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Hypothermia as anesthesia for 10-d-old rats

Dr. Greg Jeffries used zebrafish as a model for his studies on postnatal development of the central nervous system. His particular interest was in understanding how early physical or psychological trauma affected adult behaviors. He knew he had to ascertain if his findings in fish were also valid in a mammalian model, and so he proposed a study in which he would use outbred neonatal rats, 10 d old, in place of fish. One part of the study involved moderate physical trauma to the animal; therefore, Jeffries proposed a method he knew from the biomedical literature. In

those studies, a defined weight was dropped from a defined height onto the cranium of an anesthetized animal. The anesthetic used was isoflurane, but Jeffries was aware of literature indicating that isoflurane could impact cerebral cortical activity of neonatal rats and lead to long-term cognitive dysfunctions in rats. He also knew that drugs such as ketamine could lead to apoptosis in the central nervous system of young rodents. Therefore, he proposed the use of crushed ice for anesthesia.

The veterinarian pre-reviewing the protocol told Jeffries that the IACUC policy

allowed the use of crushed ice for anesthesia in animals up to 7 d old. After that, general anesthesia via inhalant gas or injectable drugs had to be used. Jeffries said that was not acceptable for the research he did, and when he submitted the final draft of his IACUC protocol, he wrote that the young rats would be given a single injection of morphine prior to the traumatic insult but any additional analgesia could not be used.

Do you believe that Jeffries has adequately resolved the issue of pain that might be caused to the rats, or are there additional concerns for the IACUC to address?

RESPONSE

Many potential options

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The *Guide for the Care and Use of Laboratory Animals* states that selection of appropriate analgesia and anesthesia should be based on veterinary judgment and whether it "...best meets clinical and humane requirements as well as the needs of the research..."¹. A common and effective (when done correctly) method of anesthesia in neonatal rodents is hypothermia. Jeffries wishes to use hypothermia for his blunt trauma research in 10-d-old rats, which falls outside of the commonly accepted age limit of 7 d for this technique. As a neonate becomes more mobile, the safety and practicality of hypothermia anesthesia decreases and alternative methods should be explored².

Although Jeffries' concern about the potential effect of isoflurane on cerebral cortical function is justified, other considerations should accompany this concern. First, the previous traumatic injury studies he found used isoflurane as an

anesthetic. Its effects notwithstanding, it may be beneficial for Jeffries to use isoflurane so that his results can be compared to the results obtained in previous studies. Second, isoflurane is the most common inhalant anesthetic used in rodents but is not the only one that can be used. Halothane or sevoflurane might be appropriate alternatives to provide anesthesia without affecting cortical function in the same way as isoflurane. Similarly, injectable anesthetics other than ketamine (e.g., alpha 2 agonists, barbiturates) should also be explored.

These alternative methods of anesthesia bring up the importance of pilot studies. Literature on rodent anesthesia is robust but far from exhaustive. Therefore, it is often advisable that researchers carry out well-designed pilot studies prior to beginning their larger experiments to specifically address the needs of their projects, especially when animal welfare is in question. In Jeffries' case, a pilot project could be used to determine whether the parameters that are of interest to him would be affected by an inhalant or injectable anesthetic. A pilot study could also be used to determine whether 7-d-old rats could be used in place of 10-d-old neonates, which

would allow him to use hypothermia anesthesia.

Despite best efforts to minimize variables in research, it is impossible to control for everything. In this scenario, the pain and distress caused by the procedure might be an experimental variable required for the goals of Jeffries' research (is this experiment designed to evaluate physical trauma, psychological trauma or both?). However, Jeffries' concern regarding the effect of anesthesia on the experiment could mostly be alleviated by ensuring that proper control animals are included in the study design to address the effects of strain, age, anesthetic, etc.

1. Institution for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
2. Cunningham, M.G. & McKay, D.G. A hypothermic miniaturized stereotaxic instrument for surgery in newborn rats. *J. Neurosci. Methods.* **47**, 105–114 (1993).

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