Bias in Rating of Rodent Distress during Anesthesia Induction for Anesthesia Compared with Euthanasia

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Selection of an appropriate method of euthanasia involves balancing the wellbeing of the animal during the procedure with the intended use of the animal after death and the physical and psychologic safety of the observer or operator. The recommended practices for anesthesia as compared with euthanasia are very disparate, despite the fact that all chemical methods of euthanasia are anesthetic overdoses. To explain this disparity, this study sought to determine whether perception bias is inherent in the discussion of euthanasia compared with anesthesia. In this study, participants viewed video-recordings of the anesthesia of either 4 rats or 4 mice, from induction to loss of consciousness. Half of the participants were told that they were observing anesthesia; the other half understood that they were observing euthanasia. Participants were asked to rate the distress of the animals by scoring escape behaviors, fear behaviors, respiratory distress, and other distress markers. For mice, the participants generally rated the distress as high when they were told that the mouse was being euthanized, as compared with the participants who were told that the mouse was being anesthetized. For rats, the effect was not as strong, and the distress was generally rated higher when participants were told they were watching anesthesia. Because the interpretation of distress showed bias in both species—even though the bias differed regarding the procedure that was interpreted as distressing—this study demonstrates that laboratory animal professionals must consider the influence of potential perception bias when developing policies for euthanasia and anesthesia.

Abbreviation: VDR, volume displacement rate

Euthanasia is defined as “ending of the life of an individual animal in a way that minimizes or eliminates pain or distress.” Selection of an appropriate method of euthanasia involves evaluating multiple criteria to balance the wellbeing of the animal during the euthanasia process with the intended use of the animal after death and the physical and psychologic safety of the observer or operator. After the release of the most recent edition of the AVMA Guidelines on Euthanasia, we learned that some groups reported that the recommendation for very low volume displacement rates (VDR) for carbon dioxide led to visible distress experienced by the animals, whereas other groups indicated that the new guidelines resulted in a more peaceful death. Multiple laboratories are evaluating the science behind the effect on the wellbeing of the animal during euthanasia (including differences between injectable anesthetics, such as pentobarbital, and inhalant anesthetics, such as carbon dioxide and isoflurane), but our current study sought to determine whether observers interpret a rodent’s behavioral responses to the euthanasia process in an unbiased manner.

Human interpretation of observations and interactions is affected by a multitude of factors. The brain filters acquired information and processes it by using neural pathways that have been created through past experiences and current mental states. These pathways are highly fluid and constantly adapting to the circumstances that are encountered. Cultural and social pressures can affect the processing of information by shaping the perspective that the person uses during processing. Perception bias is well recognized as a phenomenon in the human medical profession, especially with regard to traits such as race, socioeconomic status, and age. In addition, the care provided in response to a specific diagnosis can be biased by the perspective of the clinician. For example, clinicians rated quality of life as much lower for patients with lung cancer than those with other solid tumors, such as breast, prostate, and colon cancer, even after controlling for variables including stage of cancer and patient-reported outcomes.

On a more acute basis, the affective state of a person can affect how events and interactions are interpreted. This phenomenon is best illustrated by the lack of reliability reported with eyewitness testimony. For example, the use of a police line-up can create the situation where the witness assumes that the actual perpetrator is in the line-up, leading them to strive unconsciously to identify the person who is the closest to the actual perpetrator, even when the actual perpetrator may not be present, thus leading to false identification. Research has shown that young adults are generally more reliable eyewitnesses than children and older adults.

Veterinary medicine is frequently compared to pediatric medicine, because both veterinary and pediatric patients are unable to use verbal communication to express their pain and distress. However, caregivers, including parents and medical professionals, bring their own preconceptions and biases to their assessment of the pain experienced by young children. The effect of shaping on perception is illustrated by a study that demonstrated that, when a face scale included a smiling ‘no pain’ face, both parents and children were more likely to rate pain higher than when the no-pain face was not smiling. The observation of acute pain results in higher parental reports...
Methods of anesthesia induction were recorded for evaluation by the participants.

