Date: August 14, 2014

To: Dr. David M. Anderson  
Executive Director  
Health Sciences Administration  
Box 356355

From: Institutional Animal Care and Use Committee

Subject: Semiannual Program and Facility Review of Animal Use Program by the IACUC

The attached report represents the semiannual report of the Institutional Animal Care and Use Committee (IACUC) for the period of January 1, 2014 through June 30, 2014.

Please let me know if you have any questions.

Sincerely,

Tena Petersen  
Manager, Regulatory Affairs  
Office of Animal Welfare

cc: Dr. Sally Thompson-Iritani  
   Director, Office of Animal Welfare

   Dr. Susanna Cunningham  
   Chair, Institutional Animal Care and Use Committee
Date: July 16, 2014

To: University of Washington Institutional Animal Care and Use Committee

From: Tena Petersen
Manager of Regulatory Affairs

Subject: Summary of items identified as part of the Semiannual Program and Facility Review of Animal Use Program by the IACUC

This represents the semiannual report of the Institutional Animal Care and Use Committee (IACUC) for the period of January 1, 2014 through June 30, 2014 as required by the PHS Policy on Humane Care and Use of Laboratory Animals and as a condition of this institution’s Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), and USDA Animal Welfare Regulations, 9 CFR Chapter I, subchapter A, as applicable. The report has been signed by a majority of the IACUC members and there were no minority opinions.

Evaluation of the Animal Care and Use Program and Inspection of Facilities

The IACUC has conducted its semiannual evaluation of the Institution’s Animal Care and Use Program, using the 2011 8th edition of the Guide for the Care and Use of Laboratory Animals (Guide), and, as applicable, 9 CFR Chapter I, 2.31.

A brief overview of the deficiencies identified during is noted below and included in the attached summary (appendix A). Full details follow in the sections on “Reports to the IACUC” and “Reports to the IACUC and OLAW”.

Significant Deficiencies

There were no Significant Deficiencies identified during this reporting period. This is unchanged from the previous reporting period.

Major Deficiencies

There were 4 Major deficiencies noted. These deficiencies were found in the category of Husbandry, Drug Storage/Expiration/Documentation, Occupational Health and Animal Health. During the previous reporting period 1 citation was identified as Major.
Minor Deficiencies

There were multiple deficiencies that were individually classified as “minor”. These deficiencies, along with the major deficiencies, are detailed in a table (Appendix B) so that the groups of issues can be more easily reviewed by location and type of deficiency. A deficiencies analysis is also attached. Corrections for deficiencies noted during the current reporting period have been completed or are in progress. Individual site visit reports for those sites with deficiencies are attached, along with the documentation of corrections or plans for corrections.

Events Reported to the IACUC during this reporting period.

Details of the following reports are included in the Semiannual Report to the Institutional Official.

- There were 12 adverse events that were reported to the IACUC. Nine of these items were also reported to OLAW.
- There were 3 occupational health exposures reported to the IACUC.
- There were 9 Facility and Temperature Events reported to the IACUC.

Animal Welfare Concern: None

IACUC Protocols active during the semi-annual period with multiple major surgery, potential unrelieved pain or exceptions to the Guide or USDA Animal Welfare Regulations:

Details of the following reports are included in the Semiannual Report to the Institutional Official.

1. Site Visit Deficiency Summary
   See Appendix A

2. Performance of multiple major operative procedures – There were 55 protocols approved for multiple major operative procedures.
   See Appendix B

3. IACUC approved variances – There are four types of variances that have been approved on animal use protocols. There are approximately:
   16 Cage type variances
   13 temperature variances
   15 wire bottom cages
   39 weaning variances
   See Appendix C
4. Exemptions approved by IACUC and USDA – There is one protocol (2340-01) that is approved for a USDA exemption to allow for up to 5 major operative procedures involving cranial implants for animal A09044. This exemption is approved by the USDA for the period of March 1, 2014 through March 1, 2016 and is required to be reviewed annually by the UW IACUC.

5. USDA Category E Studies - During the past cycle, the number of protocols classified as a USDA Category E study has increased from 31 protocols to 62 protocols. This increase reflects a change in the consideration of the classification of protocols and it is anticipated that this number will continue to increase as the protocols are reviewed as part of their normal review cycles.

6. IACUC approved cage size variances for USDA species – There is one cage size variance for non-human primates. The IACUC approved an exemption to house infant monkeys (age 2 weeks to a body weight of 1 kg) in cages that meet floor space requirements but are only 24" high. These animals climb up well, but are not adept at climbing down. The lower cage height reduces the risk of injury if an animal should fall.

7. Environmental Enrichment Exemptions – There are 124 EEC exemptions that were reviewed and approved by the IACUC.

See Appendix D
Appendix A

Analysis of deficiencies found
University of Washington IACUC Site Visits January-April 2014.

No Significant Deficiencies were found.

Major Deficiencies

<table>
<thead>
<tr>
<th>Arena</th>
<th>PI Labs</th>
<th>DCM</th>
<th>WaNPRC</th>
<th>Grand Total</th>
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<tr>
<td><strong>Grand Total</strong></td>
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<td><strong>3</strong></td>
<td><strong>0</strong></td>
<td><strong>4</strong></td>
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</table>

Animal Health

Two cages found on lower level of cart were overlooked by group when returning cages to racks. Animals were without water for no more than three hours. The cages were moved to the rack during the visit and animals appeared to be OK. Please develop an SOP to ensure this will not happen in the future.

Animal Health

Chicks need to be supplied with potable water rather than lab water. Please update the IACUC with the status of a permanent solution.

Occupational Health

Cage Wash Safety training is usually performed and logged quarterly, but no records for 3rd or 4th quarters of 2013.

Drug Storage/Expiration/Documentation

Lab uses reusable Hamilton needles for intraocular injections that have been sterilized. Used needles are currently rinsed with ethanol and saline. Please confirm sterile needles will be used and specify how sterility will be achieved.

Minor Deficiencies

<table>
<thead>
<tr>
<th>Arena</th>
<th>PI Labs</th>
<th>DCM</th>
<th>WaNPRC</th>
<th>Grand Total</th>
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<tbody>
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<td>Sanitation</td>
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<td>6</td>
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<tr>
<td>Facility</td>
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<tr>
<td>Drug Storage/Expiration/Documentation</td>
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<td>Occupational Health</td>
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<td>Husbandry</td>
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<td>Animal Health</td>
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<td>Equipment</td>
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<td>Environment</td>
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<td>Pest Control</td>
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<td>2</td>
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<tr>
<td><strong>Grand Total</strong></td>
<td><strong>206</strong></td>
<td><strong>72</strong></td>
<td><strong>38</strong></td>
<td><strong>316</strong></td>
</tr>
</tbody>
</table>

Running tallies for the past three years are shown in the graph on the next page.
Appendix A

Breaking down the top four categories

Sanitation
Deficiencies related to sanitation remain high, though on a downward trend. The two big players continue to be equipment (tables, chambers, stands, etc.) that is damaged, inappropriately stored, or in need of cleaning; and the large number of taped up paper signs. There is good news: the incidence of inappropriate cardboard storage boxes remains low for the second period in a row.

Drug and Chemical Storage and Documentation
The Drugs/Chemicals category remains a major source of deficiencies, with the same top causes as previous reports: expired drugs, improper labeling of drugs, and expired supplies (e.g. sutures). Cleaning supplies and other chemicals account for less than 14% of deficiencies in this category, and share the same causes -- expired products or incomplete or missing labeling.

Facility
The past two periods have seen upticks above the three year average for this category. The top causes continue to be walls, doors, and fixtures (built-in cabinets, counters, racks, etc.) needing repair or having unsanitizable surfaces (rusty, scratched, etc.).

Occupational Health
The uptick in OH deficiencies was due almost exclusively to problems with eyewashes and sharps. We found 28 eyewash deficiencies, the largest number since 2012. Most were for incomplete or missing flushing logs, though we also found two locations without adequate access to an eyewash station. The 11 sharps deficiencies included recapped needles, overfilled sharps containers, and used needles being stored other than in a sharps container.
## Appendices

### Multiple Major Surgeries

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Procedures and Rationale</th>
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</thead>
</table>
| Multiple Major Surgery:  
1. Craniotomy to inject neurotrophins  
2. Either dorsal cochlear tract cut or cochlear ablation, 2-4 days following #1  
Species: Chickens  
Interval: 2-4 days  
Rationale: Surgery 1 is needed to treat with the plasmids to label the proteins of interest. Surgery 2 is needed to do the manipulation (dorsal tract cut) to observe the effect on the labeled proteins. | |
| Multiple Major Surgery:  
1. Craniotomy to inject TTX  
2. Craniotomy to inject TTX 4 hours after #1  
3. Terminal cochlear ablation and ABR test before perfusion, 4 hours to 7 days after #2  
Species: Chickens  
Interval: 4 hours to 7 days  
Rationale: Surgeries 1 and 2 are needed to treat with TTX (8 hours of blockade is required, and a single TTX injection lasts 4 hours). Surgery 3 is the test for function following the prolonged blockade. Maintaining anesthesia for 12 hours would be too risky for the chicks, and a recovery is required for intervals up to 7 days. | |
| Multiple Major Surgery:  
1. Orthotopic implantation of the prostate  
2. Ablation of the dorsal prostate, a minimum of 3 weeks following #1  
Species: Mice  
Interval: 3 weeks minimum  
Rationale: These two procedures are required to allow for metastatic sites to grow to the level of detection and provide adequate amounts of tissue for in vitro studies. Without removal of the primary tumor, the health of the animal would be compromised before metastatic sites become macroscopic.  
OR  
1. Castration  
2. Sub-renral capsule implantation, a minimum of 2 weeks following #1  
Species: Mice  
Interval: 2 weeks minimum  
Rationale: Castration will be done first followed by sub-renral capsule implantation of tumor tissue. By performing the castration and allowing the testosterone levels to nadir, the tumor won’t have the opportunity to revert back to androgen dependence.  
OR  
1. Castration  
2. Intra-tibial injection OR Subcutaneous implantation, a minimum of 2 weeks following #1  
Species: Mice  
Interval: 2 weeks minimum  
Rationale: Implanting tumors into already castrated mice will produce androgen independent human prostate cancer xenograft lines.  
OR  
1. Intra-tibial injection OR Subcutaneous implantation  
2. Castration, a minimum of 1 week following #1  
Species: Mice  
Interval: 1 week minimum  
Rationale: Performing castration after intra-tibial or subcutaneous injection mimics clinical cases of androgen dependent cancer treatment. |
Multiple Major Surgery:
1. Electrode implantation for EEG (epidural or depth), may include other minor surgeries such as ECG, EMG.
2. Electrode implantation for EEG (epidural or depth), more than 48 hrs following #1
Species: Mice
Interval: >48 hrs
Rationale: Individual animals will be followed over an extended period of time collecting several EEG/video recordings in order to test the effects of special diet, anti-epileptic drugs and/or thermal induction over time. This may require 1 repair surgery so that valuable data is not lost due to early termination of the experiment resulting from the electrode being dislodged.

OR
1. Implant of Emitter
2. Repair and/or replacement of Emitter, more than 48 hrs following #1
Species: Mice
Interval: >48 hrs
Rationale: Individual animals will be followed over an extended period of time collecting simultaneous activity, temperature, heart rate, and video data in order to evaluate whether hyperthermia induced seizures and spontaneous seizures occur at the same temperature, and how seizures/anti-epileptic drugs affect normal sleep behaviors. This may require 1 repair surgery so that valuable data is not lost due to early termination of the experiment resulting from the Emitter being dislodged.

Multiple Major Surgery:
1. Laparotomy
2. Laparotomy, approximately 4 months following #1
Species: M. mulatta
Interval: 4 months
Rationale: This study is looking at the degree of gastrointestinal inflammation in infant rhesus macaques at two time-points, pre- and post-MMR vaccination. In order to determine that any inflammation observed in the GI biopsies at the second surgery is associated with the MMR, a pre-MMR biopsy must also be examined. In a previous pilot study of 12 vaccinated rhesus infants, 10 animals demonstrated chronic gastrointestinal inflammation at the second surgery, 2 of which also had some minor inflammatory markers at the first surgery. It is therefore critical to the interpretation of the data to have both pre- and post-MMR tissue collected. Furthermore, there were no complications of surgery in any of these animals. Noninvasive techniques, such as endoscopy, are not feasible because the GI tract of the young animals to be used is too small to accommodate a flexible endoscope (the Primate Center currently has the smallest diameter endoscope with 4-way deflection and a biopsy channel available).

Multiple Major Surgery:
1. Baseline laparoscopy
2. Upper Reproductive Tract (URT) assessment laparoscopy, minimum of 12 weeks following #1
3. Upper Reproductive Tract (URT) assessment laparoscopy, 9-10 weeks following #2
4. Upper Reproductive Tract (URT) assessment laparoscopy, 4 weeks following #3
5. Upper Reproductive Tract (URT) assessment laparoscopy, 4 weeks following #4
6. Upper Reproductive Tract (URT) assessment laparoscopy, 20 weeks following #5
7. Upper Reproductive Tract (URT) assessment laparoscopy, 11-12 weeks following #6
8. Upper Reproductive Tract (URT) assessment/tissue collection laparotomy, minimum of 6 months following #7
Species: M. Nemestrina
Interval: 4 weeks - 6 months minimum
Rationale: The researcher compares and documents progression to upper tract chlamydial disease in animals challenged with candidate chlamydia isolates. Ten weeks after the primary cervical challenge, each macaque will undergo laparoscopy to assess URT after a single exposure to chlamydia, followed by 4 weekly cervical chlamydia inoculations to promote disease progression to
the URT. Macaque URT tissues will be assessed and disease progression documented by laparoscopy monthly for 3 months after the final cervical challenge.

Each macaque will undergo a final URT assessment and tissue collection (laparotomy/hysterectomy) to ascertain whether the vaccine candidate(s) prevent URT disease in naive individuals and/or exacerbate disease progression in individuals previously exposed to chlamydial infection.

Version 11

Multiple Major Surgery:
1. Baseline laparoscopy
2. Cervical biopsy, >6 weeks following #1
3. Upper Reproductive Tract (URT) assessment laparoscopy, 2 weeks following #2
4. Upper Reproductive Tract (URT) assessment laparoscopy, 2 weeks following #3
5. Upper Reproductive Tract (URT) assessment laparoscopy and hysterectomy, 12 weeks following #4

Species: M. Nemestrina
Interval: 2-12 weeks

Rationale: To demonstrate attenuation of the CT-Minus strain, we will compare the acute inflammatory response to LRT tissues (cervix biopsies) if a difference is noted colposcopically after primary infection; and of URT tissues after primary and repeated infections, to the tissues of animals exposed to the CT_Plus strain. We will allow a minimum of 2 weeks between any surgical procedures in a single animal.

Each macaque will undergo a final URT assessment and tissue collection (laparotomy/hysterectomy) to ascertain whether the attenuated strain results in diminished or absent disease process of reproductive tract tissues (demonstrated through histology and IHC assessments of LRT & URT tissues), compared to the wild-type strain in the macaque model.

Multiple Major Surgery:
1. Partial renal ablation of right kidney by electrocautery
2. Complete nephrectomy of left kidney, 2 weeks following #1

Species: Mice
Interval: 2 weeks

Rationale: It is necessary to perform a 2-step renal surgery model because the animals will not recover if both surgeries are done at the same time. Two surgeries provides for better survival and better induction of uremia.

OR

1. Total right nephrectomy
2. Partial ablation of left kidney via bilateral resection of poles, 1 week following #1

Species: Mice
Interval: 1 week

Rationale: It is necessary to perform a 2-step renal surgery model because the animals will not recover if both surgeries are done at the same time. Two surgeries provides for better survival and better induction of uremia.

Multiple Major Surgery:
1. Left thoracotomy to induce cardiac injury
2. Thoracotomy to administer virus or cells, 1-4 days (generally 4 days) following #1

Species: Mice and Rats
Interval: 1-4 days

Rationale: In many cases it is necessary to perform 2 survival surgeries. These experiments involve one surgery to induce cardiac injury and a second surgery to administer cells, an engineered myocardium, or a scaffold directly into the injury during the healing phase.
Multiple Major Surgery:
1. Thoracotomy and myocardial infarction
2. Thoracotomy and intramyocardial injection, 1 week following #1
Species: Rabbits
Interval: 1 week
Rationale: These studies involve one surgery to induce myocardial infarction and a second to inject cells into the injury during the healing phase. Rabbits are not large enough to administer cells through a catheter, and injecting cells at a later time point is a more relevant model for future clinical studies.

Version #9
Multiple Major Surgery:
1. Myocardial infarct via percutaneous creation with tether insertion
2. Sternotomy or left lateral thoracotomy for cell therapy injection and transplantation; possible insertion of cardiac monitor (version #9), 14 days following #1
3. Tether re-insertion (only if initial tether fails), time depending on circumstances following #1 or #2
Species: M. nemestrina
Interval: 14 days and interval dependent on need for tether re-insertion
Rationale: These studies are designed to test the feasibility of Pluripotent stem cell derived cardiomyocyte therapy for the treatment of post-myocardial cardiac dysfunction. Therefore it is necessary to create the initial cardiac insult before delivering the therapy. The group has designed the infarct creation to be percutaneous in nature to minimize complications and distress to animals. It is necessary to document the degree of cardiac dysfunction and the effect of the cell therapy by echocardiography. This is a minimally invasive procedure that is not anticipated to cause significant distress to the animals.

OR
Version #10
Multiple Major Surgery:
1. Myocardial infarct via percutaneous creation with tether insertion; possible insertion of cardiac monitor (version #10)
2. Sternotomy or left lateral thoracotomy for cell therapy injection and transplantation, possible placement of leads in thorax (version #10), 14 days following #1
3. Tether re-insertion (only if initial tether fails), time depending on circumstances following #1 or #2
Species: M. nemestrina
Interval: 14 days and interval dependent on need for tether re-insertion
Rationale: These studies are designed to test the feasibility of Pluripotent stem cell derived cardiomyocyte therapy for the treatment of post-myocardial cardiac dysfunction. Therefore it is necessary to create the initial cardiac insult before delivering the therapy. The group has designed the infarct creation to be percutaneous in nature to minimize complications and distress to animals. It is necessary to document the degree of cardiac dysfunction and the effect of the cell therapy by echocardiography. This is a minimally invasive procedure that is not anticipated to cause significant distress to the animals.

