

Summary of Studies (Animal) Listed in Column E

VOLES

In protocol PROTO201700811, a total of 19 animals were used in projects involving mild stressors that are categorized as class "E". In protocol PROTO201900151, a total of 34 voles were used in projects involving mild stressors categorized as class "E". For both protocols the same mild stressor procedure was performed. 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. In these experiments, animals 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 consoling behavior (e.g. grooming) delivered by the cage mate was quantified. 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.

Protocol #	Name of Study	# Animals Reported
PROTO201700811	Development of Viral Vector Mediated CRISPR/Cas9 Editing of the Prairie Vole Oxytocin Receptor Gene	19 Voles
PROTO201900151	Generation of Oxytocin Receptor (Oxtr) tdTomato Knock-in (KI) Prairie Voles by CRISPR/Cas9 for Visualization of Expression of Oxtr in the Brain and Analysis of Transgenic Prairie Voles.	34 Voles

In PROTO201900094 a total of 293 voles were used in projects involving mild stressors that are categorized as class "E". 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. Animals are exposed to conditions which induce mild stressors including the following procedures: the resident intruder test, water restriction (as a stressor), experimental "divorce", and social stress exposure. A human observer is present at all times with the animals during the resident intruder and social stress exposure tests. In social stress exposure, the stimulus animal is exposed to 30 min of stress due to being isolated and being unable to huddle with their cage mate. For the water restriction animals are provided 50% or their normal water as a model of drought conditions. In the experimental divorce experiment breeding pairs are separated for three days to simulate partner loss. Treatment to relieve the temporary stress was withheld because the investigator was studying the response of the experimental animal to a mildly stressed stimulus. The IACUC reviewed and approved these Class E procedures.

Protocol #	Name of Study	# Animals Reported
PROTO201900094	Mechanisms of Social Behavior	293 Voles

Hamsters

In protocol PROTO201700074 a total of 30 hamsters were used to produce *Schistosoma haematobium* eggs for mesocosm experiments that require snail exposure to miracidia. Hamsters were exposed to 350 *S. haematobium* cercariae in 2 cm deep room temperature water for 1 hour for infection. Hamsters are required as surrogate definitive hosts for *S. haematobium*. It is expected that the parasite infection levels will have only subtle effects on the animals' behavior and survival. Nevertheless, infection by any parasite may cause pain, discomfort or distress and methods described will be continuously refined so as to reduce

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animal discomfort. Palliative treatment options such as NSAIDs and analgesics are not recommended because they impair the growth of infecting schistosomes, reducing the number of viable eggs that are produced (Farag et al. Annals of Tropical Medicine and Parasitology, 1995, 89:497-504, Nessim and Mahmoud, International Journal of Infectious Diseases, 2007, 11:161-165), which would then require the use of additional animals and potentially affect the validity of research projects by increasing variability in egg viability or infectivity after hatching. Animals were housed with appropriate enrichment and monitored for clinical symptoms of schistosomiasis daily. The IACUC reviewed and approved these Class E procedures

Protocol #	Name of Study	# Animals Reported
PROTO201700074	Schistosome life cycle	30 Hamsters

Rabbits

In PROTO201700130, a total of 12 class E 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. 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

Protocol #	Name of Study	# Animals Reported
PROTO201700130	Rabbit model of uveal melanoma	12 Rabbits

Gerbils

In PROTO201900126 a total of 43 class E Gerbils were used in a project involving mild stressors that are categorized as class "E". 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. Animals are exposed to conditions which induce mild stressors including the following procedures: the resident intruder test, Latency to feed in presence of a novel object (food restriction), and social stress exposure. A human observer is present at all times with the animals during the resident intruder and social stress exposure tests. In social stress exposure, subjects will be removed from their home cage using a beaker and will be placed alone in a novel, clean cage for 1 hour. In the latency to feed experiment, animals will be food deprived for 3 hours before the initiation of the test. Animal subjects will be placed in a novel open arena (63cm W x 63cm L x 30cm H) containing a novel object (a 3D printed 'giant' red/blue/yellow lego person, 16cm tall) with sunflower seeds at the feet of the object. The latency to approach the novel object to obtain the sunflower seeds will be recorded. The test will be terminated when the subject eats the seeds or after 30 minutes. Treatment to relieve the temporary stress was withheld because the investigator was studying the response of the experimental animal to a mildly stressed stimulus. The IACUC reviewed and approved these Class E procedures.

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Protocol #	Name of Study	# Animals Reported
PROTO201900126	Spiny Mouse Sociality	43 Gerbils

Spiny Mouse

In PROTO201900126 a total of 40 Spiny mice were used involving mild stressors that are categorized as class "E". 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. Animals are exposed to conditions which induce mild stressors including the following procedures: the resident intruder test, Latency to feed in presence of a novel object (food restriction), and social stress exposure. A human observer is present at all times with the animals during the resident intruder and social stress exposure tests. In social stress exposure, the stimulus animal is exposed to 30 min of stress due to being isolated and being unable to huddle with their cage mate. In the latency to feed experiment, animals will be food deprived for 3 hours before the initiation of the test. Animal subjects will be placed in a novel open arena (63cm W x 63cm L x 30cm H) containing a novel object (a 3D printed 'giant' red/blue/yellow lego person, 16cm tall) with sunflower seeds at the feet of the object. The latency to approach the novel object to obtain the sunflower seeds will be recorded. The test will be terminated when the subject eats the seeds or after 30 minutes. Treatment to relieve the temporary stress was withheld because the investigator was studying the response of the experimental animal to a mildly stressed stimulus. The IACUC reviewed and approved these Class E procedures.

Protocol #	Name of Study	# Animals Reported
PROTO 201900126	Spiny Mouse Sociality	40 Spiny Mice

Approved Exceptions:

9 C.F.R. 2.31(d)(1)(x)(C): On December 11, 2019 Emory University received an exception letter for permitting more than one survival surgery involving three Rhesus Macaques in order to conserve overall animal use. The letter states the following "The request for an exception is **approved from December 11, 2019 to July 17, 2022** as a Special Circumstance. These animals that underwent craniotomies under one study, will be used in a different study for which craniotomies are required, reducing the need to sacrifice additional animals to achieve the same purpose. The surgeries are to be performed under protocol #201900088 during the approval period. Emory University must reflect this exception on its Annual Reports for each year within the approval period as required under Title 9 Chapter 1 Subchapter A Section 2.36(b)(3)." All 3 animals were assigned, and the research completed by July 2020.

Annual Report for Research Facilities, Emory University,

Atlanta, GA Certificate 57-R-0003, Customer 896

Attachment 2 to APHIS Form 7023

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