Institutional Animal Care and Use Committee Minutes

VCRC - 76D

01.11.22

Meeting Convened: 12:00 PM	Quorum Requirement: 10
Meeting Adjourned: 1:40 PM	Members Present to Vote: 14

Voting Members			Alternates		
1		(Chair - M, S)			
2	x	(M, V)	A		(A, V)
			B	x	(A, V)
			C	x	(A, V)
			D		(A, V)
			E	x	(A, V)
			F		(A, V)
			G		(A, V)
			H	x	(A, V)
			I		(A, V)
3		(M, S)	J		(A, S)
			K		(A, S)
4	x	(M, U)	L	x	(A, U)
			M	x	(A, U)
			N	x	(A, U)
			O	x	(A, U)
			P	x	(A, U)
5	x	(M, S)	Q		(A, S)
6		(M, V)	R	x	(A, V)
7		(M, S)	S		(A, S)
8		(M, S)	T	x	(A, S)
9		(M, St)	U	x	(A, St)
10		(M, S)	V	x	(A, S)
11	x	(M, S)	W		(A, S)
12	x	(M - NA, NS)	X		(A - NA, NS)
13	x	(M, S)	Y		(A, S)
14		(M, S)	Z	x	(A, S)
15		(M, S)	AA	x	(A, S)
16		(M, St)	BB		(A, St)
17		(M, S)	CC	x	(A, S)
18		(M, S)	-		
19	x	(M, U)	DD		(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	x	(O, U)

Institutional Veterinarian:

3	x	(M, V)

Correlates to Version **v9.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion Items

1. The committee was updated on the November 2021 inspection summary. There were no significant findings and no reports to OLAW for the month of November.

2. The committee discussed a question from an investigator regarding the suitability for adoption of a specific research dog. The [redacted] veterinarian's professional opinion was that the dog is not in good health and is therefore not a candidate for adoption. The committee concurred with the veterinarian and voted that the investigator should euthanize the dog according to the previously agreed upon timeline of January 31, 2022.

3. The committee was updated on the work of the subcommittee on teaching using client owned animals. USDA has indicated that client animals receiving standard of care that would be covered under the MN Veterinary Practice Act are not USDA/AWA-R covered, but the committee does not want to relinquish all oversight of these activities. Further discussion is needed on how the IACUC can improve and streamline the process while retaining oversight.

4. The committee was updated on the work of the subcommittee on SR-buprenorphine. The subcommittee recommends updating the posted dosing recommendation for the ZooPharm product from 2 mg/kg to 0.5-1 mg/kg for mice, consistent with the manufacturer's recommendation. The new recommendation will be rolled out gradually via new protocol submissions, continuing reviews, and inspections. Further study and discussion are needed regarding a newer, FDA-indexed product, Ethiqa.

5. The committee discussed the need for ongoing work to review and update IACUC Policies and Guidelines. Several members volunteered to assist with this work.

6. The committee discussed two self-reports in which daily checks or husbandry tasks were missed in [redacted] housing areas. No animal health or welfare issues were identified as a result of these incidents. The committee endorsed the corrective action plans and considers the matter closed.

1. **IACUC-R1S1(# Protocols: 7)**

1. **IACUC-R1S1 - NEW(# Protocols: 7)**

1. **Protocol Title:** 2112-39692A Modulating attention and decision making with closed loop control of low frequency oscillations
Species &Pain Class:(B) Nonhuman Primate (Macaques)
Question the Research Addresses: Synchronous low-frequency brain activity, as measured by human EEG, has been implicated in both normal cognition and in disease states such as schizophrenia. However, we still do not know whether changes in such rhythms directly alter neuronal information processing, or are merely epiphenomenal. To address this issue, we will measure how single and multi unit activity linked to task performance in non-human primates is altered by endogenous alpha rhythms, and how that activity is changed when alpha rhythms are directly modulated via closed-loop electrical stimulation.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: This section says that animals will be transferred to [redacted] for training. Is there an alternate plan in the event that [redacted] cannot accommodate this? The [redacted] protocol number listed here is expired and needs to be updated. There is information in this section about the thermoplastic face mask, but it says in the "updates" section that this did not work. Please remove this information if you will not be pursuing the thermoplastic masks anymore.

Comment: In the description of restraint, there is still a reference to thermoplastic masks. Please remove this if

they won't be used. This procedure also has a reference to the expired [redacted] protocol. Please update. Please provide more details on how the animals are acclimated to the head fixation.

Comment: The IACUC protocol number in the following description from the Experiment Design section may have expired and needs to be updated; please revise accordingly. Cooperative Training: Because of past success in training animals , we will employ a seperate protocol to qualify animals for this study, NHPs will be trained to cooperate with basic procedures to facilitate care (e.g. implant managment, health examination, working with handlers, cooperative chairing) and husbandry (e.g. cage and transport shifting); IACUC protocol-1603-33558A, PI [redacted].

Comment: Protocol number 1603-33558A will need to be updated in this procedure if it has expired and been renewed with a new protocol number.

Comment: In the Rationale section in response to the query for 3 year renewal of the protocol, you explain that no research results were obtained during the previous approval period, in part due to problems with head restraint because thermoplastic masks proved ineffective. Your Experiment Design Section still presents a plan for using the thermoplastic masks, and you stiill include a procedure for thermoplastic mask fitting. Could you clarify somewhere, perhaps in the Experiment Design section, your plan for either continuing to try to incorporate this equipment or to abandon its use going forward?

Comment: There is no "primate water restriction" attachment. Please include this.

Comment: It is recommended to rotate through different margin cleaning solutions (chlorhexidine, iodine, Vetericyn, etc) in 7-10 day intervals to maintain efficacy The Association of Primate Veterinarians has Cranial Implant Care Guidelines that may be helpful: https://www.primatevets.org/guidance-documents These guidelines also recommend the use of iodine-based solutions rather than chlorhexidine inside the recording chamber, because there is a risk of neurotoxic side effects of chlorhexidine. Periodic gas sterilization (or autoclaving if the material permits) of the chamber caps may also be beneficial in reducing infection risks.

Comment: Under the aseptic technique section, it says that research staff will be trained by RAR or PCRC. If this is no longer happening, then please remove it from your protocol. Under the "parameters monitored" section, it says that pinch reflex and mucous membrane color will only be examined when the animal isn't under the surgical drape. In general, it is possible to maintain a sterile field and still assess these parameters while the animal is under a drape.

Comment: In the section that explains agents used in combination, it says animals will be sedated with ketamine and dexmedetomidine. However, midazolam is also listed in the drug table. please include that animals may be sedated with ketamine alone, or ketamine in combination with either dexmedetomidine or midazolam. In the record-keeping section, it says "We do not record anesthesia duration <15m." While this is a sedation event (not general anesthesia), vitals should still be recorded at least once any time an animal is sedated.

Comment: If temporalis muscle has to be removed, what percentage of the muscle remains? Does this affect the animal's ability to chew or change its facial expression?

Comment: For surgical procedures, the protocol says that absorbable sutures will be used for the fascia. What type of suture is used on the skin?

Comment: Please remove this procedure if it will no longer be performed.

Comment: Will the animal be placed into a stereotax for the CT? If so, please include this (and also include that lidocaine cream will be used in the ears). Unless RAR staff will be hired for this procedure, please remove the sentence that says "It is expected that RAR staff will be present for this procedure and will suggest an alternate intervention." For the question about record-keeping, please describe the records that will be maintained for this procedure. Although the CT scan is short, vital parameters should be monitored and should be recorded at least once during the sedation.

Comment: Atropine is not recommended for use as a premedication.

Comment: Question 2 says that if pain or distress are observed, the animal will be directly monitored until resolution. If pain is observed, your vet should be contacted so you can come up with a treatment plan together. Question 3 says that fluids are given at 5-10 ml/kg/hr. Please update to 3-5 ml/kg/hr to match your procedures. Question 3 says the oxygen saturation level should be >85%. Normal saturation is >95%.

Comment: You mention having [redacted] perform your cooperative training. Please reach out to [redacted] and verify [redacted] has the space and staffing to perform this for your study. Also, you have an older protocol listed which would need updated if [redacted] agrees to perform this service.

Comment: You still have the thermoplastic mask procedure listed but mentioned this proved to be ineffective. Please update your protocol accordingly.

Comment: Under the database you say to see attachment "Alternative Search rev 01_11_2019" but your attachment has been properly updated to 12_27_2021 so please correct this in the Alternative search section.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0
Member Z out

2. **Protocol Title:** 2111-39559A Integrated Science Education for Discovery in Introductory Biology part two for Biology 3004 and EEB 3411/3412W student research projects
Species &Pain Class:(A,C) Fish (Zebra fish)
Question the Research Addresses: The research with the zebrafish will address two questions: 1. Will student led projects increase science proficiency? 2. How does the zebrafish environment (for example nutrition and or environmental conditions) affect zebrafish development and/or behavior?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: As this is a three year renewal, please address point "e" of this section.

Comment: As currently written, the design reads as though you want to set up an expedited review process for subprojects on this protocol. Our understanding from communciation with IACUC staff is that in the past you have submitted amendments for any work that proposed new treatment combinations or implied prolonged pain (see also comment under Species). Please clarify that amendments will be sought as necessary.

Comment: Several members of the IACUC were concerned by the idea of approving pain class C animals for the purposes of undergraduate education. However, as written the protocol suggests that very few animals would actually qualify for pain class C. As an alternative, we suggest that all animals be moved either to class A or B, with more stringent endpoint criteria applied or analgesia offered. For instance, animals with a behavior score of 6 or higher might be euthanized--the distribution of behavior scores itself could be used as an outcome variable. Given that this is a three year renewal, it would also be beneficial if the PIs could provide some information about the frequency and severity of adverse health outcomes under some of these treatments. If possible, treatments with more severe effects could be restricted to use on embryos, in which case those experiments would not be covered by protocol.

Comment: Given that many fish will be removed from the [redacted] for experimental manipulation, some accounting of care and monitoring in detached aquaria in classrooms would be of use (e.g., pH, ammonia, temperature, etc.). Also, care for embryos and fry in petri dishes is fairly clear, but no note is made of temperature control--do these animals experience ambient temperature for extended periods? Please clarify

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0
Members T, Z out

3. **Protocol Title:** 2111-39551A Neural control of hypertension in the mouse model.
Species &Pain Class:(B) Mice
Question the Research Addresses: This research works to address the mechanism and importance of the neural-cardiovascular-renal interaction in the development and maintenance of hypertension.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: In Justification for Number of Animals, the PI selects "Group sizes determined statistically". However, the PI does not describe the statistical analysis used to estimate the number (N) of animals needed.

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0
Members T, Z out

4. **Protocol Title:** 2112-39640A Neural control of hypertension in the rat
Species &Pain Class:(B) Rat
Question the Research Addresses: This research works to address the mechanism and importance of the neural-cardiovascular-renal interaction in the development and maintenance of hypertension.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: This protocol is a 3 year renewal. Therefore, the PI need to provide a brief summary of the research results from animals obtained during the prior approval period.

Comment: In the Experimental Design" The PI states "Total number of rats is based on a varying rate of success based on our past experience for all studies. 650 experimental rats + 92 potential extra rats = 742 rats" and "Total number of mice = 160 for aims 4A and 4B". Nevertheless, In the Species, the PI only requests 346 rat. Clearly, there are errors in calculation of rat numbers. Moreover, mice are not used in this protocol.

Comment: I apologize if I did not catch this already but I am unsure how the renal denervation procedure fits within the experimental design of this protocol. Please articulate when this procedure will be done and for what aim. If this procedure will not be used, please clarify whether it could be used for future experiments or omit.

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0
Members T, Z out

5. **Protocol Title:** 2112-39682A Protein O-GlcNAcylation in physiology and disease: CON-73425; CON-89584; CON-94750.
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: We are interested in defining molecular mechanisms by which hormonal and nutritional cues orchestrate metabolic physiology across tissues.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Please clarify why toe clipping is preferred to other methods of identification and what makes toe clipping superior.

Comment: Please clarify why the tail needs to be amputated rather than nicked for blood collection.

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0
Members T, Z out

6. **Protocol Title:** 2111-39547A GPCR regulation of stimulant and opioid drug effects
Species &Pain Class:(A,B) Mice
Question the Research Addresses: G protein-coupled receptors (GPCRs), including the neurotensin receptor 1 (NTSR1), are promising targets for the treatment of stimulant and opioid use disorders. We have recently identified a novel class of GPCR ligands, [redacted], which may serve as the basis for therapeutic development. The mechanism by which these ligands interact with their receptors and the consequences of this interaction for individual cells and brain circuits have yet to be elucidated. Determining the mechanism of action and therapeutic potential of this new class of ligands at the [redacted] is the major objective of this protocol.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: The rationale / study objective include abbreviations (GPCR, D2R, NTSR1, etc) which are not defined. Please update the protocol to define these at first use.

Comment: The housing location is selected as [redacted], please clarify which animals will be housed in the described [redacted] housing

Comment: Please update the protocol to include how many mice in total will require SPECT/CT imaging? Is it 208 x 3 (experiment #9)? Please be advised that the capacity of [redacted] is limited. Please update the protocol to clarify how many SPECT/CT sessions will occur per mouse. How many sessions in total?

Comment: IV drug self-administration: Please clarify a drug and a dose for the Reinstatement procedure (Currently, it reads that "... mice will receive a noncontingent drug (e.g., cocaine, remifentanil) dose (i.v., i.p., or s.c.) prior to the start of the session". The procedure location is not clear. Please clarify from where to where the animals will be transported.

Comment: PET imaging of mice using the Sophie G8 PET/CT imaging system: The type of radiotracer and route of injection must be specified, please PET imaging of mice using the Sophie G8 PET/CT imaging system: Please update the procedure to specify the type of radiotracer and route of injection, please be consistent with the DEHS description.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

7. **Protocol Title:** 2111-39600A Peytant Airway Stent Pilot Study
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: The aim of the study is to assess and compare immune/tissue response of three different airway stents via bronchoscopy, gross necropsy, histology, and potentially biopsy of the AMStent, Bare Nitinol, and Merit AERO stents.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Surgery Implant Stents -no dose for neostigmine is listed, please update the procedure to include the dose.

Comment: For the "stent check" procedure, the protocol mentions that instruments will only be cleansed and not sterilized. How about the bronchoscope? You mentioned earlier (Induction and surgical prep for initial stent implant) the use of cidex. Are you planning to sterilize your bronchoscope before the procedure using cidex, and especially between animals? Please update the procedure to describe how the bronchoscope will be cleaned/disinfected between animals. Also, I am not sure what kind of "surgical" sites you are planning to clip and prep here? In your previous answer you indicated that you are likely using indirect blood pressure measurement for this procedure. Please clarify or update as needed. Lastly, for all procedures, it is mentioned that the anesthetic depth will be monitored using "...eye reflex &position, lack of jaw tone, heart rate ..." What do you mean by "eye reflex" Palpebral reflex? Corneal reflex? Pupillary light reflex? Or perhaps the position of the globe? Please update the procedures to specify.

Comment: I am confused about the post operative monitoring frequency of animals enrolled in the study. In the attached document it is stated that : "5.1.5 Animals housed in the [redacted] following a surgical procedure will require a minimum of one in-person wellness-check by [redacted] staff each weekday." This, I think, is reasonable. However, in the "stent check" procedure you request an exemption from post operative monitoring for those animals that do not undergo biopsy: "Request exception to the 3 days of post op monitoring for weekly checks that do not involve biopsies." I think animals implanted with the stents should be monitored daily after the stent placement to ensure no stent failure/obstruction occurs resulting in distress of the animals. I do not think it is sufficient to monitor these animals once a week. Please update the protocol to comply with this monitoring requirement.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0
Member Z out

Institutional Animal Care and Use Committee
1/25/22 Minutes
VCRC - 76D

Meeting Convened: 12:00PM	Quorum Requirement: 10
Meeting Adjourned: 1:05PM	Members Present to Vote: 15

Voting Members			**Alternates**		
1.	x	(Chair - M, S)			
2.	x	(M, V)	A	x	(A, V)
			B		(A, V)
			C		(A, V)
			D	x	(A, V)
			E		(A, V)
			F		(A, V)
			G		(A, V)
			H	x	(A, V)
			I		(A, V)
3.		(M, S)	J		(A, S)
			K	x	(A, S)
4.	x	(M, U)	L	x	(A, U)
			M		(A, U)
			N	x	(A, U)
			O	x	(A, U)
			P	x	(A, U)
5.		(M, S)	Q	x	(A, S)
6.	x	(M, V)	R		(A, V)
7.		(M, S)	S	x	(A, S)
8.		(M, S)	T		(A, S)
9.		(M, St)	U	x	(A, St)
10	x	(M, S)	V		(A, S)
11		(M, S)	W		(A, S)
12		(M - NA, NS)	—		
13		(M, S)	X	x	(A, S)
14		(M, S)	Y		(A, S)
15	x	(M, S)	Z		(A, S)
16		(M, St)	AA	x	(A, St)
17	x	(M, S)	BB		(A, S)
18	x	(M, S)	—		
19	x	(M, U)	CC		(A, U)

Non-Voting, Ex-Officio:

i	x	(O, U)
ii		(O, U)
iii		(O, U)

Institutional Veterinarian:

3	x	(M, V)

Correlates to Version **v10.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion Items

1. The committee discussed the procedure for animal procedure training sessions. Under current IACUC policy trainees do not need to be added to the protocol, but this does not yet apply to outside trainers. Specific steps for adding an external person to eProtocol and obtaining a visitor waiver for UMN occupational health requirements were discussed.

1. **IACUC-R1S1(# Protocols: 9)**

1. **IACUC-R1S1 - NEW(# Protocols: 5)**

    1. **Protocol Title:** 2112-39654A Iron acquisition in Bordetella pertussis
    Species &Pain Class:(B,C) Mice
    Question the Research Addresses: We have identified multiple Bordetella iron uptake systems, and at least two of their iron receptors are important for growth in vivo. We seek to determine if these and the other receptors are important for Bordetella growth in a mouse model of pertussis and can elicit a protective immune response.

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    Comment: 1. Please update the procedure to provide the estimated protein purity and the method of estimation. 2. Please update the procedure to provide the concentration or range of concentrations for adjuvants to be used.

    Comment: It is stated in the attachments that 540 of the mice will be pain class B and then transition over to pain class C once they have been infected with any strain of bordetella. Therefore it appears that all 1500 of the requested animals should be pain class C. Please update the Species table accordingly or provide further explanation for the 540 B mice.

    Comment: There is a discrepancy between the health/monitoring section and the experimental endpoint section. "Labored breathing would necessitate euthanasia of the animal upon recommendation of an RAR veterinarian" and "...it is possible that some animals may experience some level of labored breathing. If the animals experience severe respiratory symptoms such as significant dyspnea or cyanosis, then euthanasia is recommended to relieve the suffering of the animal." Please update the protocol to resolve these two statements and clarify whether labored breathing is an endpoint to this experiment.

    Committee Decision: Stipulations must be met
    For: 15 Against: 0: Abstain: 0

    2. **Protocol Title:** 2112-39665A Tissue sampling to determine reproductive hormones, stress hormone, and immune profile of turkey breeder hens and follicle development of chicken layers under commercial farm conditions
    Species &Pain Class:(A) Turkey; (A) Chicken; (A) Turkey
    Question the Research Addresses: What factors contribute to a low/declining in fertility and disease susceptibility of avian species? Can those factors be reversed or corrected to improve fertility and lower disease incidents such as blepharitis (eye infection)? What is the difference in follicular development in different types of poultry (turkey vs chicken)?

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    Comment: Please provide a citation (ie SOP number) for the description of "Poultry Teaching and Research Facility standard operating protocol".

    Comment: For the birds that will develop blepharitis and you are keeping please list them out as Class B since eye lesions do have the potential to be painful.

    Comment: You state that the birds with eye lesions will have blood sampled every 4 weeks but can you also state

what age is their endpoint? Is it 4 weeks for the remainder of their life, or is there a set time endpoint? Are the eye lesions getting treated with antibiotics or other modalities, or are they remaining untreated for the blood samples?

Comment: In Part 1 can you please list out blood sample complications (such as hemorrhage) and if that is noted what are your monitoring parameters and then if for any reason a bird's bleeding wasn't able to be controlled and hemostasis isn't achieved what is your plan? In Part 3 Please state what your scoring system is for the eye lesions. Is there a score that would warrant euthanasia (if so please state what that score is)?

Comment: Part A: 1. How will turkey birds be selected for sampling, such as whether body weight (or size) or health status etc. will be considered in bird selection? Please update the protocol to clarify. Part B: 1. Blood samples and pictures (eye lesions) will be taken starting at 12 wks of age, and then every 4 wks. What is the end point of blood sampling and picture taken, is it at 56 wks as in Part A? Please update the protocol to clarify. 2. What happens if some birds selected (tagged) die or become sick during the sampling period? Do you have a backup plan for potential morbidity and mortality? Do you need birds to replace the dead or sick birds. Please update the protocol to clarify. Parts A and B: 1. It stated that some birds will be brought from commercial farm to [redacted] to be housed for up to 8 wks, but it is not elaborated in Part A or B. How many birds and at what age will be brought to [redacted] for each part (A and B). Please update the protocol to clarify.

Committee Decision: Stipulations must be met
For: 15 Against: 0: Abstain: 0

3. **Protocol Title:** 1903-36878A Non-hematopoietic human umbilical cord blood stem cell (nh-UCBSC) therapy in a non-human primate model of stroke
Species &Pain Class:(B,C) Nonhuman Primate (Macaques)
Question the Research Addresses: Previously, we have found that the therapeutic effect of nh-UCBSCs for stroke is tightly linked to modulation of the normal neuroinflammatory response seen after stroke. This is consistent with both our prior studies using UCBC and numerous studies using MSC for the treatment of stroke. Importantly, many experts now agree that these cellular therapies are effective in stroke. However, because of the modulation of the neuroinflammatory response, this has never been validated in large animal studies. The premise for using NHP as large animal subjects for this study is based on reports in the scientific literature that the immune system in NHP is very similar to that in humans. This is a critical point, as it is well known that the immune system of rodents is quite different than that of humans. These differences include phenotypic differences, different genomic responses, and much different rates of immune system up-regulation and recovery. It is believed that this difference is likely the reason nearly 150 clinical trials testing candidate agents, shown to block the inflammatory response in rodents, have all failed to demonstrate a benefit in critically ill septic patients. Furthermore, in human clinical trials for MS, successful therapies found in rodents (such as interferon γand tumor necrosis factor TNF-α) have not just failed but have actually exacerbated patients' MS. This difference in immune systems is also a likely reason why research has failed to translate over 1,000 experimental treatments from discovery in cells and rodents to use in humans. Recently, the much-anticipated phase II clinical trial by Athersys, using MultiStem (MSC cells) for treatment of stroke, failed to demonstrate therapeutic benefit. What is surprising (and reason for concern) regarding clinical trials testing cellular therapies based on rodent studies is that the Athersys trial followed most of the preclinical recommendations made by both the Stroke Therapy Academic Industry Roundtable (STAIR) and Stem Cell Therapeutics as Emerging Paradigm for Stroke (STEPS) translational research guidelines, with the exception that the therapeutic effect was only tested in rodents and not tested in non-human primates. We believe that a critical step in further development of nh-UCBSC therapy for the treatment of stroke is preclinical testing in our NHP stroke model. Similarities in the immune systems between NHP and humans will be important when evaluating the therapeutic efficacy of nh-UCBSCs as well as the therapeutic dose, timing of therapy, and the development of in vivo therapeutic endpoints which are likely to demonstrate a benefit in future clinical trials.

The committee concurs that this protocol can be approved via a subsequent full committee review once the following stipulations are addressed by the PI:

Comment: For Post-Operative Care Question 2, please select B. [redacted] has a designated post-op facility, which is what option A is referring to. You can still hire RAR to provide post-operative care, but you need to list in the protocol what the care/monitoring entails. In general: Animals should be monitored cage side until fully recovered from anesthesia. At that point, small amounts of food/enrichment can be provided. If animal is able to eat and is no longer ataxic monitoring can decrease to once daily for the 3 days following surgery. Daily checks include monitoring behavior, appetite, urine/fecal output and surgical site for signs of dehiscence, infections, swelling, etc. Post-operative analgesics includes meloxicam 0.1 mg/kg PO or SQ SID. Additional doses of Buprenorphine may given under veterinary guidance if animal shows unrelieved pain/discomfort.

Comment: I think there was an error with copying this procedure. Animals will not be undergoing drug self administration (e.g. cocaine studies). Change the title of the procedure and remove "Testing and drug self adminstration: In general, 1-5 hours." from the procedure description.

Comment: In general there should be a description, either as an attachment with or under the physical restraint procedure, of how animals will be trained to present limbs for VAP use. Animals need to be acclimated to having limbs held, scrubbed, poked, etc for maintaining the lines while chaired.

Comment: There is a discrepancy with how you describe euthanasia via perfusion here and how it is described in the Experimental Endpoints section. Both sections need to be edited and updated. MRI: - Include where the perfusion is taking place. Do you plan to flush fixative following the PBS flush and prior to removal of the brain? - Assuming the animal will need to be transported, especially if using formaldehyde as it requires a downdraft able, include a sentence on transport. - Is the sodium pentobarbital being used to euthanize the animal? If so the appropriate dose is >100 mg/kg IV. - Include steps to ensure death/appropriate depth of anesthesia prior to thoracotomy.

Comment: I'm not sure what you intend to do for euthanasia via exsanguination under anesthesia. Is this the perfusion procedure? If so, then the agent name should be sodium pentobarbital and the dose is >100mg/kg IV (not IP).

Comment: The language used in your response to Q5 is a bit graphic. Please remove the first two sentences entirely. Adjust the third sentence to "Following stroke, animals should be single housed until..."

Comment: [redacted] responses to IACUC concerns and possible adverse events/treatment plans should both be deleted. A new attachment, which includes the first portion of [redacted] responses to IACUC (what animals will look like post-stroke) should be included as a table and labeled "Possible adverse events/treatment plans". Work with [redacted] outside of eProtocol to create this table.

Comment: No personnel are listed as "works with animals." It's not clear who will be assisting in the surgery, much less who will be performing the "behavioral training" of accommodation with collar and chair, or any other "pre-training of behavioral studies."

Comment: There is a reference to "offering an arm for blood draw." When, where, and by whom will this be trained?

Comment: The protocol states that studies will be performed at the [redacted], and that animals "will be cared for in an intensive care setting with around the clock care." Please verify the such [redacted] and personnel exist.

Comment: Is this a blind study design with regard to dosage?

Comment: [redacted] is currently being used by the [redacted] labs. Please verify these activities can do done here, and identify who will be doing them.

Comment: Please clarify "drug self administration" which is not described in the Experimental Methods. Also describe the behaviors and whether rewards will be given.

Comment: Please clarify the purpose of the VAPs. The surgical procedure states an IV hub will be sutured to the skin. Is that was this is or is it a separate procedure.

Comment: Please clarify the purpose of blood collection.

Comment: The design section refers to a 3 T magnet, but the procedure and subsequent descriptions describe a 7 T magnet. Please verify that all animal support equipment is available at whatever scanner is being used.

Comment: Please describe anesthesia and monitoring.

Comment: It would appear the canine model is common. Please reference the advantages of the NHP model (only rodents are mentioned).

Committee Decision: Deferred

For: 14 Against: 0 Abstain: 0
Member AA out

4. **Protocol Title:** 2112-39662A XXXX Lab Breeding Protocol
Species &Pain Class:(A) Mice
Question the Research Addresses: How do animals resolve different behavioral and environmental demands by utilizing specialized brain circuits for learning and motivation? What are the key physiological properties of these brain circuits, and how do they interact during different phases of behavior?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Can you describe more of your breeding design in the experimental design? Are all breeders paired only or you using harem breeding? Any strains have known pre-weanling loss? Any strains have phenotypes or are all normal healthy lifespan? Do you have any cull criteria for your pups (microphthalmia, malocclusion, anophthalmia, runts, missing limbs, etc)?

   Comment: The number justification never mentions either 310 or 105 which are the anticipated numbers for each gene group. Please update the protocol to provide information on how these numbers were arrived upon.

   Comment: Please briefly summarize breeding outline, tissue collection procedure for genotyping, experimental endpoints, and purpose of the breeding colonies in the experimental design section.

   Comment: It would be helpful to show the math for how you arrived at your numbers of statistical significance.

   Committee Decision: Stipulations must be met
   For: 14 Against: 0 Abstain: 0
   Member AA out

5. **Protocol Title:** 2111-39576A Role of hairless (HR) in tissue homeostasis, immune regulation and tumorigenesis
Species &Pain Class:(A,B) Mice
Question the Research Addresses: Our studies focus on elucidating the function, molecular targets and pathways by which HR modulates development, tissue homeostasis and cancer pathogenesis. To do so, we have obtained several HR-mutant and HR-knockout mouse models on different genetic backgrounds to comprehensively test the various functions of HR and its target genes.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: The UMN guidelines for doses for SR-buprenorphine have been modified - please update the relevant sections of the protocol and proceed accordingly. The new recommended dose for the ZooPharm formulation for mice is 0.5 - 1 mg/kg

   Comment: Please update the protocol to indicate for the multiple gavage/injection studies - when max reps are indicated, what is the timing between injections (i.e. daily gavage, twice daily..?)

   Comment: It is difficult to account for the requested number of mice needed in each pain class in the experimental design. Please update the protocol to provide a table or summary section that clearly defines the animal needs for each pain class for each section of the study.

   Comment: Please update the Experimental Design to provide the rationale and goals of the skin grafting procedure.

   Committee Decision: Stipulations must be met
   For: 14 Against: 0 Abstain: 0
   Member AA out

2. **IACUC-R1S1 - AMENDMENT(# Protocols: 4)**

1. **Protocol Title:** 2108-39345A Evaluation of a novel arterial cannula to be used for extracorporeal circulation
Status: APPROVED
Species &Pain Class:(B) Sheep (Biomedical); (B) Cow (Biomedical)
Question the Research Addresses: The purpose of this study is to assess the deliverability and use of the TFA device in a sheep model. January 2022 Amendment: We are requesting the ability to assess the deliverability and use of the TFA device in a bovine model. We are also requesting the ability to recover animals following the surgical procedure for a maximal postoperative term of 14 days.

   Committee Decision: Approved as submitted
   For: 11 Against: 0 Abstain: 0

   Members AA, 10, and 1 out

2. **Protocol Title:** 2106-39159A Role of CFTR in Colorectal Cancer The Role of KCNQ1 in Colorectal Cancer The Role of KCNQ1 in Colorectal Cancer Metastasis The Role of CFTR in Colorectal Cancer Metastasis Tumor-Infiltrating Eosinophils: A Protective Role in Colorectal Cancer Mechanisms underlying the tumor suppressor role of CFTR in colorectal cancer A novel cancer syndrome in KCNQ1-deficient Syrian Golden hamsters Investigating underlying mechanisms of KCNQ1 tumor suppression using colorectal cancer liver metastasis-derived organoid models The Role of the Intestinal Microbiota in Cancer Pathogenesis and Clinical Care The CFTR gene and colorectal cancer Preventing gastrointestinal cancer in cystic fibrosis Contribution of the oncogenic mutation BRAF V600E to poor prognosis in colorectal cancers expressing low levels of CFTR Genotoxicity of pks+ E. coli in the cystic fibrosis epithelium
Species &Pain Class:(A,B,C) Mice; (A,C) Hamster
Question the Research Addresses: What are the mechanisms underlying the tumor resistance of these genes.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Thank you for your additional comments and updates to the protocol. I agree, if KCNQ1 mutant hamster pups do not display the phenotype until 7 days of age, to minimize stress more frequent checks to ensure good health can begin at 7 days and once additional hamster pups of unwanted genotype are culled. And to clarify the monitoring, if hamster pups appear to be in good condition (feeding, growing, active, in nest with the dam/ other pups), then these daily checks can be performed cage side to minimize any stress that could be introduced from handling. However, if a hamster pup is noted to be slow/ lethargic, is out of the nest, is hunched in posture, etc, then the cage can be disturbed and treatments or euthanasia as described should be performed. Regarding the monitoring of the KCNQ1 mutant hamsters, as this is a phenotype secondary to experimental goals, lab staff should also be involved in the daily (or more) monitoring of hamsters, rather than only animal care staff as described. The animal care staff perform general well being checks on all animals daily, however, it is the responsibility of research staff to check hamsters for ill health secondary to experimentally related aims and initiate/perform interventions as necessary.

   Comment: Thank you for the thoughtful and detailed response regarding this phenotype and the mortality expected. The information provided was very informative. Please include the information related to what has been seen/ the expected mortality (your response to Q2) within the breeding procedure of the protocol as it adds important information.

   Comment: In humans with mutations in KCNQ1 arrhythmias can occur that can lead to sudden death. As your hamsters have this mutation, it is possible this could also be an issue in the hamsters as well. Has this been documented to occur in hamsters with this mutation or are a certain incidence of sudden deaths seen? If so I recommend including that information as well; if not I would recommend including a statement within the breeding section as some additional information for this genotype that in humans a mutation in KCNQ1 can lead to arrhythmias and sudden cardiac arrest. While this has not been studied in the KCNQ1 mutant hamsters at this time, it is a possibility that sudden death could occur.

   Committee Decision: Stipulations must be met
   For: 15 Against: 0 Abstain: 0

3. **Protocol Title:** 2109-39401A Cardiac fibroblast-targeted therapies to treat fibrosis of the heart
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: Our work will address 1) whether mitochondrial signaling in cardiac fibroblasts develops cardiac fibrosis and dysfunction under pathological conditions and 2) whether we can target this mitochondrial signaling to prevent (or stop the progression of) cardiac fibrosis and dysfunction under pathological conditions.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: "All tail biopsies are performed before 21 days of age to reduce pain/stress, but may be done at a later age due to circumstances." For the later age genotyping, isoflurane does not provide analgesic properties - only anesthetic. The animals will still feel pain. Please add a local analgesic agent (such as topical lidocaine) or remove the later age comment from your protocol.

   Comment: Retro-orbital injections classify as pain class C without analgesic. Please provide an analgesic such as a proparacaine hydrochloride ophthalmic solution to your mice to provide pain relief. Please add the chosen solution to your protocol procedures. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3158461/

   Comment: I understand through your protocol that you are also requesting 30 additional training C57BL/6 mice and are maintaining 20 flox breeders. Please include these groups as brief sentences within your experimental design for clarity purposes. (120 flox mice x 3 aims/experiments, 20 breeders, 30 training mice = 410 total requested mice).

