OMB APPROVED 0579-0036

Interagency Report Control No. 0180-DOA-AN

Fiscal year: 10/01/2021-09/30/2022

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Annual Report of Research Facility Column E Explanation

(TYPE OR PRINT)

This information is required by law (7 U.S.C. 2143 and 9 C.F.R. §2.36). Failure to report according to the regulations can result in an order to cease and desist.

| 1. REGISTRATION NUMBER | 2. Research Facility Headquarters address |
|---|--|
| E7 D 0003 | Emory University 954 Gatewood Road |
| 57-R-0003 | Atlanta, GA 30329 |
| | , |
| 3. Number of animals used in the study. | 4. Species (common name) of animals used in the study. |
| 24 | Hamster |

5. Explain the procedure producing pain and distress.

Procedures to be performed comprise of intranasal inoculation with SARS-CoV-2 and collection of samples by nasal lavage as a means of monitoring viral load following infection. Animals will be monitored daily for clinical signs and weight loss. Anesthesia will be used prior to both intranasal inoculation and nasal lavage. To test the impact of ambient temperature on SARS-CoV-2 transmission, it will be necessary to house animals at a range of temperatures to span conditions that would be experienced by humans indoors and outdoors across the seasons.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The goals of this study include establishing a model to study SARS-CoV-2. The infection with SARS-CoV-2 causes respiratory illness in hamsters. To evaluate the impact of relative humidity and temperature on the efficiency of transmission of SARS-CoV-2 in the hamster model system, different temperatures are used in this study to inform as to whether SARS-CoV-2 spread will be perpetuated through warm weather months and whether more efficient spread should be expected in the winter months. Anesthetics and tranquilizers are not ordinarily used in the treatment of pneumonia and continuous administration of analgesic agents may alter immune function and confound the study goal to characterize virus transmission as related to seasonality. Housing at 5-10oC may also be distressful to hamsters, however administration of analgesic agents likewise will influence thermoregulation and therefore virus transmission at the warm and cold extremes. The IACUC reviewed and approved these Class E procedures.

| Agency | CFR |
|--------|-----|
| NA | NA |
| | |

APHIS Form 7023B

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0036. The time required to complete this information collection is estimated to average .5 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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| | 954 Gatewood Road |
| | Atlanta, GA 30329 |
| | |
| 3. Number of animals used in the study. | 4. Species (common name) of animals used in the study. |
| | Hamster |
| 18 | |
| | |

5. Explain the procedure producing pain and distress.

Animals will be infected with C. difficile. The goal is to understand how C. difficile colonizes the intestine and survives in the host to cause disease. To achieve this goal, it is necessary to observe the animals throughout the duration of an experiment (up to 10 days). Animals receive anti-C. difficile polymers to be tested in these studies are designed not to be systemically bioavailable prior to the infection. Animals are weighed daily and monitored at least daily. Animals will be assessed for signs of infection and distress (diarrhea, poor fur coat, inactivity, hunched posture). If the animals become moribund before the conclusion of the experiment, they will be humanely euthanized.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

Clostridioides difficile infection results in diarrhea and colitis. These acute studies lasting 10 days seek to characterize how C. difficile colonizes the intestine and survives in the host to cause disease. Specifically, these experiments seek to test anti-C. difficile polymers for inhibition of C. difficile infections in vivo, which have shown low toxicity in vitro. Anesthetics and tranquilizers are not used in the treatment of diarrhea or colitis. The use of analgesics may affect gut motility and local immune function and thus are contraindicated in these acute experiments. The IACUC reviewed and approved these Class E procedures.

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|---|--|
| | Emory University |
| 57-R-0003 | 954 Gatewood Road |
| | Atlanta, GA 30329 |
| | |
| 3. Number of animals used in the study. | 4. Species (common name) of animals used in the study. |
| 2 | D 11% |
| _ | Rabbit |

5. Explain the procedure producing pain and distress.

Rabbits were used in a project to study Retinoblastoma tumor formation and progression. Animals were injected with tumor cells and then treated with different agents including cyclosporin A to see if tumor formation and progression is affected. Retinoblastoma usually does not metastasize to the distant organs; most of the expected complications arise from the daily injection of cyclosporin A. Animals are monitored during the study using standard IACUC approved guidelines, and if animals reach humane endpoint prior to study completion they are euthanized.

6. Provide the scientific justification for not providing the appropriate anesthetics, analysesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

Animals were injected with tumor cells and then treated with different agents including cyclosporin A to see if tumor formation and progression is affected. Treatment to relieve pain and stress due to tumor formation and cyclosporin A treatment was withheld because the administration of pain-relieving drugs would affect inflammatory responses which could affect the study results. The protocol has a well-established plan to alter cyclosporin-A treatment levels, or sacrifice animals if toxicity is expected.

