors. The ultimate goal of the implantation of HD connectors into cats is to provide supporting bio-compatibility and bio-stability data in living animals that will support future follow-on human clinical studies. The performance of and tissue responses to our optimized HD connectors are unknown and will be the focus of this study.

For this study, a total of 7 cats are required. The cats will be used for a survival surgery for an implantation of the HD connector for a period of 6 months, followed by explantation in a second, survival surgery. My VA co-investigator Dr. [redacted] will perform the surgeries. In our study, we will monitor the connector's size and space compatibility in the intended implant locations (HD connector will be implanted in the hamstring muscles of the hindlimb with leads exiting the skin between the shoulder blades – see diagram below), as well as its stability in the presence of motion and muscle activity. Post-operatively, gross external observation of animal physiology will be observed semi-monthly.

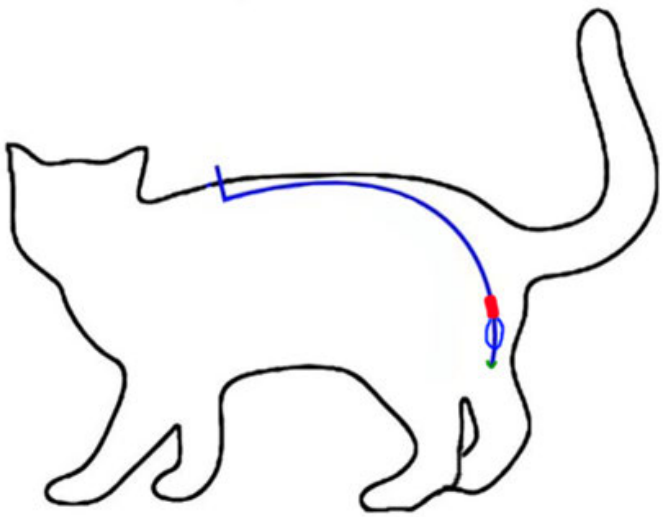

Figure 1: Schematic diagram of a feline subject having leads exiting the skin between the shoulder blades, with the leads going to an implanted high-density connector (in red); the distal half of the connector would have some leads looped back to the package, and some dummy terminated leads.

From an animal welfare perspective, it is anticipated that the use of the inline connectors will not cause any significant pain or distress based on preliminary implantation experiments in feline subjects of prior generations of HD Connector designs that have been conducted over the last two years. Our goal is to test the biocompatibility and electrical robustness of the in-line connectors, which are not expected to significantly hinder movement of the animals. The leads to the HD connectors can also be implanted in a 'loop-back' configuration (where the inline connector does not attach to an electrode) in order to test only the biocompatibility and connectivity of the connector components and leads. When implanted with 'dummy leads' going to the in-line connectors, any electrodes used would again be for testing biocompatibility only. The 'dummy leads' would not be connected to an actual stimulator at any time. In-line connectors will be tested for biocompatibility simply by being implanted within the body, by monitoring the electrical resistance between the leads, and by histology experiments on the explanted tissue capsule surrounding the removed hardware after the end of the six-month post-op observation period. The explanted tissue capsule, connectors and leads will

undergo histopathological processing and will be stained with Masson's trichrome to analyze the maturity of the collagen, and hematoxylin & eosin to analyze cellularity. Sections will be examined at 60x with light microscopy.

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

► In this study, a total of 7 cats will be used for chronic experiments. We have developed a scoring system for performing evaluation of the biocompatibility of the implanted HD Connectors, which is outlined as follows.

Scoring System. A quantitative assessment of the tissue responses around the connector capsule surfaces will be made by scoring each of the features listed below. A score of '+1' will be assigned to each feature that is present in the histological sections, or present during the period between the implantation and explantation surgeries. Photographs and scans will impart landmark information to assist in the interpretation of the histology slides. It is presumed that a mean score of zero will be found for the following features, meaning that we do not anticipate that any of these features or complications will be present:

(i) Evidence of general behavioral change of the animal. This might be judged from changes in normal activity, or of eating patterns.
(ii) Evidence of excessive post-operative inflammation, either on the surface or under the skin, that persists for more than one week and which is significant enough to warrant continued use of anti-inflammatory medications. (Some degree of inflammation is part of the normal healing process after surgery. This routine inflammation is normally easily treated and is rarely a problem. Inflammation that persists for much beyond one week, or that remains obvious by clinical examination, has the potential to injure the adjacent tissue and/or cause other adverse effects. This more significant type of inflammation will be considered a surgical complication. The decision about the post-operative degree of inflammation will be made at regularly scheduled examinations of all animals, which will be performed by our surgical team.)
(iii) Hemorrhage. (Smaller hemorrhages almost always resorb within a week or two without obvious adverse consequences). Large or persistent hemorrhages would be considered a complication.
(iv) Mechanical problems causing damage to the device, such as broken metal leads or joints.
(v) Evidence of damage to deeper tissues near where the connector is placed, e.g. due to any sharp edges, that might cause licking of the area or other observable behaviors or physical changes.
(vi) Evidence of inflammation or infection that develops after an early period of apparent success.
(vii) Evidence of diminished functioning of the connector over time due to physical damage from the implantation procedure, again after an early period of apparent success.
(viii) Evidence of "extrusion" of any part of the implanted device, e.g. if a component of the implanted device breaks through the skin.
(ix) Possible abnormal tissue pathology near the HD Connector implant site or its leads, such as excessive inflammation, abnormal capsule collagen maturity, cell hypertrophy near the connectors, or abnormal scarring

In sum, a healthy animal with normal tissue capsule formation around the HD Connectors during the post-operative period would have a 'score' of 0, which is anticipated; the worst possible score would be +9, but we have in practice never seen more than one of the above complications at once. If one or more do occur, the ARF Veterinarian will be consulted as to appropriate treatments.

While our proof-of-concept animal trials are intended primarily as pre-clinical biocompatibility studies, considerable statistical power can be achieved with a small animal cohort, as we demonstrate below.

In the absence of biocompatibility, we would expect at least one of the nine features or complications to be present in all cases, and thus we consider a mean score of 1 as a "threshold of incompatibility". By contrast, in the presence of biocompatibility, we would expect most subjects to experience no complications from the surgery as outlined above, but some may experience one. These assumptions are based on the investigators' previous experience with similar connector devices, procedures, and assessment scales/rubrics.

We considered our power to reject incompatibility (the null hypothesis) using a one-sided binomial test for comparing proportions, looking at the proportion of subjects with a score of 0, versus the proportion with a score of greater than or equal to 1. With an assumed $\alpha = 0.05$, we would reject the null (i.e. p-value < 0.05) with:

- N = 5 with up to 1 subject experiencing 1 complication, others experiencing none; power of 92%
- N = 6 with up to 1 subject experiencing 1 complication, others experiencing none; power of 99%
- N = 7 with up to 1 subject experiencing 1 complication, others experiencing none; power of 99%
- N = 7 with up to 2 subjects experiencing 1 complication, others experiencing none; power of 69%

These are based on performed binomial tests of the described data performed using the statistical analysis software program "R 3.5.0" ; the tests were analyzed by independent biostatistician Dr. [redacted] of the Louis Stokes Cleveland VA Medical Center.

Note that the request for seven animals takes into account the possibility of one failed surgery (however unlikely) that might detrimentally affect the statistical power of the study. In that case, N would be 6 for the above analyses, which will still provide strong statistical power for the study. In light of this, we maintain our request for N=7 cats, which accounts for the possibility of scattered engineering complications, and, the possibility of one potential surgical failure. Reducing the group to only four cats lowers the power to less than 80%. Since we are supposed to have a statistical power of at least 80%, we will need at least 5 successfully implanted cats. The possible complications that may well result from unanticipated engineering issues with the assembly of the connectors may not be discoverable until the parts are actually used in surgery; those issues may also possibly result in one or two failed surgical trials, which is why we are requesting a total of 7 animals.

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

Connector Implant Surgery: All cats will be anesthetized, surgically prepped, and will have a HD connector and associated leads implanted and secured between muscle planes, adjacent to the biceps femoris and semitendinosus muscles. Electrical leads will be tunneled subcutaneously from the implanted connector, past the hip, and will exit the skin in the inter-scapular region to facilitate testing. Dummy intra-muscular electrodes connected to electrical leads of the implanted connector may be implanted in the nearby muscles of the leg as an example of typical applications of the connector in practical use. The surgical wound around the lead exit will be closed in layers. Cats receive postoperative care and analgesics during the recovery period.
Note: Cats that display aggression toward their peers will be neutered (males) or spayed (females) if recommended by the veterinary staff. The neuter or spay surgery will be performed at the same time as the connector implant surgery, so that the subject is only anesthetized once. See Appendix 5 for additional details.

Jacket placement: Prior to surgery, the cats will be acclimated to wearing jackets on a near-continuous basis. Initially, the leads that exit the skin will be covered with a bandage until all skin incisions are healed. When the bandage is removed, each cat will then wear their vest-like jacket, which protects the leads, fits comfortably, and does not impede movement.

Assessing Neural Health In-Vivo: The cats will be observed daily for indications of possible damage to nerves near the HD Connector locations as evidenced by hunched posture, drop foot, and/or general disuse of an implanted limb. We have also developed a scoring system for performing evaluation of the biocompatibility of the implanted HD Connectors, outlined in section (b) above, and biocompatibility will be assessed according to that rubric regularly during the post-operative period.

Chronic Testing Sessions: The cats will be given 3-4 weeks to recover from implantation surgery. After recovery, impedance testing sessions will take place in each cat as often as weekly for up to 6 months of data collection. The goal of these experiments is to monitor the ongoing functionality of the connectors while they are subjected to typical stresses related to normal animal activity. Isoflurane anesthesia is required for approximately 15 minutes to keep the cats immobile and relaxed during testing sessions.

Explantation Surgery: The final surgery will be a survival surgery in which the implanted HD Connector and lead wires will be removed along with the thin tissue capsules which are anticipated to form around the implanted units. The surgical wound will be closed in layers, and postoperative care including analgesics will be provided. After recovery and suture removal 2 weeks post operatively, the animals will then be transferred to an ARF protocol with the intention of adopting them out of the facility.