OR
Version #17
Multiple Major Surgery:
1. Baseline EP study, Myocardial infarct with tether insertion
2. Cell therapy injection, cardiac monitor and possible lead placement in the thorax, 14 days following #1
3. Post infarct EP study, 14 days following #2
4. Tether re-insertion (only if initial tether fails), time depending on circumstances following #1 or #2 or #3
Species: M. nemestrina
Interval: 14 days and circumstance dependent on need for tether re-insertion
Rationale: These studies are designed to test the feasibility of Pluripotent stem cell derived cardiomyocyte therapy for the treatment of post-myocardial cardiac dysfunction. Therefore it is necessary to create the initial cardiac insult before delivering the therapy. The group has designed the infarct creation to be percutaneous in nature to minimize complications and distress to animals. It is necessary to document the degree of cardiac dysfunction and the effect of the cell therapy by echocardiography. This is a minimally invasive procedure that is not anticipated to cause significant distress to the animals. The EP studies are designed to study the possible mechanism of ventricular arrhythmias that occur in infarct heart after cell therapy. Therefore, it is necessary to do the baseline EP study, to create myocardial infarct before cell therapy. The EP studies and infarct surgery will be performed percutaneously to minimize complications and distress to animals.

OR
Version #18
Multiple Major Surgery:

1. Baseline EP study, Myocardial infarct with tether insertion
2. Cell therapy injection, cardiac monitor and possible lead placement in the thorax, 14 days following #1
3. Post infarct EP study, 14 days following #2
4. Removal of the telemetry transmitters and leads (only if DSI telemetry is used to monitor heart rhythm), 13 days following #3
5. Tether re-insertion (only if initial tether fails), time depending on circumstances following #2 or #3 or #4

Species: M. nemestrina
Interval: 13 or 14 days and circumstance dependent on need for tether re-insertion

Rationale: These studies are designed to study the possible mechanism of ventricular arrhythmias which occur in infarcted heart after cell therapy. Therefore, it is necessary to do the baseline EP study, to create myocardial infarct before cell therapy. The EP studies and infarct surgery will be performed percutaneously to minimize complications and distress to animals. Besides, in order to accurately assess cardiac functions and the therapeutic effects of hESC-CM transplantation, cardiac MRI is necessary. However, the transmitter is not compatible with MRI machine and may induce the transmitter migration to hurt the animals. Therefore, we have to perform a survival surgery to remove the transmitter and its leads prior to 2nd cardiac MRI study.

Multiple Major Survival Surgery:

Path A
Pre-surgical procedure: MRI (anesthesia only)
1. Skull Surgery: HS
2. Skull Surgery: CI, minimum 1 week after #1
3. Arm EMG Implant I, minimum 1 week after #2
4. Subcutaneous (SubQ) wires from head-back I, minimum 1 week after #3
5. Repair Surgery I, amount of time will depend on need, but will be preceded by veterinary and pre-survival assessment

Interval: minimum of 1 week

Rationale for the above surgeries in a single animal: Where possible, multiple procedures are performed during a single surgery. For some animals, the BCI implant is custom-machined to a physical mold of each monkey’s skull, to optimize fit and minimize weight and size. This machining process necessitates a delay between surgeries 1 and 2. In some animals we will also use this period to map the physiological properties of a brain area to aid placement of permanent electrodes in surgery 2. Surgeries 3 and 4, if performed, require separate surgeries due to their length and complexity. Veterinarians at the Primate Center concur with the use of multiple surgeries to implant these devices. These surgeries involve exposure of separate areas, so no time would be saved by combining either with any other procedure. Such a combined surgery would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of
mortality during surgery, and prolong post-surgical recovery.
Subcutaneous wires (surgery 4) are needed only if transcutaneous EMGs are used, in order to connect to the implant in Skull Surgery: CI, to monitor EMG activity during free behavior.
Specific experiments that will use this surgical chain  This path concerns experiments that involve cortical recording or stimulation, in the booth or in free behavior, sometimes in conjunction with muscle (EMG) recording.

1. Operant conditioning of cortical activity, in the booth or in free behavior (Experiment group A-I)
2. Cortical activity-triggered cortical stimulation, in the booth or in free behavior (Experiment group A-I)
3. Paired-pulse cortical stimulation, in the booth or in free behavior (Group A-I)
4. EMG-triggered cortical stimulation, in the booth (Experiment group A-II)
5. EMG-triggered cortical stimulation in free behavior (Experiment group A-III)

Path B.
1. Skull Surgery: HS (Halo Straps only)
2. Skull Surgery: CI, minimum 4 weeks after #1
3. Arm EMG implant, minimum 1 week after #2
4. Subcutaneous (SubQ) wires from head-back, minimum 1 week after #3
5. Spinal implant, minimum 1 week after #4
6. Repair surgery, amount of time will depend on need, but will be preceded by veterinary and pre-surgery assessment
Interval: minimum 1-4 weeks
Rationale for the above surgeries in a single animal: Where possible, multiple procedures are performed during a single surgery.
For some animals, the BCI implant is custom-machined to a physical mold of each monkey's skull, to optimize fit and minimize weight and size. This machining process necessitates a delay between surgeries 1 and 2. In some animals we will also use this period to map the physiological properties of a brain area to aid placement of permanent electrodes in surgery 2.
Surgeries 3, 4 and 5, if performed, require separate surgeries due to their length and complexity.
Veterinarians at the Primate Center concur with the use of multiple surgeries to implant these devices. These surgeries involve exposure of separate areas, so no time would be saved by combining either with any other procedure. Such a combined surgery would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of mortality during surgery, and prolong post-surgical recovery.
Subcutaneous wires (surgery 4) are needed only if transcutaneous EMGs are used, in order to connect to the implant in Skull Surgery: CI, to monitor EMG activity during free behavior.
Specific experiments that will use this surgical chain  This path includes experiments that may involve spinal recording and/or stimulation in the booth or in free behavior, sometimes in conjunction with cortical recording and/or stimulation or muscle (EMG) recording.

1. Operant conditioning of cortical activity, in the booth or in free behavior (Experiment group B-I)
2. Cortical activity-triggered cortical stimulation, in the booth or in free behavior (Experiment group B-I)
3. Paired-pulse cortical stimulation, in the booth or in free behavior (Group B-I)
4. EMG-triggered cortical stimulation, in the booth (Experiment group B-II)
5. EMG-triggered cortical stimulation in free behavior (Experiment group B-III)
6. Cortical activity-triggered spinal stimulation, in the booth or in free behavior (Experiment group B-IV)
7. Spinal activity-triggered spinal stimulation, in the booth or in free behavior (Experiment group B-IV)
<p>| | |</p>
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<tbody>
<tr>
<td>8.</td>
<td>EMG activity-triggered spinal stimulation, in the booth or in free behavior (Experiment group B-IV)</td>
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<tr>
<td>9.</td>
<td>Paired-pulse cortical and spinal stimulation, in the booth or in free behavior (Experiment group B-IV)</td>
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<tr>
<td>10.</td>
<td>Measurement of spinal stimulation output via stimulus-evoked EMG activity, in the booth or in free behavior (Experiment group B-IV)</td>
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Path C
1. Skull Surgery: HS
2. VNS implant, minimum 4 weeks after #1
3. Arm EMG implant, minimum 1 week after #2
4. Spinal implant, minimum 1 week after #3
5. Repair Surgery, amount of time will depend on need, but will be preceded by veterinary and presurvival assessment

Interval: minimum 1-4 weeks

Rationale for the above surgeries in a single animal: Where possible, multiple procedures are performed during a single surgery. Surgeries 2, 3 and 4, if performed, require separate surgeries due to their length and complexity. Even though surgery 4 is relatively short, surgeries 3 and 5 are particularly lengthy and complex, making it impossible to perform in combination. Veterinarians at the Primate Center concur with the use of multiple surgeries to implant these devices. These surgeries involve exposure of separate areas, so no time would be saved by combining either with any other procedure. Such a combined surgery would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of mortality during surgery, and prolong post-surgical recovery.

Subcutaneous wires (surgery 4) are needed only if transcutaneous EMGs are used, in order to connect to the implant in Skull Surgery: CI, to monitor EMG activity during free behavior.

Specific experiments that will use this surgical chain: This path concerns experiments in the booth, that involve cortical recording and/or stimulation using a cortical chamber (see description of Surgery HS), sometimes in conjunction with muscle (EMG) recording or spinal recording and/or stimulation, and VNS.

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<tbody>
<tr>
<td>1.</td>
<td>Cortical activity-triggered cortical stimulation (Experiment group C-I)</td>
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<tr>
<td>2.</td>
<td>Cortical activity-triggered cortical stimulation, with and without VNS (within subject comparison), in booth (Experiment group C-II)</td>
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<tr>
<td>3.</td>
<td>Paired-pulse cortical stimulation, with and without VNS (within subject comparison), in booth (Experiment group C-II)</td>
</tr>
<tr>
<td>4.</td>
<td>EMG-triggered cortical stimulation, with and without VNS (within subject comparison), in booth (Experiment group C-III)</td>
</tr>
<tr>
<td>5.</td>
<td>Cortical activity-triggered spinal stimulation, in the booth (Experiment group C-IV)</td>
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<tr>
<td>6.</td>
<td>Spinal activity-triggered spinal stimulation, in the booth (Experiment group C-IV)</td>
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<tr>
<td>7.</td>
<td>EMG activity-triggered spinal stimulation, in the booth (Experiment group C-IV)</td>
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<tr>
<td>8.</td>
<td>Paired-pulse cortical and spinal stimulation, in the booth (Experiment group C-IV)</td>
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<tr>
<td>9.</td>
<td>Measurement of spinal stimulation output via stimulus-evoked EMG activity, in the booth (Experiment group C-IV)</td>
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<tr>
<td>10.</td>
<td>Cortical activity-triggered spinal stimulation, with and without VNS (within subject comparison), in booth (Experiment group C-IV)</td>
</tr>
<tr>
<td>11.</td>
<td>Paired-pulse cortical and spinal stimulation, with and without VNS (within subject comparison), in booth (Experiment group C-IV)</td>
</tr>
</tbody>
</table>

Path D
1. Skull Surgery: HS (Halo Straps only)
2. Skull Surgery: CI, minimum 4 weeks after #1
3. VNS implant, minimum 1 week after #2
4. Arm EMG implant, minimum 1 week after #3
5. Subcutaneous (SubQ) wires from head-back, minimum 1 week after #4
6. Spinal implant, minimum 1 week after #5
7. Repair surgery, amount of time will depend on need, but will be preceded by veterinary and pre-survival assessment
Interval: minimum 1-4 weeks

Rationale for the above surgeries in a single animal: Where possible, multiple procedures are performed during a single surgery.
For some animals, the BCI implant is custom-machined to a physical mold of each monkey's skull, to optimize fit and minimize weight and size. This machining process necessitates a delay between surgeries 1 and 2. In some animals we will also use this period to map the physiological properties of a brain area to aid placement of permanent electrodes in surgery 2.
Surgeries 3, 4, 5 and 6 if performed, require separate surgeries due to their length and complexity.
Even though surgeries 3 and 5 are relatively short, surgeries 4 and 6 are particularly lengthy and complex, making it impossible to perform in combination. Veterinarians at the Primate Center concur with the use of multiple surgeries to implant these devices. These surgeries involve exposure of separate areas, so no time would be saved by combining either with any other procedure. Such a combined surgery would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of mortality during surgery, and prolong postsurgical recovery.
Subcutaneous wires (surgery 4) are needed only if transcutaneous EMGs are used, in order to connect to the implant in Skull Surgery: CI, to monitor EMG activity during free behavior.

Specific experiments that will use this surgical chain: This path concerns experiments that involve cortical recording and/or stimulation, in the booth or in free behavior, in conjunction with VNS or intracortical delivery of chemical substances, sometimes combined with spinal recording and/or stimulation and muscle (EMG) recording.

1. Cortical activity-triggered cortical stimulation, with and without intracortical delivery of chemical substances (within subject comparison), in booth and in free behavior (Experiment group D-I)
2. Paired-pulse cortical stimulation, with and without intracortical delivery of chemical substances (within subject comparison), in booth and in free behavior (Experiment group D-I)
3. Cortical activity-triggered cortical stimulation, with and without VNS (within subject comparison), in booth and in free behavior (Experiment group D-II)
4. Paired-pulse cortical stimulation, with and without VNS (within subject comparison), in booth and in free behavior (Experiment group D-II)
5. EMG-triggered cortical stimulation, with and without VNS (within subject comparison), in booth (Experiment group D-III)
6. EMG-triggered cortical stimulation, with and without intracortical delivery of chemical substances (within subject comparison), in booth and in free behavior (Experiment group D-III)
7. EMG-triggered cortical stimulation, with and without VNS (within subject comparison), in booth and in free behavior (Experiment group D-IV)
8. Cortical activity-triggered spinal stimulation, with and without VNS (within subject comparison), in booth and in free behavior (Experiment group D-V)
9. Paired-pulse cortical and spinal stimulation, with and without VNS (within subject comparison), in booth and in free behavior (Experiment group D-V)
10. Cortical activity-triggered spinal stimulation, with and without intracortical delivery of chemical substances (within subject comparison), in booth and in free behavior (Experiment group D-V)
Path E
1. Skull Surgery: HS (Halo Straps only)
2. Skull Surgery: CI: CI, minimum 4 weeks after #1
3. Nerve Block Implant, minimum 1 week after #2
4. Skull Surgery: CI (repeated for multiple Utah arrays, if needed), minimum 1 week after #3
5. Nerve Block Implant, minimum 1 week after #4
6. Repair surgery, amount of time will depend on need, but will be preceded by veterinary and pre-surgical assessment

Interval: minimum 1-4 weeks

Rationale for the above surgeries in a single animal: Where possible, multiple procedures are performed during a single surgery (i.e.: the halo frame is attached during Skull Surgery: CI). For animals utilizing the halo head restraint, a delay of 4 weeks or more is ideal for surgeries 1 and 2 to allow titanium plates to osseointegrate with the animal's skull, providing increased implant stability.

Repeating Skull Surgery: CI will allow us to compare multiple brain areas in the same animal. This will reduce the number of animals needed for the study and permit quantitative comparisons between brain areas while removing variations in skill among animals.

Nerve Block Implants are short-lived and may need to be re-implanted up to 3 times, allowing ample recovery time between each (typically 4 weeks or more).

Specific experiments that will use this surgical chain: This path concerns experiments that involve cortical recording in conjunction with a peripheral nerve block, sometimes in conjunction with muscle stimulation.

1. Operant conditioning of cortical activity, with and without nerve block (within animal comparison), in booth and in free behavior (Experiment groups E-I and E-III)
2. Cortical activity-triggered muscle stimulation after nerve block, in booth and in free behavior (Experiment groups E-II, E-III and E-IV)

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Multiple Major Surgery:

NOTE: A09044: The USDA multiple surgery exemption has expired for this monkey – no more surgeries can be performed.)
1. Implant eye coil and head stabilizing fitting
2. Implant recording chamber, minimum of 30 days following #1
3. Permanent cerebellar lesion, minimum of 30 days following #2
4. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment.

Interval: 30 days minimum

Species: M. mulatta

Rationale: Surgery 1 is necessary to train the monkey to make the eye movements for this study and, later, to collect the movement data for this study. Surgery 2 is necessary to record the activity of brain neurons. We need to do this to locate specific brain structures and to collect the neuron response data for this study. We combine Surgeries 1 & 2 into one surgery for animals in which we inject neuroanatomical tracers into specific brain structures, without recording the properties of neurons in these structures. In these animals we need only spontaneous eye movements. We do not combine surgeries 1 & 2 into a single surgery if we need to train monkeys to make specific eye movements. Doing so extends the duration of that surgery and gives a monkey a chamber for a month before we can use it. Surgery 3 is necessary to collect the data for this work. Surgery 4 is a potential repair surgery as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment.

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Multiple Major Surgery:

1. Thymectomy
2. Thymus grafting

Species: Mice

Interval: 3 weeks
Rationale: Removal of thymus to ascertain function of implanted thymic lobes. The time between the surgeries is required for the maturation of newly emigrated T cells, so the thymectomy and thymus grafting cannot be performed at the same time.

Multiple Major Surgery:
1. Lesion of optic tract or thalamus or enucleation
2. Craniotomy to inject anatomical tracers, minimum of 2 weeks following #1
Species: Rats
Interval: 2 weeks minimum
Rationale: The second surgery is necessary for administering the anatomical tracers that will reveal the effect that early deprivation of retinal input has on the development of the brain.

OR
1. Craniotomy to inject anatomical tracers to reveal callosal pattern in opposite hemisphere
2. Craniotomy to implant electrodes for electrophysiological recording and injection of different tracer in the same recording session, minimum of 2-3 days following #1
Species: Rats
Interval: 2-3 days
Rationale: Anatomical features revealed with the first tracer injections will be correlated with electrophysiological data. The first tracer injections are not done during the recording session because in preliminary experiments, the group discovered that the recording procedure reduces the transport of the tracer used. During the recording session, some sites will be identified electrophysiologically and injected with specific tracers. Survival time from the recording session is required to allow transport time for the tracers injected during recording. The second tracer injection will provide data about connectivity of identified sites.

OR
1. Intracortical injections of MnCl2
2. Intracortical injections of HRP, 3.5-4.5 d following #1
Species: Rats
Interval: 3.5-4.5 days
Rationale: Separate injections of MnCl2 and HRP will be done because HRP may interfere with the transport or MRI properties of MnCl2. Intracortical injections of MnCl2 reveal ocular dominance columns in contralateral hemisphere by MRI. HRP will 1) superimpose anatomical HRP data to MRI data to select region of interest, and 2) allow analysis of whether or not prior injections of MnCl2 compromise ability of neurons for transporting HRP through toxicity or physical trauma.

Multiple Major Surgery:
1. Hepatic branch vagotomy
2. Intraparenchymal injections/Brain cannulation and catheterization of carotid artery and jugular vein, a minimum of 7 days following #1
Species: Rats
Interval: 7 days minimum
Rationale: This laboratory focuses on central mechanisms regulating energy balance and insulin sensitivity. In this study, the aim is to determine whether the hypothalamic effect of leptin on peripheral insulin sensitivity involves activation of hepatic efferent vagal fibers. To accomplish this, the lab will perform selective hepatic branch vagotomy (HV) or a sham operation in leptin receptor-deficient (KO) rats. A second surgical session is needed in which animals will receive intraparenchymal injections and be implanted with a catheter to the right jugular vein and left carotid artery. Seven days later, euglycemic-hyperinsulinemic clamp studies will then be performed to measure sensitivity. The reason for this second surgical session is that the hepatic vagotomy is expected to have small but significant changes on food intake and body weight that will significantly affect insulin sensitivity. Therefore it is necessary to give sufficient time for animals to recover before performing intraparenchymal injections and implanting catheters. If all surgeries were conducted in one single session, if changes in insulin sensitivity using the clamp were detected, the interpretation of the experiments would be impossible because of the inability to distinguish whether
these effects were due to the experimental treatment or because animals simply lost weight after surgery.