<table>
<thead>
<tr>
<th>Method of anesthesia induction</th>
<th>Procedure</th>
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<tbody>
<tr>
<td>Pentobarbital</td>
<td>195 mg/kg IP (Fatal Plus, Vortech, Dearborn, MI).</td>
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<tr>
<td>Isoflurane</td>
<td>5% inhaled (Isothees, Butler Schein, Dublin, OH). Delivered by using a precision vaporizer and 0.5 L/min O.</td>
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30% / min volume displacement rate CO. 100% CO flowed through an entry port at the top of the chamber at 30% / min displacement of the chamber volume.

70% / min volume displacement rate CO. 100% CO flowed through an entry port at the top of the chamber at 70% / min displacement of the chamber volume.

Figure 1. Methods of anesthesia induction that were recorded for evaluation by the participants.

of a child’s pain experienced, but the empathy encouraged by the close bond can influence the perception of pain and distress also. However, this empathetic perception of pain can also be skewed in caretakers, as illustrated in a study in which nursing staff reported pain experienced by children in their care as higher than the children themselves had rated their pain.

The influence of perception becomes more confounding when addressing interspecies evaluations. In one study, owners were asked to evaluate their pet relative to behaviors that have previously been shown to correlate to various levels of distress. Most people were able to accurately identify and interpret behavioral cues of distress, and a significant gender-associated difference emerged, with men considering that their dogs experienced significantly less distress. Multiple studies have demonstrated that dog owners have high levels of empathy for their animals, but they confirm significant differences in distress ratings depending on the gender, childhood pet keeping, income, and education of the rater and whether the dog was kept for companionship or as a working dog. Likewise, farmers who agreed with the attitude statement that “animals experience physical pain as humans do” scored higher on an empathy score and had lower prevalence of management-agreed difference emerged, with men considering that their dogs experienced significantly less distress. Multiple studies have demonstrated that dog owners have high levels of empathy for their animals, but they confirm significant differences in distress ratings depending on the gender, childhood pet keeping, income, and education of the rater and whether the dog was kept for companionship or as a working dog. Likewise, farmers who agreed with the attitude statement that “animals experience physical pain as humans do” scored higher on an empathy score and had lower prevalence of management-associated diseases associated with their herds.

The recognition of animal distress in the euthanasia methods used for rodent species has been a predominantly controversial topic due to the ambiguity of defining these terms, which is highly dependent on the experience and training of the observer. For example, when asked to rate the distress of rats during euthanasia, neuroscientists who were accustomed to physical methods of euthanasia reported that anesthetic overdose seemed prolonged and cruel, whereas other scientists who were accustomed to chemical methods of euthanasia reported that physical methods of euthanasia appeared brutal, violent, and painful. In addition, consensus regarding what is considered ‘acceptable’ pain or distress during euthanasia is influenced by the research setting, where otherwise healthy animals are frequently euthanized at the end of studies. This discomfort with euthanizing healthy animals has led to a push to restrict the use of the term ‘euthanasia’ to situations where an animal is terminally ill and to use the term kill when discussing the euthanasia of laboratory rodents. Consequently, a person’s expectation bias might lead to identification of euthanasia, especially of an otherwise healthy animal, as a distressful and painful experience. In reality, however, with chemical methods of euthanasia (for example, pentobarbital, halogenated inhalant agents, and carbon dioxide), the animal is always anesthetized prior to euthanasia, as part of the process. This situation means that the experience of induction to loss of consciousness (stage I of anesthesia) is the same for a rodent that is anesthetized for surgery as for a rodent that is anesthetized as part of the euthanasia process, with potential differences between methods (for example, pentobarbital compared with halogenated inhalant agents compared with carbon dioxide). Personnel working with these animals may perceive different levels of distress during anesthesia compared with euthanasia, and the distress may vary depending on their previous exposure to the anesthetic agents. However, the distress experienced by the rodent should not change when the procedure is anesthesia as compared with euthanasia. Yet this disconnect in the human interpretation of what is happening to the animal is clearly exhibited by recommendations for euthanasia, which require use of the home cage and minimal disruption, as compared with recommendations for anesthesia, which consider the use of an induction chamber to be appropriate when using an inhalant anesthetic.