D. **Species.** Justify the choice of species for this protocol.

► We need an animal large enough for implanting our HD connectors, and one that will walk, run, and jump in order to properly test the connector's biocompatibility and durability. Rats and mice are far too small for this connector, and rabbits will not jump unless they are chased, which rabbits find distressing. Male rabbits can also become aggressive in group housing situations, even with enriched environments. Furthermore, rabbits do not walk in the same manner that cats do; their hopping motion would not be nearly as good a model of typical stresses applied to the connector as the motions that cats perform. Cats are the right size and are happy to run and jump after a laser pointer or other toy. Their flexibility during routine activities (stretching, grooming), and the athletic activities of walking, running and jumping will let us realistically test whether the connector will be suitable for people who may do activities such as jogging, yoga, playing basketball, etc.

Although, the physical size of pigs, sheep, and goats is sufficient to host our small connectors; all three are prey species that typically do not run unless being chased by a predator (distressful). Adult large animal models could be trained and encouraged to walk on a treadmill but getting the animals to run on the treadmill would probably require considerable effort (i.e., forced exercise). Goats, especially young goats, engage in play that involves spontaneous bouts of running and jumping but, unlike the cat, they can't be easily enticed to display these behaviors. Housing and management of young large animals would be easier than housing adults because of their smaller size. However, they will grow rapidly over a six-month period, which is likely to dislodge and possibly damage the connector.

Collectively, adult cats are of the proper size to accommodate the implanted HD connector and their natural behavior is to run, jump, twist, and turn when at play, which simulates movement and stressors on the connectors similar to a human playing a sport such as basketball. At the end of the study the connectors will be removed, and the cats will be offered for adoption into private homes. Therefore, the cat is the only animal model in which our connector can be properly tested to meet FDA requirements before we can apply to test the connectors in human patients.

Personnel

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.)

1. Co-I

Name► [redacted], PhD

Animal research experience ► Dr. [redacted] is a co-investigator for this project and will be serving the main advisory role for the animal portion of this research. He will also be the primary surgeon in implantation of HD Connectors. He has been working in animal research for the Neural Engineering Center for over 15 years developing novel stimulating electrodes for Spinal Cord Injury uses. He has chronically implanted electrodes in dozens of animals, including dogs, cats, and rats, over that period and to the present day. He has completed all necessary training for this protocol.

Qualifications to perform specific procedures

Specific procedure(s) that the Co-I will perform personally	Experience with each procedure in this species described in this ACORP
Surgical implant of chronic HD Connector	Performed several times for chronic experiments on cats in the last 3 years since 2015.
Euthanasia	There is no euthanasia planned for this protocol; even though no euthanasia endpoint is planned, there may be some instances, i.e. when criteria for interventional euthanasia are met, that euthanasia must be performed.

PI

Name► [redacted], PhD

Animal research experience ► Dr. [redacted] will supervise the production and acquisition of new implanted connector components, and oversee the laboratory experiments to evaluate the new connectors. He has more than 20 years of experience in the development and assessment of neuroprostheses and related components. Because he has only limited recent work with animal experiments, Dr. [redacted] will oversee the surgical implant, testing, and euthanasia of cats in this project. Dr. [redacted] will observe, but not assist in the animal implantation surgeries and subsequent monitoring and chronic testing.

Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this ACORP
Observation of animal surgery and testing, operation of testing instrumentation	N/A

2. Other research personnel (copy the lines below for each individual)

Name► Graduate Student TBN

Animal research experience ► A graduate student, to be named, will be trained and qualified to assist in the surgical protocol and subsequent daily monitoring and testing of the HD Connectors; they will

be added to this protocol when training is complete. ARF Staff can also be hired to perform daily monitoring and to administer medications, should a graduate student be unavailable.

Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this ACORP
To Be Determined	Needed training will be provided by experienced members of the investigative staff, by ARF veterinary staff, and online

3. VA animal care and veterinary support staff personnel (copy the lines below for each individual)

Names► [redacted], [redacted], RVT; [redacted], DVM

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
Induction of anesthesia, intubation, extubation, anesthesia monitoring during surgical implant, chronic testing, and euthanasia, training of personnel, technical services relating to anesthesia, surgery, post op care and monitoring	[redacted] is LATG certified and an RVT Registered Veterinary Technician. [redacted] also has 3 years of experience at VA, 5 years of experience at CWRU and 3 years of experience at the Cleveland Clinic. [redacted] also has experience working with this team and with these methods of implant during 3 prior procedures. [redacted] is also an RVT. [redacted], DVM is the ARF Veterinarian and will perform feline neutering or spaying procedures as described here if medically necessary. Dr. [redacted] is certified to perform these procedures.

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course

Name of Individual	Working with the VA IACUC	ORD web-based species specific course (Identify the species)	Any other training required locally (Identify the training)
[redacted], PhD	5/08/18	07/15/18 (Working with Cats in Research Settings, Basic Course)	07/16/15 (VA ARF Orientation), In person ARF orientation
[redacted], PhD	02/01/18	10/03/18 (Working with Cats in Research Settings, Basic Course)	07/16/15 (VA ARF Orientation), Observation only
[redacted]	09/25/18	09/09/18 (Working with Cats in Research Settings, Basic Course)	N/A, ARF Veterinary Technician
[redacted]	3/4/2018	03/03/18 (Working with Cats in Research Settings, Basic Course)	N/A, ARF Veterinary Technician
[redacted], DVM	3/4/2019	06/28/17 Working with Cats	N/A, ARF Veterinarian

F. **Training to be provided.** List here each procedure in Item E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, enter "N/A"

►Dr. [redacted] will provide training to a graduate student TBN who will then be added to this protocol. [redacted] and/or [redacted] will provide training on cat handling and restraint, substance administration, animal anesthesia, monitoring, recovery and post-operative care.

G. Occupational Health and Safety.

1. Complete one line in the table below for each of the personnel identified in Item E:

Name	Enrollment in OHSP		Declined optional services	Current on Interactions with OHSP? (yes/no)
	VA program	Equivalent Alternate Program – identify the program		
[redacted]	(X)	( )	(X)	Yes
[redacted]	(X)	( )	(X)	Yes
[redacted]	(X)	( )	(X)	Yes
[redacted]	(X)	( )	(X)	Yes
[redacted], DVM	(X)	( )	(X)	Yes

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?

► (X) Yes. Describe them ► All cats will be vaccinated for rabies. Nonetheless, all personnel handling cats must be aware of rabies precautions in the event of a cat bite. These include prompt notification of the ARF in the event of a cat bite, and quarantine of the cat for 10 days.

► ( ) No.

Animals Requested

H. **Animals to be Used.** Complete the following table, listing the animals on separate lines

according to any specific features that are required for the study (see ACORP Instructions, for guidance, including specific terminology recommended for the "Health Status" column):

Description (include the species and any other special features not shown elsewhere in this table)	Gender	Age/Size on Receipt	Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony)	Health Status
Cat, domestic short hair	**F/M**	**Adult**	[redacted]	**Purpose Bred and vaccinated for rabies prior to arrival at VA**

I. **Numbers of animals requested.** See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

USDA Category B

Procedures►						
Species / Experimental Group / Procedures(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category B TOTAL**

USDA Category C

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category C TOTAL**

USDA Category D

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category D TOTAL**
Cat (all listed procedures)		**7**				**7**

USDA Category E

Procedures►						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category E TOTAL**

TOTALS over all Categories

Species / Experimental Group /Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**GRAND TOTAL**
Cat		**7**				**7**

J. **Management of USDA Category D procedures**. Indicate which statement below applies, and provide the information requested.

► ( ) This protocol does NOT include any Category D procedures.

► (X) This protocol INCLUDES Category D procedures. List each Category D procedure and provide the information requested. (For surgical procedures described in Appendix 5, only identify the procedure(s) and enter "See Appendix 5 for details.)

Procedure	Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery)	Person(s) responsible for the monitoring	Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered)
Chronic Connector Implant	See Appendix 5 for details	See Appendix 5 for details	See Appendix 5 for details
Explant Surgery	See Appendix 5 for details	See Appendix 5 for details	See Appendix 5 for details

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

► (X) This protocol does NOT include any Category E procedures

► ( ) This protocol INCLUDES Category E procedures. Identify each Category E procedure included in this ACORP and justify scientifically why the pain or distress cannot be relieved.
►

Veterinary Care and Husbandry

L. **Veterinary Support**.

1. Identify the laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

   Name► [redacted], DVM, DACLAM
   Institutional affiliation►VA
   email contact► [redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted► [redacted], DVM, DACLAM
   Date of the veterinary consultation (meeting date, or date of written comments provided by the

veterinarian to the PI) ► 12/30/18

M. **Husbandry.** As a reference for the animal husbandry staff, summarize here the husbandry requirements of the animals on this protocol. (Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals. Use Appendix 9 to document any aspects of the husbandry that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

1. Caging needs. Complete the table below to describe the housing that will have to be accommodated by the housing sites for this protocol:

a. Species	b. Type of housing*	c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)	e. Estimated maximum number of housing units needed at any one time
Cat	Standard, see below	3- 7 per room, 1 per cage post operatively	yes	1

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:
► Housing will be provided by the VA ARF as per the ARF standard operating procedures. The cats will be housed together and allowed to socialize with each other. Group housing of cats is currently the standard husbandry policy of the ARF and has been and currently is being used to house cats. In the event of any illness or other issue with the animals, the affected animal(s) will be placed in separate cages. The cat housing room features include individual cages with food and water as well as shelves at multiple elevations which provide the cats the opportunity to retreat into for solitude if preferred or in the event of any conflict. Moreover, frequent positive human contact will be provided to the cats throughout the experimental period.

Animals require separate housing while in post-operative recovery, minimum 3 days up to several weeks, due to increased aggression while wound healing and the presence of percutaneous leads that can be pulled out or damaged by other animals. The ARF personnel will advise when the animals are ready to be reintegrated to community housing. See section 3, Item 2 below.

** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a justification if any animals will be housed singly (if species is not considered "social", then so note)
► See above, only housed separately during post – operative recovery or if found to be

socially incompatible with other cats.

