

Overview

Ethical and IACUC Considerations Regarding Analgesia and Pain Management in Laboratory Rodents

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Scientists have ethical and regulatory commitments to minimize pain and distress during their use of sentient laboratory animals. Here I discuss pain as a special form of distress and the long history of ethical and regulatory standards calling on scientists to prevent, minimize, treat or terminate animal pain. Scientists, veterinarians, and IACUC face 2 challenges: knowledge of effective analgesic doses and regimens for all sexes, ages and genotypes of rodent is incomplete, and concerns regarding the effects of analgesic drugs on research outcomes push scientists to request approval to withhold analgesics and leave animal pain unalleviated. IACUC thus conduct what I call an ‘ethics of uncertainty,’ in which they factor in the limits of available ethically relevant information on the amount of expected animal suffering, the usefulness of analgesics to mitigate this suffering, and the eventual benefits that come from the research. IACUC must factor in current limitations in severity assessments of various experimental manipulations in various strains, inaccurate pain diagnosis, in known effective analgesic and other refinements, and on effects of pain medications and untreated pain on data outcomes, when deciding to allow potentially painful experiments and animal care practices. This article focuses on 3 areas of concern: the limits of veterinary “professional judgment” when the animal model’s degree of pain and the efficacy of pain medications are not yet known; the review of proposals with known, unalleviated significant pain and distress (that is, Category E experiments); and the attempt to review the balance between animal welfare harms and scientific objectives. I propose no new regulations, standards, or ethical norms herein but rather explore some of the implications when existing ethical principles are applied to evolving scientific knowledge (and vice versa). I conclude that applying current animal pain management knowledge to prevailing ethical principles will shift IACUC toward greater caution in allowing potentially painful animal experiments, with heightened caution regarding the ability of analgesics to mitigate the animals’ pain.

Abbreviation: AWA, Animal Welfare Act

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In this special edition of *Comparative Medicine*, several authors come together to update animal researchers, veterinarians, and ethics committee members on the science of laboratory animal pain recognition and management. This new knowledge will influence how scientists conduct their animal experiments and how oversight bodies conduct reviews of the scientists’ work.

Just as animal welfare science has been advancing over the years, so too have regulations and ethical standards, sometimes influenced by new welfare knowledge, sometimes driving the need to conduct welfare studies. For example, emerging information on the development of pain sensation in zebrafish has resulted in official NIH guidance in their use. The NIH Office of Laboratory Animal Welfare updated its regulatory requirements for IACUC oversight of these animals.⁶ They defined 72 h postfertilization (hpf) as “hatching,” which thus classifies 72-hpf zebrafish as animals under the Public Health Service Policy. But NIH’s internal policies, endorsed by OLAW, claim—without cited references—that zebrafish cannot experience pain in the

4- to 7-d postfertilization period, thus apparently suggesting IACUC should count zebrafish that are at this stage as animals but not worry much more about them. This conclusion is ethically odd if pain in sentient animals is the driving concern and if the NIH’s claim that these stages cannot experience pain, in fact, holds up to further investigation.^{34,40} The issue illustrates how animal welfare science can shape regulation and practice.

New knowledge can also lead to less formal changes in standards, possibly at the local IACUC level. For example, one group found that automated conditioned place preference testing detected changes in mouse behavior due to growing bladder tumors earlier than less sensitive or later-occurring measures, such as weight loss, hematuria, and human observation of behavior.⁴⁹ Although that work⁴⁹ has changed no formal standards, regulations, or guidelines, IACUC should certainly consider such data in reviewing humane endpoints for studies and in determining how much to rely on cageside pain assessments during the conduct of a study.

Just as animal welfare data can drive ethical standards, so too can regulatory and ethical changes lead to the quest for new animal data. This event happened after Congress amended the Animal Welfare Act (AWA) in 1985 to include provisions for

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psychologic wellbeing of NHP.⁴⁸ The new mandate required real animal welfare data for its responsible deployment and accelerated welfare scientists' efforts in this area.^{7,8,36,43,46,58} Likewise, the 2011 *Guide's* increase in recommended cage size for mother mice with litters similarly spurred studies to better characterize space needs.^{12,26,28,38,57} We can expect a similar wave of animal welfare studies if AAALAC and the next *Guide* increase their coverage of cephalopods in American laboratories. Evidence-based welfare standards are valuable, and when the welfare standards change ahead of the available evidence, welfare scientists must catch up; conduct valid, reproducible welfare assessments; and fine-tune the regulatory standards as needed.

