

**Registration Number: 58-R-0001**

**Customer Number: 873**

**Florida State University  
Laboratory Animal Resources  
107 Chieftan Way  
101 Biomedical Research Facility**

**Category E Explanation**

**Species: Prairie voles**

**Number of Animals: 689**

**Procedures Producing Pain or Distress**

**Forced Swim Test:** The forced swim test is a 1-day procedure in voles (up to 6 minutes duration). They are forced to swim under conditions in which they cannot escape. At the start of the experiment, subjects are placed in a large clear tank (25 x 45 x 20 (H) cm) filled with tap water at a temperature of  $32 \pm 1^\circ\text{C}$  to a height of 13 cm, a level ensuring that the animal can neither touch the bottom of the container (with its paws and/or tail) nor climb out of the container. The subject is allowed to swim for up to 6 min and its behavior is video-recorded for later analysis. At the end of the session, the subject is taken out of the tank, dried with towels, and kept in a warm enclosure for 30 min.

**Restraint Stress:** Restraint tubes are constructed from 10.5 cm (length) by 1.75 cm (radius) PVC pipes with air vent holes in front for animal respiration and a 1.75 cm (radius) opening in the back for animal placement - a design used in our previous studies (Smith et al., 2016; Smith and Wang, 2014). 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 for up to 1 hr, after which they will undergo behavioral testing. Before placing into restraint tubes, subjects will be monitored to assure unrestricted respiration. During the restraint treatment, animals will be closely watched during the first 10 mins and checked regularly thereafter (every 10 mins) to ensure that the signs defined in our answer to question 5 below are not present. If any of those happens, they will be released from the restraint tube, removed from the study, and returned to their home cages. Only a single restraint session will be used.

**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, voles are forced to swim in a tank from which they cannot escape. At the beginning of the test, voles 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 voles; 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 voles, the following procedural techniques have been implemented. To prevent hypothermia, the water temperature will be  $32 \pm 1^\circ\text{C}$ . This temperature is relatively close to the body temperature of voles ( $37.9^\circ\text{C}$ ) 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, this treatment-induced stress cannot be alleviated by pharmaceutical intervention prior to the treatment, as the purpose of such treatment is to induce stress responses to examine the effects of social buffering on stress responses. The restraint stress is able to induce the desired conditions using a controlled environment. The protocol mirrors published and validated protocols in the vole literature [(DeVries et al., 1997); (Pournajafi-Nazarloo et al., 2007); (Pournajafi-Nazarloo et al., 2009)] including studies performed by this research group (Smith et al., 2016; Smith and Wang, 2014). 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.