***Use Appendix 9 to document "departures" from the standards in the *Guide*.

2. Enrichment. Complete the table below to indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required (See ACORP Instructions, for more information on enrichment requirements. Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards in the *Guide*.):

a. Species	b. Description of Enrichment*	c. Frequency
Cat	Standard, see below	See below

*If enrichment will be provided according to a local SOP, enter "standard (see SOP)" and enter the SOP into the table in Item Y. If the local standard enrichment is not described in a SOP, enter "standard, see below", and describe the standard species-specific enrichment here.
► The VA ARF will provide environmental enrichment to the cats. Cats will be provided environmental enrichment including manipulatable objects such as rubber and plastic balls. Individual cages as well as shelves at multiple elevations are provided for the cats as well. The cages and room also provide ample vertical surfaces so that the cats are able to use these vertical stretches for stretching, jumping, and other activities. The rooms in which the cats are housed also provide ample room for the cats to move freely both horizontally and vertically. Animals will also be enriched by human contact at least twice daily.

3. Customized routine husbandry. Check all of the statements below that apply to the animals on this protocol, and provide instructions to the animal husbandry staff with regard to any customized routine husbandry needed.

► ( ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals, and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.
►N/A

► (X) Devices that extend chronically through the skin WILL be implanted into some or all animals on this protocol. Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.

► The leads to the connectors will exit the skin through the scapular region. They will be bandaged for the first 3 days following implant and then monitored for infection. If site shows signs of irritation or infection, the ARF veterinary staff will be called in for advice on treatment. After bandages are removed, cats will be placed in jackets to protect the leads and exit sites from pulling or chewing. Cats will be monitored daily for signs of distress. During the first couple weeks after surgery, when the lead exit site is healing, the cats will be caged separately so that the other cats cannot adversely affect the site. Following this healing period, the cats will again be housed together and allowed to socialize with each other. However, they will be

monitored daily for any signs of adverse effects caused by other cats on the lead exit sites. If there is any evidence of this problem, the cats will be caged separately. Lead exit sites will be cleaned once weekly with chlorhexidine by the graduate student TBD or by ARF Staff.

► ( ) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.
►

► (X) This ACORP does NOT include use of any animals that will require customized routine husbandry.

N. **Housing Sites**. Document in the tables below each location where animals on this protocol may be housed.

► (X) Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU.

Building	Room number	Inside of VMU?	
		Yes	No
ARF, [redacted]	To be assigned	(x)	( )

► ( ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table.

Name of Non-VA Facility	Is this facility accredited by AAALAC?		Building	Room Number
	Yes -- enter status*	No**		
	( )	( )**		

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

Special Features

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check “Appendix 2” in Item Y, below, and complete and attach Appendix 2, “Antibody Production”.

► (X) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

► (X) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".

► ( ) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Q. **Locations of procedures.** Complete the table below, listing the location(s), inside or outside of the animal facility, for each of the procedures to be performed on animals on this protocol.

Procedure	Surgical?		Bldg/Room Number	Requires transport through non-research areas?	
	Yes	No		Yes – describe method of discreet transport	No
Chronic connector implant	**(X)**	**( )**	**ARF [redacted]**	**( )** All survival surgery will take place in the ARF [redacted].	**(X)**
Testing session	**( )**	**(X)**	**TBD**	**( )** If taken outside of the ARF, cats will be contained in a draped carrier to prevent viewing by the public.	**(X)**

R. **Body Fluid, Tissue, and Device Collection.** List each body fluid, tissue, or device to be collected, and complete the table below to indicate the nature of the collection. Check the relevant Appendices in Item Y, below, and complete and attach them, as shown in the column headings.

Body Fluid, Tissue, or Device to be Collected	Collected AFTER Euthanasia	Collected BEFORE Euthanasia		
		Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production")	Collected as Part of a Surgical Procedure (Appendix 5, "Surgery")	Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection")
Implanted connector and surrounding capsule of glial tissue	**( )**	**( )**	**(X)**	**( )**

S. **Surgery.** Does this protocol include any surgical procedure(s)?

► (X) Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".

► ( ) NO animals on this protocol will undergo surgery.

T. Endpoint criteria. Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
► The cats will be weighed immediately prior to implant surgery and daily post operatively until weight has stabilized and appetite has returned to normal. Beyond the initial ~10 day recovery period, they will be

weighed weekly on the same day each week. A veterinarian will be consulted, and treatment initiated anytime the cats have clinical signs of illness. In the event a cat has weight loss of greater than 10% of body weight, infection of the implant or skin where the leads exit that is not responsive to antibiotics, or signs of neurologic dysfunction, weakness, or pain secondary to the implant, the veterinarian will determine the treatment plan, or if interventional euthanasia is necessary. In our experience to date, euthanasia has not been necessary, and any health problems have resolved with treatment. Our intention is that after explantation of the connectors and leads, the animals will be transferred to an adoption-oriented protocol adhering to VA Adoption guidelines.

U. **Termination or removal from the protocol**. Complete each of the following that applies:

► (X) Some or all animals will NOT be euthanatized on this protocol. Describe the disposition of these animals. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these methods of disposition. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

► It is the intention that all animals under this protocol will be transferred to a Holding ARF SOP with the intention of adopting the animals out of the facility, at the conclusion of the 6 month implantation period and after recovery from survival explantation surgery. Euthanasia is limited only to animals meeting health criteria for interventional euthanasia; it is not anticipated that euthanasia will become necessary.

► ( ) Some or all animals MAY be euthanized as part of the planned studies. Complete the table below to describe the exact method(s) of euthanasia to be used. (Use Appendix 9 to document any departures from the standards in the *Guide* represented by these methods. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

Check each method that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable
( )	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death► Method for verifying death► Secondary physical method►		( )	( )	( )

(X)	Anesthetic overdose Agent► Pentobarbital Dose► 1ml/ 10lbs of ~390mg/ml drug Route of administration: IV Death will be confirmed by a sustained absence of vital signs (absence of heart beat and respiration).		(X)	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
( )	Exsanguination under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
( )	Other (Describe) ►		( )	( )	( )

1. For each of the methods above that is designated as "Conditionally Acceptable" by the AVMA, describe how the conditions for acceptability will be met:
   ►N/A

2. For each of the methods above that is designated as "Unacceptable" by the AVMA, give the scientific reason(s) that justify this deviation from the AVMA Guidelines:
   ►N/A

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.
   ► [redacted] and/or [redacted] will monitor anesthesia and verify euthanasia if it is performed due to an unexpected determination of medical necessity by the ARF Veterinarian. Both are LATG certified and RVTs (Registered Veterinary Technicians). [redacted] also has 2.5 years of experience at CWRU and 3 years of experience at the Cleveland Clinic. If unexpected euthanasia should become necessary, [redacted] will perform the euthanasia. He has over 15 years of experience using this method of euthanasia on rats, cats, and dogs.

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If

disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
►Carcass will be bagged in a red bag and placed in refrigerator to be saved. PI's staff will retrieve the carcass and perform tissue fixation immediately so that histology of tissue can be obtained. Again, it is not anticipated that euthanasia will become necessary.

b. Describe how the PI's staff should be contacted.
► (X) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
Name► [redacted]
Contact Information► [redacted]

► ( ) There is no need to contact the PI's staff immediately. Describe the routine notification procedures that will be followed. If the routine notification procedures are described in a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
►

V. **Special Procedures.** List each special procedure (including special husbandry and other special procedures) that is a part of this protocol, and specify where the details of the procedure are documented. See ACORP Instructions, for examples.

Name of Procedure	Identify Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP -- specify the Item letter(s)	Appendix 6
Testing Session: Electrical Impedance Measurements	N/A	Items: C	(X)**
Jacket Acclimation	N/A		X

*If any special procedure is detailed in a SOP, identify the SOP and enter the information requested about the SOP in the table in Item Y.

**If any special procedure is detailed in Appendix 6, check "Appendix 6" in Item Y, below, and complete and attach Appendix 6.

(Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

W. **Consideration of Alternatives and Prevention of Unnecessary Duplication.** These are important to minimizing the harm/benefit to be derived from the work.

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ►Chronic implantation of high-density connectors, tunneled leads chronically exiting skin,

   Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
Pubmed	01/30/2019	2002-19	Chronic implantation of high-density connectors	("High Density Connector implant" OR "Intramuscular electrode") AND (safety OR "chronic cats" OR anesthesia OR analgesia) AND cats	(X)	(X)	(X)	(X)
Agricola Data Base (Nat'l. Ag. Library)	01/30/2019	2002-19	Chronic implantation of high-density connectors	("High Density Connector implant"OR "Intramuscular electrode") AND (safety OR "chronic cats" OR anesthesia OR analgesia) AND cats	(X)	(X)	(X)	( )
Pubmed	01/30/2019	2002-19	Tunneled leads chronically exiting skin	"Percutaneous leads" AND ("wound care" OR "skin care") AND cats	( )	( )	(X)	( )
Agricola Data Base (Nat'l. Ag. Library)	01/30/2019	2002-19	Tunneled leads chronically exiting skin	"Percutaneous leads" AND ("wound care" OR "skin care") AND cats	( )	( )	(X)	( )

ALTBIB citations with animal use alternatives as the main topic	04/16/19	All available years	Search specifically for non-animal models for testing biocompatibility of the connector	biocompatibility, connector	(X)	( )	( )	( )

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.
► The focus of this project is on the behavior and biological response of live animals to the presence of high density connectors, so computer models are not a sufficient replacement for physiological studies. The high level of complexity of the neural and muscular physiology impedes the development of computational models to replace intact behaving animals. The results of this study will, however, contribute to the understanding of these mechanisms and may help to facilitate future development of appropriate computational models.

   We ran a search specifically looking for non-animal models for testing the biocompatibility of the connector and found no papers at all. There are no in vitro or computer models available for this work. Furthermore, the FDA requires that the testing be done in a suitable animal model before we can move into clinical trials.

   Rats and mice are far too small for the connector. We also considered using rabbits, pigs, sheep, and goats. None of these were suitable (see the discussion in section D above).

   Cats are the right size and are happy to run and jump after a laser pointer or other toy. Their flexibility during routine activities (stretching, grooming), and the athletic activities of walking, running and jumping will let us realistically test whether the connector will be suitable for people who may engage in activities such as jogging, yoga, playing basketball, etc.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.
► In this study, a total of 7 cats will be used for chronic experiments. The first goal of the experiments is to determine the biocompatibility of the connectors to be studied. A power analysis has been performed and is outlined in this document, which assumes surgical success in 6 of the 7 animals and an effect size that is consistent with that which might be expected based on preliminary studies (which indicated that the prior HD connectors were well tolerated). Please see section C2b for details.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
► The methods described here are similar to those found in other studies of biocompatibility in animal models. Anesthesia is used for all surgeries so that the animal does not feel any pain or distress. Advanced pain management is used to control surgical pain after surgeries. The cats are never forced to exercise to test the connector. Instead, all exercise is in the form of play (chasing laser pointers and such). Lastly, the cats are gradually acclimated to wearing the protective jackets so they will not find the jackets distressing.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
►These high-density connectors are novel and have not been tested for in vivo biocompatibility before. In addition to providing data for FDA approval to begin clinical trials, this study aims to expand upon previous work in this area, giving us insights into how these connectors could be improved even further.

