
Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Continuing review * * *

Renewal Form

To renew this protocol, answer the following renewal questions. If you would like to make changes to the information in the protocol, add/remove/update personnel, add/modify a procedure, etc., click on the appropriate section on the left side menu.

1) How many animals (of each approved species) were used as part of this protocol during the previous project year?

Project Summary

	Number of Animals Used		Reported Used	Total Approved	Remaining Approved	Additional Requested
Species	Year 1	Year 2				
Monkey, Rhesus	6	0	6	10	4	0

2) N During the past year, have any alternatives to the use of animals (e.g., in vitro models) become available that could be substituted to achieve your research goals? (If yes, explain)

3) N During the past year, have potentially less painful or distressful alternatives (e.g., procedures, drugs) become available that you could use and still achieve your research goals? (If yes, explain)

4) N During the past year, did you encounter any unexpected adverse outcomes with the approved animal procedures that were performed? (If yes, explain)

5) N Have your experimental procedures required you to keep live animals in your laboratory for longer that 12 hours during the past year?

6) N Did any problems arise during implementation of your research or while teaching the course?

7) Do You wish to amend/change/modify any sections of the protocol?

If you would like to make changes to the information in the protocol, add/remove/update personnel, add/modify a procedure, etc., click on the appropriate section on the left side menu.

Remember, ANY change in the care and use of animals involved in this protocol that would affect animal welfare must be promptly forwarded to the IACUC for review. Such changes must not be implemented until approval is obtained from the IACUC. Animals will not be transferred between investigators without prior approval.

Protocol Title: [redacted]
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* * * Personnel Information * * *

Principal Investigator

(Must have PI status or Exceptional PI status at UC Berkeley)

Name [redacted]

Title [redacted]

Email [redacted]

Office Phone [redacted]

Lab Phone [redacted]

Emergency Phone [redacted]

Department [redacted]

Mail Code [redacted]

Campus Mailing Address [redacted]

Will this individual be working directly with animals on this protocol? X Yes No

If "Yes" complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, surgery, monitoring, post-procedural care.

Describe the experience/training this person has had with this/these species and procedures.

Started working with NHP at UCB in 2010.

8/19/10 : OLAC Herpes B training refresher given by [redacted]
1/16/2012: refresher training for Herpes B safety by [redacted]
11/3/2010:CITI Training completed on
06/25/10: Certified by [redacted] to transport rhesus macaque into a customized restraint chair using the pole and collar method. OK'd to train [redacted] staff.
6/21/06: Approved by OLAC veterinarian to use isoflurane to anesthetize rats.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, "Investigators, Staff and Students - Basic Course" and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

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Laboratory Contact

Name [redacted]
Title [redacted]
Email [redacted]
Office Phone [redacted]
Lab Phone [redacted]
Emergency Phone [redacted]
Department [redacted]
Mail Code
Campus Mailing Address [redacted]

Will this individual be working directly with animals on this protocol? X Yes No

If "Yes" complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, surgery, monitoring, post-procedural care.

Describe the experience/training this person has had with this/these species and procedures.

Started working with NHP in 2018. [redacted] will work with NHPs under the training of the PI or other lab members until competent.
8/1/18: CITI Training completed
8/8/18: [redacted] basic safety training completed
8/14/18: Mandatory OHC medical clearance completed
8/6/18: CITI Working with Non-Human Primates in Research training completed
8/21/18: Herpes B training with [redacted] to be completed
[redacted] will be certified by OLAC prior to performing anesthesia, surgery or euthanasia independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, "Investigators, Staff and Students - Basic Course" and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Alternate Lab Contact

Name
Title

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Protocol Title: [redacted]

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[redacted]

Email
[redacted]

Office Phone
[redacted]

Lab Phone
[redacted]

Emergency Phone
[redacted]

Department
[redacted]

Mail Code

Campus Mailing Address
[redacted]

Will this individual be working directly with animals on this protocol? X Yes No

If "Yes" complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, monitoring, post-procedural care.

Describe the experience/training this person has had with this/these species and procedures.

Started working with NHP in 2014. [redacted] will work with NHPs under the training of the PI or other lab members until competent.
[redacted] completed Mandatory OHC medical clearance on 2/23/2016.
2/12/2016: CITI Training completed
2/25/2016: Herpes B training with [redacted]
3/8/2016: [redacted] basic safety training.
[redacted] will be certified by OLAC prior to performing anesthesia, surgery or euthanasia independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, "Investigators, Staff and Students - Basic Course" and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Other Personnel

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Name	Department
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Other Personnel

Name
[redacted]

Title
[redacted]

Email
[redacted]

Office Phone

Lab Phone
[redacted]

Emergency Phone
[redacted]

Department
[redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Handling, behavioral testing, surgery, monitoring, post-procedural care, euthanasia

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Describe the experience/training this person has had with this/these species and procedures.

Started working with NHP in 2014.

06/03/2014: CITI Training completed
06/03/2014: Working with Non-human primates in Research Setting
06/03/2014: Streaming videotape - working safely with non-human primates
06/10/2014: Mandatory OHC
06/19/2014: Completed Herpes B Safety Training with [redacted]
06/05/2014: EH&S [redacted] Safety Training
11/25/2014: Aseptic surgery

[redacted] will be certified by OLAC prior to performing surgery independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]
Title [redacted]
Email [redacted]
Office Phone [redacted]
Lab Phone
Emergency Phone [redacted]
Department [redacted]
Mail Code [redacted]
Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Handling, behavioral testing, surgery, monitoring, post-procedural care, euthanasia

Describe the experience/training this person has had with this/these species and procedures.

Protocol Title: [redacted]

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Started working with NHP in 1999.

[redacted] is OLAC-certified to perform listed procedures.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title [redacted]

Email [redacted]

Office Phone [redacted]

Lab Phone

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address [redacted]

Will this individual be working directly with animals on this protocol? X **Yes** **No**

If Yes complete the following:

What species will this person use?: NHPs

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Species: Rhesus macaques
Procedures: animal handling and behavioral training, neural recordings with chronic microwire arrays and semichronic arrays.

Describe the experience/training this person has had with this/these species and procedures.

Started working with NHP in 2016. [redacted] will be trained and supervised by the PI and lab personnel.

5/3/2015: Completed Herpes B and NHP Safety training with [redacted]

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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Name [redacted]	**Title** [redacted]
Email [redacted]	**Office Phone** [redacted]
Lab Phone [redacted]	**Emergency Phone** [redacted]
Department [redacted]	**Mail Code** [redacted]
Campus Mailing Address [redacted]	
Will this individual be working directly with animals on this protocol?	X Yes No
If Yes complete the following:	
What species will this person use?:	NHP
Briefly list what procedures this person will perform (a full description of procedures is asked for later).:	Pole and collar training, handling, behavioral testing, monitoring, post-procedural care. Chronic and semichronic neural recordings.

Describe the experience/training this person has had with this/these species and procedures.

[redacted] started working with NHP in 2015. [redacted] will work with NHPs under the training of the PI or other lab members until competent.
[redacted] completed [redacted] safety training and Mandatory OHC
8/7/2015: CITI Training completed
8/26/2015: Herpes B training by [redacted]

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Name [redacted]
Title

Email [redacted]
Office Phone [redacted]

Lab Phone
Emergency Phone [redacted]

Department [redacted]
Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus monkeys

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Anesthesia, peri-procedure care

Describe the experience/training this person has had with this/these species and procedures.

OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

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Name [redacted]
Title [redacted]

Email
Office Phone

Protocol Title: [redacted]
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[redacted]

Lab Phone
[redacted]

[redacted]

Emergency Phone
[redacted]

Department
[redacted]

Mail Code
[redacted]

Campus Mailing Address
[redacted]

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, monitoring, post-procedural care. Chronic and semichronic neural recordings.

Describe the experience/training this person has had with this/these species and procedures.

[redacted] started working with NHP in 2017. [redacted] will work with NHPs under the training of the PI or other lab members until competent.
[redacted] completed Mandatory OHC medical clearance on 8/7/2017.
7/15/2017: CITI Training completed
8/16/2017: [redacted] basic safety training completed.
8/21/2017: [redacted] facility orientation completed.
8/25/2017: Herpes B training with [redacted]
[redacted] will be certified by OLAC prior to performing anesthesia, surgery or euthanasia independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name
[redacted]

Title
[redacted]

Email
[redacted]

Office Phone
[redacted]

Lab Phone

Emergency Phone

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[redacted]

Department

[redacted]

[redacted]

Mail Code

[redacted]

Campus Mailing Address

[redacted]

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, chronic neural recordings.

Describe the experience/training this person has had with this/these species and procedures.

[redacted] started working with NHP in 2017. [redacted] will work with NHPs under the training of the PI or other lab members until competent.
[redacted] completed Mandatory OHC medical clearance on 8/7/2017.
7/3/2017: CITI Training completed
7/5/2017: [redacted] facility orientation completed.
8/11/2017: Herpes B training with [redacted]
8/28/2017: [redacted] basic safety training completed.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name

[redacted]

Title

Email

[redacted]

Office Phone

Lab Phone

[redacted]

Emergency Phone

[redacted]

Department

[redacted]

Mail Code

Campus Mailing Address

Protocol Title: [redacted]

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Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Macaques

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Water regulation ([redacted] lab member that will help with giving water to our animals when [redacted] lab personnel is out of town/unavailable)

Describe the experience/training this person has had with this/these species and procedures.

Trained in procedures since 2017

12/6/16: Mandatory OHC
12/19/2016: CITI Training "Working with the IACUC"
1/31/2017: Herpes B training with [redacted]
1/27/17: EH&S/[redacted] Safety Training

[redacted] will be certified by OLAC prior to performing surgery independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title

Email [redacted]

Office Phone

Lab Phone

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
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What species will this person use?: Rhesus Macaque

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, surgery, monitoring, post-procedural care.

Describe the experience/training this person has had with this/these species and procedures.

[redacted] started working with NHP in 2017. Will be trained by [redacted] and [redacted].
CITI training "working with the IACUC": 08/17/17
Occupational Health:
Primate health screening (TB/measles titer): 11/15/17
Measles follow-up vaccine #1: 12/8/17
Measles follow-up vaccine #2: 1/5/17
Risk assessment form: 12/26/17
Herpes B:
EHS202 online bloodborne pathogens training: 12/11/17
Herpes B in-person training: 1/8/17
OLAC training:
EHS201 in-person biosafety training: 8/15/17
OLAC Basic Safety Training: 12/11/17

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title [redacted]

Email [redacted]

Office Phone [redacted]

Lab Phone

Emergency Phone [redacted]

Department [redacted]

Mail Code [redacted]

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Non-human primates

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Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, monitoring

Describe the experience/training this person has had with this/these species and procedures.

[redacted] started working with NHP in 2019. [redacted] will be trained by OLAC and lab staff until competent.

4/22/19: OHSS assessment/primate-user medical clearance complete
5/1/19: CITI training complete
5/1/19: EHS205 complete
5/30/19: Herpes B training with [redacted]

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title [redacted]

Email [redacted]

Office Phone [redacted]

Lab Phone [redacted]

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Rhesus macaque

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: Pole and collar training, handling, behavioral testing, monitoring, post-procedural care. Chronic and semichronic neural recordings.

Describe the experience/training this person has had with this/these species and procedures.

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[redacted] will start working with NHP in 2019. [redacted] will work with NHPs under the training of the PI or other lab members until competent.

5/28/2019: CITI Training completed
5/31/2019: [redacted] facility orientation completed.

[redacted] will be certified by OLAC prior to performing anesthesia, surgery or euthanasia independently.

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]	**Title** [redacted]
Email [redacted]	**Office Phone** [redacted]
Lab Phone [redacted]	**Emergency Phone** [redacted]
Department [redacted]	**Mail Code**
Campus Mailing Address	
Will this individual be working directly with animals on this protocol?	X Yes No
If Yes complete the following:	
What species will this person use?:	rhesus macaque
Briefly list what procedures this person will perform (a full description of procedures is asked for later).:	headpost implantation, dural maintenance, and chamber implantation surgeries

Describe the experience/training this person has had with this/these species and procedures.

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[redacted] began working with non-human primates in 2014 at UC Berkeley. [redacted] is now a professor at UT Austin with [redacted] own NHP lab. [redacted] is certified to perform headpost implantation, dural maintenance, and chamber implantation surgeries.

9/25/2017 Measles titer performed at UCB (not required at [redacted], please contact if a more recent one is necessary)
4/12/2019 Working Safely with Macaques in Research (class) taken at [redacted], proof sent to [redacted]
8/8/2019 Tb test, results sent from [redacted] to UCB Tang center
9/4/2019 OHSS assessment completed
9/9/2019 Updated CITI training completed

Prior to approval, all individuals listed on an Animal Use Protocol (AUP) are required to complete the Collaborative Institutional Training Initiative (CITI) course entitled, Investigators, Staff and Students - Basic Course and the Occupational Health Surveillance System (OHSS). See the Training and Education and Animal Occupational Health and Safety Program (AOHSP) policies for more information.

Name [redacted]

Title

Email [redacted]

Office Phone

Lab Phone

Emergency Phone [redacted]

Department [redacted]

Mail Code

Campus Mailing Address

Will this individual be working directly with animals on this protocol? X Yes No

If Yes complete the following:

What species will this person use?: Non-human primate

Briefly list what procedures this person will perform (a full description of procedures is asked for later).: pole and collar training, handling, behavioral testing, monitoring, post-procedural care

Describe the experience/training this person has had with this/these species and procedures.

Started training in 2019.
CITI Training completed: 8/30/2019
OHSS assessment completed: 9/9/2019

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Protocol Title: [redacted]

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* * * Species * * *

Species to be Used

Common Name	Genus & Species	Source
Monkey, Rhesus	Macaca mulatta	OLAC Approved Vendors

Species to be Used

Common Name Monkey, Rhesus

Genus & Species Macaca mulatta

Strain(s) or Breed(s)

Animal Sex Male

Source OLAC Approved Vendors

Proposed Housing Location [redacted]

Building Name [redacted]

Room Number

Maximum number of animals for three year project period 10

Note: If breeding animals, the maximum number should include breeders plus all offspring produced.

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* * * Are You Using? * * *

NOTE: Select either "yes" or "no" for each question. If you select "yes", click on the "Add" button to provide required information.

Are You Using?

NOTE: The questions below are used to identify special circumstances where:

1) Animals are used in teaching

2) Additional oversight by regulatory agencies may be required

3) Coordination with campus compliance committees may be required

4) Personnel health and safety issues need to be addressed

1. Are you using live vertebrate animals for teaching?* N

2. Collaboration with Other Institution(s)* N

Animal transfers or changes in animal ownership between UC Berkeley PIs and collaborators at other institutions must comply with the ACUC policy on Changes in Animal Ownership and ACUC Guidelines on Animal Transportation.

3. Hazardous Agent(s) in Laboratory Animals

a) Infectious Agent(s) * N

Use of BSL-2 or 3 infectious agents in animals (including viral vectors; human cells, tissues or bodily fluids; and infectious select agents) requires approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site .

b) Recombinant DNA * N

The introduction of recombinant DNA/RNA into animals and the generation of transgenic animals require approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site .

NOTE: If breeding animals, create a "Breeding/Genotyping" Procedure and provide additional information and justification (including specific strains and phenotypes).

c) Human Embryonic Stem Cells * N

NOTE: Use of Human Embryonic Stem Cells in animals requires approval by the UC Berkeley Stem Cell Research Oversight Committee (SCRO) and Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the SCRO web site and the EH&S Biosafety Program web site.

1. Do you have SCRO approval? *
2. BUA # *
3. Used In Which Species?

d) Biological Material/Animal Product(s) Not Described Above* N

NOTE: The use of biological materials in rodents must comply with the ACUC Policy on Testing Biologicals used in Laboratory Rodents. The use of human cells, tissues or bodily fluids requires approval by the UC Berkeley Committee for Laboratory and Environmental Biosafety (CLEB) prior to ACUC approval. For guidance, please refer to the EH&S Biosafety Program web site.

e) Toxic Agent(s) * N

This includes the use of carcinogens, reproductive hazards, and other biological toxins (including select agents) in laboratory animals. Standard Operating Procedures (SOPs) must be in place. For guidance, please refer to the EH&S SOP web site.

f) Controlled Substance(s) * Y

NOTE: The Principal Investigator and any individuals using controlled substances in animals must be registered with EH&S prior using these agents. For guidance, please refer to the EH&S Controlled Substance Program web site.

Controlled Substance

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Species	Agent
Monkey, Rhesus (OLAC Vivarium)	Buprenorphine (Buprenex)
Monkey, Rhesus (OLAC Vivarium)	Tramadol
Monkey, Rhesus (OLAC Vivarium)	Midazolam
Monkey, Rhesus (OLAC Vivarium)	Diazepam (Valium, Midazolam)
Monkey, Rhesus (OLAC Vivarium)	Ketamine

g) Radiological Agent(s) * N

NOTE: Use of radiological agents in animals, radiation producing devices or lasers requires an approved Radiation Use Authorization(RUA) or Laser Use Registration (LUR) be in place prior to ACUC approval. For further guidance, please refer to the EH&S Radiation Safety Programs web site or Laser Safety Program web site.

4. Non-pharmaceutical Grade Compounds * Y

NOTE: Federal regulations require the use of pharmaceutical grade compounds in animals used for research and teaching unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. Please refer to the ACUC Policy on Use of Non-Pharmaceutical Grade Compounds

Non-pharmaceutical Grade Compounds

Species	Specify Material	Please provide justification for use of non-pharmaceutical compounds
Monkey, Rhesus (OLAC Vivarium)	N-methyl-β-carboline-3-carboxamide	A pharmaceutical grade compound is not available.

5. Field Study or Wildlife Study* N

NOTE: Additional procedure-based information for field studies is requested under the Protocol Information section of the Protocol.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Funding Sources * * *

Funding Checklist

If the research is not funded, check the "Not Funded" box below.
If the research is funded, add the funding source to the appropriate table below.

NOTE: Only the Principal Investigator (PI) of the grant or subcontract can add his or her own SPO Funding information in this section. The PI of the grant or subcontract must also be listed in the Personnel Information section of the protocol in one of the following roles: Principal Investigator or Faculty Sponsor, Student or Postdoctoral Investigator, Co-Principal Investigator, Administrative Contact, or Other Contact. Training Grants can be added by anyone in one of the aforementioned roles. For step-by-step instructions, see eProtocol IACUC Quick Guides

Not Funded

SPO - Funding

SPO ID	Sponsor	Sponsor Award ID	Project Title
20150357	Johns Hopkins University		Meta-Learning In Humans, Monkeys, and Robots
043413-001	NIH National Institute of Neurological Disorders and Stroke	R01NS097480	The Role of Ipsilateral Cortical Control of the Upper Limb in Monkey and Man
044374-001	NIH National Institute of Neurological Disorders and Stroke	R01NS106094	Neurophysiologically-informed Design of Flexible, 2-learner Brain-Machine Interfaces for Robust and Skillful Performance

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Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Rationale * * *

Rationale

As you answer the questions in this section, please use language that can be understood by a layperson. r Avoid overly technical terms and define abbreviations.

1. STUDY OBJECTIVES

a) What is the overall aim and purpose of this research or teaching demonstration/exercise?*

I aim to translate this research into the clinical realm through the development of neuroprosthetic systems. To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. For cortical structures we typically use fixed arrays (typically referred to as chronic recordings). For subcortical structures we use movable microelectrodes (semichronic arrays or acute recording arrays). The use of these techniques has several advantages over classical single unit recording techniques, including: 1) significant increase in the amount of data we can gather from a single animal at a single period of time, 2) the ability to record from the same neurons for the long time periods required for behavioral paradigms with multiple contingencies, 3) the ability to record from new populations of cells by adjusting electrode position (in the case of semichronic or acute recording arrays) and 4) the opportunity to study spatiotemporal patterns of neural activity.

b) How will the information gained be important to human or animal health, the advancement of knowledge, or the good of society?*

The scientific benefit of this work is to gain deeper understanding about how sensory and motor information is coded in the brain. The clinical benefit is to improve the quality of life of people with motor disabilities. As such, neuroprosthetic systems will enhance or restore functional motor and communication capabilities in patients with ALS, stroke, quadriplegia, and other neurological disorders.

2. RATIONALE FOR USE OF ANIMALS

a) Why do you need to use animals? Discuss why non-vertebrate alternatives (e.g., tissue culture, invertebrate animal models, computer simulations) are inappropriate or implausible to answer your scientific questions or meet your educational goals.*

The research conducted under this protocol includes neurophysiological recordings from cortical and subcortical areas in animals trained to carry out specific behavioral tasks. Neurophysiological experiments provide data that cannot be acquired by any other means, so there are no alternatives to the use of animals in these studies. For example, neuroimaging studies using PET or fMRI can identify major processing pathways, but provide almost no information about the neural computations underlying motor function. Theoretical studies are not helpful in this regard, because these processes are too complicated to be modeled computationally without direct access to experimental data.

The design of a theoretical model of the macaque somatosensory system was considered as a possible alternative to investigate changes in neural network properties. Nevertheless, it became obvious that not enough experimental data are available to create a realistic enough model of the mammalian somatosensory system for simulations. Even if that were feasible, new experimental data would still be needed for validation of the prosthesis performance. Therefore, it is our conviction that there is no reasonable alternative to an experimental paradigm to investigate how the mammalian central nervous system correlates with changes in motor behavior.

b) Why have you selected these particular species (and not others)?*

Experiments will be carried out on juvenile and/or adult macaques (M. mulatta) of either sex. The macaque has several advantages over other experimental animals for such studies. First, its motor system is similar in many respects to that of humans, so that the data obtained are directly applicable to humans. Second, these animals can be trained to perform very sophisticated behavioral tasks. Third, the same animals can learn multiple tasks over the course of several years. Last, the somatosensory and motor systems of these animals have been extensively used in neuroanatomical and neurophysiological studies.

The ability to learn and perform sophisticated behavioral tasks over several years requires a certain degree of sentience, e.g. the ability to hold items in working memory, delay gratification, control impulses etc. Other primates share this degree of sentience; macaques are an optimal choice among these options given the other reasons provided (similarity of motor systems and extensive prior study), as well as availability and perhaps hardiness. Beyond primates, rats are the next most phylogenetically related species (more so than cats, dogs, etc.), but these lack the ability to perform all but the most rudimentary visuomotor tasks.

3. JUSTIFICATION OF ANIMAL NUMBERS

For complete instructions and guidance on how to complete the section on justification of animal numbers, please refer to the ACUC guideline on Justification for Animal Numbers found on the ACUC website.

a) How did you determine that the numbers provided in the Species section of this protocol are the smallest number of animals needed to

Protocol Title: [redacted]

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fulfill the study goals over a three-year period? Please use the table below to graphically describe for reviewers how you arrived at your animal numbers. Regardless of species, please briefly describe the Experiments included in your protocol and complete the table below, FOR THE THREE-YEAR PERIOD OF THE PROTOCOL. Note: Experiments may consist of multiple procedures. For breeding colonies, enter these as a line item, with the total consisting of breeding stock plus offspring NOT used in any studies.

Animal Groups for Procedures

Experiment	Maximum number of groups (Control)	Maximum number of groups (Experimental)	Maximum number of animals per group	Maximum number of replications needed	Total number of animals needed
Electrophysiology and behavior in macaques	0	1	10	1	10

b) Please justify the proposed number of animals being used:

For the macaque experiments, our modern electrophysiological techniques allow tens to hundreds of neurons to be simultaneously recorded in each animal. These methods yield an extremely high sample size of neurons, while minimizing the number of subjects required. Therefore, we will be able to collect far more information per subject than is normally possible in these types of studies. In addition, since we propose to carry out multi-level recordings in these animals, multiple neuronal structures (cortical and subcortical) can be simultaneously investigated in each subject.

There are 4 rigs in the laboratory and each study requires at least 2 NHPs, which means that typically 8 NHPs will be used at a given time. We indicate 10 NHPs as in some cases a third animal may be needed to increase the number of recorded cells in the event of poor cell yield in the recordings.

In order to minimize the total number of animals used per year, macaques will be used in more than one study when possible. The maximum number of animals per group will be 10, the maximum number of groups per year will be 10, and the maximum number of macaques at a given time will be 10.

c) Method Used to Determine Group Size (check all that apply):

X **Statistical estimates; please describe the power analysis and all other statical analyses used:**

A statistical power analysis would not be appropriate for macaque studies. Two macaques per experiment are the minimum necessary according to the standards of the field, and to provide some validation of each individual's results. A third macaque may be needed to increase the number of recorded cells in the event of poor cell yield in the recordings.

This is a pilot study, as similarly established studies do not exist. The proposed study will use a small number of animals to determine the feasibility of a larger study.

