University at Buffalo
State University of New York
Office of Research Compliance

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
MINUTES OF THE MEETING
May 16, 2022

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ATTENDANCE:

[redacted]

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CALL TO ORDER:

The IACUC Chair commenced the meeting at 12:32 PM.
If any member of the IACUC has submitted a protocol or amendment for review and approval, that member is not present during the discussion of and voting on their protocol or amendment. Quorum is maintained.

PRESENTATION OF THE MINUTES:
The minutes of the 2022 IACUC April meeting were presented. The April meeting minutes were voted on and unanimously approved with the correction of two typos.

ANNUALS

A list of annual renewal submissions are provided in the CLICK system.

For protocols involving Pain Category E procedures, the PIs have been contacted and asked to provide the number of animals used over the past year for these procedures, summarize the monitoring regimen for these animals, indicate whether or not a monitoring chart/record is used, provide a copy of this record, indicate if there were any unexpected occurrences (i.e., problems, deaths), and confirm that these occurrences were reported to the LAF veterinary staff.

BUSINESS:

Category E annual submissions: Submissions are listed under protocol review.

AAALAC Site Visit: The committee's attendance was requested for an AAALAC site visit to review the committee's functions, documentation, policies, and compliance on May 24, 2022.

Semi-Annual Spring 2022:
The committee discussed humidity issues in the Biomedical Education Building (BEB) has been experiencing low humidity in various animal handling rooms since January-April. As per federal guidelines, the rodent holding room's humidity levels must be maintained at 30%-70%, and the humidity levels in numerous rooms have been below 30% from December through April. As a result of the low humidity, health issues have been documented in 25 rats. Several rats developed ringtail whereby annular constrictions/necrotic tails tips are found along the tail and or feet are dry, and the distal toes tips may slough off. The LAF called in the low humidity level issue numerous times to UB Customer Service to generate work orders, and over the past few months, per the LAF, these calls have occurred regularly 2-3 times a week. The issue was reviewed at the April IACUC 2022 meeting.

The clinical Vet retracted the statement regarding the litter of 13 pups. All had identical crusted skin lesions over the ventral abdomen. The dame was relocated to an animal housing room that did not have low humidity, and the dam in question had another litter with similar crusted skin. The Clinical Vet determined the issue genetic and could not be attributed to the low humidity.

The spring 2022 Semi-Annual Inspections and Report to the IO:
Most of the deficiencies have been resolved, and one significant deficiency was found. The low humidity issue was a significant deficiency since it has caused an animal welfare concern. The Laboratory Animal Facilities and the IACUC are working on getting the issue resolved.
OLAW will be notified of the animal welfare concern.

The draft of the spring 2022 Semi-Annual Report to the IO was included for review. The committee reviewed the document, and it will be distributed to be signed digitally. Once all the deficiencies have been resolved, the report will be sent to the IO.

Non-compliance: PROTO201900182: The PI responded to the committee's corrective action request. The PI said they would replace the tail vein injections with jugular vein cannulation. The PI also stated that they met with their staff to review the protocol and ensure that all staff members understood the procedures outlined in the protocol. The PI will also have staff review procedures and experimental timelines at the start of new experiments. The PI stated they would review humane endpoints and the lab's response time in which they need to respond to Laboratory Animal Facilities Staff. The committee accepted the corrective action plan and explanation of events. The committee determined that no further corrective action is needed, and the PI is sincere in ensuring the non-compliance does not reoccur.

Non-compliance: PROTO201800154: The PI responded to the committee's corrective action request. The PI stated that 11 mice were affected by the non-compliance. The PI also said that Laboratory Staff preforming the "Live Tissue Collection: Tail Biopsy" will retake the CITI course "Reducing Pain and Distress in Laboratory Mice and Rats," breeding colony management documentation will need to be submitted monthly. The PI also stated that they would personally work with staff until they were confident with protocol and SOPS. They will also switch to ear punching instead of tail biopsy. The committee determined that the PI must also include an attestation statement stating the PI and their staff have read and understood LAF SOP 2.A.9. The PI must also submit breeding colony management records before the non-compliance can be closed.

Non-compliance: BME13028Y: The PI responded to the committee's corrective action request. The PI has reiterated that staff must work closely with the LAF clinical Vets to help ensure responsible research with the least possible harm to the animals.

Animal Welfare Concern Facilities: The Director of Facilities Operations has not responded to the IACUC letter requesting corrective action for the animal welfare concern.