X. **Other Regulatory Considerations**.

1. **Controlled drugs**.

a. Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions, for explanations about the information requested.

Controlled substances	Storage		Personnel Authorized to Access	Location for Use		Procurement	
	Double-locked	Not Double-locked*		VA Property	Not on VA Property	VA Phar-macy	Non-VA
Buprenorphine	(X)	( )*	[redacted], ARF Vet Techs, or Study Staff TBN	(X)	( )	(X)	( )
Ketamine	(X)	( )*	[redacted], ARF Vet Techs, or Study Staff TBN	(X)	( )	(X)	( )
Pentobarbital	(X)	( )*	[redacted], ARF Vet Techs, or Study Staff TBN	(X)	( )	(X)	( )
Midazolam	(X)	( )*	[redacted], ARF Vet Techs, or Study Staff TBN	(X)	( )	(X)	( )

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary.
►N/A

b. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

► (X) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy.

► ( ) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.

► ( ) Other. Explain►

2. **Human patient care equipment or procedural areas**. Does this protocol involve use of any human patient care equipment or procedural areas?

   ► ( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".

   ► (X) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

3. **Explosive agents**. Does this protocol involve use of any explosive agent?

   ► ( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".

   ► (X) No explosive agent(s) will be used as part of this protocol.

Y. **Summary of Attachments.** To assist the reviewers, summarize here which of the following apply to this ACORP.

Appendices. Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

► ( ) Appendix 1, "Additional Local Information"
► ( ) Appendix 2, "Antibody Production"
► (X) Appendix 3, "Biosafety"
► ( ) Appendix 4, "Ante-mortem Specimen Collection"
► (X) Appendix 5, "Surgery"
► (X) Appendix 6, "Special Husbandry and Procedures"
► ( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
► ( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
► ( ) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"

Standard Operating Procedures (SOPs). List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

Item	SOP		Approval Date
	Title	ID	
C.2.c	N/A		
M.1	N/A		
M.2	N/A		
U.4.a	N/A		

U.4.b	N/A		
V	N/A		
N.1	Husbandry – AAALAC Program Description associated SOPs		4/2018
N.2	Enrichment – AAALAC Program Description associated SOPs		4/2018
X.4a	Euthanasia – AAALAC Program Description associated SOPs		4/2018
Appendix 6	Special Husbandry		4/2018
X.4b	N/A		
W	N/A		

Z. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol. Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

a. **Certification by Principal Investigator(s):**

I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

I understand that further IACUC approval must be secured before any of the following may be implemented:

- Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
- Changing any procedure in any way that has the potential to increase the pain/distress category to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
- Performing any additional procedures not already described in this ACORP;
- Use of any of these animals on other protocols, or by other investigators.

I further certify that:

- No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC. When new or additional

personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
- I will provide my after-hours contact information to the animal care staff for use in case of emergency.

Name(s) of Principal Investigator(s)	Signature	Date
[redacted], **Ph.D.**	[redacted]	01/30/2019
[redacted], **PhD.**	[redacted]	5/17/19

b. **Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:

► (X) No minority opinions were submitted by any IACUC participant for inclusion.
► ( ) Minority opinions submitted by IACUC participants are copied here

► ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages►

Name of Attending Veterinarian (VMO or VMC)	Signature	Date
[redacted], **DVM**	[redacted]	
Name of IACUC Chair	Signature	Date
[redacted], **PhD**	[redacted]	

2. **Appendix 3. Biosafety.**

a. **Certification by PI(s) and IACUC Officials:**

We certify that:

- Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;

- All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted], **Ph.D.**	[redacted]	5/17/19
[redacted], **PhD.**	[redacted]	5/17/19
Name of Institutional Veterinarian	Signature	Date
[redacted], **DVM**	[redacted]	
Name of IACUC Chair	Signature	Date
[redacted], **PhD**	[redacted]	

b. **Certification by Biosafety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is “toxic”, “infectious”, “biological”, or “contains recombinant nucleic acid”;

- The use of each of the agents thus identified as “toxic”, “infectious”, or “biological”, or “contains recombinant nucleic acid” is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;

- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee	Signature	Date

[redacted] **or** [redacted]**, PhD**	[redacted]	

3. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
- The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
- The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
- The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
- Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:

    - Identification of each animal such that care for individual animals can be documented.
    - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
    - Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
    - Daily records covering at least the period defined as “post-operative” by local policy.
    - The signature or initials of the person making each entry.

. Name(s) of Principal Investigator(s)	Signature(s)	Date
[redacted]**, Ph.D.**	[redacted]	5/17/19
[redacted]**, PhD.**	[redacted]	5/17/19

ACORP APPENDIX 3
BIOSAFETY
VERSION 4

See ACORP App. 3 Instructions, for more detailed explanations of the information requested.

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:

Material (Identify the specific agent, device, strain, construct, isotope, etc.)	**Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors)	**Nature of Material**						
		Toxic Agent (Item 4)	Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4)	Biological Agent (Item 6)	Radioactive Agent (Item 7)	Contains Recombinant Nucleic Acid (Item 8)	Routine Pre- or Post-Procedural Drug	Euthanasia agent
Ketamine	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Propofol	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Isoflurane	VA Pharmacy	(X)	( )BSL_	( )	( )	( )	(X)	( )
Convenia® / Cefovecin	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Clavamox®	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Buprenorphine	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Midazolam	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Acepromazine	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Marcaine® / Bupivicaine	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Lidocaine	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Puralube eye ointment	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Meloxicam	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )

Gabapentin	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Heparin	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Onsior	VA Pharmacy	( )	( )BSL_	( )	( )	( )	(X)	( )
Lactated Ringers	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Implantable electrode and connector assembly with leads	Manufactured by PI Dr. [redacted] and Co-I Dr. [redacted] at VA and Vendor Sites	( )	( ) BSL_	( )	( )	( )	( )	( )
Pentobarbital	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	( )	(X)

Summary of How Materials will be Administered. Complete the table below for each of the materials shown in the table in Item 1 above:

Material* (Identify the specific agent, device, strain, construct, isotope, etc.)	**Dose** (e.g., mg/kg, CFU, PFU, number of cells, mCi) and **Volume** (ml)	**Diluent* or Vehicle***	**Route** of admin	**Frequency or duration** of admin	**Reason** for Administration and **Expected Effects**	Location of **Further Details** in this ACORP (specify " Main Body" or " App #" , and identify the Item)	Administration Under **Anesthesia, sedation, or tranquilization** (Y/N)
Ketamine	10 mg/kg 100 mg/ml	Sterile Water	IV	Once pre-surgery	Induction of anesthesia. Expected to do that.	C.2, X.1, App 5.2, App 5.5, App 6.3	N
Propofol	Up to 7mg/kg 10mg/ml	Sterile Water	IV	Once pre-surgery	Induction of anesthesia.	C.2, X.1, App 5.2, App 5.5, App 6.3	N
Isoflurane	1-4%, ~12 ml/hr	Oxygenated air	Inhaled	Up to 4 hrs per surgery/ testing session	Maintenance of anesthesia during surgeries and testing sessions. Expected to provide that	C.2, U, App 5.2, App 5.5, App 5.6, App 6.1, App 6.3	Y
Convenia® / Cefovecin	8mg/kg	Sterile Water	SC	Once pre-surgery	Antibiotic taken night before surgery. Reduce risk of infection	App 5.5, App 5.7	N

Clavamox®	62.5 mg/cat	Pill Form	PO	q12 for 7 days	Antibiotic. Reduce risk of infection	App 5.5, App 5.7	N
Buprenorphine	.01-.03 mg/kg, 0.3 mg/ml	Sterile Water	Buccal/ Sublingual	q12hr	Possible pain from connector implant and percutaneous leads. Expected to lessen pain	C.2, X.1, App 5.5, App 5.7	N
Buprenorphine	0.01mg /kg, 0.3 mg/ml	NA	IM or SC	Q12hr	Expected to lessen postoperative pain	C.2, X.1, App 5.5, App 5.7	N
Midazolam	0.1-0.4 mg/kg	Sterile Water	IM	Once pre-anesthesia	Relaxes animal in preparation for anesthetic	C.2, X.1, App 5.5, App 5.7	N
Heparin		NA	IV	Once pre-surgery	To prevent blood clotting	App. 5	N
Onsior	2 mg/kg,	Sterile Water	SQ	Q24hr	Possible pain from connector implant and percutaneous leads. Expected to lessen pain	C.2, X.1, App 5.5, App 5.7	N
Lactated Ringers	5-10 mls/kg/ hr	NA	IV	during surgery	To prevent low blood pressure during surgery	App. 5	Y
Acepromazine	0.05-0.1 mg/kg	Sterile Water	IM	Once pre-anesthesia	Relaxes animal in preparation for anesthetic	C.2, X.1, App 5.5, App 5.7	N
Marcaine® / Bupivicaine	Up to 2 mg/kg	Sterile Water	Local SQ	Once pre-surgery	Local analgesia pre-incision. Reduction of local, especially cutaneous, pain signals from incisions during surgery. Shown to reduce post-operative pain.	App 5.5	Y
Lidocaine	Up to 2 mg/kg	Sterile Water	Local SQ	Once pre-surgery	Local analgesia pre-incision for neutering or spaying	App 5.5	Y

Puralube eye ointment	85% white petrolatum , 1 drop	15% mineral oil	Topical	Once pre-surgery	Eye may dry out and become irritated under anesthesia. Expected to help lessen discomfort.	App 5.5, App 6.3	Y
Meloxicam	0.1 or 0.2 mg/kg, 5mg/ml	Sterile Water	SC	q24hr	Possible pain from connector implant and percutaneous leads. Expected to lessen pain	C.2, App 5.7, App 6.3	Y, N
Gabapentin	3-10 mg/kg, 1 50mg tablet q12hr	Pill Form	PO	q12hr	Possible neurogenic pain from implant. Expected to lessen pain	App 5.7	N
Pentobarbital	1 ml/ 10lbs, ~390 mg/ml	Sterile Water	IV	Once	Euthanasia. (Not expected)	U, X.1	Y
Implantable connector and electrode assembly*	1 per animal	N/A	Implant	Once	Evaluation of Biocompatibility	Main Body	Y

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade per http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847) except for the implantable connector and electrode assembly, which is not currently approved but the biocompatibility of the device for future FDA approval is the very subject of this study; good manufacturing practices and approved sterilization procedures (ethylene oxide) will be performed on the assemblies prior to implantation.