Ethics and regulations are closely bound. At their best, regulations reflect and operationalize societal ethics regarding the consideration that animals deserve. But regulations cannot cover every detail of every situation, and ethical principles underlie and are implicitly part of the many decisions an oversight committee makes.

In this article, I describe some of the ethical and regulatory concerns that guide laboratory animal care and use decisions, specifically on matters of animal pain. I describe how some IACUC actions that appear to be simple applications of existing rules are, in fact, ethical decisions, even if not always described as such. I discuss how our still-partial knowledge of animal pain recognition and effective analgesics must factor into our ethical reviews. I raise concerns about the most explicitly ethical job for the IACUC—weighing the objectives of the research against the potential animal welfare concerns—and discuss the challenges of doing so in a meaningful way.

Case Study: Orthotopic Brain Cancer Studies in Naked Mole Rats

We start with a hypothetical case, reviewing the ethical, factual, and regulatory questions the IACUC asks when reviewing a scientist's proposal to use animals in her research. Intrigued by the resistance of naked mole rats (NMR; *Heterocephalus glaber*) to cancers and eager to test novel anticancer drugs, Dr Glaber at Great Western University proposes to anesthetize NMR, surgically drill a small hole in the skull, inject tumor cells into the brain, close the surgery site, and study the animals' response to drug or placebo over several months.

Although the species choice may be novel, this hypothetical protocol would not be unusual for an IACUC that covers a busy cancer biology program. The IACUC applies standards of the Animal Welfare Act and of the Public Health Service Policy to review the work. The scientist must consider nonanimal models or presumably nonsentient animals, such as nematodes, as replacements. She must consider nonpainful experimental designs, such as injecting tumors under the skin instead of performing brain surgeries. The IACUC can standardize its reviews by conducting a birth-to-death pain and welfare inventory, highlighting those points where pain may be a significant welfare risk for the animals. At the start of life, are there painful methods of animal identification or sample collection? At the end of life, is there a euthanasia method that minimizes pain and distress? Between these extremes, 2 pain-associated time periods merit special attention: the potential days-long subacute pain related to the initial surgery to place the tumor cells, and later, the chronic pain that a developing brain cancer might cause the animals. The possibly painful side effects of cancer chemotherapy drugs are another concern.

Having identified the high points of concern regarding animal pain, the IACUC considers ways to prevent whatever pains

can be prevented, to treat whatever unpreventable pains can be treated, and to set humane endpoints when serious, ongoing pain is beyond prevention and treatment. To review pain management in this hypothetical protocol, the IACUC, research scientist, and attending veterinarian must consider how a clinician would decide the pain diagnosis, degree of pain, availability of effective analgesics, most effective and safe dosing regimens for those analgesics, and side effects and stresses of analgesic administration and use, and then balance analgesic effects and side effects with the animal's welfare as the sole consideration. They then must go beyond the welfare considerations and also evaluate the influence of analgesics on the experiment, justification for any pain that exceeds what the analgesics can treat, and signs that will indicate the time for humane euthanasia.

Principles of IACUC Reviews

These are standard IACUC review items, the details of which may fully occupy the IACUC's attention. But it's good to take a step back and ask, "Why is the IACUC doing this?" Brain cancer is a serious disease. Might not the time spent writing an IACUC protocol be better spent doing the research? Do IACUC not jeopardize important research with the requirements for animal pain management they impose? Ethics committees and IACUC may not often ask these basic why-are-we-doing-this questions, given that the ethical principles and current regulatory mandates for IACUC oversight at this point in the 21st century are so universal as to be almost invisible.

Decades have now passed since the first editions of several seminal works in laboratory animal welfare and policy. Russell and Burch, Bernard Rollin, the 1966 and 1985 Animal Welfare Acts, the United States Government Principles, NASA's Sun-downer Principles, the Brambell Report, and the early editions of the *Guide for the Care and Use of Laboratory Animals* have all shaped thought and practice.^{4,5,13,31,39,47,50,55,56} After decades of minimal regulation in the United States in the early 20th century, the paradigm shifted from the mid 1960s to the late 1980s, establishing the basic premise of IACUC and ethics committee functions: that a committee of veterinarians, nonscientists, and community members works with scientists to minimize harms to sentient animals in laboratories. Pain and distress (as compared with, for example, death, lack of self-determination, thwarting of an animal's *telos*) are the harms of concern in most of these seminal works and in the laws, standards, and policies under which researchers operate.