Non-compliance: PMY41040Y: The committee determined that the issue was a non-compliance. Per LAF Director: "I am reporting an incident of non-compliance regarding tail snipping mice over 30 days old without the use of analgesia. Dr. [redacted] lab conducted tail snips of 23 mice on May 6, 7 mice were 3 ½ months old, and 16 were ~ 5 weeks old. All should have had topical Marcaine and a systemic analgesic according to our SOP 2A9. These mice did not receive any analgesia. They did receive general anesthesia (Isoflurane) and styptic powder on the cut tail. The LAF staff discovered this issue on Saturday AM, and the lab was asked to administer analgesia. All mice received Ethiqa XR (long-acting buprenorphine) on May 7 at 1 PM, and all are doing fine." The PI references the LAF SOP, and details written in number six under the procedure are consistent with tail snipping of mice 10-21 days old. The PI is currently not approved to perform tail biopsies on mice greater than 28 days old.
An IACUC letter will be emailed to the PI outlining the following corrective actions. The PI must provide a statement stating how they will provide their staff with appropriate oversight and an attestation statement that each individual in your lab performing the tail biopsies has understood the procedures as outlined in the protocol.

Continued Education Training: Several IACUC members attended The IACUC Administrators Association webinar on "Back to Basics: Compliance Administration and Veterinary Care."
The webinar discussed reducing the administrative burden on PIs and creating a standard IACUC protocol template.

APPROVALS:
A list of the submissions approved since the last meeting has been presented to the committee.

PROTOCOL REVIEW:

In addition to IACUC review, Environment, Health & Safety (EHS) has also reviewed all protocols and amendments submitted this month. For protocols involving the use of hazardous agents in live animals, their use will be approved by the appropriate EHS authority and, as appropriate, laboratory SOPs will be placed as recommended by EHS prior to IACUC approval.

1. **AR2022000029 (Annual Review for PMY05072Y)**
   Annual Summary: The PI used a total of 281 category E mice. From PI's annual summary: We have published two papers from the mice used in this protocol (PMID: 34608164; PMID: 34917858). We have used a total of 317 mice since the approval of this protocol (May 24, 2021) until now. The Magi2 floxed line we obtained from Japan gave us problems. When breeding pairs were separated, or mice were individually housed, these mice tended to stop drinking and eating and would die. We have now figured out the issue. In the construction of this transgenic line, a Magi2 isoform (called Magi2 gamma) is deleted. We believe this isoform is very important for synaptic function. We now breed the mice in heterozygous pairs and use of offspring for our proposed studies. The heterozygous pairs are normal and do not suffer from isolation.

Committee Discussion: In the PI's annual summary they indicated that individually housed animals would stop eating and drinking when separated. The committee requested the number of animals lost to the issue. The PI stated that 4 in total were lost in March/April of 2021 and they had not had the issue since then.
Committee Action: The committee unanimously voted to approve the annual submission.

2. **AR202200035 (Annual Review for OPT01112Y)**
Annual Summary: From PI's annual summary: We have made the following progress over the last year: Exp. 1: The goal of the experiment is to determine the effect of genetic deletion of XBP1 in retinal neurons or endothelial cells on retinal function and neurovascular degeneration in diabetic retinopathy. Over the last year, we have expanded mouse colonies of XBP1 fl/fl, Chx cre line; XBP1 fl/fl, Rho cre line; and XBP1 fl/fl, Opn cre line. We confirmed specific cre expression in retinal rod photoreceptors in XBP1 fl/fl, Rho cre mice and cre expression in cone photoreceptors in XBP1 fl/fl, Opn cre mice. To determine if XBP1 plays a role in rod photoreceptor development, we examined retinal phenotype in XBP1 fl/fl, Rho cre mice. Our results show that XBP1 deletion in rod photoreceptors does not affect retinal function and structure in young adult mice, which suggests that XBP1 is not required for photoreceptor development. To investigate the role of XBP1 in photoreceptor dysfunction and degeneration in diabetic retinopathy, we induced diabetes in XBP1 fl/fl mice; XBP1 fl/fl, Chx10 cre mice; and XBP1 fl/fl, Rho cre mice. Retinal function and retinal morphology will be evaluated by electroretinogram (ERG), optical coherence tomography (OCT), and immunohistochemistry for neuronal characterization. This experiment is currently ongoing. Exp. 2: The goal of this experiment is to determine the role of endothelial cell (EC) Nox4 in the development and progression of diabetic retinopathy by a temporally controlled deletion of EC Nox4 in mice at various times after diabetes onset. Over the last year, we have expanded mouse colonies of mNox4 fl/fl, Cdh5 creERT2 line and characterized the vascular phenotype of this mouse line. We confirmed specific cre expression in vascular endothelial cells. We are currently confirming the knockout efficiency of mNox4 gene in vascular endothelial cells in mNox4 fl/fl, Cdh5 creERT2 mice. We have also established and expanded the colony of hNox4 TG, Cdh5 creERT2 mice. We induced diabetes in mNox4 fl/fl, Cdh5 creERT2 mice. These mice will be used for the experiment to determine the role of Nox4 in retinal vascular dysfunction and vasculopathy in diabetic retinopathy. The experiment is ongoing. A total of 384 mice have been generated and 269 mice have been used to date. No unexpected events occurred.
Committee Discussion: The PI stated that 37 category E mice were used over the last 12 months and not unexpected events occurred. The committee reviewed the monitoring charts and found everything to be performed as the written in the protocol
Committee Action: The committee unanimously voted to approve the annual submission.

