RESPONSE

Justify, justify, justify

Jennifer Osborn, MS &
Rebecca K. Holt, DVM, MPH

We believe that Jeffries has not adequately resolved the issue of pain that might be caused to the rats during his study. This protocol also raises several other concerns that the IACUC should address.

It isn't clear from the scenario description if, after the pre-review, Jeffries eventually submitted the protocol to the IACUC continuing to use crushed ice for anesthesia or if he proposed to substitute a single dose of morphine as a stand-in for both anesthesia and analgesia. We assume Jeffries planned to continue to use crushed ice, with the addition of morphine. But he did not provide justification to support an exemption from the IACUC policy prohibiting the use of ice as the sole anesthetic in 10-d-old rats. He also did not address ancillary issues, such as the need to keep the neonates protected from direct contact with the ice or monitoring during the post-hypothermia recovery period.

Additionally, it seems that Jeffries did not carry out a sufficient literature search that considered all anesthetic alternatives for his proposed procedures. His protocol addresses only isoflurane and ketamine; the former might affect cerebral cortical activity, leading to long-term cognitive dysfunction, and the latter might trigger apoptosis in the rats' central nervous system. Current literature indicates that ketamine might have this effect only when administered chronically, not necessarily with acute administration such as Jeffries intends[1]. It isn't possible to determine whether Jeffries' protocol specifically outlined the neurobiological effects he was interested in validating (spontaneous locomotor activity, decline of learning and memory function, etc.) or how he would measure the effects of trauma over time (Morris water maze, open field test, rotarod test, etc.). In the absence of this information, it wouldn't be possible for the IACUC to determine whether isoflurane and ketamine were truly contraindicated. Finally, Jeffries' protocol did not address why the neonatal rat specifically would be an appropriate alternative model for validating study results from his primary model, the zebrafish.

Jeffries' protocol also lacks justification for the use of analgesics. Jeffries planned to administer only one, pre-trauma dose of morphine, stating that "additional analgesia could not be used." No explanations were given as to why other analgesics could not be used (e.g., non-steroidal anti-inflammatories, sustained-release opioids or multi-dose morphine). It may be accepted among neurobiologists that administration of morphine after traumatic brain insult can improve cognitive deficits (as evidenced by improved scores on the Morris Water maze test), but it is still incumbent on a principal investigator to address this in a protocol. IACUCs are charged with weighing the objectives of a proposed study against potential animal welfare concerns, and it is the principal investigator's obligation to provide sufficient proactive justification to the IACUC for a proposed painful or distressful procedure and to address how that pain and distress will be mitigated, given the constraints of the study[2].

Neurotrauma studies are a sensitive subject within the research and animal welfare community. Because analgesics and anesthetics have the capacity to confound the natural course of traumatic brain injuries through neuroprotective effects or other actions, their use must be considered with care, not rejected out-of-hand. It would not be unreasonable for the IACUC to require a pilot study or an intra-study anesthesia and analgesia protocol to ascertain the confounding effects of these drugs on the specific areas Jeffries is studying.

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1. Rowe, R.K. *et al.* Using anesthetics and analgesics in experimental traumatic brain injury. *Lab Anim. (NY)* **42**, 286–291 (2013).
2. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC, 1996).

Osborn is the IACUC/IBC/Safety Administrator and Holt is a senior staff veterinarian and Alternate Attending Veterinarian at the National Biodefense Analysis and Countermeasures Center, Frederick, MD.

RESPONSE

Protocol is acceptable

Mary Ellen Goldberg, BS, LVT, CVT, SRA, CCRA

Hypothermia is a safe and effective method for anesthetizing rodents up to 7 d old[1,2]. Hypothermia provides both immobilization and mild analgesia when body temperature is reduced to 10–20 °C (ref. 3). Newly born rat pups cannot maintain their body temperature and are functionally poikilothermic, with thermoregulatory ability developing only during the third week of life. Therefore, they are tolerant of low temperatures and can recover by re-warming, even from near 0 °C body temperature[4]. I believe this information is key to this scenario. Hypothermia will be acceptable for anesthesia for as long as rat pups remain functionally poikilothermic (until 21 d of age). To reduce possible unintended pain associated with cooling, the technique for inducing hypothermia should include partial insulation of the pup (e.g., wrapping it in a latex blanket or aluminum foil). Aluminum foil can be placed on crushed ice and molded to form a groove wide enough to accommodate a single pup, thus maximizing its exposure to low temperature. Pups could be placed in the groove in a row, avoiding body contact, the start time recorded and their surface body temperature recorded every minute using a thermocouple probe[4].

Opioid drugs provide effective analgesia against thermal, inflammatory and mechanical pain in neonatal rodents as young as 1 d of age and should be considered for use whenever analgesia would be provided for an adult animal[1]. Morphine targets the nociceptive pathway during transduction, modulation and perception, whereas hypothermia targets perception as a general anesthetic[1,5]. Therefore, Jeffries'