

University of Kansas Medical Center – Customer ID: 1460
Column E Explanation

The justification for the animals listed in column E of the annual report is listed below.

1. Registration Number: 48-R-0003
2. Number of animals used in this study: 6
3. Species (common name) of animals used in this study: non-human primates
4. Explain the procedure producing pain and/or distress.

The potentially painful or distressful procedures involved in this study include disease progression, morphine withdrawal and desensitization to morphine.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

As stated in the IACUC-approved protocol, morphine has a profound effect on the immune system by its ability to prevent development of cell-mediated immunity (CMI) responses against intracellular pathogens. This effect has important implications for the pathogenesis of Human Immunodeficiency Virus (HIV) infection, especially NeuroAIDS. First, morphine modifies the receptor used by the virus to infect macrophages, and this potentially causes enhancement of infection in these cells. Because macrophages are the main cells in the brain that support replication of the virus, the effect of morphine would be to enhance the infection in the brain. Second, morphine-mediated suppression of production of IFN γ would abolish anti-HIV CMI responses, the major component of the immune system, responsible for controlling replication of the virus. Loss of CMI would therefore predict continuous and more robust replication of the virus in the brain. Third, cessation of morphine intake results in recovery of previously abolished CMI responses. Such an occurrence in the HIV-infected individual would result in reconstitution of the antiviral CMI response in brains that have large amounts of viral antigen, and this could precipitate severe encephalitis. The researcher uses the morphine-Simian Immunodeficiency Virus (SIV) model to explore these concepts.

All non-human primates in this protocol are infected with simian immunodeficiency virus (SIV). Additionally, a subgroup receives injections of morphine 3 times/day to produce well maintained morphine dependence, and another control subgroup receives saline injections. Use of analgesic and/or anxiolytic drugs as supplements to morphine, or in the subgroup of non-human primates not receiving morphine, would compromise the results. These analgesic and anxiolytic drugs are known to interact with the immune system (e.g. Mitrova & Mayer, 1976; Ferrarese et al., 1993; Ghosh & Chattopadhyay, 1993; Burdo et al, 2006; Cho 2007). Because the saline subgroup of monkeys serves as a

control for the morphine group, treating the saline subgroup with analgesic/anxiolytic drugs would compromise the interpretation of data.

References

1. Burdo TH, Katner SN, Taffe MA, Fox HS (2006) Neuroimmunity, drugs of abuse, and neuro-AIDS. *J. Neuroimmune Pharmacol.* 1: 41-49.
2. Cho JY (2007) Immunomodulatory effect of nonsteroidal anti-inflammatory drugs (NSAIDs) at clinically available doses. *Archives of Pharmacal Research* 30: 64-74.
3. Ferrarese I, Appollonio I, Bianchi G, Frigo M, Marzorati C, Pecora N, Perego M, Peirpaoli C, Frattola L. (1993) Benzodiazepine receptors and diazepam binding inhibitor: a possible link between stress, anxiety and the immune system. *Psychoneuroendocrinology* 18: 3-22.
4. Ghosh N, Chattopadhyay U (1993) Enhancement of immune response by phenothiazine administration in vivo. *In-Vivo* 7: 435-440.
5. Mitrova E and Mayer V (1976) Phenothiazine-induced alterations of immune response in experimental tick-borne encephalitis: morphological model analysis of events. *Acta Virol.* 6: 479-485.
6. 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 _____

N/A

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IACUC Approved Exceptions

IACUC-approved exceptions are provided below.

1. Species: Non-human primates

Exception: Group housing

- Number of animals affected: 6

The IACUC approved an exception to group housing and animal-to-animal contact as part of the environmental enrichment for Simian Immunodeficiency Virus (SIV)-infected and Simian Human Immunodeficiency Virus (SHIV)-infected non-human primates. All non-human primates on this protocol are infected with SIV. This exemption was requested because the animals are immunocompromised and are more prone to secondary infections. Paired housing would introduce the possibility of viral transmission from one monkey to the other and compromise the data resulting from the approved experimental design. It is anticipated that single housing will reduce this risk. Additionally, because the non-human primates used in these studies have little previous exposure to group contact, the possibility of injury and/or death in a group setting is high.

- Number of animals affected: 51

The IACUC approved an exception to group housing for non-human primates involved in behavioral studies following ischemia. This exemption is necessary to facilitate the conduct of experimental procedures. The behavioral tasks involve the direct retrieval of food pellets or squeezing interactions, such as squeezing manipulanda that generate delivery of food pellets. Because the animal's home cage also serves as its behavioral testing cage, a pair housed non-human primate would have to compete with its cage mate for task participation and delivered food rewards.

- Number of animals affected: 7

The IACUC approved an exception to group housing for non human primates with head chambers. The veterinarians believe group housing these non-human primates increases the risk of chamber dislodgement and opportunity for infections, because they may pick at others' chambers with more destructive manipulation than a single primate can do to himself or herself.

Total number of non-human primates approved for an exception to group housing: 58

2. Species: Non-human primates

Exception: Manipulation Devices

- Number of animals affected: 58

After consultation between the veterinarian and the investigator, the IACUC approved an exemption to restrict environmental enrichment, specifically manipulation devices for animals on this study. The consistency of the physical environment is a primary concern, as motor skills and behaviors are the primary variable being manipulated and studied. Therefore, physically manipulatable enrichment items and procedures must be limited, because the degree to which individual animals will interact with such items or situations cannot be predicted, controlled or assessed but may have significant consequences for motor skill acquisition or recovery of function after ischemia. Additionally, studies conclude with post mortem examination of anatomical connections between brain regions. Such connections are influenced by the experimental and environmental factors that affect motor behavior.

3. Species: Non-human primates

Exception: Multiple survival surgeries

- Number of animals affected: 58

These experiments are designed as within-subject comparison studies, in part because the researchers have previously observed that the inter-subject variation in the experimental endpoints is often greater than within-subject variation. As such, the use of map-remap surgical procedures and repeated-measures statistical techniques is required. Further, the scientific aims of tracking the time course of post-injury events, such as axonal sprouting or genetic signals in specific subsets of neurons, cannot be achieved with only a single surgery design.

The minimum proposed interval between major surgeries is two weeks, with some intervals ranging up to three months. This lab has extensive prior experience with such intervals and has observed no adverse effects on animal behavior as a consequence of these repeated procedures. In almost every experiment, at least two survival surgeries are needed, and in some cases three surgeries are needed.

4. Species: Gerbil

Exception: Group Housing

- Number of animals affected: 68

Following consultation with the veterinarians, the IACUC approved an exemption from group housing for gerbils whose cage mates have been removed from study. The justification (from the 8th edition of the "Guide for the Care and Use of Laboratory Animals" page 51) is "Social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility". The default is 2 animals per cage and that both animals should be euthanized the same day (as suggested by veterinarian consult). This was problematic, because the researchers performed an acute preparation (surgery + physiologic recording) on animals and then sacrificed them immediately for histology. Performing the procedures on 2 animals in a given day is not feasible because of the length of the experiment. Sacrificing the second animal without obtaining any data would require us to use more animals to obtain the same objectives, and bring into question "reduction". The IACUC approved that the maximum time frame an individual animal will be housed is 1 week (most often, it will only be 2-3 days).

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