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Collaborative studies and animal reuse

Toby Pfeiffer, PhD, was well known for her contributions to the field of learned helplessness and its relationship to prisoner-of-war captivity stress in humans. She used squirrel monkeys for much of her research, subjecting them to mild but frequent electrical shocks, such as 0.75 milliamps for 30 s, repeated 20 times in 1 hour. She justified her methodology to the IACUC by noting that she had subjected herself to the same shock intensity and duration and had felt only moderate pain. She said that the monkeys also appeared to have experienced only moderate discomfort, as evidenced by the shifting of their position and related evasive movements, but no vocalizations or other indicators of severe pain. Furthermore, she presented data showing that cortisol levels in shocked monkeys were only slightly higher than those in unshocked control monkeys. These statements were typically included in all of Pfeiffer's protocol applications. Her studies included behavioral tests followed by euthanasia and subsequent brain structural and biochemical analyses.

Pfeiffer's most recent protocol submission was somewhat different. She planned

to use a modified animal shock protocol (fewer repetitions) and then carry out her behavioral testing. Instead of euthanizing the monkeys, however, she proposed sending them to a nearby colleague who would carry out multiple physiological samplings (metabolomics) with equipment available only in his laboratory. As part of his work he would reshock the animals, using the same methodology used by Pfeiffer. Pfeiffer's protocol went into great detail about the science behind both studies, the methods of transporting the animals, the means of accounting for shipping and other stressors, the need for approval by the receiving university's IACUC and many other details. Initially, this seemed somewhat reasonable to the IACUC, but after a discussion with Pfeiffer, the committee became firm in its contention that the two studies were independent and could be done with two groups of squirrel monkeys, not just Pfeiffer's. The committee decided that it was inappropriate to subject the animals a second time to the inescapable electrical shocks. In other words, the IACUC concluded that the reuse of the animals was

more of a convenience and cost savings than a required extension of Pfeiffer's research. Approval of the study was withheld.

Pfeiffer responded to the committee's action with three related arguments. The first was that the IACUC was essentially forcing the use of twice as many squirrel monkeys because her colleague would now have to purchase the same number of animals that she already had. The second argument was that the learned helplessness, not the shocks, was the real source of animal distress and would not be induced in this study. Her final argument was that there is no federal regulation against subjecting an animal to repeated mild (or even severe) pain or distress in the same or in unrelated experiments. In her proposed protocol, Pfeiffer argued, there was only moderate and transient pain, and she disagreed with the IACUC that the studies were unrelated.

Should the IACUC reconsider its decision and allow the animals to be sent to Pfeiffer's colleague, based on the arguments she presented?

RESPONSE

One study, two institutions

Betsy L. Bashaw, BS, LATG, CMAR, Jeffrey Carlson, PhD & Karen Krause, DVM

Learned helplessness is an animal model of human depression. The subject gives up trying to escape a noxious situation and learns that any action on his part is fruitless¹. The NRC *Guidelines for the Care and Use of Animals in Neuroscience and Behavioral Research* state, "In all those behavioral tests of depression, proposed procedures for monitoring, recordkeeping, and humane intervention should be described in the associated animal-use protocol and approved by the IACUC"². Pfeiffer seems to have satisfactorily assured the IACUC that

her original protocol was in compliance with these guidelines.

Metabolomics is defined by the National Institutes of Health (NIH) as a science whose goal is "to identify, measure, and interpret the complex time condition-dependent concentration, activity, or flux of metabolites in cells, tissues, and other biosamples, such as blood, urine, and saliva" (<http://grants1.nih.gov/grants/guide/rfa-files/RFA-RM-06-010.html>). The NIH issued Requests for Applications for grants supporting metabolomic studies in this and recent years in a roadmap initiative to encourage the use of innovative technologies in metabolomics.