OR
1. Subdiaphragmatic vagal deafferentation
2. Intra-parenchymal injections/Brain cannulation and catheterization of carotid artery and jugular vein, a minimum of 7 days following #1
Species: Rats
Interval: 7 days minimum
Rationale: This laboratory focuses on central mechanisms regulating energy balance and insulin sensitivity. In this study, the aim is to determine whether afferent vagal fibers are required for the effects of hypothalamic effect of leptin on peripheral insulin sensitivity. To accomplish this, the lab will to perform selective vagal deafferentation or a sham operation in leptin receptor-deficient (Koletsky) rats. A second surgical session is needed, in which animals will receive intra-parenchymal injections and be implanted with a catheter to the right jugular vein and left carotid artery. Seven days later, euglycemic-hyperinsulinemic clamp studies will then be performed to measure sensitivity. The reason for this second surgical session is that the selective vagal deafferentation is expected to have small but significant changes on food intake and body weight that will significantly affect insulin sensitivity. Therefore it is necessary to give sufficient time for animals to recover before performing intra-parenchymal injections and implanting catheters. If all surgeries were conducted in one single session, if changes in insulin sensitivity using the clamp were detected, the interpretation of the experiments would be impossible because of the inability to distinguish whether these effects were due to the experimental treatment or because animals simply lost weight after surgery.

OR
1. Brain cannulation
2. Catheterization of carotid artery and jugular and portal veins, a minimum of 7 days following #1
Species: Rats
Interval: 7 days minimum
Rationale: To determine the role of the sympathetic nervous system in mediating the central effects of leptin on glucose metabolism, animals will receive an ICV cannula. In addition animals will receive catheters to the jugular vein, carotid artery and portal vein. For some experiments, the surgeries will be separated by at least one week to enable animals to recover from surgery and verify cannula placement. The main reason for the separate surgical sessions is that the patency of the catheters reduces over time and if the surgeries were completed in the same surgical session, a greater percentage of catheters would be blocked and those animals would not be able to be used for the experiment.

OR
1. Brain cannulation
2. Peripheral parenchymal injections and implantation of osmotic minipump, a minimum of 7 days following #1
Species: Rats
Interval: 7 days minimum
Rationale: In this study, the aim is to determine the peripheral mechanisms whereby leptin suppresses hepatic glucose production (HGP) in uDM. The lab’s hypothesis is that in uDM, increased sympathetic outflow through sympathetic post-ganglionic neurons located in the celiac ganglion stimulates glucagon release, while increasing sympathetic outflow to the liver, thereby increasing HGP, and this effect is suppressed by leptin action in the brain. This study requires the brain cannulation and the implantation of a mini-pump to be in separate surgical sessions. The reason for this is that the drug being infused by the mini-pump is expected to have small but significant changes on food intake and body weight. Therefore it is necessary to give sufficient time for an animal to recover after the brain cannulation surgery and return to normal food intake and
body weight before investigating the actions of the drug administered by mini-pump. If not, it is impossible to distinguish whether changes in feeding or body weight are due to the drug or simply differences in recovery after surgery.

or version 174
1. Catheterization of carotid artery and jugular veins (to be done by vendor).
2. Brain cannulation at least 7 days post #1
Species: Rats
Interval: 7 days minimum
Rationale: To determine whether the CNS glucose-lowering effects in uncontrolled diabetes are due to a suppression of hepatic glucose production or an increase in tissue glucose uptake, animals are required to have catheters implanted to the jugular vein and carotid artery and also be implanted with a cannula to the brain. The rationale and justification for reversing the surgical order for this experiment and purchasing rats already bearing catheters to the jugular vein and carotid artery from an approved rodent vendor and subsequently implanting a brain cannula are several fold: 1) It allows us to study a larger cohort of animals with all the necessary controls as one group. This significantly reduces variation when performing these sensitive metabolic studies over two or three groups. We are otherwise limited on the time required to perform the necessary catheter surgeries. 2) It minimizes the potential loss of viable animals due to either complications with the surgical implantation of catheters or the failure to have two successfully patent catheters and 3) Harlan has developed and uses a rounded tip catheter which increases the patency of the catheter and reduces maintenance requirements (please see attached) which previously was a concern. We have validated this from our own experience in catheterized animals from Harlan that did not receive brain surgery.

OR (version 192)
1. Intracerebroventricular Cannulation
2. Vertical Sleeve Gastrectomy and Indwelling Jugular Vein and Carotid Artery Catheter Placement, 7 days after #1
Species: Rats
Interval: 7 days
Rationale: Indwelling catheters are patent for ~14-21 days after placement. Since animals are allowed to recover for 14 days after VSG surgery, placement at the time of VSG surgery ensures that most catheters will be patent at the time of terminal experiment and euthanasia. Performing icv cannulation at the same time as VSG and catheter placement would prolong anesthesia, be overly stressful for animals, and increase perioperative morbidity and mortality.

Multiple Major Surgery:
1. Laparotomy, Week 4 (~4 weeks post inoculation)
2. Laparotomy and Cervical Biopsy, approximately 4 weeks following #1 (Week 8)
Species: M. nemestrina
Interval: 4 weeks
Rationale: This experiment is to establish a model of ascending cervical infection with M. genitalium in an animal closely resembling human. In women M. genitalium infection is associated with serious upper tract sequelae including chronic pelvic pain, infertility and preterm birth. The hypothesis being tested is that once cervical infection is established in the primates M. genitalium will ascend to Fallopian tube tissues and can be recovered from this site in the weeks following inoculation. In order to increase the likelihood of recovering M. genitalium from tubal tissues tissue biopsies at two time points. One biopsy is collected from each Fallopian tube: the right side will be biopsied at Week 4 and the left side will be biopsied at Week 8 in five M. genitalium-inoculated primates. The optimum time for Fallopian tube biopsy will be established from this set of experiments (i.e., 4 or 8 weeks post Mg inoculation). A single cervical biopsy will be collected at the end of the experiment to assess immune cell infiltrates and to determine where M. genitalium is localized in the cervical tissue. The cervical biopsy will be collected at the same time as the Fallopian tube biopsy (i.e., during the same sedation), although the biopsies will be collected via different routes to avoid contamination of
upper tract tissues with lower tract bacteria.

OR
1. Laparotomy, Week 4 (~4 weeks post PBS-inoculation)
2. Cervical Biopsy, approximately 4 weeks following #1 (Week 8)
3. Laparotomy, approximately 8 weeks following #2 (Week 16)
4. Cervical Biopsy, approximately 4 weeks following #3 (Week 20)
Species:  M. nemestrina
Interval:  4-8 weeks

Rationale: The primate will receive two inoculations, the first with PBS then a second with M. genitalium, and a total of four biopsy procedures. Cervical biopsies will occur at Week 8 (8 weeks after PBS inoculation) and Week 20 (8 weeks after Mg inoculation). The time points for collection of the two Fallopian tube biopsies will be guided by results from the five Mg-inoculated primates described above. For example, if Mg is found in the Fallopian tube biopsies at Week 4 following Mg inoculation then this primate will have Fallopian tube biopsies done at Week 4 (4 weeks post PBS inoculation) and Week 16 (4 weeks post Mg inoculation). However, if Week 8 is the optimum time to detect Mg in Fallopian tube biopsies then the Fallopian tube biopsies in this primate will occur 8 weeks post PBS inoculation and then 8 weeks post Mg inoculation (Week 8 and Week 20, respectively). In the latter case, the cervical and Fallopian tube biopsies will occur at the same time (under the same anesthesia), thus combining Surgery 1 with Surgery 2 and Surgery 3 with Surgery 4. This primate will therefore undergo at least two surgeries but not more than four surgeries; this table outlines the maximum number of surgeries this primate will experience.

<table>
<thead>
<tr>
<th>Procedure</th>
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<tr>
<td>Multiple Major Surgery:</td>
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<tr>
<td>1. Partial Renal Ablation of Right Kidney</td>
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<tr>
<td>2. Total Left Nephrectomy, 2 weeks following #1</td>
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<tr>
<td>Interval:  2 weeks</td>
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<tr>
<td>Species:  Mice</td>
</tr>
<tr>
<td>Rationale: It is necessary to perform a 2-step renal surgery model because the animals will not</td>
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<tr>
<td>recover if both surgeries are done at the same time. Two surgeries provides for better survival</td>
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<td>and better induction of uremia.</td>
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<tr>
<th>Procedure</th>
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<tbody>
<tr>
<td>Multiple Major Surgery:</td>
</tr>
<tr>
<td>1. Laparotomy for oocyte harvest</td>
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<tr>
<td>2. Laparotomy for oocyte harvest, minimum of 1 month following #1</td>
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<tr>
<td>3. Laparotomy for oocyte harvest, minimum of 1 month following #2</td>
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<tr>
<td>4. Laparotomy for oocyte harvest, minimum of 1 month following #3</td>
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<tr>
<td>5. Laparotomy for oocyte harvest, minimum of 1 month following #4</td>
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<tr>
<td>6. Terminal laparotomy for oocyte harvest, minimum of 1 month following #5</td>
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<tr>
<td>Species:  Xenopus</td>
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<tr>
<td>Interval:  1 month minimum</td>
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<tr>
<td>Rationale: If a single animal has viable oocytes then multiple surgeries will allow researchers</td>
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<td>to collect the most data possible per animal.</td>
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<th>Procedure</th>
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<tr>
<td>Control Animals</td>
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<tr>
<td>Multiple Major Surgery:</td>
</tr>
<tr>
<td>1. Endoscopy # 1, Colon pinch biopsies collected at baseline</td>
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<tr>
<td>2. Endoscopy # 2, Colon pinch biopsies, approximately 30 weeks (baseline to 4 weeks post</td>
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<tr>
<td>immunization) following #1</td>
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<tr>
<td>3. Laparotomy # 1, Jejunum resection (15 cm section), minimum of 9 weeks (4 weeks pre virus</td>
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<td>challenge to 4 weeks post infection) following #2</td>
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<td>Species:  M. Mulatta</td>
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<tr>
<td>Interval:  At least 9 weeks - 30 weeks</td>
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<tr>
<td>Rationale: The gut is a predominant immunological organ known to be targeted by primate</td>
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<tr>
<td>lentiviruses (e.g. SIV, HIV) early in the infection process. Assessing gut mucosa by endoscopic</td>
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<tr>
<td>pinch biopsies or small intestine resections have proven an invaluable source of affected</td>
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<td>immune cells in this sort of study. Serial biopsies are essential to profile the immune response</td>
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<tr>
<td>to immunization and</td>
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| 2856-01 | Multiple Major Surgery:  
1. ICV injection of AAV vector  
2. Middle cerebral artery occlusion 3 Months after #1  
Species: Mice  
Interval: 3 Months  
Rationale: We need to reduce HDAC expression via shRNA virus injection prior to MCAO procedure. |
| 2950-01 | Multiple Major Surgery:  
1. Ovariectomy  
2. Craniotomy, 5-14 days following #1  
Species: Rats  
Interval: 5-14 days  
Rationale: Rats must recover for 5-14 days prior to entering study so that hormone levels can normalize. |
| 2968-04 | Multiple Major Surgery:  
1. Uninephrectomy  
2. Kidney polectomy, 1 week after #1  
Species: Mice  
Interval: 1 week  
Rationale: Removal of 5/6 of the renal mass all at once is often fatal for mice. Therefore, renal mass is reduced more gradually to allow the mouse to adapt. This means that a uninephrectomy is performed first, and the animal is allowed to recover from this change. Then a second surgery to remove 2/3 of the remaining kidney is performed. Survival is much better when the reduction in renal mass is performed this way. |
| 3055-09 | Multiple Major Surgery:  
Option I  
1. MCAO +/- radio telemeter implantation  
2. Reperfusion  
Species: Rat  
Interval: 2 hours  
Rationale: The first surgery is MCAO. The second surgery allows for reperfusion and access of the |
brain tissue by the immune system. It is much safer to allow the animal to recover between these short surgeries than to keep it anesthetized for 2 hours.

OR
Option II
1. Splenic nerve dissection
2. MCAO +/- radio telemeter implantation 4 days to 2 weeks following #1
3. Reperfusion 2 hours following #2

Species: Rat
Interval: 2 hours to 2 weeks
Rationale: The first surgery is done to prepare for the animal for the experiment - i.e., to prevent sympathetic nerve discharges to the spleen at the time of the stroke. The delay between this splenic nerve dissection and MCAO is to allow the animal to fully recover from surgery and to be certain the spleen is truly denervated. The third surgery is the reperfusion that is necessary to allow blood flow to return to the MCA (and thus the immune system access to the brain tissue). It is much safer to allow the animal to recover between these short surgeries than to keep it anesthetized for 2 hours.

Multiple Major Surgery:
1. Ischemia Reperfusion/Permanent ligation of left coronary artery
2. Thoracotomy for cell injection/Fibrin scaffold implant (rats only), 5 days to 2 weeks following #1
Species: Mice and Rats
Interval: 5 days - 2 weeks
Rationale: In many cases it is necessary to perform 2 survival surgeries. These experiments involve one surgery to induce cardiac injury and a second to administer cells/scaffolds directly into the injury during the healing phase.

Multiple Major Surgery:
1. Ischemia Reperfusion/Permanent ligation of left coronary artery
2. Thoracotomy for AV injection, 2 to 8 weeks following #1
Species: Mice and Rats
Interval: 2 - 8 weeks
Rationale: In many cases it is necessary to perform 2 survival surgeries. These experiments involve one surgery to induce cardiac injury and a second to administer AV directly into the injury during the healing phase.

Multiple Major Surgery:
1. Craniotomy for fluid percussion injury (or sham or naive)
2. Headset and ECoG electrode implantation, minimum 3 days following #1
3. Headset/ECoG electrode repair, if needed, minimum 1 week following #2
Species: Rats
Interval: 3 days - 1 week minimum
Rationale: Later implantation of electrodes in a second surgery is typically necessary in some animals for one of two reasons. 1) Age is a factor that affects epileptogenesis in humans. Children are more at risk of posttraumatic epileptogenesis. Also, neuronal death is age dependent. We want to study epileptogenesis as it applies to the younger human population. 2) Acute post-injury righting time may be tested as an acute predictor of later epileptogenesis. Therefore, some animals need to emerge from anesthesia after FPI for the righting time to be assessed, in which case electrodes cannot be implanted immediately after FPI. Animals previously implanted with ECoG electrodes may later lose the original headset. This may occur because the animals manage to dislodge it. The longer the study post-injury the more likely it is that the rat will manage to dislodge it. In our experience, ~20% of animals manage to dislodge the headset by 3 months post-injury. If an animal loses the headset before 3 weeks post-FPI, it will be euthanized because the loss is likely unrecoverable because the FPI craniotomy may not have fully healed. If rats lose the headset 3 weeks after FPI or later, then the skull has healed, and gentamycin will be applied daily until the scalp has healed, and then the rat will undergo another ECoG electrode implantation. No more than
one re-implantation is ever performed per each rat.

OR
1. Craniotomy for fluid percussion injury (or sham or naive)
2. Headset and ECoG electrode and cooling coil implantation, 12 hrs to 6 weeks following #1
3. Headset/ECoG repair, if needed, minimum 1 week post #2
Species: Rats
Interval: 12hrs - 6 weeks
Rationale: ECoG electrode implantation plus cooling coil implantation, which is meant to implant EEG electrodes and cooling coil 1 week after FPI, rather than in one single procedure together with FPI. This has become necessary because, despite no adverse neurological signs, histopathology obtained from rats injured with procedure ECoG electrode and cooling coil implantation showed acute brain pathology more severe than that induced by FPI alone. The acute implantation of cooling coil on the edematous brain may worsen the pathology induced by FPI. ECoG electrode implantation repair - animals previously implanted with ECoG electrodes may later lose the original headset. This may occur because the animals manage to dislodge it. The longer the study post-injury the more likely it is that the rat will manage to dislodge it. In our experience, ~20% of animals manage to dislodge the headset by 3 months post-injury. If an animal loses the headset before 3 weeks post-FPI, it will be euthanized because the loss is likely unrecoverable because FPI craniotomy may not have fully healed. If rats lose the headset 3 weeks after FPI or later, then the skull has healed, and gentamycin will be applied daily until the scalp has healed (typically 10-14 days), and then the rat will undergo procedure ECoG electrode implantation. No more than one re-implantation is ever performed per each rat.

OR
1. Craniotomy for fluid percussion injury (or sham or naive)
2. Headset and ECoG electrode and cooling coil implant, minimum 12 hrs following #1
3. Cooling piece replacement, minimum of 1 week post #2
4. Cooling piece replacement, minimum of 1 week post #3
Species: Rats
Interval: 12 hrs - 1 week minimum
Rationale: Cooling device replacement - since the cooling device cannot be turned on/off, as it cools by heat dissipation, to test its antiepileptogenic effect it needs to be removed from the headset and replaced with an equally sized biocompatible sterile silicone rod that does not dissipate heat. To test the antiepileptic effect, animals must first become epileptic with a dummy device implanted, then switch the dummy with the cooling device to test the effect, and then switch the cooling device with the dummy rod to acquire a post-cooling baseline. The second baseline is necessary to determine whether seizures return, as expected for a true antiepileptic effect, and to put the antiepileptic effect of cooling in context with the one of the other antiepileptic drugs.

OR
1. FPI (or sham or naive) + Headset and ECoG electrode implantation
2. Headset and ECoG electrode implantation repair, minimum of 1 week following #1
Species: Rats
Interval: 1 week minimum
Rationale: ECoG electrode implantation repair-animals previously implanted with ECoG electrodes may later lose the original headset. This may occur because the animals manage to dislodge it. The longer the study post-injury the more likely it is that the rat will dislodge it. About 20% of animals manage to dislodge the headset by 3 months post-injury. If an animal loses the headset before 3 weeks post-FPI, it will be euthanized because the loss is likely unrecoverable because FPI craniotomy may not have fully healed. If rats lose the headset 3 weeks after FPI or later, then the skull has healed, and gentamycin will be applied daily until the scalp has healed (typically 10-14 days), and then the rat will undergo procedure ECoG electrode implantation repair. No more than one re-implantation is ever performed per each rat.
OR
1. FPI (or sham or naive) + cooling coil
2. ECoG electrode and cooling coil implantation, minimum of 3 days following #1
Species: Rats
Interval: 3 days minimum
Rationale: ECoG electrode implantation following FPI - Later implantation of electrodes in a second surgery is typically necessary in some animals for one of three reasons. 1) Age is a factor that affects epileptogenesis in humans. Children are more at risk of posttraumatic epileptogenesis. Also neuronal death is age dependent. We want to study epileptogenesis as it applies to the younger human population. 2) Acute post-injury righting time may be tested as an acute predictor of later epileptogenesis. Therefore, some animals need to emerge from anesthesia after FPI for the righting time to be assessed, in which case electrodes cannot be implanted immediately after FPI. The timing establishes a balance between the need to start recording EEG as early as possible after injury, with the need to wait to not lose the animal's headset. Indeed, the younger the age at which the electrodes get implanted, the more likely the headset will be dislodged as the animal grows. 3) Because some studies involve determining whether a correlation exists between acute abnormalities in rat EEG after head injury and the later probability to develop posttraumatic epilepsy, there is need for both FPI + ECoG electrode implantation and FPI + later ECoG electrode implantation.