Studies cited in the literature; please provide the literature citations here or as an attachment:

X **Previous experience by this PI. Please describe and cite references here or as an attachment.**

Some examples of studies that use 2 macaque monkeys:

Taylor DM, Helms Tillery SI, and Schwartz AB, Direct Cortical Control of 3D Neuroprosthetic Devices, Science 296: 1829-1832 (2002)

Santhanam G, Ryu SI, Yu BM, Afshar A, Shenoy KV (2006) A high-performance brain-computer interface. Nature. 442:195-198.

Ganguly K. and Carmena J.M. (2009). Emergence of a stable cortical map for neuroprosthetic control. PLoS Biology 7(7): e1000153. doi:10.1371/journal.pbio.1000153

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]
Approval Period: 10/15/2019-10/31/2020
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Procedures * * *

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Positioner Implantation
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 10

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Procedure Description * * *

Procedure Description

In animals receiving a head positioner this will be placed no less than five weeks prior to the microwire array surgery.

Surgery preparations: Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.

The animal is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in stereotax and final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.

After sterile draping, the skin is incised along the midline from the orbital ridge to the occipital ridge with a #10 scalpel blade. The skin, muscle and fascia are reflected from an approximately 4-5 cm diameter area of the calvarium. Blunt scissors and a bone chisel are used to dissect and reflect the fascia and muscle respectively. The surface of the skull is cleaned with sterile saline. Hemostasis is achieved with gentle pressure with cotton tipped applicators or gauze pads. Tissues are moistened with sterile saline throughout the procedure to maintain viability and aid in healing. Suction is used to remove any excess saline and increase visibility in the surgical field. The titanium post (weight=10g) is positioned on the calvarium and the radial straps secured using titanium orthopedic screws. Small holes are made using an orthopedic hand-drill, then tapped using an orthopedic tap. The head positioner is finally secured by screwing the titanium, orthopedic screws into the tapped holes. The skin and subcutaneous tissue layers will be sewn completely over the orthopedic hardware (except for a small opening for the positioner itself) using 3-0 or 4-0 sutures. The positioner is intended to be permanent, but if necessary it can be removed in a separate procedure. The positioner will be placed no less than five weeks prior to the microwire array surgery.

Any deviation from any of the procedures or limits discussed here is only to be done in consultation with and with approval of OLAC veterinary staff and the ACUC.

How does this procedure fit into or address your overall research goals?

To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. In order to maximize the performance of subjects during behavior, which ultimately yields the best scientific results, it is critical to have the subjects attend to the visual stimuli (e.g. computer screen) in front of them. This is accomplished through head-fixation, which is common practice for many animal models and in particular non-human primate models. In order to head-fix the subjects, an implanted head positioner is required.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

Protocol Title: [redacted]

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience
[redacted]	Y	10+ years experience with all procedures in this protocol 8/19/10 : OLAC Herpes B training refresher given by [redacted] 1/16/2012: refresher training for Herpes B safety by [redacted] 11/3/2010:CITI Training completed on 06/25/10: Certified by [redacted] to transport rhesus macaque into a customized restraint chair using the pole and collar method. OK'd to train [redacted] staff. 6/21/06: Approved by OLAC veterinarian to use isoflurane to anesthetize rats.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg).	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~1cm long) of ointment is applied to each eye during surgical prep. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	May be administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

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* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]
Approval Period: 10/15/2019-10/31/2020
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably take meds orally.
Tramadol	3-5 mg/kg	Oral (PO)	Administered as a supplement to or a replacement for the injectable analgesic buprenorphine once the animal is eating reliably post-op.
Meloxicam	0.1mg/kg	Oral (PO)	Once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-

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Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

*** * * Other Agents Utilized * * ***

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Imaging

Procedure Type:	Imaging	**Procedure Title:**	MRI scan in macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	

Protocol Title: [redacted]
Approval Period: 10/15/2019-10/31/2020
Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Use Location: [redacted] **Building Name:** [redacted]
Room Number: [redacted]

* * * Procedure Description * * *

Procedure Description

Magnetic Resonance Imaging (MRI)

There are substantial interindividual differences between macaques in the size of the brain and its precise layout. Therefore at least one MRI must be performed before implantation in order to know where specific brain structures are in stereotactic space. MRI images are necessary to accurately locate place the recording implants and for the construction of the skull model to enable manufacture of semichronic implants. These scans will be obtained on the 3T magnet in the [redacted] Details regarding the procedure for obtaining MRI scans, including anesthetic induction, are included in a separate SOP.

An MRI scan is required to construct a 3D printed skull model that we will then use to ensure the accurate surgical placement of our recording electrodes. A further scan may be necessary to verify that the electrodes are in the correct position following implantation. Additional scans may be required, for example, if scanning quality is poor. Consequently, we may scan an animal up to five times total. Because the animal must be anesthetized for each scan, a scan will not be performed within two weeks of a previous anesthetic event. This interval may be extended should there be concerns about the animal's health (e.g. loss of more than 10% of the animal's body weight).

The absolute decibel limit of the magnet is 102 dB, based on the fastest rise time the scanner can do. Most sequences are significantly lower than that, with maximum peaks around 85-90 dB (Hurwitz R, Lane SR, Bell RA, Brant-Zawadzki MN. Acoustic analysis of gradient-coil noise in MR imaging. Radiology 1989;173:545-548). We will use hearing protection (ear muffs or ear plugs) which can reduce noise up to 30dB.

In addition, we plan to use one animal as a pilot animal to help us optimize and troubleshoot MRI scanning parameters and increase the reliability of our scans. This animal will have up to ten scans, with the same caveat that the animal will not be scanned within two weeks of a previous anesthetic event with the interval extended if there are health concerns.

How does this procedure fit into or address your overall research goals?

To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. For cortical structures we typically use fixed arrays (typically referred to as chronic recordings). For subcortical structures we use movable microelectrodes (semichronic arrays or acute recording arrays). Since there is subject to subject variability in localization of different brain regions, imaging the brain is an important step in order to optimize surgical planning for array or electrode implantable.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Describe post procedure monitoring that will be performed.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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See attached MRI SOP.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC is responsible for the anesthesia and anesthesia recordkeeping during this procedure.

Anesthetic Agents

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Ketamine hydrochloride	2--3mg/kg	Intramuscularly (IM)
Dexmedetomidine	0.015--0.04 mg/kg	Intramuscularly (IM)
Midazolam	0.25mg/kg	Intramuscularly (IM)
Isoflurane	1.5%-3.0%	Inhalation (IN)

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Atipamezole	0.15mg/kg	Intramuscularly (IM)	Once to reverse demedetomidine if necessary.

Behavior Study Assay

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Neural recordings and microstimulation during natural motor control
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Procedure Description * * *

Procedure Description

Natural motor control tasks entail the animal manually controlling a manipulandum, exoskeleton etc to perform a motor task, such as reaching for targets in a computer screen or physical objects in the workspace.

Training is performed using operant conditioning techniques. Whenever the animal makes a correct response they will receive a reward; the animal then quickly learns to repeat this response to get another reward. In some instances, a perinasal airpuff (0 – 50 psi) may be delivered as a cue of a negative outcome (e.g. incomplete or incorrect response). The airpuff strength is consistent with previous experiments in monkeys (Amemori and Graybiel, Nature Neuroscience 15:776 – 785, 2012) and may be delivered up 1000 times per day. This value is chosen since typically the airpuff will be administered once per behavioral trial and subjects tend to complete up to 1000 trials per experimental session. The animal is seated facing a video display upon which visual cues are presented. The animal's task is to respond to the cue though arm movements, eye movements, or button presses. Some task examples are; 1) Using a joystick to move a cursor to hit a target stimulus; 2) Reaching to target stimuli on a touch-screen panel; 3) Reaching to touch buttons directed by a cue; 4) directly interacting with a robot exoskeleton to move a cursor to a target and 5) fixating the gaze on a point on the screen for 2 seconds.

Examples of exoskeletons are the Kinarm (BKIN Technologies, Ontario, Canada), in which the shoulder and elbow are restricted to move in the horizontal plane, giving two degrees of freedom. Motors in the Kinarm allow torques to be applied to both the elbow and shoulder independently. In addition, we will use exoskeletons with up to 5 DOF developed in collaboration with the [redacted] Lab ([redacted]) as part as our ongoing NSF EFRI grant. In a given day, one animal may work with an exoskeleton for up to 6 hours.

Training for each task varies depending on how the cue stimulus is delivered, how the animal is to interact with the actuator and how the final goal/target is to be reached. The underlying theme for training is that each task is extremely easy at the beginning and becomes more specific and difficult as the animal learns how to earn the reward and becomes more proficient at performing the motor task. Training can continue for a period from one to several months, depending on the particular task required, the previous experience and inherent trainability of the animal, and the skill of the trainer. Animals are taught to perform the behavioral experiments required under this protocol, though the speed of training varies. Typically the length of the study varies from 3 months after surgery to several years in the case of the animal maintaining a stable population of neurons in the arrays of microelectrodes.

The location of the macaque's wrist, elbow and shoulder may be monitored with a tracking system consisting of small sensors on the subject’s arm that are detected by a series of cameras (e.g. PhaseSpace Inc., San Leandro, CA). Both neuronal and muscle activity may be monitored simultaneously. Self-adhesive fabric (e.g. Coban wrap, Biopac Systems Inc., Goleta, CA) is used to hold the sensors on top of the hair of the arm of the animal. The arm of the animal does not need to be shaved. The process is painless and animals tolerate it well.

In selected days we will perform EMG recordings. Chronic multiple EMG recordings allow us to measure whether the modifications in neuronal activity observed during learning of a motor task are due to correlated changes in patterns of muscle activation or derive solely from intrinsic and unique modifications in the central nervous system. For these type of recordings the arm of the animal is shaved with a regular battery-operated trimmer. Skin prep gel (e.g. ELPREP, Biopac Systems Inc., Goleta, CA) is applied gently to the skin to provide low impedance. Surface gold disc EMG electrodes (Grass Technologies Inc., West Warwick, RI) are filled with conductive paste (e.g. Ten20, Weaver Inc., Aurora, CO) and mounted on medical adhesive tape (e.g.ADD200, Biopac Systems Inc., Goleta, CA) or Coban wrap, and placed on the skin overlying six muscle groups. The muscle groups we will record from are: pectoralis major, biceps long head, biceps short head, anterior deltoid,

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muscle groups we will record from are: pectoralis major, biceps long head, biceps short head, anterior deltoid, triceps long head and triceps lateral head. Recordings may be performed for up to 6 hours each day. In cases where EMG recordings will be need for more than 1 week the arm of the animal may be shaved regularly (once per week) during the length of the study. The processes of arm shaving and EMG electrode placement are painless and animals tolerate them well.

The following Standard Operating Procedure (SOP) for maintenance of Velcro straps (and other types of commercially available materials) in the KINARM (and/or other house made/commercial exoskeletons and robotic actuators) has been developed: 1. At the start of a new experiment, Velcro will be purchased and placed on the Kinarm. 2. All Velcro strap will be replaced a minimum of once every month 3. Velcro will be replaced more frequently, as needed, in the event it gets dirty or damaged.

Direct electrical stimulation into the brain may be used as another way of providing feedback from the artificial actuator (e.g. computer screen, robot, etc.), entrain networks, or to elicit movement by stimulating in motor cortex. To encode particular sensory information in the brain, different spatiotemporal patterns are applied in specific cortical areas of the macaque's brain using biphasic stimulation. The stimulation current is applied to the same arrays of microwires. Current is applied up to 100 microamps, with a voltage of –10V to 10V, a maximum frequency of 300Hz and a maximum duration of 500ms. 100 uA is a standard current for stimulation in macaques. Higher values were not found to be very useful in eliciting neural responses (Cohen and Newsome, Current Opinion in Neurobiology 14:1–9, 2004, Edgar et al., J Neurophysiol 94: 3443–3450, 2005, Romo et al., Nature 392, 387–390, 1998) and rats (Berg and Kleinfeld, J Neurophysiol 90(5):2950-63, 2003; Venkatraman S., Long J.D., Elkabany K., Yao Y. and Carmena J.M. A system for neural recording and closed-loop intracortical microstimulation in awake rodents. IEEE Transactions on Biomedical Engineering, 2009). The animals perform a set of different visuo-motor tasks that involve the use of touch-screens, selection buttons, and hand-held manipulators.

How does this procedure fit into or address your overall research goals?

The goal of this work is to gain deeper understanding about how sensory and motor information is coded in the brain. By interrogating neural activity during motor control, we can better decode how motor commands are represented at the neural level and how sensory information is integrated into decisions of how to move. Simultaneously performing EMG recordings are also useful for correlating the neural activity with the muscle groups themselves.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected. Air puffs have been found to be well-tolerated at the indicated strengths in macaque subjects. However, if the behavioral performance of the animal decreases, the air puff strength will be reduced and/or the position of the air puff may be moved to be an increased distance from the eye. Shaving of the arm is unlikely to cause irritation since it will be performed at most 1 time per week. However, if any irritation occurs from shaving and/or wearing of electrodes, we would reduce the number of recording sessions per week.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure and are monitored daily.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Behavior Study Assay

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Neural recordings and microstimulation during neuroprosthetic control
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

Neuroprosthetic control entails the animal controlling the actuator (e.g. computer cursor, robotic arm, exoskeleton) under volitional control of neural activity digitally streamed through a mathematical decoder that transforms this activity into control signals for the prosthetic device.

Training is performed using operant conditioning techniques. Whenever the animal makes a correct response they will receive a reward; the animal then quickly learns to repeat this response to get another reward. In some instances, a perinasal airpuff (0 – 50 psi) may be delivered as a cue of a negative outcome (e.g. incomplete or incorrect response). The airpuff strength is consistent with previous experiments in monkeys (Amemori and Graybiel, Nature Neuroscience 15:776 – 785, 2012) and may be delivered up 1000 times per day. This value is chosen since typically the airpuff will be administered once per behavioral trial and subjects tend to complete up to 1000 trials per session. The animal is seated facing a video display upon which visual cues are presented. The animal's task is to respond to the cue by controlling the prosthetic device (e.g. computer cursor, exoeskeleton) through volitional control of neural activity. The task-related arm will be either restrained from the exoskeleton (e.g. enclosed in the primate chair) or will be kept inside the exoskeleton to provide natural proprioceptive feedback. Training for each task varies depending on how the cue stimulus is delivered, how the animal is to interact with the actuator and how the final goal/target is to be reached. The underlying theme for training is that each task is extremely easy at the beginning and becomes more specific and difficult as the animal learns how to earn the reward and becomes more proficient at performing the motor task. Typically the length of the study varies from 3 months after surgery to several years in the case of the animal maintaining a stable population of neurons in the arrays of microelectrodes.

Direct electrical stimulation into the brain may be used as another way of providing feedback from the artificial actuator (e.g. computer screen, robot, etc.), entrain networks, or to elicit movement by stimulating in motor cortex. To encode particular sensory information in the brain, different spatiotemporal patterns are applied in specific cortical areas of the macaque’s brain using biphasic stimulation. The stimulation current is applied to the same arrays of microwires. Current is applied up to 100 microamps, with a voltage of –10V to 10V, a maximum frequency of 300Hz and a maximum duration of 500ms. 100 uA is a standard current for stimulation in macaques. Higher values were not found to be very useful in eliciting neural responses (Cohen and Newsome, Current Opinion in Neurobiology 14:1–9, 2004, Edgar et al., J Neurophysiol 94: 3443–3450, 2005, Romo et al.,

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Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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Nature 392, 387–390, 1998) and rats (Berg and Kleinfeld, J Neurophysiol 90(5):2950-63, 2003; Venkatraman S., Long J.D., Elkabany K., Yao Y. and Carmena J.M. A system for neural recording and closed-loop intracortical microstimulation in awake rodents. IEEE Transactions on Biomedical Engineering, 2009). The animals perform a set of different visuo-motor tasks that involve the use of touch-screens, selection buttons, and hand-held manipulators.

How does this procedure fit into or address your overall research goals?

The goal of this work is to gain deeper understanding about how sensory and motor information is coded in the brain. By interrogating neural activity during neuroprosthetic control, we can better investigate how a specific set of neurons change activity in order to generate motor commands and demonstrate learning. This supports the development of both neuroprosthetics and our general scientific understanding of how neural activity encodes a variety of actions.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected. Air puffs have been found to be well-tolerated at the indicated strengths in macaque subjects. However, if the behavioral performance of the animal decreases, the air puff strength will be reduced and/or the position of the air puff may be moved to be an increased distance from the eye.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure and monitored daily.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Surgical Procedure

UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Procedure Type:	Surgical Procedure	**Procedure Title:**	Chronic Microwire Arrays Implantation
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 10

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Procedure Description * * *

Procedure Description

The microwire arrays consist of biologically compatible, chronic microwire assemblies in array form (CD Neural Engineering, Durham, NC), built to be either stationary or moveable. The moveable electrodes can be adjusted in increments of 50 microns. Due to the fact that the brain has no pain receptors, adjustments to these electrodes can be made while the animal is awake in the chair. The number of wires in the array, the materials used and the separation between wires may vary. In some surgeries, cannula electrodes may be used for recording from subcortical regions. Gas sterilization is used for the arrays of microwires.

Surgery preparations: Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.

On the day of the surgery, the monkey is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in stereotax and final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.

Craniectomies: The skin is incised along the midline from the orbital ridge to the occipital ridge with a #10 scalpel blade. The skin, muscle and fascia are reflected from an approximately 4-5 cm diameter area of the calvarium. Blunt scissors and a bone chisel are used to dissect and reflect the fascia and muscle respectively. The surface of the skull is cleaned with sterile saline. Hemostasis is achieved with gentle pressure with cotton tipped applicators or gauze pads. Tissues are moistened with sterile saline throughout the procedure to maintain viability and aid in healing. Suction is used to remove any excess saline and increase visibility in the surgical field. 15-20 self-tapping screws, which are designed to not penetrate the cranium, are inserted into the skull. These screws play two important roles: to sustain the acrylic cap and to provide multiple ground points and hence maximize recording quality. A series of craniectomies are created in the skull at precise coordinates, measured stereotaxically, for the microwire electrode arrays. The maximum number of craniectomies per animal will be 10, 5 per hemisphere. A variety of drills may be used, including a dental drill and/or dremmel. Craniectomies usually range from 3X4mm to 5X8mm. As individual animals vary slightly from the standard stereotaxic map, craniectomies may be modified to better access a cortical and/or subcortical area. A given craniectomy may be extended for the purposes of localizing important landmarks on the surface of the brain, such as the central sulcus or the arcuate sulcus. Standard physiological localization is performed, using either tactile stimulation in a stroking pattern on the animal's limb while searching for receptive fields in somatosensory areas, or by using surface macro-stimulation of cortical motor areas that evokes muscle activation in the animal's limb. In addition, MRI images will be obtained before surgery for precise localization of the recording implants as well as for the construction of the skull model to enable manufacture of semichronic implants (see 'MRI scan in macaques' procedure and separate SOP).

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Bleeding is controlled with Gelfoam soaked in Thrombin or sterile saline. After the craniectomies have been created, the dura of each craniectomy is dissected. Microwire Array Insertion: The arrays are positioned above the brain and lowered stereotaxically with a micromanipulator. As the wires make contact with the surface of the brain, a “zero” level is established, from which the depth of implantation is measured. As the arrays are lowered, qualified laboratory members monitor neuronal electrical activity detected by the microwires. Any noise interference from the room will be resolved to determine the array depth that records peak neuronal activity. A significant amount of noise comes from the various monitors used during surgery, including ECG and pulse oximetry, for example. A variety of strategies are employed sequentially to minimize noise such that recordings are acceptable. First, attempts are made to ground all equipment to the electrodes using sterile wires. Further noise may be resolved by putting electrical equipment in the room on battery operation for up to 20 minutes per implant. In extreme cases, all equipment using A/C electricity, except ECG, will be turned off for approximately 10 minutes to eliminate noise and improve recordings. Array depth is usually best between 1.5-2.5mm. Gel glue, liquid glue and glue accelerator may be used to initially secure the array, none of which can harbor biological contaminants. After each array is glued in position, the site is covered with acrylic up to the connector. The acrylic is applied in layers of liquid. As each layer dries quickly, additional layers can be applied at a reasonable rate. After the last connector is stabilized with acrylic, the exposed skull is covered with acrylic and acrylic is built up in between the
connectors to stabilize all of them into the resulting acrylic cap. If it is decided during surgery that a craniectomy will not be used for inserting a microwire array, glue will be applied and acrylic will cover the craniectomy, just as for the others with arrays.

Acrylic Cap: The acrylic cap is made smooth where it may come in contact with the skin. 2 small, stainless steel nuts and a small, stainless steel pin will be embedded in the top of the acrylic cap for securing a protective layer above the connectors. Caps have an oval shape with maximum dimensions of 10cm along the anterior-posterior axis, and 8cm along the interaural axis. Approximate weight of the cap including stainless steel nuts is 100 grams. If the skin around the acrylic is loose or if bone is exposed, the skin is sutured using 3-0 or 4-0 sutures. Sutures are inspected daily and removed after 10-14 days. A topical antibiotic is applied to the skin at the border of the new acrylic. The wound is checked daily for signs of infection and/or bleeding. If necessary, the wound is cleaned with hydrogen peroxide and a topical antibiotic is applied. A light, protective cap is custom built to fit over the new acrylic headcap to keep the electrodes and the connectors clean. Any deviation from any of the procedures or limits discussed here is only to be done in consultation with and with approval of OLAC veterinary staff and the ACUC.

Long-term Care for Acrylic Cap Margin: The cap margin will be cleaned as necessary, up to three times weekly as documented in a cleaning log kept together with the animal’s weight and water intake log. No anesthesia should be required for this cleaning procedure, although the animal will be monitored for any unanticipated distress. The margin is cleaned by the lab member in charge of the animal as follows: The entire wound edge is washed with chlorheximine (e.g. Nolvasan) scrub removing all scabs and crusts. Gauze soaked in Nolvasan solution is then placed over the entire circumference of the cap and left in place during the recording or training session or as long as possible. Fur is trimmed as needed. Infections are quite rare using these procedures, but if they do occur appropriate topical and/or systemic treatments are administered in consultation with OLAC veterinary staff.

How does this procedure fit into or address your overall research goals?

A major goal is the development of neuroprosthetic systems and the investigation of the corresponding neural activity that is used to control a neuroprosthetic device. To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. For cortical structures we typically use chronic microwire arrays for chronic recordings. This enables us to record from the same neurons for the long time periods required for behavioral paradigms with multiple contingencies and gives the opportunity to study spatiotemporal patterns of neural activity.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience
[redacted]	Y	10+ years experience with all procedures in this protocol 8/19/10 : OLAC Herpes B training refresher given by [redacted] 1/16/2012: refresher training for Herpes B safety by [redacted] 11/3/2010:CITI Training completed on 06/25/10: Certified by [redacted] to transport rhesus macaque into a customized restraint chair using the pole and collar method. OK'd to train [redacted] staff. 6/21/06: Approved by OLAC veterinarian to use isoflurane to anesthetize rats.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg)	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intravenous (IV)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~ 1 cm long) of ointment is applied to each eye during surgical preparation. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.
Fentanyl	2-50 mcg/kg/hour	Intravenous (IV)	It may be used adjunctively to lower the gas anesthetic requirement during surgery.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

Protocol Title: [redacted]
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Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably take meds orally.
Tramadol	3-5 mg/kg	Oral (PO)	1-3 times daily up to 3 days.
Meloxicam	0.1mg/kg	Oral (PO)	Administered once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and

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these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Behavior Study Assay

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Acclimation to Restraint
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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Use Location: [redacted] **Building Name:** [redacted]

Room Number: [redacted]

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* * * Procedure Description * * *

Procedure Description

Each animal is initially trained to be handled with the pole and collar method and to sit in a specially designed chair that permits both limb movements and postural adjustments. Once the animal is comfortable sitting in the chair, it is transported to the experimental room where it is trained to carry out progressively more complex behavioral tasks for a liquid reward. During task training, all trials are initiated by the animal, and it may generally work for as long as it wishes in a single session.

Animals will receive a substantial period of training (at least 1 month) in the chair before undergoing head positioner implantation and/or chronic implant surgical procedures. To condition animals to any head restraint that may be used, session duration is gradually increased from very short to normal length sessions. Head restraint, if used, will consist of the chair fixation post attached to the animal's headpost (see Positioner Implantation procedure). Training sessions last from 5 minutes to 6 hours, and are conducted from 0-7 days/week. During behavioral training, it is rarely necessary for head restraint to be greater than 3-4 hours per day. During electrophysiological recording, however, it is often necessary to extend the period of head restraint up to (but not beyond) 6 hours per day. Because of the length of head restraint, the chair is adjusted each day for maximum comfort, and the animal is free to move its limbs and adjust its posture in the chair. In addition, tasks have been designed to minimize stress on the animal by giving it as much control over its behavior as possible. The behavioral tasks consist of many short (typically <5-s) trials each of which, if performed successfully, rewards the animal with fluid. Animals become quite used to the laboratory environment. When animals get tired or have had enough fluid, they simply stop performing the task, in which case they will be returned to their home cage.