Because personal expectation and a person’s capacity for empathy can modify the evaluation of animal welfare in mice and rats, we designed this study to explore how the perception bias of laboratory animal professionals affects their evaluation of the wellbeing of animals that are used in research. In this study, we showed recordings of the induction phase of anesthesia (stage I) to multiple volunteers who were laboratory animal professionals. One group of participants was told that they were observing euthanasia, whereas the second group was told that they were observing anesthesia. Participants were not provided with any guidance regarding how to score the rodents, thus allowing their subjective interpretations to be measured. We hypothesized that American laboratory animal professionals would consider this period of anesthesia induction to be more distressing when they were told the animal was being euthanized than when they were told the same anesthesia was for a survival procedure.

Materials and Methods

Study design. To evaluate the hypothesis, 4 experimental groups were established. The first 2 groups of participants were told that they would be observing the anesthesia of rats or mice (rats–anesthetized, mice–anesthetized). The second 2 groups of participants were told that they would be observing the euthanasia of rats or mice (rats–euthanized, mice–euthanized). However, all groups watched the same 4 rat videos or the same 4 mouse videos, regardless of their assignment to treatment groups. This design allowed for a by-species comparison between scores assigned by participants who were told they were observing anesthesia compared with euthanasia.

Animals. Mice. Adult male ICR mice (n = 4, Mus musculus, approximately 50 g) were housed in groups of 2 to 4 in static filter-topped caging (Alternative Design, Siloam Springs, AR) with corncob bedding (Bed-o’Cobs, The Andersons, Maumee, OH) and paper toweling as nesting material. Standard operating procedures for the animal facility required that all mouse cages be changed at least every other week. Animal caretakers wore gloves while changing the cages and disinfected their hands with dilute bleach solution between cages. Soiled cages were sanitized in a mechanical cage washer with a final rinse.
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Figure 2. To avoid respondent bias when the recordings were viewed, the anesthesia chamber was set up identically for all anesthesia methods. Carbon dioxide gas was introduced through the diffuser located in the center top of the lid of the chamber. Isoflurane gas was introduced through the hose located at the front left and top of the chamber.

Figure 3. Instructions from the questionnaires that were provided to each participant on this study. Note that the bolded words differed depending on the treatment group to which the participant was assigned. These were the only differences between the treatment groups.

Figure 4. Survey questions. The demographic questions were asked first, and then questions 5 through 15 were repeated for each of the 4 videos that the participants watched.

All mice had previously been used in other noninvasive studies and then transferred to this study for euthanasia.

Rats. Adult male Sprague–Dawley rats (*n* = 4, *Rattus norvegicus*, approximately 300 g) were housed in pairs in static filter-topped caging (Alternative Design) with corn cob bedding.
(Bed-o’Cobs, The Andersons) and paper toweling as nesting material. Standard operating procedures for the animal facility required that all rat cages be changed at least once weekly. Animal caretakers wore gloves while changing the cages and disinfected their hands with dilute bleach solution between cages. Soiled cages were sanitized in a mechanical cage washer with a final rinse temperature of 180 °F (82 °C) and autoclaved prior to reuse. The rooms were kept on a 12:12-h light:dark cycle, and rats were provided rodent chow (LabDiet) and tap water without restriction. Temperature and humidity were maintained at 72 °F (22 °C) and at least 30%, respectively. Rat colonies were screened quarterly for Kilham rat virus, Mycoplasma pulmonis, pneumonia virus of mice, rat coronavirus, reovirus 3, rat parvovirus, Sendai virus, transmissible murine encephalomyelitis virus, and endoparasites and ectoparasites by using dirty-bedding sentinels. At the time of this study, the facility was free of all of the listed pathogens. All rats had previously been used in other noninvasive studies and then transferred to this study for euthanasia.

