Using Rat Behavior to Assess Aversion to Euthanasia Agents

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Summary

Rodents are commonly euthanized with carbon dioxide (CO_2), but increasing evidence suggests that rodents find CO_2 aversive and that induction of unconsciousness with an inhalant anesthetic is more humane. Despite this evidence, some continue to argue that CO_2 is not aversive. This view may be due, in part, to observations of rats euthanized in a closed chamber. Our first impression of such observations is that neither CO_2 nor isoflurane causes much aversion, and that isoflurane may be more aversive. We review the results of a variety of sophisticated tests (dwelling time, approach-avoidance, aversion-avoidance) showing that rats find CO_2 considerably more aversive than isoflurane. We scored rat behavior during euthanasia in a closed chamber and found few differences in behavior between the two agents. We discuss why this could happen and conclude that observations of gross behavior during euthanasia in a closed chamber are a poor indicator of aversion.

Keywords: euthanasia, behavior, carbon dioxide, isoflurane, rat

1 Introduction

The most common method of euthanasia for laboratory rats and mice is exposure to carbon dioxide (CO_2) gas. Increasing evidence suggests that rodents find CO_2 aversive (e.g., Leach et al., 2002a; Niel and Weary, 2007) and that induction of unconsciousness with an inhalant anesthetic before delivery of CO_2 is more humane (e.g., Leach et al., 2002a; Makowska and Weary, 2009). In Canada, the Canadian Council on Animal Care (CCAC) now lists the use of CO_2 alone as only conditionally acceptable and recommends instead that rodents be rendered unconscious with an inhalant anesthetic before euthanasia with another agent (CCAC, 2010). Despite increasing evidence that CO_2 may not be humane, some continue to argue that this method is not aversive. Euthanasia guidelines in most countries still support the use of CO_2 (e.g., Australia and New Zealand: ANZCCART, 2001; European Union: Council and European Parliament, 2010).

The ongoing debate surrounding the use of CO_2 and its potential replacement by inhalant anesthetics may be due, in part, to observations of rats euthanized in a closed chamber (e.g., Hackbarth et al., 2000). When people are shown videos of rats euthanized with CO_2, most conclude that the procedure does not seem to cause much distress to the animals and that CO_2 appears to be humane. When asked which of two methods appears to cause more distress to the animals – CO_2 or isoflurane – many choose isoflurane.

Are these initial responses to the videos correct? In more recent years, scientists have developed and applied a range of methods that allow them to assess aversion to CO_2 and isoflurane. We briefly review the results of these studies below.

2 Methods of assessing rat aversion to CO_2 and isoflurane

Total dwelling time

In a series of experiments, Leach et al. (2002a,b, 2004) allowed rats to travel either between a cage that contained air and one that contained CO_2, or a cage that contained air and one that contained isoflurane. The concentrations of CO_2 and isoflurane in the cage were matched so that they took 10, 20 or 30 s, respectively, to induce the same degree of ataxia. The authors then compared the total dwelling time in each of the cages. Results showed that rats would spend the most time in cages containing air, followed by those containing isoflurane, and finally those containing CO_2. For example, when the cages were filled with the medium concentration of CO_2 or isoflurane (inducing ataxia within 20 s), rats spent 13 s in isoflurane but only 1 s in CO_2. Rats spent an average of 47 s in cages filled with air.

Approach-avoidance testing

Approach-avoidance testing allows researchers to compare rats' motivation for a reward against their motivation to avoid gas exposure. In studies assessing rat aversion to euthanasia agents, animals were housed in an apparatus that consisted of two cages connected by a sloped tube, such that one cage was higher than the other. Rats were trained to run down the tube and to expect a sweet food reward in the bottom cage. As soon as rats started eating the reward, either air, CO_2, or isoflurane started to flow into the chamber. When tested with air, rats remained in the bottom cage until they had consumed all reward items. When tested with CO_2, rats left the test cage and abandoned their food reward when CO_2 concentration reached on average 16-18% (Niel and

Weary, 2007; Niel et al., 2008b), regardless of flow rate (Niel et al., 2008a) and even if they had been food deprived for up to 24 hours (Kirkden et al., 2008). When tested with isoflurane, rats left the test cage once they were ataxic (falling over and showing difficulty going up the tube) and thus already in a state of conscious sedation (Makowska and Weary, 2009). We suggest that forced exposure beyond this point may be more humane than forced exposure to CO_2 because rats become progressively more sedated and therefore less likely to experience distress.