Training is performed using operant conditioning techniques. Whenever the animal makes a correct response they will receive a reward; the animal then quickly learns to repeat this response to get another reward. In addition, clicker training is used for positive reinforcement.

See "Acclimation to Restraint" attachment for training summary.

How does this procedure fit into or address your overall research goals?

To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. Since subjects will spend up to several hours in an experiment room performing behavior, it is important that they are first habituated to the space and apparati (e.g. primate chair) used in the experiments. Additionally, a head positioner is implanted prior to all neural recordings and head-fixation is performed following recovery (6 weeks) to ensure that a subject is attentive to the behavioral task. This procedure of head-fixation additionally requires habituation in order to ensure minimal stress or discomfort of the animal.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Protocol Title: [redacted]

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Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Chamber Placement for Semichronic Arrays or Acute Recordings in Macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any

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surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 10

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* * * Procedure Description * * *

Procedure Description

Acute recordings involve lowering electrodes into the brain each day and then removing them at the end of the session. In contrast, in semichronic recordings, although the electrode position remains adjustable, the electrodes remain in the animal's brain for several months. Both types of recording require chambers to enable access to the animal's brain and to control the position of the electrodes.

Each animal will have up to four recording chambers depending on the experiment. This enables us to record simultaneously from multiple brain areas in order to determine the flow of information through the brain during the performance of the behavioral task. All the chambers will be placed at the same time. The sites of the chambers are determined stereotactically from the MRI scans to ensure that we hit the brain sites of interest for the experimental aims. This procedure has been developed and refined over the last two decades and is in use in multiple neurophysiological laboratories.

Surgery preparations

Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water. The animal is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for preemptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head fur is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivacaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in the stereotax and the final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.

Surgical procedure

After sterile draping, the skin is incised along the midline with a #10 scalpel blade. The skin, muscle and fascia are reflected using blunt scissors and a bone chisel. The surface of the skull is cleaned with sterile saline. Hemostasis is achieved with gentle pressure with cotton tipped applicators or gauze pads. Tissues are moistened with sterile saline throughout the procedure to maintain viability and aid in healing. Suction is used to remove any excess saline and increase visibility in the surgical field. The chamber is positioned on the calvarium and attached using bone cement and titanium orthopedic screws. The location of the chamber is determined by stereotactic coordinates (Paxinos G, Huang X-F, Toga A (2000) The rhesus monkey brain in stereotaxic coordinates. San Diego (California): Academic Press). Small holes are made using an orthopedic hand-drill, and then tapped using an orthopedic tap. In the case of the implantation of semichronic arrays, a tight seal is ensured between the cranium and the chamber by using MR images (see 'MRI Scan in Macaques' procedure) to construct a custom recording chamber that is shaped to the contours of the skull. Metabond bone cement will be used to create a water-tight seal at the interface between the cranial bone and chamber.

Chamber size will vary from 1.7 cm – 2.7 cm across, and 1.0 – 3.2 cm height from the midpoint of the chamber. The weight depends on the chamber size, with a maximum weight of 50g. Bone cement (Palacos, a

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methylmethacrylate methylacrylate copolymer) is applied in thin fluid layers directly to the perimeter of the base of the chamber where it comes in contact with bone. Once the initial layer has cured (20 sec – 5 mins), a second thin fluid layer will be applied around the perimeter of the chamber and bone screws positioned around the chamber. Additional bone cement is applied, layer by layer, until a cap has been formed. The bone cement is built up to the bottom lip of the chamber to ensure appropriate structural integrity of the chamber. The acrylic cap is made smooth where it may come in contact with the skin. Caps have an oval shape with maximum dimensions of 10cm along the anterior-posterior axis, and 8cm along the interaural axis, and an approximate maximum weight of 100 grams. The skin and subcutaneous tissue layers will be sewn around the cap using 3-0 or 4-0 sutures.

Long term care of skin margin

The cap margin will be cleaned when the animal is taken out of the cage to perform tasks. The cap margin will be cleaned as necessary, up to three times weekly as documented in a cleaning log kept together with the animal's weight and water intake log. The margin is cleaned by the lab member in charge of the animal as follows: The entire wound edge is washed with chlorhexidine solution (e.g. Nolvasan) scrub removing all scabs and crusts. Gauze soaked in Nolvasan solution is then placed over the entire circumference of the cap and left in place during the recording or training session or as long as possible. Fur is trimmed as needed. Infections are quite rare using these procedures, but if they do occur appropriate topical and/or systemic treatments are administered in consultation with OLAC veterinary staff. Typically these may include a topical antibiotic (e.g. Elase Chloromycetin gel, Nitrofurazone or an alternative agent), topical Chloramphenicol (or an alternative agent), or a systemic antibiotic (e.g. Cephalexin or an alternative agent).

Long term care of cranial implants

Chambers are maintained per APV Guidelines (attached) in terms of Routine Recording Cylinder Care or in consultation with an OLAC veterinarian.

Repositioning of chambers

On occasion, the position of the chambers may need to be adjusted, should they prove unsuitable for accessing the required areas. This might occur for a number of reasons. First, there is the inherent inaccuracy in placing the chambers. Small differences in the angle of the chamber can have large differences in the final position of the electrodes, particularly when one is aiming for structures that are only several millimeters in size. Second, neurons encoding selective information may be detected on the edge of the area of accessible brain. Third, new information might arise in the scientific literature indicating that another brain structure might be important for the cognitive process that the behavioral task taxes. Fourth, after extended recording from the same location there can be difficulty obtaining viable neurons, necessitating recording from the opposite hemisphere. Fifth, we occasionally detect laterality effects (since we record from opposite hemispheres in the two animals that we use for each study) which may necessitate recording from the opposite hemisphere. Finally, there is sometimes breakdown of integrity of the acrylic cap necessitating removal and re-implantation at a later date. Any of these reasons could necessitate a change in position of the recording chamber. Each of these reasons is infrequent and it is unlikely that all will occur in a single subject. However, taken together it is likely that all subjects will at some point need the chambers repositioned at least once, at a maximum of one reposition per year, and a maximum of 3 per animal. Adjustment of the chamber position will take place in a single surgery. First, the old chamber and cap will be removed using drills and rongeurs to remove overlying bone cement, and then we unscrew the orthopedic screws. Then a new chamber will be attached as described above. Any deviation from any of the procedures or limits discussed here is only to be done in consultation with and with approval of OLAC veterinary staff and the ACUC.

How does this procedure fit into or address your overall research goals?

To investigate the correlation between behavior and brain function, we perform simultaneous electrical

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recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. For subcortical structures we use movable microelectrodes (semichronic arrays or acute recording arrays). The advantage of this technique is that it gives us the ability to record from new populations of cells by adjusting electrode position. Semichronic or acute arrays both require a chamber to be implanted over the brain areas of interest in order to either house the microdrives used to lower the individual microelectrodes.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience
[redacted]	Y	10+ years experience with all procedures in this protocol 8/19/10 : OLAC Herpes B training refresher given by [redacted] 1/16/2012: refresher training for Herpes B safety by [redacted]. 11/3/2010:CITI Training completed on 06/25/10: Certified by [redacted] to transport rhesus macaque into a customized restraint chair using the pole and collar method. OK'd to train [redacted] staff. 6/21/06: Approved by OLAC veterinarian to use isoflurane to anesthetize rats.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

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Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg).	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~1cm long) of ointment is applied to each eye during surgical prep. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	May be administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

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* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

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Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam.
Tramadol	3-5 mg/kg	Oral (PO)	Administered as a supplement to or a replacement for the injectable analgesic buprenorphine once the animal is eating reliably post-op.
Meloxicam	0.1mg/kg	Oral (PO)	Once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and

Protocol Title: [redacted]
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and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Behavior Study Assay

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Neural Recordings and Microstimulation using Semichronic Arrays in Macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C

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Maximum number of animals to be used in this procedure for a THREE-YEAR period: 10

Was a veterinarian consulted (for D or E studies)?:

Use Location: [redacted]

Building Name: [redacted]

Room Number: [redacted]

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* * * Procedure Description * * *

Procedure Description

One problem with chronic microarrays is that over time recording quality diminishes as gliosis occurs around the electrode contacts. Semichronic arrays provide a potential solution to this problem. The implant assembly is similar to that used for acute recordings. However, the electrodes and microdrives are designed to remain in place chronically. Thus, although the implant is chronic, individual electrodes remain adjustable. Therefore, if gliosis occurs the electrode can be simply advanced to establish new neuronal recordings. The leading manufacturer of these devices is Gray Matter Research Inc. [1].

The number of implanted electrodes varies depending on the specific study (range: 32-256 electrodes). A fraction of these electrodes will target cortical areas whereas other electrodes will target subcortical areas. Note: 256 is a typical number in chronic implant procedures (see for example: Ganguly K. and Carmena J.M. (2009) Emergence of a stable cortical map for neuroprosthetic control. PLoS Biology 7(7): e1000153. doi:10.1371/journal.pbio.1000153).

Following the Chamber Implant and Calvarium Opening procedures, the electrodes are lowered into the brain. Although once the microdrive assembly is placed in the chamber it remains in position (under replacement is required, as described in the Calvarium Opening for Semichronic Arrays in Macaques procedure), the electrodes encased inside the assembly are able to lowered into the brain and have their positions adjusted over time. Sterility of the electrodes is ensured via a silicone barrier that sterile electrodes must pierce in order to enter the brain. Due to this barrier, cleaning is not required for up to 12 months as the chambers remain completely sealed during this time. As electrodes are inserted into the brain (depth varies from 2mm in cortical structures (e.g. M1) to 20mm in basal ganglia structures such as the caudate nucleus), signals are monitored during the initial electrode lowering until all electrode tips are past the dura and have entered cortex. The precise electrode positions will be adjusted on that day using the microdrives until the maximum number of electrodes yield high quality neuronal recordings. At this time, the electrodes will remain in the brain for a period of a minimum of several weeks, but often as long as six to twelve months. During this time, micro adjustments in electrode position (using the microdrives) will allow for optimal electrophysiological recordings --and the microdrive adjustment is achieved while maintaining the tight seal of the system. At the end of each experimental session, the chamber system is covered with a titanium cap. No cleaning is required at the end of a recording session due to the sealed and enclosed design of the system. If microelectrodes need to be replaced (or upon completion of the experiment and recordings), all electrodes and the microdrive system will be removed as per the procedure outlined in Calvarium Opening for Semichronic Arrays in Macaques.

A critical step in the preparation of the microdrive prior to implantation involves the sterilization of the electrodes and the sealing of the bottom surface to prevent fluids from traveling back into the guide holes of the plunger block. The microdrive is sterilized in a two step sequence. After it is loaded with electrodes, it is gas sterilized. Then the guide holes are backfilled with sterile biocompatible silicone grease and the bottom surface is sealed with sterile silicone sealant. Then the assembly is gas sterilized a

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second time. Subsequently the sterile silicone grease is added to the electrode guide holes and the sterile silicone sealant is applied in fine layers to the base of the drive in a sterile environment. This technique ensures the base of the drive is properly sealed and sterility of the parts is maintained. Finally the entire assembly is gas sterilized a second time and kept in sterile packaging to ensure that sterility is maintained until implantation in a sterile environment. If any electrode needs to be replaced, the entire microdrive is removed from the chamber and is processed in the same manner as initial electrode placements. Currently, there have not been any reported complications or infection/inflammation to the tissue in the Gray Matter recording chamber system (personal communication with [redacted] at the [redacted], [redacted] at [redacted] and [redacted] at the [redacted]).

Direct electrical stimulation into the brain may be used as a way of providing feedback, to entrain neural networks, and to change behavior as causal intervention. For example, preferences for particular actions (e.g. choosing between two targets on a screen) are encoded in patterns of neural activity and this encoding can be altered using stimulation due to the fact that neurons communicate to each other through electrical signaling. Electrical stimulation is safe tool widely used by the electrophysiology community [2],[3],[4],[5],[6]. Different spatiotemporal patterns are applied in specific electrodes of the semichronic array using biphasic stimulation. Current is applied up to 300uA, with a resulting voltage of –10V to 10V, a maximum frequency of 300Hz and a maximum duration of 1000ms [4],[5],[6].

[1] http://www.graymatter-research.com/
[2] K. Nakamura and O. Hikosaka. Facilitation of saccadic eye movements by postsaccadic electrical stimulation in the primate caudate. J. Neurosci. 26(50), 12885 – 12895 (2006).
[3] T.D. Hanks, J. Ditterich, M.N. Shadlen. Microstimulation of macaque area LIP affects decisionmaking in a motion discrimination task. Nat. Neurosci. 9(5), 682 – 689 (2006).
[4] Z.M. Williams and E.N. Eskandar. Selective enhancement of associative learning by microstimulation of the anterior caudate. Nat. Neurosci. 9(4), 562 – 568 (2006).
[5] K. Amemore and A.N. Graybiel. Localized microstimulation of primate pregenual cingulate cortex induces negative decision-making. Nat. Neurosci. 15(2), 776 – 787 (2012).
[6] S.A. Overduin, A. d'Avella, J.M. Carmena and E. Bizzi. Microstimulation activates a handful of muscle synergies. Neuron 76(6), 1071-1077 (2012).

How does this procedure fit into or address your overall research goals?

To investigate the correlation between behavior and brain function of subcortical structures, we perform simultaneous electrical recordings of many neurons in multiple deep areas of the brain of macaques using movable microelectrodes (semichronic arrays or acute recording arrays). Recording with a semichronic array allows us to record from new populations of cells by adjusting electrode position. In some cases, microstimulation may be performed during behavior. Microstimulation is a common investigative tool used to modulate the electrical activity of surrounding neurons in order to create a functional response, such as a chance in strategy used during the trained behavior. This enables us to investigate the critical roles of different brain structures during behavior by characterizing how the induced changes in neural activity with stimulation may result in behavioral changes.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

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In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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Approval Period: 10/15/2019-10/31/2020

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Chlorhexidine solution	0.05%	Irrigation	If microelectrodes need to be replaced all electrodes and the microdrive system will be removed so the chamber can be thoroughly irrigated with sterile saline and Nolvasan

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Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Calvarium Opening for Semichronic and Acute Arrays in Macaques
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Protocol Title: [redacted]

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Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 10

Protocol Title: [redacted]

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* * * Procedure Description * * *

Procedure Description

Acute recordings involve lowering electrodes into the brain each day and then removing them at the end of the session. In contrast, in semichronic recordings, although the electrode position remains adjustable, the electrodes remain in the animal's brain for several months. Both types of recording require chambers to enable access to the animal's brain and to control the position of the electrodes.

After the chamber placement procedure, a separate surgery is performed at a later date (typically between 1-2 weeks) to make an opening in the calvarium. The underlying dura is left intact or slightly reduced using suction to remove any granular tissue. This is a minor procedure since the periosteum (which contains sensory nerves) has already been removed, and because there is no dissection of the dura. Thus, the animals typically recover extremely rapidly from this procedure. The animal is returned to a restricted water schedule when they are bright, alert, and responsive, and have a normal appetite. This enables us to begin recording soon after the opening has been constructed, and thus when little granulation tissue has formed, increasing the quality of the data.

Surgery preparations: Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.

On the day of the surgery, the monkey is sedated in the home cage with an intramuscular injection of ketamine and midazolam, weighed, and transported to the surgical prep area. Upon arrival, buprenorphine is administered for pre-emptive analgesia as well as either atropine or glycopyrrolate to reduce salivation. Baseline vital signs are obtained and recorded. The animal's head fur is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). A local anesthetic, such as lidocaine or bupivicaine, is injected subcutaneously at the site of the surgical incision. The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in the stereotax and the final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures.

To make the opening in the calvarium, a variety of drills may be used, including a dental drill and/or dremel.The opening(s) in the calvarium are precisely the size of the inner perimeter of the corresponding chamber(s). For example, for a cylindrical chamber of outer perimeter 1.7 cm, the inner perimeter is approximately 1.4 cm and thus a craniotomy of 1.4 cm diameter is necessary. The openings will initially be made using a dental drill and/or dremel. The burrs used with the dental drill and dremel are of size ¼ (0.5 mm diameter) to 8 (2.3 mm diameter). The opening will then be expanded to the correct size using either a dental drill, dremel or rongeurs, a standard neurosurgical tool for widening a bone window. The rongeurs have a 2 mm bite for precise removal of bone to expand the craniotomy window. The underlying dura is left intact or slightly reduced, using suction to remove any granular tissue. Bleeding is controlled with Gelfoam soaked in Thrombin or sterile saline.

Following the calvarium opening procedures, if the chamber will be used for recording with a semichronic array in most cases we will place the microdrive in the chamber during the same procedure. In some instances we will perform brief sedation and perform microdrive placement in a separate procedure one week following the craniotomy. In this case, the animal will be put under brief sedation with the already listed anesthesia protocol

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and the chamber will be cleaned using a diluted betadine solution prior to placement. In some cases dura reduction may be necessary to facilitate replacement of the microdrive. Once the microdrive is placed, a watertight seal is formed between the microdrive and the chamber, and the electrodes inside the microdrive remain sterile. Sterile silicone sealant is used to create a barrier between the base of the actuator block, the microdrive component which contains the electrodes, and the cerebral spinal fluid (CSF). The sterile silicone sealant is applied in fine layers during microdrive assembly prior to the procedure. At least three layers are applied to the base of the microdrive in a sterile setting, with twelve hours of setting time between application of each layer and a final thickness of approximately 0.5 mm. This technique ensures the base of the microdrive is properly sealed and impermeable to CSF.

Sterile biocompatible silicone grease is injected into the electrode guide holes to prevent fluids from traveling back into the guide of the actuator block up to the top of the microdrive. The silicone grease is sterilized prior to injection in the guide holes and then sterile technique is used for all phased of the silicone grease injection sequence. The bottom surface of the microdrive is then cleaned in the sterile environment and then a fine layer of sterile silicone sealant is applied to coat the bottom of the microdrive, creating the waterproof barrier at the neural interface.

Once the microdrive is placed in the chamber, inset metal screws in the microdrive are used to attach it to the chamber and seal the two components together. Additional acrylic is added to the existing acrylic cap in layers. The acrylic is built up to the bottom of the retaining cap of the microdrive, ensuring that the assembly is fully sealed and that the microdrive is also held in place with acrylic. Finally, a titanium cap is screwed over the assembly to keep the microdrive free of debris.

On occasion, the position of the chambers may need to be adjusted, should they prove unsuitable for accessing the required areas. This might occur for a number of reasons. First, there is the inherent inaccuracy in placing the chambers. Small differences in the angle of the chamber can have large differences in the final position of the electrodes, particularly when one is aiming for structures that are only several millimeters in size. Second, neurons encoding selective information may be detected on the edge of the area of accessible brain. Third, new information might arise in the scientific literature indicating that another brain structure might be important for the cognitive process that the behavioral task taxes. Fourth, after extended recording from the same location there can be difficulty obtaining viable neurons, necessitating recording from the opposite hemisphere. Fifth, we occasionally detect laterality effects (since we record from opposite hemispheres in the two animals that we use for each study) which may necessitate recording from the opposite hemisphere. Finally, there is sometimes breakdown of integrity of the acrylic cap necessitating removal and re-implantation at a later date. Chambers are also sometimes removed for clinical reasons, such as the chamber becoming loose or an infection developing underneath the implant.

Any of these reasons could necessitate a change in position of the recording chamber. Each of these reasons is infrequent and it is unlikely that all will occur in a single subject. However, taken together it is likely that all subjects will at some point need the chambers repositioned at least once, at a maximum of one reposition per year, and a maximum of 3 per animal. Adjustment of the chamber position will take place in a single surgery. First, the old chamber and acrylic will be removed using drills and rongeurs to remove overlying acrylic, and then we unscrew the orthopedic screws. Then a new chamber will be attached (see “Chamber Placement for Semichronic Arrays” procedure). One to two weeks after this surgery a new craniotomy will be performed.

Chambers are also sometimes removed for clinical reasons, such as the chamber becoming loose or an infection developing underneath the implant. In addition, there can be some growth of both granulation tissue and bone inside the chamber, although this varies considerably between individual animals. In such cases it may be necessary to perform a surgical procedure to reduce the dura to remove the additional bone and granulation tissue. Explantations for clinical purposes as well as dura reductions are not included in the limit of 3 per animal. Since we will at most reposition a chamber 3 times, the semichronic microdrive assembly if used in conjunction with the chamber will at most be replaced that number of times plus 3 additional times to account for instances when electrodes need to be replaced but the chamber remains in position. Taken together it is likely that all

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subjects will at some point need the microdrive replaced at least once (e.g. when a chamber if repositioned), at a maximum of two replacements per year, and a maximum of 6 per animal.

How does this procedure fit into or address your overall research goals?

To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. For subcortical structures we use movable microelectrodes (semichronic arrays or acute recording arrays). The advantage of this technique is that it gives us the ability to record from new populations of cells by adjusting electrode position. Semichronic or acute arrays both require a chamber to be implanted over the brain areas of interest in order to either house the microdrives used to lower the individual microelectrodes. Inside the chamber, an opening is made in the calvarium in order to permit access to the neural tissue.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience
[redacted]	Y	10+ years experience with all procedures in this protocol 8/19/10 : OLAC Herpes B training refresher given by [redacted] 1/16/2012: refresher training for Herpes B safety by [redacted]. 11/3/2010:CITI Training completed on 06/25/10: Certified by [redacted] to transport rhesus macaque into a customized restraint chair using the pole and collar method. OK'd to train [redacted] staff. 6/21/06: Approved by OLAC veterinarian to use isoflurane to anesthetize rats.

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

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Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg)	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intravenous (IV)
Lidocaine/bupivicaine	0.5-1.5mLs of 2% solution (10-30mg).	Subcutaneous (SC)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~1cm long) of ointment is applied to each eye during surgical preparation. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

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* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

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Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam.
Tramadol	3-5 mg/kg	Oral (PO)	1-3 times daily up to 3 days.
Meloxicam	0.1mg/kg	Oral (PO)	Administered once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

*** * * Other Agents Utilized * * ***

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Surgical Procedure

Procedure Type:	Surgical Procedure	**Procedure Title:**	Dural Maintenance
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			D
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	Y
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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Surgery Info

For guidance, please refer to the ACUC Guidelines for Anesthesia and Analgesia in Laboratory Animals, Guidelines for Surgical Procedures, Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals, and Multiple Partial Ovariectomies on Xenopus (MPOX) Policy.

Specific room number where surgery is performed: [redacted]

Surgery Type: Survival

MULTIPLE MAJOR SURVIVAL SURGERY: The Guide defines major survival surgery as a surgical procedure that penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. The USDA defines a major operative procedure as any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

If a major surgical procedure is performed on an animal prior to obtaining it (e.g., surgerized animals obtained from a vendor), and a subsequent major survival surgical procedure is performed on the same animal, this is considered Multiple Major Survival Surgery.

Will this project include Multiple Major Survival Surgery (MMSS)? Y

PLEASE NOTE: If multiple major survival procedures are to be performed, you will be asked for specific justification in Procedure Relationships section of this form.

Number of animals that will undergo MMSS per year: 10

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Procedure Description * * *

Procedure Description

On occasion it is necessary to remove granulation tissue and scar tissue from the dural surface. This arises when the tissue begins to prevent us from inserting our electrodes into the brain. During acute recordings, when electrodes are lowered and retracted in the neural tissue on an up to daily basis, this occurs approximately every 4-8 weeks. Thus, in a typical recording session an animal will undergo one surgery to maintain the dura. The maximum number of times an animal would require dural maintenance is 6. The minimum time between procedures would be 2 weeks. This would occur if the first procedure was not successful in thinning the dura.

Weeks before surgery, the inventory is updated for supplies and equipment is checked. A meeting is arranged with OLAC veterinary staff to set a surgery date and to review the procedure and post-operative care schedule. The week before surgery, animals are typically given free water and fresh fruit daily. A minimum of at least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin again until post-surgical analgesics are no longer being given. All necessary tools and supplies are either autoclaved or gas sterilized. The animal will fast for at least 8 hours before surgery, but will have free access to water.
On the day of the surgery, the monkey is sedated in the home cage with an intramuscular injection of ketamine (10 mg/kg) and midazolam (0.1 – 0.25 mg/kg), weighed, and transported to the surgical prep area. Ketamine and midazolam are typically administered together as a single injection. Upon arrival, buprenorphine (0.01 – 0.03 mg/kg) is administered via an intramuscular injection for pre-emptive analgesia, as well as either atropine (0.04 mg/kg) or glycopyrrolate (0.1 mg/kg) to reduce salivation. Atropine and glycopyrrolate may be given either subcutaneously or intramuscularly. Baseline vital signs are obtained and recorded. The animal's head fur is clipped and a preliminary surgical scrub is performed. The collar is removed and ophthalmic ointment is applied bilaterally. An appropriately sized IV catheter is placed (20-25g). Lidocaine is applied topically to the larynx and the animal is intubated with an appropriately sized endotracheal tube (usually size 3.0-5.0). The animal is transported to the surgical suite and connected to monitoring equipment. Warmed IV fluids are administered as well as IV antibiotics (cefazolin 25mg/kg). Thermoregulation is managed with various warming blankets over/under the patient or both if necessary. The animal is placed in the stereotax and the final surgical scrub is performed in accordance with ACUC Guidelines for Surgical Procedures. The surgical procedure itself is straightforward: we use aspiration with micropipettes to remove extraneous tissue.