**Preparation for videorecording.** One male ICR mouse and one male Sprague–Dawley rat were euthanized for each of the 4 methods of euthanasia (Figure 1). The induction chamber was a rat cage (43 cm × 35.6 cm × 22.9 cm; Tecniplast, West Chester, PA) with a volume of approximately 35 L. During the anesthesia process, each chamber was configured exactly the same, to prevent the induction of bias in the observers by visual cues (Figure 2). Personnel who were skilled in each method of euthanasia performed the procedures while the chamber was digitally recorded from the side. All procedures using animals were reviewed and approved by the Indiana University School of Medicine IACUC prior to initiation of the study.

The recordings were edited (Adobe Premiere Pro CS6, Microsoft, Seattle, WA) such that the recording started at the initiation of the euthanasia method and continued until the mouse or rat had lost its normal posture (defined as the point where the animal stopped ambulating and its head dropped until the nose touched the bottom of the cage). This stipulation meant that the participants evaluated potential pain or distress in rodents that had likely not yet lost consciousness. The personnel handling the animals were not visible in any of the recordings. A series of compact discs were created for the execution of this study. Each disc had a single species with recordings of all 4 of the methods of anesthesia (A through D), but the methods were shown in different orders on each disc (for example, mouse BADC, rat CADB).

For both the mice and rats, the recordings for the pentobarbital and 70%/min VDR CO2 were approximately 60 s, as was the recording of the mouse anesthetized with isoflurane. The recordings of the mouse and rat anesthetized with isoflurane were approximately 5 to 6 min, whereas the recording of the rat anesthetized with 30%/min VDR carbon dioxide was approximately 4 min. The times were

![Figure 5](image-url).
not changed or normalized because participants were asked to evaluate the potential distress experienced by the animal prior to its loss of consciousness.

**Collection of data.** Direct email from the principal investigator was used to solicit volunteers in the laboratory animal medicine and science field by contacting veterinary colleagues at 6 different research institutions. In addition, the call for volunteers strongly encouraged people to circulate the call for volunteers to their research community and husbandry staff, as well as the veterinary and management professionals at their home institution. These people assisted the study director (DH) in advertising for volunteers who were willing to participate in a study that evaluated how laboratory animal professionals evaluated the potential distress of rats or mice being manipulated through blood collection, euthanasia, and anesthesia and in coordinating a room where the participants could meet with the study director.

Approximately 50 people volunteered to participate in the study, and 47 completed the survey (35 for the mouse recordings and 38 for the rat recordings). A range of 4 to 7 participants observed the videos in each group in the same order. All participants were provided with a sheet that described the purpose of the study, its possible benefits, and its potential pitfalls and were informed that they could withdraw from the study at any time. No compensation was provided for participation. The study was reviewed and approved by the Indiana University Institutional Review Board prior to the initiation of the study.

When participants arrived at the evaluation location, the study director randomly assigned them to either rat or mouse and either anesthesia or euthanasia. At least 5 packets of paperwork and compact discs were prepared in stacks before each session, and one stack was selected randomly when a participant arrived. Participants were directed to a dedicated computer where they could observe their assigned videos alone. Because data collection occurred at multiple locations, a few configurations were implemented during this process. In all cases, a cubicle wall or physical separation of at least 40 ft was kept between participants to provide them with privacy while watching the videos and completing their score sheets. No more than 5 participants were present at a time, and all start times were staggered by at least 5 min. On return of the completed paperwork, the group assignment was recorded on a tally sheet of species, treatment, and video order to ensure that all available combinations were used equally to achieve a random distribution among the 4 treatment groups and that the videos were observed in a variety of configurations to minimize the potential effect of video order on the responses collected.

Each participant was provided with a questionnaire, a score sheet, and a compact disc that contained 4 videos of either a rat or a mouse. The study director verbally instructed each participant to carefully read the instructions at the top of the

**Figure 6.** Distribution of scores for fear behavior by number of respondents observing a mouse anesthetized with the various anesthetic methods. The box indicates responses where the participant reported that the animal exhibited no fear behaviors. (A) Pentobarbital. (B) Isoflurane. (C) 30%/min VDR CO₂. (D) 70%/min VDR CO₂.
questionnaire and to provide the requested demographic information on the scoresheet (Figure 3). Before watching the first video, participants were further instructed to read questions 5 through 15 (Figure 4), so that they would know what they would be asked to evaluate while watching the video. After watching each video, participants were instructed to complete the scoresheet for that recording before proceeding to the next video. Participants were told that the questions were the same for each of the 4 videos, and they were encouraged to provide comments on the reverse of the scoresheet.