   Committee Decision: Stipulations must be met
   For: 15 Against: 0 Abstain: 0

4. **Protocol Title:** 2012-38672A Role of the immunoproteasome in TSC pathogenesis and therapeutics Start-Up account for [redacted] Mechanism of mTOR signaling to autophagy machinery Mechanisms of mTOR signaling to protein degradation pathways Selective Mitochondrial Autophagy in the Development of Insulin Resistance Mechanisms of mTOR signaling to early and late stages of autophagy Roles of immunoproteasomes in metabolism and insulin sensitivity Pathological role of c-Abl in alpha-synucleinoapathy Mechanism of Aging Regulation by Nutrient-Sensing Pathways Mechanisms of immunoproteasome-mediated metabolic disorders Role of hepatic immunoproteasome in NAFLD and insulin resistance
Species &Pain Class:(A,B) Mice; (A,B,C) Mice
Question the Research Addresses: Protocol 1: The project will study the role of autophagy in the regulation of metabolism and insulin resistance. Protocol 2: We are asking whether SH3BP4 suppresses TSC and tumor growth. Protocol 3: The project is to determine the role of the immunoproteasome in onset and progression of neurodegenerative diseases, such as Alzheimer's. Protocol 4: We are asking how the AMPK-ULK1 axis regulates cell growth and survival and tumor growth. Protocl 5: Do the CD4 T cell immunoproteasome and microglia immunoproteasome play important roles in EAE autoimmune diseases?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: While you have done a nice job explaining disease progression, and how you will monitor daily, I do not see a scientific justification for why animals need to be allowed to die vs. euthanizing once moribund. What data is being collected for animals found dead and how does it fit into your experimental aims (determining the role of CD4 T cell immunoproteasome and microglia immunoproteasome in EAE). In the health and monitoring section you state "If we need to keep and watch the mice with paralysis for a few days..." which implies that there are earlier endpoints for a subset of animals. Again, explain why death as an endpoint is needed and which subset of animals it applies to.

   Comment: I think there needs to be more detail on how the EAE scores (0-5) are informing your monitoring plan and supportive care strategy. If the following plan works for your experiment, please copy/paste it into the protocol. If it does not, please elaborate. 0: continue with daily monitoring 1: continue with daily monitoring 2: provide moistened chow and long water bottle sipper; monitor daily 3: Start checking mice daily for hydration (via skin tent test) and bladder function. Provide SQ fluids if evidence of dehydration and express bladder 2-3 times daily if animal is unable to urinate. 4: Check mice 2-3 times per day for hydration and ability to urinate. Provide

SQ fluids and dextrose PO if evidence of dehydration and express bladder 2-3 times daily if animal is unable to urinate. 5: immediate euthanasia

Comment: There is no strong rationale provided that death as an endpoint provides any valuable information, and that more detailed rationale needs to be provided within the protocol (Q4) to justify that moribund animals can not just be euthanized.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

Member AA out

Institutional Animal Care and Use Committee
1/26/21 Minutes
VCRC - 76D

Meeting Convened: 12:01PM	Quorum Requirement: 9
Meeting Adjourned: 2:00 PM	Members Present to Vote: 13

Voting Members			Alternates		
1	X	(Chair - M, S)			
2	X	(M, V)	A	X	(A, S)
			B		(A, S)
			C	X	(A, S)
			D		(A, S)
			E		(A, S)
			F		(A, S)
3		(M, S)	G		(A, S)
4	X	(A, U)	H	X	(A, U)
			I	X	(A, U)
			J	X	(A, U)
			K	X	(A, U)
			L	X	(A, U)
5		(M, S)	M	X	(A, S)
6	X	(M, V)	N	X	(A, S)
7		(M, S)	O		(A, S)
8	X	(M, S)	P		(A, S)
9		(A, St)	Q	X	(A, St)
10		(M, S)	R	X	(A, S)
11		(M, S)	S		(A, S)
12	X	(M - NA, NS)	-		
13		(M, S)	T	X	(A, S)
14		(M, S)	U	X	(A, S)
15	X	(M, S)	V		(A, S)
			W	X	(A, S)
16	X	(M – St)	X		(A, St)
17		(M, V)	-		

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, S)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v2.108** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the December 2020 Inspection Findings-Notes to File-Veterinary Recommendations.

2. The committee reviewed the policy and guidelines for "Use of Neuromuscular Blocking Agents (NMBA) in Animals". The policy and guidelines had gone through the Policy and Guidelines Subcommittee for initial review and updates. The committee updated the documents further and the approved drafts will be posted on the UMN IACUC website.

3. The committee discussed ongoing training for a lab that is working with RAR to receive additional anesthetic training. The lab currently is using a ventilator that may need to be updated as there were complications with two recent imaging procedures. RAR and the lab are investigating the equipment and incident further. The lab also made a request to record the heart rate of animals on this study at a 15 minute interval versus an 8 minute interval. The committee has had difficulty with pulse oximeters during high resolution imaging. The committee was amenable to the request to change the recording frequency as long as the lab was still monitoring vital signs continuously to ensure anesthetic depth. The committee also recommended that the lab contact additional researchers with experience in imaging to identify options for a pulse oximeter that would be MRI compatible. The committee will be updated on these discussions at upcoming meetings.

4. The committee was updated on efforts to adopt a particular dog that had been on study recently. An internal candidate has been identified and the committee was amenable to managing this adoption through an internal process rather than with the typical foster/adoption agency that is typically used. The committee also discussed options for adopting out cats that may be used on a study. The committee will review the policy and guidelines for IACUC processes surrounding adoption at the next IACUC meeting,

5. The New Guide for the Care and Use of Agricultural Animals in Research and Teaching has been published. The new edition has been posted to the IACUC website.

6. The committee reviewed a self-report in which there was a calculation error on the ketamine/xylazine cocktail used leading to adverse effects. In a second round of experiments the animals received the correct dose, but there were still complications. The lab had recently switched from using Avertin to this anesthetic. The lab is working with RAR to determine if this particular anesthetic could be causing laryngeal or bronchospasms. The lab has since had more success using the ketamine/xylazine anesthetic. The committee considers the matter closed.

7. The committee reviewed a self-report outlining an adverse event that was encountered during a routine spay. The animal developed a hemoabdomen following the procedure. RAR was contacted and the animal was hospitalized in the ICU. The animal has recovered and is doing well now. The committee considers the matter closed.

8. The committee reviewed a self-report outlining procedures not followed during euthanasia. The lab will have the staff involved undergo retraining on proper euthanasia methods with RAR. The committee considers the matter closed.

9. The committee was updated on an RAR self-report in which a deceased mouse was found following cage washing. RAR will update processes and training of LAA/LACTs and cage wash technicians to prevent this event from happening again moving forward. The committee considers the matter closed.

1. **IACUC-NEW (# Protocols: 2)**

   1. **Protocol Title:** 2012-38678A Adeno-associated virus vector intrathecal and intravenous delivery in mouse
      Species &Pain Class: (A,C) Mice
      Question the Research Addresses: We want to enhance peripheral analgesia by modulating the downstream receptors of mu opioid receptors (MORs) in the nervous system. We will use genetic and behavioral techniques to examine the activity of KATP channels in the nervous system in SUR1/Kir6.2 floxed neuropathic animals after administration of AAV-Cre. We will also use genetic and behavioral techniques t

examine how changing the activity of adenylyl cyclases (AC1, AC2, etc.) through AAV-shRNA also changes pain and opioid sensitivity.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Please update the protocol under "e." on the Rationale page to provide a summary of the research (if any) that has occurred on the expiring protocol in the last 3 years.
- The numbers listed on the species table, 440 Class A and 1100 Class C, do not currently match with the numbers specified in the Experimental Design: Pain Class C: 400 (Exp 1-8) + 140 (Exp 9-10) + 360 (Exp 11-12)= 900 Pain Class A: 440+200 = 640 Please update the protocol to reconcile this discrepancy, .
- All the procedures mention HR (which is very hard to measure in mice), but don't specify an acceptable range. Please update to include a range and method for monitoring. If this will not be monitored, please remove from the protocol.
- Please update the protocol to clarify whether the daily monitoring only applies to "immediate post-operative" and provide a definition of immediate (healing of surgical site?). Please also clarify whether euthanasia is going to be done if during 120 day post-operative period, severe distress is observed.
- Please list mannitol as a support agent for the AAV injection and clarfiy what determines whether itrathecal, intraplantar, or IV will be done.
- Please clarify whether xylazine is only used in combination with ketamine.
- Please update the experimental design section, as the body has not been updated to reflect the current state of the experimental design, and is still pieced together by prior amendments. Please incorporate the past amendments into one overview of the study which clearly outlines which animals are undergoing which procedures and which treatments.
- Please clarify which groups are receiving morphine and their duration on morphine treatment.
- It appears that some animals may undergo spinal nerve ligation and intracranial injections as two separate surgeries. If so, please update the "Intracranial Injections" procedure details by marking "yes" to the question "Will any animal undergo more than one survival procedure?" and answer the resulting questions.
- Gerald Sakamaki is no longer in our Occupational Health system so we are unable to verify if his requirements are complete. If Gerald is no longer in the lab, please remove him from the protocol and confirm here. If you would like to keep him on the protocol, please respond affirmatively and we'll contact Occupational Health to have him added back.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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2. **Protocol Title:** 2012-38672A Role of the intestinal immunoproteasome in metabolic diseases (ADA postdoctoral fellowship grant)
Mechanisms of Nutrient-Regulated mTOR signaling
Start-Up account for [redacted] lab in [redacted]
Mechanism of mTOR signaling to autophagy machinery
Selective Mitochondrial Autophagy in the Development of Insulin Resistance
SH3BP4 as a novel negative regulator of mTORC1 signaling and insulin resistance

ULK, mediator of mTORC1 signaling to aging
Characterization of Adiponectin-CRE/ULK1 flox mice in Metabolism
Mechanisms of mTOR signaling to early and late stages of autophagy
Distinct Functions of ULK1 and ULK2 in Mitochondrial and Metabolic Regulation
Roles of immunoproteasomes in metabolism and insulin sensitivity
Pathological role of c-Abl in alpha-synucleinoapathy
Development of Mouse Models for Autoinflammatory Rare Diseases
Mechanism of Aging Regulation by Nutrient-Sensing Pathways
Targeting Amino Acid-mTORC1 Signaling Limb for TSC Suppression
Roles of the mTORC1-UVRAG-Endocytic Pathway in Cancer
Mechanisms of immunoproteasome-mediated metabolic disorders
Species &Pain Class: (A,B,C) Mice
Question the Research Addresses: Protocol 1: The project will study the role of autophagy in the regulation of metabolism and insulin resistance, Protocol 2: We are asking whether SH3BP4 suppresses TSC and tumor growth, Protocol 3: The project is to determine the role of the immunoproteasome in onset and progression of neurodegenerative diseases, such as Alzheimer's, Protocol 4: We are asking how the AMPK-ULK1 axis regulates cell growth and survival and tumor growth.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Rats with tumor was mentioned in this section but not anywhere else. Please remove to avoid confusion or provide description in other appropriate sections.

- 1. Please provide viral load in viral genome or genome copy units similar to tail vein procedure. 2. Please specify what AAV is expressing each gene or shRNA as it currently looks like both methods of over expressing and knockdown are used in Experimental design.

- Please specify what AAV is expressing each gene or shRNA as it currently looks like both methods of over expressing and knockdown are used in Experimental design.

- Please provide list of cell line used for xenograft experiments under Human Blood, Body fluids, normal or neoplastic tissues (including human cell lines):

- The committee recognizes that your current protocol is expiring and you will need to get this submission approved in order to avoid disrupting ongoing experiments, as such we are not requesting that you reorganize this protocol immediately. However, as written the protocol has become difficult to follow due to the number of disparate aims. At annual review, please separate this protocol into multiple submissions that are organized by topic.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 1)

1. **Protocol Title:** 1905-37090A "Integrated fMRI Methods to Study Neurophysiology and Circuit Dynamics at Laminar and Columnar Level", "Neurons, Vessels and Voxels: Multi-modal Imaging of Layer Specific Signals" and "Technology to Realize the Full Potential of UHF MRI"
Species &Pain Class: (B) Cat
Question the Research Addresses: Our proposals aim to push the technology envelope beyond the current level by developing innovative multimodal fMRI approaches capable of simultaneous neural stimulation, recording and fMRI acquisition with functional mapping specificity and resolution down to the mesoscopic scale. The cutting-edge technology and developed tools will allow us to investigate brain function and connectivity at cellular columnar and laminar levels—the two most fundamental neural computational units for micro-circuits essential for brain function, and still cover large networks through thalamo-cortical and

cortico-cortical connections in the cat brain. In addition, we will elucidate the link between neural and vascular signals across laminae by combining two-photon imaging (performed in our collaborator's lab at Medical University of South Carolina) of neural and vascular responses with ultra-high-field (UHF) fMRI (performed here at UMN). In addition, we will apply various neurometabolic imaging techniques developed in our lab and elsewhere to study cerebral glucose, oxygen and energetic metabolism using the cat model.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- The committee approves the request to notate monitoring of heart rate every 15 minutes rather than every 8 minutes. However, the expectation and requirement is still to have continuous monitoring of heart rate or another sufficient parameter to ensure adequate anesthetic depth during the entire imaging procedure. Animals must have continuous monitoring during anesthetic events especially when paralytics are being used. Please confirm that animal's anesthetic depth will be continuously monitored and clarify what parameters will be measured continuously to ensure that the animal remains at an adequate level of anesthesia at all times.

- The committee requests that you consult with [redacted] to discuss methods used to continuously monitor anesthetic depth during image procedures. Both have offered their assistance and have stated that they have successfully been able to use pulse oximeters during imaging events. Please report back to the committee about the outcome of these conversations. You may also want to contact Jeremy Kulesa (ande2445@umn.edu) , the operations director [redacted], as he may also have some potential solutions for you in this regard.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee
Minutes
February 8, 2022
VCRC - 76D

Meeting Convened: 12:07 PM	Quorum Requirement: 10
Meeting Adjourned: 1:55 PM	Members Present to Vote: 10 at minimum, up to 13

Voting Members			Alternates		
1		(Chair - M, S)			
2	x	(M, V)	A	x	(A, V)
			B		(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E	x	(A, V)
			F	x	(A, V)
			G	x	(A, V)
			H	x	(A, V)
			I		(A, V)
3		(M, S)	J		(A, S)
			K	x	(A, S)
4	x	(M, U)	L		(A, U)
			M	x	(A, U)
			N	x	(A, U)
			O	x	(A, U)
			P	x	(A, U)
5		(M, S)	Q	x	(A, S)
6		(M, V)	R	x	(A, V)
7		(M, S)	S		(A, S)
8		(M, S)	T		(A, S)
9		(M, St)	U	x	(A, St)
10		(M, S)	V		(A, S)
11		(M, S)	W		(A, S)
12	x	(M - NA, NS)	—		
13	x	(M, S)	X		(A, S)
14		(M, S)	Y		(A, S)
15		(M, S)	Z	x	(A, S)
16		(M, St)	AA	x	(A, St)
17	x	(M, S)	BB		(A, S)
18	x	(M, S)	—		
19	x	(M, U)	CC		(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	x	(O, U)

Institutional Veterinarian:

3	x	(M, V)

Correlates to Version **v10.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

1. **Discussion Items**

   1. The committee discussed a recent issue at an agricultural unit. An outside welfare concern was received, and an immediate investigation was initiated by IACUC leadership. Several issues were identified related to animal health, recordkeeping, and staff training and the site was sent an inspection report detailing these issues and responses needed. In addition, the previous site PI has left the University and the SOP and research protocols for the site had lapsed. A new SOP has been submitted and will be reviewed by the committee. The IACUC will perform increased monitoring of the site until substantial improvement in animal health and records is seen and the committee will be kept updated on their progress.

2. The committee discussed an adverse event report in which expired anesthetic was found and is believed to have been used in a shared vaporizer. A lab using this vaporizer experienced compilations including animal deaths. The expired anesthetic has been disposed of. IACUC policy and guidelines for anesthetic equipment will be updated to include a requirement that a filling log be kept, to include person filling, date, expiration date and lot of anesthetic. This information will be distributed to animal users after language is finalized by the committee.

3. The committee discussed a self-report in which animals were tail snipped for genotyping after 21 days of age but IACUC policy and guidelines on anesthesia for this procedure were not followed. The lab states that local anesthetic will be used going forward and staff will be retrained on the guidelines. The committee requested personnel training records to confirm this retraining.

4. The committee discussed a self-report in which a local anesthetic was not used for a surgical procedure. This was consistent with one section of the protocol but not another. The lab will update the protocol to ensure consistency between sections and will follow the updated protocol going forward. The committee considers the matter closed.

5. The committee discussed a self-report in which two cages of animals were found without access to water, with one animal death. The facility has changed their practices related to changing water bottles and conducting room checks. The committee requested clarification as to whether the changes will apply to all facilities or only to the building in which the recent incidents occurred.

6. The committee discussed updated Policies and Guidelines for (1) Participants in Animal Procedure Training Sessions and (2) Hypothermia as Anesthesia for Neonatal Rodents. The committee approved the updated documents, which will be distributed to animal users via email and posted on the IACUC website.

7. The committee discussed allowing exemptions for staff listed on a protocol who will only work with a subset of the approved species on that protocol. The committee approved exempting these staff from IACUC mandated species-specific training when requested; these exemptions will be tracked.

8. The committee was updated on the December 2021 inspection findings.

1. **IACUC-R1S1(# Protocols: 10)**

   1. **IACUC-R1S1 - NEW(# Protocols: 8)**

      1. **Protocol Title:** 2106-39199A Development of monoclonal and polyclonal antibodies to pathogens, including Viral hemorrhagic septicemia virus (VHSV), Spring viremia of carp (SVCV), Infectious hematopoietic necrosis virus (IHNV), Infectious pancreatic necrosis virus (IPNV), porcine respiratory and reproductive syndrome virus, and Mycoplasma hyopneumoniae; Developing multiplex Giant magnetoresistance (GMR) biosensors for the detection of swine respiratory pathogens Developing multiplex biosensors for the detection of multiple pathogens Development of nanotechnology-based diagnostic tests for COVID-19
         Species &Pain Class:(B) Rabbit; (B) Mice
         Question the Research Addresses: The research involves development of diagnostic tests for early and easy diagnosis of porcine respiratory disease complex (PRDC) pathogens by simultaneous detection of swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV), and Mycoplasma hyopneumoniae in swine or Viral hemorrhagic septicemia virus(VHSV), Spring viremia of carp virus (SVCV), infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic nectrosis virus (IPNV) infections of fish by GMR biosensor assay. Antibodies to SIV and PRRSV are commercially available, where as

commercial availability of antibodies to VHSV, SVCV, IHNV, IPNV and M. hyopneumoniae are limited. This nanotechnology-based detection is based on antigen and antibody interaction and we will need to use large quantity of polyclonal and monoclonal antibodies to spike and nucleocapsid protein of SARS-CoV.. Although commercially available, these antibodies are expensive. Therefore, we will generate these antibodies in the lab. For this purpose, we will immunize mice and rabbits with purified recombinant spike and nucleocapsid proteins and generate monoclonal and polyclonal antibodies. Antibodies obtained will be purified and used to develop the diagnostic test.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: There appears to be a potential copy paste error with regards to COVID 19 study. Please remove this from your protocol or add adjoining information. The magnetic particle spectroscopy technology is not outlined in your protocol. Please provide a 1-2 sentence rationale (likely on how this technology makes it easy to quickly identify all these diseases that you are getting antibodies for) within the study objective section for clarity purposes.

Comment: In the ancillary information section you state " Lidocaine gel will be applied to the ear of rabbits about 30 min prior to each blood draw." Lidocaine is not listed in your procedure details as an analgesic agent. Please update your procedure if you plan on using lidocaine.

Comment: Please include a narrative describing the alternative search and attach a copy of the search results as requested for using USDA animals (rabbit) in this study.

Comment: Rabbits are listed as being euthanized with beuthasia but also are listed as being euthanized via cardiocentesis in the procedure section. Please add cardiocentesis to this method list.

Comment: Under the immunization section of the polyclonal antibody protocol, it states that the immunization will be administered subcutaneous to 4 to 5 different locations. Please describe where each of these injections will occur.

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

2. **Protocol Title:** 2111-39594A Purkinje Cell Representation of Motor Errors in Monkeys
Species &Pain Class:(B) Nonhuman Primate (Macaques)
Question the Research Addresses: The project tests several hypotheses regarding the signaling of motor errors in the firing of Purkinje cells and neurons in the deep cerebellar nuclei (DCN). First, are the predictive and feedback signals in the Purkinje cell simple spike (SS) firing the neural substrate for sensory prediction errors (SPEs) and how do these signals adapt to changes in sensory feedback? Second, are Purkinje cell complex spikes activated by SPEs, specifically when there is a mismatch between the SS predictive and feedback signals? Third, does the SS discharge store motor errors between movements, both as a working memory of past movement errors and to predict the consequences of upcoming movements? Finally, does the firing of DCN neurons integrate the predictive and feedback error signals in Purkinje cells to provide a measure of the overall match or mismatch? To test these hypotheses, we propose to record Purkinje cells and DCN neurons in monkeys tracking a pseudo-randomly moving target under varying conditions.

Committee Decision: Approved as submitted
For: 11 Against: 0 Abstain: 0
Members U, AA out

3. **Protocol Title:** 2102-38878A XXXX UTI Mouse Protocol
Species &Pain Class:(B) Mice
Question the Research Addresses: This project is designed to help us understand whether these more resistant inner colony subpopulations arising during fosfomycin susceptibility testing remain virulent and able to cause urinary tract infection.

Committee Decision: Approved as submitted
For: 11 Against: 0 Abstain: 0
Members U, AA out

4. **Protocol Title:** 2110-39514A Combined NRTL NHP Protocol
Species &Pain Class:(B,C) Nonhuman Primate (Macaques)
Question the Research Addresses: PD Project: The goal of this study is to investigate therapeutic outcomes of experimental deep brain stimulation for treating parkinsonian motor and non-motor signs in a non-human primate model. This study will examine how targeting individual brain pathways through current steering and optogenetic approaches affects the manifestation of parkinsonian signs and how each relates to changes in network dynamics in the brain. These signals will in turn be used to evaluate novel electrode arrays for stimulation and closed-loop stimulation-sensing algorithms for improved management of parkinsonian signs. ET Project: The goal of this study is to investigate the current steering abilities of a directional DBS arrays implanted in the thalamus of non-human primates. This study will examine how targeting specific brain pathways affects thalamic, cerebellar, and motor and sensory cortex activity, and how one can use computational models and optimization / machine learning algorithms to predict the spatiotemporal network dynamics resulting from spatially targeted DBS for treating Essential Tremor.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Is harmaline potentially toxic to the experimenters? If so, is there a plan in case of exposure? Similarly, is there permanent damage if used repeatedly in the same NHP subject? Is there a limit on how many times an NHP may be exposed? If not, please justify.

   Committee Decision: Stipulations must be met
   For: 11 Against: 0 Abstain: 0
   Members U, AA out

5. **Protocol Title:** 2112-39630A Novel mechanisms of analgesia for chronic pain Neural Mechanisms of Cancer Pain and Sickle Cell Disease Cannabinoid Modulation of Hyperalgesia.
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: We will study mechanisms of pain development during nociceptive bone cancer growth and sickle cell disease (SCD) and modulation of spinal nociception by descending projections from the brain stem. We are continuing determination of central and peripheral antinociceptive effects of Resolvin D1 (RvD1) (derivatives of omega-3 polyunsaturated fatty acids) and evaluate its analgesic mechanisms during cancer and sickle cell disease with it's analgesia for neuropathic and inflammatory pain. We will evaluate role of extracellular vehicles (EVs) that are released from cancer cells and red blood cells in the development of hyperalgesia and how lipid mediator affect development of cancer and SCD pain through reduction of nociceptive effects of EV.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Do the investigators expect sex differences? If yes, then they may want to consider increasing the sample size (currently n=5) in the acute s.c. resolvin and EV behavior studies as n=5 for analgesic studies is unlikely to be sufficient.

   Comment: There are numerous inconsistencies within the protocol. For example, animals are requested for multiple CFA experiments in the exp design but the procedure only mentions use of CFA for the bone cancer vs. Resolvin study. It is also unclear where and when the CB1 mice will be used. Please review the entire application to address these inconsistencies - they make it difficult to evaluate the protocol.

   Comment: It appears that some of your procedures currently listed as class A should be reclassified as class C: -Cold exposure: described as inducing pain crisis in sickle mice -Intraplantar injection: described as inducing inflammatory hyperalgesia -Intrathecal injection: depending on what is injected and whether it will lead to pain If this affects the overall pain class of any mice, please also update the Species table.

   Comment: In cases where anesthesia is not deep enough, it is proposed to remove them from the isoflurane for 10 min, then reanesthesitize with a lower concentration. While this is acceptable prior to invasive procedures (e.g. exposure and teasing of tibial nerve) an alternative that does not allow the animal to wake up is needed for mid-procedure.

Comment: RVM injection - the protocol proposes to inject into the RVM via a cannula in unanesthesitzed mice. Do the investigators have experience with this method? Please describe how the animals will be restrained and justify not performing this under brief anesthesia.

Comment: There are some inconsistencies in your monitoring and your experimental end points. At one point you mention that the mice will be euthanized if they lose 10% of their body weight, but in the end point section you mention 25%. Additionally there is not a definition of what is deemed to be severe pain vs mild pain, etc. Please address these issues. Since you answered that the IACUC endpoint criteria interfere with your study, please also add other signs that will determine if the animal is at endpoint, i.e. body condition score of less than 2/5, hunched posture, inappetence, etc.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 13, U, AA out

6. **Protocol Title:** 2112-39670A Effect of N-acetylcysteine on wound healing in a murine model of inhalational tobacco smoke exposure
Species &Pain Class:(B) Mice
Question the Research Addresses: To measure the clinical phenotype of N-acetylcysteine on cutaneous wound healing after cigarette smoke exposure, we will measure clinical outcomes in a mouse model: 1. Tissue perfusion via thermography and 2. Tissue viability necrosis and wound closure with serial photography.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please update the protocol to provide a more robust justification of the animal numbers. Why are 10 mice/group and 28 biopsies sufficient? How have these numbers have been calculated?

   Comment: Please update the sedated procedures to provide a plan for animal recovery after anesthesia including recovery on warm pad, monitoring until mobile, etc.

   Comment: Please update the endpoints section to describe in more detail unanticipated adverse health conditions and criteria for their monitoring which might lead to mice being euthanized prior to the 14 day post-op endpoint. There is information in the Health and Monitoring section that could be copied into the Endpoints as well.

   Comment: Under the rationale for species section, it is stated that this mouse model has been used previously. Please update this section of the protocol to cite which study that is.

   Comment: Under the experimental design, mice will be treated with NAC (1000mg/kg) via their drinking water. Please add a new Fluid/Diet Modification procedure (look for Dietary/Fluid Modification in the dropdown when adding a procedure) with the details of this medicated drinking water.

   Comment: Please update the procedure to correct your buprenorphine SR dose to 0.5-1 mg/kg SC. What do you mean by in saline? Please correct your bupivicane dose to 1-2 mg/kg (Do not exceed 6 mg/kg total dose). Will this be used for local infiltration of the wound or are you giving this systemically Please provide a source for your dose of dexmedetomidine. Are you planning to close the skin flap? If yes please update the procedure to describe how.

   Committee Decision: Stipulations must be met
   For: 11 Against: 0 Abstain: 0
   Members U, AA out

7. **Protocol Title:** 2112-39666A Porcine RV Pressure Overload
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: How does GP130 antagonism modulate right ventricular function in a banded pulmonary artery porcine model.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Under post-operative care section, it is stated that "A monitoring plan will be created ...." Should you have a monitoring plan before the procedure and provide it in this protocol?

Comment: Under section 2: it is stated that "animals will be closely observed... during the first 3 post-operative days ....". Please define "closely observed", such as how many times per day, including weekend and holidays. Additionally, I would suggest observing and checking animals at least twice daily after day 3 until the end of the study (including weekend and holidays) because the surgery can cause complications in pigs.

Comment: Please define "animals are not responsive to supportive care". For instance, what is "not responsive", not eating or drinking? For how long, a day or two days?

Comment: How old (or what body weight of) the pigs will be at the start of the study? Will be pigs housed individually or in groups pre- and post-operation?

Comment: The recommended dose of xylazine is 0.2-1 mg/kg when used in conjunction with Telazol alone at 2-7 mg/kg.

Comment: The cephalic vein, listed as an alternative to the ear vein, is difficult to access in pigs without a cut-down.

Comment: It is likely unnecessary to maintain pigs at a "deep plane of anesthesia" for procedures that do not induce pain such as imaging with CT or MRI. The pig must be immobile, but that should be achieved with a lighter plane of anesthesia.

Comment: What is the volume of the test article to be injected daily for 3-4 weeks depending on treatment group? What is the age or approximate weight of the pigs at the beginning and end of the study? Also, physical restraint for a brief IM injection can be classified in pain/distress category A. Why are antibiotic and multimodal pain management needed for 2 weeks prior to starting these IM injections?

Comment: How often will the pigs be assessed for signs of heart failure? Some signs can be evaluated by a complete physical exam, however a fluid wave test for ascites is not very sensitive. Will abdominocentesis or ultrasound be used?

Comment: You imply that the thoracotomy tube will be removed once there is no longer any fluid drainage and the animal is breathing normally and that this will happen before the pig is taken to the [redacted] area. Is there a certain volume of fluid that is acceptable or will you primarily be checking for negative pressure?

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 13, U, AA out

8. **Protocol Title:** 2111-39587A Mononuclear Phagocytes in inflammatory disease
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: What mechanisms are utilized by mononuclear phagocytes (myeloid cells, including macrophage, dendritic cell, and monocyte) to promote inflammation associated with systemic and chronic inflammatory diseases, specifically in cardiovascular disease/atherosclerosis and diseases that are directly linked such as obesity, diabetes, and chronic stress?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Please check the animal numbers and update as needed. There is discrepancy between the tables in the "breeding" procedure and "species" .

Comment: Please remove the sentence "No animals are expected to experience unrelieved pain or distress during our study" since it contradicts with your description of category "C" studies. Please provide clear criteria for early endpoint of experiments due to health complications for these category "C" studies and surgical procedures.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 13, U, AA out

2. **IACUC-R1S1 - AMENDMENT(# Protocols: 2)**

1. **Protocol Title:** 2004-38045A "Characterizing the role of CD38 in pain and addiction" "Characterization of a novel spinal astrocyte-neuron signaling system in chronic pain" "Role of astrocytes and astrocytic CD38 in spinal opioid signaling"
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: The research seeks to more fully characterize the role of CD38 in the relief of chronic pain, its role (if any) in opioid abuse disorder as well as the reward liability of CD38-generated metabolites.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: The summary of amendments includes errors including the identity of the new staff. Please correct.

   Comment: Please update the animal numbers to account for the new strains.

   Comment: Please integrate the new strains into the experimental design - it is currently not clear where and how they will be used.

   Comment: Mouse numbers in the species section appear unchanged. Are you planning to add mice to your project or are you substituting one strain for another but keeping the numbers the same?

   Comment: It does not appear that you have included your new strains in your experimental design. Please add them in this section.

   Comment: The referenced SOP for housing in [redacted] has expired and was not renewed as an [redacted] SOP. You will need to fill out the answers to the [redacted] questions within your protocol. The Behavioral Core staff will likely be able to help you with some of the details.

   Committee Decision: Stipulations must be met
   For: 10 Against: 0 Abstain: 0
   Members 13, U, AA out

2. **Protocol Title:** 2006-38199A Cortical-hippocampal brain network and associative memory in rats
Species &Pain Class:(B) Rat
Question the Research Addresses: Brain stimulation targeting the hippocampal network can produce long-lasting improvements in memory ability. Further development of stimulation techniques to enhance memory in humans would be greatly enhanced by mechanistic understanding of how functional improvements result from stimulation of network cortical areas. This project aims to identify the effects of varying stimulation within the hippocampus-projecting cortical areas on network function using rodent models.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please clarify the duration of single housing for this protocol--is it for the entire study after surgery (and therefore increased with the new timeline? Please include any enrichment provided for singly-housed animals to mitigate this potential long period.

Comment: The increase in timeline for the study is changed in the food restriction and surgery amendments but isn't reflected in your timeline attachment, experimental design, or the experimental endpoints. Please also add changes to those sections to prevent confusion in your protocol.

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 13, U, AA out

Institutional Animal Care and Use Committee Minutes

February 22, 2002

Meeting Convened: 12:03PM	Quorum Requirement: 10
Meeting Adjourned: 2:00PM	Members Present to Vote: 14

Voting Members			Alternates		
	x	(Chair - M, S)			
	x	(M, V)	A	x	(A, V)
			B	x	(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E	x	(A, V)
			F	x	(A, V)
			G	x	(A, V)
			H		(A, V)
			I		(A, V)
		(M, S)	J		(A, S)
			K	x	(A, S)
	x	(M, U)	L	x	(A, U)
			M	x	(A, U)
			N	x	(A, U)
			O	x	(A, U)
			P	x	(A, U)
	x	(M, S)	Q		(A, S)
	x	(M, V)	R	x	(A, V)
		(M, S)	S	x	(A, S)
		(M, S)	T	x	(A, S)
	x	(M, St)	U	x	(A, St)
		(M, S)	V		(A, S)
		(M, S)	W		(A, S)
	x	(M - NA, NS)	—		
		(M, S)	X	x	(A, S)
		(M, S)	Y		(A, S)
	x	(M, S)	Z		(A, S)
		(M, St)	AA		(A, St)
		(M, S)	BB	x	(A, S)
	x	(M, S)	—		
	x	(M, U)	CC		(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii		(O, U)

Institutional Veterinarian:

3	x	(M, V)

Correlates to Version **v10.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion Items

1. The committee was updated on the ongoing investigation of an agricultural site at which animal health and management issues had been previously identified. The initial necropsy report on one animal was inconclusive and further diagnostics are pending. IACUC leadership and a consulting veterinary expert will be visiting the site in the immediate future. The site will need to identify a primary veterinarian for their current and future needs. IACUC visits will take place monthly until the committee is satisfied with the status of the site; these will be a combination of announced and unannounced in nature. The committee will continue to be updated on this matter.

2. The committee was updated on the progress of two subcommittees:

    a. The SR-Buprenorphine subcommittee reported that the dosing recommendation for ZooPharm in mice has been updated and this is being communicated to users via the RAR website, IACUC email newsletter, and in an ongoing manner via inspections. A recommendation for Ethiqa (FDA-indexed version) will be developed after further study.
    b. The subcommittee on teaching with client owned animals reported that it has been confirmed that client owned animals used in teaching are not USDA covered. The subcommittee will continue to work on a streamlined process for IACUC review of these activities.

3. The committee discussed a self-report in which anesthetic records were not available for a non-surgical anesthetic procedure in rats. The lab initially identified missing information within a record, and in follow up found that other records were missing. The lab will ensure that records are kept going forward by assigning a dedicated team member to keep documentation during procedures, retraining staff, and performing periodic checks to ensure that records have been filed appropriately. The committee requests that the lab receive training from IACUC office staff on recordkeeping and that the next set of records be submitted for committee review.

4. The committee was updated on a previous self-report regarding mouse genotyping that did not follow IACUC guidelines. The lab has submitted the requested records documenting training of lab staff on the guidelines. The committee now considers the matter closed.

5. The committee was updated on a previous self-report involving mice found without water bottles. RAR answered the committee's question regarding applying the proposed corrective actions throughout campus; these are limited to the affected building initially, but broader implementation will be considered if they are successful there. The committee now considers the matter closed.

