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Chloral Hydrate Is Not Acceptable for Anesthesia or Euthanasia of Small Animals

To the Editor:—A recent paper¹ reported experiments, using laboratory rats, on the effect of intraamygdala infusion of a γ -aminobutyric acid type A antagonist on propofol-induced amnesia for inhibitory avoidance training, as well as on expression of activity-regulated cytoskeleton-associated protein in the hippocampus. This work may elucidate the neural mechanisms of the amnesic effects of propofol, as well as the neurobiological mechanisms of general anesthesia and memory more generally.

The experiments reported in this paper required stereotaxic neurosurgery to implant cannulae aimed at the basolateral amygdala, and rats were subsequently euthanized for determination of activity-related cytoskeleton-associated protein levels or histologic verification of cannula placement. The authors used chloral hydrate in both procedures; for surgical anesthesia in the first and euthanasia in the second. Chloral hydrate is not a suitable drug in either case. Chloral hydrate is regarded by many to produce hypnosis and not anesthesia.² It does not provide analgesia and causes marked respiratory depression at doses required for surgical anesthesia.³ Apart from its inadequate anesthetic properties, 20% chloral hydrate is extremely irritating and therefore unsuitable for intraperitoneal use. It is associated with ileus in rats,⁴ as well as peritonitis and gastric ulcers.⁵ Its use by intraperitoneal injection for survival surgery is not recommended.⁵ Thus, it is not the most refined choice of agent for the surgical procedure in which cannulae are chronically implanted to make drug infusions into the amygdala. The authors also used a higher dose of chloral hydrate for euthanasia. However, chloral hydrate is not an acceptable agent for euthanasia according to the guidelines of the American Veterinary Medical Association[†]; its use for this purpose has been proscribed for some time.⁶

There are no scientific justifications for using chloral hydrate for these experiments, as many other agents would be more suitable for both surgical anesthesia and euthanasia without interfering with the experimental endpoints. Indeed the chloral hydrate-induced hypox-

emia which must occur during euthanasia as respiration becomes depressed[†], may compromise the experimental aims in terms of measuring protein and messenger ribonucleic acid levels of an activity-related protein. The noxious stimulus of an intraperitoneal irritant is not only inhumane, but if it leads to peritonitis the rats will be abnormal at the time of testing.

It seems that chloral hydrate has traditionally been used to provide anesthesia where the avoidance of agents with known receptor interactions is desirable. But it is likely that chloral hydrate has unknown receptor interactions. Therefore choosing a different agent whose receptor interactions are better characterized could be beneficial, not only in terms of animal welfare but also in terms of data interpretation.

The publication of this paper in ANESTHESIOLOGY concerns us, because the standard of laboratory animal anesthesia used in this research is not acceptable.

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† AVMA Guidelines on Euthanasia, 2007. http://www.avma.org/issues/animal_welfare/euthanasia.pdf. Last accessed 11-10-08.

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In Reply:—We sincerely thank Baxter *et al.* for their interests in our article and their valuable information about the use of chloral hydrate for rats' anesthesia and euthanasia in our experiment.

First, we would like to emphasize that we do not think the reliability of our experimental results was influenced by chloral hydrate. Chloral hydrate was used in all the experimental groups, thus its interpretations were comparable among these groups. Our significant findings could not be simply induced by it. In addition, the mechanisms of most anesthetics,

including their effects on Arc expression, are still obscure. Furthermore, sevoflurane has even been proved to inhibit Arc transcription.¹ Under this condition, choosing any other anesthetic for rat euthanasia may produce the similar unpredicted interpretations. Therefore, we believe that to a great extent, our results and conclusions are reliable.

Second, we designed our experiment on the basis of a great deal of published articles on authority journals. The method as intraperitoneal injection of chloral hydrate was widely used to rats for some kinds of surgeries, particularly with the word as "anesthesia." For example, Rodríguez Manzanares *et al.*, Bredeloux *et al.*, and Sammut *et al.* all use chloral hydrate to anesthetize rats for stereotaxic neurosurgery to implant cannulae.²⁻⁴ Actually, in recent years, chloral hydrate is still widely used to anesthetize rats. However, we agree with the view of Baxter *et al.* that some other anesthetics (like Phenobarbital sodium) may be more suitable in this type of surgery because of the side effects of chloral hydrate illustrated by them. Fortunately, the overwhelming

As noted by the letter from Dr. Baxter, chloral hydrate is unacceptable for anesthesia or for euthanasia, although it has been used for these purposes in the past. We regret that the peer review system, both in this journal and in other prominent journals as noted by Dr. Yu, or the institutional animal care and use committees do not always catch this animal care issue, and we will strive to do so and not publish work using chloral hydrate for these purposes in the future. —James C. Eisenach, M.D., Editor-in-Chief.