2. **Anesthesia, Sedation, or Tranquilization.** Complete 3.a. and 3.b. below:
   a. For each material with "Y" entered in the last column of the table in Item 2 above, describe the anesthesia, sedation, or tranquilization to be used, identifying the anesthetic, sedative, or chemical tranquilizer, and detailing the dose, volume, and route of administration (Make sure that these agents are also included in Item 1 of this appendix, as materials to be administered):
   ► Isoflurane will be administered as induction prior to surgical procedures as described in the table above, and prior to nonsurgical procedures described in Appendices 5 and 6, respectively. Maracaine/Bupivicaine, Lidocaine, Puralube eye ointment, and Meloxicam will be administered after anesthesia induction as described in the table above and under surgical procedures described in Appendix 5.

   b. For each material with "N" entered in the last column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.

► Isoflurane (via anesthesia box) will be used as an agent for induction. It will be administered by a trained veterinary technician as explained in the main body of this protocol so as to minimize any discomfort of administration. Buprenorphine and Meloxicam and/or Onsior will be given postoperatively to reduce any potential pain within days after surgery. Gabapentin will be given as needed if there are any signs of neurogenic pain (indications of this pain are detailed in Appendix 5, 7f1). Although these are injections, it would be more distressing to the cat to be sedated to give these medications than to give them without sedation. Either Convenia / Cefovecin OR Clavamax will be given to reduce infection risk following surgery at incision sites as well as at the location where the leads are exiting the skin chronically. The antibiotics will begin prior to surgery and continue postoperatively as described in Appendix 5 while not under sedation. Clavamax is also an oral medication; no anesthesia required.

3. **Toxic Agents.** Complete the table below for each of the materials listed as a "toxic agent" in the table in Item 1 above, checking the all of the properties that apply (see ACORP App. 3 Instructions, for details).

Name of Toxic Agent	a. Mutagen	b. Carcinogen	c. Teratogen	d. Select Agent?			e. Other – specify toxic properties
				Not a Select Agent	Select Agent Used in Sub-threshold Quantities	Select Agent that Requires Registration/Approval	
Isoflurane							Organ toxicity and reproductive hazard

*For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ►

Registered with CDC or USDA ►
Registration Number ►
Registration Date ►
Expiration Date of Registration ►

Name of official who granted approval on behalf of VACO►
Date of approval►

4. **Infectious Agents.** Complete the table below for each of the materials listed as an "infectious agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

		b. Drug Sensitivity Panel Available? (Describe)	c. Select Agent?

Name and BSL Number of Infectious Agent	a. ABSL Number *		Not a Select Agent	Select Agent used in Sub-threshold quantities	Select Agent that Requires Registration/Approval
N/A		(Yes/No)	( )	( )	( )**

*Complete the following for each agent for which the ABSL Number given is less than the BSL Number shown (copy the lines below for each agent):

Name of agent ►
Justification for applying ABSL measures that are less protective than those recommended ►

**For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ►

Registered with CDC or USDA ►
Registration Number ►
Registration Date ►
Expiration Date of Registration ►

Name of official who granted approval on behalf of VACO►
Date of approval►

5. **Biological Agents.** Complete the table below for each of the materials listed as a "biological agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Biological Agent	Screening for Infectious Agents
N/A	

6. **Radioactive Agents.** Complete the table below for each of the agents listed as a "radioactive agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

Name of Radioactive Agent (specify the isotope)	Authorized Individual	Approving Committee or Official
N/A		

7. **Agents Containing Recombinant Nucleic Acid.** For each of the materials checked in the table in Item 1, above, as "contains recombinant nucleic acid", indicate which of the conditions applies (see ACORP App. 3 Instructions, for details).

Name of Agent that Contains Recombinant Nucleic Acid	Subject to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*	Exempt
N/A	( )	( )

8. **Potential for Pain or Distress**. Complete the table below for each of the agents listed in Item 1, above, that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3 Instructions, for details).

Name of Agent	Nature of Potential Pain/Distress	Measures to Alleviate Pain/Distress
Implanted Connector – Electrode Assembly	See full description in this Main Body and in Appendix 5 for details	See full description in this Main Body and in Appendix 5 for details

9. **Protection of Animal Facility Staff from Hazardous Materials.** Complete Items 10.a and 10.b, below, for each of the agents listed in the table in Item 1, above, as "toxic", "infectious", "biological", "radioactive", or "contains recombinant nucleic acid" (detailed in Items 4 – 8). This item specifically addresses members of the animal facility staff; protection of the research staff from each of these agents must be addressed in Item G of the main body of the ACORP. See ACORP App.3 Instructions, for details.

   a. Complete the table below.

Name of Hazardous Agent	Approving Committee or Official	Institution (VA or affiliate)	Names of Animal Facility Staff Members at Risk
N/A			

   b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents.
   ►

10. **Signatures.** Provide the applicable signatures on the signature pages (Item Z.3) of the main body of this ACORP.

ACORP Appendix 5
SURGERY
VERSION 4

See ACORP App. 5 Instructions, for more detailed explanations of the information requested.

1. **Surgery Classification.** Complete the table below for each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). See ACORP App. 5 Instructions, for details.

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	Chronic Connector implant / with or without neutering or spaying	( )	( )	(X)	(X)
2	Chronic Connector explant	( )	( )	(X)	(X)

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:
► Adequate data on the biocompatibility of the implantable connectors can be obtained without euthanizing the cats; therefore, the connectors and their leads must be explanted from the animals in a second survival surgery. The anticipated glial tissue capsules surrounding the implanted components must also be retrieved in order to conduct histological analysis. Once the cats have recovered from the explant surgery, they will be transferred to an ARF protocol for the purposes of adopting the animals out of the facility.

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
► A minimum of a six month survival period will be used to allow sufficient time for a) recovery from the first surgery and b) post-op monitoring of the connector status. This is a sufficiently long time to observe longer-term effects of the connector's that may not be initially apparent, and is typical of biocompatibility studies.

2. **Description of Surgeries.** Describe each surgery listed in Item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

Surgery 1 ► **Chronic Connector implant and possible Neutering or Spaying:**

The night before the survival surgery, the cat will start on antibiotics with either Convenia®/Cefovecin (8 mg/kg SQ) or initial dose of Clavamox® (62.5 mg/cat PO q12 hours).

Anesthesia animal prep: The cat will have one of the following pre-anesthetic procedures:

OPTION 1

- Pre-Med: Buprenorphine (0.3 mg/mL) – 0.01 mg/kg IM/SC OR 0.01-0.03 mg/kg buccal
- Pre-Med: Midazolam (5 mg/mL) – 0.2-0.4 mg/kg IM
- Pre-Med: Acepromazine (10 mg/mL) – 0.0250-0.1 mg/kg SQ or IM (*if needed*)
- Wait for 30-45 mins
- Induction: Isoflurane – 1-4% to effect via anesthesia box
- Cephalic Vein Catheter: The forelimb will be shaved and cleaned with alcohol +/- betadine or equivalent and an IV catheter will be placed in the cephalic vein and secured. One drop of blood will be collected through the catheter to test blood glucose.
- Endotracheal Intubation

- **OPTION 2**
  - Pre-Med: Buprenorphine (0.3 mg/mL) – 0.01 mg/kg IM/SQ OR 0.01-0.03 mg/kg buccal
  - Pre-Med: Midazolam (5 mg/mL) – 0.2-0.4 mg/kg IM
  - Pre-Med: Acepromazine (10 mg/mL) – 0.0250-0.1 mg/kg SQ or IM (*if needed*)
  - Wait for 30-45 mins
  - Cephalic Vein Catheter: The forelimb will be shaved and cleaned with alcohol +/- betadine or equivalent and an IV catheter will be placed in the cephalic vein and secured. One drop of blood will be collected through the catheter to test blood glucose.
  - Induction: Propofol (10 mg/mL) – up to 7 mg/kg IV slowly to effect (dose requirement may be reduced up to 25% when used with Midazolam pre-med
  - Endotracheal Intubation

- **OPTION 3**
  - Pre-Med: Buprenorphine (0.3 mg/mL) – 0.01 mg/kg IM/SQ OR 0.01-0.03 mg/kg buccal
  - Induction: Midazolam (5 mg/mL) – 0.1-0.3 mg/kg IM
  - Induction: Ketamine – 3-5 mg/kg IM
  - Wait for 30-45 mins
  - Cephalic Vein Catheter: The forelimb will be shaved and cleaned with alcohol +/- betadine or equivalent and an IV catheter will be placed in the cephalic vein and secured. One drop of blood will be collected through the catheter to test blood glucose.
  - Endotracheal Intubation

Once intubated, Isoflurane will be administered for the duration of the procedure. A registered veterinary technician will gently palpate the abdomen and empty the bladder by gently applying pressure, if urine is present. The paws will be shaved to allow for the placement of EKG electrodes for monitoring if there is no esophageal EKG available. The animal will be moved to a surgical table and placed on an anesthesia monitor for isoflurane anesthesia. A monitor will also be used to monitor vital signs. Lactated ringers solution will be provided throughout anesthesia at 5-10mg/kg IV. Fur is removed and incision sites are surgically prepped prior to making skin incisions.

Feline orchiectomy surgery (if deemed medically advisable by the ARF Veterinarian for male cats): With the cat in dorsal recumbency and the hindlimbs pulled cranially, the first testicle is mobilized in the scrotum by applying pressure with the thumb and index finger at the bone of the scrotum such that the skin is taut. A small skin incision (up to 1 cm) is made over the testicle at the end of the scrotum in a cranial to caudal fashion and the testicle and intact spermatic cord is

extruded through the incision using manual pressure. A hemostat is placed on top of the cord and the distal (testicle) end is wrapped over the hemostat once. The wrapped hemostat is then directed ventral to the cord while the testicle is held in the opposite hand. Open tips of the hemostat then grasp the distal end of the cord. The spermatic cord is then transected near the testicle using a scalpel blade and the severed end is manipulated through the loops around the hemostat and pulled snug into a knot and excess cord is resected and inspected for bleeding. The hemostat is then opened and the knotted cord is replaced through the scrotal incision. This process is then repeated on the opposite side. This entire surgery typically takes 5-7 minutes.