The IACUC reviewed and approved these Class E procedures.

| Agency | CFR |
|--------|-------------------------------|
| NA | NA Obtained by Rise for Anima |

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| | Emory University |
| 57-R-0003 | 954 Gatewood Road |
| | Atlanta, GA 30329 |
| | |
| 3. Number of animals used in the study. | 4. Species (common name) of animals used in the study. |
| | Spiny Mouse |
| 71 | |
| | |

5. Explain the procedure producing pain and distress.

Spiny mice were used in a project involving mild stressors that are categorized as class "E". Animals are exposed to conditions including the following procedures: the resident intruder test, latency to feed in presence of a novel object (food restriction), and social stress exposure. In the resident intruder test, the animal is acclimated for 1 h in the test cage, then a novel, same-sex conspecific of the approximate body weight of the subject is placed into the test cage for 5 min. Spiny mice in the resident intruder test are observed continuously with intervention and immediate termination in the event of wounding or severe distress. For the social stress exposure, subjects are removed from their home cage using a beaker and are placed alone in a novel, clean cage for 1 hr. In the latency to feed experiment, animals are food deprived for 3 hours before the initiation of the test. Animal subjects will be placed in a novel open arena containing a novel object (a 3D printed 'giant' red/blue/yellow lego person) with sunflower seeds at the feet of the object. The latency to approach the novel object to obtain the sunflower seeds will be recorded.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The goal of these studies is to deconstruct social behavior in order to have a comprehensive understanding of the factors that influence social interactions and to identify similarities and differences in the mechanisms underlying them. In these tests, animals are exposed for a limited time to an intruder, food restriction, or removed to single housing. Treatment to relieve the temporary stress is withheld because the investigator studies the response of the experimental animal to a mildly stressed stimulus. Although the food restriction is short, not unlike that experienced in nature or for meal-fed animals, and the likelihood of injury was low and the period of stress was limited, the IACUC reviewed and approved these as Class E procedures.

| , | . What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulatio | ns |
|---|--|----|
| | CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102): | |

| Agency | CFR |
|--------|-----|
| NA | NA |

57-R-0003 FY22_E.pdf OMB APPROVED 0579-0036

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| 1. REGISTRATION NUMBER | 2. Research Facility Headquarters address |
| 57-R-0003 | Emory University 954 Gatewood Road Atlanta, GA 30329 |
| 3. Number of animals used in the study.34 | 4. Species (common name) of animals used in the study. Spiny mouse |

5. Explain the procedure producing pain and distress.

The subject will be placed in a standard rat cage and allowed 5 minutes to acclimate. A conspecific (either familiar same-sex, novel same-sex, or novel opposite-sex) will then be placed inside the cage with the subject. The interacting spiny mice are observed continuously with intervention and immediate termination in the event of wounding or severe distress. Behavior will be video recorded for 20 minutes. At the end of the test, all animals are returned to their home cages with their siblings (with whom they've been housed with for the entirety of the experiment/their lives).

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

In order to understand the factors that influence and the neural mechanisms underlying social behavior, it is necessary to observe animals engaging in social interactions. Social interactions include a range of behaviors that may be displayed including positive investigation, positive side-by-side contact, huddling, allogrooming, following, negative side-by-side contact (i.e., threatening posture), chasing, pacing. Capturing positive and negative social behavior is crucial for understanding the social phenotype of an animal. Although the likelihood of injury was low and the period of stress was limited, the IACUC reviewed and approved these as Class E procedures.

7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102):

| Agency | CFR |
|--------|-----|
| NA | NA |

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|--|--|
| 57-R-0003 | Emory University |
| | 954 Gatewood Road |
| | Atlanta, GA 30329 |
| | |
| 3. Number of animals used in the study | 4. Species (common name) of animals used in the study. |
| 31 | Gerbil |
| | |
| | |

5. Explain the procedure producing pain and distress.

A large, four-sided arena (120 x 120 x 60 cm) is divided in two with a removable divider. Each side contains food, water, and two plastic rodent igloos. Groups of Gerbils acclimate to their respective side for 3 hours, and then the divider is removed so that groups can interact for 1 hour. Introducing two novel groups can result in aggressive behavior, including chasing, aggressive grooming or posture, lunging, swiping, sniffing, pinning, and attack behavior. During the acclimation phase for 3 hours, animals will be only interacting with their cagemates, and thus will be monitored every 30 minutes. However, all 1-hour group interactions will be constantly monitored by the experimenter. The test will be immediately terminated if injury occurs, and veterinary staff will be consulted.