How does this procedure fit into or address your overall research goals?

To record from subcortical structures we use movable microelectrodes (semichronic arrays or acute recording arrays). Semichronic or acute arrays both require a chamber to be implanted over the brain areas of interest in order to either house the microdrives used to lower the individual microelectrodes. A calvarium opening is made inside of the chamber in order to permit access to the neural tissue. For acute arrays, a microdive is used to lower microelectrodes into the tissue at the beginning of a recording session. Microelectrodes are lowered through the dura mater into the brain, and then retracted at the end of the session. On occasion it is necessary to remove granulation tissue and scar tissue from the dural surface in order to allow microelectrodes to successfully penetrate through the dura.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

Animals may experience mild discomfort when chewing food. Moistened biscuits, soft fruit and fruit juice may be offered. Analgesics are described under post-procedure care.

Describe post procedure monitoring that will be performed.

In the hours following any surgery the animal is monitored closely by the surgical team, to ensure normal recovery from anesthesia and an appropriate level of analgesia. Immediately after surgery the animal is checked

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constantly by the principal investigator and/or another qualified member of the lab until it is able to maintain a normal sitting posture. The level of post-operative alertness may vary somewhat, because of the use of buprenorphine as post-operative analgesics. For example, buprenorphine causes some animals to sleep for several hours after surgery, while others are up and eating within the hour.

After initial recovery the animal is checked at least 3 times per day (usually more), at which time appropriate analgesics and antibiotics are administered. Monitoring continues for one to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC veterinary staff.

Protocol Title: [redacted]

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* * * Surgeon Details * * *

Surgeon Details

Surgeon Name	Does the Surgeon have prior specific experience with this surgery on this species? Indicate whether the surgeon has been certified by an OLAC veterinarian.	Describe the previous experience and/or training plan to assure surgical proficiency.
[redacted]	Y	10+ years experience
[redacted]	Y	10+ years experience with all procedures in this protocol 8/19/10 : OLAC Herpes B training refresher given by [redacted] 1/16/2012: refresher training for Herpes B safety by [redacted] 11/3/2010:CITI Training completed on 06/25/10: Certified by [redacted] to transport rhesus macaque into a customized restraint chair using the pole and collar method. OK'd to train [redacted] staff. 6/21/06: Approved by OLAC veterinarian to use isoflurane to anesthetize rats.

Protocol Title: [redacted]

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* * * Anesthetic Regimen * * *

Anesthetist(s)

Anesthetist Name	Describe previous experience and training in anesthesia.
[redacted]	OLAC veterinary staff have specialized training in laboratory animal medicine, and include licensed/board-certified veterinarians and registered veterinary technicians. All OLAC vet staff have experience performing and training others in anesthesia and peri-procedure care.

X Respiratory Rate
X Heart Rate
X Body Temperature
X Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
X PO2
X ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

OLAC veterinary staff perform anesthesia and are responsible for maintaining the anesthesia record, including: date and time of procedure, animal's identification number, animals' weight as recorded on the day of surgery, the name, dose, route and time of each drug administered, all major surgical or anesthetic events, and measurements of the animal's physiologic parameters including heart rate, respiratory rate, and body temperature. These physiological measurements are assessed and recorded at least every 15 minutes throughout the procedure. The anesthesia record is maintained in the animal's health record.

Anesthetic Agents

Protocol Title: [redacted]

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Agent Name	Dosage (in mg/kg if possible) and volume	Route
Isoflurane	1.0%-3.0%	Inhalation (IN)
Ketamine hydrochloride	10 mg/kg	Intramuscularly (IM)
Lidocaine/bupivicaine	0.1-0.3 mLs of 2% solution (2-6mg)	topical (Topical)
Midazolam	0.1--0.25mg/kg	Intramuscularly (IM)

Other premedications not already listed above

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Ocular Lubricant	N/A	topical (Topical)	A thin strip (~1cm long) of ointment is applied to each eye during surgical prep. It is reapplied as necessary intraoperatively.
Glycopyrrolate	0.1 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated in place of atropine.
Atropine	0.04 mg/kg	Intramuscularly (IM)	Administered once after the animal has been sedated.

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* * * Peri procedure Care/Analgesics * * *

Pre-emptive Agents (analgesics given prior to/during procedure)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	Administered once prior to surgery and every 4 hours intra-operatively.

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Respiratory rate, heart rate, ECG, and capnography are monitored to assure a proper level of analgesia.

Antibiotics or Anti-Microbials

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Cefazolin	25 mg/kg	Intramuscularly (IM)	Administered twice daily (every 12 hours) for at least 7 days post-operatively.
Cefazolin	25 mg/kg	Intravenous (IV)	Administered every 2 hours intraoperatively.
Cephalexin	25mg/kg	Oral (PO)	Administered twice daily as a replacement for the injectable antibiotic cefazolin once the animal is eating reliably post-op for the remainder of the treatment course.

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Post-procedure Monitoring

Post-procedure Analgesics

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency & duration of administration
Buprenorphine	0.01-0.03 mg/kg	Intramuscularly (IM)	1-3 times daily (minimum 48hrs)
Meloxicam	0.2 mg/kg	Intramuscularly (IM)	It is injected upon extubation and recovery the day of surgery and then the morning after surgery. May continue at 0.1mg/kg SQ/IM once daily for 3-5 days if the animal does not reliably ingest oral formulation of meloxicam.
Tramadol	3-5 mg/kg	Oral (PO)	1-3 times daily up to 3 days.
Meloxicam	0.1mg/kg	Oral (PO)	Administered once daily for 3-5 days when the animal has returned to eating reliably post-op in place of injectable meloxicam.

Recovery Location Building Name [redacted] **Room Number** [redacted]

Responsible Personnel

OLAC veterinary staff, PI, and/or another qualified member of the lab.

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

All post-operative monitoring and administration of medication is managed by the OLAC veterinary staff and is recorded in the animal's health record.

Several indicators of post operative pain are considered, including the animal's level of alertness and responsiveness, movements in the recovery or home cage, appetite, and social interactions with conspecifics and the laboratory staff. In general all appropriate measures are taken to minimize post-operative pain, and these must necessarily be tailored to some extent for each animal. Therefore, to ensure adequate control of post-operative pain, choice of analgesia and frequency of administration are made in consultation with OLAC veterinary staff.

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Monitoring Duration

One to two weeks following surgery (including weekends and holidays), depending on the rate of recovery.

Monitoring Frequency

Several times per day (as necessary).

Describe what actions will be taken if parameters monitored fall outside normal ranges:

OLAC veterinary staff will be immediately notified.

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Moistened biscuits, soft fruit and fruit juice may be offered.

Describe record keeping/documentation methods for post-procedure monitoring:

Post-procedure notes are maintained in the animal's health record.

*** * * Other Agents Utilized * * ***

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Behavior Study Assay

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Neural recordings and microstimulation in-cage during natural behavior
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]

Protocol Title: [redacted]

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Room Number: [redacted]

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* * * Procedure Description * * *

Procedure Description

Natural behavior in-cage refers to untrained, spontaneous behavior of the animal in the home cage environment. For example, this behavior includes periods of grooming, eating and sleeping.

Following the Calvarium Opening for Semichronic Array and placement of the semichronic array, a wireless neural recording device ("OMNI" device) may be used to record neural activity during natural behavior in the home cage. The device will be placed subsequent to recovery from these procedures, and will be performed while the animal is awake and in a primate chair. The OMNI device has capabilities similar to other wireless neural recording devices previously used with nonhuman primates, such as the HermesC [1] and Neurochip [2,3] devices. The OMNI device is designed to mate with connectors on the semichronic recording arrays that are located outside of the seal maintaining sterility of the tissue-array interface, which means the OMNI device need not be sterile. Thus, the OMNI device will interface directly with connectors on previously implanted arrays and will not require any additional surgical procedures to use. The OMNI device will fit inside the chamber cap that the semichronic array is housed inside. However, depending on the height and material of the original cap used with the semichronic array a new chamber cap may be used. This cap may be made of hard plastic or titanium, standard materials used by the manufacturer of the semichronic array (Gray Matter Research, Inc.), and have an increased height up to 10 mm from the original cap height. If a taller cap is used, the subject will be habituated to this cap before the OMNI device is placed inside. This taller cap size is comparable to what has been used in other primate labs with similar implants (e.g. the [redacted] Lab).

The OMNI device is capable of recording neural activity or delivering electrical microstimulation for up to 12 hours at a time. Since recordings are during untrained behavior in the home cage, this device may be used in subjects who are additionally participating in trained behavioral experiments. Direct electrical microstimulation may be administered in-cage in order to promote particular neural states following or proceeding a particular behavioral experiment. For example, stimulation may be use to enhance activity in the neural population used for neuroprothetic control so that subjects may become proficient at neuroprosthetic control at a faster rate. Current is administered via the semichronic array implant. The maximum current amplitude used will be 300 uA, which is the absolute max current the OMNI device is capable of delivering and is consistent with stimulation performed during trained behaviors while the monkey is chaired (see "Neural recordings and microstimulation during natural motor control"). Safety mechanisms have been implemented redundantly both in software and firmware to ensure that current delivery above the 300 uA limit is impossible. Additionally, safety shutoff features have been added in software so that if there is a software malfunction, such as a clock or command error, the device will default to an OFF state. Current will be delivered in a series of biphasic (i.e. positive and negative phases) pulses with a maximum pulse duration of 500 ms and maximum frequency of 300 Hz, consistent with microstimulation delivered during trained behaviors (see "Neural recordings and microstimulation during natural motor control"). Trains of current pulses may be applied for up to 12 hours per day and up to 7 days per week. In humans, electrical stimulation with similar parameters is used chronically and safely for periods exceeding 12 hours (typically 24 hrs/day), such as in the case of deep brain stimulation to treat Parkinson's disease (e.g. [4,5]) where patients receive electrical stimulation treatment for 24 hrs/day for years. Electrical stimulation is not anticipated to affect the subject's behavior, but the first two times stimulation is performed with the OMNI device the animal will be separated from its cagemate and OLAC staff will be alerted on these occasions so that any unforeseen effects can be safely and swiftly resolved.

The OMNI device is battery-powered and has a transmit antenna to wirelessly transmit neural activity to a nearby receiver. The range of wireless transmission is less than 10 m, well within the NHP area of the facility,

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and is comparable to Bluetooth devices. There is no risk that an outside individual might be able to gain access to the wireless transmissions due to this limited range of wireless transmission. The receiver will be housed in the colony room of the subject and placed in consultation with OLAC staff so as not to interfere with daily husbandry duties or other activities in the colony room. For example, the receiver may be mounted on the ceiling above the subject's home cage.

The OMNI device weight is 20 - 30 grams. The temperature of the device may heat up to 2 degrees Fahrenheit above room temperature when in use. It does not make any audible or ultrasonic sound, and thus is not anticipated to disrupt normal behavior of the subject or other animals in the same colony room. The OMNI battery will be replaced as necessary during regular chairing.

[1] C. A. Chestek, V. Gilja, P. Nuyujukian, R. J. Kier, F. Solzbacher, S. I. Ryu, R. R. Harrison, and K. V. Shenoy, "HermesC: Low-Power Wireless Neural Recording System for Freely Moving Primates," IEEE Trans. Neural Syst. Rehabil. Eng., vol. 17, no. 4, pp. 330–338, Aug. 2009.
[2] Mavoori, J., Jackson, A., Diorio, C., Fetz, E.E. (2005) An autonomous implantable computer for neural recording and stimulation in unrestrained primates. J. Neurosci. Methods 71, 71 – 77.
[3] Jackson, A., Mavoori, J., Fetz, E.E. (2006) Long-term motor cortex plasticity induced by an electronic neural implant. Nat. 444, 56 – 60.
[4] de Hemptinne, C., Swann, N.C., Ostrem, J.L., Ryapolova-Webb, E.S., San Luciano, M. Galifianakis, N.B., Starr, P.A. (2015) Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease. Nat. Neurosci. 18, 779 – 786.
[5] Johnson, M.D., Ghosh, D., McIntyre, C.C., Zhang, J., Vitek, J.L. (2012) Neural targets for relieving parkinsonian rigidity and bradykinesia with pallidal deep brain stimulation. J. Neurophysiol. 108, 567 – 577.

How does this procedure fit into or address your overall research goals?

An overarching research goal in our studies is to investigate the correlation between behavior and brain function. While we assay behavior and perform simultaneous neural recordings during experiments with the subjects in primate cages, this can only serve an approximation of behaviors within their natural repertoire. Neurophysiological studies performed in-cage has the advantage of allowing for the acquisition of neural data during spontaneous behavior and the opportunity to study native behaviors not typically displayed in-chair, such as sleep.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected.

Describe post procedure monitoring that will be performed.

Animals will be chaired to remove the in-cage recording device and then returned to their cages at the end of the procedure. During chairing the device battery will be replaced, if necessary, and the device inspected to ensure normal operation.

We will discontinue administration of electrical stimulation if there is unacceptable weight loss (more than 10% of baseline weight) or changes in social behavior.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Behavior Study Assay

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

Procedure Type:	Behavior Study Assay	**Procedure Title:**	Salivary Cortisol Monitoring
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

Cortisol hormone levels can be used as a physiological marker of the stress. Basal cortisol levels naturally fluctuate throughout the day with species-dependent variability though levels may be modulated by acute events. Cortisol levels can be measured using plasma, serum, urine, interstitial fluid, and saliva, among others. We propose to monitor cortisol levels by analyzing saliva. Salivary cortisol collection is non-invasive and can be easily performed with a head-restrained subject while in a primate chair in the lab. Saliva collection will be performed using either cotton dental rope (Lutz et al., 2000; Tiefenbacher et al., 2003), a cotton swab, or cotton roll (Thomas et al., 1995) with an attached dental rope tie. The dental rope, swab or cotton roll will be placed in the subject's mouth for a period of up to 60 s, depending on the amount of saliva necessary for the assay and the saliva present in the mouth, and then removed. If using dental rope or the cotton roll with an attached dental rope tie, part of the dental rope will be left outside of the subject's mouth and held by the researcher so that the sampling materials may be easily removed. In the unlikely occasion that the dental rope or roll has been swallowed, the animal will be subsequently monitored to ensure that the item is easily passed. Previous studies have found that levels of immunoreactive cortisol are unaffected by either cotton dental rope or cotton swabs. Saliva collection may occur multiple times per day with at least 10 minutes between each sample collection and at most 10 samples collected daily. All materials will be used a single time and then discarded appropriately.

References:

Lutz, C.K., Tiefenbacher, S., Jorgensen, M.J., Meyer, J.S., Novak, M.A. (2000) Techniques for collecting saliva from awake, unconstrained, adult monkeys for cortisol assay. American J. of Primatology, 52, 93 – 99.

Thomas, B.W., Champoux, M., Suomi, S.J., Gunnar, M.R. (1995) Salivary cortisol in nursery-reared rhesus monkeys: reactivity to peer interactions and altered circadian activity. Developmental Psychobiol., 28, 257 – 267.

Tiefenbacher, S., Lee, B., Meyer, J.S., Spealman, R.D. (2003) Noninvasive technique for the repeated sampling of salivary free cortisol in awake, unrestrained squirrel monkeys. American J. of Primatology, 60, 69 – 75.

How does this procedure fit into or address your overall research goals?

An overarching goal of this research is the development of neuroprosthetic systems. To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons

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in multiple areas of the brain of macaques over an extended period of time. To additionally investigate how neural activity may correlate with mood or emotional state, cortisol levels are tracked to determine how the stress level of the subject may or may not change throughout the performance of a behavior.

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

No clinical effects or changes for the normal health are expected. However, in the unlikely case of ingestion of sampling materials, the subject will be monitored for the passing of the passing of the materials.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure and monitored daily.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Compound/Drug Administration

Protocol Title: [redacted]

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Procedure Type:	Compound/Drug Administration	**Procedure Title:**	Pharmacological testing during behavior with neural recording and microstimulation
Species:	Monkey, Rhesus (OLAC Vivarium)		
Pain/Distress Category:			C
Maximum number of animals to be used in this procedure for a THREE-YEAR period:	10	**Was a veterinarian consulted (for D or E studies)?:**	
Use Location:	[redacted]	**Building Name:**	[redacted]
		Room Number:	[redacted]

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* * * Procedure Description * * *

Procedure Description

The aim of this technique is to assess the electrophysiological effects of pharmacologic compounds administered during trained behavior. Effects of these manipulations are assessed while the animal subjects perform simplified motor control or neuroprosthetic control tasks that are designed to test various aspects of cognitive performance in order to obtain rewards. For example, anxiolytic drugs are known to increase the subjective value of “good” choices in simple cost-benefit decision making.
The class of agents that will be administered are known as benzodiazepines (BZs) and are known to produce anxiolytic-like effects. BZs act by allosterically binding to GABAA receptors and enhancing the ability of GABA to increase chloride conductance. The precise dosages that we use have been determined from the existing literature, and we will use multiple concentrations to ensure that a reasonable proportion of the dose-response curve is measured. We specifically wish to test diazepam and midazolam, which are conventional BZs, flumazenil, a BZ antagonist, and beta-carbolines, which are a class of BZ inverse agonists. All agents will be pharmaceutical grade if available and, if not available, have a grade >99% purity.

We do not anticipate any effect on behavior beyond a potential change in strategy implemented in the cognitive task. Indeed, such an effect would be detrimental to the interpretation of our results, since we would not be sure whether the change in neuronal activity was a direct result of the drug on the neuron, or an indirect effect on neuronal activity because the drug has altered the animal’s behavior. If there were behavioral effects they would be subtle and only evident using an appropriate behavioral assay. For example, we might expect a reduction in motivation or attentional deficits, but these could only be detected if the animal were performing a task that taxed these cognitive processes.

Dosages will be based on a combination of experimentation and guidance from the literature. Diazepam has been shown to produce changes in decision-making strategies in a standard approach-avoidance task with dosages of 0.1 – 0.4 mg/kg intramuscular (IM; Amemori & Graybiel, 2012). A similar dosage, 0.5 mg/kg IP, was found to decrease anxiety-like behaviors in marmosets (Cagni et al., 2012). We will begin with the smallest effect dosage, 0.1 mg/kg IM, and then increase the dosage as necessary by increments of 0.05 mg/kg to a maximum dosage of 0.5 mg/kg IM. Diazepam is metabolized faster in monkeys relative to humans and we anticipate it will be mostly metabolized within 3 hrs of administration (Seddon et al., 1989). Midazolam has been safely administered in adult rhesus monkeys in dosages of 0.032 – 1 mg/kg subcutaneous (SC; McMahon & France,

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2006; Zanettini et al., 2014). Flumazenil has been shown to reverse the effects of BZs using dosages in the range of 0.032 – 1 mg/kg SC (McMahon & France, 2006; Zanettini et al., 2014). With midazolam and flumazenil, we will begin with dosages of 0.032 mg/kg SC and increase the dosage as necessary up to a maximum dosage of 1 mg/kg SC. Beta-carbolines have been shown to produce anxiety-related behaviors with dosages ranging from 0.1 to 1 mg/kg IM (Major et al., 2009). Drugs that are administered IM or SC will be injected in the back or hind limb in a volume ranging from 0.2 to 1.2 ml. Injection volumes will vary due to the solubility of the agents and the concentration of agents available commercially. Since injecting volumes such as these multiple times per day for multiple days may result in discomfort and lameness in that limb, typically only one injection will be performed per day. Daily injections may be performed for up to 60 consecutive days. Injections will be performed while the subject is head-fixed in a primate chair in the lab. If the effects of the pharmacological agent extend beyond the time of the experiment, the subject will be separated from its cage mate upon return to the cage and monitored until baseline behavior is restored.

In summary, we will minimize the chances of seeing averse behavioral effects by using small quantities and small concentrations of short-acting drugs. In addition, prior to beginning the use of pharmacological agents we will habituate the animals to receiving IM and SC injections while sitting in a primate chair through saline injections. Small volumes, 0.5 – 1.2 ml, of 0.9% saline will be injected in either the hind limb or back to habituate the animal to this process. Over the course of experiments, saline may also be injected as a control agent.

We will discontinue administration of pharmacological agents if there is abnormal weight loss or changes in social behavior.

References:
Amemori, K., & Graybiel, A. (2012) Localized microstimulation of primate pregenual cingulate cortex induces negative decision-making. Nature Neuroscience, 15(5), 776 – 785.

Cagni, P., Komorowski, M., Melo, G.C., Lima, T., Barros, M. (2012) Diazempam-induced decrease in anxiety-like behaviors of marmoset monkeys exposed to a novel open-field. Pharmacology Biochemistry and Behavior, 100(3), 518 – 521.

Major, C. A., Kelly, B. J., Novak, M. A., Davenport, M. D., Stonemetz, K. M., & Meyer, J. S. (2009). The anxiogenic drug FG7142 increases self-injurious behavior in male rhesus monkeys (Macaca mulatta). Life Sciences, 85(21–22), 753–758. https://doi.org/10.1016/j.lfs.2009.10.003

McMahon, L.R., & France, C.P. (2006) Differential behavioral effects of low efficacy positive GABAA modulators in combination with benzodiazepines and a neuroactive steroid in rhesus monkeys. British J. of Pharmacol., 147, 260 – 268.

Seddon, T., Michelle, I., Chenery, R.J. (1989) Comparative drug metabolism of diazempam in hepatocytes isolated from man, rat, monkey and dog. Biochemical Pharmacoloy, 38(10), 1657 – 1665.

Zanettini, C., France, C.P., Gerak, L.R. (2014) Quantitative pharmacological analyses of the interaction between flumazenil and midazolam in monkeys discriminating midazolam: determination of the functional half life of flumazenil. Eur. J. Pharmacol., 723, 405 – 409.

How does this procedure fit into or address your overall research goals?

An overarching goal of this research is the development of neuroprosthetic systems. To investigate the correlation between behavior and brain function, we perform simultaneous electrical recordings of many neurons in multiple areas of the brain of macaques over an extended period of time. To additionally investigate how neural activity and cognitive performance may be affected by mood or emotional state, anxiolytic compounds are administered.

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Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

While clinical effects from (IM/SQ) injections are not expected, hematoma formation, tissue trauma, and infection may occur. Changes from normal health are not anticipated with the dosages of pharmacological agents, however possible side effects of the agents include drowsiness, dizziness and nausea.

Describe post procedure monitoring that will be performed.

Animals will be returned to their cages at the end of the procedure and are monitored daily.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

In the event of any animal exhibiting clinical (e.g. signs of infection, loss of weight) or behavioral changes (e.g. lack of motivation during training) we would consult with OLAC.

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* * * Anesthetic Regimen * * *

Respiratory Rate
Heart Rate
Body Temperature
Blood Pressure
Corneal/Palpebral Reflex
Pedal Reflex
Capillary Refill
PO2
ETCO2
Other (Describe)

Describe recordkeeping methods during anesthesia. For guidance, please refer the ACUC Recordkeeping Guidelines for Surgical Procedures on Laboratory Animals.

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* * * Peri procedure Care/Analgesics * * *

Describe what parameters will be monitored during the procedure to assure proper analgesia (e.g., respiratory rate, corneal/palpebral reflex, pedal reflex, etc.):

Post-procedure Monitoring

Recovery Location Building Name

Room Number

Responsible Personnel

Parameters Monitored (e.g., appetite, body weight, body condition score, posture, etc.)

Monitoring Duration

Monitoring Frequency

Describe what actions will be taken if parameters monitored fall outside normal ranges:

Describe any non-pharmaceutical support provided during recovery (e.g., heating pads, soft/palatable foods, food provided on cage floor, etc.):

Describe record keeping/documentation methods for post-procedure monitoring:

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* * * Other Agents Utilized * * *

Note: Pharmaceutical grade compounds must be used in animals unless those compounds are not available or are otherwise inappropriate for the aims of the proposed animal use. If proposing to use non-pharmaceutical grade compounds, please complete the appropriate questions on the "Are You Using" section of the protocol. For guidance, please refer to the ACUC policy on Use of Non-pharmaceutical Grade Compounds.