Feline ovariohysterectomy surgery (if deemed medically advisable by the ARF Veterinarian for female cats): A 1.5-2 inch abdominal incision will be made on skin of the ventral midline. The abdominal wall will be opened along the linea alba. A spay hook is used to exteriorize one uterine horn from the abdomen. The ovarian suspensory ligament including the ovarian vessels is clamped and double ligated with 3-0 vicryl or monocryl, transected and excised. The procedure is repeated for the left uterine ovary. The uterine body and associated vessels is clamped, double ligated with 3-0 vicryl or monocryl, transected and excised. The abdominal wall is closed with 3-0 vicryl or monocryl simiple interrupted sutures. The subcutaneous tissue is opposed with 3-0 vicryl or monocryl continuous mattress. The skin is closed with either simple interrupted with 3-0 vicryl or monocryl or continuous subcuticular with 3-0 vicryl or monocryl.

Connector-Electrode assembly implant: A 3-4 inch incision will be made on the back upper portion of the leg just proximal to the popliteal fossa. The connector and its leads will be implanted and secured between the muscle planes, adjacent to the biceps femoris and semitendinosus muscles. Once implantation and subcutaneous tunneling of the leads is complete, the musculature will be brought together and the skin will be closed in two layers. Subcutaneous tissue will be closed with an absorbable suture such as Vicryl or Dexon. Skin will be closed with staples and possibly one or two non-absorbable suture such as polypropylene. Staples, if used, will be removed between post-op day 10 - 14. The electrode leads will be tunneled subcutaneously near the dorsum, to emerge through a stab incision in the inter-scapular region of the animal. The surgical wound will again be closed in layers. Both surgical sites will be covered with sterile gauze and bandaged.

Surgery 2 ► **Chronic Connector-Electrode Assembly Explant:**

This survival surgery will be performed after approximately 6 months of post-op data collection from the implantation surgery above. The cats will receive the same pre-procedural care as described above under anesthesia. Incisions will be made near the same locations as were used to implant the HD Connector and its leads as above, and the implanted components and the glial tissue capsules surrounding them will be removed; closure of the wounds will again follow the same procedure as above.

During the approximately two-week post-removal observation period, we will collect observational data to assess the impact of removal of the connector and its leads, and subsequent nerve function. This will give us needed data to successfully perform this procedure in patients in the future.

3. **Personnel.** Complete the table below for each individual who will be involved in any of the surgeries on this protocol.

Name	Surgery #(s) (see Item 1)	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	1, 2	(X)	( )	( )	( )
[redacted], DVM	1	(X)	( )	( )	( )
[redacted]	1, 2	( )	( )	(X)	( )
[redacted]	1, 2	( )	( )	(X)	( )

4. **Location of surgery.** Complete the table below for each location where surgery on this protocol will be performed.

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
VA, ARF	[redacted]	1, 2	(X)	( )*	( )*

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
► N/A

5. **Pre-operative protocol.**

   a. **Pre-operative procedures.** Complete the table below for each pre-operative procedure that will be performed to prepare the animal(s) for surgery.

Surgery #(s) (see Item 1)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1, 2	12 hours	N/A	Cephalic	( ) --

b. **Pre-operative medications.** Complete the table below. Include agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered prior to preparation of the surgical site on the animal.

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Convenia®/ Cefovecin	**1**	**8 mg/kg**	**SQ**	**1**	**12 hours**
Clavamox®	**1**	**62.5 mg/cat**	**PO**	**q12**	**12 hours, immediate**
Buprenorphine	**1,2**	**0.01-.03 mg/kg Or .01 mg/kg**	**Buccal Or IM or SQ**	**1**	**45 minutes**
Midazolam	**1,2**	**0.2-0.4 mg/kg 5 mg/mL**	**IM**	**1**	**45 minutes**
Onsior	**1,2**	**2 mg/kg**	**SQ**	**Q24**	**45 minutes**
Acepromazine	**1,2**	**0.025-0.1 mg/kg 10 mg/mL**	**SQ or IM**	**1**	**45 minutes**
Ketamine	**1, 2**	**10 mg/kg**	**IV**	**1**	**immediate**
Propofol	**1, 2**	**Slowly to effect Up to 7 mg/kg**	**IV**	**1**	**immediate**
Isoflurane	**1,2**	**1-4% to effect**	**Inhalation /mask**	**1**	**immediate**
Puralube eye ointment	**1, 2**	**1 drop**	**Topical**	**1**	**immediate**
Marcaine® / Bupivicaine	**1**	**Up to 2 mg/kg**	**Local SQ**	**1**	**Immediate**
Lidocaine	**1**	**Up to 2 mg/kg**	**Local SQ**	**1**	**immediate**

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ► Surgical site for neutering of male cats (perineum) or spaying of female cats (ventral abdomen), if these surgeries are determined to be necessary.
Surgical site for HD Connector implantation: 3-4-inch region on the back upper portion of the leg just proximal to the popliteal fossa.
Surgical site for the lead exit region: a 2-inch long area in the inter-scapular region of the cat.

Ophthalmic lubricant will be placed in both eyes to help protect against corneal desiccation.

If the neutering procedure is to be performed, the cat will be placed in dorsal recumbency and hair will be removed (plucked) from the scrotum for the castration procedure. For the connector implant procedure, the cat will then be placed in ventral recumbency and the leg area and the region of the port/wires (mid-shoulder area) will be shaved and cleaned with alcohol, prior to moving the patient to the surgical suite. Once in the O.R., the subject will be placed on a water circulating heating pad in dorsal recumbency. Maracaine and Lidocaine (up to 2 mg/kg each, local SQ) will be injected SC at the incision site. The scrotum will be scrubbed with at least three alternating wipes of betadine (or equivalent) and alcohol in preparation for the neutering. Additionally, the cat will be monitored using a temperature probe, EKG, a blood pressure cuff on forelimb not catheterized, and a SPO2 sensor on the tongue. Immediately following the castration procedure, the cat will then be placed in ventral recumbency and the shaved surgical areas for the connector implantation procedure will be scrubbed with at least 3 alternating wipes of alcohol and a surgical scrub such as betadine. The subject will be covered with a sterile drape exposing only the prepared, sterile surgical sites.

If the spaying procedure is to be performed, the cat will be placed in dorsal recumbency and hair will be shaved from the ventral abdomen. The cat will then be placed in ventral recumbency and the leg area for the connector and the region for the port/wires (mid-shoulder area) will also be shaved and cleaned with alcohol, prior to moving the patient to the surgical suite. Once in the O.R., the subject will be placed on a water circulating heating pad in dorsal recumbency. Maracaine and Lidocaine (up to 2 mg/kg each, local SQ) will be injected SC at the incision site. The ventral abdomen will be scrubbed with at least three alternating wipes of betadine (or equivalent) and alcohol in preparation for the ovariohysterectomy. Additionally, the cat will be monitored using a temperature probe, EKG, blood pressure cuff on forelimb not catheterized, and a SPO2 sensor on the tongue. Immediately following the ovariohysterectomy procedure, the cat will then be placed in ventral recumbency and the shaved surgical areas for the connector implantation procedure will be scrubbed with at least 3 alternating wipes of alcohol and a surgical scrub such as betadine. The subject will be covered with a sterile drape exposing only the prepared, sterile surgical sites.

Surgery 2 ► Anesthesia induction/prep will occur in the same manner as that described for surgery 1.

6. **Intra-operative management.**

a. **Intra-operative medications.** Complete the table below for each agent that will be administered to the animal <u>during</u> surgery.

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
Isoflurane	( )*	**1, 2**	**1% - 4%**	**Inhaled**	**To effect**

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.
►N/A

b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals <u>during</u> surgery (e.g., warming, cushioning, etc.).
Surgery 1 ► The chronic survival surgery is expected to last no longer than 4 hours.

Cats will be intubated at the beginning of the procedure with the appropriate size tracheal tube to keep airways open.

Cats will receive IV fluids (Lactated Ringer's solution) at a maintenance rate or increased, if there seems to be blood loss during the surgery) throughout the procedure.

Ophthalmic lubricant will be applied to the eyes every hour if the eyelids are not closed during the procedure to protect the corneas from desiccation.

Cats will be placed on a water circulating heating pad with towels covering the core of the body. Fluids may also be warmed in the IV line before entering the body if necessary.

Surgery 2 ► The animal will receive the same intra-operative physical support as described for Surgery 1 above.

c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.
Surgery 1 ► The following parameters will be monitored and recorded at least every 15 minutes throughout any anesthetic procedure: temperature, respiration rate and quality, heart rate and rhythm, oxygen saturation, end title CO2, peripheral blood pressure, and anesthetic depth via jaw tone, anal tone and/or blink reflex. Animals will routinely be maintained under positive-pressure ventilation.

Surgery 2 ► The animal will receive the same intra-operative monitoring as described for Surgery 1 above.

7. **Survival surgery considerations.** For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a. – 7.g.

a. Complete the table below for each survival surgery listed in Item 1, above.

Surgery # (see Item 1)	Survival Period	Measures for Maintaining Sterility							
		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
1,2	6 months (~1 month recovery from surgery & ~5 months of testing after Surgery 1)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*

* Describe any “other” measures to be taken to maintain sterility during surgery.
►N/A

b. For each surgery, describe the immediate post-operative support to be provided to the animals.

Surgery 1,2 ► Cats will be given 0.2 mg/kg of meloxicam or 2 mg/kg Onsior SQ and will continue to be monitored as during surgery until extubation. Once extubated a Registered Veterinary Technician will oversee anesthetic recovery and continue to record at least temperature, heart rate and respiratory rate every 15 minutes until animals are sternal and maintaining body temperature. Animals will then continue to be observed at least every 45-60 minutes until fully awake and eating.

Meloxicam (0.1 mg/kg PO or SQ) will be given 24 and 48 hours after the first dose for continued pain relief. This dose will also be given during anesthetic recovery from testing sessions and may be repeated 24 and/or 48 hours after that, if indicated based on veterinary direction. Buprenorphine (0.01-0.03 mg/kg Buccal or .01 mg/kg IM or SQ) may also be given every 8-12 hours, or Onsior (2 mg/kg SQ) at veterinary direction for break-through pain during surgery recovery for 2-4 days; however, whichever NSAID is used initially will be the same one that is continued for the 2-4 days.