OR
1. FPI
2. ECoG electrode and dummy cooling, or cooling device implantation (+Peltier cell in some cases; #16.4). Animals that receive the peltier cell will not receive any additional survival surgeries.
Minimum 12 hrs after #1.
3. Cooling Device, or dummy cooling device replacement, minimum 1 week after #2.
4. Replacement may be repeated once to switch cooling piece, or acquisition of post-cooling baseline, minimum 1 week after #3.
Species: Rats
Interval: 12 hrs to 1 week.
Rationale: ECoG electrode implantation plus cooling device implantation. (+peltier cell in some cases) - Later implantation of electrodes in a second surgery is typically necessary in some animals for one of three reasons: a) Age is a factor that affects epileptogenesis in humans. Children are more at risk of posttraumatic epileptogenesis. Also neuronal death is age dependent. We want to study epileptogenesis as it applies to the younger human population. b) acute post-injury righting time may be tested as an acute predictor of later epileptogenesis. Therefore, some animals need to emerge from anesthesia after FPI for the righting time to be assessed, in which case electrodes cannot be implanted immediately after FPI. In some experiments we have to balance between the need to start recording EEG as early as possible after injury, with the need to wait not to lose the animal's headset. Indeed, the younger the age at which the electrodes get implanted, the more likely the headset will be dislodged as the animal grows. Because part of our studies involve determining whether a correlation exists between acute abnormalities in rat EEG after head injury and the later probability to develop posttraumatic epilepsy, we need to use both FPI + ECoG electrode implantation and FPI + later ECoG electrode implantation.

In some cases, since passive cooling needs to start as early as 12hrs after injury, to simulate treatment of head injury patients in the intensive care unit, the cooling piece, which works passively by dissipating heat without power, must also be implanted as early as 12hrs after injury.

In some cases, at the time of electrode and cooling device implantation, in order to see if cooling by more than 2.5 degrees will have a greater impact on the epileptic focus, some animals will receive a peltier cell on the exterior side of the scalp of the animal. This device will allow us to cool the brain area of interest by up to 6oC which has been shown to be safe (Rothman et al 2005). The peltier is removed only if it gets sloughed or at the end of the study. In either case, its removal occurs at time of euthanasia of the animal.
Cooling device replacement- In animals that do not receive the peltier cell the cooling device cannot be turned on/off, as it cools by heat dissipation, to test its anti-epileptogenic effect we need to remove it from the headset and replace it with an equally sized biocompatible sterile silicone rod that does not dissipate heat. To test the antiepileptic effect, we need to first let the animals become epileptic with a dummy device implanted, then switch the dummy with the cooling device to test the effect, and then switch the cooling device with the dummy rod to acquire a post-cooling baseline. The second baseline is necessary to determine whether seizures return, as expected for a true antiepileptic effect, and to put the antiepileptic effect of cooling in context with the one of the other antiepileptic drugs.

or

(v81)
1. FPI (or sham or naive)
2. Headset and ECoG electrode implantation, minimum of 3 days following #1
3. Osmotic mini-pump implantation, minimum of 3 days post 1 and may be done in same session as #2.
4. Osmotic mini-pump removal/implantation, 1-4 weeks after #3.

Species: Rats
Interval: 3 days to 4 weeks
Rationale: The fluid percussion injury is the first surgery to be performed this is followed 3 days to 3 weeks by the electrode implant. To study the effect of antiepileptic drugs on FPI-induced seizures in some cases drug levels need to be maintained at specific levels over the course of weeks, s. cu mini-pumps are used. Pumps are implanted 3 days to 5 weeks after injury. The pump may be replaced 1-4 weeks after the initial pump implantation for prolonged drug exposure. If the osmotic mini-pump and electrode implant fall on the same day they will be done during the same surgical session. All surgical sessions will be separated by at least 3 days.

<table>
<thead>
<tr>
<th>Multiple Major Surgery:</th>
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<tbody>
<tr>
<td>1. Lami/Spinal Contusion</td>
<td></td>
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<tr>
<td>2. Craniotomy/Cortical Injection and/or Intraspinal Injection with Wave guide/Ferrule implant* (Injections with differentiated virally transfected cells or virus).</td>
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<tr>
<td>3. Tracer Injection (cortical and/or spinal cord) (BDA, FluoroRuby, FastBlue, Microspheres, Fluorogold)*</td>
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<tr>
<td>4. Intramuscular Tract Tracer Injection (PRV)*</td>
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<tr>
<td>5. Repair Surgeries In case a repair to skin, skull, and superficial muscle layers is required we will contact Veterinary Services for their advice about whether it is clinically appropriate to proceed, and with their concurrence and advice we will attempt to reattach the ferrule. If erosion of the spinal implants occurs the animal will be sacrificed. The lab’s standard SOP for spinal injuries (attached) will be followed.</td>
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</table>

Species: Rat
Interval between 1 and 2: 10-14 Days
Interval between 2 and 3: 2+ weeks
Interval between 3 and 4: 1-2 weeks
Interval between 4 and 5: Varies, see above.
Rationale: When differentiated cells are injected, two spinal surgeries must be performed because differentiated cells cannot be implanted at the time of injury due to the pro-scaring and reactive astrocyte cues present during acute injury.

The brain and spinal cord tracers must be injected after recovery has taken place due to our experimental intervention. This is a 20-minute procedure involving only a small bur hole through the skull given that the animal will already have an acrylic head cap placed during the skull surgery. During these long-term studies, it may be occasionally necessary to add sutures to the skin near the head implant to protect a catheter after the skin has relaxed or eroded. Head caps also become loose over time, and it may be necessary to add several skull screws and new acrylic in a surgical procedure. We will consult with veterinary services before performing such repairs. Note that we will not re-approach the spinal cord in these procedures. Repairs will only involve the skin, skull,
and superficial muscle layers.
* Some of these surgeries will be omitted for some animals.

OR
1. Lami/Spinal Contusion
2. Window Chamber Implantation
   Spinal Injection or Craniotomy/Red Nucleus Injection
3. Spinal Injection Imaging Session #1
4. Spinal Injection Imaging Session #2
5. Spinal Injection Imaging Session #3
6. Spinal Injection Imaging Session #4
7. Spinal Injection Imaging Session #5
Species: Mouse
Interval between 1 and 2: 5-10 days
Interval between 2 and 3: 11-16 days for 1st imaging session
Interval between 3 and 4: 3-7 days
Interval between 4 and 5: 3-7 days
Interval between 5 and 6: 3-7 days
Interval between 6 and 7: 3-7 days
Rationale: For this study, it is critical to implant the window chamber at 5-10 days post-injury to measure properties of myelinating cells acutely as they begin the process of repair and regeneration and to allow us to ultimately establish the process of internodal plasticity. The multiple imaging sessions are required because the process of myelin repair following injury occurs over many weeks.

OR
1. Postnatal spinal injection of NpCre virus
2. Laminectomy/contusion spinal cord injury 8-12 Weeks
Species: Mouse pup
Interval between 1 and 2: 8-12 Weeks
Rationale: For this study, it is critical to inject the NpCre virus at P2-5 to catch myelinating cells before they mature. The contusion spinal cord injuries will occur in these animals only once they have reached adulthood (aged 8-12 weeks) as this is consistent with our model of SCI in mice. These two surgeries will enable us to measure the properties of mature myelin acutely after an injury.

OR
1. Laminectomy/Spinal Cord Contusion
2. Spinal Injection
Species: Mouse
Interval between 1 and 2: 5 days
Rationale: To optimally capture development of myelin repair after injury, a minimum of 5 days after injury is required before the lentivirus should be injected into the injured spinal cord.

Multiple Major Surgery:
1. Laparotomy, Hepatic subcapsular tumor injection
2. Laparotomy, Hepatic artery infusion pump 1-3 weeks after #1
Species: Rats
Interval: 1-3 weeks
Rationale: 1 to 3 weeks between surgeries is necessary to ensure that the laparotomy was successful before proceeding with the pump surgery

Multiple Major Surgery:
1. Minipump IP implant
2. Minipump IP explant
Species: Mice
Interval: 10 days
Rationale: Exhausted pumps continue to absorb fluid and begin to leak concentrated salts after the
<table>
<thead>
<tr>
<th>Reservoir is empty, so removal is recommended by manufacturer.</th>
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</thead>
<tbody>
<tr>
<td><strong>Multiple Major Surgery:</strong></td>
</tr>
<tr>
<td>1. Intracerebral microinjector cannulation, injectrode or voltammetry electrode implantation (with or without intracranial microinfusion of AAV vectors)</td>
</tr>
<tr>
<td>2. Intravenous (jugular) catheter implantation, 1-4 weeks after #1</td>
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<tr>
<td><strong>Species:</strong> Rats</td>
</tr>
<tr>
<td><strong>Interval:</strong> 1-4 weeks</td>
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<tr>
<td><strong>Rationale:</strong> Indwelling jugular catheter surgeries will be performed separately from intracranial implantation surgeries a) to minimize the total time of anesthesia per surgery, and b) because the longevity of catheter patency is limited and thus needs to be performed in closer proximity to the behavioral studies. To allow for adequate recovery from the intracranial implantation surgery, catheter surgeries will be performed at least 1 week and up to 4 weeks after intracranial implantation surgery.</td>
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</tbody>
</table>

| Multiple Major Surgery:                                     |
| 1. Steroid implant procedure                                |
| 2. Auditory physiology recordings, 23-35 days following #1  |
| **Species:** Fish                                           |
| **Interval:** 23-35 days                                     |
| **Rationale:** Multiple surgical sessions are required to test for the effects of steroids on auditory physiology. |

| Multiple Major Surgery:                                     |
| 1. Cardiac cryo-injury with or without implantation of Telemetric ECG |
| 2. Thoracotomy for intra-cardiac cell transplantation, 10 days or 28 days following #1 |
| **Species:** Guinea Pigs                                    |
| **Interval:** 10-28 days                                    |
| **Rationale:** This sequence of events better models one context in which human patients could potentially receive a comparable cell therapy. |

| Multiple Major Surgery:                                     |
| 1. Thoracotomy for ischemia/reperfusion with or without implantation of Telemetric ECG |
| 2. Thoracotomy for cell implant, 7 days following #1         |
| **Species:** Rabbits                                        |
| **Interval:** 7 days                                        |
| **Rationale:** (1) The rabbit is known to reach peak granulation tissue formation at 7 days post infarction. Transplanted cells are more likely to survive if given during this period, rather than at the time of infarction (when the tissue is less vascular and highly inflamed). (2) This sequence of events better models the context in which human patients would receive a comparable therapy. |

| Multiple Major Surgery:                                     |
| 1. Laparotomy for bile duct partial ligation to dilate duct or percutaneous access to bile duct |
| 2. Percutaneous bile duct injection of therapeutic agents and/or placement of stents, 1 to 4 weeks following #1 as determined by noninvasive imaging of bile duct for injection of agents or stenting |
| **Species:** Pigs                                           |
| **Interval:** 1-4 weeks                                     |
| **Rationale:** Bile duct must be dilated to establish the disease model before therapeutic agents can be administered. Version 1 amendment. Percutaneous access to bile duct may be substituted for surgery I. This percutaneous surgery must be done by Dr. Yang or Dr. Zhang. |

| Multiple Major Surgery:                                     |
| 1. Implant rat orthotopic pancreas tumor into mesentery.    |
| 2. Imaging-guided radio frequency heating enhanced chemotherapy treatment 3-4 weeks after #1. |
| **Species:** Rats                                          |
| **Interval:** 3-4 weeks                                     |
| **Rationale:** Treatment for the pancreatic tumors can be applied only after the implanted tumors have had time to grow. |
Multiple Major Surgery:
1. Head post implantation
2. Array implantation or V4 chamber implant in hemisphere 1, at least 3 months following #1
3. First craniotomy/porthole (V4 implant 1), 2 months following #2
4. Second craniotomy/porthole (V4 implant 1), at least 1 month following #3
5. V4 chamber implant in hemisphere 2, at least 2 months following #4
6. First craniotomy/porthole (V4 implant 2), 2 months following #5
7. Second craniotomy/porthole (V4 implant 2), at least 1 month following #6
8. IT chamber implant, at least 2 months following #7
9. IT chamber craniotomy, 2 months following #8
10. Array implantation (optional for chamber naïve animals), at least 3 months following #1
11. Potential repair surgery #1, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary presurgical assessment
12. Potential repair surgery #2, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary presurgical assessment
Species: M. mulatta
Interval: 1-3 months minimum
Rationale: These experiments require a minimum of 3 surgeries to obtain any experimental data using the standard chamber method - implantation of a head post, implantation of a recording chamber and a craniotomy. These three surgeries are incompatible to being performed as one, because the head post surgery requires a recovery period of 6 weeks, while a craniotomy requires that experiments start the very next day.

If using the array method, experiments require a minimum of 2 surgeries to obtain any experimental data - implantation of the head post and implantation of the array, which are incapable of being done as a single surgery for the same reasons as with the chamber surgeries. However, these surgeries are not sufficient for successfully achieving the group’s research goals due to three reasons:

First, when the goal of research is to understand higher order brain function, it is important to compare the roles of multiple different brain areas in the same animal, since brain areas seldom operate in isolation to effect higher order cognitive tasks. This requires the placement of multiple recording chambers.

Second, when the areas of interest are on the surface of the cortex, it is important for the dural surface to be fresh for successful recordings. This necessitates i) performing the porthole surgery immediately prior to experiments to increase the success and ii) exposing small regions of the cortex periodically to facilitate an extensive period of successful data collection.

Third, a huge investment in time and effort is required to prepare these animals for recording, as animals typically spend 1-1.5 years getting trained on a behavioral task. Replacing an animal with another naïve animal (because experimental hardware came undone, etc.) would be a loss of valuable effort and time.

OR

Version #37 (Animal A05066 ONLY)
Multiple Major Surgery:
1. Head post implantation
2. Array implantation or V4 chamber implant in hemisphere 1, at least 3 months following #1
3. First craniotomy/porthole (V4 implant 1), 2 months following #2
4. Second craniotomy/porthole (V4 implant 1), at least 1 month following #3
5. V4 chamber implant in hemisphere 2, at least 2 months following #4
6. First craniotomy/porthole (V4 implant 2), 2 months following #5
7. Second craniotomy/porthole (V4 implant 2), at least 1 month following #6
8. PFC chamber implant, at least 2 months following #7
9. PFC chamber craniotomy, 1 month following #8
11. Potential repair surgery #1, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment
12. Potential repair surgery #2, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment

Species: M. mulatta
Interval: 1-3 months minimum

Rationale: These experiments require a minimum of 3 surgeries to obtain any experimental data using the standard chamber method - implantation of a head post, implantation of a recording chamber and a craniotomy. These three surgeries are incompatible to being performed as one, because the head post surgery requires a recovery period of 6 weeks, while a craniotomy requires that experiments start the very next day.

If using the array method, experiments require a minimum of 2 surgeries to obtain any experimental data - implantation of the head post and implantation of the array, which are incapable of being done as a single surgery for the same reasons as with the chamber surgeries. However, these surgeries are not sufficient for successfully achieving the group’s research goals due to three reasons:

First, when the goal of research is to understand higher order brain function, it is important to compare the roles of multiple different brain areas in the same animal, since brain areas seldom operate in isolation to effect higher order cognitive tasks. This requires the placement of multiple recording chambers.

Second, when the areas of interest are on the surface of the cortex, it is important for the dural surface to be fresh for successful recordings. This necessitates i) performing the porthole surgery immediately prior to experiments to increase the success and ii) exposing small regions of the cortex periodically to facilitate an extensive period of successful data collection.

Third, a huge investment in time and effort is required to prepare these animals for recording, as animals typically spend 1-1.5 years getting trained on a behavioral task. Replacing an animal with another naïve animal (because experimental hardware came undone, etc.) would be a loss of valuable effort and time.

---

Multiple Major Surgery:
1. Eye coil and lug implantation
2. Chamber implantation, at least 1 month after #1
3. Vestibular prosthesis implantation, at least 1 month after #2
4. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment

Species: M. Mulatta
Interval: 1 month

Rationale: A single surgical procedure would be too long and too stressful for the animals. Furthermore, a single surgical procedure would compromise the positioning of the vestibular prosthesis or the recording chamber, or both. This would need to be repaired/revised surgically.

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Multiple Major Surgery:
1. Transient middle artery occlusion
2. ICV injection, 24-48 hours following #1
3. Middle artery reperfusion, 24 hours following #2

Species: Mice
Interval: between 1 and 2 24-48 hours
Interval: between 2 and 3 24 hours
Rationale: Ischemic preconditioning requires genomic reprogramming and gene expression. Classically, these changes require 48-72 hours to take effect and last several weeks. These are the earliest time point possible (48-72 hours) for scientific/experimental design reasons and in order to minimize post-surgical time for the animal.

Multiple Major Surgery:
1. Maternal-fetal vascular catheterization
2. C-section, up to 8 weeks following #1
Species: Macaca nemestrina
Interval: up to 8 weeks
Rationale: The second major survival surgery is performed in order to obtain fetal and placental tissues under controlled conditions. If the dam is allowed to deliver vaginally, the fetus may die during the birth, rendering experimental results from the fetal tissues difficult to interpret. If the delivery occurs at night, the dam often ingests the placental tissues, preventing scientific use of these tissues.