6. The committee discussed updated IACUC Policy and Guidelines on the Use and Calibration of Anesthetic Machines and Monitoring Equipment. The committee voted to remove specific language regarding monitoring equipment and instead default to the manufacturer's recommendation for this equipment. The Policy and Guidelines were approved and will be shared with investigators.

7. The committee was updated on RAR's implantation of IACUC mandated training, including number of staff trained and average waiting time to enroll. The committee voted to approve two modifications to the training requirements:

    a. Rodent Anesthesia will be required for all new surgeons using mice or rats. Those previously approved as a surgeon (prior to 1/1/21) are exempt.
    b. Since there are not specific RAR courses for anesthesia in USDA-covered species, new surgeons using these species will be directed to contact RAR for an assessment of their anesthetic management skills by veterinary staff or their designee, in lieu of a course. Those previously approved as a surgeon (prior to 1/1/21) are exempt.
    c. These requirements are in addition to the existing training requirements.

IACUC-R1S1(# Protocols: 9)

IACUC-R1S1 - NEW(# Protocols: 8)

1. **Protocol Title:** 2201-39717A Role of Brain-Sympathetic-Gut Microbiome Axis in Hypertension
Species &Pain Class:(B) Rat
Question the Research Addresses: What is the role of the organum vasculosum of the lamina terminalis (OVLT) sympathetic-gut microbiome axis in DOCA-salt-induced hypertension?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Telemetry transducers are sterile immediately out of the original packaging and can be reused multiple times depending on the life of the battery. If these transducers are used for multiple animals, how are they sterilized prior to implantation into each new animal?

   Comment: Please indicate the dose of DOCA to be implanted.

   Comment: Please expand on why [redacted] housing is being requested for this protocol. Have experiments been done successfully during the time in which rats have been housed in [redacted]? If animals can be housed in [redacted] and transported to the lab for experiments, please update the housing to [redacted]. If [redacted] housing is needed, please update the protocol to provide a more robust scientific justification for this request.

   Committee Decision: Stipulations must be met
   For: 14 Against: 0: Abstain: 0

2. **Protocol Title:** 2201-39720A Maintenance of a mosquito colony Expression of Wolbachia genes in the mosquito ovary
Species &Pain Class:(B) Hamster
Question the Research Addresses: I am studying the genetics of the bacterium, particularly with respect to the WO phage that occurs in the bacterial genome. The bacterium is specific to insects and does not infect humans.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: The description of what actually happens to the animal during anesthesia and mosquito feeding is hard to follow. Is the entire procedure done under a fume hood? How is a test tube used as a nose cone? What keeps mosquitoes from escaping while the hamster is placed in the net cradle? A little more detail on the equipment and process would be helpful.

   Comment: Please add some details for what steps will be taken to prevent mosquitoes from escaping and potentially spreading pathogens.

   Committee Decision: Stipulations must be met
   For: 14 Against: 0: Abstain: 0

3. **Protocol Title:** 2202-39802A Linking neuronal, metabolic, and hemodynamic responses across scales
Species &Pain Class:(B) Nonhuman Primate (Macaques)
Question the Research Addresses: While functional magnetic resonance (fMRI) has proved invaluable for identifying where in the brain activation is occurring during a particular task, it has had less to say about how the dynamics of that activation actually contribute to task performance. Indeed, because of the belief that fMRI signals are sluggish and temporally imprecise, fMRI experimental paradigms traditionally have used sustained block designs which deliberately preclude measuring the rapid changes in sensory and motor signals that underlie everyday actions. Recent evidence, however, suggests that there is considerable temporal information present in the blood oxygen level dependent (BOLD) signal, opening the possibility that fast neuronal dynamics can be revealed by fMRI. In this proposal, we will examine this possibility with a series of multimodal experiments in

which a consistent experimental paradigm is applied across spatial and temporal scales to quantify responses to transient inputs.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: In the "Estimated Timeline" portion of the experimental design part of the protocol, please describe how much time there will be between recording chamber implantation procedures and the resumption of task performance/start of recording

Comment: It is mentioned in the rationale section that thermoplastic masks have proved ineffective, but in the head fixation procedure's description of method of restraint, use of the thermoplastic mask is still described. Please clarify if thermoplastic masks will still be in use for restraint on this protocol.

Comment: The experimental design section mentions animals performing tasks within the magnet. Please add a procedure for awake MRIs and include a description of acclimation, restraint, how the animal will be monitored, and actions that will be taken if the animal shows signs of distress.

Comment: In the selected procedure (Chairing with head fixation) an outdated protocol number is listed for restraint training. Please update with a currently active protocol number.

Comment: In the question about recordkeeping, it says "We do not record anesthesia duration <15 min." Even if animals are sedated for less than 15 minutes, at least one set of vital parameters should be recorded.

Comment: The procedure description says that animals will be sedated with "ketamine and/or dexmedetomidine." This should say "ketamine with or without dexmedetomidine" since dexmedetomidine shouldn't be used alone for sedation.

Comment: Atropine is not recommended for use as a premed. SPO2 should also be measured during anesthesia and the normal range provided. In the question about parameters measured, it says that the depth of anesthesia will be tested by foot pinch at the start and end of an imaging sequence. Will you be removing the animal from the scanner at the end of each imaging sequence to do this? If not, please clarify that this testing will only occur before the animal goes into the scanner and after it comes out.

Comment: In the question about acclimation, it says that you do not use poles and collars. However, in the question about animals showing distress, it says "If the animal does not quickly acclimate to the pole, we will slow down..." Please remove the statement about acclimating to the pole if you won't be using it.

Comment: In the experimental design, the following is stated: "We use standard operant conditioning techniques with positive reinforcement (delivery of fruit juice or water for correct responses). Please refer to attached document ("Water Restriction") for details on the water restriction protocol." When referring to the Water Restriction attachment there is no description of a plan to use positive reinforcement to test NHPs during training before moving on to water restriction. Please outline the process for determining if fluid reward without restriction will be used and recorded.

Comment: Do you keep documentation of restraint acclimation trials and maintenance? In the "Chairing with head fixation" procedure the description of acclimation is somewhat vague in regard to timelines and how events are recorded to better formulate plans for updated/altered acclimation. Can you provide details on how restraint training is performed and recorded? This would also apply to restraint used for awake MRI procedures as well

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0
Member 15 out

4. **Protocol Title:** 2112-39642A MVAP: Training, instrumentation, and qualification of nonhuman primates for sensible enrollment in preclinical trials
Species &Pain Class:(B) Nonhuman Primate (Macaques)
Question the Research Addresses: The primary qualification aspect of this protocol is designed to evaluate individual NHPs planned for use and subsequently prepare them for preclinical studies based on this assessment. Unlike small animal populations which are generally homogeneous phenotypically to a small number of founders,

nonhuman primate populations are heterogeneous. This is with the perspective of employing small numbers of highly informative animals in NHP modeling and also ensuring proper comparison in efficacy and safety studies by attempting to eliminate sources of bias e.g. animals that differ considerably in behavior, health status, or condition. Animals are selected using incoming vendor data then fully characterized using behavioral data, veterinary exam data, clinical pathology, immune, and metabolic data obtained during the qualification phase. During basic qualification, all of our animals participate in our NHP training program with the purpose of reinforcing beneficial behaviors and developing basic coping skills. Essentially all training is designed to reframe negative experiences associated with medical management in complex disease modeling. Depending on intended study designation, NHPs may undergo additional preparation (e.g. disease induction, instrumentation, advanced task training). The intended strength of this approach is twofold 1) the ability to stage procedures in a way that minimizes burden on our animals (e.g. cumulative effect/recovery) and 2) support of a 'pool' of ready eligible animals so that animals can complete the program at their own individual pace rather than study-forced timing and avoiding selection bias since training is successful in equalizing animals coping and skills across temperament. In addition, healthy animals in training provide a valuable source of NHP control/reference blood on request, which supports the overall reduction of laboratory use of valuable nonhuman primates by maximizing information obtained per animal through collaboration.

Committee Decision: Approved as submitted
For: 13 Against: 0 Abstain: 0
Member 15 out

5. **Protocol Title:** 2111-39618A Evaluation of standard and novel therapies in glioblastoma
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: 1. Does RBBP4/p300 complex control DNA repair genes to impact the repair proficiency of TMZ induced DNA damage? 2. Will targeting RBBP4/p300 complex, biochemically by shRNA or pharmacologically by p300/BRD4 inhibitors, be efficacious alone and in combination with TMZ in GBM?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Note that your total number of animals in the Species section is 582, but your total in the Experimental Design section is 584. The same is true for the Animal Number Justification attachment -- the total says 582 but when you add the numbers below it equals 584.

   Comment: Your attached Drinking water analgesic SOP does not entirely match what you have written in the protocol for dosing. In the Intracranial tumor cell injection procedure and surgery procedure you state that mice will be on ibuprofen water 2 days pre- to 72 hrs post surgery. The first sentence of the SOP states it will be administered "72 hrs pre- and 48 hrs post-surgery" and the last sentence of the first paragraph says "bottles containing drinking water with analgesics will be placed on the cage at least 12-24h prior to the painful procedure and continued during the post-op period for at least 48 hours." Please update the attachment to match the text in the body of the protocol. I also do not see a scientific justification for administering analgesics in the drinking water. In the last round of stipulations it was stated that "Analgesics may be administered via the drinking water if the following conditions are met: 1. A justification is provided in the IACUC-approved animal use protocol..." Please add that as you are updating this attachment.

   Comment: Please include a maximum volume that will be gavaged.

   Comment: In the attachment you mention using nude mice, which are immunocompromised, for this project. Please address potential health concerns related to their weakened immune systems (i.e. at greater risk for opportunistic infections) and how you will adjust your housing/husbandry practices to care for them. Standard of care would be housing them in full autoclaved cages, handling them prior to handling immunocompetent mice, only opening cages in a hood, etc.

   Committee Decision: Stipulations must be met
   For: 14 Against: 0: Abstain: 0

6. **Protocol Title:** 2112-39700A Pathogenic Mechanisms of alpha-synuclein pathology in transgenic mice
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: These rodents will provide valuable resources to define mechanistic

associations between α-Syn abnormalities and neurodegeneration. First, we will ask how alpha-synuclein pathology causes neurons to die in vivo, including identification of toxic oligomers. Second, we will ask whether manipulation of neurodegenerative pathways can directly impact the progression of disease in mice

Committee Decision: Approved as submitted
For: 14 Against: 0 Abstain: 0

7. **Protocol Title:** 2201-39770A Analysis of cognitive deficits in alpha-synucleinopathy
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: These rodents will provide valuable resources to define mechanistic associations between α-Syn abnormalities, memory deficits, and neurodegeneration. The studies are designed to determine cell intrinsic (e.g. tau expression, mutant aS expression) factors, cell extrinsic factors, and or circuitry mechanisms.

   Committee Decision: Approved as submitted
   For: 14 Against: 0 Abstain: 0

8. **Protocol Title:** 2201-39718A Optogenetic stimulation of transplanted neural progenitor cells and investigation of neural activity with GCaMP in a chronic spinal cord injury model.
Species &Pain Class:(B) Rat
Question the Research Addresses: Our goal is to determine the activity level of these transplanted neural progenitor cells in chronic spinal chord injuries. In other words, are these transplanted cells able to function as a central nervous system, thereby healing a spinal cord injury?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please clarify the nature of the viral agent used for gene transfer upon intracerebral administration.. Based on the Specific aims and Procedure description it is the Adeno-Associated Vector (AAV), however the IBC section is listing it as an Adenovirus.

   Committee Decision: Stipulations must be met
   For: 13 Against: 0: Abstain: 0

IACUC-R1S1 - AMENDMENT(# Protocols: 1)

1. **Protocol Title:** 2106-39160A Improving safety profile of flexible bronchoscopy: Novel device to allow intermittent oxygen insufflation
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: This project is a proof of concept / feasibility study of an innovative bronchoscopy device on a swine model. The overarching goals of the study include testing the safety of the device, efficacy, ease of use, and visual field.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please add a statement to confirm that the IACUC's policy and guidelines for photography, video and audio recordings of animal use in research and teaching will be followed. See documents:
   https://drive.google.com/file/d/1AeD4DpNX9IE0H0Vwlel_JTIQt7TzV5Hu/view
   https://drive.google.com/file/d/1U4E1qwfPcjKO0iUrc_EgEu8yLHwkMafd/view

   Comment: Please clarify what exactly is going to be recorded. Will it just be a close up of the device or will the animal be able to be identified by species?

   Committee Decision: Stipulations must be met
   For: 13 Against: 0: Abstain: 0

Institutional Animal Care and Use Committee
2/9/21 Minutes
VCRC - 76D

Meeting Convened: 12:01PM	Quorum Requirement: 9
Meeting Adjourned: 2:00 PM	Members Present to Vote: 14

Voting Members			Alternates		
1	X	(Chair - M, S)			
2	X	(M, V)	A	X	(A, S)
			B	X	(A, S)
			C	X	(A, S)
			D	X	(A, S)
			E	X	(A, S)
			F		(A, S)
3		(M, S)	G	X	(A, S)
4	X	(A, U)	H	X	(A, U)
			I	X	(A, U)
			J	X	(A, U)
			K	X	(A, U)
			L	X	(A, U)
5		(M, S)	M		(A, S)
6	X	(M, V)	N	X	(A, S)
7		(M, S)	O	X	(A, S)
8		(M, S)	P	X	(A, S)
9	X	(A, St)	Q	X	(A, St)
10		(M, S)	R	X	(A, S)
11		(M, S)	S	X	(A, S)
12	X	(M - NA, NS)	-		
13	X	(M, S)	T		(A, S)
14	X	(M, S)	U		(A, S)
15		(M, S)	V		(A, S)
			W	X	(A, S)
16		(M – St)	X		(A, St)
17		(M, V)	-		

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, S)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v2.108** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the policy and guidelines for "Adoption of Teaching or Research Animals Owned by the University of Minnesota". The policy and guidelines had gone through the Policy and Guidelines Subcommittee for initial review and updates. The committee updated the documents further and the approved drafts will be posted on the UMN IACUC website.

2. The committee reviewed the policy and guidelines for "Tumor Endpoint Criteria in Research Rodents". The policy and guidelines had gone through the Policy and Guidelines Subcommittee for initial review and updates. The approved drafts will be posted on the UMN IACUC website.

3. The committee discussed requests for use of expired surgical gloves due to shortages caused by COVID-19. The committee determined that this could be approved for now on a case by case basis as long as the integrity of the gloves had been maintained when donning them.

4. The committee discussed the new IACUC Mandated RAR training that is required beginning on January 1, 2021. The initial notice and request for additional required training included:

    a. "Mouse/Rat Basics" including euthanasia methods for personnel using mouse or rat models
    b. Species specific basics/handling and anesthesia training for personnel using animal models that include USDA regulated species
    c. "Husbandry" for personnel with Investigator Managed Housing Areas (IMHAs)
    d. "Basic surgery training: aseptic technique and suturing" for personnel conducting surgery

    Currently Husbandry courses are not finalized, so this required course has been delayed. RAR trainers requested that the IACUC provide feedback on the content, so that expectations are being met for the training modules. As such, the committee determined that it would form a subcommittee to review the content of these modules.

    The committee also determined that since there is a limited size for training courses due to COVID-19 restrictions, approval of protocols would not be held up due to completion of these new training requirements. RAR may however, withhold RAR access until all training is completed.

5. The committee was updated on completion of euthanasia training by lab staff that had recently submitted a self-report regarding euthanasia of mice.

6. The committee reviewed a self-report in which a lab had requested animals that had been fighting to be euthanized without separating the animals. The lab responded to the concern by stating that it would separate animals immediately in the future if fighting was noticed between animals that were to be euthanized. The area vet will follow up with staff to ensure that everyone is on the same page and that there have not been significant issues with this lab before regarding fighting animals. Barring additional concerns identified, the committee considers the matter closed.

7. The committee reviewed a self-report in which mice were identified in cages after autoclaving. The lab believes that these mice were euthanized and then missed when returning the cages to be cleaned. The PI has met with the lab staff to discuss the importance of making sure that all animals are removed from cages when returning them to RAR. The committee considers the matter closed.

8. The committee discussed a request to monitor heart rate at 15 minute intervals for a study where animals are on paralytics and undergoing high resolution imaging. The lab has reported that there are technical difficulties in making these measurements consistently during the imaging. The committee has asked the lab to consult with a veterinarian who has had previous success getting oximeters to work in similar animal models undergoing challenging imaging procedures and to attempt using a mouse oximeter. The veterinarian will work with the group and report back to the committee.

1. **IACUC-NEW (# Protocols: 3)**

1. **Protocol Title:** 2003-37916A Inhibition of Opioid Tolerance
Species &Pain Class: Mice (A,B,C); Rat (C)
Question the Research Addresses: My proposed experiments will address the question(s) What is the role that endogenous agmatine plays in the development of opioid tolerance and what is the mechanism by which it exerts its activity.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - The rationale refers to a cancer pain model that is no longer included in the protocol. Please remove.
   - This procedure refers to tumor-induced pain that is no longer included in the protocol. Please remove.
   - This procedure does not include carrageenan as an inflammatory agent for mice. Add here if you anticipate using this agent in mice. E.g., Study 1A of your electroacupuncture study implies potential use of carrageenan as an inflammatory agent in mice.
   - Please submit a new controlled substances protocol. The one we currently have on file will expire next month.

   Committee Decision: Stipulations must be met
   For: 12 Against: 0 Abstain: 0
   Members 13 and 14 out

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2. **Protocol Title:** 2010-38514A Development of Immune Tolerance; Tolerance and Immunity in acute lymphoblastic leukemia; Interferons in Treg development and function; Regulation of central tolerance and Treg development by recirculating Treg
Species &Pain Class: Mice (A,B,C)
Question the Research Addresses: There are three studies covered by this application. The first will focus on immunotherapy and leukemia. In those studies we will examine whether depletion of regulatory T cells and/or other immune-modulation improves the adaptive immune response to BCR-ABL+ leukemia. In the second study we will examine how two IFN-signature Tregs contribute to protecting mice against autoimmune disease. The third study will examine how recirculating Tregs contribute to immune tolerance.

   Committee Decision: Approved with suggestions
   For: 14 Against: 0 Abstain: 0

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3. **Protocol Title:** 2010-38553A Parabiosis of mice for immunological studies
Species &Pain Class: Mice (A,B)
Question the Research Addresses: We wish to know whether the memory T cell populations recirculate through the blood into tissues, including lymphoid organs (spleen, lymph nodes, Peyer's patches) and non-lymphoid tissues (lung, small intestine, brain, salivary gland, etc.). This has significance for understanding the way in which immune cells may protect some sites against local challenge (e.g. infection) by producing dedicated long-term tissue resident populations.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - The Rationale section does not have the response completed indicating that this protocol is a 3 year renewal. It can be left blank if this is a new protocol, however, in the Experiment Design

Section response to the justification for number of animals, you indicate that this protocol is a 3 year renewal of protocol 1801-35432A. Please clarify and complete the query in the Rationale section if applicable.

- Guidelines for UMN researchers advise that mouse eyes remain open under anesthesia. This can lead to corneal drying and trauma. Apply ophthalmic ointment (e.g., Paralube® or Lacrilube®) to eyes if the anesthetic event lasts longer than five minutes, or if the anesthesia is being delivered by facemask. I do see that in the checklist for surgery in your detailed SOP that there is a prompt to apply ophthalmic ointment, but it indicates that the ointment is applied after a 20 minute surgery and not before when the mouse is anesthetized. Please make 2 corrections: 1) Add eye lubrication to the surgical procedure under the prompt for other support agents used during the procedure, and 2) Update the checklist in the SOP to indicate that eye lubrication will be applied following administration of injectable anesthetics and not when the surgery is complete

- Procedures for recovery from anesthesia indicate that mice may be placed on a heating pad for recovery. Please confirm that mice are not placed directly on the heating pad without padding or toweling to protect from burns or overheating while they are immobilized.

- Please update the procedure to indicate that when survival blood collection is done via the retro-orbital method that topical anesthetic ophthalmic eyedrops will be used for the procedure in addition to the isoflurane anesthesia (Proparacaine HCL ophthalmic solution, 0.5% USP or equivalent).

- The Experiment Design explains that some mice will undergo a second surgery to separate the two animals joined during parabiosis surgery and that these animals will be maintained for up to another 17 days. Please either confirm that these animals are able to be pair or group housed after the second survival surgery or, if not, note any requirement for singly housed mice in the health and monitoring section where justifications for exceptions to the social housing policy are recorded.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 2)

1. **Protocol Title:** 2001-37801A Development and Translation of an Intracranial Nerve Implant
Species &Pain Class: Nonhuman Primate (B)
Question the Research Addresses: This study will build and evaluate the safety and design needs of a new type of intracranial auditory prosthesis that targets the auditory nerve (auditory nerve implant, ANI).

   Committee Decision: Approved with suggestions
   For: 14 Against: 0 Abstain: 0

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2. **Protocol Title:** 1905-37090A "Integrated fMRI Methods to Study Neurophysiology and Circuit Dynamics at Laminar and Columnar Level", "Neurons, Vessels and Voxels: Multi-modal Imaging of Layer Specific Signals", and "Technology to Realize the Full Potential of UHF MRI"
Species &Pain Class: Cat (B)
Question the Research Addresses: Our proposals aim to push the technology envelope beyond the current level by developing innovative multimodal fMRI approaches capable of simultaneous neural stimulation, recording and fMRI acquisition with functional mapping specificity and resolution down to the mesoscopic scale. The cutting-edge technology and developed tools will allow us to investigate brain function and connectivity at cellular columnar and laminar levels—the two most fundamental neural computational units for micro-circuits essential for brain function, and still cover large networks through thalamo-cortical and cortico-cortical connections in the cat brain. In addition, we will elucidate the link

between neural and vascular signals across laminae by combining two-photon imaging (performed in our collaborator's lab at Medical University of South Carolina) of neural and vascular responses with ultra-high-field (UHF) fMRI (performed here at UMN). In addition, we will apply various neurometabolic imaging techniques developed in our lab and elsewhere to study cerebral glucose, oxygen and energetic metabolism using the cat model.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- The committee appreciates not only your efforts to find a solution to this monitoring issue, but also your willingness to reach out to additional researchers for advice on measuring the HR during high resolution imaging. The committee has reached out to them as well for their feedback. The committee requests that in addition to these consults, you work with Dr. Whitney McGee (mcgee291@umn.edu) to confirm that the current pulse oximeters will not work during these procedures. Finally, as a suggestion, you may want to consider a mouse pulse oximeter as this may allow for a better fit for small kittens when adapted to use around the leg or ankle.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee
2/23/21 Minutes
VCRC - 76D

Meeting Convened: 12:00 PM	Quorum Requirement: 9
Meeting Adjourned: 1:48 PM	Members Present to Vote: 12

Voting Members			Alternates		
1	X	(Chair - M, S)			
2	X	(M, V)	A	X	(A, S)
			B	X	(A, S)
			C	X	(A, S)
			D	X	(A, S)
			E	X	(A, S)
			F		(A, S)
3		(M, S)	G	X	(A, S)
4	X	(A, U)	H	X	(A, U)
			I	X	(A, U)
			J	X	(A, U)
			K	X	(A, U)
			L	X	(A, U)
5		(M, S)	M	X	(A, S)
6	X	(M, V)	N		(A, S)
7		(M, S)	O		(A, S)
8		(M, S)	P	X	(A, S)
9		(A, St)	Q		(A, St)
10		(M, S)	R	X	(A, S)
11		(M, S)	S		(A, S)
12		(M - NA, NS)	-		
13		(M, S)	T	X	(A, S)
14	X	(M, S)	U		(A, S)
15		(M, S)	V		(A, S)
			W		(A, S)
16	X	(M – St)	X		(A, St)
17	X	(M, V)	-		

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, S)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v2.108** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the January 2021 Inspection Findings-Notes to File-Veterinary Recommendations.

2. The committee extended the current exception to require members only for inspections with regulatory requirements for IACUC members to be present. The extension is due to COVID-19 and the committee will reevaluate in June.

3. The committee reviewed the policy and guidelines for "Use of Non-Pharmaceutical Grade Compounds in Research Animals". The policy and guidelines had gone through the Policy and Guidelines Subcommittee for initial review and updates. The committee updated the documents further to remove the specification for filter sizes and to limit the time that Avertin solutions may be used to 14 days rather than 30 days. Updates will be made to the documents and will then be brought back to the committee for final approval.

4. The committee reviewed a revised lab supervision plan. The committee had required a supervision plan in 2017 for this lab due to concerns over animal care practices. The current IACUC chair had requested a revised simplified plan that would also outline any updates or changes that the lab foresees implementing. The committee endorsed the overall plan but requested updates regarding the experience of new lab staff and will also require that an RAR veterinarian shadow work five anesthetic events done by the PI and the lab's new personnel who will be providing anesthesia support. After the five events the veterinarian will report back to the committee for further deliberations.

5. The committee was updated on the ownership of space currently being used by a PI who had their IMHA privileges revoked. At the time of moving the animals to this space, the IACUC and RAR were under the impression that the new area was owned by RAR, further information has come to light that this space is actually not owned by RAR. Consequently the animals are still housed in a non-RAR space. However, all animal care and husbandry is managed by RAR. Since RAR manages all care and husbandry the committee determined that even though the rooms are not owned by RAR, this is acceptable.

6. The committee received an update on ongoing efforts to assist a lab in determining a method to monitor anesthetic depth for animals on a study that utilizes high resolution imaging and paralytics. Software for the oximeter was unfortunately not available on the computer in use, so consequently the test of the oximeter was delayed. The committee will be updated at a later meeting when the group is able to test the oximeter.

7. The committee reviewed a self-report in which complications during surgery ultimately led to the death of an animal. For future procedures on this study surgical access to the carotid artery will be performed on the right side to avoid violating the esophagus running on the left side of the neck. The committee endorsed this refinement and considers the matter closed.

8. The committee reviewed a self-report in which mice were lethally irradiated (500 rads) and received T-cell depleted bone marrow transplants two hours following irradiation. The mice underwent tail vein injections and difficulty with this procedure ultimately led to the loss of some animals. Staff has since undergone additional training for tail vein injections and RAR trainers are comfortable with the competency of the lab's staff in conducting these procedures moving forward. The committee considers the matter closed.

9. The committee reviewed a self-report outlining a group of animals that did not have free access to water for approximately four hours. None of the animals exhibited any signs of distress. The protocol did not list that animals could have limited access to water. The lab reviewed the protocol and will make sure that there is not a reoccurrence of this event. The committee considers the matter closed

1. **IACUC-NEW (# Protocols: 5)**

    1. **Protocol Title:** 2101-38779A (Prevalence of Gastroduodenal Erosions in Dogs with Critical Illness)
    Species &Pain Class: Dog (A)
    Question the Research Addresses: Do gastroduodenal erosions occur in dogs in critical illness and are they clinically relevant if they occur?

Committee Decision: Approved as submitted with personnel stipulations
For: 12 Against: 0 Abstain: 0

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2. **Protocol Title:** 2101-38800A (Xpg Mouse Model Colonies – Breeding)
Species &Pain Class: Mice (A)
Question the Research Addresses: This breeding protocol is to provide a model of CS to a future experimental study (yet to be submitted or approved) that will enable us to both understand the underlying disease mechanisms underlying Cockayne Syndrome and develop a gene therapy to treat it.

Committee Decision: Approved as submitted
For: 12 Against: 0 Abstain: 0

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3. **Protocol Title:** 2012-38717A (MegF10 Mouse Model Colony - Breeding)
Species &Pain Class: Mice (A)
Question the Research Addresses: This breeding protocol is to provide a model of early onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD). The Megf10+/- mouse line provides a knockout line of mice that replicates the genetic defect in patients affected by EMARDD.

Committee Decision: Approved as submitted
For: 12 Against: 0 Abstain: 0

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4. **Protocol Title:** 2101-38755A A Preclinical Study to Evaluate a Biologically Engineered Vascular Graft in a Growing Lamb Model
Species &Pain Class: Sheep (Biomedical (A,B)
Question the Research Addresses: We have previously shown our biologically-engineered vascular grafts grow with young lambs into adulthood when implanted as a (16mm diameter) pulmonary artery replacement. Now we seek to show that clinical repair of discontinuous branching arteries and hemitruncus, two common congenital cardiac artery defects, can be emulated in the same growing lamb model using smaller 6mm diameter grafts**.**

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Please add butorphanol to the controlled substance list (used for sedation during TTE).

Committee Decision: Stipulations must be met
For: 10 Against: 0 Abstain: 0
Members 1 and R out

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5. **Protocol Title:** 2012-38723A (Antifouling Airway Stent)
Species &Pain Class: Pig (Biomedical) (B)
Question the Research Addresses: The aim of the study is to determine whether or not special coatings on an airway stent will prevent mucous adhesion as compared to a non-coated airway stent. Examples of airway stent prescription are lung transplants and lung-related diseases in which the trachea and/or bronchial tubes must be kept patent.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Although study is pilot please provide satisfactory rate of new stent performance (5 out of 8, 4 out of 8, etc). This will help to determine any necessity for additional animals.

- Please update the procdeure details section to include a dosage for neostigmine (recommended dosage is 0.04 mg/kg IM).

- It is unclear how proper ventilation will be maintained and ensured during the bronchoscopy procedure (after the ET tube is removed). Further, it is my understanding that during the use of a paralytic agent while under adequate anesthesia depth, there is a loss of independent respiratory effort and therefore proper ventilation must be maintained. Please include in the stent implant procedure a detailed description of how the animal will be properly ventilated and monitored during the bronchoscopy.

- In the case a cut down is needed to achieve arterial or venous access, please include a description of cut down repair.

- Please address the impact of repetitive open surgery during short period of 6 weeks.

- In the health and monitoring section, it is mentioned that the animal will receive prophylactic antibiotics daily for the duration of the study to prevent stent infection. In the post-operative section of the stent implant procedure, it is stated that: " Ceftiofur (3 mg/kg, IM) or Ceftriaxone (1g, IM) SID for 7 days or as recommended per veterinarian. Animals may be alternatively treated with oral amoxicillin/clavulante (Calavamox, 13.75 mg/kg PO BID) as soon as they start accepting medications orally." Please clarify the duration the animals will receive prophylactic antibiotics during the study.

- Endpoints are not well defined. Please provide clear criteria of " the performance of the stent" or "adverse events".

- Personnel must complete the medical and online requirements, as well as the (NEW!) RAR trainings prior to working with animals on the protocol. Personnel include: Wong, Jennifer: • RAR Training: ”Pig Basics”. • RAR Training: ”Pig Overview” • RAR Training: ”Pig Anesthesia” (Please note, anesthetic training is only required if personnel intend to carry out anesthetic procedures on the protocol. If personnel will not be administering anesthesia or monitoring animals under anesthesia, please disregard the anesthetic training/s.) • RAR Training: ”Aseptic Technique and Suturing” Register for all RAR trainings at https://eforms-tst.oit.umn.edu/xfp/form/1382). Personnel required to complete the in-person RAR training have received directions via email. Principal Investigators have also been copied on these emails to aid in the understanding and compliance of the new training requirements. Note: Starting on January 1, 2021 the IACUC implemented additional RAR training requirements for personnel new to our university research program. The goal of the expansion is to provide proactive training to new lab members to prevent pain & distress in animals. Please complete online and medical health requirements first, and then RAR trainings as soon as possible.

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 1)

1. **Protocol Title:** 1810-36465A (Display Turtle at [redacted])
   Species &Pain Class: Yellow bellied slider (Trachemys scripta scripta) (A)
   Question the Research Addresses: Display turtle at [redacted]

   Committee Decision: Not accepted or approved. Alternate housing must be found.
   For: 12 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee
Minutes
March 8, 2022

Meeting Convened: 12:01pm	Quorum Requirement: 10
Meeting Adjourned: 1:47pm	Members Present to Vote: 14

Voting Members			**Alternates**		
1.	x	(Chair - M, S)			
2.	x	(M, V)	A	x	(A, V)
			B	x	(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E	x	(A, V)
			F	x	(A, V)
			G	x	(A, V)
			H	x	(A, V)
			I		(A, V)
3.		(M, S)	J		(A, S)
			K		(A, S)
4.	x	(M, U)	L		(A, U)
			M	x	(A, U)
			N	x	(A, U)
			O	x	(A, U)
			P	x	(A, U)
5.		(M, S)	Q	x	(A, S)
6.		(M, V)	R	x	(A, V)
7.		(M, S)	S		(A, S)
8.		(M, S)	T	x	(A, S)
9.		(M, St)	U	x	(A, St)
10	x	(M, S)	V		(A, S)
11		(M, S)	W		(A, S)
12		(M - NA, NS)	—		
13	x	(M, S)	X		(A, S)
14	x	(M, S)	Y		(A, S)
15		(M, S)	Z	x	(A, S)
16		(M, St)	AA		(A, St)
17	x	(M, S)	BB		(A, S)
18	x	(M, S)	—		
19	x	(M, U)	CC	x	(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	x	(O, U)

Institutional Veterinarian:

3	x	(M, V)

Correlates to Version **v10.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion Items

1. The committee was updated on the January 2022 inspection summary. There were 3 significant and 21 minor findings; one finding was reported to OLAW.
2. The committee discussed the possibility of discontinuing the requirement for annual continuing reviews now that they are no longer required by USDA. The matter was tabled for further consideration and discussion at semi-annual Program Review.
3. The committee was updated on the progress of the subcommittee on teaching with client owned animals. The subcommittee recommended that protocols describing these activities still be submitted for review and approval by the IACUC. Regarding flexibility in instructional personnel, two options were proposed: creation of a roster protocol, or allowing any staff defined by the CVM as qualified to participate. Regarding veterinary care, the subcommittee proposed that CVM clinicians be allowed to make clinical decisions without consulting RAR, consistent with the MN Veterinary Practice Act, but that self-reporting to the IACUC be required in cases where animal welfare might have been impacted due to unexpected circumstances. The subcommittee's recommendations will be formalized for further committee review.
4. The committee discussed updated IACUC Policy and Guidelines on Social Housing of Research Animals. The committee voted to approve the updated documents, and these will be shared with investigators.
5. The committee was updated on an agricultural site at which animal health and management issues had been previously identified. A follow-up visit by IACUC representatives and a University Extension veterinarian raised additional, serious concerns regarding animal health and veterinary practices. Because of the immediate concern for animal welfare, the Institutional Official used his authority to suspend animal activities at the site, effective 2/24/22. All research activities were ordered to cease immediately and all animals to be sold as quickly as can be arranged. The committee will be kept updated on the sale of the animals and their care in the interim.
6. The committee was updated on a procedure in hamsters that had raised questions at a previous meeting. An RAR veterinarian observed the approved tick feeding procedure and found no signs of irritation or other concerns. The committee considers this matter closed.
7. The committee discussed a self-report of an adverse event in which a pig experienced complications in the immediate post-operative period. Lab staff took several actions to treat the animal but did not contact RAR veterinary staff. The pig recovered and has not had further health issues. The committee requested that the corrective action plan be updated to include adding emergency drugs to the protocol and providing RAR with medical records when transferring an animal.
8. The committee discussed a self-report in which a viral vector different from the one approved on the protocol was given to rats. No health concerns were associated with the alternative vector and the investigator has submitted a protocol amendment adding it as an option. The committee considers the matter closed.
9. The committee discussed a self-report in which two ferrets were found out of their cage but within their housing room. RAR veterinary staff examined the animals and verified that there were no veterinary or animal welfare concerns. Staff have been reminded to verify that cage doors are securely latched, reminder signs will be posted in the room, and cages are being examined and repaired to ensure they are easy to close. The committee considers the matter closed.