Aversion-avoidance testing

Aversion-avoidance testing is the newest method used to address the issue of humane euthanasia of laboratory rats. This method allows researchers to compare which of two aversive stimuli rats find less aversive. In a recent study (Wong et al., 2011) albino rats were placed in a light/dark box where they had the choice between remaining in the preferred dark compartment filling with either CO_2 or isoflurane, or escaping to the light compartment illuminated at a light level aversive to rats (1600 lux). When the dark compartment was filling with CO_2, none of the rats tested remained in the dark (CO_2) compartment, choosing instead to escape into the brightly lit compartment. When the dark compartment was filling with isoflurane, all rats tested remained in the dark (isoflurane) compartment until they had become recumbent.

3 Gross behavior during euthanasia in a closed chamber

Results from these studies using a range of methods to assess aversion to CO_2 and isoflurane all show that rats find CO_2 strongly aversive and that isoflurane is preferred. We argued earlier that initial impression of videos of rats euthanized with CO_2 and isoflurane is that neither method causes much aversion, and that isoflurane may be more aversive than CO_2. The aim of this study was to determine if more thorough analysis of the gross behavioral responses during euthanasia are consistent with this impression.

Male Sprague-Dawley rats were euthanized with either CO_2 (n = 13) or isoflurane (n = 13) delivered in oxygen from a vaporizer. The flow rate in each case was set to 23% of the cage volume per minute. Rats were placed in the euthanasia cage for 30 min before gas flow began, during which time air was delivered to the cage at a rate of 23% of the cage volume per minute. Of the several behavioral measures taken, only two were observed consistently: activity (defined as rat's front paws crossing a line that divides the length or a line that divides the width of the chamber in half) and rearing (defined as raising of the upper body with both front paws off the ground). These behaviors were scored from 90 s before the euthanasia agent was introduced until animals ceased all purposeful movement. Activity increased in rats euthanized with each agent, and there was no difference in peak activity between the two agents. Rearing also increased in rats euthanized with each agent, and peak rearing was higher with isoflurane than with CO_2.

Multiple experiments have shown that rats prefer exposure to isoflurane to exposure to CO_2 – why, then, were there mostly no differences in behavior between rats euthanized with these two agents, with the difference in rearing going in the direction opposite to what was found using the other methods? It is possible that at least part of the increase in activity with isoflurane was due to an excitatory phase associated with induction with anesthetics (Guedel, 1951; Ma et al., 2002). Moreover, part of the increase in rearing may be an artifact of ataxia – it seemed that sometimes rats would attempt to move forward (activity) but that because of muscle incoordination, they would lose balance and their front paws would come off the ground (a behavior scored as a rear). It is also possible that we simply do not understand behavior exhibited by a rat in a closed chamber without an escape route, and that the behaviors we are scoring are not appropriate. Differences in behavior may have more to do with the quality than the quantity of behavioral changes. For example, the jaggedness of movement or the pattern in which these behaviors are exhibited may be more accurate indicators of aversion.

4 Conclusion

The results from a variety of sophisticated tests have shown that rats find CO_2 considerably more aversive than isoflurane. Observations of gross behavior during euthanasia in a closed chamber reveal very few differences in behavior between the two agents. The one difference we found was in rearing, and this behavior was more common in response to isoflurane (a response inconsistent with the other methods). Induction with isoflurane is associated with an excitatory phase and with ataxia, perhaps accounting for this difference. We conclude that observation of gross behavior during euthanasia in a closed chamber is a poor indicator of aversion.

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