4165-01

Multiple Major Surgery:
1. Implant of head post and eye coil
2. Craniotomy and implantation of recording device #1, minimum of 6 months following #1.
3. Craniotomy and implantation of recording device #2, minimum of 1 year following #2.
4. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment.
Species: Macaca mulatta
Interval: At least 6 months
Rationale: The eye coil and head post are implanted in a single surgical session and the first recording device is implanted during a subsequent surgical session. The justification for not including the first recording device in the first surgical session arises from consideration of the timing of the behavioral training and the desire to guard animal health. Specifically, the animal is prepared for behavioral training as soon as the eye coil and head post have been implanted. Training can take up to a year. During this time, invasive manipulations are not made, obviating the necessity for recording chambers, craniotomies, or multielectrode arrays. Indeed, these hold the potential to compromise the health of the animal: A recording cylinder or multielectrode array connector increases the area of the cranial implant, thereby increasing the likelihood of osteopathy, and a craniotomy raises the risk of intracranial infection. For these reasons we do not generally implant the recording device nor make the craniotomy until after behavioral training is completed. If the behavioral task is sufficiently simple (i.e. ocular fixation) such that training is expected to take less than two months we may implant the recording device during the same surgical session as the eye coil and head post. The second recording device is intended as a replacement for the first recording device. It is not implanted until the first one fails. Implantation of a second recording device allows us to increase the amount of data we can obtain from each animal, thereby reducing the total number of animals used in the completion of the project aims.

OR Version #62 (2 approved M mulatta ONLY)

Multiple Major Surgery:
1. Implant of head post and eye coil
2. Craniotomy and implantation of two recording devices (area V1, bilateral), minimum of 6 months following #1.
3. Craniotomy and implantation of recording device (area SC) and removal of one of the V1 chambers, minimum of 6 months following #2.
4. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment.

4167-01
Species: Macaca mulatta  
Interval: At least 6 months  
Rationale: The eye coil and head post are implanted in a single surgical session and the first recording device is implanted during a subsequent surgical session. The justification for not including the first recording device in the first surgical session arises from consideration of the timing of the behavioral training and the desire to guard animal health. Specifically, the animal is prepared for behavioral training as soon as the eye coil and head post have been implanted. Training can take up to a year. During this time, invasive manipulations are not made, obviating the necessity for recording chambers, craniotomies, or multielectrode arrays. Indeed, these hold the potential to compromise the health of the animal: A recording cylinder or multielectrode array connector increases the area of the cranial implant, thereby increasing the likelihood of osteopathy, and a craniotomy raises the risk of intracranial infection. For these reasons we do not generally implant the recording device nor make the craniotomy until after behavioral training is completed. If the behavioral task is sufficiently simple (i.e. ocular fixation) such that training is expected to take less than two months we may implant the recording device during the same surgical session as the eye coil and head post. The second recording device is intended as a replacement for the first recording device. It is not implanted until the first one fails. Implantation of a second recording device allows us to increase the amount of data we can obtain from each animal, thereby reducing the total number of animals used in the completion of the project aims.

Multiple Major Surgery:  
1. Skull Implant  
2. Muscle Implant* (in some cases combined with Skull Implant), at least 2 weeks following #1  
3. Spinal Injury* (in some cases combined with Spinal Implant), at least 10 days following #2  
4. Spinal Implant*, at least 2 weeks following #3  
5. Brain tracer injections*, at least 10 days following #5  
6. Repair Surgery (if needed)*  

Species: Rats  
Interval: 10 days - 2 weeks minimum  
Rationale: Multiple surgeries are required to test the ability of different applications of intraspinal electrical stimulation to restore motor function following spinal cord injury. The initial skull surgery must be separated from the remaining surgeries in order to determine the effects of brain recording and/or stimulation prior to injury of the spinal cord. The experimental protocol will compare the effects of starting stimulation soon after injury and several weeks after injury (acute vs. chronic injury conditions). To prevent possible malfunction of the spinal implant between implant and use in the chronic treatment groups the spinal injury and spinal implant procedures will be performed in different surgeries in these animals. The brain tracer must be injected after recovery has taken place due to our experimental intervention. During these long-term studies, it may be occasionally necessary to add sutures or acrylic to protect wires entering the skull implant after the skin has relaxed or eroded. Head caps also become loose over time, and it may be necessary to add several skull screws and new acrylic in a surgical procedure. We therefore request one potential repair surgery for each animal to cover these possibilities. Note that we will not re-approach the spinal cord in these procedures. Repairs will only involve the skin, skull, and superficial muscle layers. *These surgeries will not be performed in some animals.

Multiple Major Surgery:  
Option I:  
1. Skull implant MM  
2. Skull Implant S+E, 1 at least week following 1  
3. Arm implant, 1 at least week following 2  
4. Vertebral implant, at least 7 days following #3  
5. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment  
Species: Macaques  
Interval: at least 7 days
Rationale: Skull, arm and vertebral implants are necessary to achieve the scientific aims of the project - to correlate the electrical activity of spinal neurons with identified inputs and outputs. Combining the implant surgeries would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of death during surgery, and delay post-surgical recovery. Skull Implant MM is needed to identify targets for electrodes in the brain with MRI.

OR

Option II:
1. Skull implant S + E
2. Arm implant, at least 7 days following #1
3. Vertebral implant, at least 7 days following #2
4. Skull implant C + E, at least 2 weeks following #3
5. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment

Species: Macaques
Interval: at least 7 days

Rationale: Skull, arm and vertebral implants are necessary to achieve the scientific aims of the project - to correlate the electrical activity of spinal neurons with identified inputs and outputs. Combining the implant surgeries would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of death during surgery, and delay post-surgical recovery. Skull implant C+E is needed to study brain neurons in the same animal after data collection from spinal neurons is completed.

OR

Option III:
1. Skull implants S+E and C+E combined in one surgery
2. Arm implant, at least 7 days following #1
3. Vertebral implant, at least 7 days following #2
4. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment

Species: Macaques
Interval: at least 7 days

Rationale: Skull, arm and vertebral implants are necessary to achieve the scientific aims of the project - to correlate the electrical activity of spinal and brain neurons with identified inputs and outputs. Combining the implant surgeries would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of death during surgery, and delay post-surgical recovery. This sequence is used when simultaneous recordings are performed from both spinal and brain neurons to identify directly connected cells.

OR

Option IV:
1. Skull implants S+E and C+E combined in one surgery
2. Arm implant, at least 7 days following #1
3. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment

Species: Macaques
Interval: at least 7 days

Rationale: This sequence is performed if additional data is needed from brain, but not spinal, neurons. Skull and arm implants are necessary to achieve the scientific aims of the project - to correlate the electrical activity of brain neurons with identified inputs and outputs. Combining the implant surgeries would require an excessive period of continuous anesthesia that could adversely affect cardiac or respiratory function, increase risk of death during surgery, and delay post-surgical recovery.
Multiple Major Surgery:
1. Implant head post or head post holder and recording chamber or recording chamber holder
2. Intracocular injection, at least 1 month following #1
3. Craniotomy, at least 3 months following #2
4. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment

Species: S. sciureus or C. apella (Squirrel Monkeys or Capuchin Monkeys)
Interval: 1-3 months minimum

Rationale: The craniotomy is performed later so that the animal does not have a craniotomy during the extended training period, since this can increase the risk of infection. The head post or head post holder and recording chamber or recording chamber holder is implanted in a single surgical session and the intraocular injection and craniotomy are performed during subsequent surgical sessions. The justification for doing the implant and intraocular injection at separate times is that pre-injection color vision data must be collected using a restrained color vision test, which requires the head post. The justification for not including implantation of the head post or head post holder and recording chamber or recording chamber holder in the same surgical session as the craniotomy is to protect the animal's health. Specifically, the animal is prepared for behavioral training as soon as the head post and recording chamber holder has been implanted. Training can take up to 1.5 years. During this time, invasive manipulations are not made, obviating the necessity for craniotomies, which have the potential to compromise the health of the animal. Surgery 4 will only be performed if one or both implants fail.

Multiple Major Surgery:
1. Eye coil and head-stabilizing fitting implant
2. Subretinal injection of the 1st eye or intravitreal injection of both eyes. At least 30 days after 1.
3. Subretinal injection of the second eye. At least 30 days after 2.
4. Subretinal injection of the 1st eye for the 2nd time with a modified virus, will occur only if 1st virus fails to express. At least 30 days after 3.
5. Subretinal injection of the 2nd eye for the 2nd time with a modified virus, will occur only if 1st virus fails to express. At least 30 days after 4.
6. Recording chamber implant and craniotomy. At least 30 days after 5.
7. Repair/reimplantation of head post and/or recording chamber and/or eye coils. Will be performed only if necessary and at least 14 days after any other surgical procedure.

Species: M. mulatta
Interval: At least 30 days will separate experimental surgeries except for a repair surgery which might need to be done at least 14 days after any other surgery.

Rationale: Surgery 1 is necessary to train the monkey to make the eye movements for this study and to collect the color vision data pre and post-gene therapy. Surgeries 2 and 3 are the gene therapy treatments designed to alter the animal's color vision. Surgery 3 will be done only on animals that receive the subretinal injection. Surgeries 4 and 5 are gene therapy treatments by subretinal injection that will be done only if the animals that received subretinal injections in surgeries 2 and 3 show no signs of the therapeutic virus expressing. Surgery 6 has the potential to compromise the health of the animal and so must be done after the training, which can take up to 1.5 years.
animals by repairing damaged implants and therefore minimizing the number of animals used in the project.

<table>
<thead>
<tr>
<th>Multiple Major Surgery:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Coronary artery ligation without occlusion</td>
</tr>
<tr>
<td>2. Closed chest myocardial ischemia-reperfusion injury, 1 week following #1</td>
</tr>
<tr>
<td>Species: Mice</td>
</tr>
<tr>
<td>Interval: 1 week</td>
</tr>
<tr>
<td>Rationale: The coronary artery ligation sets up the experiment for the closed-chest myocardial ischemia-reperfusion injury, this initial operation must heal prior to the second experimental surgery. Closed-chest mouse model of IR allows ligation of the LAD coronary artery posterior to the acute surgical trauma providing a unique opportunity to study the structure, function and energetic metabolism of the mouse heart by MRI/MRS after IR injury in vivo.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Eye muscle surgery</td>
</tr>
<tr>
<td>2. Growth factor pellet implantation on extraocular muscle, 1 week to 3 months following #1</td>
</tr>
<tr>
<td>3. Restraining hardware, recording chamber, scleral search eye coil implantation in one eye, or no eye coil placement with both eye coils placed in next surgery, 1.5 to 2 years following #1</td>
</tr>
<tr>
<td>4. Scleral search eye coil implantation in second eye, or eye coil in both eyes, at least 2 weeks following #2</td>
</tr>
<tr>
<td>5. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment</td>
</tr>
<tr>
<td>Species: Macaques</td>
</tr>
<tr>
<td>Interval: 2 weeks - 2 years</td>
</tr>
<tr>
<td>Rationale: To achieve the goals, surgical adjustment of eye muscles must be made during infancy (critical period for visual-oculomotor development). For some eye muscle surgery cases, muscles may be treated with growth factors in pellet form. Because the growth factor is delivered to specific muscles it requires a surgical procedure. When these animals reach at least 1.5 - 2 years of age, they...</td>
</tr>
</tbody>
</table>
will be subjects in studies of neural control of eye movements as described under juvenile protocols. The first juvenile surgeries, consisting of coil implant, chambers and head stabilization post all in one surgery. Even an experienced team needs 4-6 hours to accomplish all of this. Therefore, a second juvenile surgery is performed on the second eye (at least 2 weeks after the first), to reduce the trauma to the monkey. Placing the second coil two weeks or more after the first allows the monkey to recover optimally. In order to reduce the time required for the surgery to place head stabilization, recording chambers and the first eye coil, the two eye coils may be placed together in the following surgery. A broken coil might need to be replaced. A coil may break due to defective insulation or metal fatigue.

OR

1. Eye muscle surgery (possible growth factor placement)
2. Restraining hardware, recording chamber, scleral search eye coil implantation in one eye, or no eye coil placement with both eye coils placed in next surgery, 1.5 to 2 years following #1
3. Scleral search eye coil implantation in second eye, or eye coil in both eyes, at least 2 weeks following #2
4. Growth factor pellet implantation on extraocular muscle, 1 week to 3 months following #3
5. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment
Species: Macaques
Interval: 1 week - 2 years

Rationale: To achieve the goals, surgical adjustment of eye muscles must be made during infancy (critical period for visual-oculomotor development). Growth factor injections will follow surgery or sham within 3 months of the initial surgery. When these animals reach at least 1.5 - 2 years of age, they will be subjects in studies of neural control of eye movements as described under juvenile protocols. The first juvenile surgeries, consisting of coil implant, chambers and head stabilization post all in one surgery. Even an experienced team needs 4-6 hours to accomplish all of this. Therefore, a second juvenile surgery is performed on the second eye (at least 2 weeks after the first), to reduce the trauma to the monkey. Placing the second coil two weeks or more after the first allows the monkey to recover optimally. In order to reduce the time required for the surgery to place head stabilization, recording chambers and the first eye coil, the two eye coils may be placed together in the following surgery. A broken coil might need to be replaced. A coil may break due to defective insulation or metal fatigue.

OR

1. Eye muscle surgery
2. Restraining hardware, recording chamber, scleral search eye coil implantation in one eye, or no eye coil placement with both eye coils placed in next surgery, 1.5 to 2 years following #1
3. Scleral search eye coil implantation in second eye, or eye coil in both eyes, at least 2 weeks following #2
4. Growth factor pellet implantation on extraocular muscle, 1 week to 3 months following #3
5. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment
Species: Macaques
Interval: 1 week - 2 years

Rationale: To achieve the goals, surgical adjustment of eye muscles must be made during infancy (critical period for visual-oculomotor development).
order to reduce the time required for the surgery to place head stabilization, recording chambers and the first eye coil, the two eye coils may be placed together in the following surgery. For some eye muscle surgery cases, muscles may be treated with growth factors at the juvenile stage. Because the growth factor is delivered to specific muscles it requires a surgical procedure. A broken coil might need to be replaced. A coil may break due to defective insulation or metal fatigue.

<table>
<thead>
<tr>
<th>4244-01</th>
<th>Multiple Major Surgery:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unilateral ischemia and reperfusion injury of kidney</td>
<td></td>
</tr>
<tr>
<td>2. Unilateral nephrectomy 2-3 weeks later</td>
<td></td>
</tr>
<tr>
<td>Species: Mice</td>
<td></td>
</tr>
<tr>
<td>Interval: 2-3 weeks</td>
<td></td>
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<tr>
<td>Rationale: The model induces a chronic ischemic nephropathy in the kidney. It is not possible to achieve this model without the two-step procedure. Multiple surgeries are required since the diseased kidney can only be established by initially performing surgery on a single kidney but leaving a healthy kidney in place. If we perform the experiment all in one go, we do not get chronic kidney disease.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4246-01</th>
<th>Multiple Major Surgery:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Craniotomy (EEG, thermistor, ICV guide cannula)</td>
<td></td>
</tr>
<tr>
<td>2. Cecal Ligation Puncture, 1 week following #1</td>
<td></td>
</tr>
<tr>
<td>Species: Mice</td>
<td></td>
</tr>
<tr>
<td>Interval: 1 week</td>
<td></td>
</tr>
<tr>
<td>Rationale: Some experiments require sleep recordings to be obtained from animals subjected to CLP and they first require baseline readings prior to CLP.</td>
<td></td>
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<tr>
<td>OR</td>
<td></td>
</tr>
<tr>
<td>1. Telemeter Implant - Laparotomy</td>
<td></td>
</tr>
<tr>
<td>2. Cecal Ligation Puncture, 2-4 weeks following #1</td>
<td></td>
</tr>
<tr>
<td>Species: Mice</td>
<td></td>
</tr>
<tr>
<td>Interval: 2-4 weeks</td>
<td></td>
</tr>
<tr>
<td>Rationale: Some experiments require sleep recordings to be obtained from animals subjected to CLP and they first require baseline readings prior to CLP.</td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td></td>
</tr>
<tr>
<td>1. Telemeter Implant - Laparotomy</td>
<td></td>
</tr>
<tr>
<td>2. Craniotomy for ICV guide cannula implant, 2-4 weeks following #1</td>
<td></td>
</tr>
<tr>
<td>Species: Mice</td>
<td></td>
</tr>
<tr>
<td>Interval: 2-4 weeks</td>
<td></td>
</tr>
<tr>
<td>Rationale: The recovery from the telemeter implant is longer than the average patency of an ICV guide cannula.</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>4253-01</th>
<th>Multiple Major Surgery:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cardiac ischemia/reperfusion thoracotomy</td>
<td></td>
</tr>
<tr>
<td>2. Cardiac CM cell implantation thoracotomy 4 days later</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>Species: Rats</td>
<td></td>
</tr>
<tr>
<td>Interval: 4 days</td>
<td></td>
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<tr>
<td>Rationale: Coronary arteries are too narrow for catheter based infusion of cardiomyocytes so they must be delivered by a second surgery. The model mimics a clinical situation when cells would be engrafted during the healing phase after infarction.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4254-01</th>
<th>Multiple Major Surgery:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Titanium restraining hardware and recording chamber (option 1) or along with scleral search coil implant (option 2)</td>
<td></td>
</tr>
<tr>
<td>2. Scleral search coil implantation for two eyes (option 1) or second eye (option 2), at least 2 weeks after #1</td>
<td></td>
</tr>
<tr>
<td>3. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human</td>
<td></td>
</tr>
</tbody>
</table>
Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary pre-surgical assessment
Species: M. mulatta
Interval: 2 weeks minimum
Rationale: We need to implant recording chambers and scleral search coils (both eyes). We implant head posts and recording chambers in one surgery taking 4-5 hours. If time allows, we also place one eye coil, but a surgery to implant both eye coils would be too long for the monkey. In Surgery #2 we implant either one or two eye coils as needed. A broken eye coil might need to be replaced. Stabilization hardware and recording chambers can become loose or break requiring replacement.

Multiple Major Surgery:
1. Skull surgery (with optional tracer/muscle implant/wireless device implant)  
2. Spinal injury (with optional ChABC/cell injections)*, minimum of 1 week following #1  
3. Spinal surgery* (electrodes/waveguide and ChABC/cells/virus/tracer) with optional muscle/wireless device implant minimum of 1 week following #2  
4. Brain tracer injections*, minimum of 1 week following #3  
5. Spinal Tracer Injections*, minimum of 1 week following #4  
6. Superficial repair surgery*, minimum of 1 week following any of the above surgeries  
* Some of these surgeries will be omitted for many of the animals

Species: Rats
Interval: Minimum 1 week
Rationale: Multiple survival surgeries are required to create this complex animal model to test the combination of stimulation and differentiated cell therapies at restoring movement following spinal cord injury.  
- The initial skull surgery must be separated from the remaining surgeries in order to determine the effects of brain recording and/or stimulation prior to injury of the spinal cord  
- When differentiated cells are injected, two spinal surgeries must be performed because differentiated cells cannot be implanted at the time of injury due to the pro-scaring and reactive astrocyte cues present during acute injury.  
- The brain tracer must be injected after recovery has taken place due to our experimental intervention. This is a minor, 20-minute procedure involving only a small bur hole through the skull given that the animal will already have an acrylic head cap placed during the skull surgery. Spinal tracers must often be injected in a separate surgery than brain tracers due to different propagation times.  
- During these long-term studies, it may be occasionally necessary to add sutures to the skin near the head implant to protect a catheter after the skin has relaxed or eroded. Head caps also become lose over time, and it may be necessary to add several skull screws and new acrylic in a surgical procedure. We will consult with veterinary services before performing such repairs. Note that we will not re-approach the spinal cord in these procedures. Repairs will only involve the skin, skull, and superficial muscle layers.