No names were collected, but each scoresheet was identified with a code that communicated the species, order in which the videos were watched, and treatment group. For example, ‘rat-BCDA-1’ meant that the participant received the rat compact disc; watched method B first, C second, D third, and A last; and that the participant was told that he or she was observing anesthesia, whereas ‘Mouse-DCAB-2’ indicated that the participant received the mouse compact disc; and watched method D first, C second, A third, and B last; and that that the participant was told that he or she was observing euthanasia. The scoresheets were provided to research staff who were responsible for the input of data into an Excel (Microsoft) spreadsheet for analysis. During data entry, these personnel were blinded to the significance of the 1 or 2 in the code, thus preventing them from identifying a scoresheet as correlating to the anesthesia (code, 1) or euthanasia (code, 2) treatment groups.

Data analysis. Multiple logistic regression was used to compare responses between the anesthesia and euthanasia respondents. The responses were compared by identifying the median score (1 through 5) for each parameter that the participants were asked to evaluate. The higher the median score, the greater the distress reported by the participants. A Wilcoxon test (JMP, Cary, NC) was used to compare the medians between the 2 treatment groups. In addition, a $\chi^2$ analysis was performed to evaluate the likelihood of a yes or no response to the questions of appropriateness and comfort with depicted method between treatment groups.

The population of people available for this study was anticipated to be small because our laboratory had earlier performed a study that used a similar format and enrolled more 1000 people, who were therefore ineligible for inclusion on this study. Therefore, prior to initiation of the study, we determined that we would set significance at $P < 0.1000$ and ensured that we reported the actual $P$ values to allow readers to make their own determinations of significance.

Results

Mice. When participants were asked whether the mouse appeared to be engaging in escape behavior during anesthetic induction, $\chi^2$ analysis demonstrated no significant differences in the responses between the group told they were observing
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Anesthesia as compared with those told that they were observing euthanasia by using isoflurane ($X^2_{1, 10} = 1.498, P = 0.2210$), 30%/min VDR of carbon dioxide ($X^2_{1, 10} = 0.00, P = 1.000$), and 70%/min VDR of carbon dioxide ($X^2_{1, 10} = 0.403, P = 0.5257$).

Responses of participants observing the pentobarbital method differed significantly ($X^2_{1, 10} = 3.278, P = 0.0702$), with those told that they were observing euthanasia responding 'yes' more frequently. Median scores of the rankings of escape behavior did not differ between groups for pentobarbital ($Z = 1.13389, P = 0.2568$), isoflurane ($Z = –1.51851, P = 0.1289$), 30%/min VDR of carbon dioxide ($Z = –0.11952, P = 0.9049$), and 70%/min VDR of carbon dioxide ($Z = 0.69282, P = 0.4884$). The ranges and medians of scores for each anesthetic are provided in Figure 5.

When participants were asked whether the mouse appeared to be fearful during the induction, responses did not differ between the 2 groups for the mice exposed to pentobarbital ($X^2_{1, 10} = 0.483, P = 0.4870$), isoflurane ($X^2_{1, 10} = 0.000, P = 1.000$), 30%/min VDR of carbon dioxide ($X^2_{1, 10} = 0.483, P = 0.4870$), and 70%/min VDR of carbon dioxide ($X^2_{1, 10} = 0.383, P = 0.4870$). Median scores of the rankings of fear behavior did not differ between the groups for pentobarbital ($Z = 0.34017, P = 0.7337$), isoflurane ($Z = 0.00000, P = 1.0000$), 30%/min VDR of carbon dioxide ($Z = 0.00000, P = 1.0000$), and 70%/min VDR of carbon dioxide ($Z = 0.76681, P = 0.4432$). The ranges and medians of scores for each anesthetic are provided in Figure 6.

