**VERTEBRATE ANIMALS**

1. **Description of Procedures:** A goal of this project is to XXXX. All procedures involving mice (males and females) are in accordance with the National Institute of Health guidelines for use of live animals (NIH Publication No. 85-23, revised in 1985).

*Aim 1.* In Aim 1, we will use two mouse strains - chosen because xxxx. We will use male and female mice, and ages xxxxx.

# Table 1. Groups for Aim 1

|  |  |  |
| --- | --- | --- |
| Total: | # mice |  |
| **Group** | **Treatment Age start treatment** | **Time of Sac (# of mice)** |  |
| 1. | Control xx weeks | xx month (n = 20: 10 male, 10 female) |  |
| 2. | Treatment xx weeks | xx month (n = 20: 10 male, 10 female) |  |

In collaboration with our statistician, we carried out a ***power analysis***, and determined the appropriate sample sizes for the study. The number of mice to be used for each study is based on parameters from our previous studies.

***Power***. Desired sample size for each factor-level combination to achieve 80% power when N=20 for an analysis of variance test with 5% level of significance (Put this also into main text. Power calculation has to be in main text too)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| N=20 | 1.0 | 1.25 | 1.5 | **2.0** | **2.5** |
| n = sample size | 33 | 28 | 23 | **20** | **10** |

We assumed that there will be equal numbers of experimental units (n) in each of the 8 factor-level combinations (Groups). Furthermore, we assumed that the level of significance that will be utilized in the analysis of variance test will be 5%, and we desired a power of at least 80% whenever the ratio between Δ, the range of the means among the five factor-level combinations, and σ, the standard deviation of the error term in the model is at least k, where the value of k is specified in the sample size table (Table 1). The appropriate sample size, according to these power requirements, is well-known from statistical theory [see for instance Chapter 26 of Neter, Kutner, Nachtsheim, and Wasserman (1990)], and a table of these desired sample sizes is provided in Appendix B12 of the aforementioned book. Therefore, if at least 80% power is desired whenever >2, then for each combination of type and treatment, at least xx experimental mice will be required per group for each time point. A total of xx mice for Aim 1.

*Aim 1.* In Aim 1, we will use two mouse strains - chosen because xxxx. We will use male and female mice, and ages xxxxx.

# Table 2. Groups for Aim 2

|  |  |  |
| --- | --- | --- |
| Total: | # mice |  |
| **Group** | **Treatment Age start treatment** | **Time of Sac (# of mice)** |  |
| 1. | Control xx weeks | xx month (n = 20: 10 male, 10 female) |  |
| 2. | Treatment xx weeks | xx month (n = 20: 10 male, 10 female) |  |

# TOTAL NUMBER OF MICE FOR THIS PROJECT: xxxx mice

1. **Justifications:** The experiments in this project are aimed at determining the ability of xxxx in male and female mice xxx mice. These experiments cannot be replicated in an in vitro system and must be done in intact animals where conditions mimic those found in human (name disease). Thus, these experiments can only be carried out in the context of the whole animal. If successful, our approach will ultimately be tested in humans in clinical trials, but the approach is too preliminary at the present time to be tested in humans.
2. **Minimization of Pain and Distress:** Describe the interventions to minimize discomfort, distress, pain, and injury. These include analgesia, anesthesia, sedation, palliative care, and humane endpoints. All procedures in this proposal are approved by the UofSC Institutional Animal Care and Use Committee. All procedures are limited to that which is necessary to achieve the objectives of the proposed project. We do not anticipate any toxic or adverse side effects from the treatment, however, we will carefully monitor the mice for any signs of sickness, pain, or discomfort during the treatment weeks. Mice will be weighed daily during the treatment period. We will consult the university veterinarian if animals show signs of distress. Mice that are deemed moribund will be removed from the study and sacrificed by cervical dislocation after administration of anesthesia using isoflurane by inhalation. This method of euthanasia is humane and consistent with the recommendations of the Panel of Euthanasia of the American Veterinary Medical Association. This same method of euthanasia will be used to sacrifice mice at the end of the experiments.
3. **Euthanasia:** Mice will be euthanized by cervical dislocation under isoflurane anesthesia. Isoflurane ensures that mice are unconscious before cervical dislocation. These procedures minimize animal distress, is efficient and effective and is consistent with the AVMA Guidelines for the Euthanasia of Animals.