Multiple Major Surgery:
1. Up-to 15 cm transection of the jejunum and associated mesenteric lymph nodes  
2. Up-to 15 cm transection of the jejunum and associated mesenteric lymph nodes a minimum of 6 weeks following #1  
3. Up-to 15 cm transection of the jejunum and associated mesenteric lymph nodes a minimum of 14 weeks following #2
Species: M. mulatta
Interval: 6 weeks - 14 weeks minimum
Rationale: Up-to 15 cm transection of the jejunum and associated mesenteric lymph nodes will be collected to measure virus and immune responses in the gut. The timepoints selected will provide analysis of a baseline immunological and virological data, which must be established prior to vaccination, so that any alteration in the immune response can be attributed to vaccination. These biopsies are essential to profile the immune and virological response to vaccine-induced protection.

(Changed per Ver #8, mn)
Multiple Major Surgery:
1a. Chondrocyte harvest (ear)
1b. Auricular Chondrocyte harvest (ear only if contamination of culture of 1a), 1-8 weeks following #1a (only if necessary, may skip second harvest)
2. Epithelial harvest and Neotrachea implantation with epithelial lining using skin, tracheal or buccal mucosa, a minimum of 4 weeks following #1a or #1b
Species: Rabbits
Interval: 1-8 weeks
Rationale: Surgery 1a and possibly 1b are needed to obtain autologous chondrocytes to engineer cartilage sheets. These cells normally take between 4-16 weeks to form a viable cartilage sheet. Surgery 2 is needed to form a neotrachea with an epithelial lining.

OR
1a. Chondrocyte harvest (ear or shoulder)
1b. Auricular Chondrocyte harvest (ear only if contamination of culture of 1a), 1-8 weeks following #1a (only if necessary, may skip second harvest)
2. Neotrachea implantation, a minimum of 4 weeks following #1a or #1b
3. Epithelial harvest and Lining of neotrachea, 2-16 weeks following #2
Species: Rabbits
Interval: 1-16 weeks
Rationale: Surgery 1a and possibly 1b are needed to obtain autologous chondrocytes to engineer cartilage sheets. The option of using shoulder surgery is there as an alternative approach if ear cartilage, which is elastic cartilage and not hyaline, does not prove to be effective long-term. Surgery 2 is needed to form a vascularized neotrachea. Surgery 3 is needed to determine the optimum epithelial lining.

OR
1. Placement of tracheal stent or t-tube
2. Endoscopic cleaning of stent or t-tube (only if necessary)
3. Trans-cervical replacement of stent or t-tube (only if necessary)
4. Endoscopic cleaning of stent or t-tube (only if necessary)
5. Endoscopic cleaning of stent or t-tube (only if necessary)
Species: Rabbits
Interval: 1 hour minimum
Rationale: Surgery 1 is needed to determine if the rabbit will tolerate a tracheal appliance. Procedure 2,4,5 is needed only if the rabbit develops respiratory distress from obstruction of the tracheal appliance. Surgery 3 is needed only if the rabbit develops respiratory distress from obstruction of the tracheal appliance.

Please note that in those animals that have had a tracheal appliance placed the PI requests one surgical attempt at replacement of the appliance and three endoscopic attempts at cleaning the appliance if the appliance becomes obstructed and causes respiratory distress. Although it is unlikely to happen in this short of a time frame, the appliance can obstruct with secretions at anytime after placement so the PI is requesting a minimum duration of 1 hour between interventions. These animals will undergo a maximum of 2 survival surgeries and three survival procedures.

OR
1a. Chondrocyte harvest (ear or shoulder)
1b. Auricular Chondrocyte harvest (ear only if contamination of culture of 1a), 1-8 weeks following #1a (only if necessary, may skip second harvest)
2. Epithelial harvest and Neotrachea implantation with epithelial lining using skin, tracheal or buccal mucosa, a minimum of 4 weeks following #1a or #1b
3. Tracheal resection and neotracheal implantation, 2-16 weeks following #2
Species: Rabbits
Interval: 1-16 weeks
Rationale: Surgery 1a and possibly 1b are needed to obtain autologous chondrocytes to engineer cartilage sheets. Surgery 2 is needed to form an epithelialized and vascularized neotrachea. Surgery 3 is needed to test whether the neotrachea tissue can function as a replacement tissue for normal trachea.

OR
1a. Chondrocyte harvest (ear or shoulder)
1b. Auricular Chondrocyte harvest (ear only if contamination of culture of 1a), 1-8 weeks following #1a (only if necessary, may skip second harvest)
2. Neotrachea implantation, a minimum of 4 weeks following #1a or #1b
3. Epithelial harvest Lining of neotrachea, 10 days -16 weeks following #2
4. Tracheal resection and neotracheal implantation, 2-16 weeks following #3
Species: Rabbits
Interval: 10 days-16 weeks
Rationale: Surgery 1a and possibly 1b are needed to obtain autologous chondrocytes to engineer cartilage sheets. Surgery 2 is needed to form a vascularized neotrachea. Surgery 3 is needed to epithelialize the neotrachea. Surgery 4 is needed to test whether the neotrachea tissue can function as a replacement tissue for normal trachea.

Multiple Major Surgery:
1. Craniotomy - EEG, thermistor
2. CCI, osmotic pump, guide cannula implant, 25 days following #1
Species: Mice
Interval: 25 days
Rationale: Animals are used in a within-subject design to study sleep-wake behavior, EEG, and brain temperature both before and after controlled cortical impact (CCI). To obtain the control data the EEG and thermistor implants must be made before the CCI is conducted.

Multiple Major Surgery:
1. Cardiac injury
2. Intramyocardial cell transplantation, 7 days following #1
Species: Guinea Pigs and Rats
Interval: 7 days
Rationale: These procedures must be performed serially to ensure cell survival (graft survival would be expected to be reduced during the very acute phase following cardiac injury) and to model the expected situation in human patients (in which cells would likely be grafted into the heart in the healing phase of infarction, rather than immediately after coronary occlusion).

Version 3 changed surgery 2 from '1 or 2' recording chambers to '1 to 3' recording chambers.
Multiple Major Surgery:
1. Eye coil and Head lug implantation
2. Craniotomy and implantation of 1 to 3 recording cylinder/chamber, minimum of 2 weeks following #1.
3. Potential repair surgery, as described in the approved UW IACUC Guidelines for Non-human Primate Neuroscience Studies, interval depends on need, but is always preceded by a veterinary presurgical assessment.
Species: Macaca mulatta
Interval: At least 2 weeks
Rationale: Multiple surgical sessions minimize craniotomy exposure time, repair surgeries maximize the use of animals (by repairing damaged implants), and, therefore, minimize the number of animal used in the project. We are requesting a repair surgery per the IACUC Guidelines for Non-human Primate Neuroscience Studies.

ACTIVE
Version 3
Bacterial vaginosis study, Aim I
Aim I, Group I (control)
Multiple Major Surgery:
1. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection
2. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #1
3. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #2
4. Colon and jejunum resection, lymph node, vagina, and rectum biopsy, and CSF collection, at least 2 weeks after #3
5. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 4 weeks after #4
6. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #5
7. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #6
8. Colon and jejunum resection, lymph node, vagina, and rectum biopsy, and CSF collection, at least 2 weeks after #7
9. Vagina, jejunum, colon, and rectum biopsy and CSF collection, at least 4 weeks after #8
10. Lymph node, vagina, jejunum, colon, and rectum biopsy and CSF collection, at least 1 week after #9

Species: Macaques
Interval: At least 1 week to at least 4 weeks
Rationale: The importance of this study is to understand the kinetics of the mucosal and peripheral immune system in relation to changes in hormone levels and the microbiome. Multiple biopsies and colon/jejunum resection surgeries will be essential to determine the kinetics of the mucosal and lymphoid immune response over time and in response to differing treatments and phases of the menstrual cycle. Due to the nature of mucosal immunity and the limited sampling techniques available, biopsies and resection are our only options to collect immune cells for this study. Ten biopsy/CSF/resection time points were selected in order to perform the needed experiments to assess mucosal immune changes with limited cell numbers isolated from biopsies in a longitudinal manner prior to treatment and SIV, during treatment prior to SIV, and after SIV infection in this control group.

ACTIVE
Version 3 continued
Bacterial vaginosis study, Aim I, continued
Aim I, Groups II-III (BV,BV+Probiotics)

Multiple Major Surgery:
1. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection
2. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #1
3. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #2
4. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #3
5. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #4
6. Colon and jejunum resection, lymph node, vagina, and rectum biopsy, and CSF collection, at least 2 weeks after #5
7. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 4 weeks after #6
8. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #7
9. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #8
10. Colon and jejunum resection, lymph node, vagina, and rectum biopsy, and CSF collection, at least 2 weeks after #9
11. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 4 weeks after #10

Species: Macaques
Interval: At least 1 week to at least 4 weeks
Rationale: The importance of this study is to understand the kinetics of the mucosal and peripheral immune system in relation to changes in hormone levels and the microbiome relative to vaginosis,
and to develop a model of bacterial vaginosis. Multiple biopsies and colon/jejunum resection surgeries will be essential to determine the kinetics of the mucosal and lymphoid immune response over time and in response to differing treatments and phases of the menstrual cycle. Due to the nature of mucosal immunity and the limited sampling techniques available, biopsies and resection are our only options to collect immune cells for this study. Eleven biopsy/CSF/resection time points were selected in order to perform the needed experiments to assess mucosal immune changes with limited cell numbers isolated from biopsies in a longitudinal manner prior to treatment and SIV, during treatment prior to SIV, and after SIV infection in these BV and BV + probiotic groups.

**ACTIVE**  
Version 4 (Note: Version 4, Replaces Aim II timelines of version 3 (and modified in version 5))  
(Note: There is no multiple major surgery in this time line; included for improved understanding of timelines)  

**Group A: Pre-SIV treatment**  
*Multiple Surgery*  
1. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection  
2. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #1  
3. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #2  
4. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #3  
5. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #4  
6. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #5  
7. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #6  
8. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #7  
9. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #8  
10. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #9  
11. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #10  
12. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #11  
13. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #12  
14. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #13

**Species:** Macaques  
**Interval:** At least 1 week  
**Rationale:** The importance of this study is to determine the effects of Lacto/IL-22 treatment on mucosal immunity and markers of mortality during SIV infection. Multiple biopsies and CSF collections will be essential to determine the kinetics of the mucosal and lymphoid immune response over time and in response to Lacto/IL-22 treatments, SIV infection, and phases of the menstrual cycle. Due to the nature of mucosal immunity and limited sampling techniques available, biopsies and CSF are our only options to collect immune cells for this study. Fourteen biopsy/CSF timepoints were selected in order to perform the needed experiments to assess mucosal immune changes with limited cell numbers isolated from biopsies in a longitudinal manner prior to treatment and SIV (timepoint 1), during acute SIV infection (timepoints 2-6), and during chronic SIV infection and treatment (timepoints 7-14). Fourteen timepoints were selected in order to determine the kinetics of mucosal changes prior to SIV, acute infection, chronic infection and during treatment, and late/chronic disease progression. These time points are essential in order to be able to get enough cells for different assays and to effectively characterize each stage. Given the length of time throughout this study, the large number of time points is adequately spread out.

**ACTIVE**  
Version 4 continued (Note: Version 4, Replaces Aim II timelines of version 3 (and modified in version 5))
Group B: Chronic/ART
*Multiple Surgery*

1. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection
2. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 2 weeks after #1
3. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #2
4. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #3
5. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #4
6. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #5
7. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #6
8. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #7
9. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #8
10. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #9
11. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #10
12. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #11
13. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #12
14. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #13

Species: Macaques
Interval: At least 1 week
Rationale: The importance of this study is to determine the effects of Lacto/IL-22 treatment on mucosal immunity and markers of mortality during SIV infection. Multiple biopsies and CSF collections will be essential to determine the kinetics of the mucosal and lymphoid immune response over time and in response to Lacto/IL-22 treatments, SIV infection, and phases of the menstrual cycle. Due to the nature of mucosal immunity and limited sampling techniques available, biopsies and CSF are our only options to collect immune cells for this study. Fourteen biopsy/CSF timepoints were selected in order to perform the needed experiments to assess mucosal immune changes with limited cell numbers isolated from biopsies in a longitudinal manner prior to treatment and SIV (timepoint 1), during acute SIV infection (timepoints 2-6), and during chronic SIV infection and treatment (timepoints 7-14). Fourteen timepoints were selected in order to determine the kinetics of mucosal changes prior to SIV, acute infection, chronic infection and during treatment, and late/chronic disease progression. These time points are essential in order to be able to get enough cells for different assays and to effectively characterize each stage. Given the length of time throughout this study, the large number of time points is adequately spread out.

ACTIVE
Version 4 continued (Note: Version 4, Replaces Aim II timelines of version 3 (and modified in version 5)
(Note: There is no multiple major surgery in this time line; included for improved understanding of timelines)

Group C: Chronic/ART Treatment
*Multiple Surgery*

1. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection
2. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 2 weeks after #1
3. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #2
4. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #3
5. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 2 weeks after #4
6. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #5
7. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #6
8. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #7
9. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 2 weeks after #8
10. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #9
11. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #10
12. Lymph node, vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #11
13. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #12
14. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #13
15. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #14
16. Vagina, jejunum, colon and rectum biopsy and CSF collection, at least 1 week after #15

Species: Macaques
Interval: At least 1 week

Rationale: The importance of this study is to determine the effects of Lacto/IL-22 treatment on mucosal immunity and markers of mortality during SIV infection. Multiple biopsies and CSF collections will be essential to determine the kinetics of the mucosal and lymphoid immune response over time and in response to Lacto/IL-22 treatments, SIV infection, and phases of the menstrual cycle. Due to the nature of mucosal immunity and limited sampling techniques available, biopsies and CSF are our only options to collect immune cells for this study. Sixteen biopsy/CSF timepoints were selected in order to perform the needed experiments to assess mucosal immune changes with limited cell numbers isolated from biopsies in a longitudinal manner prior to treatment and SIV (timepoint 1), during acute SIV infection (timepoints 2-3), then if UNINFECTED, another pre-SIV timepoint (surgery 4), re-infect SIV then acute SIV #2 (surgeries 5-8), and during chronic SIV infection and treatment (timepoints 9-16). Sixteen timepoints were selected in order to determine the kinetics of mucosal changes prior to SIV, re-infection if not infected, acute infection, chronic infection and during treatment, and late/chronic disease progression. These time points are essential in order to be able to get enough cells for different assays and to effectively characterize each stage. Given the length of time throughout this study, the large number of time points is adequately spread out.

ACTIVE Version 9

Multiple Major Surgery:
1. Colon, rectal and lymph node biopsy
2. Colon, rectal and lymph node biopsy, at least 1 week after #1
3. Colon, rectal and lymph node biopsy, at least 1 week after #2
4. Colon and rectal biopsy, at least 1 week after #3
5. Colon, rectal and lymph node biopsy, at least 1 week after #4
6. Colon, rectal and lymph node biopsy, at least 1 week after #5
7. Colon and rectal biopsy, at least 1 week after #6
8. Colon, rectal and lymph node biopsy, at least 1 week after #7

Species: Macaques
Interval: At least 1 week

Rationale: The importance of this study is to understand the kinetics of vaccine-specific immune responses in mucosal tissues. Multiple biopsies will be essential to determine the kinetics of the mucosal and lymphoid immune response prior to and after vaccination. Due to the nature of mucosal immunity and the limited sampling techniques available, biopsies are our only options to collect immune cells for this study. Surgery 8 may only occur if long-term follow up is deemed necessary as determined by high levels of vaccine responses that may confer resistance for an extended duration.
Groups 1, 2b, 3, 4

Multiple Major Surgery:
1. Lymph node biopsy
2. Lymph node biopsy, at least 1 week following #1
3. Implantation of indwelling catheter (1 or 2 ver 2), at least 1 week following #2
4. Repair of indwelling catheter, at least 1 day following #3
5. Lymph node biopsy, at least 1 week following #4
6. Repair of indwelling catheter, at least 1 day following #5
7. Nephrectomy, at least 1 day following #6
8. Renal Transplant including removal of the second native kidney, at least 30 days following #7
9. Lymph node biopsy, at least 1 week after #8

Species: M. Mulatta
Interval: At least 1 day to at least 30 days

Rationale: These multiple surgeries are required for the longitudinal analysis of the impact of NK depletion (Group 1) CAR+ T cells (Group 2b), costimulation blockade (Group 3) and Tregs (Group 4) on immune tolerance induction. The lymph node biopsies allow us to analyze the impact of the experimental protocol on the primary lymph organs, to determine if the immunologic response that we measure in the peripheral blood is also occurring in the sites of antigen presentation (the lymph nodes). The indwelling catheter placement allows to provide optimal clinical supportive care for the transplant recipients. Given that these catheters are expected to stay in place for as long as 100 days, we have included two potential repairs in our multiple surgery plan, which may be necessitated for a mechanical problem. Finally, renal transplantation is performed to most rigorously test whether immune tolerance is induced by our experimental protocol. Recipients first undergo a single nephrectomy and then, at least two weeks later, a renal transplant and concomitant removal of the second native kidney.

Groups 1, 2b, 3, 4 Using animals that were previously used as leukopheresis donors as transplant recipients.