When asked if the mouse appeared to experience signs of respiratory distress during the induction, participants who were told that the mouse was being euthanized were significantly more likely to say 'yes' for the mouse representing 30%/min VDR of carbon dioxide ($X^2_{1, 10} = 3.278, P = 0.0702$) and 70%/mon VDR of carbon dioxide ($X^2_{1, 10} = 5.487, P = 0.0192$). The group told that the mouse was being anesthetized was significantly more likely to say 'yes' for the mouse representing isoflurane ($X^2_{1, 10} = 5.487, P = 0.0192$). Responses did not differ between groups for the mouse representing pentobarbital ($X^2_{1, 10} = 0.00000, P = 1.0000$). Median scores of the rankings of respiratory distress did not differ between the groups for pentobarbital ($Z = 0.43818, P = 0.6613$) and 30%/min VDR of carbon dioxide ($Z = 1.35000, P = 0.1770$). Median scores for respondents told they were observing euthanasia were significantly higher for the mouse representing 70%/min VDR of carbon dioxide ($Z = 2.04101, P = 0.0413$) and significantly lower for the mouse representing isoflurane ($Z = –1.83303, P = 0.0668$). The ranges and medians of scores for each anesthetic are provided in Figure 7.

When participants were asked whether the mouse exhibited other distress during the induction, responses did not differ between the group told they were observing anesthesia as compared with those told they were observing euthanasia for the mouse representing isoflurane ($X^2_{1, 10} = 0.403, P = 0.5257$). Those who were told the mouse was being aneste-
tized were more likely to say ‘yes’ for the mouse representing 30%/min VDR of carbon dioxide ($X^2_{1,10} = 3.72, P = 0.0702$) and the 70%/min VDR of carbon dioxide ($X^2_{1,10} = 5.487, P = 0.0192$) but more likely to say ‘no’ for the mouse representing pentobarbital ($X^2_{1,10} = 3.278, P = 0.0702$). Median scores of the rankings of other distress did not differ significantly between the groups for the mice representing pentobarbital ($Z = 1.35000, P = 0.1770$), isoflurane ($Z = 0.11547, P = 0.8174$), 30%/min VDR of carbon dioxide ($Z = 0.00000, P = 1.0000$), and 70%/min VDR of carbon dioxide ($Z = –1.56374, P = 0.1179$). The ranges and medians of scores for each anesthetic are provided in Figure 8.

When observers were asked if the method depicted was an appropriate and efficient induction of anesthesia, responses did not differ between the group told they were observing anesthesia as compared with those told they were observing euthanasia for the mice representing pentobarbital ($X^2_{1,10} = 0.00000, P = 1.0000$), isoflurane ($X^2_{1,10} = 0.2210$), and pentobarbital ($X^2_{1,10} = 0.00000, P = 1.0000$). Those who were told they were watching euthanasia were more likely to say ‘yes’ for the mouse representing pentobarbital ($X^2_{1,10} = 3.379, P = 0.0702$). When participants were asked to rank their willingness to perform the method depicted, median scores did not differ between groups for pentobarbital ($Z = 0.00000, P = 1.0000$), isoflurane ($Z = –0.80000, P = 0.4237$), 30%/min VDR of carbon dioxide ($Z = 0.00000, P = 1.0000$), and 70%/min VDR of carbon dioxide ($Z = 0.43164, P = 0.1797$). The ranges and medians of scores for each anesthetic are provided in Figure 9.