These efforts established most of the general principles under which modern scientists and IACUC operate. The principles cover pain and distress together. In this special issue of *Comparative Medicine*, and in this article, however, the focus is specifically on pain. Pain and distress often track together but are not inseparable companions. Not all pains are of sufficient duration or intensity to rise to the level of distress. Nor is pain the only source of distress for laboratory animals; hunger, thirst, social stresses, and restraint may all cause distress that does not involve pain.^{15,37,41} Both pain and distress should be prevented if possible; if they cannot, pain is typically treated by using medications (analgesics), and simply stopping the procedure may not stop the pain. Distressors are more often ameliorated by stopping the source of distress, rather than by treating with drugs. This simple distinction warrants isolating pain and analgesics for special focus in this issue.

With regard to animal pain, some established principles are: 1) "Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of

society;⁵⁶ 2) More than minor or momentary pain requires special consideration; 3) Conversely, minor and momentary pain is not a significant enough harm to warrant special treatment; 4) Absent good animal data, scientists must extrapolate from the human situation and assume that what is painful to a person is painful to an animal; and 5) IACUC may approve unalleviated significant animal pain if research data require it. These principles do not derive directly from any particular ethical framework. Discussions of competing ethical theories—whether deontology, utilitarianism, contractarianism, or ethics-of-care—are not particularly illuminating for the applied ethics of IACUC work. Committed utilitarians can argue for complete abolition of most animal research or for the abolition of constraints on animal research, depending, for example, on how they weigh current NMR in the laboratory against generations of potential human brain cancer patients and how they weigh the suffering of prolonged untreatable human brain cancer against the temporary pains of surgery and tumor growth in the NMR. The same goes for deontologists and rights theorists, who argue from a starting position of granting rights to all sentient beings or solely to humans. Instead, we live in a time of a widespread societal and legal consensus that harmful uses of research animals are permissible, but with ethical constraints on how much animal harm we cause.

Principles of applied animal ethics are consistent with some weak versions of rights theories, or with utilitarian or consequentialist reasoning, as long as people agree to recognize some moral status for sentient nonhuman animals.^{9,10,21-23} This concern for sentience, more or less, the ability to have pleasant and unpleasant feelings that matter to them, does not derive from the various ethical theories. It is instead a starting concern, a value judgment. In most situations, we focus on animals' experiential wellbeing, that is, how they are feeling right now.^{21,32} Few laboratory animals live long enough for people to worry about balancing current good or bad feelings against longer-term health and welfare outcomes. In current times, animals have a moral status that affords them some protection but nonetheless allows human interests in knowledge and its fruits to outweigh animal interests.

Simply put, scientists have an ethical and regulatory commitment to avoid causing significant pain to laboratory animals. Ethics and regulations are not synonyms, but they intersect. In the best of all worlds, our rules (and for our purposes, I use the word 'rules' here for the constellation of regulations, *Guide* guidelines, and accreditation standards) are based on ethical principles that have broad societal acceptance. Standards writers should combine ethical norms with the best available animal welfare science, balance those with consideration of financial and other practicalities, and produce rules for scientists and their IACUC.

The rules for laboratory animal pain management set minimal standards that look simple enough: avoid doing painful procedures; treat the pain of unavoidable procedures with the veterinarian's recommended medicines assuming that will not interfere with the study; and euthanize animals with untreatable pain as soon as possible.

So why review the ethics? Why not simply follow the rules? Naming a legal or ethical imperative to respect animal interests is important but lacks specificity. Even an explicit mandate to treat pain and to justify untreated pain risks being a hollow platitude unless scientists and IACUC review the case-by-case specifics and apply the ethical principles to every protocol they review. The IACUC is not simply witness to the scientist and veterinarian working together to produce a protocol that

follows all the rules as written. Rather, the IACUC must make ethical judgments in applying rules, and, in the case of pain management, no matter the species, must do that in the face of a seriously limited database of expert knowledge of animal pain and analgesia.