Pfeiffer can justify the use of her colleague's equipment to get metabolomics data as a continuation of her own studies of learned helplessness, but that doesn't seem to be all that's going on here. There are many unanswered

questions for the IACUC to ask. We see no reason to purchase additional monkeys. It is part of the IACUC's duty to keep animal numbers to the minimum statistically required. We do, however, feel that more information is needed to explain the changes to the original protocol Pfeiffer suggests. Will she still be euthanizing the animals and doing the brain analysis after her colleague obtains his data? Why is she reducing the number of shocks? Her argument falls down, in my opinion, when she states that these animals will not be in a state of learned helplessness when they are sent out. Can she still compare the data with that from her previous protocols? And if they are not inducing learned helplessness, then is she perhaps using the animals in this protocol to get control data for studies in the future? From the information given, we cannot tell. The notes given suggest that

the IACUC learned something from Pfeiffer that lessened their confidence in her motives, but we do not know what that is. Reducing cost, of course, cannot be the sole reason for using these animals in related studies. A clearer outline of the study sequence would be desirable, such as (i) shock treatment to induce learned helplessness (ii) behavioral testing of the monkeys (iii) shipment to the collaborating institution for metabolomics data and (iv) return of the monkeys to the original site for euthanasia and brain analyses data. We would recommend that this protocol be conditionally approved, allowing the shipment of the monkeys to the second site, pending justification of the changes in shock administration and satisfactory explanation of why and where who is doing what.

1. Seligman, M.E. Learned helplessness. *Annu. Rev. Med.* **23**, 407–412 (1972).
2. *Guidelines for the Care and Use of Mammals in Neuroscience* 141 (National Academies Press, Washington, DC, 2003).

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RESPONSE

Inter-related, not just related

Kathryn Nepote, VMD, MPH

In this scenario, there is a difference of opinion on the appropriate use of the experimental monkeys. The primary investigator wants to maximize the amount of information obtained from these monkeys. But the IACUC is not convinced that exposing the monkeys to two different studies, both of which involve unavoidable shocks, is both appropriate and scientifically necessary. The PI is obviously aware of various aspects of the animal welfare regulations, as she understood the need to obtain her IACUC's approval of the animal studies that would occur at her colleague's institution, to address transportation and shipping concerns and to obtain approval from the receiving university's IACUC. Her responses to the IACUC's concerns also show her knowledge base. She says that the reuse of the monkeys is an appropriate application of the 'reduction' alternative.

The real distress to the monkeys is the learned helplessness, and not the shock itself, and the shocks are transient and moderate. Finally, there is no federal regulation preventing repeated pain or distress in the same or unrelated experiments.

But the IACUC is correct to not allow her to carry out the studies on the same monkeys. Her response does not address the costs and benefits of painful procedures. The IACUC does not dispute the scientific validity of the individual studies. They just do not agree that the use of the same monkeys is appropriate and necessary. Just because the studies are related does not mean that the use of these monkeys for both is appropriate. The IACUC must determine in such studies whether the unrelieved pain is necessary to produce valid data. Pain is pain, and unavoidable shocks produce pain. Thus, both studies must be considered to be 'unrelieved pain' studies. Although the regulations do not specifically state that animals cannot experience unrelieved pain in experiments, they do state that this pain must be scientifically justified. One should also assume that as with major surgeries, the intent of the regulations is to limit the pain experienced by any one animal to that which is absolutely necessary.

Thus, I believe the IACUC must be convinced that the two studies are not just related but are inter-related. Specifically, the second study must require monkeys that have experienced the procedures done by Pfeiffer. These experiences must be a necessary and essential prerequisite for the studies done by her colleague. Then and only then should the IACUC permit the use of the monkeys for both studies.

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RESPONSE

The other IACUC should decide

Debra L. Hickman, DVM, MS, DACLAM, Eden Paster, DVM & Eric Tonsfeldt, BS, RLAT

Federal regulations reinforce the need for reduction of animal numbers and minimization of experimental pain and distress. This IACUC determined that although

sharing the monkeys would decrease the animal numbers, it would also increase the amount of pain and distress experienced by each animal. Although we concur with the concerns raised by the IACUC, we point out that they have jurisdiction only for work done at their facility. Assuming Pfeiffer has adequately justified the scientific merit of her proposed studies, shown an appropriate search for alternatives, and taken steps to minimize the pain or distress associated with the proposed procedures, her part of the study should be approved. The evaluation of previously manipulated versus naive animals should be done by the IACUC of the collaborator's institution.