Other Agents Utilized

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Diazepam	0.1 – 0.5	Intramuscularly (IM)	Infused for 0-5 mins, 1-5 times a session
Midazolam	0.032 - 1	Subcutaneous (SC)	Infused for 0-5 mins, 1-5 times a session. It may be difficult to administer a SQ injection precisely in an awake/moving animal.
Flumazenil	0.032 – 1	Subcutaneous (SC)	Infused for 0-5 mins, 1-5 times a session. It may be difficult to administer a SQ injection precisely in an awake/moving animal.
Midazolam	0.032 - 1	Intramuscularly (IM)	Infused for 0-5 mins, 1-5 times a session. Typically this agent will be administered SQ, however, it might be given IM since the SQ space is difficult to access.
Flumazenil	0.032 - 1	Intramuscularly (IM)	Infused for 0-5 mins, 1-5 times a session. Typically this agent will be administered SQ, however, it might be given IM since the SQ space is difficult to access.
N-methyl-β-carboline-3-carboxamide	0.1 - 1.0 mg/kg	Intramuscularly (IM)	Infused for 0-5 mins, 1-5 times a session. This agent is typically administered IM.

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* * * Alternative Search * * *

For Pain/Distress Categories D or E

For any procedure that is likely to cause more than slight or momentary pain or distress, a literature search is required to determine if other methods are available that could reduce or eliminate pain or distress experienced by the animal. Instructions and examples of this literature search, appropriate databases to use, and helpful keywords can be found in the guidelines on Literature Searches for Alternatives on the ACUC website.

Search Data

Search Date	Search Range	Keywords	Databases Searched
09/17/2019	1991-2019	Head restrain, positioner, head post, rhesus macaque	PUBMED/ Web of Science/ Society for Neuroscience Abstracts
09/17/2019	1991-2019	multielectrode recordings, rhesus macaque, cortex, brain-machine interface, computational model, computer simulations, non-invasive neuroprosthetics	PUBMED/ Web of Science/ Society for Neuroscience Abstracts
09/17/2019	1991-2019	rhesus macaque, cortex, motor control, motor learning, computational model, computer simulations, motor psychophysics	PUBMED/ Web of Science/ Society for Neuroscience Abstracts
09/17/2019	1991-2019	Dural maintenance, dura scrape, durectomy, rhesus macaque	PUBMED/ Web of Science/ Society for Neuroscience Abstracts

Describe the search strategies used to conduct your search.

Methods other than a literature search:

Professional conferences/meetings attended: Society for Neuroscience annual meeting, Neural Control of Movement society, COSYNE workshop, IEEE Biomedical Engineering Society, Duke Neurobiology retreat, Christopher Reeve Paralysis Foundation annual meeting, Helen Wills Neuroscience annual retreats.

Names of other experts consulted [redacted]

List of service provided to grant review committees, panels or editorial boards:Grant reviewer for the National Institutes of Health study section NSD-A, US Department of Defense and European Union Neurobotics network. Reviewer for PNAS, Journal of Neuroscience Methods, Journal of Neurophysiology,, Neurocomputing journal and IEEE-Transactions on Biomedical Engineering.

List of journals subscribed to and/or read: Science, Nature, J. Neuroscience, J. Neurophysiology, PNAS, PLoS Biology, Current Biology, Nature-Neuroscience, Neuron, J. Neuroscience Methods, Neuroreport. Newer searches also included the following journals: Experimental Brain Research; Brain; British Journal of Anesthesia;

List of seminars/lab meetings presented/attended: University of Washington, Massachusetts Institute of Technology, Dept. Bioengineering- Stanford, Dept. Biomedical Engineering- U. Southern California, Dept. Biomedical Engineering- U. Minnesota, Cosyne Workshop on Emerging Directions in Cortical Interfaces for Control, Sensorimotor Program, Rehabilitation Institute of Chicago, Dept. Biomedical Engineering- Northwestern, School of Biomedical Engineering- Science and Health Systems- Drexel, Dept. Neurobiology and Anatomy-

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Drexel, Dept. of Biomedical Engineering- Rutgers, Dept. Biomedical Engineering- Duke, Dept. Biomedical Engineering- Yale, Dept. Electronic and Electrical Engineering- Imperial College of Science Technology and Medicine, Spinal Cord Symposium- Christopher Reeve Paralysis Foundation, Advances in Neuroscience- Neuroscience Institute of Miguel Hernandez University, Behavioral Ecological and Sociobiological Topics at Dept of Biological Anthropology and Anatomy- Duke University.

Provide the number of hits and summarize the findings of your search results.

In the search on 11/9/2017, for all keywords there were in total 737,311 hits on PubMed, 986,719 hits on Web of Science, and 35,070 hits in the search of the Society for Neuroscience Abstracts. The findings from this literature search indicate that the dural maintainence procedure is a typical procedure that accompanies an acute electrophysiology study, which is a standard method of acquiring neural data in the rhesus macaque animal model. The use of head restraint and head positioners is also standard within rhesus macaque studies.

I believe there is no alternative to further reduce, replace or refine this potentially painful/distressful procedure. Based on the following references and experience, this animal model is the most appropriate for conducting my research.

Specific Relevant Citations

Relevant citations must be listed here or provided as an attachment.

For category E procedures, explain why drugs or other ameliorative treatments cannot be used to fully alleviate pain/distress. Include the number of animals per year.

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* * * Procedure Relationships * * *

Procedure Relationships

Please describe the sequence and timing of the manipulations:

If more than one surgery or procedure will be performed on some or all animals used under this protocol, describe the sequence and timing of these manipulations. Flow charts may be helpful and can be attached to the protocol.

Animals are used in multiple experiments (sequentially) until recordings are not viable due to microelectrode malfunction and/or signal degradation.
Up to 10 macaques (with a maximum of 3 macaques per study but typically these will be 2) will be used in these experiments. Up to 4 studies may start in a given year. Each animal is initially trained to be handled with the pole and collar method and to sit in a customized chair. Once the animal is comfortable sitting in the chair, it is transported to the experimental room where it is trained to carry out progressively more complex behavioral tasks for a liquid reward. During task training, all trials are initiated by the animal, and it may generally work for as long as it wishes in a single session. Training sessions last from 5 minutes to 6 hours, and are conducted from 0-7 days/week. Training can continue for a period from one to several months, depending on the particular task required, the previous experience and inherent trainability of the animal, and the skill of the trainer. Most every animal can be taught to perform the behavioral experiments required under this protocol, though the speed of training varies.

See 'Procedures Schematic' attachment for the timeline of the manipulations.

Procedures done on a single animal:

Please indicate how many and which procedures a single animal will go through. If applicable, please identify the strain/genotype/breed of animals that will be used in each procedure. Charts are highly recommended for clarity.

The minimum number of procedures a single animal may undergo is 4.
Example: Acclimation to Restraint —> Positioner Implantation —> Chronic Microwire Arrays Implantation —> and one of the behavioral study assays (e.g. Neural recordings and microstimulation during natural motor control).

The maximum number of procedure a single animal may undergo is 15. Example:
MRI scan (skull model formation) —> Acclimation to Restraint —> Positioner Implantation —> Chamber Placement for Semichronic Arrays —> Calvarium Opening for Semichronic Arrays (+ microdrive positioning) —> MRI scan (chamber location verification) —> Neural Recordings and Microstimulation using Semichronic Arrays —> Salivary Cortisol Monitoring —> Pharmacological testing during behavior with neural recording and microstimulation —> plus up to 3 more MRI scans (if scanning quality is poor) —> plus up to 3 chamber repositions and up to 6 microdrive replacements.

Multiple Major Survival Surgery Description:

Describe why it is necessary to perform multiple major surgical procedures on the same animal. Indicate the length of time between surgeries.

Multiple survival surgeries are necessary and unavoidable for these experiments. Our experiments require a positioner to orient the animal to the task and prevent head movements during neurophysiological recording. All of our experiments require other surgical procedures to place the chronic or semichronic microwire arrays. These implantations could theoretically be performed at the same time. However, both are lengthy procedures. In consultation with OLAC veterinarians, we determined that recovery is easier and safer for each animal if these are performed as separate procedures. An additional advantage of separation is that several months might elapse between each procedure, allowing better healing.

The interval between the implantation of the head positioner and the recording chamber or chronic microwire array implant is very variable since it depends on the type of behavioral task the subject is performing, which in turn constrains where within the subject's training we need to implant the head positioner. Consequently, it can be many months (up to 18) until the subject has learned the full behavioral task and is ready for implantation of recording chambers or chronic microwire array implant. The interval between placing the chambers and opening the calvarium depends on the speed of the subject's recovery. We wait until the subject is again working on the behavioral task. This typically takes about 7 to 10 days. The interval between opening the calvarium and repositioning the chamber is very variable and depends on the reasons for repositioning the chamber. For example, if the chambers are being repositioned because of inaccuracy in their original placement this would occur approximately 2 weeks after the previous surgery to open the calvarium. However, if additional behavioral training is required then the interval can be much larger.

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* * * Husbandry * * *

Animal Transportation None

If animals will be transported between facilities, laboratories or institutions (e.g., hand carried, vehicular, etc.), describe the methods and containment measures to be utilized. Transportation of animals must conform to the ACUC Animal Transportation Guidelines.

OLAC has already established procedures for transporting animals from the vendor.

For behavioral testing, monkeys are moved in commercial primate chairs from their housing room to the testing room covered with opaque fabric.

For MRI scanning, monkeys are sedated and moved to the scanner room in a cart by the Vet and AHT staff.

FIELD STUDIES: If animals (live or dead) will be transported to or from the field, describe how they will be transported and measures to be taken to avoid potential disease transmission to researchers and other animals. Transportation of animals must conform to the ACUC Animal Transportation Guidelines.

N/A

Non-Standard Housing Requirements X None

Please check and describe all non-standard housing requirements that apply. Provide justification for each. For guidance, please refer to ACUC's Guidance on Exceptions Regarding Housing or Husbandry of Laboratory Animals, Aquatic Frog Housing Density, Guidelines for Investigators Who Manage Mouse Breeding Programs, and Rat Housing Guidelines.

Non-Standard Housing Requirements

Species	Cage/Pen Size	Cage sanitation interval	Wire-bottom rodent cages or grids	Animals outside dedicated animal housing for greater than 12 hours	Exemption from exercise (dogs only)
Monkey, Rhesus (OLAC Vivarium)					

Description of Non-Standard Housing Requirements

Non-Standard Husbandry or Care X None

Please check and describe any non-standard environmental requirements, diets, husbandry equipment or animal care. Include which species are affected. For guidance, please refer to ACUC's Guidance on Exceptions Regarding Housing or Husbandry of Laboratory Animals and Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Investigator Care of Animals (describe below and provide justification). For guidance, please refer to the ACUC Guidelines on Investigator Care of Vertebrate Animals.

Non-Standard Lighting Cycles, e.g., greater than twelve-hour light or dark cycles (describe below and provide justification).

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Non-Standard Housing Temperature Ranges (describe below and provide justification).

Non-Standard Diets (describe below and provide justification). For guidance, please refer to the ACUC Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Telemetry or Tether Devices (describe below and provide justification).

Running Wheels (describe below and provide justification).

Individually Housed (describe below and provide justification).

Exemption from Standard Enrichment (describe below and provide justification). For guidance, please refer to the ACUC Environmental Enrichment Guidelines.

Other - Please describe and provide justification.

Non-standard Experimental Requirements

Food or Fluid restriction None

Complete all section below that apply. For guidance, please refer to the ACUC Fasting Animals, Special/Regulated Diets/Water/Housing Policy.

Food or Fluid restriction

Species	Food Restriction	Length of Restriction	Fluid Restriction	Length of Restriction	Reason for Restriction
Monkey, Rhesus (OLAC Vivarium)			X	18-22 hrs per day	See attached

Restraint of Conscious Animals None

Complete all section below that apply. For guidance, please refer to the ACUC guidelines on Physical Restraint of Unanesthetized Animals.

Restraint of Conscious Animals

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Species	Type restraint (manual, commercial, manual and commercial)	Describe acclimation to restraint	Length of restraint
Monkey, Rhesus (OLAC Vivarium)	Commercial	See attached	4-6hrs

Description of Restraint

During training and recording, animals sit in a commercial, specially designed chair that permits both limb movements and postural adjustments. Vendors include bKin, Primate Products and Crist Instruments. They adapt readily to chair training procedures. Animals who are required to learn a complex behavioral task may receive a substantial period of training in the chair before surgery. In some experiments, animals may work with an exoskeleton, such as the commercially available KINARM exoskeleton, which is used to restrain movement of a single forelimb to be within a horizontal plane. To condition animals to any head restraint that may be used, session duration is gradually increased from very short to normal length sessions. Head restraint, if used, will consist of the chair fixation post attached either to the animal's headpost. During behavioral training, it is rarely necessary for head restraint to be greater than 3-4 hours per day. During electrophysiological recording, however, it is often necessary to extend the period of head restraint up to (but not beyond) 6 hours per day. Because of the length of head restraint, the chair is adjusted each day for maximum comfort, and the animal is free to move its limbs and adjust its posture in the chair. In addition, tasks have been designed to minimize stress on the animal by giving it as much control over its behavior as possible. Daily length of chairing times is recorded along with the total fluid intake of the animal. The behavioral tasks consist of many short (typically <5-s) trials each of which, if performed successfully, rewards the animal with fluid. When an animal gets tired or has had enough fluid, it simply stops performing the task. Animals typically become quite used to the laboratory environment and often fall asleep if their task is not available (i.e. if the program is not running) or after they have had all the fluid they are willing to work for in a given day. Animals are never left unattended while chaired.

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* * * Animal Disposition * * *

Please consult the ACUC Euthanasia Guidelines. Physical methods of euthanasia must be performed under anesthesia. Following euthanasia and prior to carcass disposal, an additional physical means of ensuring euthanasia must be performed. These physical methods vary by species but may include cervical dislocation for small rodents, bilateral thoracotomy, decapitation, exsanguination, double pithing for amphibians and reptiles, freezing for small ectotherms, or another AVMA-approved method. These must occur after the animal has been rendered non-responsive to noxious stimuli by the primary euthanasia agent.

Euthanasia

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Species	Method of Euthanasia: Primary	Route of Administration	Dosage (in mg/kg if possible) and volume	Site	Building Name	Room Number	Method of Euthanasia: Secondary	Briefly describe the euthanasia procedure
Monkey, Rhesus (OLAC Vivarium)	Pentobarbital Overdose	Intravenous (IV)	At a minimum 1 mL per 10 pounds of body weight (after isoflurane overdose).		[redacted]	[redacted]		If used, the euthanasia procedure will take the following form. Animals will be euthanized with a large overdose of Pentobarbital. This is achieved with a pentobarbital-based euthanasia solution (390 mg pentobarbital sodium and 50 mg phenytoin sodium per mL), after isoflurane anesthetic overdose, verified by checking for absence

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								of heartbeat. Euthanized animals are perfused and their brains removed for later analysis. All tissue not critical for experimental verification and analysis will be made available to other laboratories.

Provide specific details for carcass disposal.

Remains are placed in red bags/barrels and placed in appropriate cold room for carcass storage until proper disposal as biohazardous waste.

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* * * Attachments * * *

NOTE: The following types of files can be attached here: pdf, gif, jpeg,jpg, docx, xlsx.

Other Documents

Document Type	Document Name	Attached Date	Submitted Date
Other Documents	Acclimation_to_Restraint	01/27/2015	08/19/2015
Other Documents	Water_Regulation	05/14/2015	08/19/2015
Other Documents	Procedures Schematic	01/02/2017	01/18/2017

References

Document Type	Document Name	Attached Date	Submitted Date
References	Insel et al 1984 A benzodiazepine receptor-mediated model of anxiety	05/15/2018	08/20/2018

SOPs

Document Type	Document Name	Attached Date	Submitted Date
SOPs	APV Cranial Implant Care Guidelines.pdf	09/27/2016	11/15/2016
SOPs	[redacted]_R299_MRI NHP SOP_9.28.18	10/09/2018	10/09/2018

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* * * Certifications * * *

Certification

As Principal Investigator, I have ultimate responsibility for this study, the protection of animal subjects, and strict adherence by all co-investigators and research personnel to federal regulations, state statutes, and University of California (UC) Office of the President (UCOP) and UC Berkeley (UCB) policies pertaining to animal use in research and teaching.

I hereby assure the following:

1) As per the ACUC's Policy and Procedures on Protocol Review, any changes in the care and use of animals involved in this protocol will be promptly forwarded to the ACUC for review. Such changes will not be implemented until approval is obtained from the ACUC. I understand that the ACUC and Institutional Official (IO) have the authority to suspend a previously approved protocol if an activity is performed differently from that outlined in the protocol.

2) All procedures involving animal subjects will be performed under my supervision or that of another qualified professional listed on this protocol. Individuals listed on this protocol are qualified or will be trained to conduct procedures involving animals outlined under this proposal as per the ACUC's Training and Education Policy.

3) As per the ACUC's Training and Education Policy, all individuals listed on an this protocol have completed the required Collaborative Institutional Training Initiative (CITI) course, "Investigators, Staff, and Students - Basic Course".

4) As per the ACUC's Animal Occupational Health and Safety Program (AOHSP), all individuals working on this protocol have enrolled in the AOHSP by submitting an Occupational Health Surveillance System (OHSS). I understand that further participation in the AOHSP is voluntary unless required by the Occupational Health Physician or if the individual is working with specific species or research material.

5) The research proposed herein is not unnecessarily duplicative of previous reported research.

6) I ascribe to all of the responsibilities outlined in the ACUC's Principal Investigator Responsibilities policy.

X As Principal Investigator, I have read and agree to abide by the above obligations.

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Disclaimer: The generated PDF may not duplicate the original format completely. We do not warrant the accuracy of the changed format.

* * * Attached Document * * *

Document Name	Created Date
Acclimation_to_Restraint.pdf	09/17/2019

Training Summary

Phase 1

Pole and collar training and chair training using food rewards. Free waterline is attached. Fresh fruit is only given during training.

Habituation to:

1) The handler
2) Coming to the front of the cage
3) Pole on collar
4) Coming out of the cage
5) Climbing up the chair
6) Having the collar secured in the chair
7) Adjusting the back and leg panels to the animal's body
8) Being covered and rolling in the chair

Phase 2

Habituation to experimental setup using food and fluid rewards. Free waterline is attached. Fresh fruit is given only during training.

Habituation to:

1) Laboratory
2) Helmet or positioner (if present)
3) Limbs in exoskeleton
4) Application of EMG surface electrodes
5) Juice dispenser

Phase 3 (procedures: neural recordings and stimulation during natural motor control and neuroprosthetic control)

Training on progressively more difficult behavioral tasks. Animal may drink to satiety during session. Free waterline is disconnected. Fresh fruit is given Friday and Saturday. Dry treats are given on weekdays.

1) Move arm/cursor/exo
2) Reach to center target
3) Hold position at center target
4) Reach to peripheral target
5) Reach to peripheral target and return to center target
6) Hold center target until "go" cue
7) Cue association with forcefield (indicates how much resistance the animal will feel during that reach)

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*** * * Attached Document * * ***

Document Name	Created Date
Water_Regulation.pdf	09/17/2019

WATER REGULATION

During behavioral training and recording, animals are kept on a water schedule. Access to water is regulated for 18-22 hours in the day while the animal is in the cage. Daily training or recording sessions can last up to 6 hours/day. During this period liquid rewards are used as positive reinforcement in shaping the animal to perform the required task, using operant conditioning techniques. Extreme care is taken to ensure that these procedures do not stress the animals.

Naïve animals can be chair-trained using food or juice rewards alone, without water restriction. Once habituated to the chair and experimental setup with operant conditioning, the free-water line will be removed from the home cage. This means that the animal will already be very familiar with the delivery of fluid through the juice dispenser. The animal will then determine its fluid allotment daily by performing the behavioral task for fluid rewards until satiated. Animals will be returned to their cage when they no longer perform the task for fluid. On weekend days if the animals are not run, they will receive water in their cages, equivalent to their average consumption. If the animal does not earn its minimum daily allotment in the session, the difference will be supplemented in a water bottle on the home cage.

Whenever an animal is on a water schedule, detailed records of fluid intake are maintained. During this period animals are also weighed at least weekly, and usually several times per week. When animals are not on a water schedule but they have been chair trained they will get weighted at least once a month. Skin and stool condition and general health are also monitored closely. Records are maintained in the animals' quarters and in the laboratory where they are available to veterinary staff and inspectors. Pair-housed animals that are not on water schedule, but which are housed with a partner that is on such a schedule, are offered water freely during the partner's laboratory session. Each monkey's "normal" body weight is determined at least once annually, by taking its weight after a period of at least one week of ad libitum food and water access.

The fluid intake of animals on a water schedule is monitored extremely closely to ensure that the animal gets sufficient daily fluid. Supplementary hydration is provided if the animal's weight falls below 90% of normal (determined as stated above), if it appears listless or unduly distressed, if the skin is loose and inelastic, or if stools are hard and dry or if the animal is constipated. Normally rehydration is achieved by suspending training and/or recording for several days so that the animal can be placed on ad libitum water.

Recording is typically carried out 3-7 days per week. Typically, training will take place 5 days per week. When an animal has a single day off or is moving from a water schedule back to ad libitum water, care is taken to prevent behavioral stress. When experiments are not being performed during weekends, the animal may be given an amount at least equivalent to their average consumption.

UCB Investigator Guidelines for Special Food/Water must be followed for water-regulated animals.

At least 24 hours prior to surgery, animals must have ad lib water. Water regulation will not begin until post-surgical analgesics are no longer being given.

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Document Name	Created Date
APV Cranial Implant Care Guidelines.pdf	09/17/2019

Association of Primate Veterinarians
Cranial Implant Care Guidelines for Nonhuman Primates in Biomedical Research

PURPOSE

Use of nonhuman primates (NHPs) in biomedical research may include performing invasive cranial surgeries with chronic implantation of research devices. The Association of Primate Veterinarians (APV) supports the responsible use of NHPs in neurobiological research. Such research must meet specific criteria, such as the institutional animal care and use committee (IACUC) review and approval, verification of the investigator's skill and experience, and establishment of a close working relationship with institutional veterinary staff (Guide 2011). The following text aims to provide nonhuman primate researchers, IACUCs, and veterinary staff with guidelines for conducting research involving chronic cranial implants and for assessing their routine and non-routine care.

BACKGROUND

Success in maintaining a chronic cranial implant in operational condition is a function of how the implant is placed and the types of materials used, coupled with the animal's physiology and healing responses. The laboratory animal veterinarian should interface closely with the research group to ensure the adequacy of training and use of optimal surgical technique. Cranial implantation surgery must be conducted with consideration of normal host anatomy and physiology, as well as the maintenance of aseptic technique. With some surgical implant procedures, it is common to stage placement of cranial implants. Waiting to place each attachment (e.g. recording chamber) until the data recording is required helps to preserve the integrity of the chamber and safeguard the health of the animal. The total number of allowable cranial surgeries should be reviewed and approved by the IACUC.

GUIDELINES

Pre-surgical procedures

Clipping the hair liberally around the surgical site while avoiding clipper burns and cuts will help minimize unwanted irritation and infections. Small scissors or commercial depilatory products can be used in areas inaccessible for clipping. The skin must be surgically prepared, disinfected, and draped in a sterile fashion.

Surgical procedures

1. Cranial implantation surgeries must employ techniques that minimize trauma and preserve tissue architecture. A combination of aseptic technique, appropriate instrument and suture use, isotonic fluid lavage, and skillful and gentle tissue handling is highly recommended.

2. A neat and sterile cranial surgical site provides the best bonding surface, promotes bone remodelling, and facilitates anchoring of the cranial implant to the skull.

3. Use of a high-powered drill by an inexperienced surgeon may lead to thermal cranial damage and secondary local bone necrosis with loosening of the screws and eventual implant detachment. Hand drills do not cause thermal damage, but their use can lead to larger than necessary holes due to their poorer stability. Continuous lavage with cold isotonic fluids during drilling or application of thin layers of exothermic compounds (e.g., methacrylate) may help prevent or minimize thermal damage to the bone and periosteum. This kind of damage may be particularly important in younger or smaller NHPs with a thinner cranium.

4. Titanium or high quality stainless steel orthopedic screws are often used to anchor cranial implants. Drilling pilot holes combined with the use of bone taps and blunt tipped screws will minimize or even eliminate bone damage while contributing to implant longevity (Abee 2012).

5. Hemostatic materials such as Gelfoam®, are effective in stopping acute bleeding but they must not be left inside the cylinder indefinitely. To remove Gelfoam® the cylinder should be filled with sterile saline for approximately 10 min to soften residual foam pieces for removal and the process repeated, if needed. Forceful removal of Gelfoam® residue may produce additional hemorrhage and should be avoided. Cranial bone edges are the most common source of bleeding within the cylinder and this can be controlled by sealing the edges with bone wax. The implanted cylinder may be opened after the surgery for visual examination and carefully cleaned 1-2 days after surgery. After assuring adequate hemostasis, 2-3 ml of sterile saline should be placed in the cylinder followed by aseptic replacement of the cap for another 2 to 3 days. Routine cleaning and maintenance of a non-infected cylinder should be initiated in 1 week post-op.

