

Jerald Silverman, DVM, Column Coordinator

When reducing numbers might increase pain

Neuropathic pain, or pain that arises from disorders of the nervous system, is a challenge to treat. One of the more common research techniques used to induce neuropathic pain is known as the chronic constriction injury model¹. The model involves placing a series of loosely tied sutures around the sciatic nerve of a rat. Within about a week after surgery this results in signs of pain in the rat's affected leg, such as licking or biting of the leg, avoiding use of the leg, and limping if the leg is used at all.

Dr. John Foxworthy studies the mechanisms of action of drugs that were used or proposed to be used to treat neuropathic pain. For an upcoming study he proposed using the chronic constriction model on twenty rats that would also receive a drug

that was believed to modulate calcium ion conductance. Another twenty rats would be treated with the same drug and undergo the same surgical manipulation but without any constriction of the sciatic nerve. One additional group of five rats would only be treated with the drug. During the IACUC review of Foxworthy's protocol one of the reviewers questioned the need for one of the study groups, suggesting that the surgical procedure without the nerve constriction could be performed on the same rat's other hind limb. That way the number of animals to be used would be significantly reduced, and one leg would serve as a control for the other leg. Foxworthy replied that he would rather use the extra twenty rats, thereby causing less pain to

more animals rather than more pain to fewer animals. He also said that even without constricting the sciatic nerve of the contralateral leg, a second surgery on the same animal would confuse the interpretation of his findings.

Do you agree with the opinion of Foxworthy or the IACUC reviewer? Assuming a sample size of $n = 20$ is appropriate for each of the two surgical groups, do you believe that $n = 5$ is an appropriate number of animals for the non-surgical control group?

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).

RESPONSE

Request for more information

Michele M. Bailey, DVM, MRCVS, DACLAM & Michelle Loh, BVSc

The IACUC reviewer asks a good question, but we do not feel that the contralateral leg would serve as a good control. Rats receiving surgery on both hind legs would ambulate differently from rats undergoing unilateral ligation or unilateral sham surgery, since the former would not have the option of compensating with an unaffected hind leg. This could interfere with the assessment of pain responses. Previous research indicates that "unilateral constriction of the left common sciatic nerve... gives rise to a marked increase in sensitivity to normally innocuous tactile stimuli in both the nerve-injured as well as the intact contralateral hind paws. Although less in magnitude and duration, surgery alone without nerve constriction also produces

a decrease in withdrawal threshold of each of the hind paws"¹. Accordingly, this study "demonstrated that the contralateral hind paw and either hind paw in sham-operated rats are inappropriate as 'controls'."

Additionally, it is unclear which parameters Foxworthy will be monitoring to assess whether the drug treatment is effective. Will he use behavioral, biochemical or other tests? For example, if measuring serum cortisol levels as an indicator of effectiveness, Foxworthy would not be able to distinguish between the drug's effectiveness after nerve constriction and its effectiveness after sham surgery if both legs are on the same animal. If, during publication, reviewers should deem that sham surgery on the contralateral leg is not a scientifically valid control treatment, the experiment might need to be repeated using new and separate groups of rats for nerve constriction and sham surgeries, which would relegate the original group of rats to wasted animals.

Regarding the size of each group, no data have been provided to explain and support the use of 20 animals per experimental group. The investigator should provide

justification for the group size. The *Guide for the Care and Use of Laboratory Animals* states that the number of animals and size of experimental groups should be statistically justified whenever possible². Assuming that the IACUC is given sufficient justification for the group size and finds that 20 rats per group is a valid sample size, it is then unlikely that a control group of 5 rats will reach statistical significance if all groups are assessed according to the same methods. It is important that control groups be of a statistically valid size.

In addition, we feel that the IACUC should query the rationale behind using as controls rats that underwent sham surgery and were treated with the drug and also rats that underwent no surgery and were treated with the drug. If the purpose of the study is to determine a drug's effectiveness in controlling neuropathic pain then a better control might be a single group that undergoes nerve constriction but does not receive the drug treatment. Although such an untreated nerve-constricted group is a less palatable option, it would reduce the number of animals used in total.