Sutures (or staples) will be removed 10-14 days post-surgery. The leads to the connectors will exit the skin through the scapular region after Surgery 1. The leads will not make use of a percutaneous connector, but will simply exit through a narrow incision that will be closed in layers. The incision will be covered with bandage material for the first 3 days following implant and monitored for infection or inflammation. If the site shows signs of irritation or infection, the veterinary staff will be consulted. After bandages are removed, cats will wear jackets or other protective clothing to protect the leads and exit sites. Lead exit sites will be inspected and cleaned with chlorhexidine once weekly by the graduate student TBD or by ARF staff. Cats will be monitored daily for signs of distress by these same personnel, who will perform all post-op care. Cats may be housed individually during the initial 5-10 days post-op period.

c. Post-operative analgesia. Complete the table below for each surgery listed in item 1, above.

Surgery # (see Item 1)	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1,2	**Buprenorphine**	**.01-.03 mg/kg Or .01 mg/kg**	**Buccal Or IM or SQ**	**Q8-12hr**	**2 days with additional days for all cats PRN as decribed above**
1,2	**Onsior**	**2 mg/kg**	**SQ**	**Q24hr**	**2 days with additional days as needed**
1,2	**Meloxicam**	**0.1 mg/kg**	**PO or SQ**	**q24hr**	**2 days with additional days as needed**
1,2	**Gabapentin**	**3-10 mg/kg, 1 50mg tablet**	**PO**	**q12hr**	**If needed for any neurogenic pain**

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:
►N/A

d. Other post-operative medications. Complete the following table to describe all other medications that will be administered as part of post-operative care.
Only one of the two antibiotic options listed below will be used depending on circumstances

Surgery # (see Item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
1,2	**Clavamox®/**	**62.5 mg/cat**	**PO**	**q12 hr**	**7 days**
1,2	**Convenia®/ Cefovecin**	**8 mg/kg**	**SQ**	**Once, day before surgery**	**12-14 days**

e. Post-operative monitoring. <u>After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency</u>.

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1,2	5 minutes	Until cat is extubated	[redacted], [redacted] or onnell
1,2	15 minutes	Until cat is sternal	[redacted], [redacted] or [redacted]

(2) Post-operative monitoring after the immediate post-operative period

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1,2	Daily	10 Days	[redacted], [redacted] or [redacted]

f. Post-operative consequences and complications.

(1) For each surgery, describe any common or expected post-operative consequences or complications that may arise and what will be done to address them.

Surgery 1,2 ► Complications could include soreness and difficulty walking. Minor complications will be mitigated with good pain management with buprenorphine, onsior and/or meloxicam until soreness is gone.

As with all animals after surgery, there is a risk of self-mutilation at incision sites. Self-mutilation is very rare and not expected to occur.

Neurogenic pain is not expected as connectors of similar design have been employed in human and animal subjects with intact sensation, and chronic implantation testing of like connectors has been carried out in cats without ill effects. However, if there is any evidence of neurogenic pain following implantation, gabapentin will be given as needed. Evidence of any neurological damage would include any weakness, lameness, or pain. In particular, evidence of neurogenic pain would include any self-mutilation or chewing of the affected dermatomes, avoidance of use of the affected limb, decreased ambulation and grooming, shaking or twitching of the skin, muscle atrophy, decreased appetite, decreased interaction and hiding, increased tension or withdrawal of the affected region, elevated heart rate, pupil dilation, and vocalization. If there are issues of self-mutilation an e-collar and/or jacket will be placed and gabapentin administered.

Symptoms from weakness and lameness caused by potential damage to the nearby sciatic nerve would include hyperextension of the tarsus, knuckling, or walking on the hock of the leg, any difficulty squatting (for instance, while using a litter box), any difficulty trotting, any leg dragging or limp. Any changes in sensation, including any numbness, paresthesia, and pain, would present along the back of the leg and the paw; if present, the ARF Veterinarian will be consulted as to the appropriate treatment protocol.

There is also a risk of infection due to the incisions as well as the leads chronically exiting the cat's skin. These are mitigated preoperatively by the administration of Covenia immediately before the first incision, by good sterile technique, good technical skill, and minimal time with open incisions during surgery, and by daily postoperative checks for any signs of infection as well as administration of cefazolin postoperatively.

Surgery 2 - Unanticipated adverse effects from the removal of connectors/leads may include:

1) Lead fragments. Because we will minimize the incisions, we will be pulling wires to a few exit points. It is possible that some parts of the wire will break and be left in the animal. If that occurs, it is possible that there will be granulation tissue, there may be an increased risk of infection, they may cause irritation to the animal (or pain, but this is not likely), and/or the pieces may eventually work themselves out of the skin. These are not threatening or do not warrant treatment, unless infection is present. If the pieces are irritating, they may require a minor procedure to remove, and if they protrude through the skin, they can be removed by gentle tractions.

2) Surgical site dehiscence: Will be treated by the attending veterinarian and the ARF veterinary staff as needed.

3) Temporary or permanent nerve damage: While unlikely, if this occurs it will be treated by the attending veterinarian and the ARF veterinary staff.

All post-operative medical care and, if needed, interventional euthanasia will be based on the descriptions contained herein. All post-operative care, treatments, observations and decisions for interventional euthanasia will be performed by the attending veterinarian and the ARF

veterinary staff. The ARF veterinarian will have final authority to determine medical treatments and interventional euthanasia.

(2) List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1,2 ► The cats will be weighed immediately prior to implant surgery and once every 3 days for the 10 days following implant procedure. Otherwise they will be weighed weekly on the same day each week. A veterinarian will be consulted in any of the following cases: if any animals have an unresolved infection or weight loss of greater than 10% body weight; if there is a chronic infection of the implant that is not responsive to antibiotic treatment; if there is infection of the skin or tissue near where the leads are exiting the skin that is not responsive to antibiotic treatment; there are signs of chronic neurologic dysfunction, weakness, or pain secondary to the implant. The veterinarian will advise whether treatment is indicated or interventional euthanasia is necessary.

(3) In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)
►N/A

g. Maintenance of post-surgical medical records. Complete the table below for each surgery, specifying where the records will held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

Surgery # (see Item 1)	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1,2	VA ARF, Cat Housing Room	[redacted], [redacted] or [redacted]	(X)	(X)

8. **Certification.** The PI must sign the certification statement in Item Z.5 of the main body of the ACORP.

ACORP APPENDIX 6
SPECIAL HUSBANDRY AND PROCEDURES
VERSION 4

See ACORP App. 6 Instructions, for more detailed explanations of the information requested.

1. **Description of Procedures.** Complete the table below for each procedure listed in Item V of the main body of the ACORP that is not detailed in a SOP or in another item or Appendix of the ACORP. For each special procedure, check all features that apply.

Special Procedure		Features							
Number	Brief Description	Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Conditioning	Irradiation	Imaging	Other**
1	Electrical Impedance Measurement	( )	(X)	( )	( )	( )	( )	( )	()
2	Jacket Acclimation								(X)

*Husbandry refers to all aspects of care related to the maintenance of the animals, including (but not limited to) provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

**Describe any "Other" features that are involved.
►

a. Provide a complete description of each special procedure listed above, including the duration of the procedure, how frequently it will be repeated in any one animal, and any effects it is expected to have on the animal:

Special Procedure 1 ► Testing sessions involve making a connection to the leads exiting the animals with prepared external wires leading to impedance monitoring equipment in the lab. The goal of these experiments is to monitor the ongoing functionality of the connectors while they are subjected to typical stresses related to normal animal activity.

The cats will be given 3-4 weeks to recover from implantation surgery. After recovery, testing sessions will take place in each cat as often as weekly for up to 6 months of data collection. The purpose of the testing sessions is to measure resistance between the leads that exit the animal's skin that are in turn connected to the implanted HD Connector. During such testing, animals will be anesthetized with isoflurane (chamber induction followed by mask). The implants in this study do not make direct contact with any nerve, nor do they involve any active electrical stimulation of any tissue. The HD connector will be configured so that there is a through-connection between some of its pins, while other pins will simply be terminated at the HD connector itself. For example, pin 1 may be terminated, but pins 2 and 3 may be connected electrically through the HD connector, hence the term "loop-back." This is to measure the resistance between the pins, similar to taking a weight or length measurement, which is made by connecting wires coming from the measurement instrument to the free ends of the leads that exit the animal's skin. It is less invasive than taking temperature. The testing sessions should require

the cat to be immobile for less than 15 minutes. An ARF Vet Tech will administer the anesthesia for the cat while the measurements are performed. The measurements will be taken as needed, up to once per week, so long as the animals show no undue signs of stress due to the procedure, and so as long as anesthesia limitations would not be exceeded.

Special Procedure 2 ► The vest-like jackets are used to protect the leads that exit the skin between the shoulder blades. Cats are acclimated to wearing the jacket by slowly introducing them in the days and/or weeks prior to surgery, gradually increasing the time between donning and doffing of the garments in a stepwise manner, as follows. Acceptance of the jackets by the animals will vary from subject to subject, so sensitivity to the jackets will be judged by merely placing the jackets on the cat's back at first, and then, once comfortable with that, the cats will wear the jackets for 5-15 minutes, depending on their reactions as the minutes go by. Time of wearing will then be increased in increments depending on the tolerance of the first wearing experience; for example, if 5 min were tolerated at first, the duration might be increased to 10 min the second time, and so on. Eventually, through gradual increase in wearing time, near-continuous use will be achieved prior to the first surgery date. In this manner, wearing the jackets post-op to protect the leads will not be a new experience for the animal subjects. Petting and food treats will be used as positive reinforcement. Near-continuous protection of the lead wires during the post-op period is necessary to prevent the leads from being chewed or pulled out. The jackets will be washed at least once per week in the washer and dryer in the ARF facility, or sooner if they become soiled.

b. <u>Explain why each of these special procedures is necessary</u>:

Special Procedure 1 ►These procedures are necessary to collect experimental data regarding the effects of normal animal activity on the ongoing connectivity of the implanted components.

Special Procedure 2 ► Acclimation to the jackets will be necessary in order that the cats are not distressed when wearing the jackets post-operatively. Jackets are necessary to protect the surgical sites and the electrical leads that exit the skin.