Post-surgical procedures

While tending to the newly placed or chronic cranial implants one should be vigilant about potential pain. If there is any evidence of pain or distress associated with routine cleaning the underlying cause should be investigated, addressed, and appropriate analgesia given. One or a combination of the following agents is recommended: EMLA cream, lidocaine jelly, lidocaine or bupivacaine local block, or systemic NSAIDs or opioids.

1. <u>Wound Margin Care</u>

    a. An uninfected surgical wound that is healing well is best left alone for a period of 7-14 days post-operatively. Sterile saline rinses can be used if needed to clean the wound. Use of H_2O_2 is not recommended for 2-3 weeks post-op as it can interfere with normal healing process. Dry, non-infected, hard and crusty scabs formed during normal healing may cause local irritation or pruritus, inviting self-trauma. Petroleum jelly or wet dressings applied every 2-3 days will keep the scabs soft and facilitate healing. There is no universally recommended frequency of cleaning. Rigorous, over exuberant, unwarranted cleaning can result in inflammation and infection. Wound margins should be closely inspected a minimum of once each week and cleaned as often as needed.

b. Re-growing hair should be carefully removed on an as-needed basis.

c. The wound margin adjacent to an implant requires regular observation and attention as it may become infected leading to suture dehiscence or necrosis, resulting in areas of skin devitalization or retraction away from the implant. Daily cleaning may be necessary as the serous, serosanguinous or purulent secretions will dry up at the wound margin producing a protein-rich crust that may serve as a nidus of infection. Cleaning of the skin/implant interface involves gentle removal of loose crusts and of unwanted hair with a scissors and rinsing wound margins. The following solutions or their combinations should be considered for cleansing: sterile saline, chlorhexidine diacetate 0.05% solution (1:40 dilution of stock chlorhexidine with water) (Slatter 2003), povidone-iodine 1-2% solution (1:10 – 1:5 dilution of stock povidone solution to saline), Dakin's solution (0.5% sodium hypochlorite in water) can be used particularly in the presence of necrotic tissue or 1.5 - 3% hydrogen peroxide (to remove dried up blood and other secretions followed by copious saline irrigation). **None of the above compounds is effective indefinitely or against all pathogens and a 7 to 10 day rotation of different disinfectants should be employed.**

d. Enzymatic debriding compounds facilitate the process by which devitalized tissue is softened or liquefied and removed (e.g. Trypzyme ®, an enzymatic soaking solution).

e. Infected sites should be cleaned frequently (e.g. daily). Where mild but chronic skin/implant problems are evident, a minimum twice a week inspection and cleaning 3-4 days apart, are recommended. Culture and sensitivity should be done to ascertain the nature of the infectious agent. The indiscriminate use of systemic or local antibiotics may contribute to the development of bacterial resistance and is strongly discouraged.

2. Cranial Head-post Care

The skin may retract away from the head-post over a period of weeks to months post-operatively and this is usually a gradual process. In the absence of local infection, skin repair surgery may be attempted. If the skin retraction is significant, an addition of bone cement may be considered.

3. Routine Recording Cylinder Care

Most recording cylinders are anchored with screws and methacrylate products and have a tight fitting cap secured with 1-3 small screws. The inside of a chronic recording cylinder is not sterile but it must be maintained aseptically. Recording cylinders are routinely opened in the non-sterile environment of the research laboratory or procedure room. Careful cleaning of the recording cylinder as described below has been demonstrated to minimize or prevent active cylinder infections. Ideally, no smell should be detectable in the recording cylinder and the underlying dura should appear creamy white, smooth, and shiny.

a. The outside of the cylinder is typically contaminated and must be cleaned before the cylinder is opened for cleaning and/or recording. Povidone-iodine scrub (soap) should be

used for the initial scrub and washed off with saline or 70% alcohol. Residual blood may be removed with 0.75% to 3% hydrogen peroxide (H_2O_2). Care must be taken to avoid contact between alcohol or H_2O_2 and viable soft tissues that are in the process of re-epithelialization.

b. Sterile draping and aseptic techniques while opening a recording cylinder are recommended.

c. Uninfected cylinders should be cleaned as often as possible, but no less than twice a week 3-4 days apart. Sterile instruments (e.g., aspirator/suction tips, forceps) and supplies (e.g., gauze, drapes, gloves) should be used while working inside the recording cylinder. After cleaning, it is recommended that the old cap be replaced with a new sterilized (i.e. autoclave, Cidex®) cap each time. Although it may be less effective, but cleaning of the used cap with povidone-iodine scrub, alcohol, and rinsing or soaking it in a 1:10 sodium hypochlorite solution is used by some programs.

d. Known or suspect infected cylinders should be cleaned 5 to 7 days a week (Gografe & Niekrasz 2009), regardless of whether animals are treated with antimicrobial agents. If there are multiple cylinders, they should each be thoroughly cleaned sequentially rather than simultaneously. No materials (e.g. forceps, suction tips, etc.) should be shared between cylinders during multiple cylinder care. Uninfected cylinders should always be cleaned before suspect or known infected cylinders. Cleaning should always begin with a sterile saline lavage followed by suction. The dura must be carefully examined for the presence of focal infection, necrosis, cuts or tears before any cleaning agents are applied. Disinfectants and antibiotics (e.g. cephalosporins) may contribute to unwanted toxic events that manifest clinically as neurological deficits. The following compounds have proven useful:

    i. Use of a 3% H_2O_2 solution or a 1:1 mixture of H_2O_2 and povidone-iodine facilitates removal of biofilm and proteinaceous material from the interior surface of the cylinder wall.

    ii. Rinsing several times with a dilute povidone-iodine solution at 1-2 % (dilution is necessary for ionization of bound iodine). After cleaning, a few drops of 2% povidone-iodine solution may be left inside the cylinder.

    iii. Although some programs have reported the use of chlorhexidine inside the recording cylinder for routine maintenance without problems, its use is controversial, as the compound has been demonstrated to have neurotoxic properties (Henschen & Olson 1984, Perez, et. al. 2000, Lai, et. al. 2011). The US Physician Desk Reference (PDR), as of 1984, warns that "*chlorhexidine gluconate is for external use only. Keep out of eyes and ears and avoid contact with meninges*". Since other disinfectants (i.e. chlorine, iodine, etc.) have been demonstrated to be efficacious for cylinder maintenance, the use of chlorhexidine should be carefully evaluated. At a minimum, care should be taken to evaluate the

dural integrity prior to using chlorhexidine and to thoroughly rinse the cylinder free of the compound after each use. Leaving residual chlorhexidine in the cylinder for extended periods of time is also not recommended.

iv. Dakin's solution may be used cautiously when addressing infections refractory to other treatments and when the integrity of the dura has not been compromised.

v. Chlorine dioxide is typically not used in routine cleaning but it may be effective in short-term treatment of mycotic infections (Lee 1998).

vi. In the majority of cases involving a durotomy or durectomy, the underlying cortex is covered with artificial dura combined with the use of silicone membranes, collagen matrix, or aliphatic polyether polyurethane sheets. Where the dura has been cut, it should be sutured to protect the cortex. The cylinder cleaning process is the same as with intact dura. It is critical to rinse with copious volumes of sterile water or saline if any disinfectant was used.

4. Granulation tissue.

Granulation tissue (GT) formation is part of the normal healing process, but it is not always desired when maintaining chronic cranial implants. Budding GT on the wound margin and the dura is typically highly vascular and bleeds easily, oozes serum, and may interfere with healing if it becomes infected. Dural GT that is not removed on a regular basis may bleed and eventually result in dural fibrosis. Thick granulation tissue pads can harbor bacteria and become a source of chronic chamber infections.

a. 5-Fluorouracil (5-FU) may be helpful in reducing or delaying the GT growth (Spinks 2003). 5-FU is an antimetabolite, antimitotic agent that reduces tissue re-growth, vascularization, and bacterial overgrowth by interfering with nucleic acid synthesis, thus preventing mitosis. 0.5-1.0 ml of 25 mg/ml aqueous 5-FU should be instilled into the cylinder three times weekly to bathe the dura for 5 minutes. At the end of 5 minutes the cylinder should be rinsed with copious volumes of sterile saline. 5-FU must never be used on compromised dura as subdural leaks may contribute to complications. 5-FU decreases fibrinolytic activity and enhances the risk of thromboembolic events (Kessler 1994). Care must be used when handling 5-FU because it is a known carcinogen.

b. Early GT deposits may be removed using suction. Local anesthesia can be provided via instillation of 0.25-0.5 ml of 1-2% lidocaine or 0.25% bupivacaine or a 50:50 mixture for a few minutes before removal. Post-procedural systemic analgesics should be considered.

c. Chronic growth of GT typically leads to the formation of a firm fibrous layer requiring "dural scraping", which must be conducted under general anesthesia with the post-operative use of analgesics. GT deposits on the wound margin may be addressed by surgical debridement followed by a V-plasty, regular cleaning, treatment of local infections, and chemical or electrical cauterization under systemic or local anesthesia.

5. Treatment of Implant Margin and Cylinder Infections

While assessing the skin/implant interface, care must be taken to determine if the interface infection is topical or originating from under the cranial implant. In addition, the inability to retain fluid within the recording cylinder is often the result of open tracts between the cylinder and wound margin. The ideal interface should be smooth and free of "pockets" and abrupt changes in the contour of the implant. Reshaping of the interface and performing a "V-plasty" should be considered. Culture of purulent exudate, cleaning/debridement of the area, and administration of topical or systemic antibiotics have also been used.

Infections inside the recording cylinders are common and can be prevented and treated with careful cleaning and maintenance as outlined. Systemic antibiotics should be reserved for treating cylinder infections in which the dura or bone are severely compromised or where the infection has been unsuccessfully treated with the frequent cleanings and use of halogen solutions. Indiscriminate use of antibiotics can result in bacterial resistance and additional problems. The use of halogen solutions within the cylinder (e.g. Povidone-iodine, Dakin's solution, etc.) has been demonstrated to clear infections in many cases. These solutions have been used as part of the cleaning regimen and have been left in the chamber after cleaning for extended periods to treat chronic infections. Chronic infections should always be treated in consultation with a veterinarian.

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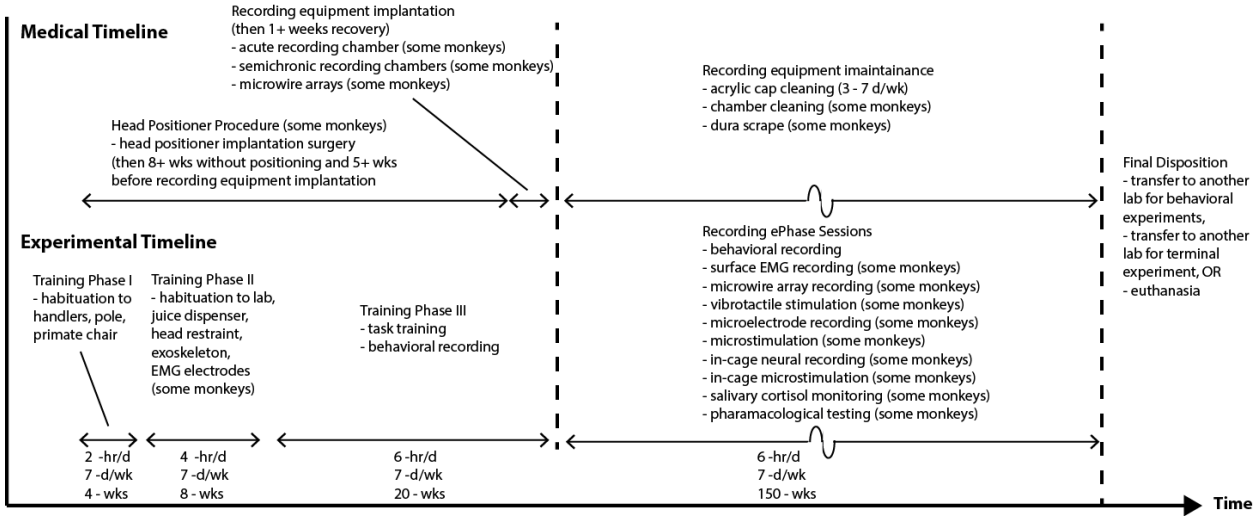
Procedures Schematic.docx	09/17/2019

PROCEDURES SCHEMATIC



An MRI scan will be performed to some monkeys at the beginning of training. A further scan may be necessary to verify that the electrodes are in the correct position following implantation. Additional scans may be required, for example, if scanning quality is poor or if the position of the recording chambers in the case of semichronic arrays needs to be adjusted and re-verified.

Alternatively, animals may be implanted with a Semichronic Array or Acute Array instead of the chronic arrays. In such case, 2 to 3 surgical procedures to place a chamber and open the calvarium respectively will be performed. In this case, the sequence of procedures will be the following:

MRI scan (skull model formation) → Acclimation to Restraint → Positioner Implantation → Chamber Placement for Semichronic Arrays or Acute Arrays [1-2 weeks] → Calvarium Opening for Semichronic Arrays or Acute Arrays [1+ weeks] → Neural Recordings and Microstimulation using Semichronic Arrays or Acute Arrays → plus possible in-cage neural recording and/or microstimulation→ plus possible salivary cortisol monitoring and pharmacological testing during in-chair behavior → plus up to 3 more MR scans (if scanning quality is poor) → plus up to 3 chamber repositions → plus dural maintenance if required

In addition, an MR scan for chamber location verification could take place in between 'Calvarium Opening for Semichronic and Acute Arrays' and 'Neural

Recordings and Microstimulation using Semichronic Arrays’.

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Document Name	Created Date
Insel et al 1984 A benzodiazepine receptor-mediated model of anxiety.pdf	09/17/2019

A Benzodiazepine Receptor–Mediated Model of Anxiety

Studies in Nonhuman Primates and Clinical Implications

Thomas R. Insel, MD; Philip T. Ninan, MD; Joseph Aloi;
David C. Jimerson, MD; Phil Skolnick, PhD; Steven M. Paul, MD

• β-Carboline-3-carboxylic acid ethyl ester (β-CCE) binds with high affinity to brain benzodiazepine receptors and has potent behavioral and physiologic effects in primates. Dose-related increases in behavioral agitation, plasma cortisol level, BP, and heart rate were observed after administration of doses between 50 and 500 μg/kg of β-CCE to rhesus monkeys. All of these effects were blocked by pretreatment with diazepam. Pretreatment with clonidine hydrochloride and propranolol hydrochloride, both of which have been reported to have anxiolytic actions in man, attenuated only selective aspects of the response to β-CCE. The behavioral, endocrine, and physiologic effects of low doses of β-CCE in monkeys are similar to those observed in anxious patients or normal subjects under anxiety-provoking or stressful situations. Administration of benzodiazepine receptor active antagonists such as β-CCE to primates may, therefore, provide a valid and reproducible model of human anxiety that could be used to investigate specific biologic aspects of anxiety disorders.

(*Arch Gen Psychiatry* 1984;41:741-750)

. . . . One thing is certain, that the problem of anxiety is a nodal point, linking up all kinds of most important questions; a riddle, of which the solution must cast a flood of light upon our whole mental life.[1]

FREUD, 1917

Accepted for publication Dec 26, 1983.

From the Clinical Neuropharmacology Branch (Dr Insel and Mr Aloi), the Laboratory of Clinical Science (Dr Jimerson), and the Clinical Neuroscience Branch (Dr Paul), National Institute of Mental Health; the Laboratory of Biorganic Chemistry (Dr Skolnick), National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Md; and the Department of Psychiatry (Dr Ninan), Emory University and Veterans Administration Hospital, Atlanta.

Reprint requests to Clinical Neuropharmacology Branch, National Institutes of Health Clinical Center 10-3D 41, Bethesda, MD 20205 (Dr Insel).

Two recent developments in the psychopharmacology of the benzodiazepines promise to greatly advance our understanding of the biology of anxiety. First, in 1977, two independent research groups described high-affinity stereospecific receptors for benzodiazepines in the mammalian CNS.[2,3] The benzodiazepine receptor was later shown to be functionally (and perhaps structurally) coupled to both a recognition site for γ-aminobutyric acid (GABA) and a chloride ionophore, existing as part of a "supramolecular receptor complex".[4] Considerable evidence supports the notion that the central pharmacologic actions of the benzodiazepines (ie, anxiolytic, anticonvulsant, sedative, and muscle relaxant actions) are mediated through this receptor.[5,6]

Research on benzodiazepine receptors has been facilitated more recently by a second major advance, the development of several novel high-affinity benzodiazepine receptor ligands (Table 1) such as β-carboline-3-carboxylic acid ethyl ester (β-CCE). Although first thought to be a naturally occurring component of human urine,[7] β-CCE has been shown to be produced artifactually from the Pictet-Spengler condensation of tryptophan during the extraction process and thus does not appear to be an endogenous ligand.[8] Nevertheless, β-CCE's affinity for the benzodiazepine receptor in vitro is roughly equivalent to the most potent benzodiazepines (eg, flunitrazepam, clonazepam) and is approximately eightfold greater than diazepam.[9] Unlike diazepam or clonazepam, however, β-CCE lowers the threshold for pentylenetetrazol-induced seizures[10,11] and blocks the anticonvulsant effects of benzodiazepines in rodents.[12] These observations led to the proposal that β-CCE acts as a benzodiazepine receptor antagonist. Because β-CCE is rapidly metabolized in rodents[13] and because measurements of stress are inherently more difficult in this species compared with primates, we examined the pharmacologic profile of β-CCE in the chair-adapted rhesus monkey.

Table 1.—Benzodiazepine Receptor Ligands

	IC_{50} (nM)*
Agonists	
Diazepam	13.5
Chlordiazepoxide	1,300
Flunitrazepam	5.1
Clonazepam	1.2
Antagonists	
RO-15-1788	2.0
CGS-8216	0.3
Active antagonists (inverse agonists)	
β-Carboline-3-carboxylic acid ethyl ester	1.6
FG-7142	200†

*Represents concentration giving 50% inhibition of tritiated clonazepam binding (5nM) at 0 °C to synaptic membrane from rat cerebral cortex (from Mohler and Richards[9]).

†Represents 50% inhibition tritiated flunitrazepam binding to rat cerebral cortex (from Braestrup[59]).

Table 2.—Behavioral Activation Scale

Spontaneous behaviors (rated 0 if absent to 3 if extreme)
Head turning
Body turning
Vocalization
Jerky movements*
Freezing or immobilization*
Scratching*
Picking
Response to approach (rated 0 if cannot be elicited, 1 if mild and <3 s, 2 if moderate and >3 s, 3 if prolonged >5 s or repeated)
Observer across room, no eye contact
Observer at two-thirds meter, eye contact attempted
Observer touching animal's foot, eye contact attempted

*Not observed at β-carboline-3-carboxylic acid ethyl ester doses of 25 to 500 μg/kg.

In a recent report,[14] we described the effects of administering a rather high dose of β-CCE (2.5 mg/kg intravenously [IV]) to rhesus monkeys. In these experiments, profound behavioral arousal developed with marked increases in BP, heart rate, and plasma catecholamine and cortisol levels. These effects lasted about two hours and peaked within the first hour after drug administration. All of the behavioral and physiologic effects of β-CCE were blocked by pretreatment with the benzodiazepine receptor antagonist Ro 15-1788. This latter compound had no behavioral or physiologic effects when administered alone. It appeared therefore that the benzodiazepine receptor "recognized" at least two different kinds of antagonists (Table 1): those such as Ro 15-1788, which compete for benzodiazepine receptors but have little or no intrinsic activity, and "active" antagonists (or inverse agonists) such as β-CCE, which in primates possess activating and possibly "anxiogenic" properties. An important implication of these experiments is that the benzodiazepine receptor may mediate not only the anxiolytic effects of the benzodiazepines but may also play a role in the development of anxiety. (There has been some confusion about whether β-CCE should be labeled an agonist or an antagonist at the benzodiazepine receptor. Classically, a pharmacologic antagonist is a drug that binds to the same receptor as an agonist but reverses or prevents the agonist's effects. If diazepam is an agonist, then Ro 15-1788, for example, is clearly an antagonist. Ro 15-1788 binds to the benzodiazepine receptor with high affinity and antagonizes the principal pharmacologic actions of the benzodiazepines. We have chosen the term *active antagonist* for drugs such as β-CCE because like traditional antagonists they bind to benzodiazepine receptors and reverse or prevent benzodiazepine effects, but in addition they have potent intrinsic effects opposite to those of the agonists. Confusion arises because Ro 15-1788 which blocks benzodiazepine effects also blocks the effects of β-CCE. Some investigators have concluded that drugs such as β-CCE should therefore be considered "inverse agonists"—agonists because they have intrinsic effects and inverse because these effects are opposite to those of benzodiazepines. Ultimately, the discovery of an endogenous ligand for the benzodiazepine receptor should help to resolve the question of whether β-CCE is an agonist or antagonist.)

The current series of studies was undertaken to investigate whether β-CCE could provide a pharmacologic model of anxiety. Although our initial report demonstrated that high doses of β-CCE elicited profound behavioral effects, many of the observed behaviors as well as the marked autonomic activation are not usually witnessed in patients with generalized anxiety. In addition, diazepam, even in doses as high as 1 mg/kg, did not consistently prevent the behavioral, cardiovascular, or endocrine effects of β-CCE. Furthermore, in subsequent experiments we occasionally observed generalized seizures in younger monkeys, suggesting that the initial dose of β-CCE used may have produced a state of generalized activation and not anxiety per se.

These observations suggested that lower doses of β-CCE might produce a more clinically relevant behavioral syndrome. In the present report, we investigated the dose-response relationships for the behavioral, cardiovascular, and endocrine effects of β-CCE. Consistent and reproducible effects on each of these factors were observed at doses of β-CCE as low as 100 μg/kg. To further establish the possible clinical relevance of this syndrome, we studied the effects of pretreatment with each of three pharmacologically distinct anxiolytics on the behavioral and physiologic activation elicited by low doses of β-CCE. Our results strongly support the hypothesis that administration of β-CCE provides a receptor-mediated model of anxiety with applications for the study of normal and pathologic anxiety states in man.

METHODS

Dose-Response Studies

Adult male rhesus monkeys (5 to 8 kg) were studied in restraining chairs. Each had been carefully adapted to chair restraint during the previous year. Twenty-four hours before the first experiment, monkeys were anesthetized with ketamine hydrochloride (10 mg/kg), removed from their home cages, fitted with femoral venous catheters, and put in chairs in the experimental room. In preliminary experiments (unpublished observations) the "adaptation" of monkeys to their restraining chairs was essential for reducing basal plasma levels of stress-related hormones such as cortisol and catecholamines. The β-CCE was administered IV over two minutes in a 5-mL solution of 20% diluted Emulphor.[14] Each monkey received 25, 50, 100, and 500 μg/kg of β-CCE in randomized order. Vehicle (20% diluted Emulphor) alone was administered at the beginning and again near the end of each series of β-CCE infusions.

An observer, blind to the identity of the infusion, rated behavior at −5, +5, +30, and +60 minutes following drug or vehicle administrations using an activation rating scale developed from the combined observations of our previous β-CCE studies as well as the behaviors of rhesus monkeys in other anxiety-related paradigms.[15] The activation scale (Table 2) combines items of spontaneous behavior with response to approach by the rater. The interrater reliability for two observers serially rating three monkeys was $r = .85$.

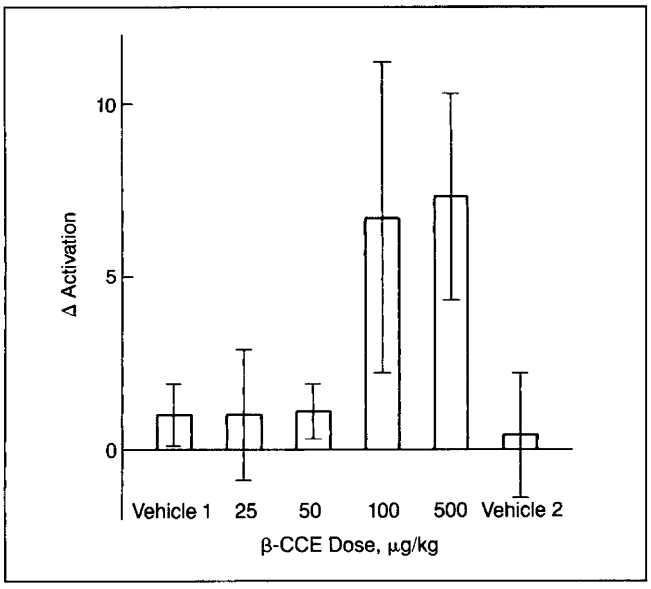

Fig 1.—Change from baseline in activation scores during first hour after β-carboline-3-carboxylic acid ethyl ester (β-CCE) administration. Increase following 500 μg/kg does not reach statistical significance (n = 3).