Multiple Major Surgery:
1. Surgical approach to the femoral vein for placement of a pheresis catheter
2. Surgical approach to the femoral vein for placement of a pheresis catheter, at least 4 weeks after #1
3. Lymph node biopsy, at least 4 weeks after #2
4. Lymph node biopsy, at least 1 week after #3
5. Implantation of Indwelling Catheter (1 or 2, ver 2), at least 1 week after #4
6. Repair of Indwelling Catheter, at least 1 day after #5
7. Lymph node biopsy, at least 1 week after #6
8. Repair of Indwelling Catheter, at least 1 day after #7
9. Nephrectomy, at least 1 day after #8
10. Renal Transplant including removal of the second native kidney, at least 2 weeks after #9
11. Lymph Node Biopsy, at least 30 days after #1

Species: M. Mulatta
Interval: At least 1 day to at least 30 days

Rationale: These multiple surgeries are required for the longitudinal analysis of the impact of NK depletion (Group 1) CAR+ T cells (Group 2b), costimulation blockade (Group 3) and Tregs (Group 4) on immune tolerance induction in animals who have previously been utilized as peripheral blood stem cell donors. Utilizing previous leukopheresis donors in this fashion is necessary because transplant pairs are chosen based on rare MHC compatibility criteria. Thus, there are occasions where animals that had previously been donors are found to have these rare MHC compatibilities and must be used. The lymph node biopsies allow us to analyze the impact of the experimental protocol on the primary lymph organs, to determine if the immunologic response that we measure in the peripheral blood is also occurring in the sites of antigen presentation (the lymph nodes). The indwelling catheter placement allows to provide optimal clinical supportive care for the transplant recipients.
## Protocol Number | Weaning Variance
--- | ---
4009-01 | Weaning Variance: Wean by 30 days of age. In General mice are weaned around 25 days when they look large enough to survive without mother. Some latitude works well for the mice. We have 2 females and 1 male in a breeding cage. We remove the older pups as soon as possible if the other dam delivers. If additional litters are born i.e. if a second litter is born to a dam prior to the older pups being weaned then the older pups are weaned at that time.  
Species: Mice, D1 & D2-GFP heterozygotic BAC-transgenics  
Rationale: Mice are underweight at 21 days wean mice at 30 days instead of 21 days for health of pups, based on recommendations from Jackson labs.
3333-01 | Weaning Variance: Up to 28 days. Maximum number of adult mice in a cage is 5 with one litter counting as 1 adult mouse.  
Species: All MDX (dystrophic) mice.  
Rationale: Dystrophic mice have smaller litter sizes and less robust weanlings than the wild type controls. The lab has implemented this weaning variance for the health of the mice because there was a fair amount of mortality when the dystrophic mice were weaned at 21 days. While most of the colony (mdx) mice will be weaned between 21 to 24 days, the more fragile strains (dystrophin:utrophin double knockout [mdx:utrn-/-; or DKO]) requiring a longer time with the parents and will be evaluated on a litter by litter basis. The stronger/larger members of the same litter will be weaned as early as possible. The double knockout mice are the most fragile and breeding cages will be set up with one male and one female to help prevent overcrowding. With careful vigilance, the intent is to reduce both weanling and neonatal mortality.
4134-01 | Weaning Variance: Wean at 28 days of age  
Species: Mice, CHF1/Hey2 KO  
Rationale: These mice are generally small at 3 weeks and have decreased survival when weaned at this age. Better success is achieved when weaned up to 28 days postpartum.
2242-08 | Weaning Variance: Wean at 28 days instead of 21 days  
Species: Mice, Id1+/Id3-/-  
Rationale: Because of their transcriptional defects, growth rate of Id1+/Id3-/- pups is also slower so that at 21 days their body size resembles that of a 15-16 day old wild type pup. Weaning of such pups clearly smaller in body size is delayed from 21 to 28 days post-birth. Because these knock-out mice are usually represented at low frequency within a litter (i.e., 1 out of 8-12 pups), it is expected that no more than 1 or 2 pups per breeder cage may be left for weaning at 28 days at any given time.
4231-01 | Weaning Variance: Wean at 28 days, maximum in cage 1 male and 2 female adults with 1 litter.  
Species: CAR-null mice  
Rationale: This mouse strain has lower body weight compared to age-matched wild type mice.
4339-01 | Weaning Variance: Wean no later than 25 days onto breeder chow 5058.  
Species: Mice, line Th fs/fs  
Rationale: Homozygotes Th fs/fs mice are adipsic, aphagic, hypoactive, small, and need to be left with the mothers a little longer than normal. If delayed weaning overlaps with the
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<td>Birth of a new litter, then we will wean remove all but the homozygous mutants from the cage. Due to the expected genotype frequency of 1:22 for Th fs/fs mice we expect no more than 1 or 2 homozygous mice with extended weaning would remain with the new litter.</td>
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| Weaning Variance: Wean at 28 days of age.  
Species: Mice, TCR transgenic, RAG null, TCRa null, TCRbg null, plt and Bim null  
Rationale: These lines develop more slowly than standard strains and at 21 days are too small and weak to self-feed. Will have (a) 1 adult male, 2 adult females, and 2 litters up to 21 days of age, or (b) 1 adult female, 1 litter up to 28 days of age and 1 litter under 21 days of age. May need to separate out a female and pups into a separate cage until the pups are weaned, e.g., if one female has two litters and the other female has one litter. In rare cases in which 13 or more pups 14 days of age or older exist in a cage of breeders, we will separate out one female and her pups to reduce the total number of mice per cage to an acceptable level. |

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| Weaning Variance: Wean at 28 days. Breeding cages will contain 2 adult mice and potentially 2 litters of mice.  
Species: Mice, alpha syntrophin KO, the NPC knockout, and the KN2 mice  
Rationale: Mice in this study tend to be smaller at the standard weaning age. |

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| Weaning Variance: Wean at 22-28 days of age. 
Species: Mice, backcrossed B6.129  
Rationale: Pups are frequently too small at 21 days to wean. |

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| Weaning Variance: Wean at 28 days in cases where mutated mice experience delayed development and reduced viability.  
Species: Mice, CX3CR1-/-, CX3CR1+/-, CX3CL1-/-, CX3CL1+/-  
Rationale: If pups develop slower than average they will need to stay with the dam for improved viability. Additionally, maternal care has long-term effects for offspring development, and the duration of that maternal care has been shown to influence physiological and social growth. This is important since our protocol entails behavioral testing. |

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| Weaning Variance: Triplicate breeding where one mom is WT. Late weaning by 5-7 days if pups look smaller than 21 days or recommended by Vet Services. Late weaning/separating of the first litter by 5-7 days (i.e., at the time the second litter is 5-7 days old) if a new litter is born in the cage prior to weaning the first litter. Late weaning applies to entire litter. Latest weaning will be 28 days. 
Species: Mice Pit-1 and Pit-2  
Rationale: Neonatal death is increased within 5-7 days after birth due to a cage disturbance or loud noise/vibration nearby. Early (prior to 5-7 days old) separation of the neonates into a new cage is likely to result in neonate lethality in these lines. A weaning variance is required to reduce stress on the animals and promote breeding with these lines. |

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| Weaning Variance: floxed-Runx2, SM22Cre, LDLr-/-, MGP+/- wean 5-7 days late in standard pair breeding. 
Species: Mouse  
Rationale: We had better neonate survival if postponed weaning of these lines of mice. Late weaning/separating of the first litter by ~5-7 days (i.e., at the time that the second litter is 5-7 days old) if a new litter is born in cage prior to weaning the first litter. |

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| Weaning Variance: Wean between 28 - 35 days of age. The maximum number of mice in one cage will be 1 adult male, 2 adult females, and 2 litters less than 35 days old. 
Species: Mice, TCR transgenics, Rag-/-, B cell-deficient, IFN-g-/-, IFN-g receptor-/-, IL-17 receptor-/-, Fas-/-, perforin-/-, GM-CSF-/-  
Rationale: Many of our TCR transgenic and other immunodeficient strains have pups that are too small to be weaned at 21 days of age. If we leave these pups with their dam until
they are 28 to 35 days old, they thrive and do much better.

<p>| Weaning Variance: Wean at 28 days of age. Breeding cages may house a trio (one male, two females) and no more than 12 pups up to 28 days old. If additional litters are born prior to weaning the older litter(s), the older litter will be moved to a new cage. Species: Mice, various strains: TCR gag Rag1-/- X Albgag Rag1-/-; AST X AlbCre; AST GAG Thy1.1; AST X TamCre; SV40-1 Thy1.1 X SV40-1 Thy1.1; SV40-4 Thy1.1+-/-; SV40-1 PD1KO; P14 X P14 Thy1.1; P14 Thy1.1+-/- Cre Flox+/-; aBeta TCR Thy1.1 Cre Flox; P14Thy1.1+-/- clb-b-/-; P14 Thy1.1 X GMIL-2; P14 Thy1.1+-/- PD1KO; P14+-/- Thy1.1+-/- Ly5.1+-/- IL10+-/- X P14 Thy1.1+-/- Cre; P14 Thy1.1 X Ly5.1+-/- Cre F/F; P14 Thy1.1+-/- TGFBeta+-/- Cre; Micro150+-/- TCR Thy1.1+-/-; TCR Thy1.1+-/- clb-b-/- Flox+-/- Cre; TCR Thy1.1+-/- clb-b-/- PD1KO; aBeta TCR Thy1.1+-/- PD1KO; TCR Thy1.1+-/- Micro150+-/- X Micro150+-/-; P14 Thy1.1+-/+ F/F Cre; SMARTA PD1KO+-/-; SMARTA Thy1.1+-/- clb-b-/-; POET^2; IL10+-/- Cre Ly5.1+-/-; OT-1 Ly5.1+-/- F/F Cre; OT-1 Ly5.1 Cre IL10+-/-; SMARTA Thy1.1; Flox+-/- Cre; TRAMP Rationale: Mice of these strains are sometimes relatively small and show increased mortality if weaned at 21 days of age. Variance: House 6 mice &lt; 25 grams per cage for a 4 week period between weaning and genotyping. Species: Mice, see strains under Weaning Variance above Rationale: The strains of mice being genotyped are less than 25 grams so technically 6 animals per cage is within the requirements in the GUIDE. | 2013-01 |
| Weaning Variance: Pups of CaBP1 KO and CaBP2 KO to be weaned between 21 - 28 days of age. For trio breeding, when a new litter is born in the cage prior to weaning of the old litter, the old litter will be separated immediately. For quad breeding, 2 females are separated when apparent that one of them is pregnant. Species: Mice, CaBP1 KO and CaBP2 KO Rationale: Pups with CaBPs targeted genes are still very small at a weaning age of 21 days. In addition these mouse lines produce small litters (~5-6 pups). To increase viability, weaning age will be between 21 - 28 days of age. | 3274-02 |
| Weaning Variance: Wean at 28 days of age. Breeding cages include 1 adult male and 2 adult females and up to 2 litters. Remove one female and litter(s) if additional litters are born. Species: Mice, PiT1 mutant strains (Pit1fl/fl;MxCre and Pit1fl/fl;EpoRCre) Rationale: Delay weaning out to 28 days because the 3 strains have low weight/size and would benefit from receiving extended maternal nutrition. | 4274-01 |
| Weaning Variance: Wean at up to 28 days of age. Number of mice maintained in breeding cages will be per relevant IACUC policy. Species: Mice, PKC KO Rationale: Mice are smaller than normal at 21 days of age of age and therefore need to delay weaning by up to 1 week. | 4261-01 |
| Weaning Variance: Wean between 24 - 35 days of age. The maximum number of mice in one cage will be one male, 2 females, and 2 litters less than 35 days old; if unusually large litters are born making it so that there are more than 12 pups, 2 females and 1 male, present in the cage at one time, one of the two females with her litter will be separated to a new cage before the pups reach two weeks of age. In the event that a new litter is born to a cage in which pups greater than 19 days of age are present, the older litter will be separated with their dam immediately. If both dams have new litters while older pups are present or we are unable to determine which dam to separate with the older litter, the older pups will be weaned immediately. Species: Mice, Smad3-/-, Rag2-/- double knockout, B6.Smad3-/-, TLR4-/- | 2436-12 |</p>
<table>
<thead>
<tr>
<th>Date</th>
<th>Rationale</th>
<th>Weaning Variance</th>
<th>Species</th>
<th>Notes</th>
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<tbody>
<tr>
<td>2022-01</td>
<td>Pups are not able to feed themselves at 21 days of age due to genetic mutation. Breeding is done in trios so the cage can have an adult male, two adult females, and two litters less than 35 days of age. If a female has a second litter, one of the females and litter(s) should be removed to another cage.</td>
<td>Wean between 28 - 35 days of age.</td>
<td>Mice, RII mutant, Cbeta, RIIbeta, Calpha, AKAP7 and crosses of these strains.</td>
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<td>Weaning Variance: After weaning, up to 6 male siblings may be housed together for up to 35 - 42 days of age.</td>
<td>Wean at 30 days of age.</td>
<td>Species: Mice. Rationale: Animals are genotyped at 21 days of age, and the genotyping process can take from 1 - 2 weeks. Animals that do not have the desired genotyped are euthanized. Housing the males together alleviates the problem that once separated, males cannot be caged together again without fighting.</td>
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<tr>
<td>2856-01</td>
<td>Tumor growth in RIIalpha KO and mutant mice usually happens after 1 year and mice are euthanized prior to that age.</td>
<td>Wean at 30 days of age.</td>
<td>Species: Mice, any strains that may have retarded growth but need to be kept into adulthood, especially Bax-/-, Bif-1/-/, Bif-1c (added in version 74). Tumor growth in RIIalpha KO and mutant mice usually happens after 1 year and mice are euthanized prior to that age.</td>
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<tr>
<td>2225-04</td>
<td>Weaning Variance: Wean at 38 days for DBA/2J alphaMHC GFP mice. Harem breeding. Species: Mice, DBA/2J alphaMHC GFP.</td>
<td>Wean at 30 days of age.</td>
<td>Species: Mice, muscarinic receptor knockouts. Rationale: It has been our experience over the last 10 years that a large percentage of the</td>
<td>Weaning Variance: Wean at 30 days of age. For a single breeding pair (1 male plus 1 female per cage) pups to remain with their mothers for 30 days rather than the standard 21 day, with potentially a new litter also in the cage. Breeding trio (one male, two females) and 1-2 litters of pups up to 30 days old. In this case, if additional litters are born prior to weaning the older litter(s), the new litter will be removed for use in our tissue culture studies.</td>
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<tr>
<td>2239-01</td>
<td>Species: Mice, DBA/2J alphaMHC GFP. Rationale: We have found that some of the pups are small (~6 g) at 21 days of age. The veterinary staff agrees that the pups may benefit by staying with the adult mice until 28 days. Our plan will be to separate the older litter from the dam when a new litter is born.</td>
<td>Wean at 30 days of age.</td>
<td>Species: Mice, DBA/2J alphaMHC GFP. Rationale: We have found that some of the pups are small (~6 g) at 21 days of age. The veterinary staff agrees that the pups may benefit by staying with the adult mice until 28 days. Our plan will be to separate the older litter from the dam when a new litter is born.</td>
<td>Weaning Variance: Wean at 30 days of age. For a single breeding pair (1 male plus 1 female per cage) pups to remain with their mothers for 30 days rather than the standard 21 day, with potentially a new litter also in the cage. Breeding trio (one male, two females) and 1-2 litters of pups up to 30 days old. In this case, if additional litters are born prior to weaning the older litter(s), the new litter will be removed for use in our tissue culture studies.</td>
</tr>
<tr>
<td>2239-01</td>
<td>Weaning Variance: Wean at 30 days of age. For a single breeding pair (1 male plus 1 female per cage) pups to remain with their mothers for 30 days rather than the standard 21 day, with potentially a new litter also in the cage. Breeding trio (one male, two females) and 1-2 litters of pups up to 30 days old. In this case, if additional litters are born prior to weaning the older litter(s), the new litter will be removed for use in our tissue culture studies.</td>
<td>Wean at 30 days of age.</td>
<td>Species: Mice, muscarinic receptor knockouts. Rationale: It has been our experience over the last 10 years that a large percentage of the</td>
<td>Weaning Variance: Wean at 30 days of age. For a single breeding pair (1 male plus 1 female per cage) pups to remain with their mothers for 30 days rather than the standard 21 day, with potentially a new litter also in the cage. Breeding trio (one male, two females) and 1-2 litters of pups up to 30 days old. In this case, if additional litters are born prior to weaning the older litter(s), the new litter will be removed for use in our tissue culture studies.</td>
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Rationale: These mice are much smaller than heterozygote or wild type mice and if left with their dam until they are 24 - 35 days old they thrive and do much better.
<table>
<thead>
<tr>
<th>Code</th>
<th>Weaning Variance</th>
<th>Species/Strain</th>
<th>Rationale</th>
</tr>
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<tbody>
<tr>
<td>4246-01</td>
<td>Wean at 28 days of age.</td>
<td>Mice, double knockout mice</td>
<td>Weaning at a later age is beneficial for mice with reduced viability. Double knockout mice often have reduced viability. Gel and diet on the bottom of the cage will be provided at weaning and the sign &quot;Gel/Toggle&quot; attached to the front of weaned cage. To avoid trampling newborn pups by previous litter, cages with P15-P18 pups will be checked for pregnant female(s) and pregnancy stage. Pups will be weaned earlier (starting at 19 days) if next litter is to be born within 1-2 days.</td>
</tr>
<tr>
<td>2183-02</td>
<td>Wean at 28 days of age.</td>
<td>Mice, Th fs/fs</td>
<td>These mice are small and need to be left with their mothers a little longer than normal (but no longer than 28 days).</td>
</tr>
<tr>
<td>2650-05</td>
<td>Wean at 28 days of age.</td>
<td>Mice, apo E/-</td>
<td>Litters are smaller and pups grow at a slower pace</td>
</tr>
<tr>
<td>2650-07</td>
<td>Wean at 28 days of age for apo E/- strain.</td>
<td>Mice, apo E/-</td>
<td>The pups of the apo E/- mice are smaller than normal C57Bl/6.</td>
</tr>
<tr>
<td>2650-08</td>
<td>Wean at 28 days. Mice are bred as 2 females and 1 male. If a dam drops a second litter, wean the older pups immediately.</td>
<td>Mice apoE/-</td>
<td>Mice are undersized at 21 days and do not thrive well if weaned.</td>
</tr>
<tr>
<td>2456-06</td>
<td>Wean at up to 27 days of age.</td>
<td>Mice, PPARgNKO and WT</td>
<td>Weaning time is an experimental variable (20 vs 27 days). PPARg controls body weight in a manner that is dependent on time of weaning might suggest that this receptor is an important factor in the post weaning development of neurocircuits involved in the regulation of body weight, food intake and energy homeostasis. Assess post-weaning body and food intake in PPARgNKO and WT mice both after a normal weaning time (20 days) and after prolonged weaning (27 days).</td>
</tr>
<tr>
<td>4196-01</td>
<td>Wean at 28 days of age. Cage will contain 2 adult females and 1 adult male and 2 litters of up to 28 days of age.</td>
<td>Mice, AKAP150 and others.</td>
<td>Some strains of mice (e.g., AKAP150, AKAP220, Wave1KO, crosses of these) are undersized for their age.</td>
</tr>
<tr>
<td>4236-02</td>
<td>Wean at 28 days of age. Breeding trio (one male, two females) and 1-2 litters of pups up to 28 days old. We request leaving these pups with their dam until they are 28 days old, we will wean these mice at 28 days with 2 normal</td>
<td>Mice LDLR/- and RAGE/-</td>
<td>According to our past work with the strains proposed in this protocol, neonatal death is increased within 5-7 days after birth due to a cage disturbance or loud noise/vibration nearby. Considering smaller average litter size for C57BL6 strain and sometimes smaller pup size for LDLR/- and RAGE/- mice, we request a weaning variance for the standard (pair) breeding.</td>
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Muscarinic receptor knockout pups die if separated from their mothers at 21 days.
| Littermates and 1-2 runts. We will reduce the litter size to 3-4 pups per litter at 21 days. In the event that a new litter is born to a cage in which pups greater than 21 days of age are present, the older litter will be separated with their dam immediately. If a dam has a new litter prior to weaning its older pups then the older pups will be weaned immediately. | Weaning Variance: Weaning age up to 28 days with no more than 3 adult mice (2 females and one male) and two litters (12 pups) in a cage at any time. When there are two litters in a cage at the same time the older litter will not be > 18 days old. When a litter is born in a cage that already has a litter > 18 days either the older litter will be weaned immediately (if 21 days or older and appear to be doing well) or the younger litter will be removed and sacrificed immediately or before the older litter reaches 19 days old. | 3410-01 |
| -- | Species: Mice (strain AC3/-) Rationale: The AC3/- mice are runts and too small to be weaned at 21 days of age. We usually get 1-2 runts per litter and will keep the runted pups with their two normal littermates until 28 days; the normal littermates are to be used as controls for the runted litter mates. | |
| -- | Species: Mice on background strain of C57BL/6;129Sv Rationale: All of the protocol's mice are on a background strain of C57BL/6;129Sv. This inbred strain is known to have small litters of slow developing pups with relatively long inter-litter intervals. | 2199-09 |
| -- | Species: Mice, Deafwaddler and quivering strains, Potassium channel mutants Kcna1KO, Kcna1EA1, Kv1.2KO PMCA2 mutants Atp2b2dfw, Atp2b2dfw2J, Atp2b2dfw5, Atp2b2Tg-dox, Atp2b2V586M β-spectrin4 mutants Spnb4qv, Spnb4qv4J Claudin9 mutant Cldn9Tg-dox Rationale: Several of our strains have smaller pups and reduced litter sizes often of only 4-5 pups. These pups do not develop motor skills sufficient for them to be weaned at 21 days. We find that maintaining stable trios (1 adult male and 2 adult females) and allowing the pups to stay with their moms until 28 days for weaning helps the pups be able to reach/eat the food and water. Given the small pups and reduced litter size this variance should not cause overcrowding. | |
| -- | Species: Mice, Tet1/- strain Rationale: There is a developmental delay in the Tet1/- genotype, which means that the mice are very small at the time for normal weaning at 21 days. Veterinary Services recommends that they be weaned between 21 and 28 days to allow mice to thrive without their mother. | 4057-01 |
| -- | Species: Mice, transgenic on C57BL/6 background Rationale: These mice tend to be smaller than other strains of mice. Weaning at 28 days of age results in more robust mice. We normally place 1 male and 2 females in cage for mating. We will place one litter with dam only per cage during the extended weaning period (between 3-4 weeks after pups are born) to avoid potential crowding. | 3041-04 |
| -- | Species: Mice, Rag 1/-, mdx and alpha7 integrin Rationale: Because of the small size of these animals, there is an increase in deaths post-weaning if they are weaned at 21 days of age. The animals at 28 days of would be a more typical size for weaning. | 2488-10 |
Weaning Variance: Wean Drd1a-null mice at up to 28 days of age. Breeding trio (one male, two females) and 1-2 litters of pups up to 28 days old may be in the cage at any one time. If additional litters are born prior to weaning the older litter(s), the new litter will be removed.
Species: Mice, Drd1a-null strain
Rationale: These mice are smaller than littermate controls and require one extra week before weaning.