IACUC-R1S1(# Protocols: 8)

1. **IACUC-R1S1 - NEW(# Protocols: 6)**

    1. **Protocol Title:** 2201-39764A Evaluation of a Pediatric Annuloplasty Ring for Annular Support - BAR Study
    Species &Pain Class:(B) Sheep (Biomedical)
    Question the Research Addresses: The novel mitral annuloplasty ring will be evaluated in this study to determine if it maintains shape and supports normal cardiac function in a growing lamb model in terms of biocompatibility and effectiveness.

Committee Decision: Approved
For: 12 Against: 0: Abstain: 0
Members 1, 10 out

2. **Protocol Title:** 2202-39787A An Evaluation of the Performance of the LifeCradle Heart Transport System in a Porcine Orthotopic Transplant Model - BLT Study
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: The purpose of this study is to evaluate the safety and performance of the LifeCradle test article as compared to the standard of care preservation in a porcine cardiac transplant model.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: The request is for 50 animals - 20 donors, 20 recipients and 10 additional in case replacements are needed. Since this is a pilot study, the data necessary to perform a power calculation are not available. However, the investigators should provide more detail on why 20 donor/recipient pairs are needed vs. 5 or 10 or 100. Is this based on experience? Something from the literature? If they obtain the necessary information with n=10 pairs will they stop? The attachment that further defines the groups as GLP compliant vs. not is helpful but the origin of the numbers is still not clear.

   Comment: There will be two blood collections prior to surgery - one the day before and one the day of. It is unclear if the one the day of surgery or the repeated draws during and after transplantation will be under Xylazine or does that refer just to the day before? Please clarify.

   Comment: It is noted in Section 2 that you may (and most likely will) administer opioids and NSAIDs to mimic the clinical scenerio. In what instances would the decision to administer or not be made, and should all animals receive the same compound? In addition, what opioids and NSAIDS? How does this fit in with the use of SR Buprenorphine?

   Committee Decision: Stipulations must be met
   For: 12 Against: 0: Abstain: 0
   Members 1, 10 out

3. **Protocol Title:** 2201-39750A Stress associated with agricultural fairs in Minnesota dairy cows: farm to fair comparison
Species &Pain Class:(A) Cow (Agricultural)
Question the Research Addresses: Does showing cattle at fairs, either from transportation or from fair activities, increase stress in cattle?

   The committee concurs that this protocol can be approved via designated member review once the following stipulation is addressed by the PI:

   Comment: Please add a client consent form as an attachment to the protocol. An example template has been sent via email.

   Committee Decision: Stipulations must be met
   For: 14 Against: 0: Abstain: 0

4. **Protocol Title:** 2201-39736A Distribution &life history studies of Minnesota mollusks, fishes, and amphibians
Species &Pain Class:(A,B,C) Fish (Other); (A,B) Amphibian (Other); (A) Fish (Other)
Question the Research Addresses: We will conduct life history and distribution studies to improve our

understanding of the needs of various aquatic species.

Committee Decision: Approved
For: 14 Against: 0: Abstain: 0

5. **Protocol Title:** 2201-39730A Evaluation of induced pluripotent stem cell derived chondrocytes for focal cartilage repair in a goat model.
Species &Pain Class:(B) Goat
Question the Research Addresses: Do induced pluripotent stem cell (iPSC) derived organoids generate chondrocytes that are suitable to repair focal cartilage defects?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please verify that all scanner rooms indicated are capable of supporting animal work and monitoring. Also please indicate where euthanasia will occur.

   Comment: Is temperature, PO2, and CO2 monitoring possible within the magnet? Such monitoring would also be desirable during surgery.

   Comment: Please indicate the typical duration of the scan.

   Comment: Please add SpO2, CO2, and temperature to the MRI scan monitoring criteria, as I believe the [redacted] monitoring equipment is able to adequately capture these in the scanner.

   Comment: 1. Please include the dosage or dose range of the medications or anesthetic agents outlined in the description of each surgical procedure. ie. Midazolam (0.1 mg/kg, IV) ; Isoflurane (1-2%)

   Comment: Please elaborate on the transport methods in more detail as it relates to comfort and safety for the animals. Will RAR be in charge of animal transport to each facility?

   Comment: Please clarify if this is a renewal or new pilot study.

   Committee Decision: Stipulations must be met
   For: 13 Against: 0: Abstain: 0
   Member T out

6. **Protocol Title:** 2201-39727A Pre Verification and Validation Device Study
Species &Pain Class:(B) Rabbit
Question the Research Addresses: This study will provide pre-verification and pre- validation of the test device performance in an in vivo model.

   Committee Decision: Approved
   For: 12 Against: 0: Abstain: 0
   Members 1, 10 out

2. **IACUC-R1S1 - AMENDMENT(# Protocols: 1)**

1. **Protocol Title:** 2005-38135A Connectivity of motivational brain circuitry Posteromedial cortex circuits in depression and schizophrenia Revealing functional networks and circuits of the posteromedial cortex with anatomical connectivity Academic Investment Research Program (“AIRP”), Center for Neural Circuits in Addiction "Center for Neural Circuits in Addiction" NIDA P30 grant

Species &Pain Class:(B) Nonhuman Primate (Macaques)
Question the Research Addresses: We aim to characterize the connections between brain regions involved in motivation. We examine whether particular regions connect to one another, how strongly, and with what organization.

Committee Decision: Approved
For: 14 Against: 0: Abstain: 0

3. **IACUC-R1S1 - CONTINUING REVIEW(# Protocols: 1)**

    1. **Protocol Title:** 2001-37746A Hearing and Sound Communication in Frogs
    Species &Pain Class:(A,B,C) Amphibian (Other)
    Question the Research Addresses: This research program seeks answers to the following questions about frog communication: how do their vocalizations evolve, how are they used to make adaptive behavioral decisions, and what are the biophysical and physiological mechanisms that underly auditory perception and decision making?

    Committee Decision: Approved
    For: 14 Against: 0: Abstain: 0

Institutional Animal Care and Use Committee
Minutes
March 22, 2022

Meeting Convened: 12:00pm	Quorum Requirement: 10
Meeting Adjourned: 2:15pm	Members Present to Vote: 12

Voting Members			Alternates		
1	X	(Chair - M, S)	A		(A, S)
2	X	(M, V)	B	X	(A, V)
			C		(A, V)
			D		(A, V)
			E		(A, V)
			F		(A, V)
			G		(A, V)
			H		(A, V)
			I		(A, V)
			J		(A, V)
3		(M, S)	K		(A, S)
			L	X	(A, S)
4		(M, U)	M		(A, U)
			N		(A, U)
			O	X	(A, U)
			P		(A, U)
			Q		(A, U)
5	X	(M, S)	R		(A, S)
6	X	(M, V)	S		(A, V)
7		(M, S)	T		(A, S)
8		(M, S)	U		(A, S)
9		(M, St)	V	X	(A, St)
10		(M, S)	W		(A, S)
11		(M, S)	––		
12	X	(M - NA, NS)	X		(A, NA, NS)
13		(M, S)	Y	X	(A, S)
14		(M, S)	Z		(A, S)
15		(M, S)	AA		(A, S)
16		(M, St)	BB	X	(A, St)
17		(M, S)	CC	X	(A, S)
18		(M, S)	–		
19	X	(M, U)	DD		(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, U)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v11.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Affiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee discussed the Veterinary Verification and Consultation process (VVC) and approved the policy, guidelines, and internal process SOP provided by the policy subcommittee.

2. The committee finalized the policy for teaching with client owned animals. The policy will now be sent to investigators for any questions, comments, or concerns regarding the ongoing changes made to this policy.

3. The committee discussed a self-report in which mice with large amounts of dehiscense were discovered and subsequently euthanized. The committee requested further information about the training and experience of the surgeon to assess the need for additional training.

4. The committee discussed a self-report where a pig experienced severe respiratory depression during which the lab did not contact their RAR veterinarian. Moving forward the lab will add refinements to the procedure, ensure RAR is notified of any procedural complications, and provide records to post-op personnel immediately after procedures. The committee endorses the lab's corrective actions and considers the matter closed.

5. The committee discussed a self-report where a PI administered a hazardous chemical to a rat which was not outlined on their protocol and did not notify RAR prior to administration. The committee also reviewed anesthetic records for a previous self-report submitted by the lab. Given the repeated and serious nature of findings, the committee voted to suspend all animal work until a full investigation can be conducted.

IACUC-R1S1 (# Protocols: 5)

1. **IACUC-NEW (# Protocols: 3)**

    1. **Protocol Title:** 2203-39863A Tuberculosis pathogenesis and immunology
    Species &Pain Class: (A,C) Mice
    Question the Research Addresses: PI requests 184 class A and 562 class C mice for studies to examine the critical mechanisms by which bacteria cause infection and evade immunity; and how can these mechanisms be targeted to improve the treatment of TB? What are the crucial antigenic targets of protective immunity in TB, and what are the essential functions of immune cells that mediate this protection?

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    Comment: The experimental design says that there are 9 key experiments, but only 7 are described. If there are 2 additional studies, please describe them here.

    Comment: In experiment 3 of the experimental design section, it is stated that mice will be infected with a metA CRISPRi strain. Please include a procedure that outlines the details of how mice will be infected with this metA CRISPRi strain, including the route of administration, the dose administered, vehicle used, volume administered and frequency and duration of administration?

    Comment: In several spots you describe moribundity as an endpoint for a subset of mice, but you did not select Moribundity as an endpoint in response to Q4. I think your justification and monitoring plans elsewhere in the protocol sound appropriate, but for clarity it is best to select "Yes" here.

    Committee Decision: Stipulations must be met
    For: 12 Against: 0 Abstain: 0

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    2. **Protocol Title:** 2202-39800A Principles and applications of neuro-technologies
    Species &Pain Class: (A,B,C) Mice; (B) Rat
    Question the Research Addresses: PI requests 1075 class A, 417 class B and 557 class C mice, and 30 class

B rats for studies to understand how brain cells work together to compute emotions, decisions, sensations, and movements. Ideally one would be able to analyze how these cells work together in cognition, and how they go awry in brain disorder states. Currently, interfacing with the brain at the cellular level is a daunting task. Our group, accordingly, is setting out to develop a series of new technologies to enable the analysis of brain circuits which greater precision specificity.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: The PI frequently refers to the development and optimization of 'algorithms' in describing the the ultimate goals and aims of these studies. To the uninitiated, please clarify what is exactly meant by the term 'algorithm.'

Comment: The PI refers to performing cognitive and behavioral tasks in animals in the presence and absence of water or food deprivation. There is some vague reference to auditory or visual stimuli in several of the Procedures listed as 'Behavior.' However, there is no clear or specific description or explanation as to what those specific cognitive/behavioral tasks are.

Comment: The author writes, ' Any animal that cannot move, eat, drink, defecate, and urinate without discomfort will be promptly euthanized.' Obviously, difficulty with eating and drinking will result in weight loss or a definable body appearance. How does one define difficulty in defecating or urinating?

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0

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3. **Protocol Title:** 2201-39753A Neurobiology of Female Sexual Desire
Species &Pain Class: (A,B) Hamster
Question the Research Addresses: PI requests 534 class A and 812 class B hamsters to pursue a novel hamster model of female sexual desire to identify molecular and cellular mechanisms applicable to the human condition.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: The procedures do not include a description of how the therapeutic drugs and/or saline will be administrated. For example, the SA 1 experimental design includes "a total of 18 treatment conditions: 3 (DREADD injection site) X 2 (saline/CNO) X 3 (behavioral testing group), with a target of 10 female hamsters per treatment group". Accordingly, please create a procedure (category: other) outlining all drug administrations on the protocol. For each drug outline the dose, route of administration, volume, vehicle, and frequency of administration.

Comment: The SOP for Steroids states that estradiol and progesterone are administered systemically in a vegetable oil solution, but administration details are not provided. The systemic administration in hamsters should be reflected as a separate procedure. If it is systemic: is it IV or IP? Are hamsters anesthetized? If it is IV, via what vein? As stated in the previous stipulation, please create a procedure (category: other) outlining all drug administrations on the protocol. For each drug outline the dose, route of administration, volume, vehicle, and frequency of administration.

Comment: You stated in the first cycle of stipulations that antibiotics will not be administered on this protocol. However, there were still sections of the protocol that outlined antibiotic use. Accordingly, the IACUC office has administratively removed antibiotic administration details from this protocol to remedy any confusion. As a reminder, the decision to give animals antibiotics is under the purview of the RAR veterinarian in the area and cannot be written into the protocol for use. In theory, all surgeries should have been done with full aseptic technique including no breaks in sterile field, and if there is a concern that sterility might have been compromised, the RAR veterinarian should be consulted to assess the risk of

infection. Please confirm your acknowledgment of this update to the protocol and confirm that antibiotics will not be administered without prior RAR consultation.

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 2)

1. **Protocol Title:** 2003-37950A Antibody-based countermeasures against fentanyl and its analogues
Species &Pain Class: (A) Mice, (A) Rats
Question the Research Addresses: Request for change of Principal Investigator from Marco Pravetoni to Carly Baehr. The purpose is to change PI to Carly Baehr, Ph.D., as the site-PI for the U01 sub-award supporting this work. Dr. Baehr is not currently faculty at UMN; we are requesting an exception and her CV is included in Attachments to support her role as PI.

   Committee Decision: Approved
   For: 10 Against: 0 Abstain: 0
   Members 2, V out

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2. **Protocol Title:** 2001-37801A Development and Translation of an Intracranial Nerve Implant
Species &Pain Class: (B) Nonhuman Primate (Macaques)
Question the Research Addresses: PI requests to amend the protocol to provide more detail concerning possible post-surgical side-effects and the monitoring and treatment associated with those side effects. Accordingly, the primary changes are to the Health and Monitoring section and attachments describing our care during vestibular accommodation. We have also added personnel that have experience with the proposed care to help with the care, and train existing staff.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: For you Hydration and Nutritional Support SOP, I have some concerns regarding the description of the methodology for human safety. For an awake and otherwise BAR monkey with vestibular defects, placing food in front of or near a mouth may pose a bite hazard. Please update the protocol with how you will alleviate this risk (likely gripper claw/other long reaching item). Similarly, for the "syringe water into his mouth" please update it to reflect a long-reach item or syringe pole. These animals should not be sedated for food/water administration for risk of aspiration

   Comment: Please create an SOP regarding nasal tube feeding making sure to list sedative, dosages and specific food slurry w/volumes per kg.

   Comment: Please clarify what the expectations are for the Endpoint Action Plan. Will animals that are not recovering after ANI be euthanized or is there something else that can be done to alleviate their distress that has not been provided to date? If there is something else that can be done, why not write that into the proposal rather than waiting for this to happen?

   Comment: Please confirm you will notify RAR in advance (by two weeks if possible) prior the next planned survival surgery. Additionally, the committee has requested that you submit surgical and post-op records following the next survival surgery so that they may further evaluate the procedure. Accordingly, please confirm you will send the records to Dr. Ilana Cohen (iecohen@umn.edu) and the IACUC inbox (iacuc@umn.edu).

   Committee Decision: Stipulations must be met
   For: 10 Against: 0 Abstain: 0
   Members 2, V out

Institutional Animal Care and Use Committee
3/9/21 Minutes
VCRC - 76D

Meeting Convened: 12:00 PM			Quorum Requirement: 9		
Meeting Adjourned: 12:56 PM			Members Present to Vote: 17		
Voting Members			Alternates		
1	X	(Chair - M, S)			
2	X	(M, V)	A		(A, S)
			B	X	(A, S)
			C	X	(A, S)
			D	X	(A, S)
			E	X	(A, S)
			F		(A, S)
3		(M, S)	G	X	(A, S)
4	X	(A, U)	H	X	(A, U)
			I		(A, U)
			J	X	(A, U)
			K	X	(A, U)
			L	X	(A, U)
5	X	(M, S)	M		(A, S)
6	X	(M, V)	N	X	(A, S)
7		(M, S)	O	X	(A, S)
8	X	(M, S)	P		(A, S)
9		(A, St)	Q	X	(A, St)
10	X	(M, S)	R	X	(A, S)
11		(M, S)	S	X	(A, S)
12	X	(M - NA, NS)	-		
13		(M, S)	T	X	(A, S)
14	X	(M, S)	U		(A, S)
15		(M, S)	V		(A, S)
			W	X	(A, S)
16	X	(M – St)	X		(A, St)
17	X	(M, V)	-		

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, S)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v2.108** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the updates to the policy and guidelines for "Use of Non-Pharmaceutical Grade Compounds in Research Animals". The committee approved all changes and the new documents will be posted to the IACUC website.

2. The committee was updated from the training subcommittee on efforts to implement more robust training for researchers. The subcommittee proposed using the CITI training modules for species specific "overview" courses rather than RAR. The subcommittee also suggested deputizing certain labs or cores to assist in the hands-on training modules. Finally the subcommittee reminded the committee that approval of IACUC protocols is not currently held up by completion of the new training modules. The subcommittee will continue to update the IACUC as we continue to work on the implementation of the new training modules.

3. The committee reviewed a self-report in which a pregnant animal on pasture fell on an icy hill. Moving forward husbandry staff will be sure all pregnant cows are on flat ground throughout the winter and if need be, sand will be spread if there are any issues with ice. The committee considers the matter closed.

4. The committee reviewed a self-report in which a lab used hazardous chemicals without notifying RAR husbandry staff beforehand. Moving forward the lab will be sure to inform RAR. The committee considers the matter closed.

5. The committee reviewed a self-report in which wound clips were removed four days past the removal date. The lab will conduct formal training on wound clip removal at an upcoming lab meeting and establish a more effective system for tracking of upcoming wound clip removal. The committee considers the matter closed.

6. The committee reviewed a self-report in which food restriction of animals on study was extended beyond the approved 24 hour period to 26-28 hours. Personnel received additional training on the importance of following the study as approved in the protocol and will implement a system to ensure that the restriction period does not exceed the 24 hours approved within the protocol. The committee considers the matter closed.

7. The committee received an update on necropsies for animals that had died following imaging. Lung damage and a bacterial infection were identified in the respiratory tract. The area vet will review the necropsy results with the lab and consider ways to prevent damage due to problems with the ventilation of animals. The committee will continue to be updated on these efforts.

8. The committee reviewed a self-report in which animals were found outside of an isolator. The RAR area veterinarian and the RAR area vet tech also inspected the isolator in question. From the examination of the isolator and reports from the RAR animal care staff, it appears that the cover that is used around a section of plastic pipe that goes down through the tenderfoot deck got somehow dislodged. It seems that sometimes these covers get warped due to the heat, which in turn can cause them to be more easily dislodged by the birds moving around, etc. To prevent a recurrence of this issue, the following corrective actions will be implemented:

    - The covers around the pipe will be checked for integrity and proper placement by the RAR staff during the twice a day health checks.
    - Weights on or by the covers will be used to decrease the chances of them getting dislodged.
    - A sign will be posted on the room door stating to not move the bricks by the covers for the safety of the animals.

    The committee considers the matter closed.

9. The committee reviewed a self-report in which two animals who are typically pair-housed in two cages were confined to one cage together overnight. The area supervisor will provide re-training to the individual employee about the importance of checking locks, latches and dividers at the end of the day (a task that is

currently on the daily check sheet). Additionally, the supervisor will double check that this task has been performed each day until the employee and supervisor are confident that the employee can perform this task on[redacted] own again. The committee considers the matter closed.

1. **IACUC-NEW (# Protocols: 3)**

    1. **Protocol Title:** 2101-38777A High Rate Pacing and Heart Failure Model Development for VAD testing
    Species &Pain Class: Pig (Biomedical) (C)
    Question the Research Addresses: Medtronic would like to create a model for heart failure in order to test devices and algorithms in a relevant hemodynamic conditions.

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    - The committee was unclear why animals were categorized as Class C. Please remember that class C is for animals that may experience Pain/distress WITHOUT analgesia/anesthesia/tranquilizers and Class B is for animals that will undergo procedures with the Potential pain/distress WITH appropriate analgesia/anesthesia/tranquilizers. If animals on this study are actually Class C, please clarify what procedures are expected to cause pain/distress without analgesia or anesthesia. If animals are actually Class B, please update the species table and procedure details to reflect Class B

    Committee Decision: Stipulations must be met
    For: 17 Against: 0 Abstain: 0

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    2. **Protocol Title:** 2101-38746A Neural basis of reward seeking, consumption and addiction
    Species &Pain Class: Rat (A,B)
    Question the Research Addresses: Our objective is to determine the neural mechanisms of reward-seeking and motivation, and how they are altered in models of addiction, using the rat as a model system. We will investigate which pathways and cell populations in the brain are recruited in vivo while rats are seeking of alcohol and food rewards, and which are necessary and sufficient to drive changes in reward seeking behaviors and reward consumption.

    Committee Decision: Approved as submitted
    For: 17 Against: 0 Abstain: 0

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    3. **Protocol Title:** 2012-38718A Chemoprevention of colon cancer by targeting the Wnt/β-catenin pathway
    Species &Pain Class: Mice (C)
    Question the Research Addresses: Colorectal cancer (CRC) is the third most common cause of cancer death in the USA. Up to 80% of tumors have nuclear accumulation of β-catenin due to inactivating mutations in the gene for adenomatous polyposis coli (apc). Based on our preliminary data, we will combine in silico, in vitro, ex vivo and in vivo studies to identify novel β-catenin inhibitors that effectively prevent or treat colon cancer. Directly targeting the β-catenin/Tcf-4 complex could be an effective means to prevent or treat colon cancers that exhibit mutations in apc, β-catenin or axin, while avoiding interference with the normal functions of β-catenin. We have hypothesized that Bestatin may inhibit intestinal tumorigenesis in the APC min/+ mouse model.

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    - The Rationale section of the protocol references Bestatin and skin cancer, while the body of the protocol, does not appear to outline this as an Aim that is currently included in the protocol. If these studies are no longer part of this protocol, please remove the reference from the rationale page. If these are still part of the aims of this protocol, please clarify what aims they refer to.

- In the Xenograft section, the author writes that up to 6 compounds WILL be tested (HI-B1, HI-B5, HI-B9, HI-B16 plus 2 unnamed) before the top 2 will be chosen for xenograft studies. The emphasis is on the word will. If it is the author's intention to test these compounds during the course of this described project, then the in vivo methods that will be used to screen these 6 compounds will need to be described and the number of animals accounted for.

- In the APC mouse model section, the author describes performing dose-escalating maximum tolerated dose studies in order to identify an optimal dosing regimen. Although it appears that the study is described as part of the APC mouse protocol, a seperate protocol tab for these studies needs to be added as well as increasing the number of animals requested (100) in the Species Table. In additon, the sequence of studies appears to be sub-optimal in that the MTD studies will not be performed until after the xenograft studies. It would seem that MTD studies would be appropriate for all compounds prior to testing in any of the tumor models. I believe that this point was raised in Cycle 1 but the response was not satisfactory. The author responded that the doses for the xenograft study were essentially fixed but how do they know if those doses are not toxic? Futhermore, these doses are not even listed.

- In the AOM/DSS section, the author proposes pilot studies with specific doses listed. These doses should be removed since the results of the MTD study are unknown.

- This procedure seems to simply repeat the experimental design. Please modify to specify particular manipulations (e.g., oral gavage) rather than describing the experiment again.

- The author states that the AOM/DSS cycle may be repeated TWICE. Does that mean a total of 3 treatment cycles or does the author just mean that the treatment will be repeated once (for a total of 2 cycles)? In additon, what determining factors will indicate the necessity for re-treatment?

- It was previously suggested that AOM/DSS mice be weighed weekly. Having had prior experience with colitis models, I think that weights should be taken at least 3x/week because of diarrhea and dehydration.

- The euthanasia criteria refer to "weight loss of 20", which I assume is 20%? Please correct.

- Before personnel can be approved to work with animals, they must complete the remaining medical and online requirements. Please have Yifei Xie complete the: • Animal Exposure Questionnaire (https://eresearch.umn.edu/researchforms/animalExposure.html) Once all requirements been documented as complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 17 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 1)

1. **Protocol Title:** 1808-36261A Targeted delivery technologies for modulating tumor-associated and inflammatory immune components
Species &Pain Class: Mice (A,B); Rat (B)
Question the Research Addresses: Does RXRB-targeting provide better navigation of therapeutic payloads to TAMs or IAMs, and thus improve antitumor or anti-inflammatory efficacy?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - For the intradermal collagen injection (100ug) in the mouse model, please describe the volume of the collagen emulsion to be injected to achieve this dose

- In section 2 of the "Health and Monitoring" section of the protocol, please clarify that the first sentence - "after the treatment studies begin we will monitor the mice daily, ..." applies to the new aim 10 as well as to aim 2. If so, please move this sentence so it is not under the header of "Aim 2". If not, please clarify how frequently the arthritis-induced mouse model will be monitored.

Committee Decision: Stipulations must be met
For: 17 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee
3/23/21 Minutes
VCRC - 76D

Meeting Convened: 12:03PM	Quorum Requirement: 9
Meeting Adjourned: 1:00PM	Members Present to Vote: 13

Voting Members			Alternates		
1	X	(Chair - M, S)			
2		(M, V)	A	X	(A, S)
			B		(A, S)
			C	X	(A, S)
			D		(A, S)
			E	X	(A, S)
			F		(A, S)
			G	X	(A, S)
			H		(A, S)
3		(M, S)	I		(A, S)
4	X	(A, U)	J	X	(A, U)
			K		(A, U)
			L	X	(A, U)
			M	X	(A, U)
			N	X	(A, U)
5		(M, S)	O	X	(A, S)
6	X	(M, V)	P		(A, S)
7		(M, S)	Q		(A, S)
8	X	(M, S)	R		(A, S)
9		(A, St)	S	X	(A, St)
10		(M, S)	T	X	(A, S)
11		(M, S)	U		(A, S)
12	X	(M - NA, NS)	V		(A - NA, NS)
13	X	(M, S)	W	X	(A, S)
14		(M, S)	X	X	(A, S)
15	X	(M, S)	Y		(A, S)
16	X	(M – St)	Z		(A, St)
17		(M, V)	-		

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, S)

Institutional Veterinarian:

3		(M, V)

Correlates to Version **v2.110** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the February 2021 Inspection Findings-Notes to File-Veterinary Recommendations.

2. The committee was updated on the progress of recategorizing the pain classes used at the University to align with the USDA pain classes. It was noted that because there is not a one-to-one correspondence between the current categories and the USDA categories, this will take a substantial amount of time to implement. An option was presented to revert to the software vendor's stock form in place of the custom form currently in place, however this would require a new format for protocols.

3. The committee reviewed a self-report in which animals were weaned early due to confusion regarding the weaning cards placed on the cages. Moving forward, the lab will be sure to read the cards carefully to ensure animals are weaned at the correct time. The committee considers the matter closed.

4. The committee reviewed a self-report in which animals were left in a procedure hood overnight without access to water. The animals were returned to the rack with water by RAR the next morning and no health issues were identified. Moving forward, the lab will institute a checklist to ensure that animals have been returned to the proper area before leaving the room. The committee considers the matter closed.

5. The committee reviewed a self-report in which a lab failed to notify RAR before administering a chemotherapeutic agent to animals. Moving forward, the lab will be sure to notify RAR beforehand. The committee considers the matter closed.

6. The committee reviewed a self-report in which more animals than approved were used for a teaching lab to allow for social distancing of students, and the timing and method of animal transport for the teaching lab were not as described in the protocol but did not pose any additional risks to the animals. Moving forward, the lab will update the protocol accordingly. The committee will consider the matter closed once the amendment is received.

7. The committee reviewed a self-report in which an equipment failure caused animals to be without access to water and animals were subsequently found dead. Moving forward, RAR will remind staff of proper decontamination of the watering system during rack swaps and change out, as any issues are most likely to be found during these processes. The committee requested additional information regarding the timing of the incident and the process for daily observations of animals. The committee will be updated on the responses.

8. The committee was notified that semi-annual program review will take place at the normally scheduled meeting time on 4/20/21 and attendance was encouraged

1. **IACUC-NEW (# Protocols: 2)**

    1. **Protocol Title:** 2102-38852A Pentraxin 3 in age-related thermogenic adipose degeneration and glucose intolerance Lipocalin 2 as a regulator of phospholipid metabolism in adipose mitochondrial bioenergetics
    Species &Pain Class: Mice (A,B,C)
    Question the Research Addresses: To investigate the role of PTX3, Lipocalin 2, and other genes in diet-induced adipose tissue inflammation, insulin resistance and metabolic homeostasis during obesity and aging.

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    - Only PTX3 knockout (ko) mice were mentioned in the "rationale for species selection" section of the Species page, but the Experimental Design describes the use of additional genetically modified animals such as Lipocalin 2 (Lcn2) ko, and Lcn2 transgenic (tg) mice. Please update the protocol to be consistent as to which models are used

    - Please update the Health and Monitoring section to provide a statement that genetically modified animals (PTX3 knockout (ko), Lipocalin 2 (Lcn2) ko, and Lcn2 transgenic (tg) mice) do not have preexisting health complications, if this is the case. Otherwise please describe these health conditions

and plans for monitoring.

- Please update the Health and Monitoring section to provide a statement that genetically modified animals (PTX3 knockout (ko), Lipocalin 2 (Lcn2) ko, and Lcn2 transgenic (tg) mice) do not have preexisting health complications, if this is the case. Otherwise please describe these health conditions and plans for monitoring.

- Please expand and clarify the term "moribundity" by including easy to monitor criteria such as weight loss over 20%, lack of mobility, tremor, etc.

- Please update this procedure to clarify the "warm environment" that will be used. Please provide a description of body temperature support (i.e. placing cage with animal half on heat pad or under lamp) after surgery.

- Please create new procedures for LPS, recombinant PTX3, beta3-adranergic receptor agonist and retinoic acid administration and move relevant information from "experimental design" to these new procedures.

- Please create procedure type "Dietary or Fluid Modification" to describe the Antibiotic treatment and move relevant information from "experimental design" to that procedure. Use "experimental design" page to describe the scientific need for the procedure and animal number justification

- Many procedures described in the protocol require an individual mouse to be separated from cagemates for some period of time. C57BL6 mice, particularly males, can be aggressive to intruders or mates wakened during procedures. Please update the protocol to provide a statement describing how you will monitor for and address any fighting.

- In the health and monitoring portion of the protocol, please describe any potential adverse conditions (and steps to respond to them) that may result from the osmotic mini pump implant surgery (for example, the possibility of infection that is mentioned in the osmotic pump procedure section).

- For experiments #14, #15, #16, please describe the experimental timeline for animals involved in these behavioral tasks as was done for the other experiments (how often will each animal be exposed to the behavioral task, what experimental interventions will be done in the animals to create different experimental groups, what are the experimental endpoints, etc.)

- The description for experiment 8 describes the use of wild type and PTX3 KO mice for this part of the study, but the corresponding animal number flow chart shows the use of wildtype, Lcn2 KO, and Lcn2 Tg mice. Please fix the appropriate section for consistency.

- Please confirm that the "custom restraint tube" mentioned in the procedure is supplied by the manufacturer of the instrument. If the restraint device is made by the lab, please include details of its construction including access to air for the mouse and ability to be sanitized.

- The SOPs attached are due for review (required every three years). Please use the Toxic Hazard Class SOP template (located here: https://dehs.umn.edu/node/129581/attachment) and attach it to your protocol. Thank you.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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2. **Protocol Title:** 2103-38899A Next Generation Attenuated Rickettsial Vaccines
Species &Pain Class: Mice (A)
Question the Research Addresses: The central hypothesis to be tested is that a rickettsial species attenuated by mutagenesis can be complemented with antigenic epitopes from other species of intracellular, tick-borne

pathogens (Anaplasma phagocytophilum) to stimulate broad immunity against spotted fever group rickettsiae and A. phagocytophilum. This platform could be exploited to develop vaccines against other vector-borne pathogens that occupy the intracellular niche

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- In question 10 under Mice, I believe it mistakenly refers to hamsters instead of mice. In question 10 , 11 - The protocol references Dr. Angela Craig. I would suggest replacing with RAR vet on call.

- Under procedures,please provide a description of the IP injection as a separate procedure; as opposed to combining them as one but not including any description about the IP injection

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 3)

1. **Protocol Title:** 1912-37675A Evaluation of the Safety of a Commercial Colostrum IgG Replacement Product
Species &Pain Class: Cow Biomedical (A)
Question the Research Addresses: Is the commercial colostrum replacement being evaluated safe for calves?

The committee concurs that this protocol can be approved via designated member review once the following stipulation is addressed by the PI:

- Please update the IMHA section questions 6 and 8 (under Species) to include the square footage/dimensions for [redacted]. Is it equivalent to that of [redacted]? Is it sufficient to house and separate the calves? We did not get access to the line drawing of the [redacted] floor plan.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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2. **Protocol Title:** 1812-36628A Neural Control of Hypertension and Cardio-Renal Diseases. Afferent renal nerves, renal inflammation, and hypertension. Renal denervation to treat hypertension: Mechanisms and mediators 1K99HL141650
Species &Pain Class: Rat (B); Mice (B)
Question the Research Addresses: This research works to address the mechanism and importance of the neural-cardiovascular-renal interaction in the development and maintenance of hypertension.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Please add an additional aim specifying the goal and use of these transfer animals from the [redacted] lab.

- 1. In experiment 4A, please describe how will you be measuring the effects of these "cytokine cocktails" in these mice. 2. Please describe in detail the 2K1C procedure/model in Experiment 4B or transcribe the detailed procedure and add it to the attachments section. A written document of the protocol is appreciated in addition to the visual of the 2K1C timeline in the attachment section. 3. In addition to the timeline provided, please outline in detail in the Experimental Design the stepwise procedures/treatments these transfer animals will undergo. Please include if the animals will start in

the [redacted], be transferred to the [redacted] for OVLT surgery and then back to the [redacted] lab? Once animals are transferred, what is the timeline for when they will undergo transplant surgery? Also, please address what is the purpose of performing an OVLT in these animals and how will it contribute to your overall research?

- Under the question addressing if these animals will undergo more than one survival surgery, the phrasing could be interpreted that the pain medication will be administered at 3 days after surgery (and not before). Please update this sentence to clarify that analgesics are given daily for 3 days post op at a minimum.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

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3. **Protocol Title:** 1811-36514A Novel mechanisms of analgesia for chronic pain Neural Mechanisms of Cancer Pain Cannabinoid Modulation of Hyperalgesia.
Species &Pain Class: Mice (A,B,C)
Question the Research Addresses: We will study mechanisms of pain development during sickle cell disease and modulation of spinal nociception by descending projections from the brain stem. We are continuing determination of central and peripheral antinociceptive effects of resolvins (derivatives of omega-3 polyunsaturated fatty acids) and evaluate its analgesic mechanisms during cancer and sickle cell disease. We will compare effects of resolvins with morphine and MMG22, a chemically conjugated mu opioid agonist and CCR5 antagonist through a 22 atom spacer, which demonstrates potent analgesia at very low doses.

