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

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
MINUTES OF THE MEETING
March 21, 2022

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ATTENDANCE:

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CALL TO ORDER:

The IACUC Chair commenced the meeting at 12:34 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 February meeting were presented. The January meeting minutes were voted on and unanimously approved with the correction of two typos.

BUSINESS:

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

Annual Review for ORB17018Y: Not a category E protocol, it has DOD funding and requires an annual review. The committee requested additional information for this annual. Details are listed under protocol review.

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.

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

Annual Review for PSY03092Y
Annual Review for OPS02075Y

Training opportunities/ protocol/ amendment review reminder:
The committee was informed of an IACUC conference opportunity put on by Prim&R. The Conference is scheduled for November 2022 in Salt Lake City, Utah.
The committee also went over protocol and amendment review reminders. The reminders included the flowing items:
Committee protocol review reminder:
1. The non-scientific summary of the research is written so non-scientists can understand what is written.
2. Animal numbers: The number of animals requested by the PI is supported by a reasonable justification and a power analysis.
3. Checking the alternatives section has updated searches.
4. Checking that the Scientific Merit Form has been signed with a date
Amendments:
1. Please do not forget to add a "private comment" to amendments. Leaving a comment on an amendment even if you do not have any concerns or modification requests is a way of leaving a paper trail for AAALAC or other agencies to review.
The IACUC administrative office will send out an updated form on how to add a reviewer comment to Click.

PAM Update: The PAM officer has resigned, but they tried to reschedule one visit that was postponed due to a positive COVID test. The following information is from the PAM officer: In regards to the jugular catheter and ICV cannulation, dual surgery that I was requested to site review/observe: 1) The student, who is involved with performing the dual procedure, is leaving the lab in April, and 2) if the data they have already generated is as expected, they will not be doing that again until the Fall. However, if the data is not as expected, they may do it in the summer. If the IACUC would like to observe the ICV cannulation procedure alone, that will likely happen in early summer. The University at Buffalo is hiring a new PAM officer and will reschedule this site visit once a new individual is hired.

IACUC Policy Review: The Edits to the Policy on Review and Approval of Animal Use Protocols and Amendments were approved on March 1, 2022.

Noncompliance: Monitoring chart review PROTO202000086. These animals were supposed to be euthanized at 20% weight loss per the monitoring chart. Two mice were allowed to reach greater than 20% weight loss and were found dead on 2/17/2021. The PI completed an amendment requesting to adjust the humane endpoint to be increased to 25% weight loss, submitted the document stating how the PI will oversee laboratory staff, and explained why the animals were not euthanized when they reached the 20% weight loss. The committee unanimously voted that the incident was a non-compliance and accepted the PI's corrective actions.

Noncompliance PROTO2019001098: The noncompliance was initially reviewed at the January 2022IACUC meeting. The PI submitted a corrective action response to be reviewed at the February 2022 IACUC meeting. The committee determined that the PI's response was inadequate and requested

the missing corrective action items. The PI chose to close the protocol on 3/7/2021. The committee was notified of the PIs protocol closure and unanimously agreed that no further action was necessary.

Noncompliance PROTO201900029: The PI self-reported the noncompliance incident on March 10, 2022. The PI withheld post-surgical buprenorphine after administering atipamezole because the animal showed labored breathing during the surgical procedure on January 20, 2022. The rat was euthanized on March 7, 2022, as per the natural end of the experiment. Per the PI, the surgery went well, aside from accidental extubation at skin closure. The PI administered 60 microliters of atipamezole 1mg/kg solution. The rat was ambulating after the atipamezole, placed in a cage under a warm lamp, and received all other post-operative medication. In the report, the PI submitted corrective actions to ensure the noncompliance did not occur again. The PI will continue optimizing ketamine/xylazine dosage to use the lowest possible dose in the proposed range. The PI stated that they would consider using benzodiazepine instead of the alpha-2 agonist to help avoid respiratory depression. In addition, the PI stated that buprenorphine would be administrated as outlined in the protocol. An amendment will be submitted to include the option not to administer buprenorphine if the animal is at risk of severe respiratory depression. The PI will consult with a Clinical veterinarian for advisement before withholding analgesics and ensure veterinary staff can assist with monitoring the animal. The committee has unanimously determined the incident is a noncompliance and accepted the PI's explanation of the event and corrective action plan. No further correction action was requested.