Case Study Continued: Applying the Principles to an IACUC Review

Pain inventory in hand, the IACUC continues its deliberations. It considers the case by combining expert knowledge of animal pain biology, evaluated in the context of the general principles to minimize animal pain.

I focus on 3 elements of the review: 1) The veterinarian's applied professional judgment of the best pain management for NMR; 2) The challenge of knowing when analgesic use truly disrupts scientific studies and data; and 3) Whether it is the IACUC, scientists, peer reviewers, or a combination thereof who can best balance research objectives against animal harms and how they can do that.

These 3 elements illustrate the ethical work that IACUC do, whether they see it as explicitly ethical deliberation or not. Serious consideration of these elements may lead to shifts in practice.

Professional Judgment and Animal Pain Management: When Data Are Unavailable

For these NMR surgeries, the veterinarian, researcher, and IACUC must agree on signs of pain that will be monitored and the choice and selection of analgesic drugs. But based on what knowledge? There are but a handful of examinations of opioids or acetaminophen (paracetamol) in this species, and essentially none that I can find assess clinically relevant treatment regimens for these animals.

The veterinarian's professional judgment may lead her to extrapolate from the mouse or rat literature, but is this judgment sound? The following anecdote illustrates my concerns with privileging professional judgment. When meloxicam became available as a palatable oral NSAID for dogs and cats, I thought I could add it to my rodent formulary, alongside carprofen—but at what dose? Knowing that rodent doses of carprofen had been found to be comparable to dog and cat doses, I started prescribing meloxicam at 0.3 mg/kg for rodents, as I might for a dog.²⁵ Absent other information, my professional judgment may have been sound at the time. When published scientific evaluations revealed that meloxicam doses of 10 to 20 mg/kg were necessary for mice, I was able to change my prescription for future protocols, but I could not undo the serious undertreatment I had inadvertently been prescribing for several months.⁵⁹ Professional judgment without hard data can mislead clinicians into thinking they have successfully managed a condition when, in fact, they may not have.

Absent other data, a diligent veterinarian will use professional judgment to apply knowledge of other species to Dr Glaber's NMR. But she must remember that NMR are in the lab precisely because their biology is so strikingly different from *Mus* and *Rattus* mice and rats. The small body of literature on NMR pain biology reports striking differences from *Mus* and *Rattus* there too—striking enough to call into question the wisdom of extrapolating pain medications or even pain diagnostic methods such as grimace scales from other rodents.

Much of the IACUC's work is thus applying ethics of uncertainty, making real-world ethical decisions for immediate action when many of the relevant facts are unknown, probabilistic or

difficulty to quantify with precision. The question that the IACUC faces is, "Given how little we know about pain diagnosis and management in NMR, can we approve major survival surgery for this project?" Some IACUC might be willing to go with the veterinarian's professional judgment, or really, best guess regarding appropriate analgesia. Some might urge switching to a species where the pain database is more developed. Some might insist on a pilot study, setting the veterinarian and scientist in pursuit of pain measures (grimaces? weight loss? other behaviors?) and analgesic regimens (doses and frequencies of various classes of analgesic drugs, with an examination on how those doses affect the research). Could such a pilot study be robust enough to truly inform the animals' welfare and pain management? Clearly, the IACUC is doing ethical work that goes well beyond simply applying some simple rules to the case.

At Great Western University, the IACUC and veterinarians set a standard of care for pain management for animal surgeries. Great Western animals that undergo moderate to major surgeries receive 3-drug multimodal analgesia at the time of surgery (opioid, NSAID, local anesthetic) and continue to receive systemic analgesia for at least 2 d. This standard does not come from the *Guide* and AWA, which do not specify pain management standards with any specificity beyond exhorting scientists to treat pain and to consult their veterinarian. I call this consultative requirement a 'jurisdictional standard'; it differs from performance standards and engineering standards in that it does not dictate how to do something (an engineering standard) or specific goals to achieve through flexible routes (a performance standard) but rather who in the institution has the authority to dictate practice.¹⁶ Great Western's standard is based on the veterinarians' interpretation of the literature, their professional judgment, that this multimodal approach will likely keep most animals in minimal pain. Institutions may vary in their standards of care. Veterinarians share a common body of literature and similar veterinary training and might be expected to have a common professional judgment of which analgesic regimens minimize animal pain. That different institutions have different standards is not so likely to reflect differing levels of competency of their veterinarians but differences in the ethical standards that IACUC develop and decisions about what standard of proof and benefit of doubts they will apply in the face of hard-to-see animal pain.