In addition we will evaluate a possibility to induce analgesia through inhibition of hydrolyzes of palmitoylethanolamide (PEA), a endogenous derivative of omega-6 polyunsaturated fatty acid which is a native analgesic substances.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- In several places the authors refer to the bivalent ligand MMG22 with the description of pharmacophores that correspond rather to the bivalent ligand MCC22. This occurs in the Common Terms table and in the experimental description following the section with the subheading: "I. Hyperalgesia in mice with sickle cell disease" in the following sentence: "We will also compare effects of RvD1 with that induced by s.c. injections with 0.5 and 1 pmol of morphine and MMG22 a new substance that is a chemical conjugate of mu opioid agonist and CCR5 antagonist linked by a 22 atom spacer (MCC22)." Please clarify this language and which compound is the one that is intended for study. If the compound to be tested is MCC22, then the Common Terms Table should be corrected. If MMG22 is to be tested, then the correct 2nd pharmacophore is mGluR5 and that should replace CCR5. It is recognized that this was not the subject of the amendment, but the reviewer noticed this inconsistency and, therefore, this is an opportunity to correct the protocol.

- The investigators note that for the introduction of cold environment as a stimulus to evoke hyperalgesia that "Mice will be kept in their home cage and placed in a cold room maintained at 10 degree C (50 degrees F) for 30 or 60 minutes." The plan is to use 5 HbSS sickle cell and 5 HbAA control mice for this pilot experiment. Is it the intent to do two separate experiments, one at 30 minutes and one at 60 minutes in the same subjects? If so, is the plan to test 30 minutes of cold exposure to determine whether hyperalgesia has been induced in the HBSS mice and, if not, proceed to 60 minutes? If not, how will a sample size of 5 mice each be sufficient to test the hypothesis? It may be that this experiment is underpowered to detect the effect.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0

University of Minnesota Panel: FCR Panel
April 05, 2022

Meeting Convened: 12:02 PM	Quorum Requirement: 10
Meeting Adjourned: 2:15PM	Members Present to Vote: 13

Voting:

1	x	(Chair - M, S)	A		(A, S)
2	x	(M, V)	B		(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E	x	(A, V)
			F	x	(A, V)
			G	x	(A, V)
			H	x	(A, V)
			I	x	(A, V)
			J		(A, V)
3		(M, S)	K		(A, S)
			L		(A, S)
4	x	(M, U)	M		(A, U)
			N	x	(A, U)
			O	x	(A, U)
			P		(A, U)
			Q	x	(A, U)
5		(M, S)	R	x	(A, S)
6	x	(M, V)	S	x	(A, V)
7		(M, S)	—		(A, S)
8		(M, S)	T		(A, S)
9		(M, St)	U	x	(A, St)
10		(M, S)	V	x	(A, S)
11		(M, S)	—		
12	x	(M - NA, NS)	W		(A, NA, NS)
13	x	(M, S)	X		(A, S)
14		(M, S)	Y		(A, S)
15		(M, S)	Z	x	(A, S)
16	x	(M, St)	AA		(A, St)
17	x	(M, S)	BB		(A, S)
18	x	(M, S)	–		
19	x	(M, U)	CC	x	(A, U)

Non-Voting, Ex-Officio:

i			(O, U)
ii			(O, U)
iii	x		(O, U)

Institutional Veterinarian:

3	x		(M, V)

Correlates to Version **v12.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U =

Discussion Items

1. The committee discussed a self-report in which rats were separated by lab staff and a water bottle was not placed in the new cage. The issue was corrected by RAR staff and no animal welfare issues resulted. The committee had voted at the previous meeting to suspend the investigator's use of animals (the reported incident occurred prior to the suspension) and corrective action for this incident will be included in the lab's overall correction plan, to be submitted to the committee when it has been developed.
2. The committee discussed an update on a previous self-report involving dehiscence of surgical sites. The surgeon is experienced, and it is felt that retraining is not warranted at this time. The committee requests that the protocol be revised to change the type of suture used and will be updated when this has been done.
3. The committee discussed a cow on a veterinary teaching protocol that was found to be unexpectedly pregnant. The cow will be allowed to deliver, and a suitable adopter found for the calf. The committee will be kept updated.
4. The committee discussed a self-report of two separate incidents in which an NHP briefly escaped their cage during jumping procedures. Two NHPs sustained minor injuries which have been treated and resolved. Moving forward, RAR will lock chains and bank wheels to avoid gaps and is looking into an NHP lift and tunnel to facilitate the jumping process. The committee considers the matter closed.
5. The committee discussed a self-report in which the wrong NHP was accidentally sedated for TB testing. The animal was monitored until fully recovered from sedation and there were no welfare concerns. The staff member involved is being retrained on animal identification. The committee considers the matter closed.

IACUC-R1S1(# Protocols: 8)

IACUC-R1S1 - NEW(# Protocols: 7)

1. **Protocol Title:** 2111-39581A "Integrated fMRI Methods to Study Neurophysiology and Circuit Dynamics at Laminar and Columnar Level" and "Neurons, Vessels and Voxels: Multi-modal Imaging of Layer Specific Signals" "Technology to Realize the Full Potential of UHF MRI"
   Species & Pain Class:(B) Cat
   Question the Research Addresses: Our proposals aim to push the technology envelope beyond the current level by developing innovative multimodal fMRI approaches capable of simultaneous neural stimulation, recording and fMRI acquisition with functional mapping specificity and resolution down to the mesoscopic scale. The cutting-edge technology and developed tools will allow us to investigate brain function and connectivity at cellular columnar and laminar levels—the two most fundamental neural computational units for micro-circuits essential for brain function, and still cover large networks through thalamo-cortical and cortico-cortical connections in the cat brain. In addition, we will elucidate the link between neural and vascular signals across laminae by combining two-photon imaging of neural and vascular responses with ultra-high-field (UHF) fMRI. In addition, we will apply various neurometabolic imaging techniques developed in our lab and elsewhere to study cerebral glucose, oxygen and energetic metabolism using the cat model.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: [redacted]: For the "Protocol" document. p.14/End of Experimental Design- "isoflurane via face mask for intubation if cat is too light"- [redacted] an injectable induction would provide more optimal intubation conditions p.16/General anesthesia and preparation procedure description- cats fasted 4-10 hours, this differs from the fasting time of 2-4 hours on p.2 of the "cat Anesthesia SOP" and 2-4 hours would be the recommended time for cats of this age p.17/General anesthesia and preparation procedure anesthetic table- only lists IM route and doses, xylazine at 0.5-1.0mg/kg is different than the 2-2.5mg/kg listed on p.2 of the "Cat Anesthesia SOP" and differs from the 2.5mg/kg in the dosage table on p.8. The xylazine at 0.5-1mg/kg IM or 0.2-0.5mg/kg IV would be the recommended doses. Additionally N2O is not listed as an anesthetic drug--if this is not an option in the drop down menus it can be added as "other" p.20/General anesthesia and preparation procedure, support agents section- Dobutamine should be administered at 1-20mcg/kg/min IV. NaHCO3 should be 1mEq/kg IV. Atipamezole (0.05-0.1mg/kg) and atropine (0.04mg/kg) should also be listed as emergency drugs. There is no mention of blood pressure monitoring p.37/Health and Monitoring- no mention of blood pressure monitoring p.43/DEHS anesthetic gases section- no mention of N2O under anesthetic gases

Comment: [redacted]: For the "Cat Anesthesia SOP for Survival MR Imaging Study" document p.1- the peak inspiratory pressures on the ventilator should be <20 cm H2O to prevent barotrauma p.3- typically only need to wait 1-2 minutes after lidocaine to intubate p.3- if cat is too light to intubate then ketamine would need to be administered in conjunction with a muscle relaxant for optimal intubation conditions (i.e. midazolam, propofol, xylazine) and an injectable induction would be preferred over face mask with isoflurane (as stated above) p.3- no mention of N2O-O2 mix used for cuff inflation to prevent cuff from increasing in size during anesthesia with N2O p.4- lidocaine will penetrate mucosa but does not penetrate intact skin, would likely need to use EMLA cream for any desensitization p.6- N2O not listed as a drug used in the study p.8- xylazine dose in table differs from that found in "Protocol" document (see above)

Comment: Craniotomy and implants are mentioned in a few different sections (Health and Monitoring and the Exp Design attachment). Please remove.

Comment: There is still no justification for the age/weight of the kittens to be used. Please provide this within the protocol.

Comment: Please describe the total number of procedures/anesthetics events that each animal may receive including a max number.

Comment: The statistical justification in not appropriate and needs actual rationale. The limits per the IACUC will be placed but the total number needed with statistics/justification should be provided.

Comment: With the history of these procedures I recommend only 1 animal be used as a pilot then if that goes well with the updated SOP and procedures then additional animals can be requested via amendment.

Committee Decision: Stipulations must be met
For: 11 Against: 0: Abstain: 0

2. **Protocol Title:** 2201-39722A "In vivo MRS and MRI study of metabolism, bioenergetics and function using rodent models" "Advancing simultaneous fMRI-multiphoton imaging technique to study brain function and connectivity across different scales at ultrahigh field" "Development of predictive pharmacodynamic models of ADOR"
Species & Pain Class:(B) Rat; (B) Mice
Question the Research Addresses: In this application, we propose to apply the high-field (≥ 9.4T) MRI/MRS to study (i) the hemodynamic, metabolic, electrophysiologic and energetic characteristics and the redox state of normal rodent brains in different brain states; (ii) ischemic brain tissue and therapeutic effect of ADOR using rat model of forebrain (global) ischemia (four-blood-vessel-occlusion (4BVO) model); (iii) glucose metabolism in brain tumor (peripheral gliosarcomas); and (iv) brain function and connectivity across different scales. In addition, we will train lab personnel for the general procedures used in the studies.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please update the rationale section so it better matches the current protocol (especially part B, which itemizes experiments that don't match up with protocol).

   Comment: Will the a-chloralose be terminal?

   Comment: Please clarify what you mean by 'resting/excise muscle' in part B. Is that a model or are you removing muscles to measures something etc...

   Comment: The Guide for the Care and Use of Laboratory Animals says about the review of IACUC protocols that there should be "Clear and concise sequential description of the procedures involving the use of animals that is easily understood by all members of the committee". It's understood and accepted that it's difficult for you to know in advance exactly how many rats or mice will undergo a given procedure, but we still should be able to understand from your Experiment Design section how many procedures an individual animal might undergo and why. There are 21 separate procedures for rats on the protocol, and 6 for mice, and the Experiment Design section should explain when each procedure is used and how they are combined in individual experiments. I see that you've made a big effort do that with the attachment entitled "Examples of study design Jan 2022", but please understand that this document, while thorough. is very difficult to read. The key is separated from diagrams on a separate page, and text to give a simple description of the purpose and list of procedures in each kind of study is not provided. Could you please improve the readability of this attachment by adding some text to name the procedures used in each type of study, or at a minimum repeat the procedure key for symbols used in each set of experiments beneath each sequence,

   Committee Decision: Stipulations must be met
   For: 13 Against: 0: Abstain: 0

3. **Protocol Title:** 2103-38933A Hydrogel embolic agents for the management of bleeding, tumor embolization and potential drug delivery
Species & Pain Class:(B) Rabbit
Question the Research Addresses: Currently available embolic agents on market, including Onyx®, N-butyl cyanoacrylate (NBCA) and PHILTM, have many drawbacks including the use of cytotoxic organic solvent and potential risk of adherence of the catheter tip to the blood vessel wall. Our group has developed a series of hydrogel materials consisting of aqueous solutions of naturally occurred proteins, providing a much safer and easier-to-handle candidates as next-generation embolic agents.
The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Please expand the explanation of why the rabbit model was chosen for this study. If the reason for using rabbits is that other previous studies have used this species, please provide the papers as a reference and explain why rabbits are a superior model to other mammalian species (i.e. mice, rats, etc.)

Comment: Although this is a pilot study (hence a power analysis might not be required) please explain how the investigators have reached the conclusion of using 5 rabbits per experimental group.

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

4. **Protocol Title:** 2111-39563A XXXXX Mouse Protocol: Circuit mechanisms of dopaminergic control over flexible behavior.
Species & Pain Class:(B) Mice
Question the Research Addresses: How do animals resolve different behavioral and environmental demands by utilizing specialized brain circuits for learning and motivation? What are the key physiological properties of these brain circuits, and how do they interact during different phases of behavior?
The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Please update the protocol to describe the rationale behind performing the ovariectomy and how it pertains to the aims of this study.

Comment: It is mentioned that a mouse's head will be fixed via a head post during the "Behavior - In vivo imaging with behavior procedure" and "Behavior - In vivo chronic awake electrophysiology procedure". Please update the protocol to describe how mice will be acclimated to the head post.

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

5. **Protocol Title:** 2202-39815A Novel Immunotoxin and IGF Therapy for Strabismus Treatment Novel Pharmacologic Approaches for the Treatment of Strabismus Novel Pharmacologic Approaches for the Treatment of Nystagmus Novel Pharmacologic Approaches for the

Treatment of Congenital Nystagmus Development of Pharmacologic Treatments for Eye Movement Disorders Pitx2 Signaling and Strabismus Defining a potential molecular basis for infantile nystagmus syndrome Potential molecular basis for infantile nystagmus syndrome
Species & Pain Class:(A,B) Rabbit; (A,B) Mice
Question the Research Addresses: Our goal is to develop pharmacologic approaches to treat strabismus and nystagmus in order ultimately to improve treatment options and treatment efficacy in these children.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: It looks like the number rabbits requested (1896) is incorrect based on the information provided. Adding 360+576+960 gives a total of 1896. It doesn't appear that the 108 rabbits requested for Section 1 (Western and IHC) were included in the total. If these are added, then the number of animals requested is 2,004. Please confirm the total number of rabbits requested and confirm that the number needed for Western blotting in section 1 is 54 (9x6) and not 45 (9x5). There is also supposed to be an appendix provided for this section, yet it was not attached to the protocol. Please provide the appendix.

Comment: The following was taken from the procedure section: "This is used to ensure sufficient anesthesia, and not have access to the face and eye region restricted by inhalation bells, + not require presedation. They are kept on 2." Please update the protocol to clarify the meaning of they are kept on 2?

Comment: The lengthening surgery is mentioned in the procedures but not in the experimental design. Please clarify how many rabbits will have this procedure and how will that number be determined?

Comment: An appendix is mentioned but no attachments are present, please add any any attachments necessary. A table would be helpful in clarify the rabbit studies and numbers.

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

6. **Protocol Title:** 2201-39705A XXXXX Lab CNS blood flow and extracellular spaces and glial modulation of electrical synapses in the retina protocols 2019
Species & Pain Class:(A,B,C) Mice
Question the Research Addresses: The specific questions addressed in this study are: A) CNS blood flow 1. Is retinal blood flow regulated, in part, by constriction and dilation of pericytes, which surround retinal capillaries? 2. Does activation of glial cells result in increases and decreases in retinal blood flow? 3. Which chemical messenger systems mediate glial control of blood flow in the retina? 4. How is glial control of retinal blood flow disrupted in such pathological conditions as diabetic retinopathy and ischemia? 5. Do specific therapies, including iNOS inhibition, slow the progression of diabetic retinopathy? B) CNS extracellular space (ECS) 1. How does the ECS volume fraction of retina change during development? 2. How does the ECS volume fraction of retina change with light-evoked neuronal activity? 3. How do neurotransmitters diffuse through the ECS in the retina? 4. How does diabetes influence the ECS in the retina? 5. How do changes in the ECS volume fraction affect neuronal activity in the

retina? 6. How do changes in the ECS volume fraction affect spontaneous retinal activity during early stages of development? C)Glial Modulation of Electrical Synapses in the Retina A. Do known gliotrasmitters modulate gap junction coupling in the retina? 1. Do known gliotransmitters affect electrical coupling of gap junctions? 2. Do known gliotransmitters affect functional coupling of gap junctions? B. Do glia modulate gap junction coupling in the retina? 1. Does release of molecules by glial cells affect electrical coupling of gap junctions? 2. Does release of molecules by glial cells affect functional coupling of gap junctions?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: The total number of mice should include the mice used for breeding. The PI requests 3275 mice (2780 + 375 + 120) for breeding. The total number of mice should include these mice.

Comment: Please specify the methods for mouse identification, such as ear tag.

Comment: Both the retro-orbital injection procedure and intra-ocular injection procedure state that the animal will be provided Carprofen or Meloxicam pain management as needed but the analgesic section says it will be given SID for 4 consecutive days including the experimental day for the retro-orbital injection and 3 consecutive days for the intra-ocular injection procedure. Please update the protocol to clarify if this will be given on an as needed basis or will always be given after these procedures.

Comment: The blood collection procedure outlines the blood glucose measurements but not the blood taken during the 2-8 hour non-survival surgery--mouse retina procedure. Please update the blood collection procedure to add the blood volume taken during this procedure to measure blood pH.

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

7. **Protocol Title:** 2202-39822A Examining the microbiome at the interface of gene-environment interactions in nervous system health and disease
Species & Pain Class:(A,B) Mice
Question the Research Addresses: This protocol includes experiments that aim to test i) how the microbiome is altered in response to genetic and environmental risk factors for disease and ii) how the microbiome, select bacteria from the microbiome, or select microbial factors impact disease outcomes in mouse models for autism, stress- and obesity-related cognitive deficits, fragile X syndrome and Parkinson's disease.
The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: In order to capture all relevant details, please create a separate procedure for AAV administration and provide information on route, volume, frequency and AAV titer in viral genome or genomic copy number.

Comment: Please add a procedure of type "dietary or fluid modification" for the microglia depletion by diet, and answer all the questions within that procedure type.

Comment: Please provide IBC protocol # since viral vectors (AAV) used as well as genetically modified animals. Contact IBC for details (ibc@umn.edu). IACUC will need an approved or pending/submitted IBC protocol #.

Comment: The IMHA housing is not approved as currently proposed. A meeting has been scheduled to discuss this and the protocol should be updated accordingly after that meeting.

Comment: As Aim 1 describes that animals will experience numerous behavioral tasks over several weeks during the course of the study, please update question #2, "Will animals undergo a battery of behavioral tasks" from 'no' to 'yes' and describe the general interval expected between tasks (described as 2-3 days in the experimental design).

Comment: Please add a behavioral procedure for the novel whisker texture test as described in experimental design Aim 1

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

IACUC-R1S1 - AMENDMENT(# Protocols: 1)

1. **Protocol Title:** 2009-38457A Cell Signaling and Neurodegeneration Molecular Genetics SCA1
   Species & Pain Class:(A,B,C) Mice
   Question the Research Addresses: There is no effective treatment for SCA1 and our goal is to examine specific elements in the pathway leading from the genetic mutation to development of the disease causing protein and to investigate steps ultimately responsible for causing the disease and thus test potential therapeutics for the disease.
   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: What is the experimental design for these new procedures on the SCA1 mice? Is it happening every few months, once in a lifetime? Starting at a particular age?

   Comment: Please include the new procedures in tje experimental design and show what animals will be used and in what sequence with other procedures.

   Comment: Please include the frequency of this procedure or indicate it will be done once.

   Comment: Please indicate the frequency of this procedure or indicate that it will be done once.

   Comment: Please include distress monitoring criteria for individually housed animals. Is there a possibility of fighting when animals are returned to group housing?
   Committee Decision: Stipulations must be met
   For: 13 Against: 0: Abstain: 0

Institutional Animal Care and Use
Committee Minutes
VCRC - 76D
April 19, 2022

Meeting Convened: 12:00pm	Quorum Requirement: 10
Meeting Adjourned: 2:20pm	Members Present to Vote: 17

Voting Members			Alternates		
1	x	(Chair - M, S)	A	x	(A, S)
2	x	(M, V)	B	x	(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E	x	(A, V)
			F	x	(A, V)
			G	x	(A, V)
			H		(A, V)
			I	x	(A, V)
			J		(A, V)
3		(M, S)	K		(A, S)
			L	x	(A, S)
4	x	(M, U)	M	x	(A, U)
			N	x	(A, U)
			O	x	(A, U)
			P	x	(A, U)
			Q	x	(A, U)
5	x	(M, S)	R	x	(A, S)
6	x	(M, V)	S	x	(A, V)
7	x	(M, S)	—		
8		(M, S)	T		(A, S)
9	x	(M, St)	U	x	(A, St)
10		(M, S)	V	x	(A, S)
11	x	(M, S)	—		
12		(M - NA, NS)	W	x	(A, NA, NS)
13	x	(M, S)	X	x	(A, S)
14	x	(M, S)	Y		(A, S)
15	x	(M, S)	Z		(A, S)
16		(M, St)	AA	x	(A, St)
17	x	(M, S)	BB		(A, S)
18		(M, S)	–		
19	x	(M, U)	CC		(A, U)

Non-Voting, Ex-Officio:

i	x	(O, U)
ii		(O, U)
iii	x	(O, U)

Institutional Veterinarian:

2	x	(M, V)

Correlates to Version **v12.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion Items

1. The committee conducted the Spring 2022 Semi-Annual Program Review. All discussion is documented in the Spring 2022 Report to IO and Program Review Packet.

1. **IACUC-R1S1(# Protocols: 2)**

1. **IACUC-R1S1 - NEW(# Protocols: 2)**

    1. **Protocol Title:** 2203-39845A Field methods in ornithology
    Species & Pain Class:(A) Bird (Other)
    Question the Research Addresses: Students will learn the fundamentals of the following skills: 1) Field identification of birds by species and sex using both visual and auditory cues 2) Survey methods for birds, including transects, point counts, and area searches 3) Methods for field recording and playback of bird vocalizations 4) Use of mist nets and traps in the safe capture of birds 5) Safe handling of captured birds for the purposes of marking and sampling 6) Proper use of euthanasia methods (in theory, no direct experience will be provided)

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    Comment: In the "Justification of animal numbers" and the "List of species" attachment it states the number of animals that may be euthanized for training of of students on curatorial methods. This procedure is not. however, described in the experimental design section, and should be added.

    Comment: This procedure says that birds may undergo feather plucking and banding. Please create procedures for these to provide more details about how many feathers and which feathers may be plucked and how the banding is done.

    Comment: When birds are in permeable cloth bags, how are they monitored? For example, how can you determine that a bird is gaping or has stopped breathing if it's in an opaque bag?

    Comment: It says apparent death will be assessed by apnea and absence of reflex - which reflexes are assessed?

    Comment: Please verify that only trained personnel will perform this method of euthanasia. Please include more details regarding the mechanical small bird dispatcher (e.g. brand or model, how it works, etc).

    Comment: Over what period of time will vocalizations be played? For example, 1-10 vocalizations over the course of 5 minutes or over the course of 1 hour.

    Comment: Please remove the use of firearms as a euthanasia method since that will no longer be used.

    Comment: Please update the mist netting procedure to specify how often mist nets are checked.

    Comment: Please clarify whether you will be using a mobile CO2 setup for euthanasia or if birds will be transported for euthanasia. If you are transporting animals to be euthanized, you must arrange for your vehicle to be inspected. Also, if using CO2, do you have a flow meter to ensure rate?

    Committee Decision: Stipulations must be met
    For: 12 Against: 0: Abstain: 0

2. **Protocol Title:** 2112-39687A The development and characterization of a soft-tissue attachment to a transdermal skin-implant interface in a swine model of osseointegration
Species & Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: Porous titanium discs will be coated with biodegradable vehicle material (GelMA) in the presence or absence of ex vivo cultured cells derived from swine stem cell populations and then implanted transdermally in the dorsal lumbar region of domestic pigs. We will investigate new approaches to develop ex vivo engineered living tissues which can be transplanted and integrate with soft tissue, bone and metal to form a seal at the transdermal interface to prevent bacterial leakage. The ultimate goal of this study is optimization of the skin-implant interface, including skin-to-implant healing and attachment, remodeling of connective tissue during wound-healing processes, prevention of infection and control strategies.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Under post op care, NSAID, opioid, and local are mentioned (presumably carpofen, lidocaine, bupivicaine, and buprenorphine), but most of these drugs are not described in the context of post-operative care. Please verify that these drugs and dosages would be used and modify the text accordingly.

   Comment: Please describe what combination of anesthetics will be used and when they will be used (induction, maintenance, etc).

   Comment: Under specific steps both pupil responses and palpebral reflex are mentioned. Are those monitored at 15 minute intervals?

   Comment: The author has checked the box indicating that this model has been used in other publications but has also checked the box indicating that this is a new model. Please clarify.

   Comment: Please explain what these designations are: pMSC selected candidate/ [redacted] human iPSC-derived MSCs/Mutreja For example, are they the names of the people who are providing the cells?

   Comment: Please provide more information regarding immunosuppression: What level of immunosuppression will be used? Are there any special husbandry practices needed? Are there any related welfare considerations or special monitoring needed?

   Comment: A study fallout rate of 20% is indicated in the Experimental Design and is used to determine the requested number of replacement animals.. Please provide more information about how immunosuppression affects the expected failure rate.

   Committee Decision: Stipulations must be met
   For: 10 Against: 0: Abstain: 0
   Members 1, X out

Institutional Animal Care and Use Committee
4/6/21 Minutes
VCRC - 76D

Meeting Convened: 12:03PM	Quorum Requirement: 9
Meeting Adjourned: 1:53PM	Members Present to Vote: 15

Voting Members			Alternates		
1	X	(Chair - M, S)			
2	X	(M, V)	A	X	(A, S)
			B	X	(A, S)
			C	X	(A, S)
			D	X	(A, S)
			E	X	(A, S)
			F		(A, S)
			G	X	(A, S)
			H		(A, S)
3		(M, S)	I	X	(A, S)
4	X	(A, U)	J	X	(A, U)
			K	X	(A, U)
			L	X	(A, U)
			M	X	(A, U)
			N	X	(A, U)
5	X	(M, S)	O		(A, S)
6	X	(M, V)	P	X	(A, S)
7		(M, S)	Q	X	(A, S)
8		(M, S)	R	X	(A, S)
9		(A, St)	S	X	(A, St)
10	X	(M, S)	T	X	(A, S)
11		(M, S)	U	X	(A, S)
12	X	(M - NA, NS)	V		(A - NA, NS)
13	X	(M, S)	W		(A, S)
14		(M, S)	X	X	(A, S)
15		(M, S)	Y	X	(A, S)
16		(M – St)	Z		(A, St)
17		(M, V)	-		

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, S)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v2.110** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the March 2021 Inspection Findings-Notes to File-Veterinary Recommendations.

2. The committee was updated on changes made to the Policy and Guidelines for Transportation of Animals. The policy now clearly indicates that RAR must be contacted for transportation between disconnected buildings. The committee requested the following additions to the Guidelines and will be updated when they have been made:

    a. Vehicles must be approved in advance of use.
    b. Transportation at agricultural sites is governed by their respective unit SOPs and does not require RAR to transport between buildings.
    c. If inappropriate animal transportation is observed, it should be reported to the IACUC, as well as any applicable vendors.

3. The committee was updated on the adoption of a research dog. Pre-adoption procedures have been completed and the dog is recovering well and ready to go home with the new owner. The IACUC office will follow up with the adoptor in one month and update the committee.

4. The committee was updated on the progress of implementing IACUC mandated RAR training for new animal users and was reminded that protocol approval is not being delayed for the new training. An experienced research group will be contacted to request their assistance with hands-on training for agricultural cows.

5. The committee was updated on the upcoming AAALAC site visit, which will be scheduled sometime in summer 2021. RAR will conduct mock visits, including in IMHA areas, and RAR and IACUC will develop materials to assist labs in preparing for the site visit.

6. Semi-annual Program Review is scheduled for 4/20/21 during the normal FCR meeting time. Discussion items will include the OLAW checklist, summary of previous discussion items, inspection data, administrative data, and preparation for AAALAC. All members are encouraged to attend.

7. The committee was updated on a revised supervision plan for a lab. The plan was approved, with the inclusion of supervised anesthetic events as previously communicated to the PI.

8. The lab was updated on an NHP that is no longer a candidate for long-term studies. The labs involved would like to plan an acute-use study for the animal and several scenarios for this were discussed. The committee was supportive of the proposal to transfer the animal between the protocols on which the final procedures are approved and endorsed the suggestion for the labs to create an “end of study” protocol for future use.

9. The committee reviewed a self-report in which surgical procedures were conducted on a protocol for which they were not yet approved. Moving forward, the animals have been transferred to an appropriate protocol on which the procedures and follow-up care are approved, and these procedures will not be done on the original protocol until they are approved. The committee considers the matter closed.

10. The committee reviewed a self-report in which animals received a special diet not approved on the protocol, and food levels were found to be low by RAR and the lab did not immediately replenish the supply. Moving forward, the lab has submitted an amendment to add the diet and reminded lab staff of the need to complete weekend tasks. The committee will send the PI a letter reinforcing the importance of prompt attention to issues identified by RAR.

11. The committee reviewed a self-report in which two dogs experienced burns caused by a grounding unit for an electrocautery device used during surgery. Moving forward, the lab will use gel on the grounding device and include this on their pre-surgery checklist. The committee considers the matter closed.

12. The committee was updated on efforts to identify a suitable method for monitoring heart rate in cats while in a high field MRI environment. The Mouse Ox device worked initially but the lab reported fluctuations later

in the scan. The committee requires that the lab use the Mouse Ox device for their future procedures, to collect as much heart rate data as possible. Additionally, the committee was updated on a new staff member in this lab and will allow her to supervise cat anesthetic procedures in place of RAR/IACUC supervision, once she is added to the protocol.

13. The committee was updated on a self-report from RAR in which an equipment failure caused animals to be without access to water and animals were subsequently found dead. The committee reviewed an additional self-report in which three cages of mice were found without food and one animal died and others required medical attention. RAR will remind animal care staff to check food levels every day during health checks and to verify water sources are in working order when placing cages on the rack. The committee requests that RAR evaluate the effect of the change in daily check procedures and report whether there have been additional issues with food or water access since the change was made.

1. **IACUC-NEW (# Protocols: 7)**

1. **Protocol Title:** 2102-38855A Development of mouse models to better understand arbovirus pathogenesis
Species &Pain Class: Mice (A,B,C)
Question the Research Addresses: The overarching question driving this proposal is how do arboviruses evolve in the face of new selective pressures? A related question is why was congenital Zika syndrome not recognized earlier?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - Under "Experiment 1. Breeding", you mention that 3 to 15 breeding pairs will be utilized to maintain the colony, however, under the "Procedure section: Breeding", you mention that 44 breeding pairs will be used. Please update either section to reflect the correct number of breeding pairs.

   - Jennifer Vangorder-Braid cannot be found in the ROHP database. If she is still working on this study, please contact uohs@umn.edu to have her re-added, and confirm here in eProtocol when any outstanding requirements are complete. If she is no longer working on this study, please remove her from the Personnel list.

   Committee Decision: Stipulations must be met
   For: 15 Against: 0 Abstain: 0

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2. **Protocol Title:** 2101-38796A Ocular hyperalgesia in dry eye
Species &Pain Class: Rat (B,C)
Question the Research Addresses: This project is designed to determine the mechanisms that underlie pain-like behavior in an animal model for tear deficient DE. Emphasis is directed at understanding how tear deficiency alters the neuronal circuitry in the brain and ocular reflexes (eye wipe, eye blink, lacrimation).

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - It is described that 250 rats (classified as class C) will be initially undergoing exorbital gland removal and eye wipe behavioral testing, and subsequent to those experiments, it appears those same 250 rats (now classified as class B) will be undergoing neural/OOemg testing. However, within the species section, it appears that a total of 500 rats will be utilized. Please clarify the total amount of rats utilized within this study and ensure they are grouped to the highest pain class that they will undergo.

   - Please update this section of the protocol to include the concentration of menthol to be used.

   - Although this is a non-survival surgery, please address how you will adequately assess the depth of

the deep surgical anesthesia (first question under "Parameters monitored during procedure" section) to ensure the animal is properly anesthetized before performing the thoracotomy.

Committee Decision: Stipulations must be met
For: 15 Against: 0 Abstain: 0

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3. **Protocol Title:** 2101-38797A Mechanisms of TMJ hyperalgesia
Species &Pain Class: Rat (B,C)
Question the Research Addresses: Does sex hormone status contribute to enhanced TMJ hyperalgesia after inflammation of the joint? Do neuron-glia interactions contribute to persistent TMJ hyperalgesia?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Pentobarbital is still listed as an anesthetic agent in the Anesthetic Regimen table. Please remove it if you are no longer planning on using pentobarbital.

- Thank you for updating the procedure to include the use of ketamine/xylazine instead of pentobarbital. Please update the "Anesthetic Agents and Sedatives" table to include ketamine and xylazine and remove pentobarbital.

- If Fatal Plus will be used to euthanize the animals, please change your answer to the question "It is expected that investigators use pharmaceutical-grade compounds whenever they are available in non-experimental research. Do you need an exception to this guideline?" to 'No" (it still states "Yes").

Committee Decision: Stipulations must be met
For: 15 Against: 0 Abstain: 0

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4. **Protocol Title:** 2102-38871A Primary Title: K/BxN mouse model of arthritis and carditis.
Species &Pain Class: Mice (A,C)
Question the Research Addresses: We are determining the inflammatory pathways that drive inflammatory arthritis and valvular carditis. We are also testing novel pharmacologic compounds to treat arthritis.

Committee Decision: Approved as submitted
For: 15 Against: 0 Abstain: 0

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5. **Protocol Title:** 2101-38789A Reducing mastitis in the dairy cow by increasing the prevalence of beneficial polymorphisms in genes associated with mastitis resistance Heirloom Holsteins for Functional Genomics Studies
Species &Pain Class: Cow (Agricultural) (C)
Question the Research Addresses: This work contributes to our goal of identifying the impact of selection on the immune system of Holstein cows, especially relative to mastitis resistance. We seek to identify genetic polymorphisms associated with improved health and resistance to mastitis.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- The experimental design indicates that the SOP for treating Grade 2 mastitis condition will be withheld for 48 hrs. The Health and Monitoring section indicates treatment will be given to those cows with more severe infection lasting for 36-48 hours and then will be treated. Please update these sections of the protocol to clarify which criteria is being used.

- This section indicates cows with low-grade infection lasting more than 11 days will be treated and removed from the study. Please also include this statement in the Health and Monitoring section.

- I realize this protocol involves working with an E. coli that is a common pathogen for mastitis. However, I think administering the pathogen to 120 cows is a different situation where a pathogen is being intentionally introduced into cows producing milk for commercial sale. I also realize that the protocol indicates that this E. coli has not been detected in milk and that the SOP deals with how milk will be handled in terms of dumping the milk. I think to be on the safe side, the question regarding food chain approval needs to be answered relative to administration of an E. coli that causes infection even it is mild. Please update the Food Chain section of the protocol.

- Please include the E. coli administration under DEHS: Animal Pathogens.

- Please include IBC approval information, or clarify in the response to this comment why it is not needed for this work.