Noncompliance: PMY41040Y: The PI self-reported the non-compliance on March 7, 2022. The lab performed cannulation and microdialysis procedures that were not listed on the protocol. Per the PI, the protocol was not amended appropriately to incorporate the cannula procedure as part of the survival stereotactic surgery. The cannula implant procedures were performed from November 22, 2021, to November 26, 2021. The animals underwent a non-survival microdialysis surgery one week later. Twenty animals were involved in the incident. The PI submitted a corrective action plan, which included adding cannulation survival and microdialysis non-survival surgeries. The committee reviewed the PI's response, which detailed the non-compliance incident and the PI's corrective action. The committee unanimously determined that incident was a non-compliance and that the PI must submit a letter stating how they will ensure appropriate oversight to ensure the non-compliance does not occur again.

Noncompliance: PROTO201900203: The PI self-reported the noncompliance incident on March 12, 2022. The lab conducted cannulation procedures that were not listed in the protocol. Per the PI, the incident occurred when entering the triennial into Click from the old paper protocol form. During the switch, the cannulation procedure was not transferred into Click. The PI stated that they would be reviewing the entire protocol to ensure all procedures were present and outlined in the protocol. The committee unanimously determined that the incident was a noncompliance and reviewed the PI's corrective action plan. The committee determined that the PI must also submit a letter stating how they will ensure appropriate oversight to ensure the noncompliance does not occur again and submit the number of animals that underwent the cannulation procedure.

Click Discussion: The committee discussed if the system is working and if it is an increased burden on reviewers and PIs. There seems to be an increase in lapsed protocols and noncompliance incidents. The committee would like to do a protocol awareness month for PIs as a possible solution to lapsed protocols and noncompliance incidents.

Category E protocols: The committee would like to set up a subcommittee to discuss the category E protocols and create more explicit guidelines for what experiments are considered category E procedures

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. **AR202200005 (Annual Review for PSY03092Y)**
   Annual Summary: From PI's annual summary: Our goal is to conduct a genome-wide association study (GWAS) in 1440 heterogenous stock (HS) rats. This work has been completed by Dr. [redacted] and research technician [redacted], with help from undergraduates that assist with surgery, testing, and dissections. Last year, we had fine-tuned our surgical pipeline. This year, we have fine-tuned it even further, as evidenced by our 82-88% completion rate, meaning that for each batch of catheterized rats, only 12-18% of rats are removed from the study due to catheter patency loss and other reasons (see table attached to the accompanying comment to this report). This attrition rate is less than what we had estimated when proposing this project. In short, we've hit our stride for this project. We do have a few animals in each batch that show swelling around the catheter implants, but the incidence of this is decreasing and now only occurs in a very small subset of subjects. Thus far, we have tested 442 rats for this project; 324 have been tested since last year. Our goal is to test 360 rats per year, a rate that we are on track to achieve next year. In the next year, we hope to increase the size of each "batch" to up to 90 rats/batch, which means we will be able to partially make up for the deficit caused by the COVID-induced shutdown in 2020. We are currently testing 69 rats in the 9th batch of rats. The behavioral testing itself has proceeded without major issues. Our paradigm for cocaine self-administration is intermittent access (IntA), in which rats get 5 minutes to take drug, separated by 25-min periods of drug non-availability. After taking drug for 15 days, we assess motivation for cocaine using progressive ratio tests, a measure of punished responding, and cue seeking test which occurs after 14 days of drug withdrawal. The reason why this protocol is a category E because the animals are shocked during the punished responding test. Note that the shocks are voluntarily incurred, if the animal does not respond for the drug (cocaine) it does not receive a shock. These 0.3mA shocks are quite mild, they are barely detectable by humans, in fact we have to turn the amperage up to feel the shocks when we are testing the equipment. All 324 rats that completed testing last year received this procedure. During this procedure, animals are monitored for low activity, poor haircoat, poor posture, and weight loss. If these conditions do not subside, rats will be removed from the study. We do not use a monitoring chart for this. No unanticipated results, the swelling that we see in some animals is expected to occur in a very small subset of subjects.
   Committee Discussion: The annual was detailed and well written.
   Committee Action: The committee unanimously voted to approve the annual submission.

2. **AR202200021 (Annual Review for OPS02075Y)**

Annual Summary: From PI's annual summary: No animals have been used over the last year under this protocol.
Committee Discussion: No category E animals were used in the past 12 months.
Committee Action: The committee unanimously voted to approve the annual submission.