The danger of this hypothetical case is that it could create the impression that NMR are such unknown creatures that the ethics of uncertainty applies to them but possibly not to other, better-studied species. As my experience with meloxicam for mice shows, that would be a mistake. Several articles in this issue describe recent advances in pain recognition and pain treatment for our most common laboratory rodents. These advances in our knowledge imply recent—quite recent—serious gaps in our knowledge, raising questions about how substantially we have been under-treating pain in laboratory rodents and—quite possibly—still seriously under-recognizing and under-treating their pain.

A look back is quite discomfiting. Potent, long-acting opioid treatment only became available in the 1990s, with buprenorphine, despite decades of major surgeries in mice and rats. Textbooks and formularies listed opioids such as meperidine of dubious efficacy and short duration, with aspirin one of the only NSAID.²⁰ Retrospectively, we should classify most rat and mouse surgeries as examples of unintentionally unalleviated pain, even if they used then-available analgesic regimens. In my own clinical practice as a laboratory animal veterinarian in the 1980s, we rarely included postoperative pain management for

laboratory rodents. Such practice should have faced a high bar for approval, comparable to studies in which pain management is knowingly and intentionally withheld. The mindset in those days was that surgical pain alleviation applies solely to anesthesia for surgery, and only gradually, institution by institution, came to include effective management of postoperative pain.

The articles in this issue describe progress but also conversely illustrate the need for further progress. Pain biology and response to analgesics vary with sex and age and species, and current studies of clinical pain management are still too few in number to capture this breadth, especially when genetic modifications of relevant pain and drug-relevant genes are so widespread in so many laboratory strains.

Despite this known uncertainty, IACUC likely review protocols with confidence that the veterinarians' doses are appropriate and sufficient to truly minimize the pain of major survival experimental surgeries. As one of those veterinarians who has enjoyed IACUC's confidence in my pain prescriptions, I worry that practitioners 10 y from now may look back and judge that we were overconfident. The longstanding ethical norm to treat animals' surgical pain with analgesics meets the uncomfortable assessment that our analgesic tools have likely been inadequate for many of those animals over the years. They are now better, but still not fully developed. Even with common species, IACUC should bear in mind that they review protocols that include considerable uncertainty about the degree of pain and the success of treatments. IACUC should consider more animal surgeries, especially in strains whose pain biology is unclear, as likely instances of unalleviated pain, pushing for strong justifications and strong consideration of nonsurgical alternatives.

The Effects of Pain and Pain Medications on Data Outcomes: Category E Protocols

Dr Glaber does not want to subject NMR to major surgery only to thwart the project with the use of NSAID and opioid drugs that the veterinarian recommends. What if NSAID keep the implanted cells from growing into tumors that she can study? What if opioids affect the tumors' response to chemotherapies? Whether for the immediate postsurgical pain of the tumor implantation or the chronic pain of a growing tumor, her precautionary approach is to avoid these drugs and their possible effects, by solely using local anesthetics for her perioperative analgesia. She cites *in vitro* and *in vivo* data regarding how these drugs can affect tumor cell biology, although openly admitting none of this information comes from studies of NMR. The IACUC must decide whether to approve intentionally causing pain and leaving it untreated or undertreated.

Like many institutions, Great Western applies USDA pain categories to nonAWA-covered species. NMR are considered animals under the AWA, and Great Western has no choice but to use USDA categories for them. Although USDA's C, D, and E pain category schema is imperfect, its use does encourage scientists and committees to seriously discuss what would push a procedure over the threshold and into Category E, with occasional decisions (in my experience) to redesign the study to stay out of the E realm; I encourage its use even for nonAWA-covered species.

The *Guide* has no explicit guidance on how to decide these cases.¹⁶ The AWA is more specific, at least on first read: it requires that institutions report their animal use in columns characterizing the amount of pain (and distress) and use or nonuse of analgesics or other drugs.⁵³ In the case of pain, this reporting requires answering 2 questions, in no particular order: 1) Do

the procedures (surgery, postoperative convalescence, chronic cancer pain) cause more than momentary or slight pain? and 2) Would analgesic pain medications adversely affect research results or interpretation and so must be denied to the animals?