- Personnel must complete the medical and online requirements prior to working with animals on the protocol. Personnel include: [redacted]: • Tetanus surveillance (due 4/5/21) Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 15 Against: 0 Abstain: 0

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6. **Protocol Title:** 2101-38758A NF1 to MPNST and associated projects
Species &Pain Class: Mice (A,C)
Question the Research Addresses: We're asking what gene mutation combinations can cause cancer and what drug or drug combinations can inhibit cancer growth.

   Committee Decision: Approved as submitted
   For: 15 Against: 0 Abstain: 0

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7. **Protocol Title:** 2012-38705A Novel oncolytic adenoviruses: generation and testing (in vitro and in vivo)
Species &Pain Class: Mice (B,C); Hamster (B,C)
Question the Research Addresses: Development of innovative combination therapy strategies which allow effective treatment of cancers.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - The protocol refers to tumor size in mm rather than the more conventional mm3 in both the Experimental Design and in certain Procedure Tabs. Do mm refer to the diameter or some other dimension? Please clarify the references to mm or update the protocol to have all measurements in mm3.

   - There are several Procedures that require IV injection of hamster through the saphenous vein. Please include a Procedure Tab describing this injection procedure.

   - Please remove this Tab since it just references another Procedure.

   - The titles of several procedures do not match the descriptions in the main text. In several places, it is unclear whether cells or adenovirus will be injected. Please check and revise the procedures to ensure that titles and descriptions match.

- Personnel must complete the medical and online requirements prior to working with animals on the protocol. Personnel include: Praveensingh Hajeri: -Animal exposure questionnaire (due 3/12/21) Once complete, please confirm here in eProtocol. Nikita Sharma: -Not found in the ROHP database. If she is still working on this study, please contact uohs@umn.edu to have her re-added, and confirm here in eProtocol when any outstanding requirements are complete. If she is no longer working on this study, please remove her from the Personnel list.

Committee Decision: Stipulations must be met
For: 15 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 2)

1. **Protocol Title:** 1811-36488A Innate immune control of natural rodent pathogens
Species &Pain Class: Mice (A,B); Rat (A); Deer mice (B)
Question the Research Addresses: How does the innate immune system control diverse pathogens from the same, or closely related, hosts.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - Since the Peromyscus maniculatus might be exposed to mouse pathogens when pet store mice bedding will be added to their cage, and, as mentioned in the health and monitoring section, since this could result in significant morbidity, why are they being placed in pain class B? If these animals do get sick will any treatment be started? If not, then a more appropriate pain class for these animals would be C. Please clarify.

   - The protocol does not specify the source for Peromyscus to be included in the new experiment. If any of these animals are wild caught, there is a reasonable chance that they might be infected with a hantavirus (e.g. Sin Nombre), which would be of potential harm to other rodents in the lab as well as to humans. Please be more specific as to the origins of these mice and/or other efforts that are being made to address this possibility.

   Committee Decision: Stipulations must be met
   For: 15 Against: 0 Abstain: 0

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2. **Protocol Title:** 1911-37613A Reprogramming astrocytes into neurons in canine stroke model
Species &Pain Class: Dog (B)
Question the Research Addresses: Can astrocytes reactive after stroke in canine be reprogrammed into neurons?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - Please include ketamine, xylazine and butorphanol within the "Anesthetic Agent(s) and Sedatives(s)" section.

   Committee Decision: Stipulations must be met
   For: 15 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee Minutes
May 3, 2022
VCRC - 76D

Meeting Convened: 12:02PM	Quorum Requirement: 10
Meeting Adjourned: 1:55PM	Members Present to Vote: 11

Voting Members			Alternates		
1		(Chair - M, S)			
2	x	(Vice Chair – M, S)			
3		(M, V)	A	x	(A, V)
			B	x	(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E	x	(A, V)
			F	x	(A, V)
			G	x	(A, V)
			H		(A, V)
			I		(A, V)
4		(M, S)	J		(A, S)
5	x	(M, U)	K	x	(A, U)
			L	x	(A, U)
			M		(A, U)
			N	x	(A, U)
			O	x	(A, U)
6		(M, S)	P	x	(A, S)
7	x	(M, V)	Q		(A, V)
8		(M, S)	R		(A, S)
9		(M, S)	S	x	(A, S)
10		(M, St)	T	x	(A, St)
11		(M, S)	U	x	(A, S)
12		(M, S)	–		
13	x	(M - NA, NS)	V		(A, NA, NS)
14	x	(M, S)	W		(A, S)
15		(M, S)	X		(A, S)
16		(M, S)	Y		(A, S)
17	x	(M, St)	Z		(A, St)
18	x	(M, S)	AA		(A, S)
19	x	(M,U)	BB	x	(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	x	(O, U)

Institutional Veterinarian:

A	x	(M, V)

Correlates to Version **v13.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

1. **Discussion Items**
    1. The committee voted to approve the finalized version of the Spring 2022 Semi-annual Program Review Packet, including the OLAW checklist. The vote was unanimous and no minority views were expressed. The final document will be sent to the Institutional Official.
    2. The committee considered moving occupational health requirements related to the health of the animal rather than the protection of the researcher under formal IACUC purview. This discussion was tabled to allow for members not present at this meeting to participate in the discussion.
    3. The committee discussed the corrective actions taken by a researcher whose privilege to use animals had been suspended at a prior meeting. The investigator and lab were noted to have been cooperative and receptive throughout the process and have submitted documentation of extensive retraining on IACUC and RAR policies, animal handling and anesthesia, recordkeeping, and chemical safety. The committee voted unanimously to reinstate the researcher's use of animals. There will be increased IACUC oversight of the lab through monthly submission of procedural records and at least one unannounced inspection.
    4. The committee discussed surgical and post-operative records that were submitted for an NHP procedure that was new to the lab and had the potential for complications. There were no specific concerns about the surgery or recovery, and veterinary staff will keep the committee updated on future procedures as needed.
    5. The committee was updated on a cow that was found to be pregnant unexpectedly. The cow has delivered, and the healthy calf was adopted out.
    6. The committee discussed an update on a previous self-report involving incision site dehiscence in mice. The protocol has been amended to change the suture material. The committee considers the matter closed at this time but will reassess if future dehiscence issues are observed.
    7. The committee discussed a self-report in which lab staff treated a clinical case in a mouse without veterinary oversight. The investigator confirms that for future health conditions, no action will be taken before consultation with veterinary staff. The committee considers the matter closed.
    8. The committee discussed a self-report in which an incorrect pig was retrieved from the housing area and anesthetized. The error was discovered before a procedure was started and the animal was recovered with no health concerns. Lab staff have been retrained on verifying animal identity. The committee considers the matter closed.
    9. The committee discussed an incident in which a rabbit died during a surgical procedure. It is believed that the endotracheal tube became dislodged during surgical manipulation, and that this was not detected in time for successful intervention. The lab will add additional monitoring for future procedures and confirms that there will be prompt and detailed communication with veterinary staff in case of failure of monitoring equipment or animal complications. The IACUC requires that either veterinary anesthesiologists approved on the protocol or RAR veterinary staff be present throughout future procedures until further notice. The committee will continue to be updated.
    10. The committee discussed a self-report and adverse event report in which there was unexpectedly high mortality in mice following administration of busulfan, and cages were not appropriately labeled as a chemical hazard. The lab discontinued further busulfan doses and staff have been retrained on chemical hazard procedures. The committee requests more information regarding the what the lab believes to be the cause of the mortality and changes that will be implemented in the future.
    11. The committee discussed a self-report in which a miscommunication resulted in two sheep receiving a duplicate dose of antibiotics. No adverse clinical signs were observed. RAR has updated their discharge procedures and forms to ensure all parties are aware of which group is responsible for which medications following discharge. The committee considers the matter closed.
    12. The committee discussed a report of an adverse event in which two agricultural pigs died at the time of blood collection. Lab staff will receive additional training on alternative blood collection sites for studies with intensive blood collection. The committee considers the matter closed.
    13. The committee discussed a self-report in which mice were bred on the wrong protocol due to issues with processing of transfer paperwork. The lab is consolidating their breeding and experimental protocols to avoid the need for future transfers. The committee considers the matter closed.
    14. The committee discussed a self-report in which an unapproved second surgical procedure was performed on a rat, the staff member performing surgery was not approved as a surgeon for the procedure conducted, and dates on post-operative notes appeared inconsistent. The protocol has been amended to add the second surgical procedure and the surgeon and the lab has discussed the importance of accurate record keeping. The committee requests that the investigator submit the complete surgical and post-operative records for the case as well as training records for the surgeon.

1. **IACUC-R1S1(# Protocols: 5)**

    1. **IACUC-R1S1 - NEW(# Protocols: 3)**

        1. **Protocol Title:** 2203-39912A Co-repressors in STAT5-dependent CD4+ T cell development and Function; COREPRESSORS IN REGULATORY T CELL DEVELOPMENT AND FUNCTION
        Species &Pain Class:(A,B,C) Mice
        Question the Research Addresses: The basic question is what do NCOR1 and NCOR2 do during T cell development and how is this linked to the transcription factor STAT5.

            The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

            Comment: Please include potential complications associated with irradiation and bone marrow graft failure. What could you see, how will you monitor, and how will you treat?

            Comment: In subaim 1.1, the Experimental Design describes the use of 30 pain class C mice; however, 60 mice are listed in the summary table. Please confirm that either 30 or 60 pain class C mice are requested.

            Committee Decision: Stipulations must be met
            For: 11 Against: 0: Abstain: 0

        2. **Protocol Title:** 2204-39928A A urinary biomarker of N'-nitrosonornicotine exposure
        Species &Pain Class:(A) Rat
        Question the Research Addresses: What urinary metabolite of NNN, as identified in rats, can be used as a biomarker of human exposure to NNN.

            The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

            Comment: For the NNN water in section where it states to describe what will be done to alleviate or minimize adverse effects. Please state your monitoring frequency of the rats to ensure they are drinking the water. Do you measure out the water each day to ensure they drink enough or you assessing urine amount and quality? If you see signs of dehydration due to them not drinking the water then what do you do?

            Comment: Having rodents in a metabolism cage for 7 days is quite long. Are there other measures in place to help keep them more comfortable since they will not have bedding material to assist them with thermoregulation? Will the room be kept at higher temperatures? Can you provide them with a plastic tunnel so that urine and feces can still be collected but they have an area to rest and potentially provide some warmth?

            Committee Decision: Stipulations must be met
            For: 11 Against: 0: Abstain: 0

        3. **Protocol Title:** 2203-39881A Deciphering the immunoregulatory network governing antigen presenting myeloid cells
        Species &Pain Class:(A) Mice
        Question the Research Addresses: The principal question revolves around how key novel immunoregulatory genes coordinate and govern immunity in antigen presenting myeloid cells

            The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

            Comment: In the experimental design section of the protocol, it is described in Aim 1 that 20uL of blood will be collected weekly for up to 3 weeks, but in the procedure for blood collection, it is described that 50uL of blood will be collected weekly. Please change one of these sections so both accurately reflect how much blood will be collected each week. It is acceptable to list a range (i.e. 20-50ul) in both locations, but they should be consistent.

            Committee Decision: Stipulations must be met
            For: 11 Against: 0: Abstain: 0

2. **IACUC-R1S1 - AMENDMENT(# Protocols: 2)**

1. **Protocol Title:** 2109-39465A Non-neuronal pathology and etiology of cognitive deficits in Spinocerebellar Ataxia type 1. Etiology of cognitive decline in Spinocerebellar ataxia type 1 NIH/NINDS 1R01NS109077-01A1
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: The goal is to examine the effect that non-neuronal cells have on the progression of disease and develop strategies for new treatments for the disease. Additional goal is to investigate anatomical correlates and mechanism of cognitive deficits in SCA1. It will also explore the effect that ATXN1 has on cognition.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please consider increasing monitoring from 3x weekly to daily once an animal begins to show signs of morbidity.

   Comment: For the mice in the survival studies in Pain Class C, more clarity is needed for the health monitoring and steps taken to relieve pain and distress for the animals. There is no clear assignment of responsibility between lab staff and RAR staff sufficient to assure that the measures described will be followed as written. RAR daily checks in housing rooms do not include the degree of health monitoring for individual mice in cages described in your health monitoring procedure, and visits from lab staff three times weekly are not frequent enough to provide the level of observation needed once animals approach a moribund state. These mice require at least daily monitoring, including weekends and holidays. This monitoring may be done by either lab staff or RAR staff, but you need to specify that if it is done by RAR staff that you will arrange to have this provided for you as a contracted special service well in advance of the time when it will be needed.

   Comment: In the Health and Monitoring Section, your protocol says "To prevent pain or distress in our Atxn1154Q/2Q and Floxed 146Q line they will be euthanized if, upon daily monitoring by the lab, they meet any one of the criterion requiring euthanasia listed in our protocol including, inability to walk and to reach food and water, fluids needed for 3 consecutive days, non-responsiveness, defined as lack of sustained purposeful response to gentle stimulation following the euthanasia guidelines on the IACUC webpage." However, in the Experimental Endpoints section, an exception is requested from the weight loss criteria for euthanasia. Please reconcile these sections so that it is very clear what constitutes a moribund condition which requires euthanasia, and also who will be responsible for making this decision and how and when the responsibility will be assigned. The health and monitoring section mentions that you want this decision to be made by a veterinarian or vet tech to avoid bias by the research team, but without a very clear plan to demonstrate how you will communicate responsibility for monitoring and deciding, animals could suffer unnecessarily. If there is a specific plan in place with RAR, please clarify this here as well as in the Health and Monitoring.

   Committee Decision: Stipulations must be met
   For: 11 Against: 0: Abstain: 0

2. **Protocol Title:** 2111-39597A Pre-clinical models of low back pain
Species &Pain Class:(A,C) Mice
Question the Research Addresses: The central questions of this research are to i) understand how intervertebral disc degeneration leads to chronic low back pain and ii) explore mechanism-driven interventions for prevention and treatment.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please double check the number of breeding pairs for Pirt x Ai14 x SPARC-null intermediate strains. From your explanation it appears that the number should be 4 and not 7.

   Comment: Please add procedure details for the drug administrations-- clonidine, RG 108, anti-VGF (dose, route, frequency). You could add these details to the "drug administration--intrathecal and IP" procedure section if you did not want a specific procedure section for each.

   Comment: Please add all drugs used in this study, including Tamoxifen, to the DEHS section of this protocol for

review. Currently only Isoflurane is listed.

Comment: Please outline how animals will be acclimated to behavioral tests as some descriptions are missing from some of the tests (rotarod, tail suspension).

Comment: While I could attibute all animals in the breeding section to the total animals to be used in your species section, I could not attribute the same numbers in your experimental sections (off by 29 animals). Please adjust the total numbers used if needed or adjust the experimental animals needed for this study.

Committee Decision: Stipulations must be met
For: 10 Against: 0: Abstain: 0
Member 18 out

Institutional Animal Care and Use Committee Minutes

VCRC - 76D

May 17, 2022

Meeting Convened: 12:02PM	Quorum Requirement: 10
Meeting Adjourned: 2:40PM	Members Present to Vote: 15 (discussion), 12 (protocols)

Voting Members			Alternates		
1	x	(Chair - M, S)			
2		(Vice Chair – M, S)			
3	x	(M, V)	A	x	(A, V)
			B	x	(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E	x	(A, V)
			F	x	(A, V)
			G	x	(A, V)
			H	x	(A, V)
			I		(A, V)
4		(M, S)	J	x	(A, S)
5	x	(M, U)	K		(A, U)
			L	x	(A, U)
			M	x	(A, U)
			N	x	(A, U)
			O	x	(A, U)
6	x	(M, S)	P		(A, S)
7		(M, V)	Q	x	(A, V)
8		(M, S)	R	x	(A, S)
9		(M, S)	S	x	(A, S)
10	x	(M, St)	T		(A, St)
11		(M, S)	U	x	(A, S)
12		(M, S)	–		
13	x	(M - NA, NS)	V	x	(A, NA, NS)
14		(M, S)	W	x	(A, S)
15		(M, S)	X		(A, S)
16	x	(M, S)	Y		(A, S)
17		(M, St)	Z	x	(A, St)
18		(M, S)	AA		(A, S)
19	x	(M, U)	BB	x	(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	x	(O, U)

Institutional Veterinarian:

3	x	(M, V)

Correlates to Version **v14.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion Items:

1. The committee discussed placing occupational health requirements that are specifically for the health of animals, including influenza vaccination, tuberculosis testing, and measles vaccination/titer, more explicitly under the purview of the IACUC. IACUC leadership will arrange a meeting with Occupational Health leadership to further discuss the best way to manage these requirements.
2. The committee discussed the criteria for review of protocols via FCR vs DMR. Potential additional criteria for FCR review that were discussed were amendments adding a new aim or 25% or greater increase in animal numbers, and/or all new protocols. It was noted that the current criteria appear to be appropriate, and members can and do request specific protocols be moved to FCR. The proposed changes in criteria were tabled.
3. The committee discussed an updated policy and guidelines for mouse and rat surgery. The committee proposed several minor updates to the documents and the revised documents will be discussed at a future meeting.
4. The committee discussed a self-report in which a second skin incision was made in a pig due to incorrect placement of the initial incision, with no resulting animal welfare issues noted. The surgeon will be directly supervised until proficient in the procedure. The committee considers the matter closed.
5. The committee discussed a self-report in which a daily check was missed in an agricultural unit, resulting in chickens running out of water for a period of 6-12 hours. There were no signs of dehydration and no mortality observed. The facility manager and staff have reviewed the schedule to ensure that checks will not be missed in the future, and an additional watering device has been placed in the pen. The committee considers the matter closed.
6. The committee was updated on a previous self-report of unexpected mortality in mice following busulfan administration. The lab has updated their corrective action plan to include administration of fluids and moist food and reduced handling. They have also reviewed injection techniques to ensure that they are correct. The committee considers the matter closed.
7. The committee was updated on a previous self-report of a rat that underwent an unapproved second surgery. The committee had requested surgical and post-operative records and records of the surgeon's training. These were submitted, along with the additional corrective actions of careful review of protocols prior to procedures, timely and thorough record keeping, and a change in analgesic dosing. The committee will continue to be updated on the outcome of upcoming surgeries in the lab.
8. The committee discussed an incident in which veterinary staff observed a surgeon not following required aseptic technique practices for a surgery on a rabbit. Veterinary staff intervened to correct the sterile technique. Due to the concerns about the surgeon's aseptic practices, the committee voted unanimously to suspend the individual's privilege to perform survival surgical procedures in animals. The committee also voted unanimously to request that the principal investigator voluntarily halt survival surgical procedures on this study, until the lab develops processes to ensure proper sterile practices are followed at all times.
9. The committee was updated on the April 2022 inspection summary. There were 8 significant and 20 minor findings, with 2 reports to OLAW.

1. **IACUC-R1S1(# Protocols: 8)**

    1. **IACUC-R1S1 - NEW(# Protocols: 6)**

        1. **Protocol Title:** 2201-39776A Muscle regeneration by stem cells
        Species &Pain Class:(A,C) Mice
        Question the Research Addresses: This project aims to understand how muscle cells develop in teratomas and how muscles regenerate by stem cells and their derivatives.

        The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

        Comment: "We cannot use any agents that may interfere with inflammation. Inflammation is an important process during muscle regeneration and modulation of inflammation is likely to impact muscle regeneration." Doxycycline (and other tetracyclines) are noted to have anti-inflammatory and inhibitory effects on Matrix metalloproteases even in doses as low as yours (5mg/kgBW) (https://www.nature.com/articles/s41598-017-14408-7). In skeletal muscle, MMPs play an important role in the homeostasis and maintenance of myofiber

functional integrity by breaking down ECM and regulating skeletal muscle cell migration, differentiation and regeneration (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2802742/). Doxycycline usage is integral to your study. Please rationalize how you might address this confounder in your study.

Committee Decision: Stipulations must be met
For: 12 Against: 0: Abstain: 0

2. **Protocol Title:** 2203-39883A Methamphetamine induced dysfunction in the dorsolateral striatum
Species &Pain Class:(A,B,C) Mice
Question the Research Addresses: The primary question is: what changes in the striatum, particularly in spiny projection neurons when methamphetamine is contingently administered and how does this differ from a natural reward such as sucrose?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: "The aim of this study is to determine the effects of both methamphetamine self-administration self-administration on spiny projection neuron function and anatomy" "Identified spiny projection neurons of the direct and indirect pathway will interrogated using " Missing words/repeated words. Please update for clarity

   Comment: "local SC injection of 4mg/kg lidocaine " Please include this lidocaine under list of analgesic agents.

   Comment: Please include the possibility of infection for your chronic indwelling catheter mice. Stated in the procedure it mentioned amikacin and gentamicin treatment. How are you monitoring for possible infection?

   Comment: One of the requirements for submission of animal use protocols is that a layperson should be able to understand the procedures each animal is subjected. For the non-technical or technical reader who may not be familiar with addiction terminology, please explain what yoked animals are.

   Comment: The author describes 3 experimental groups in the Pilot Study: control, sucrose self administration and methamphetamines self-administration. Later in the narrative, the author describes that methamphetamine animals will be yoked to either another methamphetamine animal or a saline animal. Does the author mean sucrose instead of saline?

   Comment: Please describe the viral content of the PSAM/PSEMs that will be used to treat animals. In addition please add a Procedure Tab to describe this procedure.

   Comment: Please add a Procedure Tab(s) describing the injection of the L-type Ca2+ channel agonist Bay K8644 and the L-type Ca2+ channel inhibitor isradipine.

   Comment: An oral self administration procedure was described in the Procedures Section. Please clarify how this particular model will be used as there appears to be no reference to this in the Experimental Design.

   Committee Decision: Stipulations must be met
   For: 12 Against: 0: Abstain: 0

3. **Protocol Title:** 2203-39859A Development of Paratransgenic Ticks for Disease Control
Species &Pain Class:(B) Hamster
Question the Research Addresses: In order to implement paratransgenic strategies to control tick-borne diseases, it is necessary to understand the role of symbionts in tick biology, and how symbionts regulate the response of ticks to tick-borne pathogens. We will elucidate the role of tick symbionts in provision of nutrients lacking in blood, priming the tick immune system, and their interactions with tick-borne pathogens to identify new strategies for the prevention of tick-borne diseases.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please revise or expand some descriptions so that the hypothetical lay reader could follow them more easily. A step-by-step outline (e.g. "hamsters are anesthetized so ticks can feed; some animals are

infected using i-p injection with agents that may affect immune activity in the ticks; euthanasia occurs within 2.5 to 3 weeks after infection") could be added to the experimental design.

Comment: Please clarify the need to infect hamsters with the organisms that cause Lyme's disease and Anaplasmosis? A more detailed overview of the experimental flow would be helpful.

Committee Decision: Stipulations must be met
For: 12 Against: 0: Abstain: 0

4. **Protocol Title:** 2203-39910A The role of vascular failure and biomechanical stress in the pathogenesis and healing of osteochondritis dissecans
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: Is it possible to surgically induce OC-like lesions in miniature pigs? Is it possible to identify changes associated with development of early, subclinical OC using MRI? Is it possible to develop MRI sequences to successfully image subclinical OC?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please clarify whether pigs will be housed in groups or individually both before and after the surgery.

   Comment: Number of animals: The number of pigs requested (n=81, both in Experimental Design and Justification for # of animals) is different from that in Species (n=80). Please correct whichever is incorrect.

   Comment: Aim 2: Please confirm that the weight (also in Procedure –Joint Concussive Exercise) for concussive treatment is appropriate for the age group of pigs (at weaning).

   Comment: Aim 3: There is a typo "…anesthetized an imaged …..". I believe it should be: "…anesthetized and imaged …..". Please correct.

   Comment: Typo: "receive anti-inflammatory medication or 72 hours", should be "for 72 hours". Please correct.

   Comment: Due to the potential damage the experimental treatments may cause to pigs, I suggest monitoring all animals daily for at least the first 7 days after the surgery and the concussive exercise, including weekends and holidays.

   Comment: Suggest to remove human/"patient" references: "Anesthetic depth will be assessed utilizing jaw tone and eye position/reflexes. HR and blood pressure will also be factored into anesthetic depth. If the patient has a low HR, low BP, flaccid jaw tone and central eye position with dilated pupils, the patient will be considered too deep. Inhalant percentage will be decreased. Tight jaw tone with palpebral reflex present, elevated HR and blood pressure indicates too light an anesthetic depth, the inhalant percentage will be increased. A 1mg/kg dosage of propofol will be administered to the patient if movement occurs or to immediately deepen the anesthetic plane."

   Comment: For hypotension management would recommend normal saline first before using dopamine

   Committee Decision: Stipulations must be met
   For: 11 Against: 0: Abstain: 0
   Member S out

5. **Protocol Title:** 2201-39782A Evaluation of the CoLabs Ventor System in a Porcine Model
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: The purpose of this study is to evaluate the Ventor System's ability to perform as an advance airway and ventilation system as compared to commercially available airway and ventilation system ("control system") when used in an induced respiratory and cardiac arrest porcine model. Our hypothesis is that the Ventor System can optimize emergency airway management and ventilation without adverse complications.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: After reading the procedures section, I cannot find any information on what the proposed intervention and control treatments entail. What does ventor treatment (12 trachea and 12 esophagus) entail? What will be the control treatment? Please update the protocol to provide sufficient details so one can understand what kind of experiments/interventions the enrolled animals will be exposed to.

Comment: It is stated that he animals will be monitored once a day for 3 days post procedure. How frequent will be the monitoring between day 3 and 35 post operatively?

Comment: This statement requires further clarification. "If an event occurs that can't be easily managed, the animal will be euthanized." Please provide examples of qualifying events that will result in termination of the animal.

Comment: The experimental design section states that animals achieving ROSC will be kept alive for 30 days, whereas the procedures and endpoint sections mentions 35 days of survival. Please reconcile this conflict.

Comment: In the first surgical procedure has the following statement: "A modified Seldinger approach (under ultrasound guidance) will be used to cannulate the right internal jugular vein for blood pressure monitoring and emergency medications. Do you mean central venous pressures will be monitored ? The jugular vein is not an appropriate vessel for blood pressure.

Comment: Dextrose: 50% solution, 30-100 ml, IV Using full strength dextrose is likely to cause irritation to the blood vessels. Clinically, this is usually diluted to 5% or 2.5%.

Comment: Heparin: 0.5-1.0 ml/hr, IV, monitored via ACT Is there a dose for the heparin based on weight of the animal? What is the concentration of the heparin?

Comment: Could you clarify why and how much lidocaine would be administered SC instead of IV? How does that connect to an ABG? I understand lidocaine as an arrhythmic or local anesthetic. "The following drugs will be utilized based on ABG results: Lidocaine 0.25-0.5% SC as needed"

Comment: It states that a urinary catheter may be placed at the conclusion of the surgical preparation. Will a strict aseptic technique be used to place the catheter as to not indroduce pathogens into the bladder? How long will the catheter be left in the animal?

Committee Decision: Stipulations must be met
For: 12 Against: 0: Abstain: 0

6. **Protocol Title:** 2203-39892A Artificial Intelligence CPR
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: Will using machine learning algorithms to adjust compression depth, frequency, and duty cycle during ongoing CPR lead to improved coronary perfusion pressure and carotid blood flow as compared to keeping constant CPR settings?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: In the AI CPR flowchart under the sensor set ups heading, you state that millars will be placed in the "femoral artery to ascending aorta". I believe you meant descending aorta as you wrote it in the experimental design section. Also, you mention Millar placement for intracranial pressure measurement. This is the first time intracranial pressure measurement is mentioned in the protocol. It is not a part of the experimental design. Could you please reconcile this conflict.

Comment: The CSF is collected via a lumbar spinal tap as described in the surgery prep. The flow chart states CSF +/- burr hole. Will you be creating a burr hole in the skull? If so, this needs to be described in the protocol.

Committee Decision: Stipulations must be met
For: 12 Against: 0: Abstain: 0

2. **IACUC-R1S1 - AMENDMENT(# Protocols: 2)**

1. **Protocol Title:** 2103-38940A Next-Generation fMRI with MB-SWIFT: Insights into the Origins of Contrast Technology to realize the full potential of UHF MRI
   Species &Pain Class:(B) Rat
   Question the Research Addresses: Origin of MB-SWIFT fMRI signals: Our long term goal is to establish the newly developed zero echo time MRI pulse sequence MB-SWIFT as the next-generation method of choice for artefact-free, quiet, high-resolution fMRI in animals and humans. The research will establish the physiological mechanisms that contribute to the generation of the functional contrast with MB-SWIFT. Relaxation mapping and functional connectivity in Alzheimer's disease: We will apply novel non-invasive MRI modalities for quantitative brain relaxation mapping in aging and in a model of Alzheimer's disease. The research is designed to characterize the capability of the novel MRI metrics to identify brain abnormalities, and to identify the molecular basis of the proposed biomarkers in the context of aging and Alzheimer's disease.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: The box is checked for "no funding" but NIH grant numbers are provided.

   Comment: What is the maximum number of survival procedures that an animal may undergo? What purpose is served by placing two rats in the MRI simultaneously? Do they interfere with each other's scans at all?

   Comment: The procedure description says that isoflurane will be given at 1.8%, but there is a range of 0-5% in the table. Please update the procedure description to match the table. For the electrical stimulation, it says there will be a minimum of two electrodes. What is the maximum that would be placed in an animal at one time? Eye lubrication should be placed once the animal is anesthetized. In the case of animals that are too light or too deep while under urethane anesthesia, what steps do you take?

   Comment: The procedure description says that isoflurane will be given at 1.8%, but there is a range of 0-5% in the table. Please update the procedure description to match the table. Atropine is not typically indicated as a premedication and should be removed unless there is a justification for its use. What type of suture is used to secure the catheter and close the skin? Lidocaine is listed as being given at 20 mg/kg PO, which could cause toxicity. If the lidocaine is used to aid in intubation, only 1-2 drops should be applied topically to the larynx. Eye lubrication should be placed once the animal is anesthetized.

   Comment: Please confirm that if the animal is coming from the MRI, they will be anesthetized through the whole procedure, and will not wake up in between the MRI and perfusion.

   Comment: Please include the maximum volume that may be given as a daily dose. What is the maximum number of injections that a rat may experience in a day? Is there a reason why multiple injections are needed in one day? For how many days will the rats receive EdU injections?

   Comment: Item 4 is designed for training and practicing, and yet only one person in the personnel is indicated as unfamiliar with the procedures they will be performing. Please clarify

   Comment: For items 3 and 4, the group sizes are simply justified by "method driven." Please expand: why 30 and not 60 or 10?

   Comment: Brenna Knaebe is listed as experienced with all procedures, but then identified as need training with regard to catheter implantation and perfusion. Please clarify.

   Comment: While a range for respiration and end tidal CO2 is specified, no steps are identified in case these parameters are out of range. Please update to indicate what steps would be taken.

   Comment: For the safety of the animal, especially during gas challenges, pulse ox and/or end tidal CO2 monitoring seems important, but no range is given and that monitoring is described as optional.

   Comment: Please clarify whether a ventilator is being used; respiratory rate is described as a parameter to be monitored, but the gas challenge refers to a 20-30 absence of mechanical ventilation.

Committee Decision: Stipulations must be met
For: 12 Against: 0: Abstain: 0

2. **Protocol Title:** 2103-38970A Barth Syndrome Experimental
Species &Pain Class:(A,B) Mice
Question the Research Addresses: There are 2 questions that will be answered: 1) Which of several gene therapy vectors alone or in combination are the most advantageous?, and 2) What other proteins complex with tafazzin?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: If BTHS is a good model of Barth, then poor cardiac function and exercise intolerance should be present, and the findings of aims 1 and 2 in terms of optimal paradigms, might be inapplicable or dangerous to the BTHS animals of Aim 3. Please indicate how exercise intolerance will be addressed and if additional monitoring or safeguards may be required.

Comment: What is the scientific rationale for taking pictures and recording activity? It is not clear what you are trying to capture and if there is another way to record it without having photos and video. Where will photos/videos be stored? How long will they be maintained? Please include this information in the procedure.

Comment: Please update this procedure to include more information about what behavioral tests the mice will be performing while being recorded/photographed.

Committee Decision: Stipulations must be met
For: 12 Against: 0: Abstain: 0

Institutional Animal Care and Use Committee Minutes

May 31, 2022

Meeting Convened: 12:02pm	Quorum Requirement: 10
Meeting Adjourned: 2:29pm	Members Present to Vote: 15

		Voting Members			Alternates
1	x	(Chair - M, S)			
2	x	(Vice Chair – M, S)			
3		(M, V)	A	x	(A, V)
			B	x	(A, V)
			C	x	(A, V)
			D	x	(A, V)
			E		(A, V)
			F		(A, V)
			G	x	(A, V)
			H	x	(A, V)
			I		(A, V)
4	x	(M, S)	J	x	(A, S)
5	x	(M, U)	K		(A, U)
			L	x	(A, U)
			M	x	(A, U)
			N	x	(A, U)
			O	x	(A, U)
6		(M, S)	P	x	(A, S)
7	x	(M, V)	Q		(A, V)
8		(M, S)	R		(A, S)
9		(M, S)	S	x	(A, S)
10	x	(M, St)	T	x	(A, St)
11	x	(M, S)	U	x	(A, S)
12		(M, S)	–		
13		(M - NA, NS)	V	x	(A, NA, NS)
14	x	(M, S)	W		(A, S)
15		(M, S)	X		(A, S)
16		(M, S)	Y	x	(A, S)
17		(M, St)	Z		(A, St)
18		(M, S)	AA	x	(A, S)
19	x	(M, U)	BB	x	(A, U)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	x	(O, U)

Institutional Veterinarian:

3	X (A)	(M, V) Alternate "A"

Correlates to Version **v14.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion Items

1. The committee met with a researcher whose privilege to conduct survival surgeries on animals had been suspended at a previous meeting. The researcher discussed [redacted] perspective on the observed issues with sterility and [redacted] practices in human medicine. The committee will mandate retraining for the researcher on asepsis specific to animal surgery, and [redacted] surgical privileges remain suspended pending this training.
2. The committee discussed an SOP submitted by a research group, describing aseptic practices to be followed for survival surgery in their facility. The committee feels that revisions to the SOP are needed. Members were asked to submit specific suggestions, which will be disseminated to the research group.
3. The committee discussed and voted on an updated Policy and associated Guidelines for Mouse and Rat Survival and Non-survival Surgery and Post-Operative Care. The updated documents were approved and will be distributed to research labs and posted on the IACUC website for reference.
4. The committee was updated on a surgical procedure in NHPs. The first animal did fairly well post-operatively and the second even better, and no further procedures of this type are planned in the near future. The committee considers this matter closed.
5. The committee continued its discussion of a previous self-report involving an unapproved second surgical procedure in a rat. In addition to the previously submitted self-report and associated records, the area veterinarian has discussed with the research group the expectations for prompt communication. The committee considers the matter closed.
6. The committee discussed a self-report in which a mouse was inadvertently killed by crushing between the wire frame and cage lip. The PI has reminded lab staff to check the cage carefully before closing and when placing it on the rack to ensure all animals are in correct part of the cage. The committee considers the matter closed.
7. The committee discussed a self-report in which one room of mice was missed in an RAR daily check. No welfare concerns were noted. RAR will ensure that daily check sheets are on the outside of the door and added a verbal confirmation with staff that rooms have been checked. The committee considers the matter closed.
8. The committee discussed a self-report in which mice on the RAR training protocol underwent a terminal retro-orbital injection class after they had reached the maximum approved number of procedures. No welfare concerns were noted. Staff have been reminded of the procedure limit and cage stickers have been instituted to mark animals that have reached the procedure limit. The committee considers the matter closed.