2. **Personnel.** Complete the table below for each special procedure listed in Item 1, above. Identify the individual(s) who will be responsible for carrying out the procedures, and those who will be responsible for monitoring the condition of the animals during and after the procedures. <u>After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency</u>.

Procedure Number (see Item 1)	Responsible Individual(s)	
	Carrying Out Procedure	Monitoring the Animals
1	[redacted] and/or [redacted], DVM	[redacted], [redacted], Graduate Student TBD, and/or [redacted]
2	[redacted] and/or [redacted], DVM	[redacted], [redacted], Graduate Student TBD, and/or [redacted]

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so, describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

Procedure Number (see Item 1)	Expected Potential Pain and/or Distress			
	No	Yes		
		Description	To Be Relieved	Not to Be Relieved
1	(X)		( )[a]	( )[b]
2	(X)			

a. For each procedure for which potential pain and/or distress is expected, but WILL be prevented or alleviated by administration of the analgesic(s) or stress-relieving agents, complete the table below:

Procedure Number (see Item 1)	Agent	Dose (mg/kg) & vol (ml)	Route of admin	Freq of admin (times/day)	Duration of admin (days post-procedure)
1	NA				0
2	NA				

Describe any non-pharmacological measures to be taken to address the potential pain and/or distress:

Special Procedure 1 ► Safety of the connectors and the procedures herein have been established through similar prior animal trials. Moreover, testing sessions will only be carried out at most once a week to ensure less impact on the cats.

Special Procedure 2 ► Cats will be acclimated to the jackets in a stepwise fashion by the research and/or veterinary technicians; petting and food treats will be used as positive reinforcement. ARF Veterinary Staff will monitor the animals 3 times per week for potential sores arising from wearing the jackets. If a sore is noted, the on-call ARF Veterinarian will be notified,and recommended treatment will be followed, such as use of topical or oral antibiotics and adjustment of the jacket.

b. For each procedure for which potential pain and/or distress is expected and will NOT be prevented or alleviated, provide the scientific justification for this:

Special Procedure 1 ► N/A

Special Procedure 2 ► N/A

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the special procedures, and list the criteria that will be used to determine when individual animals will be removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

Procedure Number (see Item 1)	Monitoring Methods	Endpoint Criteria
1	Following testing sessions, cats will be monitored for any unexpected signs of muscle soreness, pain, or difficulties in gait	If any animals become sick such that they have unresolved infection or weight loss of greater than 10% body weight, there are signs of pain or weakness, or changes in gait lasting longer than a day after any testing session, veterinarians will be consulted.
2	During acclimation training, cats will be continuously monitored.	Jackets will be removed, if cats are biting or scratching at the jacket, if cats are rolling or immobile while wearing the jacket, or if cats refuse to eat or drink while wearing the jackets. The jacket will be re-introduced at a later time and training will continue until the cat is comfortable wearing the jacket.

Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
[redacted], Ph.D.	Cleveland, OH-541	MERIT/Summer 2018	Optimization & Pre-clinical Testing of Implantable, In-Line High Density 32-Channel Connector

	SCORE	DESCRIPTION	ACTION NEEDED BY IACUC
● 6/3/19	0	No concerns noted. Any comments provided are for information only.	*None.* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
● 5/29/19	1	Some concerns noted.	*The IACUC must review the **level 1** concerns listed below and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
○	2	Concerns are noted that must be addressed by the local IACUC and PI before funding can occur, but work described in the ACORP(s) may continue.	*A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. Upload the following at https://vaww.gateway.research.va.gov: (1) a memo addressing the concerns, dated and signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *The IACUC must review each of the **level 1** concerns listed and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).*
○	3	Significant concerns are noted that must be addressed by the local IACUC and PI before funding can occur, and work described in the ACORP(s) listed below must cease immediately.	*A response to each of the **level 3** concerns listed below must be reviewed and cleared by the CVMO* before work can resume and funding can be released. (If unusual circumstances dictate that work should continue despite concerns, notify the CVMO immediately.) *A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. For **level 2 and 3** concerns, upload the following at https://vaww.gateway.research.va.gov :

			(1) a memo addressing the concerns, signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *The IACUC must review each of the **level 1** concerns listed and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).*

Date of review: 5/29/2019 (initial)

The ACORP for Dr. [redacted] (chas received an overall score of 1, which means that it is cleared and represents no bar to funding the application, although some concerns were raised, as shown below.

Please note that a separate score is shown for each of the individual concerns (shown in parentheses under the Item number to which each of the individual concerns refers), to assist you in interpreting the review. An explanation of each of the levels of concern is shown above, in the chart on the previous page. The IACUC must review each of the **level 1** concerns listed and decide what response is needed. This action must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP, but no further correspondence with the CVMO is needed.

In case of questions about this review, please contact Dr. [redacted], Assistant Chief Veterinary Medical Officer at [redacted].

Date of review: 6/3/2019 (final)
The ACORP for Dr. [redacted] has received an overall score of 0, which means that it is cleared and represents no bar to funding the application. The investigator and the IACUC are commended for their collective efforts to ensure humane animal care and use.

In case of questions about this review, please contact Dr. [redacted], Assistant Chief Veterinary Medical Officer at [redacted].

REVIEWER FEEDBACK

ACORP Item number(s) (score)	Comments/Concerns
ACORP (cat)	This ACORP involves implanting small high density connectors in cats to provide supporting biocompatibility and bio-stability data to meet Food and Drug Administration requirements before applying to test the connectors in human patients. The use of the feline model as opposed to other animal species or other model systems is well justified. Cats used in this study will live and play in an enriched environment, experience daily positive interaction with the research and veterinary staff, and will be offered for adoption to private homes once the study is complete. Prior to the ACORP being submitted to the VA JIT Document Manager, the research team worked extensively with Dr. [redacted] (the reviewer), Dr. [redacted], and Dr. [redacted] to optimize the protocol. As a result, only, a few concerns were noted.

	A revised ACORP was received on 6/3/19; all requested corrections have been made. No further correspondence with the Office of the CVMO is needed; the ACORP is cleared and represents no bar to funding the application.
Item E (1) *(0)*	Please provide the dates that Dr. [redacted] completed "Working with the VA IACUC" and "Working with Cats in Research Settings" training. *Reconciled.*
Item F (1) *(0)*	In the sentence shown below, please insert the word "on" between training and cat. "[redacted] and/or [redacted] will provide training cat handling, and restraint, substance administration, animal anesthesia, monitoring, recovery and post-operative care." *Resolved.*
Item T (1) *(0)*	In the sentence shown below, please insert the word "will" between veterinarian and determine. " In the event a cat has weight loss of greater than 10% of body weight, infection of the implant or skin where the leads exit that is not responsive to antibotics, or signs of neurologic dysfunction, weakness, or pain secondary to the implant, the veterinarian determine the treatment plan, or if interventional euthanasia is necessary. *Resolved.*
Appendix 6 (1) *(0)*	In item 3.a – Special Procedure 2, the last sentence is garbled, please revise as follows: If a sore is noted, the on-call ARF Veterinarian will be notified, and recommended treatment will be followed, such as use of topical or oral antibiotics and adjustment of the jacket. *Corrected.*

Summary of the Literature

W. Consideration of Alternatives and Prevention of Unnecessary Duplication. These are important to minimizing the harm/benefit to be derived from the work.

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ►Chronic implantation of high-density connectors, tunneled leads chronically exiting skin,

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
Pubmed	01/30/2019	2002-19	Chronic implantation of high-density connectors	(“High Density Connector implant” OR "Intramuscular electrode”) AND (safety OR “chronic cats” OR anesthesia OR analgesia) AND cats	(X)	(X)	(X)	(X)
Agricola Data Base (Nat'l. Ag. Library)	01/30/2019	2002-19	Chronic implantation of high-density connectors	(“High Density Connector implant”OR "Intramuscular electrode”) AND (safety OR “chronic cats” OR anesthesia OR analgesia) AND cats	(X)	(X)	(X)	( )
Pubmed	01/30/2019	2002-19	Tunneled leads chronically exiting skin	“Percutaneous leads” AND (“wound care” OR “skin care”) AND cats	( )	( )	(X)	( )
Agricola Data Base (Nat'l. Ag. Library)	01/30/2019	2002-19	Tunneled leads chronically exiting skin	“Percutaneous leads” AND (“wound care” OR “skin care”) AND cats	( )	( )	(X)	( )
ALTBIB citations	04/16/19	All available	Search specifically for	biocompatibility, connector	(X)	( )	( )	( )

with animal use alternatives as the main topic		years	non-animal models for testing biocompatibility of the connector					

Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.
► The focus of this project is on the behavior and biological response of live animals to the presence of high density connectors, so computer models are not a sufficient replacement for physiological studies. The high level of complexity of the neural and muscular physiology impedes the development of computational models to replace intact behaving animals. The results of this study will, however, contribute to the understanding of these mechanisms and may help to facilitate future development of appropriate computational models.

   We ran a search specifically looking for non-animal models for testing the biocompatibility of the connector and found no papers at all. There are no in vitro or computer models available for this work. Furthermore, the FDA requires that the testing be done in a suitable animal model before we can move into clinical trials.

   Rats and mice are far too small for the connector. We also considered using rabbits, pigs, sheep, and goats. None of these were suitable (see the discussion in section D above).

   Cats are the only species that are the right size and are happy to run and jump after a laser pointer or other toys. Their flexibility during routine activities (stretching, grooming), and the athletic activities of walking, running and jumping will let us realistically test whether the connector will be suitable for people who may engage in activities such as jogging, yoga, playing basketball, etc.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.
► In this study, a total of 7 cats will be used for chronic experiments. The first goal of the experiments is to determine the biocompatibility of the connectors to be studied. A power analysis has been performed and is outlined in this document, which assumes surgical success in 6 of the 7 animals and an effect size that is consistent with that which might be expected based on preliminary studies (which indicated that the prior HD connectors were well tolerated). Please see section C2b for details.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
► The methods described here are similar to those found in other studies of biocompatibility in animal models. Anesthesia is used for all surgeries so that the animal does not feel any pain or distress. Advanced pain management is used to control surgical pain after surgeries. The cats are never forced to exercise to test the connector. Instead, all exercise is in the form of play (chasing laser pointers and such). Lastly, the cats are gradually acclimated to wearing the protective jackets so they will not find the jackets distressing.