Pfeiffer raised three concerns after the IACUC's decision. Her first was that the IACUC was advocating doubling the number of animals used. Although this is correct, we are concerned that Pfeiffer's shock protocol would induce an exaggerated response to the second shock protocol, thus confounding the experimental results obtained by her collaborator. We would propose that the procedure be modified so that the shock protocol is done only once, with both investigators collecting the data they require. This would minimize both animal numbers and the pain and distress experienced by each animal.

Second, Pfeiffer claimed that learned helplessness was the principal source of distress in her studies. She qualified this statement with the claim that she had experienced only minimal pain when applying the shock to herself, indicating that her proposal involves only moderate and transient pain. Pfeiffer then concluded by stating that no federal regulations prohibit the exposure of an animal to repeated pain or distress in the same or unrelated experiments.

The Animal Welfare Regulations define pain as "any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied." It further charges the investigator to assure that there are no alternatives to the proposed procedures and that potential pain or distress is minimized¹. The USDA Policy 11 states that "noxious electrical shock that is not immediately escapable" is a procedure that may cause more than momentary pain or distress and that animals experiencing this procedure must be reported in column

E of the USDA annual report with the IACUC-approved written justification for using this procedure². The proposal under review reportedly uses fewer repetitions, but the strength and duration of the shock are still substantial and have the potential to cause distress, requiring the IACUC to assure that the amount of distress experienced has truly been minimized. Although she applied the shock to herself, Pfeiffer experienced it a single time. Her experimental subjects experience this shock up to 20 times in an hour. Other studies with squirrel monkeys have suggested that vocalization and cortisol levels may not be reliable indicators of pain or distress in this species³.

Ultimately, the use of these animals on the second study is at the discretion of the collaborator's institution. However, the IACUC at Pfeiffer's institution is expected to evaluate the disposition of the animals. Because the disposition includes plans for further use on similar studies, discussion is appropriate.

1. 9 CFR Subchapter A – Animal Welfare, §2.31
2. Animal Care Policies 11.1-2, USDA, APHIS, AC [9 CFR, §2.31(d)(1)]
3. Weiner, S.G. *et al.* Influence of postnatal rearing conditions on the response of squirrel monkey infants to brief perturbations in mother-infant relationships. *Physiol. Behav.* **39**, 21–26 (1987).

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A word from OLAW and USDA

In response to the issues raised in this scenario, the Office of Laboratory Animal Welfare (OLAW) and the United States Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care (USDA/APHIS/AC) offer the following clarification and guidance:

The Animal Welfare Act and regulations (AWR) and the PHS Policy require research facilities to ensure that procedures involving animals will avoid or minimize discomfort, distress and pain to the animals. The responses to this scenario all provide thought-provoking questions for IACUCs to consider.

One of the responses to this scenario suggested 'conditional approval' for transportation of the animals to the second site. Though well intentioned, this suggestion is not acceptable. The AWR and the PHS Policy do not allow IACUCs to grant conditional approval for animal use protocols. Committees may only approve, require modification (to secure approval) or withhold approval of a protocol, and we highly recommend the use of this unambiguous language when interacting with the principal investigator^{1,2}. An IACUC has the authority to require more information (such as justification of the changes in shock administration) and, upon receipt of that information, may then decide whether to approve or withhold approval. All animal activities must be conducted under an approved IACUC protocol, including the transportation of animals between research sites.

1. Garnett, N.L. & DeHaven, W.R. So much work, so little time. OPRR and USDA commentary. *Lab Anim. (NY)* **27**, 18 (1998).
2. Wolff, A., Garnett, N., Potkay, S., Wigglesworth, C., Doyle, D. & Thornton, V. Frequently asked questions about the Public Health Service Policy on Humane Care and Use of Laboratory Animals. *Lab Anim. (NY)* **32**, 33–36 (2003).

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