1. **IACUC-R1S1(# Protocols: 13)**

1. **IACUC-R1S1 - NEW(# Protocols: 9)**

    1. **Protocol Title:** 2203-39890A A Preclinical Study for Evaluation of a Transcatheter Mitral Valve in the Sheep Model. WBV
    Species &Pain Class:(B) Sheep (Biomedical)
    Question the Research Addresses: This is a pilot study to evaluating a new concept in transcatheter mitral valve replacement. Animals will be used to evaluate the biocompatibility, functionality, durability, and safety of this developmental heart valve.

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    Comment: The species page and the fourth paragraph in the experimental design heading both request 25+5=30 sheep for the experiments. However, the last paragraph and the table at the end of the experimental design heading outlines the use of 20+5 sheep only. This latter table is also different from the table that is attached to the protocol (proposed study design updated fro renewal). Please reconcile.

    Committee Decision: Stipulations must be met
    For: 13 Against: 0: Abstain: 0
    Members 1, 11 out

    2. **Protocol Title:** 2204-39944A Chromatin Modifiers in MSK Tissues
    Species &Pain Class:(A,B) Mice
    Question the Research Addresses: The overall goal of the project to is understand how chromatin modifiers,

including Hdac3 and Hdac8, function within myeloid cells to promote intramembranous bone formation and skeletal healing.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: The breeding procedures do not include a procedure to generate Hdac3: Hdac8 dcKO mice. The procedure described ("Hdac3 fl/fl: Hdac8 fl/fl : LysM Cre-mice will be crossed with Hdac3 fl/+: Hdac8 fl/+ : LysM Cre+ animals to obtain Hdac3 WT (e.g., Hdac3 fl/fl: Cre-, Hdac3 fl/+:Cre-, Hdac3+/+:Cre-, Hdac3+/+:Cre+), Hdac8 WT (Hdac3fl/+:Cre+), Hdac3 cKO (Hdac3 fl/fl:Cre+) mice") only produces Hdac3 cKO mice, but no Hdac8 cKO mice or Hdac3: Hdac8 dcKO mice. Please update or clarify if needed. Note that if this results in a change in numbers, the Species table should be updated accordingly.

Comment: Please update the procedure to specify the vehicle used for Rgfp966 injection.

Comment: My only concern is a lack of details / information on the use of chemicals. It seems that most of them are hazardous. Even though the Lab-specific SOPs for general hazard are provided, they are not specific and provide little information on each chemical (e.g., a full name, if it is hazardous, etc) and the safety procedures. Please update to provide additional detail.

Committee Decision: Stipulations must be met
For: 15 Against: 0: Abstain: 0

3. **Protocol Title:** 2109-39450A Endocrine Effects in Breast Cancer
Species &Pain Class:(B) Mice
Question the Research Addresses: In patients, different types of breast cancer cells co-exist in a single tumor. For example, few cells may respond to an endocrine therapy while some other cell type may be completely resistant to it. In other words, a single breast tumor is made up of different kinds of cells with diverse sensitivity for endocrine-based therapy. Currently, we do not know how one particular cell type may influence the other cell type during the course of endocrine-based treatment and contribute towards the development of overall resistance.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: As per RAR guidelines, please allow a one-week acclimation period prior to research use instead of three days.

Comment: Anesthesia: Mouse eyes remain open under anesthesia, which can lead to corneal drying and trauma. Please update the procedures to indicate that ophthalmic ointment (e.g., Paralube® or Lacrilube®) will be applied to the eyes for all imaging and surgical procedures.

Comment: In the "Experimental Procedures" attachment, it is stated that tumors will be measured twice weekly; however, in the "Summary" section, it is stated that tumors will be measured once each week. Please confirm that tumors will be measured once or twice (preferable) per week. Will imaging take the place of tumor measurement or will the tumors be measured once or twice per week in addition to imaging? The diagrams in attached Experimental Procedures should also be changed accordingly. In the "Euthanasia" section, it is stated that that mice will be checked weekly or twice/week. Does this mean that the mice will be weighed? Other sections state that the mice will be observed daily. Please clarify the number of times each week that the mice will be weighed and confirm that the mice will be checked daily for overall health.

Comment: For Experimental Design (Attachment) it states, "Depending on the cell line, five (5) to twenty (20) weeks will be required from the time of implantation until mature (1.5 cm3) tumors have formed in control treated mice." However, for the SOP "Grafting mammary tumor cells to mouse milk ducts," it states “Animals will be maintained for maximum 12 weeks after surgery.” Please update the protocol to clarify the overall time frame needed for tumor growth is up to 20 weeks for consistency.

Comment: In this section the administration of substances via oral gavage as well as via IP route are described. These two routes are not mentioned in the attached experimental design. Please correlate which drugs will be administered via which route either in this procedure or in your attached experimental design.

Committee Decision: Stipulations must be met
For: 15 Against: 0: Abstain: 0

4. **Protocol Title:** 2204-39970A Title 1: Cryopreservation of organs for transplant Title 2: Breakthrough Tissue and Organ Preservation and Transplantation Using Scaled-Up Nanowarming Technology Title 3: Organ banking for transplant—kidney cryopreservation by vitrification and novel nanowarming technology Title 4: Hepatocyte Production from Ice Free Cryopreserved and Nanowarmed Livers Title 5: Subzero preservation of vascular composite allografts
Species &Pain Class:(A,B) Mice; (A,B) Rat
Question the Research Addresses: Cryopreservation by vitrification, which allows cooling and storage of tissues at temperatures close to liquid nitrogen (-160 °C) for indefinite time periods, is one of the most attractive approaches to achieving long- term banking of biomaterials. Cryoprotective agents (CPAs) stabilize biomaterials in the vitreous (i.e. “glassy” or “amorphous”) state, preventing significant damage to biomaterials by blocking ice crystal formation and cellular dehydration. The warming rates needed to avoid crystallization during rewarming are typically an order of magnitude higher (hundreds of °C/min) than the required cooling rates (tens of °C/min), even with the aid of CPAs. We are investigating a novel approach using radiofrequency heating and iron nanoparticles that can achieve rapid and homogeneous warming that can thaw organs from a vitrified state.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: The animal number requested for breeding requires clarification. 100 breeding pairs producing 196 offspring means less than 2 pups per pair. Please clarify and correct if needed.

   Comment: It is not clear from rat table how the number of animals needed comes up to 1600. Please clarify and correct if needed.

   Comment: In looking at total mouse numbers for experiments, the 100 breeders noted in the breeding section is missing from the species section (only the number of animals produced in house is noted). Please add the breeders as part of the overall numbers in this section.

   Comment: For the non-vascularized heart transplant procedure, how often would the EKG be performed for graft monitoring if this is the only way to confirm monitoring of the graft in case of no visible contractility.

   Comment: Please outline what anesthetic will be used for the ultrasound procedure to ensure patency of the aorta in the aortic interpostion graft surgical procedure section. How often would this be done to monitor the graft?

   Comment: Please update the surgery procedure title for the neonatal mouse hearts for transplant. These are not recovery procedures (in the sense of the mouse recovering from the procedure).

   Comment: For the liver transplant monitoring, it states that transabdominal doppler ultrasounds will be used to assess vascular patency. What anesthesia is used for this procedure and how often is this done during the course of the study.

   Committee Decision: Stipulations must be met
   For: 15 Against: 0: Abstain: 0

5. **Protocol Title:** 2204-39972A Title 1: Animal model for assessing transplant tolerance Title 2: Foundational methods for high throughput pancreatic islet cryopreservation Title 3: Engineering optimization and scaling enables high quality pancreatic islet cryopreservation for banking and transplant
Species &Pain Class:(A,B) Mice
Question the Research Addresses: Our central hypothesis is that the improved heat transfer achieved by cryomesh VR, combined with optimizations in CPA use, will enable ice-free vitrification and rewarming of islets while avoiding toxicity. We seek to determine the viability, function, and scalability of this method of cryopreservation of islets for transplantation.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Please update the Experimental Design to provide a brief overview of your study and be sure to include a clear description of the design, treatments, procedures and animal numbers. A complete, sequential, and accurate description of what procedures will be performed on/with the animals must be provided. You can also refer to your comprehensive attachment describing sequential order of the procedures.

Comment: In the breeding procedure, 1020 mice are requested. However, in Species, 500 mice Produced In-House are requested. Please update to reconcile, ensuring that all mice bred are accounted for in the Species table, even if not used for experiments.

Comment: Please update the procedure to describe post-procedure monitoring and how animal body temperature is maintained for this procedure.

Committee Decision: Stipulations must be met
For: 14 Against: 0: Abstain: 0

6. **Protocol Title:** 2204-39990A Liquid:air interfaces and transmission of infectious bronchitis virus to chicks
Species &Pain Class:(A) Chicken
Question the Research Addresses: We will simulate typical liquid-air interface activities (e.g., washing and raining) in an airtight chamber. Aerosols and bioaerosols that are generated will be collected, and naive animals will be placed in the chamber. The chicks will be exposed to generated bioaerosols and then tested to evaluate the efficacy of airborne transmission.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: The procedure for blood collection indicates being done at 2 wks of age; while the experimental design section indicates at 28 days of age although post-mortem but nothing earlier. Please confirm the blood collection is meant for 2 wks of age and update the protocol accordingly.

Comment: Please update the protocol to include in responses to these questions, holding of the chicks in the chamber for the IBV exposure. My concerns would be either a chilling effect on the chicks depending on how much moisture is transferred, or high humidity that might impact respiration and heat loss depending on chamber temperature. Would these things be monitored in the chamber as well as the chicks?

Comment: What is the average weight of your 2 or 4 week old chicks? 3 ml of blood collection is potentially greater than 1% body weight of what I would expect a 2-4 week old chick to weigh.

Comment: For the wing banding procedure as you are going through skin, please update the procedure to include cleaning the site prior to banding using alcohol or betadine prior to incision or placement of the band through the skin.

Comment: Are you performing all aspects included within the Checking the health of the birds SOP twice daily? For example, are you performing a musculoskeletal check and handling the birds each time you check on them? If not, please update the protocol to make a note that this check is only done if any concerns are noted during general observation for musculoskeletal issues, then a musculoskeletal exam will be performed.

Comment: Within the procedure "Checking the health of birds held in filtered-air isolators" twice daily observations of animals is described, however, within the health and monitoring section only once daily checks (unless a second check is deemed necessary) is described. For consistency within the protocol please update one or both sections with the same information.

Comment: As IBV can lead to respiratory distress, please also include within the clinical signs that will lead to euthanasia that if marked respiratory distress is noted chicks will be euthanized.

Comment: While RAR staff observe birds once daily and will notify if any concerns, the monitoring by RAR staff is for general health and should not be included within your IACUC protocol as monitoring for experimental concerns is the responsibility of lab staff. Please remove reference of RAR health checks within your protocol.

Comment: In the experimental design it states that blood collection will only be done post-mortem, however there is a procedure for what appears to be ante-mortem collection. Please clarify if the procedure was meant to be

post-mortem or if the experimental design needs to be changed.

Comment: While exposing the animals, will the temperature be monitored as well as the humidity? Depending on how wet the animals get, will they be dried off or placed near heat to prevent hypothermia (as well as worries about too high of humidity and respiratory distress during the showering/aerosol procedure). How will the birds be monitored during this time (60 min aerosol and then immediately after)? Please add this to the Health and Monitoring section as currently it only describes possible disease post exposure.

Comment: After birds are exposed in the chambers, how specifically will they be transferred back to the isolators? Since the animals will be exposed, isn't it possible that the room may become contaminated and thus possibly infect other animals and controls. What controls will be used to prevent cross contamination?

Committee Decision: Stipulations must be met
For: 14 Against: 0: Abstain: 0

7. **Protocol Title:** 2205-40034A Shear-Wave Elastography Evaluation of Canine Hepatic &Splenic Round Cell Neoplasia
Species &Pain Class:(B) Dog
Question the Research Addresses: Can elastography be used in canine patients to differentiate the presence or absence of round cell neoplasia in the liver and spleen?

   Committee Decision: Approved as submitted
   For: 14 Against: 0: Abstain: 0

8. **Protocol Title:** 2204-39931A Ultrasound elastography for the diagnosis of feline pancreatitis
Species &Pain Class:(B) Cat
Question the Research Addresses: The primary objective of the study is to use elastography to distinguish between normal pancreas and pancreatitis in cats.

   Committee Decision: Approved as submitted
   For: 14 Against: 0: Abstain: 0

9. **Protocol Title:** 2202-39817A COMBINED NMRC NHP PROTOCOL
Species &Pain Class:(B,C) Nonhuman Primate (Macaques)
Question the Research Addresses: This study will compare the relative efficacy and optimal location for DBS within the basal ganglia to maximally alleviate parkinsonian motor and non-motor signs in a non-human primate model. This study will seek to better understand the physiologic mechanisms which underlie parkinsonian motor signs and the effect of DBS within the basal ganglia and cortex using single and multi-cell recording techniques to characterize the changes in neuronal activity.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please change specification for SpO2 to > 95% and Heart Rate to 80-160 to make it consistent with other procedures on the protocol.

   Comment: Please change specification for SpO2 to > 95% at the bottom of the procedure page to make it consistent with other procedures on the protocol.

   Comment: This procedure has an instruction to give Mannitol (300-500 mg/kg, 20%, IV, over a 20 min period) and/or mechanically controlled hyperventilation may be given ~10-15 min before the craniotomy to prevent brain swelling. The surgical procedure for Microarray placement has a different instruction which was corrected in the previous cycle of review, please reconcile instructions in this surgical procedure. Mannitol (1.5 g/kg, 20%, IV, over 20 min) and/or mechanically controlled hyperventilation may be given ~10-15 min before the craniotomy to prevent brain swelling. Note that the attachment "Drug Table 2022" specifies the dose of mannitol 20% at 0.5-1.0 gm/kg, a range which does not include the 1.5 g/kg dose specified in the procedure; please reconcile.

   Comment: This procedure has an instruction to give Mannitol (300-500 mg/kg, 20%, IV, over a 20 min period)

and/or mechanically controlled hyperventilation may be given ~10-15 min before the craniotomy to prevent brain swelling. The surgical procedure for Microarray placement has a different instruction which was corrected in the previous cycle of review, please reconcile instructions in this surgical procedure. Mannitol (1.5 g/kg, 20%, IV, over 20 min) and/or mechanically controlled hyperventilation may be given ~10-15 min before the craniotomy to prevent brain swelling. Note that the attachment "Drug Table 2022" specifies the dose of mannitol 20% at 0.5-1.0 gm/kg, a range which does not include the 1.5 g/kg dose specified in the procedure; please reconcile.

Comment: In the Anesthetic Agent Table in the procedure, the Acepromazine dose specified is 0.05-0.1 mg/kg IM (I think this needs to be corrected to match the dose ranges in the 2 places below) Further along in the Procedure under Parameters monitored during procedure, the dose is given as 0.01-0.1mg/kg, SQ/IM The Attachment Drug Table 2022 lists the Acepromazine dose as 0.01-0.1mg/kg, SQ/IM In the Anesthetic Agent Table in the procedure, the Atipamezole Hydrochloride dose specified is 0.015-0.02 mg/kg, while in the attachment Drug table 2022 it is listed as 0.15-0.225 mg/kg; please reconcile. Both this procedure and it's attachment, Dura and Headcap Maintenance, refer to the 2021 APV drug formulary for recommended drug doses. If that has been replaced by your new Drug Table 2022, please update the reference in both the procedure and the attachment. Please also change specification for SpO2 to > 95% and Heart Rate to 80-160 to make it consistent with other procedures on the protocol in the section for Parameters monitored during procedure.

Comment: The procedure details say that the animal may be sedated with Acepromazine during the procedure (0.05-0.5 mg/kg). However, acepromazine is not listed in the Anesthetic Agents and Sedatives Table for the procedure, and the dose in the procedure details description does not match the dose range for acepromazine given in the Drug Table 2022, which is 0.01-0.1 mg/kg, IM or SQ. Please reconcile dose information and update the Anesthetic Agents Table in the Procedure. Please also change specification for SpO2 to > 95% and Heart Rate to 80-160 to make it consistent with other procedures on the protocol in the section for Parameters monitored during procedure.

Comment: In the Attachment titled Cooperative Behavioral Training II, final sentence says "Acepromazine (0.05-0.1 mg/kg, IM) or diazepam (1mg/kg, oral) may be used on a case-by-case basis to facilitate introductory acclimation to headposting." This dose is not in the same range as the one listed in the Drug Table 2022, which is 0.01-0.1 mg/kg IM or SQ. Please reconcile.

Comment: In the Attachment titled Burr Hole Procedure Description in the first paragraph under Subject Preparation, it says "If during the procedure the animal still displays signs of distress despite the standard course of sedatives outlined above, Diazepam (0.5 – 2.0 rectally, or 0.1mg/kg orally) may also be administered to mitigate potential anxiety associated with drilling" The Drug Table 2022 give diazepam dose information as 0.5-1mg/kg IV, 1 mg/kg oral. Corrections: rectal dosing should be added to the Drug Table and confirm the dose. Oral dose in the drug able says 1 mg/kg by the oral route, 10-fold higher than the dose given in the procedure. Please either explain/justify the difference or update the table. In the breached dura section of the Burr Hole Procedure as in the Chamber Cleaning SOP corrected during the previous round of review, you state that the chambers should only be rinsed with Nolvasan and saline, but the APV guidelines caution against chlorhexidine (brand name Nolvasan) as it has been shown to be neurotoxic. Please update this attachment with the recommended course of action to control bleeding, rinse with saline, and call the vet for next steps, such as systemic antibiotics.

Committee Decision: Stipulations must be met
For: 14 Against: 0: Abstain: 0

2. **IACUC-R1S1 - AMENDMENT(# Protocols: 4)**

    1. **Protocol Title:** 2101-38762A The role of anti-serpin autoantibodies in immune tolerance and diabetes
    Species &Pain Class:(A,B) Mice
    Question the Research Addresses: The first main question is: What is/are the role of serpin molecules in rejuvenation of pancreatic islets? The second main question is: What is the efficacy of therapy with monoclonal antibodies to serpins in diabetes?

    Committee Decision: Approved as submitted
    For: 14 Against: 0: Abstain: 0
    Member 14 out

    2. **Protocol Title:** 2202-39802A Linking neuronal, metabolic, and hemodynamic responses across scales
    Species &Pain Class:(B) Nonhuman Primate (Macaques)

Question the Research Addresses: While functional magnetic resonance (fMRI) has proved invaluable for identifying where in the brain activation is occurring during a particular task, it has had less to say about how the dynamics of that activation actually contribute to task performance. Indeed, because of the belief that fMRI signals are sluggish and temporally imprecise, fMRI experimental paradigms traditionally have used sustained block designs which deliberately preclude measuring the rapid changes in sensory and motor signals that underlie everyday actions. Recent evidence, however, suggests that there is considerable temporal information present in the blood oxygen level dependent (BOLD) signal, opening the possibility that fast neuronal dynamics can be revealed by fMRI. In this proposal, we will examine this possibility with a series of multimodal experiments in which a consistent experimental paradigm is applied across spatial and temporal scales to quantify responses to transient inputs.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: 5FU is considered a chemotherapeutic agent and all needles, syringes, liquid should be disposed as such. Pleaes confirm that this will be done.

Comment: Please describe what it means that disinfectants will be rotated on a 7 to 10 day basis.

Comment: Is the diluted antibiotic wash made fresh each day?

Comment: Implant Care SOP: 1) The top of page 2 mentions mitomycin and bevacizumab, but should also include 5-FU. 2) Triple antibiotic ointment should not be used inside the chamber and it should be removed from step 7 of the chamber cleaning process. 3) If bevacizumab will be used, then it should be included on the SOP and DEHS.

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

3. **Protocol Title:** 2112-39692A Modulating attention and decision making with closed loop control of low frequency oscillations
Species &Pain Class:(B) Nonhuman Primate (Macaques)
Question the Research Addresses: Synchronous low-frequency brain activity, as measured by human EEG, has been implicated in both normal cognition and in disease states such as schizophrenia. However, we still do not know whether changes in such rhythms directly alter neuronal information processing, or are merely epiphenomenal. To address this issue, we will measure how single and multi unit activity linked to task performance in non-human primates is altered by endogenous alpha rhythms, and how that activity is changed when alpha rhythms are directly modulated via closed-loop electrical stimulation.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please update the protocol to specify how often animals would undergo a repair/explantation during their time on the protocol.

   Comment: implant repair/explantation: Other support agents: I am confused why would you want to use dexamethasone up to 48hours pre surgery? Is it for controlling intracranial swelling? Did you mean to administer it up to 48 hours post operatively? Also, to ensure correct use of terminology please use the term intracranial or cerebral edema vs cranial edema.

   Comment: The "agents used in combination" gives a dose range for midazolam of 0.05-2 mg/kg, but the dose range in the drug table is 0.05-0.15 mg/kg, which is more appropriate. Please update the dose listed in the "agents used in combination" section. Since animals are not all placed on the ventilator, change the wording to say "animals may be placed on a ventilator" instead of "animals are placed on a ventilator." The "parameters monitored" question does not say that blood pressure will be monitored - please add this.

   Comment: 5FU is considered a chemotherapeutic agent and all needles, syringes, liquids, chux pads, gloves, etc... should be disposed as such. Please confirm that this will be done.

   Comment: Please describe what it means that disinfectants will be rotated on a 7 to 10 day basis

Comment: Is the diluted antibiotic wash made fresh each day?

Comment: Implant Care SOP: 1) The top of page 2 mentions mitomycin and bevacizumab, but should also include 5-FU. 2) Triple antibiotic ointment should not be used inside the chamber and it should be removed from step 7 of the chamber cleaning process. 3) If bevacizumab will be used, then it should be included on the SOP and DEHS.

Committee Decision: Stipulations must be met
For: 13 Against: 0: Abstain: 0

4. **Protocol Title:** 2004-38075A Effects of Stimulation in Fear Regulation Circuit on Conflict Resolution and Motivational Behavior
Species &Pain Class:(B,C) Rat
Question the Research Addresses: This study will aim to understand the underlying rmTBI induced changes in approach-avoidance circuitry. In the emotional domain, we aim to detect the intent/need for emotion regulation by electrical recording, then translate those intentions into stimulation in the limbic circuit. This project takes the first steps towards that by testing circuit-specific modulation of emotion regulation.

   Committee Decision: Approved as submitted
   For: 13 Against: 0: Abstain: 0

Institutional Animal Care and Use
5/4/21 Committee Minutes
VCRC - 76D

Meeting Convened: 12:01 PM	Quorum Requirement: 9
Meeting Adjourned: 1:38 PM	Members Present to Vote: 14

Voting Members			Alternates		
1	X	(Chair - M, S)			
2	X	(M, V)	A	X	(A, S)
			B		(A, S)
			C	X	(A, S)
			D		(A, S)
			E		(A, S)
			F		(A, S)
			G	X	(A, S)
			H		(A, S)
3	X	(M, S)	I		(A, S)
4	X	(A, U)	J	X	(A, U)
			K	X	(A, U)
			L	X	(A, U)
			M	X	(A, U)
5	X	(M, S)	N		(A, S)
6	X	(M, V)	O	X	(A, S)
7		(M, S)	P		(A, S)
8	X	(M, S)	Q		(A, S)
9		(A, St)	R	X	(A, St)
10		(M, S)	S		(A, S)
11		(M, S)	T	X	(A, S)
12	X	(M - NA, NS)	U		(A - NA, NS)
13	X	(M, S)	V		(A, S)
14	X	(M, S)	W		(A, S)
15		(M, S)	X		(A, S)
16	X	(M – St)			
17		(M, V)	Y	X	(A, NA, NS)

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v3.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee was updated on a recent unannounced visit to the North Central Research and Outreach Center in Grand Rapids. Cows appeared healthy and the facilities were in good condition. The staff member that the site visitors met with was knowledgeable and engaged. It was recommended that the previously required monthly meetings between NCROC and an RAR vet become optional. The committee endorsed this plan.

2. The committee reviewed the updated Policy and Guidelines on Anesthesia Monitoring in Research Animals and approved the updated documents.

3. The committee reviewed the updated Policy and Guidelines on the Transportation of Research Animals and approved the updated documents.

4. The committee reviewed a self-report in which a rat pup was inadvertently left in a temporary cage overnight. The animal was found by RAR and no adverse health effects were noted. Moving forward, the lab has implemented additional checks to ensure that all animals are accounted for. The committee considers the matter closed.

5. The committee reviewed a self-report in which blood pressure was not monitored during a non-human primate surgery due to an equipment failure. The animal, which had other underlying conditions, did not fully recover from surgery, and was euthanized. A necropsy is in process. Moving forward, the lab has ordered additional backup blood pressure monitoring equipment and will contact RAR to borrow their equipment if needed. The committee considers the matter closed.

6. The committee was updated on recent self-reports from RAR regarding animals found without access to water or food. Daily animal checks are done as carefully as possible while minimizing disruption and staff have discretion to pull out any cages for more detailed checks. No other similar incidents have been reported since the adoption of the current process for daily checks, and RAR staff regularly identify animals with low food or water and intervene. The committee thanks RAR for their detailed response and considers the matter closed.

1. **IACUC-NEW (# Protocols: 14)**

    1. **Protocol Title:** 2011-38630A Mechanisms of Transplant Rejection
    Species &Pain Class: (A,B) Mice
    Question the Research Addresses: PI requests 342 class A and 3720 class B mice for studies using experimental mouse models to discover safer and more effective means of suppressing the immune response for the prevention of transplant rejection, with the hope of eventually being able to induce durable transplantation tolerance, acceptance of a transplanted organ without the need for immunosupressive drugs.

    The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

    - Thank you for your responses and making the changes throughout the protocol. Regarding the anesthetic regimen, please incorporate some of your response regarding the use of midazolam-fentanyl-droperidol within the description of its use on the protocol within the Skin Graft procedure. Below are components that recommend including. The combination of fentanyl and midazolam provides an excellent plane of anesthesia and the mice typically sleep for an additional hour or two after the procedure which helps in the early seating/attachment of the skin graft. The addition of droperidol as an anti-nausea treatment seems to extend the post-op recovery period when the mice continue to sleep. The online publication of the Institute of laboratory animal research discusses neuroleptoanalgesia includes the combination of fentanyl, droperidol and midazolam. Neuroleptoanalgesia is carried out by combining a narcotic analgesic with a tranquilizer to produce a state of deep sedation with profound analgesia sufficient to perform short painful procedures, exactly what we need for the skin grafting procedure (http://nas-sites.org/ilarjournal/previous-issues/neurobiology-of-addictive-behaviors/mice-anesthesia-analgesia-and-care-part-1-anesthetic-

considerations-in-preclinical-research/). Additionally, here is another article discussing this method: https://pubmed.ncbi.nlm.nih.gov/24668552/

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0
Member 14 out

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2. **Protocol Title:** 2102-38818A Determining the role of oxytocin signaling in social attachment behavior related cardiometabolic function
Species &Pain Class: (A,B) Prairie Vole)
Question the Research Addresses: In prairie voles that have been gametically mutated for the oxytocin receptor gene, we will try to address the question of the role of compromised bonding due to oxytocin receptor disruption on cardiovascular and metabolic function, as well as transcriptional responses in target peripheral tissues.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - The husbandry and sentinel SOP is from another institution. At a minimum the SOP should be edited to reflect University of Minnesota and updated based on consultation with RAR. Under health observations, there should be indication that the observations occur at a minimum once daily, including weekends. Also, please specify that new, clean water bottles will be changed when the feeder is changed every two weeks at a minimum.

   - It is recommended to give meloxicam pre-operatively to prevent post-surgical pain. Giving the meloxicam during recovery may not cover the pain experienced initially. Meloxicam will not prolong recovery like opioids.

   - The blood collection procedure includes both tail tip and saphenous vein as collection sites. Why, are these separate procedures, or alternatives? Please clarify.

   - The total here is 104 animals, but the experimental design seems to indicate 102. Please reconcile.

   Committee Decision: Stipulations must be met
   For: 13 Against: 0 Abstain: 0
   Member 14 out

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3. **Protocol Title:** 2103-38887A Xenogeneic studies of Graft Vs. Host Disease "Transplant Tolerance in Nonhuman Primates"
Species &Pain Class: (A,C) Mice
Question the Research Addresses: PI requests 6150 class A and 6298 class C mice for a study to define and test novel therapeutics to 1) ameliorate graft versus host disease and 2) eradicate established tumors.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - Since both leukemia and solid tumors will be introduced, please add a procedure of type "Tumor Induction". This will generate the required question on Tumor Endpoint Criteria.

   - The attachment "IACUC mouse justification 031821" includes Inhibiting XGVHD with FMT as section 1d and NHP XGVHD model as section 1e. However the Experimental Design within the protocol itself does not include the FMT experiment and lists NHP XGVHD as section 1d. Please clarify wither the FMT model will be used in this protocol, and add it to the Experimental Design

and Procedures if so. If this model will not be used, please remove it from the attachments as well as the IBC and DEHS tabs.

- The attached SOP is for biologicals not toxins and does not contain the correct information for engineering controls, waste disposal etc. Please use the template here: https://dehs.umn.edu/node/129581/attachment.

Committee Decision: Stipulations must be met
For: 12 Against: 0 Abstain: 0
Members 13 and 14 out

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4. **Protocol Title:** 2102-38868A Dysfunctional State Representation in Psychosis: From Neurophysiology to Neuroplasticity-based Treatment
Species &Pain Class: (B) Nonhuman Primate (Macaues)
Question the Research Addresses: PI requests 4 class B nonhuman primates for a study to address two related questions: (1) how neurons in the primate prefrontal cortex and connected brain structures synaptically interact to perform computations that underlie complex cognitive behavior, and (2) how malfunction of prefrontal neurons, synapses, and networks leads to cognitive deficits in human neuropsychiatric disease, with a focus on schizophrenia.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- 1. It is stated that adapting to the chair restraint procedure can take up to two weeks, however, it is unclear whether the two week timeframe is a clear endpoint to this procedure if animals do not comply. Please clarify whether two weeks will be the maximum allotted timeframe that these animals will be expected to adapt to this procedure. 2. It is stated that the behavioral testing can take up to two years, however, it is unclear whether the two year timeframe is a clear endpoint if animals do not comply or exhibit the preferred behavior. Please clarify whether two years will be the maximum allotted timeframe that these animals will be expected to exhibit the preferred behavior.

- Since these animals will be undergoing multiple behavior tests, it is crucial they receive rest periods between behavior tests to reduce stress and allow recovery. Please specify the rest times the animals will be allotted between each behavior test.

- Please describe the route of transport [redacted] if not using the RAR transportation services. Please ensure that NHPs will be out of public view at all times.

- Personnel must complete the medical and online requirements prior to working with animals on the protocol. Personnel include [redacted] Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 13 Against: 0 Abstain: 0
Member 14 out

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5. **Protocol Title:** 2101-38763A Adapting high expansion foam for use in American systems for mass depopulation and on-farm culling
Species &Pain Class: (C) Pig (Agricultural); (C) Turkey; (C) Cow (Agricultural)
Question the Research Addresses: PI requests 400 class C agricultural pigs, 60 class C turkeys, and 8 class C agricultural cows for a collaborative study between researchers at the University of Minnesota and international consultants. The objectives of the study are to use high expansion nitrogen gas-filled foam method for mass depopulation of swine, adapt the method for on-farm euthanasia in American systems, and build acceptance and promote adaption of the technology by performing outreach and education to American

stakeholders.

The committee concurs that this protocol can be approved via a subsequent full committee review once the following stipulations are addressed by the PI:

- Videos collected as part of this study are potentially subject to FOIA request and are likely to be sensitive. Please provide justification for why videos must be collected for this study, or consider an alternative method for collecting data. Is direct visual observation possible with any of the chambers? Are there any alternative measurements that could be used to ascertain time to unconsciousness and time to death? Is video data necessary as part of the proposed submission of the method to AVMA?

- The pain class for behavioral assessment of pigs, turkey and cow due to foam production and subsequent N2 release should be as same as euthanasia of these animals, namely class C.

- Personnel must complete the medical and online requirements prior to working with animals on the protocol. Personnel include: Casey Roeker: • ROHP Rabies online training • ROHP Ringworm online training Once complete, please confirm here in eProtocol.

Committee Decision: Deferred
For: 13 Against: 0 Abstain: 0
Member 3 out

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6. **Protocol Title:** 2103-38924A Development and Translation of an Intracranial Nerve
Species &Pain Class: (B) Cat
Question the Research Addresses: PI requests 6 class B cats for a study that will build and evaluate the safety and design needs of a new type of intracranial auditory prosthesis that targets the auditory nerve (auditory nerve implant, ANI).

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- The investigator proposes to test the hearing status of the animal prior to initiation of surgery by evaluating the auditory brainstem response (ABR) through external attachment of electrodes. If an issue arises during preliminary evaluation, the procedure will be discontinued and the animal allowed to recover. A substitute animal is subsequently pursued. Since only 6 cats were requested and each cat has a specific protocol that will be required to reach the eperimental milestones, will the recovered animal be used at a future date for one of the prescribed protocols since no surgery has been initiated?

- Since no animal procedures aside from normal husbandry will be performed until the start of anesthesia and since this is a terminal procedure, it appears that Point 3 in Experimental Endpoints may be irrelevant.

- Personnel must complete the medical and online requirements prior to working with animals on the protocol. Personnel include: Steven Zuniga: • Tetanus surveillance • Animal Exposure Questionnaire • Rabies surveillance David Warren: • Tetanus surveillance • Animal Exposure Questionnaire • Rabies surveillance • ROHP Introductory Training • ROHP trainings for cats (Cat scratch, parasitic disease, rabies, ringworm, toxoplasmosis) • Animal Use Tutorial Once complete, please confirm here in eProtocol. Contact iecohen@umn.edu if there are any questions about these requirements.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

7. **Protocol Title:** 2008-38352A Preclinical development of antimicrobial peptide DGL13K
Species &Pain Class: (B) Pig (Biomedical)
Question the Research Addresses: PI requests 4 class B biomedical pigs for a study to develop a wound healing model in biomedical pigs to demonstrate efficacy of the DGL13K peptide. In preliminary experiments, we have shown that DGL13K is active in a mouse burn-wound infection model. However, the mouse model has limitations for preclinical evaluation of a topical antibiotic due differences in wound healing in the skin between mice and humans.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - Please provide the age, length, and weight of the pigs for the bacterial inoculum study. This information will be useful in determining the number of wounds on each side of the midline.

   - Please clarify why all 4 treatments are done on the same pig, rather than testing one treatment on each of the 4 pigs requested.