3. **AR202200046 (Annual Review for PMY49120)**
Annual Summary: From PI's annual summary: In the past year, we have made substantial progress on the understanding of the combination of the selective GABAa 2/3 positive allosteric modulators with clinically-used analgesics (opioids). Our results showed that the combination of the two pharmacologically diverse classes of drugs lead to improved analgesic effectiveness and reduced doses required. These results are in line with our research plan. We presented the results in 3 scientific conferences. In the past year, we used a total of 94 rats (86 male, 8 female) that belong to pain category D; a total of 138 rats (36 male, 102 female) that belong to pain category E. No unexpected events occurred.
Committee Discussion: The committee reviewed the monitoring charts and they showed no adverse effects or signs of pain. However, the committee is recommending that the actual body

weight be recorded and the percentage of weight loss (or gain) be calculated and recorded on the monitoring charts.
Committee Action: The committee unanimously voted to approve the annual submission.

4. **AR202200047 (Annual Review for ORB18018N)**
Annual Summary: From PI's annual summary: In the past year we have performed additional experiments to analyze the role of RAGE in inflammation. These includes LPS-induced neutrophil infiltration to air pouch, and extracting BM macrophages from RAGE-KO and RAGE knock-in mice for cell signaling studies. Some of the data was published in our recent publication on eLife. We have used ~40 RAGE knock-in mice and 20 RAGE-KO mice. We have generated a total of ~70 mice for these experiments.
Committee Discussion: The committee reviewed the annual and had no concerns regarding the category E experiments.
Committee Action: The committee unanimously voted to approve the annual submission.

5. **AR202200041 (Annual Review for NEU09116Y)**
Annual Summary: From PI's annual summary: No experiments were done under this protocol in the last year.
Committee Discussion: The PI stated that no experiments were conducted over the past year.
Committee Action: The committee unanimously voted to approve the annual submission.

6. **PROTO202200022**
Protocol Summary: From PI's non-scientific summary: The protozoan parasite Toxoplasma gondii latently infects 11-22% of Americans and can cause disease in immunocompromised patients and fetuses. As the parasite traffics through the body where it encounters diverse environment that it must adapt to or will die. The work proposed here is focused on learning how the parasite adapts to these environments with the ultimate goal of developing new therapies and treatments.
Committee Discussion: Edits and clarifications need to be made in Click. The protocol seems to be closely related to another one of the PIs protocols. The committee wants the PI to verify that the submission is a new protocol, not a triennial review. The funding source needs to be added. The procedure "Toxoplasma IP Infection" needs clarifications and edits tissue cysts and harvesting of these cysts. The committee would like the PI to explore alternate euthanasia methods other than CO2. An experiment needs to be added outlining the generation of the tissue cysts from the Swiss Webster mice. The experiment "Infectivity" needs clarifications regarding the substance administration route and the distribution of mice used for the experiment, and the committee wants to know what "dpid" stands for in the "expt 3.pdf" document. It is unclear if the experiment "Role of PhyA and PHYb in recruitment of and survival in innate immune cells" is meant to describe the growth of tissue from within Swiss Webster mice, acronyms need to be clearly defined, and the humane endpoints need to be added. The experiment "Tissue Dissemination" needs clarification on the substance administration route, experimental time points, the description needs to be reconciled, and acronyms need to be defined. The stain section needs Swiss Websters added. The housing section requires a vivarium location and a justification for using SPF. The monitoring chart needs clarification and edits.
Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

