

The IACUC should send this protocol back to Foxworthy and ask for a scientific justification for the selection of his control groups and for a statistical justification for his sample sizes.

1. Pitcher, G.M., Ritchie, J. & Henry, J.L. Nerve constriction in the rat: model of neuropathic, surgical and central pain. *Pain*, **83**, 37–46 (1999).
2. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8<sup>th</sup> edn. (National Academies Press, Washington, DC, 2011).

Bailey is the Director of Comparative Medicine, Attending Veterinarian and Adjunct Professor of Physiology and Loh is a Clinical Veterinarian at the National University of Singapore, Singapore.

**RESPONSE**

**The answers are in experimental design**

Felicia Duke, DVM & Kimberly Jen, DVM, MS

It is beyond the scope of an IACUC to determine the scientific merit of a study, but it is evident from Foxworthy’s proposal and the questions of the IACUC reviewer that the IACUC should consider this study’s experimental design during the review process. This scenario presents three questions. The first asks whether we can reduce the number of animals to be used without compromising scientific integrity; the second asks whether a scientifically sound reduction of animals to be used introduces concern for the welfare of the remaining animals; the third asks whether there are even enough animals in the non-surgical control group. Without any additional information, the best answer we can offer to any of these questions is “maybe.” If the reviewers ask the right questions, though, they can evaluate the proposed use of animals in the context of the overall study design, and then knowledgeably reply “yes” or “no” to each question.

The first question asks whether combining groups and carrying out two treatments on a single set of animals will confuse the study’s results, as Foxworthy claims. This depends on the outcomes he intends to measure. For example, if the study’s aim is to demonstrate the test drug’s effects on neuropathic pain using an ethogram, then

a contralateral sham surgery has significant potential to alter the animals’ pain-associated behaviors and confuse interpretation. However, previous literature does describe contralateral sham surgeries with no ill effects<sup>1</sup>, suggesting that the sham surgery might be minimally or transiently painful and might not alter behavioral metrics. This makes the possibility of combining groups an appealing option, but one that still requires rigorous and recent support from scientific literature or pilot studies. If, however, Foxworthy intends to analyze only post mortem tissue, then combined groups might be acceptable or even preferable for control purposes. These different possibilities illustrate how knowledge of an experiment’s design is necessary to determine the scientific propriety of combining groups.

If such a reduction of animals is shown to be scientifically sound, the IACUC must then address the second question: would animals undergoing bilateral surgery experience an ethically acceptable degree of pain and distress? Foxworthy and the IACUC should evaluate published or pilot data in consultation with a veterinarian to determine whether bilateral surgery is significantly more debilitating than unilateral sciatic constriction, and whether that debilitation necessitates procedural refinement. If that debilitation is deemed so severe as to warrant analgesics, but analgesics are contraindicated for research purposes, then perhaps the IACUC should favor the use of more animals so that each rat experiences less pain and distress. But if the debilitation is mild and transient, then short-term analgesic use might be permissible alongside the scientific aims of the study. This decision would also support the option of carrying out both nerve constriction and sham surgeries on only one set of animals. Here, again, knowledge of the experimental design is critical for determining the best course of action.

More information is also needed to address the third question of whether five rats comprise a sufficient control to evaluate the effects of this drug treatment. If this drug’s effects under control conditions are already well characterized through the previous work of Foxworthy or others, then a small control group might suffice, particularly if the effects are statistically rare and of little physiological significance.

Reviewers with concerns regarding the proposed number of animals and sample sizes should request justification from the investigator. They should then evaluate the propriety of the investigator’s justification and decisions, consulting with a statistician if needed. The reviewer could also consider whether Foxworthy’s design calls for an additional control group that receives only surgery.

Each of these questions could be answered yes or no, and each for multiple reasons. The correct answers depend on many factors, all of which depend on the scientific aims of the study and the procedures that are intended and proposed to achieve those aims. This, in summary, is the experimental design of the study. Foxworthy might need 25, 45 or even 100 rats to draw defensible conclusions, but more information is needed to justify that number and determine how those animals can best be used.

1. Bennett, G.J. & Xie, Y.K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **33**, 87–107 (1988).

Duke and Jen are Postdoctoral Fellows and Veterinary Residents with the Unit for Laboratory Medicine at University of Michigan, Ann Arbor, MI.

**RESPONSE**

**Welfare comes first**

Nancy A. Johnston, DVM, MS, DACLAM

Foxworthy’s proposed experiment will inherently and necessarily cause pain in rats, as this is the focus of the research. The reviewer in this scenario has asked Foxworthy to consider reducing the number of animals to be used in this experiment, in accordance with the principle of reduction from the 3Rs (ref. 1). The reviewer noted that surgery is performed on only one hind leg of each animal, thus the other leg could be used as a control on the same animal. However, this viewpoint fails to consider the animal as a whole, as each rat in this study will experience and respond to pain from sciatic nerve constriction throughout its whole body to some degree. The contralateral leg