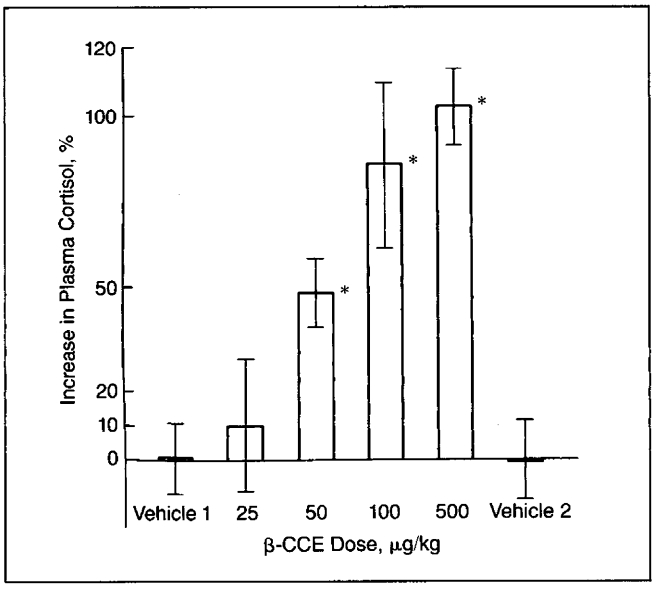

Fig 2.—Plasma cortisol level percent change from baseline during first hour after β-carboline-3-carboxylic acid ethyl ester (β-CCE) administration. Asterisk indicates $P<.05$ for Student's t test for paired data comparing mean of post–β-CCE values to mean of two baseline values in each monkey (n = 3).

Blood was collected via an indwelling femoral venous catheter into tubes containing 6.0 mg edetic acid at 20 minutes and one minute prior to drug or vehicle administration, and at 20, 40, 60, and 90 minutes thereafter. Blood was centrifuged immediately at 4 °C for ten minutes to separate the plasma, which was then stored at −20 °C. Plasma cortisol level was determined by radioimmunoassay with a sensitivity of 2 μg/dL (intra-assay and interassay coefficients of variation below 10%). Plasma free 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), a major metabolite of central and peripheral norepinephrine, was assayed with a gas chromatographic–mass spectrometric method with a sensitivity of 0.5 ng/mL and an intra-assay coefficient of variation below 5%.[16]

Heart rate and mean arterial BP were monitored every three minutes throughout the study using an ankle cuff and a research trend recorder (Dinamap). The mean of individual three-minute values during the 20 minutes before infusion was compared with the mean of values for the 20 minutes after infusion.

Results following each dose of β-CCE were analyzed separately using a t test for paired data. For behavioral scores and plasma cortisol and MHPG levels, baseline values were compared with the mean of values obtained during the 60 minutes after infusion in each monkey.

Effects of Anxiolytics on β-CCE–Induced Changes in Behavior and Physiology

To test further the validity of β-CCE as a model of anxiety, the effects of pretreatment with three pharmacologically different anxiolytics were examined. Diazepam was chosen as a prototypical benzodiazepine[6] that acts via the benzodiazepine receptor. Clonidine hydrochloride, an α_2-adrenergic receptor agonist, also has been reported to have anxiolytic activity in man,[15,17,18] and these effects are believed to be mediated by adrenergic receptors in the locus ceruleus. Propranolol hydrochloride, a β-adrenergic receptor blocker, has also been clinically useful as an anxiolytic, particularly for blocking the somatic manifestations of anxiety.[19]

On each of three days, diazepam (0.5 mg/kg), clonidine hydrochloride (10 μg/kg), or propanolol hydrochloride (3 mg/kg) was administered IV over a one-minute period 20 minutes before β-CCE infusion. Because of the "challenge" nature of our paradigm, doses of each drug were chosen that were slightly higher than those typically used for anxiolysis in man. The protocol was otherwise identical to experiments previously described, except that blood sampling began at 40 minutes before β-CCE administration, behavior ratings were not blind, and behavior rating scores were analyzed only for the last five minutes of baseline (−25 to −20 minutes) and the first five minutes following β-CCE administration.

Each study was analyzed independently using a Student's t test for paired data matching the mean of baseline and the mean of post–β-CCE values. The values following the anxiolytics alone were not included in baseline measures. Thus, a t value for heart rate in the diazepam condition represents the comparison of the mean values from −40 to −20 minutes before β-CCE compared with the mean of +1 to +20 minutes following β-CCE administration in each monkey. Subsequent to these "within-treatment" comparisons, data from all of the pretreatment experiments were analyzed by an analysis of variance for repeated measures (condition v time) to allow direct comparison across treatment conditions.

RESULTS

β-CCE, at doses between 25 and 500 μg/kg, produced incremental increases in behavioral activation (Fig 1). The intensity of behavioral response varied considerably among animals. Whereas one animal was observed to show head turning, vocalization, and prolonged grimacing when approached 30 and 60 minutes following 25 μg/kg of β-CCE, another failed to manifest any signs of behavioral activation except at the 500-μg/kg dose. At a dose of 100 μg/kg, mean (± SD) activation scores changed from a baseline of 4.0 ± 2.0 to 10.7 ± 5.6 following β-CCE. Although this difference failed to reach statistical significance because of the less reactive subject, particular behaviors such as head and body turning, defecation, and urination were consistently observed in all monkeys within five minutes of drug administration. In general, spontaneous behaviors (Table 2) were very low at baseline (overall mean, 1.4 ± 1.1), peaked at the five-minute rating point (4.7 ± 2.5 for 100-μg/kg dose; 7.0 ± 1.0 for 500-μg/kg dose), and returned to baseline levels by 60 minutes.

More striking and long-lasting changes were observed in response to approach by the rater. Although each of the monkeys was placid and unperturbed when approached following vehicle administration (mean ± SD postvehicle "response to approach" score, 3.6 ± 1.7), all became "agitated" when approached in an identical fashion during the 60 minutes following β-CCE administration

Table 3.—Mean Plasma Free MHPG Level (ng/mL) Before and After β-CCE*

	Vehicle	100 μg/kg	500 μg/kg
Before (−20, −1 min)	6.4±3.1	6.1±1.5	5.3±1.2
After (+20, +40, +60 min)	5.9±1.7	6.1±1.6	6.0±1.5
90 min after	. . .	. . .	5.8±1.1

*MHPG indicates 3-methoxy-4-hydroxyphenylethylene glycol; β-CCE, β-carboline-3-carboxylic acid ethyl ester. Values represent mean±SEM (n=3). Differences analyzed within each dose (Student's *t* test of before *v* after) or across doses (repeated measures analysis of variance) show no significant drug or drug×time effects.

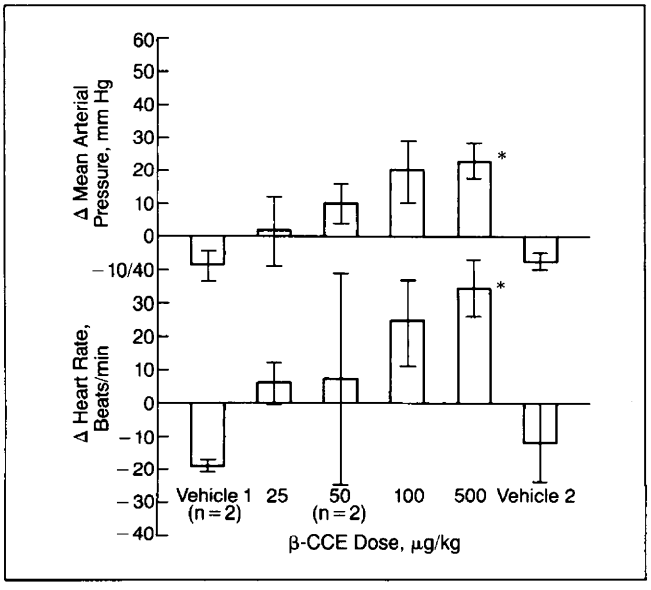

Fig 3.—Heart rate and mean arterial pressure change from baseline (mean of individual values from −20 to −1 minute pre–β-carboline-3-carboxylic acid ethyl ester [β-CCE] administration) following β-CCE administration (mean of individual values from +1 to +20 minutes). Asterisk indicates $P<.05$ for Student's *t* test for paired data comparing post–β-CCE values to baseline in each monkey (n=3).

(mean±SD post–100-μg/kg β-CCE "response to approach" score, 7.4±3.4). Monkeys varied not only in the intensity but in the character of this response. For instance, the lowest-scoring monkey would respond to approach by turning away and licking its lip whereas the others would adopt an aggressive posture with grimacing, chair clasping, and vocalizing. These individualized responses were consistent across doses. Response to a naturalistic threat, such as approach by a novel investigator, was not systematically studied in this investigation. However, extensive previous and subsequent experience with these same monkeys suggested that the repertoire of behaviors following a naturalistic threat was similar to that observed following β-CCE administration. The monkey that "fearfully" turned away from a familiar investigator following β-CCE administration had responded in the same way when approached by a stranger or presented with a mirror prior to the current investigation. Similarly, the animals with more "aggressive" responses following administration of β-CCE had a history of grimacing and chair clasping when "threatened" in the drug-free state.

A dose-response effect was also observed for the β-CCE–induced elevations in plasma cortisol level (Fig 2). Cortisol level increased significantly in only one monkey following 25 μg/kg (mean±SD plasma cortisol level, 18.1±4.9 μg/dL), transiently in all monkeys following 50 μg/kg (19.9±4.8 μg/dL), and persistently in all animals after 100 μg/kg of β-CCE (28.7±3.7 μg/dL). Following 100 μg/kg of β-CCE, the peak concentrations of plasma cortisol (38.0±8.5 μg/dL) were roughly equivalent to the peak concentrations following the 500-μg/kg dose (38.1±10.8 μg/dL); however, the higher dose elicited a more persistent elevation (plasma cortisol still elevated at the 110-minute point and mean±SD plasma cortisol level, 32.7±8.4 μg/dL). By contrast, plasma MHPG level did not change significantly following administration of even 500 μg/kg of β-CCE (Table 3).

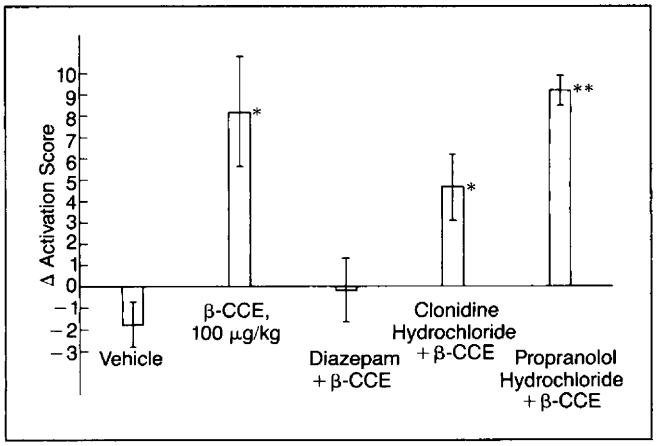

Fig 4.—Behavioral activation change following β-carboline-3-carboxylic acid ethyl ester (β-CCE) alone and following β-CCE with pretreatment with diazepam (0.5 mg/kg), clonidine hydrochloride (10 μg/kg), and propranolol hydrochloride (3 mg/kg). Student's *t* test for paired data (n=6) yields significant increases from baseline following β-CCE alone (asterisk indicates $P<.05$) and with clonidine and propranolol pretreatment (double asterisks indicate $P<.005$).

Cardiovascular effects also followed a dose-response pattern (Fig 3), with consistent elevations in heart rate and mean arterial BP following 100 μg/kg of β-CCE, although statistically significant changes were only apparent at doses of 500 μg/kg for both heart rate ($t=3.95$, $P<.05$), and mean arterial pressure ($t=4.04$, $P<.05$). Cardiovascular activation was not significantly correlated with increases in plasma cortisol level.

A comparison of the changes following initial vehicle with changes following the later vehicle administration failed to show significant differences on any of the variables measured, suggesting that the monkeys were not becoming sensitized to the infusion procedure itself.

In a larger group of animals (n=6), administration of 100 μg/kg β-CCE alone resulted in significant elevations in plasma cortisol level ($t=4.5$, $P<.025$), heart rate ($t=5.03$, $P<.025$), and behavioral activation ($t=2.78$, $P<.05$). Mean arterial pressure increased, but the change was only marginally significant ($t=2.23$, $P=.07$).

Pretreatment with diazepam effectively attenuated all of the β-CCE–induced behavioral and physiologic changes (Figs 4 through 6). In the diazepam-pretreated monkeys the effects of β-CCE could not be distinguished from the placebo condition by analysis of variance. Pretreatment with clonidine did not prevent a significant behavioral "activation" ($t=2.91$, $P<.05$) following β-CCE administration, although the magnitude of activation was attenuated in the clonidine-pretreated monkeys. Clonidine alone had potent effects on mean arterial BP and heart rate, which were only weakly reversed by β-CCE. Pretreatment with clonidine also blocked the increase in plasma cortisol level following β-CCE ($t=2.3$, not significant), an effect that could not be distinguished from the effect of diazepam by analysis of variance. In contrast, pretreatment with propranolol reversed the heart rate elevations seen following β-CCE ($t=4.58$, $P<.025$) but actually augmented the increase in mean arterial pressure ($t=9.21$, $P<.005$). Propranolol administration did not lessen β-CCE's action on behavioral activation ($t=8.49$, $P<.005$), nor did it block the β-CCE–induced increase in plasma cortisol level ($t=5.99$, $P<.01$). Moreover, the increase in plasma cortisol level following β-CCE in the propranolol-pretreated monkeys was significantly greater than the increase in plasma cortisol level in the placebo, diazepam pretreatment, and clonidine pretreatment conditions ($F=9.9$, $df=20,100$, $P<.05$ for Tukey's "honestly significant difference").

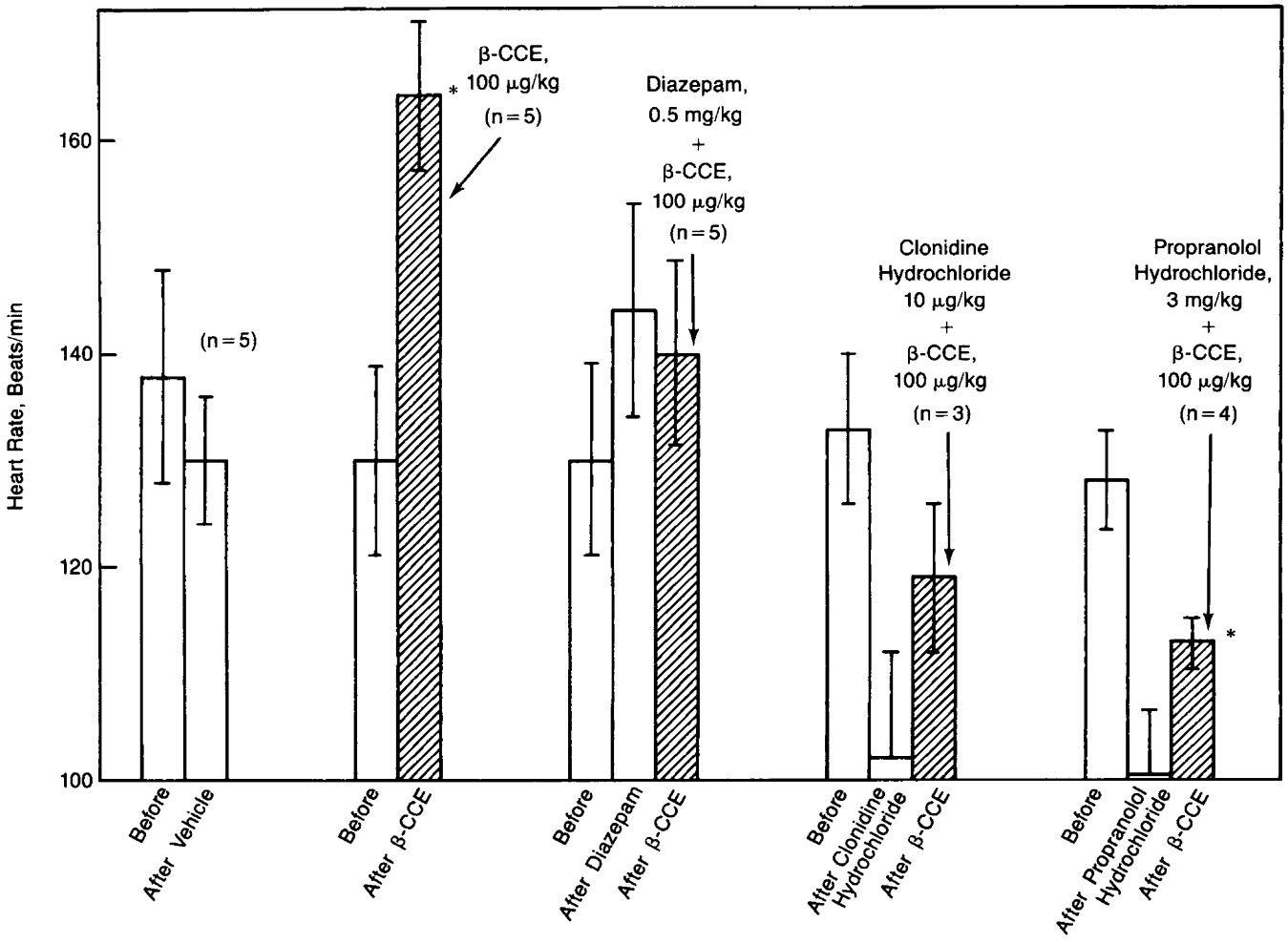

Fig 5.—Change in heart rate following β-carboline-3-carboxylic acid ethyl ester (β-CCE) alone and following β-CCE with pretreatment with diazepam, clonidine hydrochloride, and propranolol hydrochloride. For Student's *t* test for paired data comparing mean of values from +1 to +20 minutes post–β-CCE administration to mean of values during 20-minute baseline period (n=6), asterisk indicates $P<.05$.

COMMENT

These results demonstrate a dose-response relationship for the behavioral, endocrine, and cardiovascular responses to β-CCE administration in chair-adapted rhesus monkeys. Consistent elevations in behavioral activation, plasma cortisol level, and heart rate are observed with doses of β-CCE as low as 100 μg/kg.

Previous studies in rodents have failed to find such marked behavioral responses to β-CCE.[10-12] There is a striking difference in the rate of metabolism of β-CCE between rodents (plasma half-life in vitro less than one minute)[13] and primates (plasma half-life in vitro more than 60 minutes).[20] This remarkable species difference in plasma esterase activity very likely accounts for the markedly different behavioral effects of β-CCE between rodents and primates.

The increase in plasma cortisol level does not appear to result from a direct adrenal effect of the drug because high-affinity binding of β-CCE was not observed in primate adrenal membranes (unpublished data, March 1983). Furthermore, preliminary results in our laboratory have shown a robust increase in plasma corticotropin level immediately following administration of low doses of β-CCE.[21] Thus, the increase in plasma cortisol level reflects activation of the pituitary either directly or via the hypothalamus.

The failure to find a significant increase in MHPG level at doses of β-CCE causing cardiovascular and endocrine activation was surprising. As there appears to be a trend toward a decrease in MHPG following vehicle administration and an increase following the 500-μg/kg dose of β-CCE, we reanalyzed the data with analysis of variance with repeated measures to look directly for differences across conditions. No significant dose-time interactions were evident by this analysis ($F=0.78$, $df=8,16$, $P>.05$). Conceivably, a modest but significant increase in central MHPG production was obscured by dilution in plasma (where MHPG is a major metabolite of norepinephrine), or our plasma sampling times failed to catch a later peak. Current studies with serial sampling of CSF should provide a more sensitive indicator of central MHPG changes following β-CCE administration.

In the present experiments, diazepam potently blocked all the β-CCE–induced effects without, by itself, lowering BP, heart rate, or plasma cortisol level. In our earlier investigations, diazepam failed to consistently antagonize the effects of 2.5 mg/kg of β-CCE. These current results demonstrate that a 5:1 dosage ratio of diazepam to β-CCE prevents the activation seen with the latter. This ratio is in

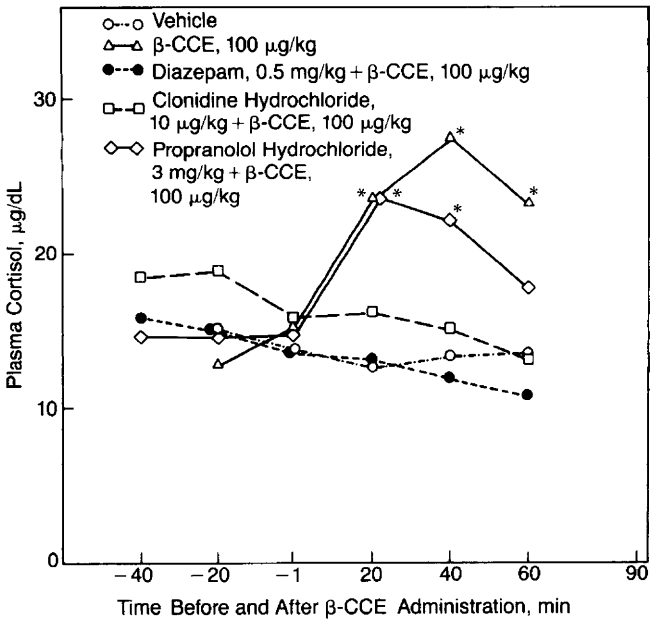

Fig 6.—Plasma cortisol values following vehicle, β-CCE, and β-CCE with diazepam, clonidine hydrochloride, and propranolol hydrochloride pretreatment. Pretreatment drugs were given at −20 minutes, β-CCE or vehicle was administered at time 0. Repeated measures analysis of variance for values obtained before and after β-CCE infusion shows significant time by condition interaction ($F[20,100] = 9.95$). Tukey's test for "honestly significant difference" for values across conditions shows plasma cortisol level to be significantly elevated only following β-CCE alone and β-CCE with propranolol pretreatment (asterisk, $P<.05$). (Adapted from Gallager and Tallman.[58])

rough agreement with the relative affinities of these two compounds for the benzodiazepine receptor (Table 1). One can predict from this ratio that lower doses of β-CCE (eg, 50 μg/kg), which are associated with significant elevations in plasma cortisol level, would be effectively antagonized by doses of diazepam comparable with the anxiolytic dose in humans (ie, 150 to 250 μg/kg).

As with diazepam, pretreatment with clonidine also prevented the cardiovascular and cortisol elevations observed following β-CCE administration. Unlike diazepam, however, clonidine had potent effects on these variables when administered alone. The hypotensive effects of clonidine, which can be demonstrated in man at a dose comparable with that used in this experiment, are believed to be mediated via the α_2-adrenergic receptor. At this dose, clonidine is an α_2-agonist that decreases norepinephrine turnover in brain, presumably through activation of the inhibitory feedback autoreceptor on neurons of the locus ceruleus as well as other noradrenergic nuclei in the pons and medulla.[22] Clonidine has been previously reported to decrease plasma cortisol level, although the site of action for this effect appears to be in the forebrain rather than the brain stem.[22] Though it might be inferred from these data that the cardiovascular and hypercortisolemic actions of β-CCE are mediated through α_2-adrenergic receptors, this conclusion is not supported by the observation that levels of MHPG, the major central norepinephrine metabolite, did not increase following β-CCE administration. If β-CCE were directly affecting neurons of the locus ceruleus, some change in MHPG level should have been evident at the same dose of drug that caused cardiovascular activation. Given the intrinsic effects of clonidine on both BP and plasma cortisol level, the blockade of β-CCE's actions by clonidine is most likely linked to the latter producing physiologic antagonism rather than a direct interaction at the α_2-adrenergic receptor.

Pretreatment with propranolol blocked the increase in heart rate following β-CCE but did not significantly alter the β-CCE–induced increases in behavioral activation, mean arterial pressure, or plasma cortisol level. At the dose used in this study, propranolol is a potent β-adrenergic receptor antagonist that, when given alone, markedly slows heart rate via a direct peripheral effect.[23] Although in man comparable doses of propranolol have anxiolytic effects,[24-26] these effects may largely result from attenuation of the somatic symptoms of anxiety such as autonomic and cardiovascular activation.[19,24] Our data, showing activation on all behavioral and physiologic measures except heart rate in propranolol-treated animals, fit with this notion of a peripheral or somatic component of anxiety that is selectively affected by propranolol. Furthermore, the β-CCE–induced rise in cortisol level, BP, and behavioral activation in the presence of a β-adrenergic receptor antagonist suggests that these effects are not mediated via the β-adrenergic system.