7. **TR202100052 (NSG02011N)**

Protocol Summary: From PI's non-scientific summary: There are 28,000 people annually who suffer intracranial hemorrhages from ruptured intracranial aneurysms in the United States. Nearly half of these patients will die despite best medical and surgical intervention. Recent technological advances may provide better results following endovascular treatment of ruptured and unruptured intracranial aneurysms. It is our goal to use the canine and swine aneurysm models to develop and test techniques for treating aneurysms that obviate the need for open surgery and improve upon current endovascular therapies. Access to the cerebral vasculature is gained by introducing small catheters into the femoral artery in pig and dog. Using x-ray fluoroscopy, these catheters can be guided directly into an aneurysm cavity. Devices can be placed into the aneurysm or parent vessel in an attempt to isolate it from the cerebral circulation, preventing growth and rupture of the aneurysm. This protocol has provided us the ongoing ability to develop the microangiographic imaging that has become the cornerstone of our laboratory and now a commercially available unit, as well as dozens of devices to be tested and evaluated over the years it has been in place. Because aneurysm endovascular intervention has not been perfected, and the micro-imager continues to be developed and improved, there is always work to be done in this field that necessitates the use of live animals.

Committee Discussion: The committee discussed the animal justification numbers and determined the justification was inadequate. There needs to be a scientific justification for the number of animals per experimental group. The PI has not adequately described the experimental design, and the committee cannot sufficiently review the animal justification numbers.

Committee Action: The committee unanimously voted to withhold approval.

8. **TR202200010 (PTH05028Y)**

Protocol Summary: From PI's non-scientific summary: After more than 40 years of investigation, the etiology and pathogenesis of depression are still unclear. Conventional antidepressants that affect monoamine (serotonin, dopamine, norepinephrine (NE)) pathways require ~14 days for efficacy, and 2/3 of patients are resistant to these treatments. Clearly there is a critical need for improved antidepressant therapies; the urgency for effective treatments of mental health disorders, including depression, is emphasized by the recent nationwide tragedies of gun violence. This research uses rat models to address this important problem by directing future development of novel antidepressants that target specific proteins with better side-effect profiles and faster onset of action. A major focus of this ongoing study is to show a common mechanism of action between non-typical and contemporary antidepressant agents, that is, establishing the role for the protein mediator tumor necrosis factor-alpha (TNF) in the cause and treatment of depressive symptoms. The general approach is to target/decrease TNF in the brain to alleviate depressive behaviors in rodents.

Committee Discussion: Edits and clarifications need to be made in Click. The committee has requested that all experiments that include a Force Swim Test (FST) be placed in a pain category E. In the experiment "Intracerebral silencing of TNF" the procedure needs to be listed as a category E since analgesics cannot be given. The reason for withholding the analgesics has been well justified scientifically. The experiment "Inducing TNF in the brain prevents antidepressant effectiveness" needs to be a category E, given that the rats will all be administered LPS. The committee has requested that the PI add the Forced Swim Test (FST) to the alternative records search.

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

9. **TR202200012 (ORB05072Y)**

Protocol Summary: From PI's non-scientific summary: This research uses mouse models to study the ill effects of colonization by a select group of harmful bacteria on tooth-supporting structures, i.e., gums and jaw bone - causing periodontitis, a common inflammatory disease leading to tooth loss. This study will evaluate the effect of one such harmful bacteria, namely Tannerella forsythia.

Committee Discussion: Edits and clarifications need to be made in Click. NPG forms need to be added for non-pharmaceutical grade substances. The committee is requesting the PI explore alternate euthanasia methods, as CO2 is no longer recommended. The breeding "Mouse breeding experiment" needs additional information regarding breeding colony management. The justification for the number of animals used needs more consideration, and the experimental justification needs to be edited, as the animal cost is not a scientific justification. The experiment "Mouse infection and alveolar bone" needs edits, husbandry exceptions identified, the strain being used needs to be defined, and excess information regarding the breeding schemes needs to be relocated to the breeding experiment. The strain section needs edits and additional information. The vivarium location needs to be added.

Committee Action: The committee unanimously voted to require modifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

The IACUC Chair adjourned the meeting at 2:43 PM.