3. **AR202200009 (Annual Review for)**
Annual Summary: From PI's annual summary: In the past year, there has been little progress on this protocol. Although new extramural funding from the Dept. of Defense has been secured, we have been focused on publishing data sets related to this study, including: In this past year, we have maintained the Dusp1/MKP-1 KO and LysMCre-Dusp1/MKP-1 conditional KO colonies, but no new implanted experiments have been performed. We have generated 124 Dusp1/MKP-1 global KO mice, and 34 Conditional MKP-1 KO. In addition, one small 4NQO study was performed with 10 WT mice for some pilot data related to the project
Committee Discussion: LAF Veterinary Records show that several mice in the 4NQO Oral Tumor Model were placed under veterinary care in July 2021. Monitoring charts need to be submitted for the mice from 2021 to complete the annual review. In addition, the PI needs to provide the number of mice used for experiments during the past year.
Committee Action: The committee unanimously voted to require clarifications to secure approval and to allow the revised protocol to be reviewed and approved by Designated Member Review.

4. **PROTO202100079**
Protocol Summary: From PI's non-scientific summary: The proposed study will examine the effects of different exercise regimens on cocaine preference.
Committee Discussion: This is the second time the PI has submitted the protocol for full committee review. Edits and clarifications need to be made in Click. The procedure "Decapitation following Anesthesia, ver. AND Procedure: Substance Administration: Isoflurane Anesthesia" needs edits to the isoflurane delivery. The procedure "Treadmill Exercise" needs clarifications on the use of the electric shock grid, acclimation of the animals to the behavioral equipment, and the sanitation of the treadmill. The procedure "Jugular Vein catheterization" needs clarifications regarding analgesics, and the committee recommends the use of non-absorbable sutures. The procedure "Catheter Patency Checks (Heparinized Saline; IV)" needs clarifications regarding the dosage of cefazolin and duration. The PI needs to provide additional justification NPG cefazolin and heparin.
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.

5. **TR202200006 (PROTO201800237)**
Protocol Summary: From PI's non-scientific summary: Evidence from clinical studies of fetal alcohol spectrum disorders (FASD) has shown that prenatal ethanol exposure could lead to many adverse effects including increased risk of addiction and attention deficit. We are using animal studies to study the neuronal mechanisms for these adverse effects. Strong evidence show that the increased addiction risk could be mediated by altered synaptic neurotransmission in dopamine (DA) neurons located in the ventral tegmental area (VTA). Therefore we will study prenatal ethanol exposure-induced effects VTA DA neuron function and addiction risk.
Committee Discussion: Edits and clarifications need to be made in Click. The needle size needs to be indicated for injections. The dosage for Fatal Plus used for euthanasia needs to be updated in all procedures. The committee recommends replacing "Hibitane" with

“chlorhexidine or iodine surgical scrub. Habitant skin cleanser is a specific brand. Using the generic name will allow the PI to utilize different name brands if Hibitane is unavailable or back-ordered. NPG forms need to be added for apomorphine, methylphenidate, urethane, and amphetamine sulfate. The committee needs clarification regarding if the animal undergoes more than one survival surgery. In the triennial review summary, the committee requested that the PI submits any monitoring charts for restraint in the past year. The committee also requested additional information regarding any lactose intolerance or other indigestion issues noted in rats receiving the cafeteria diet. The “Brain slices” procedure can be removed because it occurs after euthanasia. The procedure “Chronic unpredictable stress” needs clarifications regarding the padding used under the elevated platform and a justification for using distilled water instead of disinfectant to clean the equipment. The behavioral procedures need to answer the sanitation between uses, and the question cannot be left bank. The procedure “Optogenetic stimulation during behavior” needs clarifications in the procedural description, sanitation procedures need to be described, monitoring during behavioral testing and endpoints need to be described, and a typo needs to be corrected. The procedure “Intracerebral injection of fluorescent latex microspheres or viral vectors” needs to indicate a supplemental heat source. The substance “AAV viruses” needs to have an updated Biosafety approval. The procedure “PLX 5562 gavage” needs to indicate potential adverse outcomes, and monitoring endpoints parameters must be indicated. The substance administration “minocycline treatment” needs clarifications as to whether this substance is of pharmaceutical grade or not. If it is not pharmaceutical grade, an NPG form will need to be added. The procedure “intracerebral cannula/optrode implantation” clarifications need to be made to the sterilization procedures, the PPE and antiseptic technique needs more information, supplemental heat and sterile ophthalmic lubricant need to be added to the procedure, the surgical timeline needs clarifications, and the use of Marcaine in surgical description needs clarifications. The committee recommends replacing the brand name Marcaine with bupivacaine and adding the alternative ropivacaine if Marcaine and bupivacaine are unavailable. The procedure “Jugular vein cannulation” clarifications need to be made to the sterilization procedures, the PPE and antiseptic technique needs more information, supplemental heat and sterile ophthalmic lubricant need to be added to the procedure, the sterile draping for surgery needs clarifications, the suture material, and pattern needs clarification and additional information. In the procedure “Buprenorphine HCL/ Sigma-Aldrich administration,” the NPG form needs to be revised to remove the statement “indefinite backorder” buprenorphine is no longer on backorder. The substance also needs to have the box checked for a controlled substance. The procedure “Euthanasia: Decapitation only” needs clarifications regarding the use of general anesthesia. The substance administration “Thiamine injection” needs to have the math for mg/kg double-checked. The substance administration “Substance Administration: Prenatal ethanol and sucrose gavage” needs clarifications to dosage and volume. The experiments “Generating control and prenatal ethanol exposed animals (Breeding)” need clarifications regarding the use of the Bell Jar for anesthesia, husbandry exceptions are needed for food restriction, cage changing schedule, single housing, use of wire bottom cages, and an enrichment wavier needs to be added. A husbandry exception needs to be added for single housing after jugular cannulation. The procedure “In vivo dopamine neuron recordings” needs a husbandry exception for the enrichment waiver and a scientific justification. The experiment “3 Intra-VTA microinjection and Amphetamine self-administration” needs a procedural timeline, a husbandry expectation for single housing and use of wire bottom cages, and an enrichment waiver with a scientific justification. The experiment “4 Microglia activation after prenatal ethanol exposure” needs a procedural timeline, a husbandry expectation for single housing, and an enrichment waiver with a scientific justification. The experiment “5 Optogenetic studies for dopamine