Those 2 technical questions regarding pain, drugs, and their effects imply the ethical judgment of whether the degree of unalleviated pain can be justified by the objectives and quality of the work. The AWA is more explicit about the technical components, the *Guide* is clearer that there is a justification question before the IACUC.

The AWA obliges the scientist to review the available literature and present her case to the IACUC for approval to cause unalleviated pain. She, the veterinarian, and the IACUC must decide whether surgery and its following postanesthesia hours are significantly painful and whether advancing brain cancer is painful. If so, they must decide whether analgesic medications for acute or chronic pains will affect the biology or the interpretation of the results. So important are these evaluations, the AWA includes special rules for how to get this knowledge (primarily through database searches) and a separate column (column E, therefore the reference to Category E experiments) in a facility's publicly visible annual report.⁵⁴

Categorizing levels of pain and distress can help investigators and IACUC focus on studies most in need of 3Rs consideration. American institutions are not required to use the USDA's categories for nonAWA-covered species, like rats and mice, although doing so adds consistency for IACUC familiar with these categories for other rodents and for larger animals. But with a single yes-no threshold of "more than minor or momentary," it is easy to get caught up in the threshold question, especially when incorrect category reporting can lead to USDA citations.³ Other countries use different scales, which still require some degree of categorization, as well as some sort of severity assessment for planned projects.^{52,60}

IACUC and veterinarians might well consider South Africa's standard of care for laboratory animal pain management, with less emphasis on categories and more emphasis on treatment: "The use of analgesic, sedative, and tranquilizing agents should at least parallel usage in medical or veterinary practice."⁵¹ Of course, there are experimental procedures in laboratory animals that do not have direct parallels in therapeutic settings, and again, times when researchers will want to avoid the use of analgesics. The application of this standard requires us to remember that in human medicine and much of veterinary medicine, a human patient or the human caregiver of a veterinary patient can decide at 2 AM to take or administer some "as needed" analgesics. Most laboratory animals endure 12 h or more overnight with no ongoing pain assessment, and IACUC must figure that reality into their standard of care.

The AWA sidesteps the question of ethical justification, focusing on the technical. And unfortunately, the USDA underestimates the complexity even of the technical questions and overestimates the certainty with which the investigator can answer the questions. If the investigator, veterinarian, and IACUC are to truly evaluate the ethical justification for unalleviated pain, they must work with the soundest facts, addressing these and other questions.

- How much pain will the animals likely experience after accounting for the surgical anesthesia and the local analgesic, bupivacaine?
- What—and how great—are the differences in data outcomes when analgesics are used compared with situations where the pain is left untreated?

- How certain is it that different outcomes reflect negative consequences (in terms of data outcomes) of analgesic medicines rather than untreated pain itself?

Note that these are quantitative questions—how much pain? how strong an effect?—as much as they are qualitative—is it painful or not? will analgesics negatively affect the data or not?—although the language of the policy is a sharp yes–no qualitative line.⁵³ Sometimes research outcomes will differ if the animals received analgesics or if they did not. Although the first inclination may be to 'blame' the analgesics for the difference and decide that they must be avoided, it is by no means clear that every difference in data outcomes supports that interpretation. The relative effects of pain medications and of untreated pain on data outcomes must be defined in terms of the objectives of the research and just what it is that a study is modeling. It is testable on a case-by-case basis for every model, type of pain, and the particular analgesic under consideration.^{19,42,45} Nor is every effect on data outcomes necessarily of sufficient magnitude to justify withholding pain treatments. For example, what if NSAID treatments mean that 8 of 10—rather than 9 of 10—NMR successfully grow a brain tumor, requiring slightly higher animal numbers, some degree less pain per animal? And rather than debate whether a particular procedure crosses the threshold of more than momentary or slight, should the scientist, veterinarian, and IACUC apply these analyses to *all* pains inflicted on laboratory animals?

As another example, cancer metastatic rates may differ among rats that receive cancer cells through the tail vein, undergo surgery, and either do or do not receive opioid analgesics. For most research questions being asked, rats that receive cells only (that is, with neither surgery nor drugs) are the closest model of spontaneous metastasis in humans. If surgery must be performed, buprenorphine analgesia best keeps the model's integrity, because both surgery and some analgesics affect immune function and thus complicate the model. Therefore, in some models, analgesics not only reduce pain but also may improve outcomes compared with experiments with unalleviated pain.^{42,27,44} The IACUC's ethical evaluation of the justification for untreated or under-alleviated pain must factor in these complexities and nuances.