   - Personnel must complete the medical and online requirements prior to protocol approval. Personnel include: Beverly Norris: • Citi training: Working with Swine Craig Flory: • Citi training: Working with Swine Once complete, please confirm here in eProtocol.

   Committee Decision: Stipulations must be met
   For: 13 Against: 0 Abstain: 0
   Member 13 out

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8. **Protocol Title:** 2101-38787A 1. Role of two-component regulators in pathogenesis 2. Essential Global Regulator YeaZ: a novel antibacterial target 3. Functional CD4+ Memory T cell responses to Staphylococcus aureus infections 4. Novel Anti-Acinetobacter Multilin Derivatives and their Mechanism of Actions 5. Novel Histidine Kinase Inhibitors: Alternative Antibacterial Virulence therapeutics 6. Role of GM-CSF in impaired macrophage clearance of Mycobacterium abscessus 7. Antibiotic alternatives in food animals that attenuate the virulence of bacterial pathogens 8. Development of Alternative Growth Supplement using Bacterial Virulence Inhibitor
Species &Pain Class: (A,B,C) Mice; (A,B) Chicken; (B) Turkey
Question the Research Addresses: PI requests 1266 class A, 250 class B, and 250 class C mice; 72 class A and 122 class B chickens; and 122 class B turkeys for a study of the role of Staphylococcus aureus virulence factors and essential proteins for bacterial survival in growth and/or during infection.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - It looks like you missed some of the question from the first round of stipulation. For both bird procedures, please include the route (SQ, PO, IM, etc) and maximum volume (mls) for the antibiotic dosing. Also include what your vehicle control is; right now it says "vehicle" but it isn't clear what that is.

   - It appears as though other drugs may be given IV, such as "doxycycline and the lead compound" (per the IM procedure). Any drug that could be administered IV should be listed here. Please note that you should wipe the tails with alcohol prior to IV injection to avoid accidentally tracking bacteria into the blood stream.

   - Please provide the route for the cyclophosphamide.

   - Unfortunately it does not look like any of your edits were saved, at least I cannot see any changes to this procedure. Please address the previous stipulation (copy and pasted below). "I am confused by the comment that it may take some mice up to 30 min to recover from a brief procedure with

isoflurane. Mice should be awake within a few seconds/minutes, especially since the procedure is written in a way that makes me believe they are anesthetized with isoflurane, but are actually off iso/waking when they are inoculated. Please include volumes to be used with the MicroSprayer. Additionally, please include a reference for administering 100ul into the lungs of mice. In your "Procedure of intratracheal inoculation of Mycobacterium abscessusand treatment" attachment I see one reference (Non-surgical Intratracheal Instillation of Mice with Analysis of Lungs and Lung Draining Lymph Nodes by Flow Cytometry.) that describes the procedure but they are only administering 50ul."

- Unfortunately I cannot see any changes to the Health and Monitoring section from the previous round. Please address the following concerns from the first round of stipulations (copy and pasted below). "Admittedly I am a bit confused by your definition of Septicemia only applying to P4 and P5 mice; your pyelonephritis mice are also getting bacteria injected IV and P3 describes injecting s. aures IV and trying to maintain mice for up to 56 days. You also want to inject bacteria IP, which leads to peritonitis, which is very painful and deadly if not treated. This section also fails to capture clinical signs associated with pyelonephritis, pneumonia from IN/intratracheal infection, SQ abscess, muscle necrosis from IM injections, etc. Your monitoring section should be more robust and include how you are monitoring for the (updated) potential adverse health effects listed above. How often do you weigh mice? How are you determining hypothermia/cyanosis? What about hydration status? Respiratory rate/pattern? Mobility in the cage? Please include specifics on when you intervene with supportive care. For example, we give SQ fluids when mice are dehydrated (prolonged skin tent), we provided moistened chow on the floor immediately after inoculation and/or when there is >10% weight loss. Mice with increased respiratory effort or decrease mobility are provided heat support and extra nesting material. Based on the procedure sections for chicks and poults, it also seems like you expect weight loss in these animals, so you should list weight loss/failure to thrive as a potential adverse health effect. Please include your weighing schedule in this Monitoring section. "

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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9. **Protocol Title:** 2104-38986A Animal models for myelin disorders
Species &Pain Class: (A,B,C) Mice
Question the Research Addresses: PI requests 47872 class A, 5564 class B, and 2396 class C mice for a study to understand the effects of the unfolded protein response, autophagy, and NF-kB on myelin orders, particularly multiple sclerosis.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Please clarify whether CFA or IFA is used for the EAE model, as there are references to both in the protocol.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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10. **Protocol Title:** 2104-38982A Physiological Role of the Vitamin A Transporter RBPR2 for Vision in Mice
Species &Pain Class: (A,B) Mice
Question the Research Addresses: PI requests 2196 class A and 180 class B mice for a study to describe the role of novel dietary vitamin A carriers, that help consumed vitamin A (from food), to reach the eye, in the support of vision. This project will provide new information on the processes influencing dietary vitamin A transport and delivery to the eye. Animal models are needed to study this important process, that also occurs in humans.

The committee concurs that this protocol can be approved via designated member review once the following

stipulations are addressed by the PI:

- The question "Will you be conducting the same experiment in multiple species?" is marked Yes, but since only mice are proposed, this response should be changed to No.

- Please update the Breeding procedure to provide rationale/scientific justification for the number of offspring produced.

- The numbers in the design don't add up (group 3 numbers are unclear, is it 2*33*2=132 or 2*30*2=120?), but others are fine. Also, reconcile 1152 mice (or whatever this number should be) with numbers in totals below, as this is unclear: Total pups used : 1296 Breeders for all groups: 216 Pups euthanized: 1080 Total: 2376 Please also update the Species table to reflect the corrected numbers.

- It isn't clear whether ERG/SLO/OCT measurements will happen in a single bout of anesthesia or will require three rounds. Please update the Procedure to clarify. Assuming these are separate bouts, then administration of anesthesia should be listed as a step for SLO and OCT, right now the procedure only lists recovery.

- This procedure is unnecessary as it happens post mortem, and can be removed.

- PI needs to submit a controlled substances protocol. Please contact me at cshelp@umn.edu to discuss the process.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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11. **Protocol Title:** 2103-38958A Switchable Chimeric Antigen Receptor-Mediated Immunotherapy for Human CD19+ B Cell Malignancies
Species &Pain Class: (A,C) Mice
Question the Research Addresses: PI requests 32 class A and 160 class C mice for a study to address whether a novel CAR therapy can kill B cell tumors while minimizing normal B cell aplasia and acute toxicities.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- It is recognized in this section that "mice may become moribund and require euthanasia prior to losing 25% body weight." However, the alternative criteria, based on the clinical scoring system that takes activity level, fur texture, and posture into account, also requires a weight loss of >25% to achieve the maximum score of 8 that is proposed as an alternative endpoint. Are there any clinical criterion that would warrant euthanasia even in the absence of 25% weight loss, and if so, please describe.

- Personnel must complete the medical and online requirements prior to working with animals on the protocol. Personnel include: Christopher Pennell: • Tetanus surveillance Once complete, please confirm here in eProtocol.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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12. **Protocol Title:** 2104-39004A Phylogeography of Embayment Fishes
Species &Pain Class: (A) Pirate Perch; (A) American Pickerel; (A) Bowfin

Question the Research Addresses: PI requests 460 class A Pirate Perch, 460 class A American Pickerel, and 460 class A Bowfin fishes for a study to determine how genetic variation in the study species is partitioned across their distribution. Are phylogeographic breaks consistent between the study species? Do these taxa conform to the two existing hypotheses explaining Gulf Coast vicariance?

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- Please include capture via seine and electrofishing as a procedure in the protocol.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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13. **Protocol Title:** 2102-38860A Role of Phosphate-Responsive Signal Transduction in Mycobacterium tuberculosis Persistence PPE Export by the Essential ESX-5 Secretion System M. tuberculosis Virulence
Species &Pain Class: (A,C) Mice
Question the Research Addresses: PI requests 1035 class A and 144 class C mice for a study to define the factors that Mycobacterium tuberculosis uses to evade immune responses and promote its persistence in mammalian hosts. We previously identified a Mycobacterium tuberculosis phosphate-sensing signal transduction system that is important for immune evasion and will perform genetic analysis to identify the genes controlled by this system that directly promote resistance to adaptive immune responses. We will also directly test the role of the M. tuberculosis ESX-5 protein secretion system in virulence using strains that conditionally express components of the ESX-5 secretion apparatus.

Committee Decision: Approved as submitted
For: 14 Against: 0 Abstain: 0

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14. **Protocol Title:** 2002-37847A Field Studies in Mammalogy course at the [redacted]--[redacted]
Species &Pain Class: (C) Mice, Voles, Ground Squirrels, etc, (C) Shrews, various species; (A) Bats, various species; (A) Small to medium carnivores
Question the Research Addresses: PI requests permission for a field course dealing with identification of mammals and techniques used in studying the ecology of mammals in the field. PI will be training students in the techniques of capture, handling, and behavioral observations of free-ranging mammals. Students are expected to gain an understanding of the diversity of mammals in Minnesota, how to identify them and to understand aspects of their ecology, conservation, and management.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

- The data on the long term effects of toe clipping are equivocal at best, and I think this is hard to justify for a short term study (maximum of five weeks) for educational purposes. Since this is a method that is still sometimes used, I can see justifying this for a small number of individuals for training purposes, but not for all animals captured. Ear punches are likely much less impactful, and can be used to uniquely mark up to 99 individuals of a given rodent species (https://med.virginia.edu/genetically-engineered-murine-model-core/research/all-mice-considered/pattern-for-ear-punch-numbering-system/), and in combination with fur clipping could be expanded to uniquely marking several hundred individuals.

- Please explain why ear punches couldn't be used as an alternate to toe clipping. I am also not seeing a strong enough rationale to not use meloxicam as an analgesic if using toe clipping. All medications have some side effects but that does not mean we do not use any analgesia if we suspect more than momentary pain.

- If specifically targeting shrews, which have a high metabolism and do not do well with the stress of capture, how often will you be checking traps. Some literature states (and is cited by the Guidelines of the American Society of Mammalogists) that these traps should be checked every 1.5-2 hours to limit mortality. Please confirm for these species that the traps will be checked more often.

- The decapitation of mescocarnivores has not changed from the previous submission, and this process is not recommended for larger species, at least as a primary method. Use of an inhaled anesthetic to provide deep anesthesia or death should be used, which is not considered a controlled substance. It could be an alternative to call conservation officers to dispatch the animals appropriately. Please update the protocol with one or both of these options for the mesocarnivores.

- Please ensure that wildlife permits are updated prior to beginning work. These can be attached to the protocol or emailed to iacuc@umn.edu.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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2. IACUC-AMENDMENT (# Protocols: 4)

1. **Protocol Title:** 1810-36465A Display Turtle at [redacted]
Species &Pain Class: (A) Turtle (yellow bellied slider)
Question the Research Addresses: In response to previous deferral of request to relocate a display turtle to [redacted], PI requests a process to apply for [redacted] to become an approved animal housing area.

   The committee concurs that this protocol cannot be approved and will be closed.

   Committee Decision: Not approved
   For: 14 Against: 0 Abstain: 0

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2. **Protocol Title:** 2012-38674A "Investigating the neural mechanisms underlying motivation and emotional processing using mouse models" "Determining the unique and dynamic neural constellation belonging to acute and chronic stress states" "Stress-induced modulation of striosome circuit in decision making"
Species &Pain Class: (A,B) Mice
Question the Research Addresses: PI requests to add [redacted] for mice. The room has undergone pre-inspection with members of the IACUC. Air exchanges, temperature, humidity, positive pressure, and the HVAC system are all within acceptable limits. PI has ensured that converting positive pressure would NOT disrupt the [redacted] nearby, and the room is now at positive pressure and within acceptable limits to house mice. Per IACUC request from the pre-inspection, PI has also submitted a work request for an electrician to look at the lighting timer in [redacted]. Currently both [redacted] are controlled by the same light switch, so PI has asked for them to be placed in individual switches. PI will also coordinate with RAR to make sure the light timer outside the room is in good order and can be adjusted to a 12:12 on/off cycle. Additionally, PI has requested that the chipped walls are painted by FM.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - The protocol indicates in the species section for [redacted] is being added for the CUMS procedure, but in the procedure section of the protocol you updated the room number [redacted] for the Repeated Forced Swim Stress procedure and not for the CUMS procedure. Please update the protocol to clarify which procedure will use [redacted]

   - Please contact Jennifer Borgert in the IACUC office at borg0052@umn.edu or 612-730-7175 when

electrical work and painting are complete for final approval.

Committee Decision: Stipulations must be met
For: 14 Against: 0 Abstain: 0

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3. **Protocol Title:** 2002-37873A Neural Control of Hypertension in the Sheep: Afferent and Efferent Renal Nerves, Renal Inflammation, and Hypertension. A novel electroceutical tool for treatment of kidney-based diseases
Species &Pain Class: (B) Sheep (Biomedical)
Question the Research Addresses: PI requests to add the use of succinylcholine paralytic agent to surgical procedures. Succinylcholine was omitted from the original protocol and personnel who perform these surgical procedures have requested its use for these surgeries.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - Please see additional comment on the procedure re: justification for use and dose.

   - Please justify the use of the paralytic. Electrocautery is able to be safely used to provide hemostasis without the use of a paralytic, therefore the original response is a little unclear. Alternatively, the use of succinylcholine can be removed entirely. Have lower doses (such as the published dose previously referenced) of the paralytic been used and found insufficient?

   Committee Decision: Stipulations must be met
   For: 14 Against: 0 Abstain: 0

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4. **Protocol Title:** 2001-37778A A Preclinical Evaluation of a Biologically-Tissue Engineered Pulmonary Heart Valve in the Sheep Model
Species &Pain Class: (B) Sheep (Biomedical)
Question the Research Addresses: PI requests to add an option to the surgical procedure that would allow for either a interpositional placement of the valve (currently approved) or a right ventricle outflow tract (RVOT) reconstruction. PI also requests to allow for the implantation of a transcatheter pulmonary valve as a second surgery with a 90 +/-5 day duration of implant.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   - Under anticipated study-induced adverse health conditions, please describe that additional pain management will be given in the event of a broken rib.

   - "Valved Pulmonary Conduit Implant with RVOT Reconstruction Addition" Please list a reference for the use of steroids to stabilize pulmonary cells due to fluid overload.

   Committee Decision: Stipulations must be met
   For: 14 Against: 0 Abstain: 0

Institutional Animal Care and Use Committee
5/18/21 Minutes
VCRC - 76D

Meeting Convened: 12:00 PM	Quorum Requirement: 9
Meeting Adjourned: 2:12 PM	Members Present to Vote: 13

Voting Members			Alternates		
1	X	(Chair - M, S)			
2	X	(M, V)	A		(A, S)
			B		(A, S)
			C	X	(A, S)
			D	X	(A, S)
			E	X	(A, S)
			F		(A, S)
			G		(A, S)
			H		(A, S)
3	X	(M, S)	I		(A, S)
4	X	(A, U)	J		(A, U)
			K	X	(A, U)
			L	X	(A, U)
			M	X	(A, U)
5		(M, S)	N	X	(A, S)
6	X	(M, V)	O	X	(A, S)
7		(M, S)	P		(A, S)
8		(M, S)	Q	X	(A, S)
9		(A, St)	R	X	(A, St)
10	X	(M, S)			
11		(M, S)	S		(A, S)
12		(M - NA, NS)	T	X	(A - NA, NS)
13		(M, S)	U	X	(A, S)
14		(M, S)	V	X	(A, S)
15		(M, S)	W		(A, S)
16		(M – St)			
17	X	(M, V)			

Non-Voting, Ex-Officio:

i		(O, U)
ii		(O, U)
iii	X	(O, U)

Institutional Veterinarian:

3	X	(M, V)

Correlates to Version **v4.0** of the IACUC Roster

M = Member, A + Alternate, S = Scientist, NS = Non-Scientist, NA = Non-Afiliated, V = Veterinarian, St = Student, O = Ex-officio, U = University Staff

Discussion/Information Items

1. The committee reviewed the April 2021 inspection summary.

2. The committee voted to approve the final version of the Spring 2021 Program Review packet and send it on to the Institutional Official.

3. The committee was updated on the recent adoption of a dog that had been on study. The dog is doing well in his new home.

4. The committee reviewed a self-report involving issues with surgical and aseptic technique. The lab has met with their area veterinarian to discuss best practices and the lab member performing surgery will take the RAR aseptic technique and suture class prior to performing additional surgeries. The committee considers the matter closed.

5. The committee reviewed a self-report in which animals were weaned improperly and a staff member was brought into the animal facility before completing all training requirements. Moving forward, the lab has reviewed access requirements and proper weaning practices. The committee considers the matter closed.

6. The committee reviewed a report of an adverse event involving an animal that developed tail lesions following surgery. The tail was amputated and has healed well. Moving forward, the lab will continue to pay special attention to blood pressure and heating during surgeries. The committee endorsed the plan and has no further concerns at this time.

7. The committee reviewed two self reports involving individual animal rooms missed during RAR daily health checks. Both rooms were checked the following day and no health or husbandry concerns were noted. Supervisors have worked with staff to ensure that all rooms are checked daily going forward. The committee considers the matter closed.

8. The committee discussed the recently implemented IACUC mandated RAR training. The Aseptic Technique and Suture course required for new surgeons has limited availability due to both staffing and COVID-related constraints. To facilitate timely training of surgeons, the committee approved the following three options as alternatives to the course:
    a. Labs may train surgeons but they cannot perform surgery independently until their proficiency has been certified by an RAR or IACUC designee.
    b. A lab member can be certified as a trainer by an RAR or IACUC designee and can then train others in the lab.
    c. Waiver of the course for experienced surgeons.

    Training will be revisited at the next semiannual Program Review.

9. The committee discussed sourcing of NHPs. The matter was tabled and will be discussed again at a future meeting with input from a PI who uses NHPs.

1.
    1. **IACUC-R1S1(# Protocols: 10)**

        1. **IACUC-R1S1 - NEW(# Protocols: 6)**

1. **Protocol Title:** 2102-38847A Benefits of Intra-arrest Cardioplegia and Ketamine on Outcomes in a Porcine Model of Refractory VF Cardiac Arrest
   Species &Pain Class:(B) Pig (Biomedical)
   Question the Research Addresses: Will reversible intra-arrest cardioplegia improve CPR hemodynamics and thus the ability to achieve return of spontaneous circulation? Will the combination of reversible intra-arrest cardioplegia and ketamine lead to improved cardiac and neurological survival outcomes?

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: I have some concerns regarding the recovery of pigs after a 24h anesthetic period. Who is going to oversee the recovery of these pigs? Also, monitoring the pigs immediately after a 24h anesthetic period using "once a day monitoring at a minimum" does not seem sufficient. I think, if it is logistically feasible, the frequency of the monitoring during the first 24h after recovery should be more frequent (perhaps at 2, 4, 6, and 24h after recovery).

   Comment: Left jugular cutdown and repair are mentioned but not described. Please update the protocol to describe both the cutdown and repair.

   Committee Decision: Stipulated
   For: 13 Against: 0 Abstain: 0

2. **Protocol Title:** 2103-38904A Mechanisms, Prevention, and Treatment of Murine Chronic Graft-Versus-Host Disease
   Species &Pain Class:(A,C) Mice
   Question the Research Addresses: Our research will investigate the mechanism involved in the development and maintenance of Chronic Graft-versus-Host Disease. We will be investigating the role cell surface markers, signaling proteins / pathways, cellular metabolic pathways and cell subsets such as germinal center B cells and multiple subsets of T cells that are necessary for development or regulation of disease.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: The aim of this protocol is to define the cellular and molecular mechanism of GVHD in order to ameliorate or prevent this disease in transplant patients. In protocol 38905A, The goal is to both define the mechanisms and potential treatments for GVHD that will not prevent successful bone graft treatment of irradiated patients, i.e. can we prevent/lessen immune mechanisms driving GVHD and not significantly inhibit bone marrow engraftment and subsequent re establishment of the host immune response? Without thoroughly analyzing the hundreds of individual experimental procedures listed in these two protocols it is very difficult to interpret the novelty of the GVHD studies between the two protocols, i.e. how are they distinct in their aims and projected outcomes? Is there significant repetition/overlap between the two protocols? A statement clarifying the unique questions being asked for each protocol with respect to GVHD would be useful.

   Comment: Within your experimental design you state, "Since GVHD is a complex pathophysiological process that in its most severe state results in mortality or a moribund state, lethality evaluation is a requisite and the only accepted endpoint some of our studies." As it is indicated that these are accepted endpoints in only some of your studies, please include which specific studies require mortality as an endpoint and which studies will have additional endpoints. As death as an endpoint is best avoided, specific information within the Health and Monitoring section to minimize the number of mice who have death as an endpoint is ideal.

   Comment: Please include the total volume injected IP (max of 10ml/kg). Also, please include how sterility is ensured of the cyclophosphamide, is it sterile filtered prior to injection? Is this pharmaceutical grade?

   Comment: Please include what route(s) is used for intravenous injections. To avoid adverse events in the mice, such as tail necrosis secondary to tail vein injections, for cell injections intravenously, recommend including steps to ensure cells do not clump including mixing/ gently shaking cells prior to drawing up in the syringe and administration.

   Comment: For all therapeutics given intraperitoneally, how is sterility ensured in any non-pharmaceutical grade drugs?

   Comment: TBI procedure is stated to be administered as a single dose of 700 to 1000 cGy, however, in the

procedures section under TBI, dose is stated to be 700 to 1200 cGy. Please specify the correct dose range and update the corresponding section.

Comment: Under the question addressing "Which steps will be taken if parameters are outside of normal range", it is stated that mice will be sacrificed. Please elaborate on how the mice will be properly euthanized and disposed of.

Comment: Under the question addressing "Which steps will be taken if parameters are outside of normal range", it is stated that mice will be sacrificed. Please elaborate on how the mice will be properly euthanized and disposed of.

Comment: 1. While the use of anesthetics for the mice on this study are to minimize movements, the mice must be fully anesthetized for the procedure as intubation is stressful for the mice. Therefore, the statement, "Mice only require sufficient anesthesia to minimize movement. Their reflexes are intact and chest excursion is normal," is not appropriate for this procedure. Please remove and include that a toe pinch is performed to ensure a deep plane of anesthesia. Depth will be assessed every 15 minutes by a toe pinch. If a toe pinch is noted then additional anesthetic will be provided. 2. Please include more details on the intubation procedure in the mice including restraint for intubation and what is used as the endotracheal tube. 3. Please include that eye lubrication will be applied to the eyes of the mice. As mice are enophthalmic and do not blink while under anesthesia, eye lubrication is required to minimize the chance of corneal ulceration.

Comment: GVHD in mice can lead to severe dry eye that can lead to corneal ulceration; please include this as a parameter that is monitored for within the Health and Monitoring section. Due to this potential, once corneal lesions are noted (opacity of globe, periorbital alopecia, blepharospams, positive fluorescein stain uptake), it is recommended to either contact RAR for treatment option or begin treatment to prevent worsening of the corneal ulcer and potential rupture of the globe as these are painful conditions. Treatment would include placement of topical ophthalmic lubricant twice daily if evidence of dry eye is noted or topical ophthalmic ointment twice daily if active ulceration is present (you can contact your RAR veterinarian for specific options). Would a topical ophthalmic antibiotic ointment be contraindicated for your studies? Severe corneal ulceration or loss of the globe should be included as an endpoint if treatment is not successful. Please note enucleation as a treatment for these mice may not be a viable option due to the potential for loss of blood when mice are already receiving blood collections weekly.

Comment: Aseptic preparation of surgical site should include 3 alternating scrubs with nolvasan scrub and alcohol. Please update protocol and process accordingly.

Comment: For all anesthetic procedures (with exception of cervical dislocation training), is there a particular reason sodium pentobarbital is used over more common injectable anesthetics such as ketamine/ xylazine combinations? Specifically for the pulmonary function test pentobarbital causes a marked depression in respiratory rate; does this pose a variable to your data?

Comment: CFA has the potential to cause skin ulcerations/ reactions at the site of injections and monitoring for this within your Health and Monitoring section should be included. Have you noted this in your mice?

Comment: Please submit a new controlled substances protocol. Contact me at cshelp@umn.edu with any questions.

Committee Decision: Stipulated
For: 13 Against: 0 Abstain: 0

3. **Protocol Title:** 2103-38905A Mechanisms, Prevention, and Treatment of Acute Graft-Versus-Host Disease &Nongenotoxic Conditioning for Gene Therapy and Allogeneic Transplantation in Fanconi Anemia
Species &Pain Class:(A,C) Mice
Question the Research Addresses: Studies will address which cell surface markers, signaling proteins / pathways, cellular metabolic pathways and cell subsets are important to the pathophysiology of GvHD and which are amenable to interventional strategies to improve BMT outcome.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Please include the route of intravenous injections within the description. Is tail vein injection the only IV technique used? Additionally, to avoid adverse events in the mice, such as tail necrosis secondary to tail vein injections, for cell injections intravenously, recommend including steps to ensure cells do not clump including vortexing/mixing/ gently shaking cells prior to drawing up in the syringe and injection.

Comment: For the question, "What specific steps will be taken in case any of the measured values are outside acceptable ranges?" Please include the statement, if mice are determined to be light from anesthesia determined by movements or increasing respiration rate, isoflurane level will be increased. If the mouse is determined to be too deep from anesthesia as noted by decreased respiratory rate or shallow breaths, isoflurane level will be decreased. If none of the above works, the procedure would be aborted.

Comment: Please include the route of intravenous injections within the description. Is tail vein injection the only IV technique used? Additionally, to avoid adverse events in the mice, such as tail necrosis secondary to tail vein injections, for cell injections intravenously, recommend including steps to ensure cells do not clump including vortexing/mixing/ gently shaking cells prior to drawing up in the syringe and injection.

Comment: Paralysis as a complication of tumor growth is not an appropriate reason for a mouse to be retained on study as this condition is stressful and painful for the mouse. If wish to include these mice post paralysis scientific justification needs to be included as to why it is needed to keep mice in a paralyzed state on study. Within the current description it is described as a short duration of 2-5 days; if wish to use this time period as a monitoring criteria, euthanasia is indicated if paralysis persists longer than 5 days and please include a literature source for this condition.

Comment: For mice on the GVL studies, is death the data needed for these studies or is fresh tissue collection important for these mice? There are some general signs of tumor involvement that are not currently included within your health an monitoring that could be used to assess if a mouse is reaching endpoint from the tumor and not GVHD. For example: 1. Neurologic deficits such as head tilt, circling, paresis or paralysis would be a good indicators of CNS involvement 2. Clinically evident increased respiratory effort indicating metastasis to lungs. Per your protocol 2103-38904A "lung fibrosis in chronic GVHD generally goes unrecognized clinically but perhaps for a slightly increased respiration rate only noted if the mouse is particularly agitated upon being handled and if one is very very observant." That being the case any clinically observable respiratory effort without abdominal distention would be a good indicator of lung metastasis.

Comment: You mention the cornea may be involved with the mice on the GVHD studies. Please include what kind of corneal involvement is expected or seen in your experience. GVHD in mice can lead to severe dry eye that can lead to corneal ulceration. This being the case, once corneal lesions are noted (opacity of globe, periorbital alopecia, blepharospams, positive fluorescein stain uptake), it is recommended to either contact RAR for treatment option or begin treatment to prevent worsening of the corneal ulcer and potential loss of the globe. Treatment would include placement of topical ophthalmic lubricant twice daily if evidence of dry eye is noted or topical ophthalmic ointment twice daily if active ulceration is present (you can contact your RAR veterinarian for specific options). Would a topical ophthalmic antibiotic ointment be contraindicated for your studies? Severe corneal ulceration or loss of the globe should be included as an endpoint if treatment is not successful. Please note enucleation as a treatment for these mice may not be a viable option due to the potential for loss of blood when mice are already receiving blood collections weekly.

Comment: Without thoroughly analyzing the hundreds of individual experimental procedures listed in these two protocols (2103-38904A and 2103-38905A) it is very difficult to interpret the novelty of the GVHD studies between the two protocols, i.e. how are they distinct in their aims and projected outcomes? Is there significant repetition/overlap between the two protocols? A statement clarifying the unique questions being asked for each protocol with respect to GVHD would be useful.

Committee Decision: Stipulated
For: 13 Against: 0 Abstain: 0

4. **Protocol Title:** 2104-38998A Immunosuppressive maintenance and monitoring of in progress nonhuman primate solid organ transplant recipients transferring from Emory to University of Minnesota
   Species &Pain Class:(B) Nonhuman Primate (Macaques)
   Question the Research Addresses: This research is designed to evaluate strategies that reduce the toxic side effects of immunosuppression while protecting the organ graft to support successful graft function and survival. Novel immunosuppression strategies may allow for the possibility of transplanting organs and tissues between different species.

   Committee Decision: Approved
   For: 13 Against: 0 Abstain: 0

5. **Protocol Title:** 2104-38993A Zebrafish Core Facility General Protocol
   Species &Pain Class:(A,B) Fish (Zebra fish)

Question the Research Addresses: Our investigators are using zebrafish to study eye development, stem cell homing, organ regeneration, spinal cord development, cancer, and lung disease.

The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

Comment: Regarding the attachment 'Zebrafish Euthanasia Protocol,' is all ice melted when the fish are placed in the cool water for euthanasia? Direct exposure to the skin of the fish with ice can be painful. Please include what method is used to ensure fish do not have direct contact with the ice in the water.

Committee Decision: Stipulated
For: 13 Against: 0 Abstain: 0

6. **Protocol Title:** 2104-39008A A Single-Arm Clinical Study Evaluating the Safety and Efficacy of Neoadjuvant and Adjuvant CV01 Delivery of Sonodynamic Therapy (SDT) in Canines with Suspected Primary High-Grade Glioma
Species &Pain Class:(B) Dog
Question the Research Addresses: Is PDT using a custom ultrasound device (CV01) and 5-ALA safe and effective for treating pet dogs with primary intra-axial brain tumors?

   Committee Decision: Approved
   For: 13 Against: 0 Abstain: 13

2. **IACUC-R1S1 - AMENDMENT(# Protocols: 4)**

1. **Protocol Title:** 2002-37885A Unfolded Protein Response and Arrhythmias Mitochondria calcium handling and arrhythmias Na+ Channel mRNA Regulation in Heart Failure
Species &Pain Class:(A,B) Mice
Question the Research Addresses: This proposal aims to explore the mechanisms of arrhythmogenesis in heart failure. A variety of proteins and signaling molecules will be investigated.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please update the protocol to clarify whether there will be a period of time in which animals will be maintained in a morbid condition. If so, please update the Endpoints section to describe this, including criteria for euthanasia and maximum duration.

   Comment: Please update the relevant surgical procedures to include the new alternative endpoint.

   Comment: Please add a separate procedure using type Other to describe the added pilot study.

   Committee Decision: Stipulated
   For: 13 Against: 0 Abstain: 0

2. **Protocol Title:** 1810-36480A Eliminating breast cancer metastases by targeting cancer associated fibroblasts with anti-FAP antibody-drug conjugates
Species &Pain Class:(B,C) Mice
Question the Research Addresses: CAFs secrete a protein called FAP, which is not present in high levels on breast cancer cells or in other cells in the adult. We propose that by eliminating CAFs with cytotoxic anti-FAP ADCs we will impair the ability of CTCs to reseed metastases by eliminating cCAFs and cCAF/CTC co-clusters, reduce metastatic burden, and prolong survival in preclinical models of BC metastasis.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: It is stated that if animals show signs of pain after ulceration, that RAR will be contacted for an

analgesic plan. Please work with your RAR area vet to develop a prospective analgesic plan for these animals. Please update the protocol to reflect the agreed upon plan.

Comment: The previously-approved analgesic for surgery is Buprenorphine-SR at a dose of 2 mg/kg and the protocol states that "Animals undergoing surgery will be given analgesia (bupenorphine-SR) 2 hours prior to surgery. This should last 72 hours after surgery. If animal is still showing signs of pain after 72 hours, RAR veterinarian will be consulted to determine if another dose should be given." Please indicate what measures will be used to determine whether SR Buprenorphine is providing adequate pain relief during the postsurgical period.

Committee Decision: Stipulated
For: 13 Against: 0 Abstain: 0

3. **Protocol Title:** 1808-36256A Mechanisms of Aging - Healthspan Protocol; Additional FundingTitle: U19AG05627803 Project 2 Alzheimer's Supplement
Species &Pain Class:(A,B) Mice
Question the Research Addresses: What are the genes that influence mammalian lifespan, cancer risk and the age at onset of degenerative diseases.

   The committee concurs that this protocol can be approved by subsequent full committee review once the following stipulations are addressed by the PI:

   Comment: Please update the procedure to provide scientific justification for video recording.

   Comment: Please confirm whether death is an endpoint for this experiment. If so, please update Q4 on the Experimental Endpoints tab of the protocol with scientific justification, and update the pain class for the affected animals to C.

   Comment: Question 20 says that mice are routinely combined with other cages in case of a single mouse in the cage. This is not advised, particularly for males, because this may lead to aggression and fighting. Please complete [redacted] questions 22-32 specifically for the proposed [redacted] space.

   Comment: Question 33 states [redacted] The mice will be covered from light 6 pm to 6 - 8 am.". Please update the protocol to clarify what covered means. Are the cages covered with a drape or cloth? Or are the windows in the [redacted] space covered to shield the animals from light?

   Comment: Please update the protocol to explain what is meant by "continuous monitoring". Will somebody watch the mice at all times to ensure that heart failure symptoms are detected timely and mice are euthanized before reaching a moribund state or die?

   Comment: Please contact Jennifer Borgert at borg0032@umn.edu or 612-730-7175 to set up an [redacted] pre-inspection for this space. Please complete [redacted] questions 22-32 for [redacted] space.

   Committee Decision: Deferred
   For: 13 Against: 0 Abstain: 0

4. **Protocol Title:** 2012-38723A Antifouling Airway Stent
Species &Pain Class:(B) Pig (Biomedical)
Question the Research Addresses: The aim of the study is to determine whether or not special coatings on an airway stent will prevent mucous adhesion as compared to a non-coated airway stent. Examples of airway stent prescription are lung transplants and lung-related diseases in which the trachea and/or bronchial tubes must be kept patent.

   The committee concurs that this protocol can be approved via designated member review once the following stipulations are addressed by the PI:

   Comment: Please update the protocol to clarify why ACT is being monitored in these animals undergoing tracheal/bronchial stent placement. "When it is confirmed that the stent is in the correct position, the ACT will be monitored to confirm return to approximately baseline levels. If necessary, Protamine (10-50mg) will be administered IV until the ACT is under 1.5 times baseline. The vascular sheaths will be removed with direct pressure being applied until hemodynamic stability confirmed." I am also unsure why the ACT should change during the procedure and why it is planned to be decreased below baseline. Lastly, I am not sure what kind of vascular sheathes are removed at

the end of the procedure.

Comment: Please update the protocol to clarify why paralytic agents are necessary during the stent implantation procedure.

Committee Decision: Stipulated
For:13 Against: 0 Abstain :0