neuron activation" needs clarifications to the variable procedure, husbandry expectations for single housing, wire bottom cages, and enrichment waiver with a scientific justification. The experiment "6 prenatal ethanol effect on attention" needs clarifications to the experimental description; the husbandry exception is needed for an enrichment waiver with a scientific justification. The experiment "7 prenatal ethanol effect on mPFC synaptic function" needs an updated experimental timeline; a husbandry exception is needed for single housing. The experiment "8 prenatal ethanol exposure effects on dopamine neurons" needs husbandry exceptions for single housing and wire bottom cages. The experiment "9 Rescue attention deficits in ethanol exposed rats with environmental enrichment" needs an updated experimental timeline. The experiment "Tissue collection for mass spec and transcriptome studies" needs a substance administration added for the use of the Bell Jar. The animal justification needs clarifications, and additional information regarding the number of animals generated in the breeding experiment and the explanation of the statistical method used to generate the animal numbers 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.

6. **TR202200008 (PROTO201800154)**

Protocol Summary: From PI's non-scientific summary: The goal of the lab is to understand the role of complement in health and disease and to train the next generation of researchers.

Committee Discussion: Edits and clarifications need to be made in Click. All behavioral experiments need to indicate appropriate sanitation procedures. A husbandry exception needs to be added for any single housing. In the triennial review summary, the committee wanted to know how many mice were generated and euthanized because they were not the desired genotype. More information regarding congenital malocclusions and the statement "Unanticipated is the pandemic that caused loss of mice since we were not allowed into the building that caused mice generated for experiments to grow older than the age needed for the experiments had to be euthanized." is requested. The protocol team members section needs to be updated. Radiation safety training needs to be verified. The dosage for Fatal Plus used for euthanasia needs to be updated in all procedures. The committee recommends not using ear punching for MRL/lpr mouse strain. The committee recommends using alternative methods to CO2 euthanasia; if CO2 euthanasia is necessary, a scientific is required. The procedure "Treadmill" needs additional information regarding electric shock exposure. The procedure "Tail Biopsy" needs to be updated. The experiment "Assessment of renal blood flow" needs a substance administration for buprenorphine. The experiment "Bone marrow transplant" needs clarifications to the acronym FACS; a blood collection procedure and identification method must be added to the experimental timeline. The experiment "Cell Culture" needs to have the euthanasia procedure clarified. The experiment "Immune complex disease" needs clarifications to the strains being used in the experiment and the behavioral testing needs to be added to the experimental timeline. The experiment "Lupus" needs to describe the health monitoring for all MRL/lpr mice, the pathology due to phenotype, supportive care, and humane endpoints need to be indicated, and the procedure timing section must be completed. The experiment "PET Imaging of Lupus Mice" needs an updated procedural timeline. The experiment "pilot study for the irradiation of the MRL/lpr mice" the procedural timeline and the committee has requested information regarding it the experiment has been completed in the last three years and if the x-ray irradiation dosage has been optimized.

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 2:40 PM.