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pharmacokinetic studies.

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<th>Protocol Number</th>
<th>Temperature Variance</th>
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| 2070-06         | Temperature Variance: Mice caged from 1-6 hours at 4 degrees Celsius Species: Mice  
Rationale: Used to provide a physiological delivery of adrenergic stimulation for study of body temperature control. |
| 3104-01         | Temperature Variance: 5 degrees C for 4 hours, 14 degrees C for 3 days Species: Mice  
Rationale: For acute cold stress measurements, mice will be placed inside the test chamber and ambient temperature will be reduced from 22 degrees C to 5 degrees C over a 60 minute period. The temperature will be maintained at 5 degrees C + or - 0.5 degrees C for 240 minutes and then raised back up to 22 degrees C over the next 60 minutes. Each cohort of mice will be subjected to a single acute cold stress over a 3-day period. Following recovery (at least 5 days), an additional second chronic cold stress will be induced by reducing ambient temperature to 14 degrees C over a 60-minute period. The temperature will be maintained at 14 degrees C + or - 0.5 degrees C for 3 days and then raised back to 22 degrees C over the next 60 minutes. These measurements are necessary to determine the effect of CLA on thermogenesis in a mouse model of diet-induced obesity. |
| 3328-04         | Temperature Variance: Cool rat pups to 32C for 4 h Species: Rats  
Rationale: Determine blood and brain pharmacokinetics of dexmedetomidine to mimic hypothermic therapy in newborn. |
| 3328-05         | Temperature Variance: Use of a cooling cap for 72 hours Species: Macaca nemestrina  
Rationale: The group is studying hypothermia as part of a treatment for brain injury during or immediately following birth. |
| 4118-01         | Temperature Variance: 5 degrees C for 4 hours. Species: Mice  
Rationale: We will conduct the varied temperature experiment using environmental test chambers (incubators). All the energy homeostasis measurements can be performed within the two incubators by placing the Sable systems metabolic cages. For experiments examining the metabolic response to cold adaptation, the environmental chambers will then be set at 5°C and allowed to reach that temperature overnight. The following day, mice will be placed in individual metabolic cages that contain bedding that are housed within the environmental chamber at 5°C. Following four hours of exposure at 5°C, animals will be removed and immediately sacrificed. |
| 2057-01         | Temperature Variance: Species: Zebrafish  
Rationale: For induction of transgene expression zebrafish will be heat-shocked for 1 hour per day for the length of the experiment (up to 42 days post transection) by raising the temperature of their water from 28°C to 37°C slowly over 15-30 minutes, then returning the fish to 28°C water. The lab has found that heat-shocking transgenic fish one time per day for the length of the experiment for a duration of 1 hour leads to adequate induction of the transgene as measured by GFP expression. Heat-shock will not be administered prior to completion of the 5-day post-operative monitoring procedure. |
<p>| 4246-01         | Temperature Variance: Housing at 29 +/- C Species: Mice |</p>
<table>
<thead>
<tr>
<th>Temperature Variance</th>
<th>Species</th>
<th>Rationale</th>
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</thead>
<tbody>
<tr>
<td>Elevated housing temperatures</td>
<td>Zebrafish</td>
<td>Exposure of larval and adult fish to temperatures ranging from 29 - 33 degrees C to inactivate a particular immune receptor.</td>
</tr>
<tr>
<td>Thermoneutral</td>
<td>Mice, Rats</td>
<td>Animals undergo a series of metabolic experiments. Normally, room temperature is kept between 68° and 78° F. However, to eliminate the possibility that a defect in the maintenance of body temperature is contributing to the enhanced energy expenditure in rodents of a particular genotype, the same metabolic experiments need to be performed under thermoneutral conditions. This experiment is performed routinely in metabolic chamber systems and does not harm the animal. Room temperature will be manually raised from 76° to 90° F with a 4 ~5 degree rise per every 48 hour. Animals will be placed in the metabolic chambers for a total of 192 hours.</td>
</tr>
<tr>
<td>Heat exposure</td>
<td>Mice, Rats</td>
<td>Metabolic experiments. Ambient temperature (Ta) of the chamber will be increased from 22°C to as high as 38°C over a period of 60 min. Ta will be maintained at 38 ± 0.5°C for 120 min and then returned to 22°C over the next 60 min. Each cohort will be tested in the incubators over a 3-day period. Animals will be monitored every 15 minutes throughout the duration of the heat exposure.</td>
</tr>
<tr>
<td>Cold stress</td>
<td>Mice, Rats</td>
<td>Metabolic experiments. Ambient temperature (Ta) will be reduced from 22°C to 7°C over a 60-min period. A Ta value of 7 ± 0.5°C will be maintained for 120 min and then will be raised back to 22°C over the next 60 min. Each cohort will be subjected to cold stress over a 3-day period. Animals will be monitored every 15 minutes throughout the duration of the heat exposure.</td>
</tr>
<tr>
<td>Cool Temperature</td>
<td>Mice (C57Bl/6 and ob/ob)</td>
<td>Metabolic experiments. Ambient temperature (Ta) will be reduced from 22°C to 14°C. A Ta value of 14°C will be maintained for up to 1 week and then will be raised back to 22°C. This cool exposure time is less than that previously reported in the literature (2 weeks).</td>
</tr>
<tr>
<td>Thermal seizure induction</td>
<td>Mice</td>
<td>Thermal seizure induction - core temp potentially raised through 37C - 42.5C - maximum duration 22 minutes outside of normal housing temperatures.</td>
</tr>
</tbody>
</table>
| Mice in MPTP experiment in K017B with a temperature range of 68-81 degrees F before and after MPTP injection with an average temperature setting of 76
degrees F. The average setting falls within the range of the Guide (68-79) but there is a chance that the daily high may exceed the Guide. Temperature should not be reset unless it exceeds 81 degrees. Sensaphone still alarms at 82 degrees F. The purpose is that MPTP-treated animals have better survival if kept warmer. Controls must be kept at the same temperature as MPTP animals.

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<tr>
<td>3361-01</td>
<td>Cage Variance:</td>
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<tr>
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<td>Species: MICE</td>
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<tr>
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<td>Description: CLAMS metabolic cages are smaller than Guide recommendations.</td>
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<td>Rationale: Animals are housed in these cages up to 72 h for indirect open-circuit calorimetry.</td>
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<td>Cage Variance:</td>
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<tr>
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<td>Species: Mice</td>
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<tr>
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<td>Description: Mice are housed for 24-48 hours in CLAMS cages for metabolic screening. The CLAMS cages are 4&quot; in height.</td>
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<tr>
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<td>Rationale: The CLAMS caging system is necessary to perform a comprehensive characterization of energy homeostasis. Significant change (v.65) requesting variance was made on 11/18/2013.</td>
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<td>Description: Mice are housed in the Comprehensive Lab Animal Monitoring System (CLAMS) to measure total energy expenditure, food and water intake, and physical activity for up to 72 hours.</td>
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<td>Rationale: The CLAMS indirect calorimeter is preferred to that provided by Sable Systems (which has adequate cage dimensions) depending on the study design and the measures required to perform a comprehensive characterization of the energy homeostasis phenotype of the animal. The CLAMS cage does not contain bedding, which allows for collection and quantification of urine and feces over designated time periods. The feeding apparatus is equipped to continuously measure food intake allowing the calculation of the size of the meal, the number of meals consumed, and the time between meals, which are important measures in the study of obesity and insulin resistance. The Sable System has a flawed hopper design, which results in excess food spillage into the cage; therefore, the optimal collection of metabolic data requires use of the CLAMS unit.</td>
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## Appendix D
### Environmental Enrichment Exemptions

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<th>Principal Investigator</th>
<th>Species</th>
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<tr>
<td>Adams Waldorf, Kristina M</td>
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<td>M. Nemestrina</td>
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<tr>
<td>Agy, Michael B</td>
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To:     Institutional Animal Care and Use Committee Members

From:   Tena Petersen
        University of Washington
        Manager, Regulatory Affairs
        Institutional Animal Care and Use Committee
        Box 357160

Attached are copies of the University of Washington's Semiannual Program and Facility Review for the humane care and use of animals for the period beginning January 1, 2014 and ending June 30, 2014.

*Please note: Copies of the individual reports and the responses to any deficiencies noted in the report are on file in our office. If you would like to see the responses to any of the deficiencies please let me know and I will get a copy of it to you.*

Please sign and return this memo, if you are in agreement with the Program Review, or note below or on a separate sheet of paper, any discrepancies within the report that you find or any minority opinions that you might have regarding this report. Positive responses from a majority of the Committee members are required in order to submit the report to the Executive Director for Health Sciences Administration. Please return by July 31, 2014. Thank you

*******************************************************************************

Return To:   Tena Petersen
             Manager of Program Operations
             Box 357160
tena@uw.edu

I concur with the January 1, 2014 through June 30, 2014 University of Washington's Semiannual Program and Facility Review.

____________________________
Signature

Adam Leaché
Printed Name
July 17, 2014

To: Institutional Animal Care and Use Committee Members

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University of Washington
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Signature

Printed Name

July 17, 2014

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tena@uw.edu

I concur with the January 1, 2014 through June 30, 2014 University of Washington’s Semiannual Program and Facility Review.

Signature

Printed Name

July 17, 2014

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Manager, Regulatory Affairs  
Institutional Animal Care and Use Committee  
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tenaf@uw.edu

I concur with the January 1, 2014 through June 30, 2014 University of Washington’s Semiannual Program and Facility Review.

Signature  
4-7-2014

Printed Name  
Mark Tetrich
July 17, 2014

To: Institutional Animal Care and Use Committee Members

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University of Washington
Manager, Regulatory Affairs
Institutional Animal Care and Use Committee
Box 357160

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Manager of Program Operations
Box 357160
ten@uw.edu

I concur with the January 1, 2014 through June 30, 2014 University of Washington’s Semiannual Program and Facility Review.

Signature

Emily Clark

Printed Name
January 13, 2014

To: Institutional Animal Care and Use Committee Members

From: Tena Petersen
University of Washington
Manager of Program Operations
Institutional Animal Care and Use Committee
Box 357160

Attached are copies of the University of Washington’s Semiannual Program and Facility Review for the humane care and use of animals for the period beginning July 1, 2013 and ending December 31, 2013.

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I concur with the July 1, 2013 through December 31, 2013 University of Washington’s Semiannual Program and Facility Review.

[Signature]

[Printed Name]

July 17, 2014

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Institutional Animal Care and Use Committee
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July 17, 2014

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Institutional Animal Care and Use Committee  
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******************************************************************************

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Manager of Program Operations  
Box 357160  
tena@uw.edu

I concur with the January 1, 2014 through June 30, 2014 University of Washington’s Semiannual Program and Facility Review.

Cathy Carrier  
Printed Name

July 17, 2014

To: Institutional Animal Care and Use Committee Members

From: Tena Petersen
University of Washington
Manager, Regulatory Affairs
Institutional Animal Care and Use Committee
Box 357160

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Manager of Program Operations
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tenat@uw.edu

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Signature

Jane Sullivan

Printed Name

July 17, 2014

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tenapetersen@uw.edu

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Robert Murnane
Signature

July 17, 2014

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[Signature]

Printed Name

Box 357160 Seattle, Washington 98195-7160  http://depts.washington.edu/uawhome/index.html
July 17, 2014

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Manager, Regulatory Affairs  
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Box 357160  
tenavw.edu

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Signature

Thea Brabb
Printed Name
July 17, 2014

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Manager of Program Operations
Box 357160
ten@uw.edu

I concur with the January 1, 2014 through June 30, 2014 University of Washington’s Semiannual Program and Facility Review.

Signature

Printed Name

Subject: Re: Updated Documents for the Semiannual Program and Facility Review

Date: Wednesday, July 30, 2014 at 8:04:18 AM Pacific Daylight Time

From: Jeremy Clark

To: Tena L. Petersen

Hello Tena,
I am going to be unable to make it into the office this week. Can this email serve as my concurrence? If so, I concur.

Jeremy

Jeremy J Clark PhD
Assistant Professor
Psychiatry and Behavioral Sciences
University of Washington
Seattle WA 98195

On Jul 24, 2014, at 12:52 PM, Tena L. Petersen <tenauw.edu> wrote:

Hi Everyone,

Attached are the updated documents for the Semiannual Program and Facility Review. Also attached is the concurrence letter. Please let me know if you have any additional corrections, questions, or additions and submit your concurrence sheet, by July 31, 2014.

This is your report, so if moving forward, you have suggestions for improving this process or if there is information that you would like to be reviewed, etc., please let me know.

Thanks very much! Tena

Tena Petersen
Manager, Regulatory Affairs and Interinstitutional Collaborations
Office of Animal Welfare
University of Washington
Box 357160
tenauwashington.edu
Phone: (206) 543-9678
Fax: (206) 616-5664
OAW Website: http://depts.washington.edu/oawhome/
<Concurrence Letter January 1 - June 30, 2014.docx>
<Spring 2014 Report Summary for IACUC.pdf>
<Jan - Apr 2014 by Type.xlsx>
<Semi-annual Prog Rev Checklist - July 2014.docx>
<Semiannual Facility Inspection Checklist July 2014.docx>
Hi Tena,
I concur with the report.
Steve

On Jul 24, 2014, at 12:52 PM, Tena L. Petersen <tena@uw.edu> wrote:

Hi Everyone,
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Thanks very much! Tena
Tena Petersen
Manager, Regulatory Affairs and Interinstitutional Collaborations
Office of Animal Welfare
University of Washington
Box 357160
tena@u.washington.edu
Phone: (206) 543-9678
Fax: (206) 616-5664
OAW Website: http://depts.washington.edu/oawhome/

Stephen J. Libby, Ph.D.
University of Washington
Chair, Institutional Biosafety Committee
Department of Laboratory Medicine
HSB K451
Campus Box 357110
1959 NE Pacific St
Seattle, WA 98195-7110
206-616-4941 office
206-616-9925/4282 Lab
206-616-1575 Fax
425-765-9629 Cell
slibby@uw.edu
slibby@uw.edu
July 17, 2014

To: Institutional Animal Care and Use Committee Members

From: Tena Petersen  
University of Washington  
Manager, Regulatory Affairs  
Institutional Animal Care and Use Committee  
Box 357160

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Manager of Program Operations  
Box 357160  
ten@uw.edu

I concur with the January 1, 2014 through June 30, 2014 University of Washington’s Semiannual Program and Facility Review.

______________________________
Judith G. Stoloff  
Signature

______________________________
Judith G. Stoloff  
Printed Name

July 17, 2014

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tenaa@uw.edu

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[Signature]

Printed Name

July 17, 2014

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ten@uw.edu

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Signature

Loren M. Kinman

Printed Name
July 17, 2014

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