Finally, the AWA's Category E standards presume a level of certainty in our facts that does not exist, with implications for ethical evaluation of justification. The USDA's Policy no. 12 (currently under review and not accessible on their website) recommends "a database search as the most effective and efficient method for demonstrating compliance with the requirement to consider alternatives to painful/distressful procedures."⁵⁴ Despite the USDA's optimistic claim, the available literature on how pain and pain medications affect data outcomes for any given model is typically quite sparse.¹⁹ The 'gold standard' would be adequately powered, reproducible, side-by-side comparisons of the same species, procedures and data analyses, some with an assortment of analgesics and some without. This 'gold standard' is hard to achieve, certainly for NMR, but also for the myriad genetically modified mouse strains whose pain biology, either intentionally or as a side-effect of other desired genetic changes, may differ dramatically from the wild type laboratory *Mus musculus*. Lacking such gold standard information, a scientist may search a bit more generically; in our hypothetical case, Dr Glaber might look at the literature on orthotopic brain cancer models in mice and rats, if only to see what others who use these models report using. Unfortunately, authors of scientific articles report such details in but a minority of cases.^{11,19} A scientist may interpret silence about pain management as an

implied statement that analgesics are not or cannot be used in the model, rather than as a simple failure to include these details, and argue that the IACUC must approve unalleviated pain for her work as well.

Approval of projects with significant unalleviated pain is one of the most serious ethical judgments an IACUC makes, and so I emphasize the vital importance of full and complete animal pain information in the ethical evaluation. How the IACUC factors in this uncertainty of knowledge is an ethical decision. Absent regulatory guidance, IACUC vary in the standard of proof they require to approve a Category E study.¹⁷ Do they settle for a theoretical explanation of why various classes of analgesics might affect the model? For example, “We study cancer, and immune function affects cancer, so therefore even a single dose of immune-altering NSAID could affect our outcomes.” Do they require a very explicit comparison of treated and untreated animals undergoing the precise model, with a comparative qualitative and quantitative analysis of the effects of pain and of pain medicine? The challenge of unalleviated pain and Category E studies needs more explicit coverage in the *Guide*, and because that will be some years from now, in forums such as this special edition of *Comparative Medicine*, at veterinary and IACUC conferences, and through clearer guidance from the USDA.

Weighing the Objectives of the Study against Potential Animal Pain

In contrast to the USDA and the AWA’s focus on technical questions of analgesics and their effects on data, the *Guide* does include some language regarding evaluating justification. The IACUC “is obliged,” according to the *Guide*, “to weigh the objectives of the study against potential animal welfare concerns.”³⁰ Withholding pain medications is not one of the situations specifically mentioned for this weighing, although it seems a ripe candidate for such a review. This obligation is the first point for future editions of the *Guide* to detail more fully: where does “is obliged” fit in the *Guide*’s ‘should/must’ schema of guidance? What outcomes might follow an unsatisfactory weighing of objectives and welfare? What is the metric for “weighing objectives”? In medical research, for example, do some diseases, by virtue of their prevalence or their effects on patients, produce more valuable data that justify higher levels of animal suffering? Or is this an evaluation of the scientific caliber of the research proposal and its likelihood of producing more credible data? AAALAC International, the main organization that accredits animal research programs, repackages the *Guide*’s “objectives/welfare concerns” as “harms/benefits” and reviews how IACUC do this during accreditation site visits but without articulating or setting standards of *how* IACUC should do this.¹

Since the inception of IACUC in American law in the 1980s, animal welfare scholars have grappled with the principle that only work of sound scientific merit can justify the harmful use of animals.^{24,29,35} Over several decades, people have wrestled with the principle that inflicting animal pain requires justification, without yet quite determining who should do that—the scientist, the funding agency, the veterinarian, or the IACUC. This 2011 provision of the *Guide*, however minimal, puts some onus on the IACUC.

In their forthcoming book, Beauchamp and DeGrazia build on earlier efforts to establish principles for the ethical review of animal research, focusing on research that harms animals for human biomedical progress.^{9,39} Their 6 principles include principles of “Sufficient Value to Justify Harm,” “No Unnecessary Pain,” and “Upper Limits to Harm.” Relevant to pain, alleviated

or not, their principles combine to envision a working ethic in which research that may bring important societal benefits and that cannot reasonably be done without animals may be permissible, as long as animal pain and suffering are limited to what is “necessary” and as long as upper limits on animal suffering are set and respected. These principles are an excellent articulation of existing principles rather than a radical departure from practice.