6. Provide the scientific justification for not providing the appropriate anesthetics, analysesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

It is of great interest to understand the characteristics of group interactions that result in positive interactions and aggressive interactions. In controlled experiments (particularly those that involve neural manipulation), studies that potentially involve aggression can ethically only be conducted in non-human animals. Thus, there is the potential for translational insight to be gained from the examination of group dynamics in these experiments. Although the likelihood of injury was low and the period of stress was limited, the IACUC reviewed and approved these as Class E procedures.

| Agency | CFR |
|--------|---------------------------------|
| NA | NA Obtained by Rice for Animals |

OMB APPROVED 0579-0036

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| | | | | |

| 1. REGISTRATION NUMBER 57-R-0003 | 2. Research Facility Headquarters address Emory University 954 Gatewood Road Atlanta, GA 30329 |
|--|---|
| 3. Number of animals used in the study.48 | 4. Species (common name) of animals used in the study. Gerbil |

5. Explain the procedure producing pain and distress.

The study will use potentially painful or distressing procedures (forced-swim task, elevated plus maze, intruder task that are thought to probe human symptoms of depression or other mood disorders). In the behavioral test forced swim task, the gerbil is placed into a vat of cold water for six minutes (Wallace-Boone et al., 2008). Animals are dried off with a towel and warmed under a heat lamp at the conclusion of the procedure. In the Intruder test - A resident gerbil will be taken to a neutral cage and allowed to acclimate to the new cage. Behavioral responses to the intruder gerbil will be documented. Gerbils in the swim test and the resident intruder test are under continuous observation and will be terminated if the subjects are in distress.

6. Provide the scientific justification for not providing the appropriate anesthetics, analysesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The purpose of these tests is to assess the animal's emotional state. Animal responses to unrelieved stress is thought to correlate with human depressive-like conditions. Animal models of human psychological conditions are imperative to understanding mood and psychological issues. Thus, utilizing animal models of human behavior (learned-helplessness here) can provide powerful insights into the experimental manipulations and how they might impact the human condition. Although the period of stress was limited and not likely to result in nonadaptive distress, the IACUC reviewed and approved these as Class E procedures.

| 7. What, if any, Federal regulation | ns require this procedure? Cite | e the agency, the Code of Fe | deral Regulations |
|-------------------------------------|---------------------------------|------------------------------|-------------------|
| (CFR) title number, and the specia | fic section number (e.g., APH | IIS, 9 CFR 113, 102): | |

| Agency | CFR |
|--------|-----|
| NA | NA |

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| 1. REGISTRATION NUMBER | 2. Research Facility Headquarters address |
| 57-R-0003 | Emory University 954 Gatewood Road Atlanta, GA 30329 |
| 3. Number of animals used in the study.16 | 4. Species (common name) of animals used in the study. Gerbil |
| | |

5. Explain the procedure producing pain and distress.

In this behavioral test, the subject is placed in a standard rat cage and allowed 5 minutes to acclimate. A conspecific (either familiar same-sex, novel same-sex, or novel opposite-sex) is then placed inside the cage with the subject. Behavior will be video recorded for 20 minutes. At the end of the test, all animals are returned to their home cages with their siblings (with whom they've been housed with for the entirety of the experiment/their lives). Interacting gerbils are observed continuously with intervention and immediate termination in the event of wounding or severe distress.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

In order to understand the factors that influence the neural mechanisms underlying social behavior, it is necessary to observe animals engaging in social interactions. Social interactions include a range of behaviors that may be displayed including positive investigation, positive side-by-side contact, huddling, allogrooming, following, negative side-by-side contact (i.e., threatening posture), chasing, pacing. Capturing positive and negative social behavior is crucial for understanding the social phenotype of an animal. Although the likelihood of injury was low and the period of stress was limited, the IACUC reviewed and approved these as Class E procedures.

| Agency | CFR |
|--------|-----|
| | NA |

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|------------------------------|-------------|------------------------|--------|-----------------------|---------------------|--------------------|-------------------------|----------------|
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| 1. REGISTRATION NUMBER 57-R-0003 | 2. Research Facility Headquarters address Emory University 954 Gatewood Road Atlanta, GA 30329 |
|---|---|
| 3. Number of animals used in the study. 36 | 4. Species (common name) of animals used in the study Voles |

5. Explain the procedure producing pain and distress.

Mild stressors categorized as class "E" were used in this project. In these experiments voles were housed in pairs. One subject was placed in a chamber and mild stressors were administered. This animal was then returned to the home cage and the consoling behavior (e.g. grooming) delivered by the cage mate was quantified.

6. Provide the scientific justification for not providing the appropriate anesthetics, analgesics, or tranquilizing drugs during procedures where the animal experienced accompanying pain or distress greater than momentary or slight.

The goal of these studies is to understand the neural circuitry underlying empathy-related behavior and the role of oxytocin in these behaviors. Individuals with autism show deficits in empathy-related behaviors and oxytocin is a potential treatment for autism. Treatment to relieve the temporary stress was withheld because the investigator was studying the response of the experimental animal to a mildly stressed stimulus animal. The IACUC reviewed and approved these Class E procedures.

| 7. What, if any, Federal regulations require this procedure? Cite the agency, the Code of Federal Regulation | ons |
|--|-----|
| (CFR) title number, and the specific section number (e.g., APHIS, 9 CFR 113, 102): | |

| Agency | CFR |
|--------|-----|
| NA | NA |