Column E Explanation (Amendment)

This form is intended as an aid to complete the Column E explanation. It is not an official form and its use is voluntary. Annual Reports and explanations should NOT include PI information such as names (principal investigators and research staff), addresses, protocols, meeting notes (either in part or in full), the animals room numbers, grant information, veterinary care programs, and the like. A Column E explanation must be written so as to be understood by lay person as well as scientists.

1. Registration Number___48-R-0002, Customer ID Number: 1459__

2. Number_____384_____of animals categorized as column E used in this study.

3. Species (common name)____Prairie Voles_____of animals used in this study.

4. Explain the procedure producing pain and/or distress. Explanations should include a brief description of the procedure, but also explain what the animal's experience, examples of which may include, but are not limited to: Neurological signs, seizures, tremors, paralysis, lethargy, inappentance, respiratory signs, GI distress, vomiting and diarrhea.

   The overall scientific objectives of the research project are to evaluate the role that stress has on physiology, neurochemistry, and behavior associated with social behavior to determine the influence that stress has on sociality. In order to reach these objectives, prairie voles are utilized because of their unique social systems including mating induced formation of pair bond-related behaviors including partner preferences and selective aggression in prairie voles with male prairie voles displaying paternal behavior toward their own offspring or conspecific pups. These behaviors are modulated by stressful life events tested through various methodologies described in further detail below.

   Animals involved in these two specific experimental test methods are considered Category E due to the induced stress as part of the research objectives. They are unable to escape a known stressful situation that occurs for a duration longer than what is considered "momentary." Analgesics, topical antiseptics, and antibiotics are utilized for wounds incurred during these tests whenever possible as long as it does not interfere with study objectives. To minimize pain and distress from wounding, there is an established rubric to evaluate wound size and severity with the humane endpoint of euthanasia for significant wounding.

Resident intruder test (RIT): This is a commonly used test to examine aggressive behavior. The subject is placed in an arena (20x25x45cm) to establish "residence." After 10 min, an intruder (a same or different sex individual from the same species) is introduced into the arena and aggressive behavior on the part of the resident is quantified for a further 10 min. Aggression typically is manifested by threat displays and chases, although biting does occasionally occur. The use of large cages gives the animals sufficient room for separation. Animals are closely monitored throughout the test to ensure that neither animal is

seriously harmed. The test will be terminated if either animal appears to be unable to defend themselves. Frequency could include a single exposure only or once per day for up to seven days. Bite wounds are common. Animals with wound(s) exceeding 1 cm, wound(s) that involve the face or genitals, or full thickness wounds that expose bone or viscera will be euthanized. Mild wounds will be treated with a topical antiseptic (i.e., chlorhexidine).

Social defeat (SD): This is a commonly used test to examine the consequences of an agonistic interaction. The subject is placed in an arena (20x25x45cm), an established "residence" home cage. Aggressive behavior on the part of the resident is quantified for 30 min with an additional 30 min of non-physical contact using a divider between resident and intruder. Aggression typically is manifested by threat displays and chases, although biting does occasionally occur. The use of large cages gives the animals sufficient room for separation. Animals are closely monitored throughout the test to ensure that neither animal is seriously harmed. The test will be terminated if either animal appears to be unable to defend themselves. Frequency could include a single exposure only or once per day for up to seven days. Bite wounds are common. Animals with wound(s) exceeding 1 cm, wound(s) that involve the face or genitals, or full thickness wounds that expose bone or viscera will be euthanized. Mild wounds will be treated with a topical antiseptic (i.e., chlorhexidine).

5. Attach or include with the reason(s) for why anesthetics, analgesics and tranquillizers could not be used. (For federally mandated testing, see Item 6 below).

   There may be times when medical treatment of wounds inflicted during these tests is not possible due to interference with data collected related to stress response. When performing this type of stress modeling, behavior is a component measure that is evaluated; however, neurotransmitter levels in the brain are the most critical variable for analysis. Use of systemic drugs including analgesics or antibiotics can alter the production and thus measurement of various neurochemicals of interest, which would negatively impact research outcomes. To minimize pain and distress from wounding, there is an established rubric to evaluate wound size and severity with the humane endpoint of euthanasia for significant wounding.

6. What, if any, federal regulation 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): If the requirement is per a guidance document, such as an Agency notice or harmonization guideline, please provide specific sufficient information to identify the cited document.

   Agency_____Not applicable_____CFR_____Not applicable_____