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* 8th edn. (National Academies Press, Washington, DC, 2011).

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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 effects¹, 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).

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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

A word from OLAW

In response to the questions posed in this scenario, the Office of Laboratory Animal Welfare (OLAW) offers the following clarification and guidance:

The key issues raised in the scenario are: 1) whether the experimental design is consistent with the strategic aims of the research; 2) concerns for animal welfare in considering two of the three “Rs”, reduction versus refinement; and 3) if the statistical power of the animal numbers in the control group is appropriate for the study.

Although an IACUC’s primary focus is on animal welfare, often it must include consideration of the soundness of the research design in its review of protocols. The *Guide* states that “While the responsibility for scientific merit review normally lies outside the IACUC, the committee members should evaluate scientific elements of the protocol as they relate to the welfare and use of the animals”¹. If a rationale for the experimental design is unclear to the IACUC then the committee should request further clarification from the investigator.

Minimizing the number of animals is a worthwhile consideration, but it must allow for valid results and be balanced by the discomfort, distress and pain experienced by each individual animal². The *Guide* states that “reduction involves strategies for obtaining comparable levels of information from the use of fewer animals or for maximizing the information obtained from a given number of animals (without increasing pain or distress) so that in the long run fewer animals are needed to acquire the same scientific information” and that the goals of refinement versus reduction “should be balanced on a case by case basis”¹.

Whenever an IACUC is faced with complex issues, including the statistical justification for control and experimental groups, it should consider using consultants to provide expert counsel³.

1. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
2. Interagency Research Animal Committee. *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training*. (Office of Science and Technology Policy, Federal Register, Washington, DC, 1985).
3. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986; revised 2015).

Patricia Brown, VMD, MS, DAACLAM

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OLAW, OER, OD, NIH, HHS

the long run fewer animals are needed to acquire the same scientific information”¹.

In this scenario, the reviewer’s suggestion to prioritize reduction before welfare would reduce the number of animals used in Foxworthy’s experiment, but it would also increase the pain and distress for each of those animals, probably beyond an acceptable threshold. The Office of Laboratory Animal Welfare endorses prioritizing the welfare of individual animals above the principle of reduction, prescribing that “procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design”². Foxworthy’s preference, using more animals so that each animal experiences less pain, is a better option than the alternative, using fewer animals with each animal experiencing more pain. Foxworthy’s design will maximize the welfare of each rat in his experiment and reduce the presence of unwanted variables in the resultant dataset.

Every IACUC is charged with evaluating the experimental models and design proposed in each protocol. They must consider what level of pain and distress is acceptable for each animal and what methods are most likely to generate reliable data. IACUC members should recognize that experimental groups are made up of individual animals, and it is the IACUC’s responsibility to help ensure the welfare of each and every animal used for research at the institution. Principal investigators must strive to generate high quality data while concurrently minimizing the pain and distress of their research animals. In this scenario, Foxworthy should be allowed to perform his research as described in his protocol with group sizes large enough to minimize pain and distress for each animal.

1. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
2. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).

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is therefore not a suitable control because the limb is not separate from the whole animal and not immune to systemic pain. It would compromise both the welfare of each rat and the experiment’s data, which would introduce new unwanted variables and interactions, if Foxworthy were to follow the reviewer’s suggestion.

Amendments in adherence to the principle of reduction must be evaluated with the entire experiment in perspective, as rigid interpretation of this principle can demand that an IACUC compromise its other responsibilities. It is a clear mandate of biomedical research that investigators reduce the number of animals used in experiments, so researchers must clearly

justify their sample sizes. But they must also maintain concern for the welfare of each individual animal in their experiments. For Foxworthy’s proposal, the IACUC must consider what level of pain and distress is acceptable for each rat. The upper limit of distress for each rat must be defined and not exceeded, even if this requires compromising other principles, such as reduction. The *Guide for the Care and Use of Laboratory Animals* explicitly states that “reduction involves strategies for obtaining comparable levels of information from the use of fewer animals or for maximizing the information obtained from a given number of animals (without increasing pain or distress) so that in