CONCLUSIONS

The Benzodiazepine Receptor and Anxiety

These experiments demonstrate that β-CCE, a high-affinity benzodiazepine receptor ligand, has potent behavioral and physiologic actions that can be reversed by the benzodiazepine diazepam. In contrast to earlier studies with relatively high doses of β-CCE (1.0 to 2.5 mg/kg) that produced marked behavioral agitation, lower doses of β-CCE (50 to 500 μg/kg) induced more subtle behavioral changes also accompanied by significant increases in plasma cortisol level, and heart rate. The antagonism of these effects by diazepam together with our previous report using the selective benzodiazepine receptor antagonist Ro 15-1788[14] demonstrate the importance of central benzodiazepine receptors in mediating the behavioral and physiologic activation following β-CCE. Our results with clonidine and propranolol suggest that adrenergic receptors do not mediate β-CCE's behavioral actions but are undoubtedly involved in the peripheral manifestations of this compound's effects.

Although the notion that the benzodiazepine receptor might mediate "anxiogenic" effects is relatively new, there is already considerable evidence supporting the role of this receptor in mediating the anxiolytic actions of benzodiazepines. The evidence includes the high correlation between the affinities of a series of benzodiazepines for this receptor in vitro and their relative clinical potencies as anxiolytics.[27] Furthermore, studies of in vivo binding in mice have shown extremely high correlations ($r = .98$) between the occupancy of benzodiazepine receptors by various benzodiazepines and both anticonvulsant (protection against pentylenetetrazol-induced seizures) and anticonflict effects, although only a small percent of sites needed to be occupied for a maximal behavioral effect.[28,29] Behavioral paradigms of anxiety in rodents have also been reported to alter benzodiazepine binding in brain.[29] Finally, the Maudsley rat, selectively bred as a genetic model of "fearfulness," has been reported to have a significantly lower density of benzodiazepine receptors compared with its "nonfearful" littermates.[30]

The precise regulation of the benzodiazepine receptor "complex" has been the source of considerable interest. Benzodiazepines have long been known to potentiate the

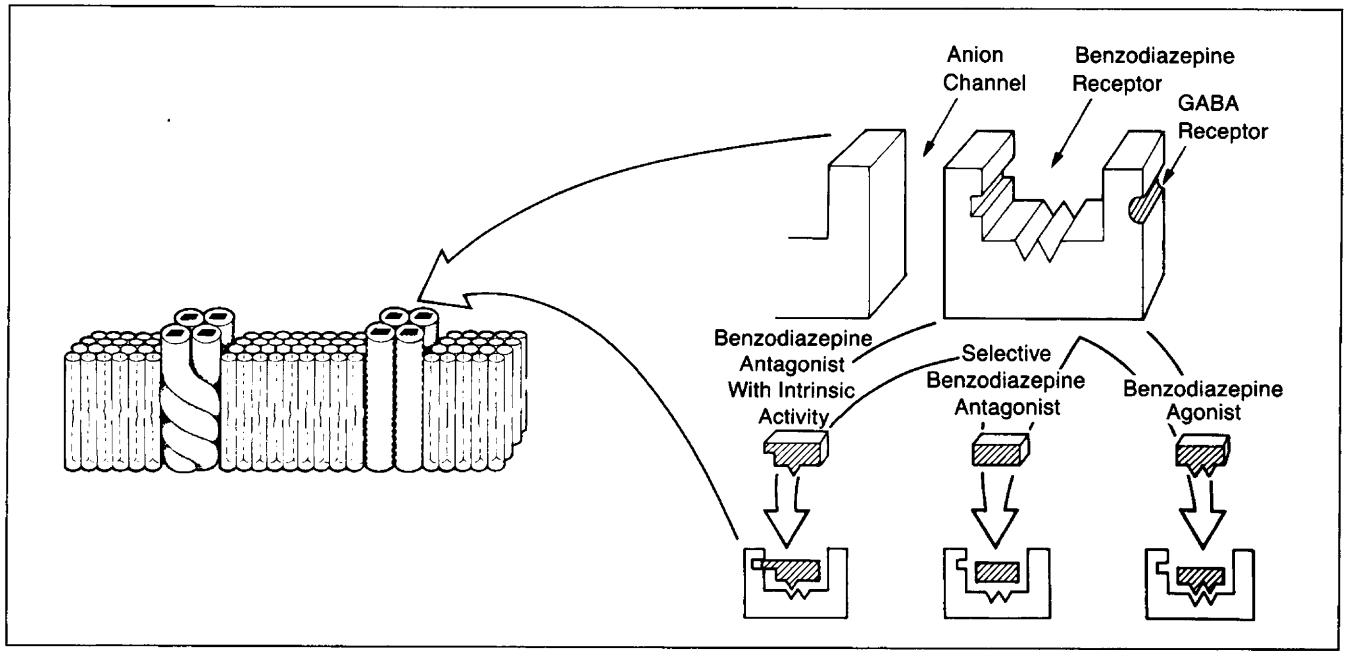

Fig 7.—Proposed model of benzodiazepine receptor showing separate domains for agonist, antagonist, and active antagonist (inverse agonist) binding. GABA indicates γ-aminobutyric acid.

effects of GABA. More recently, GABA has been shown to potentiate binding of benzodiazepines allosterically by increasing the affinity of the receptor for the drug.[4] A similar effect has been demonstrated for a number of drugs (eg, the barbiturates) as well as permeable anions, such as chloride, leading to the hypothesis that the benzodiazepine receptor is functionally and perhaps structurally linked not only to a GABA receptor, but also to a chloride ionophore or channel.[4,31] The current model (Fig 7) of the benzodiazepine receptor site (thought to be a tetramer of four proteins of approximately 51,000 daltons each) includes several binding sites or "domains" for agonist, antagonist, and "active" antagonist binding. The benzodiazepine receptor is, in turn, part of a larger supramolecular complex including the GABA receptor protein and the chloride channel itself. Binding to the benzodiazepine receptor in the presence of GABA is believed to lead to an increase in GABA-mediated chloride conductance and thus to membrane hyperpolarization.

A key issue is whether the β-CCE–induced syndrome reported herein is in fact a valid model of human anxiety. Several lines of experimental evidence suggest that it is. First, the behaviors observed following low doses of β-CCE resemble behaviors seen in more naturalistic situations of fear such as confrontation with a new investigator. Indeed, the exact form of behavioral response, though showing considerable individual variation, was consistent within the same monkey. Furthermore, as shown in Table 4, many of these behavioral and autonomic effects of β-CCE, particularly the increases in motor tension and heart rate, resemble the signs of pathologic anxiety in man.[32] Increased secretion of cortisol is also associated with anxiety states in man although the relationship of plasma cortisol level to stress has been traditionally easier to demonstrate in nonhuman primates.[33] Finally, the clinically effective anxiolytics diazepam, clonidine, and propranolol attenuated one or more aspects of the β-CCE–induced syndrome.

Because anxiety is a subjective experience, its presence can ultimately be demonstrated only in man. Although β-CCE has not yet been given to humans, a closely related analogue, FG-7142 (β-carboline monomethylamide), has been administered to healthy volunteers.[34] Subjects experienced intense motor unrest and "almost intolerable tension," which one subject described as a feeling of impeding doom. In one case these symptoms were so intense that the subject was given lormetazepam, which reversed the unpleasant behavioral effects in minutes. In addition to these behavioral changes, plasma cortisol, systolic BP, and heart rate all increased significantly following FG-7142. To compare the effects of FG-7142 with β-CCE, we have recently obtained FG-7142 (from Claus Braestrup, PhD) for administration to two rhesus monkeys. Following a dose of FG-7142 (3.0 mg/kg IV) roughly comparable with that given to the human volunteers, both monkeys showed behavior similar to that observed following administration of β-CCE (head and body turning, hyperresponsiveness to approach) and both showed robust increases in plasma cortisol level (84.3% and 134.9% of baseline, respectively). Although these data are limited, they suggest that FG-7142 has much the same effect in nonhuman primates as in man, that FG-7142 has effects similar to β-CCE, and that β-CCE will most likely produce the same effects as FG-7142 in man.

Pharmacologic Models of Anxiety

How do the behavioral effects of the benzodiazepine receptor "active" antagonists compare with other pharmacologic models of anxiety? Three alternative models have been proposed (Table 5).

In five independent double-blind trials, sodium lactate has been shown to precipitate panic attacks in patients with panic disorder but not in normal controls.[35-39] Unfortunately, the mechanism for lactate's effect is entirely unclear. With one exception (plasma epinephrine level), the physiologic measures following lactate generally fail to distinguish panic patients from controls,[40] suggesting that the difference may be entirely in the subjective interpretation of the

same pharmacologic effect. Nevertheless, the test appears to have diagnostic usefulness and may identify a group of patients with anxiety who are responsive to tricyclic antidepressants and not to benzodiazepines.[41]

In addition, caffeine, which is a methylxanthine stimulant, has been suggested as a pharmacologic model of anxiety. As caffeine is known to bind with high affinity to brain adenosine receptors,[42] an adenosine receptor model of anxiety might be hypothesized. However, as the "anxiety symptoms" associated with caffeine are generally only present at high doses, are most common in low-caffeine users, and pharmacologically probably involve both the benzodiazepine and adrenergic systems as well as adenosine receptors,[43,44] the relationship of caffeine's "anxiogenic" effects to the adenosine receptor remains unclear.

Table 4.—Effects of Benzodiazepine Receptor Agonists and Antagonists on Anxiety Symptoms

DSM-III Criteria for Generalized Anxiety Disorder	Benzodiazepine Agonist Effects*	Benzodiazepine Active Antagonist Effects†
Motor tension		
Shakiness, jitteriness, trembling	↓	↑
Muscle aches, tension	↓	↑
Fatigability	. . .	. . .
Fidgeting, restlessness	↓	↑
Autonomic hyperactivity		
Heart pounding, racing	↓	↑
Dizziness	. . .	. . .
Light headedness	↓	. . .
High respiratory rate	?	. . .
Paresthesias	?	. . .
Upset stomach	?	↑
Frequent urination	?	↑
Sweaty, cold, clammy hands	. . .	. . .
Flushing	. . .	↑
Apprehensive expectation		
Anxiety	↓	↑
Fear, worry	↓	↑
Anticipation of misfortune	↓	↑
Vigilance and scanning		
Insomnia	↓	↑

*From Greenblatt and Shader.[55] Downward-pointing arrows indicate decrease.

†From Dorow et al,[34] Mendelson et al,[13] and current data. Upward-pointing arrows indicate increase.

Another important animal model of "anxiety" has been proposed and developed extensively by Redmond and coworkers.[15,45] This model focuses on the noradrenergic system, particularly the α_2-adrenergic receptor, as being critical to the pathophysiology of anxiety. Redmond,[46] for instance, has shown that electrical stimulation of the locus ceruleus or administration of α_2-adrenergic receptor antagonists such as piperoxan hydrochloride or yohimbine to nonhuman primates elicits anxietylike or "alarm" behavior that is associated with increases in plasma levels of the norepinephrine metabolite MHPG. This effect is blocked by the α_2-adrenergic agonist, clonidine, as well as by electrical destruction of the locus ceruleus. Unfortunately, clonidine is only a partially effective anxiolytic in man, as its anxiolytic effects wear off within a few weeks.[17,18,47] Furthermore, it is not clear that the locus ceruleus is the primary structure mediating such alarm or anxietylike behavior or that this effect is entirely noradrenergic in nature.[48] Studies in rodents using social models of anxiety[49] have not demonstrated anxiolytic effects from chemical lesions of the locus, and the novel anxiolytic buspirone hydrochloride has been recently shown to increase rather than decrease the firing of locus ceruleus neurons.[50]

In support of Redmond's hypothesis, Charney and coworkers[45] have recently demonstrated that healthy volunteers challenged with the α_2-adrenergic antagonist, yohimbine, briefly have mild to moderate anxiety, which is accompanied by an increase in plasma MHPG level. The behavioral effect is blocked by diazepam or clonidine, but the increase in MHPG level is not blocked by diazepam. Diazepam thus appears to have an anxiolytic effect without decreasing noradrenergic turnover, as might be predicted from the relatively low density of benzodiazepine receptors on the neurons of the locus ceruleus.[51,52]

It seems likely that the noradrenergic and benzodiazepine receptor models of anxiety represent two very different phenomena. On a clinical level, clonidine has been reported to be effective for panic disorder, a syndrome for which most benzodiazepines are not useful.[17] In animal behavioral models of anxiety, clonidine reduces the acoustic startle reflex[53] but does not consistently show significant "anticonflict" effects in paradigms that are sensitive to benzodiazepines.[54] Clonidine at anxiolytic doses in man has both sedative and hypotensive effects, suggesting that the drug is less selective for anxiety but may have general effects on arousal mediated through the locus ceruleus. By contrast, benzodiazepines, such as diazepam, are anxiolytics at doses well below those that produce sedation.[55] The site(s) of anxiolytic action of the benzodiazepines is not known, but increasing evidence points to particular regions of the limbic system. Not only do structures such as the hippocampus and amygdala have high densities of benzodi-

Table 5.—Psychopharmacologic Models of Anxiety*

Challenge	Blocker	Physiologic Effects†	Receptor	Site of Action
β-CCE (FG-7142)	Ro 15-1788, diazepam	↑ cortisol, ↑ heart rate, no change MHPG	Benzodiazepine	Limbic (?)
Yohimbine	Clonidine hydrochloride, diazepam	↑ MHPG (not blocked by diazepam)	α_2	Locus ceruleus
Sodium lactate	Imipramine	↑ epinephrine (no change cortisol, norepinephrine)	?	?
Caffeine	?	↑ epinephrine, no change norepinephrine	Adenosine, benzodiazepine	?

*β-CCE indicates β-carboline-3-carboxylic acid ethyl ester; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol.

†Upward-pointing arrows indicate increase.

azepine receptors,[51,52] but microinjection of benzodiazepines into discrete nuclei of the amygdala produces anticonflict effects.[56] Similarly, anxiogenic effects can also be elicited by electrical stimulation of selective sites in the hippocampus.[57] In summary, these two proposed pharmacologic models of "anxiety" may correspond to different neuroanatomic sites of action and to different clinical phenomena: noradrenergic activation originating in the pons and corresponding to "alarm"; the benzodiazepine system with its dense telencephalic representation corresponding more to "fear" or "conflict." Although these two systems undoubtedly have different phylogenetic and ontogenetic histories, it seems likely that in particular clinical states, both systems would be activated.

Implications for Psychiatry

The power of any model resides in the testable hypotheses it generates. The benzodiazepine receptor model of anxiety has several immediate implications for new research avenues in psychiatry.

First and most compelling is the possibility of an endogenous ligand for this receptor. Though it may seem that the existence of such a receptor would automatically imply a natural ligand or even a family of such ligands, research up to the present has failed to confirm conclusively the presence of such a substance.[27] Conceivably, the level(s) of endogenous ligand(s) might correlate with interindividual or intraindividual variations in anxiety, level of arousal, or rate of habituation. Of course, other components of the benzodiazepine receptor system, such as the number, receptor affinity, or the GABA levels might be equally critical to the development of either normal or pathologic anxiety. The challenge will be to develop strategies for assessing each of the components in the system and to elucidate a pathophysiologic taxonomy for what is now generally labeled as "anxiety."

Not only could a potent pharmacologic model of anxiety be used as a diagnostic challenge for patients with different clinical syndromes, it could begin to dissect the various affective and physiologic components of anxiety. Our preliminary data with β-CCE in monkeys, for instance, suggest that individual animals vary in their behavioral and autonomic sensitivity to the drug. To dissociate the psychologic, endocrine, and cardiovascular aspects of the response to β-CCE may provide a first glimpse of the various functional neuroanatomic substrates of anxiety mediating each limb of the "fight or flight" response. A similar approach could be followed to study the differential effects of anxiolytics. Though our data provide preliminary evidence for the effectiveness of propranolol on the somatic but not behavioral aspects of anxiety, the effects of other anxiolytics, such as barbiturates, alcohol, and novel GABA-mimetics, remain to be studied.

In summary, we appear to be entering a new era of understanding the biologic bases of what Freud called "the problem of anxiety." Though major questions remain to be answered, these studies in nonhuman primates suggest that the benzodiazepine-GABA receptor complex is important in the mediation of both anxiogenic and anxiolytic effects. For the first time, we appear to have a pharmacologic model of anxiety for which there is a known molecular basis, a powerful pharmacologic probe, and a well-documented anxiolytic treatment. The diagnostic, developmental, and theoretic implications of this model remain to be fully explored.

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UCB
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)
NIH ASSURANCE #A4107-01
Animal Utilization Proposal Form

Protocol Title: [redacted]

Approval Period: 10/15/2019-10/31/2020

Important Note: This Print View may not reflect all comments and contingencies for approval. Please check the comments section of the online protocol.

*** * * Attached Document * * ***

Document Name	Created Date
[redacted]_R299_MRI NHP SOP_9.28.18.docx	09/17/2019

MRI NHP SOP

Ahead of time, magnet will be booked for 2 hours.

Personnel

- All personnel allowed to enter Zones III (operator: [redacted]) and IV (magnet: [redacted]) must have passed the BIC User Safety training (http://bic.berkeley.edu/scanning).
- All personnel must have undergone Herpes B training.
- Number of personnel need to be kept to a minimum to ensure safety around magnet. For each stage of the protocol, personnel should have definite roles.
- Number of personnel simultaneously in room with magnet should be kept to a minimum to avoid diffusion of responsibility.
- Protocol requires four personnel: vet, AHT, PI (or another NHP PI with a currently approved Animal Use Protocol, or another NHP trained lab member from a lab with a currently approved AUP), and one lab member. One member of the team will be a qualified scanner operator, or an additional qualified Research Associate (hired by the lab) will be used.

Equipment

- Fully enclosed transportation cart for animal
- Veterinary cart with supplementary supplies (needles, syringes, additional ketamine, atipamezole, towels, ET tube, laryngoscope, lidocaine, thermometer, portable pulse oximeter)
- Anesthesia machine with full oxygen tanks (including extra isoflurane, tubing with appropriate connectors, extra anesthetic masks)
- MRI compatible stereotactic frame
- MRI compatible monitoring equipment (pulse oximeter)
- Portable NHP exposure kit (to remain in [redacted]
- MRI cleaning cart
- All equipment designated for use within the magnet on the must be approved ahead of time by Ben Inglis and marked with MRI compatible sticker

Procedure overview

1. Induction

Procedure	Room	Responsibility
Anesthetic induction (2-3mg/kg ketamine, 0.015-0.04 mg/kg dexmedetomidine, 0.1-0.25mg/kg midazolam) and catheter placement for IV access	[redacted]	Vet, AHT
Shave area for placement of respiratory monitor	[redacted]	Vet, AHT
Remove primate collar	[redacted]	Vet, AHT
Endotracheal intubation (optional; this step performed if stereotaxic frame will be utilized)	[redacted]	Vet, AHT

	[redacted]	

2. MRI decontamination (beginning)

Procedure must be performed at least 20 minutes before the arrival of the animal to ensure sufficient air changes.

Procedure	Room	Responsibility
Transport equipment to scanner		PI , Lab member
Placement of barriers and signage	[redacted]	Lab member
Black out windows from operator room to hallway		Lab member
Lay down plastic sheeting in magnet room from doorway to scanner		PI
Tape down metal sheet in scanner doorway	[redacted]	Lab member
Remove wall panels close to doorway in magnet room		PI
Remove patient bed and insert tray for animal		PI
Surface decontamination (bed, coil and bore) using Clorox disinfectant wipes		PI
Lay down plastic sheeting in operator room from doorway to magnet room	[redacted]	Lab member
Prepare vital sign monitoring equipment	[redacted]	PI, Lab member

3. Transport of animal

Procedure	Room	Responsibility
Record baseline vitals (heart rate, respiratory rate, temperature, SpO2)	[redacted]	Vet, AHT
Call operator room in BIC before leaving ([redacted])		Vet, AHT
Transport animal to scanner in cart		Vet, AHT
Spray cart wheels with NPD on leaving vivarium		Vet, AHT
Remove shoe covers on leaving vivarium and place in biohazard bag		Vet, AHT

4. Positioning of animal in scanner

Procedure	Room	Responsibility
Animal lifted from cart to magnet	[redacted]	Vet, PI
Place animal in ventral recumbency in stereotactic device*		Vet, PI
Position pulse oximeter and respiratory monitor		Vet, AHT
Slide head into head coil		PI
Monitor vital signs at least every 10 minutes (heart rate, respiration, SpO2)		AHT
Wrap patient in towels to maintain body temperature	[redacted]	AHT
Insert anesthesia tubing through wall conduit once wall panel has been removed and tape to side of bed to ensure proper placement is maintained	[redacted]	Vet, AHT

Initiate supplemental O2 (± isoflurane) via mask or endotracheal tube at 1-3 L/min	[redacted]	AHT
Index head with bed referencing system	[redacted]	PI
Insert animal into magnet via motorized bed		PI
All personnel leave room, RF-screened door closed		

* alternately position animal using disposable foam blocks and register position using fiducial markers (vitamin E capsules) taped to ear canals and eye orbits

5. Scanning

Procedure	Room	Responsibility
Monitor of vital signs at least every 10 minutes (respiratory, heart rate, SpO2)	[redacted]	AHT
MRI operator registers monkey as a subject and initiates scan protocol	[redacted]	PI
If vital signs degrade or there is reason to check on the animal's health, scanning is suspended and one or more personnel enter the magnet as necessary*	[redacted]	Vet
Should vital signs indicate that supplemental anesthesia is required, isoflurane will be administered at a dose of 2-3% via mask	[redacted]	Vet, AHT
If there is reason to check on the animal's position, scanning is suspended and one or more personnel enter the magnet as necessary		PI

* one member of vet staff will remain gowned in the event that the patient needs to be accessed for monitoring

6.Removal of animal from scanner

Procedure	Room	Responsibility
Animal removed from magnet via motorized bed	[redacted]	PI
Head removed from head coil		PI
Vital sign sensors removed		Vet
Animal removed from stereotactic device		PI
Animal lifted from magnet to cart and transported to anteroom		Vet, PI

7. Transport of animal

Procedure		Room	Responsibility
Transport animal to holding room		[redacted]	Vet

8. MRI decontamination (end)

Procedure must be performed at least 20 minutes before end of scanning block to ensure sufficient air changes.

Procedure	Room	Responsibility
Surface decontamination (bed, coil and bore) using Clorox	[redacted]	PI

disinfectant wipes		
Remove animal tray and replace patient bed	[redacted]	PI
Collect plastic sheeting	[redacted]	PI
Collect plastic sheeting	[redacted]	PI
Remove metal sheet from scanner doorway	[redacted]	PI
Replace wall panels as needed	[redacted]	PI
Remove restricted access signs and window black out	[redacted]	PI
Transport equipment back to animal facility		PI

9. Recovery

Procedure	Room	Responsibility
Provide supplemental heat via warm water recirculating blanket or warmed towels if needed; remove catheter; monitor vitals to ensure return to normal (including pink and moist mucous membranes, CRT< 2 seconds, and rectal temperature of approximately 99-102.5 F)	[redacted]	Vet, AHT
Replace primate collar	[redacted]	Lab member
Reverse dexmedetomidine if necessary with 0.15mg/kg IV/IM atipamezole	[redacted]	Vet, AHT
Remove endotracheal tube if placed	[redacted]	Vet, AHT
Monitor animal recovery	[redacted]	Lab member

Emergency procedures

A building occupant is required by law to evacuate the building when a fire alarm sounds. Good judgment is required while working with non-human primates. The following guidelines will assist you to safely and humanely respond to an emergency alarm.

In the event of a major earthquake or fire

1. If possible, enter the magnet room and euthanize the animal prior to evacuating.
2. If at any time you deem that there is an immediate threat to your safety, evacuate immediately.

In the event of a magnet quench

The magnet could quench either due to an earthquake, because a large metal object hits the magnet, or because it is activated manually using a dedicated emergency button. The quench should be controlled (i.e. vent to the outside) and take about 30-40s. However, if the quench is uncontrolled (e.g. the vent fails in the earthquake), the magnet room could fill with freezing cold helium gas.

1. If you think the magnet is quenching, you should first open the magnet room and operator room doors and prop them open, to prevent a pressure build-up and to allow fresh air in. Then, if you can do so safely, go into the magnet room (ensuring that the magnet room door remains wide open) to remove the subject from the magnet as quickly as possible.
2. If there are indications that the quench is uncontrolled (white vapor, substantial temperature drop, animal vital signs absent, low oxygen alarm above the operator console) do not enter the room until instructed to do so by BIC personnel.

All other responses to an emergency alarm

1. As soon as you hear the alarm, send someone to speak with the Building Coordinator.
2. In the interim, begin procedures to take the animal out of the magnet and return him to the animal facility.
3. If, in the judgment of the Building Coordinator, there is an immediate threat to human safety you will be instructed to evacuate.
4. If at any time you deem that there is an immediate threat to your safety, evacuate immediately.