Justification can mean several things in an IACUC review.¹⁷ There is *scientific* justification, in which the IACUC simply verifies that a degree of animal pain appears necessary to achieve the objectives of the experiment. The articles in this issue will advance the work that I believe many IACUC currently do with excellence: applying the 3Rs and, in particular, refining animal research protocols to minimize laboratory rodents’ pain. But the 3Rs do not cover the *ethical* justification for those times when pain and distress (or *inhumanity*, in Russell and Burch’s terms) do not reduce to zero.

Ethical justification requires at least 2 more sets of considerations. There are decisions—by Congress, foundation donors, drug company executives, others—on how much of their finite resources to devote to any given area of research. Their priorities and resource allocations reflect a combination of disease severity, prevalence, the existence of currently available treatments, the likelihood of success, profitability and other factors, allowing an organization like the American Cancer Society, for example, to devote more grant funding to breast cancer than to thyroid cancer.² Working within these finite resource limitations, but separate from and requiring different expertise, are the specialized scientists conducting scientific merit peer reviews, evaluating the soundness of research hypotheses and prospects that an investigator will successfully achieve her research objectives.

Thus, 3 very separate areas of expertise—animal pain biology, societal resource priorities, scientific merit—must all be considered to truly weigh the objectives of the work against the animal welfare concerns. In our current system, IACUC may rely on study sections to conduct merit review and study sections rely on IACUC to oversee refinement of painful protocols. These reviews can happen in either sequence, so the IACUC has no quantitative information of *how much* merit a project has, and the merit peer reviewers have no detailed information on animal pain in the protocol. A challenge for the coming years is developing systems that promote the integration of this tripartite ethical analysis.^{14,18,33} Until that time, IACUC will continue their reliance on animal welfare advances such as those in this journal issue, but they will not—and neither will veterinarians, funding allocators, or peer reviewers—truly meet the *Guide*’s obligations or Beauchamp and DeGrazia’s principles.

Conclusion

The scientific advances in rodent pain biology in this issue of *Comparative Medicine* offer important contributions to the ethical use of laboratory animals. The knowledge here should lead to reduced animal pain in the laboratory and a fuller understanding of ways in which pain and pain medicines can affect data outcomes. These advances can lead to better science and greater animal welfare. They accord well with long-standing ethical principles: that pain in sentient laboratory animals must be taken seriously and must be minimized but that infliction of pain and withholding of analgesics may be justified for high-quality important research when that is the only way to achieve needed research data. They do not yet signal the day when biomedical research in animals will come without welfare costs, and significant pain, for the animals.

Our long-standing principles are hollow platitudes unless scientists, veterinarians, and IACUC have sound facts for deciding individual cases. Articles in this special issue update our knowledge base for determining when laboratory rodents are in pain, for weighing effects of analgesic pain medicines as well as pain on research outcomes, and for treating pain with effective doses and dosing frequencies. Ironically, these advances also illustrate that in many past instances, and in many current ones as well, we do not necessarily have adequate tools for recognizing and treating laboratory rodent pain. In the past, and to some extent still, this deficiency reflects insufficient knowledge of drugs, doses, efficacy, and duration of action to completely treat pain. More than ever, some of our wide variety of genetically modified mice are likely to feel pain and respond to analgesics differently than animals with 'wild type' nociceptive and cognitive anatomy and physiology. Clearly, many rodents have suffered inadvertent pain for want of good diagnostic and treatment tools.

In the face of still-limited knowledge of best practices for recognition and alleviation of laboratory rodent pain, coupled with insufficiently developed methods for a comprehensive harm-benefit weighing of objectives and animal welfare, IACUC should bear in mind the risk of inadvertent pain in animals. In addition, they should consider the limitations of information on the impact that pain medications and unalleviated pain may have on data outcomes whenever they review Category E proposals. At every meeting and with every protocol review, IACUC make an ethical judgment in the face of this uncertain information of whether to err on the side of proceeding with research and hoping the animal welfare costs are not too great or on the side of privileging animal pain treatment at the risk of impeding science.

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