

Registration Number: 58-R-0001

Customer Number: 873

**Florida State University
Laboratory Animal Resources
107 Chieftan Way**

(b) (7)(F)

Category E Explanation

Procedures Producing Pain or Distress

Forced swim test: Following an already established protocol for voles (DeVries *et al.*, 1996; Bosch *et al.*, 2009; Lieberwirth *et al.*, 2012), animals are placed into a large clear tank (25 x 45 x 20 (H) cm) filled with tap water to a height of 13 cm. This water level ensures that the animal can neither touch the bottom of the container (with paws and/or tail) nor climb out of the container. Immediately before the start of the test, the tank is filled with tap water at a temperature of 32 ± 1 °C. Hot and cold tap water are mixed to reach the target temperature and a thermometer is used to ensure the appropriate temperature. Due to the size of the tank and the water volume, it is expected that the water temperature will not drop significantly during the 5-minute test.

Restraint Stress: This is also an established protocol used in our previous studies (Smith & Wang, 2014; Smith *et al.*, 2016). Restraint tubes are constructed from 10.5cm (length) by 1.75cm (radius) PVC pipes with air vent holes in front for animal respiration and a 1.75cm (radius) opening in the back for animal placement. During the restraint stress treatment, voles will be removed from the home cage and wrapped (excluding the head) in cloth mesh to minimize handling time and risk of injury associated with handling. Subjects will be placed into restraint tubes by a skilled individual. Time of restraint is 1 hr, after which animals are removed from the tube and will undergo behavioral testing.

Scientific Justification for Withholding Pain Relieving Drugs

The forced swim test described above is essential to better understand the role of newly adult-generated cells in mediating depression-like behavior. The forced swim test is a widely accepted paradigm to study stress and passive-coping/depression-like behaviors. In this model, rodents are forced to swim in a tank from which they cannot escape. At the beginning of the test, rodents tend to swim vigorously, but will tend to become immobile over time. Although, the exposure to the forced swim test results in an increased stress level in rodents; unfortunately, this test-induced stress cannot be alleviated by pharmaceutical intervention prior to the forced swim test, since the purpose of the research is to study the effects of unmitigated stress. However, to minimize the pain and distress to the animals, the following procedural techniques have been implemented. To prevent hypothermia, the water temperature will be 32 ± 1 °C. This temperature is relatively close to the body temperature of voles (37.9 °C) and has been used previously in the forced swim test for prairie voles (DeVries *et al.*, 1996; Taymans *et al.*, 1997; Lieberwirth *et al.*, 2012). In addition, after removal from the water, the vole is dried with towels, kept in a warm enclosure (next to heat lamps) for 30 minutes, and only returned to its home cage once the fur is completely dry. During the test, the experimenter remains in visual proximity of the testing apparatus to monitor each animal and remove the animal if needed. Removal of the animal and immediate termination of the test occurs if an animal appears to be in danger of drowning either by prolonged immobility or by sinking. The test is only performed once, is of very short duration (no more than 5 minutes) and avoids any possibility for physical injury as might occur with the alternative test, the tail suspension test.

The restraint stress is an established treatment known to increase physiological stress responses (e.g., increased levels of circulating corticosterone) and anxiety-like behavior. Unfortunately, these treatment-induced stress cannot be alleviated by pharmaceutical intervention prior to the treatment, as the purpose of such treatment is to induce stress responses in order to examine the effects of social buffering on stress responses. Based on this design, the animal's respiration is not hindered, as air vent holes have been made to the front side of the tube, and the cloth mesh that is wrapped around the body of the animal is loose around the torso and abdomen. In addition, voles will be secured in cloth mesh (excluding the head) to minimize handling time and risk of injury associated with handling and placement into restraint tubes. Before placement into restraint tubes, voles will be monitored to assure unrestricted respiration. Voles will then be inserted face-forward into the restraint tube. After the cap is secure, technicians will evaluate the position of the vole through the air vent holes to confirm that the vole is positioned such that the face is next to the air vent holes, not pressed against any surfaces, and that the vole is laying on its ventral side.