**Rats.** When observers were asked whether the rat exhibited escape behaviors during anesthesia induction, responses did not differ between the group told they were observing anesthesia as compared with those told they were observing euthanasia for the rats representing pentobarbital ($X^2_{1,10} = 0.219, P = 0.6401$), isoflurane ($X^2_{1,10} = 0.026, P = 0.8719$), 30%/min VDR of carbon dioxide ($X^2_{1,10} = 0.00000, P = 1.0000$), and 70%/min VDR of carbon dioxide ($X^2_{1,10} = 0.882, P = 0.3477$). Median scores of the rankings of escape behavior did not differ between groups for pentobarbital ($Z = 0.14699, P = 0.8831$), isoflurane ($Z = –0.60830, P = 0.5430$), and pentobarbital ($Z = 0.00000, P = 1.0000$). Those who were told they were watching euthanasia were more likely to say ‘yes’ for the mouse representing 30%/min VDR of carbon dioxide ($X^2_{1,10} = 3.278, P = 0.0702$). When participants were asked to rank their willingness to perform the method depicted, median scores did not differ between groups for pentobarbital ($Z = 0.24340, P = 0.8077$), isoflurane ($Z = –1.32812, P = 0.1841$), and pentobarbital ($Z = –0.7042, P = 0.2764$). Median scores of the rankings of fear behavior did not differ between groups for pentobarbital ($Z = 0.21079, P = 0.8330$). The ranges and medians of scores for each anesthetic are provided in Figure 11.
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When observers were asked whether the rat displayed any evidence of respiratory distress during anesthetic induction, responses did not differ between the group told they were observing anesthesia as compared with those told they were observing euthanasia for the rat representing pentobarbital ($X^2_{1, 10} = 1.752, P = 0.1856$), isoflurane ($X^2_{1, 10} = 0.934, P = 0.3338$), and 70% per minute VDR of carbon dioxide ($X^2_{1, 10} = 0.760, P = 0.3834$). Those who were told they were watching anesthesia were more likely to say ‘yes’ for the rat representing 30% per minute VDR of carbon dioxide ($X^2_{1, 10} = 3.845, P = 0.0499$). Median scores of the rankings of respiratory distress did not differ between groups for pentobarbital ($Z = 1.62885, P = 0.1033$), isoflurane ($Z = –0.74983, P = 0.4534$), and 70% per minute VDR of carbon dioxide ($Z = 0.53120, P = 0.5953$). The ranges and medians of scores for each anesthetic are provided in Figure 12.

When participants were asked if the rat exhibited any other kind of distress during anesthetic induction, responses did not differ between groups for pentobarbital ($X^2_{1, 10} = 1.185, P = 0.2764$), isoflurane ($X^2_{1, 10} = 0.393, P = 0.5308$), 30% per minute VDR of carbon dioxide ($X^2_{1, 10} = 2.486, P = 0.1149$), and 70% per minute VDR of carbon dioxide ($X^2_{1, 10} = 0.760, P = 0.3834$). Median scores of the rankings of other distress did not differ between groups for pentobarbital ($Z = –0.79715, P = 0.4254$), 30% per minute VDR of carbon dioxide ($Z = –1.05396, P = 0.2919$), and 70% per minute VDR of carbon dioxide ($Z = 1.53796, P = 0.1241$). The ranges and medians of scores for each anesthetic are provided in Figure 13.

When observers were asked whether the videorecording depicted an appropriate and efficient method to induce anesthesia, responses did not differ between the group told they were observing euthanasia for the rats representing pentobarbital ($X^2_{1, 10} = 0.00, P = 1.0000$), isoflurane ($X^2_{1, 10} = 0.026, P = 0.8719$), 30% per minute VDR of carbon dioxide ($X^2_{1, 10} = 0.219, P = 0.6401$), and 70% per minute VDR of carbon dioxide ($X^2_{1, 10} = 0.26, P = 0.8719$). When participants were asked to rank their willingness to perform the method depicted, median scores did not differ between groups for the rats representing pentobarbital ($Z = 0.0000, P = 1.0000$), isoflurane ($Z = –0.64774, P = 0.5172$), 30% per minute VDR of carbon dioxide ($Z = 0.43183, P = 0.6659$), and 70% per minute VDR of carbon dioxide ($Z = 0.26684, P = 0.7896$). The ranges and medians of scores for each anesthetic are provided in Figure 14.

Demographic characteristics. Analysis by demographic is not reported because the sample sizes for each division of the demographic analysis were too small for meaningful comparisons. However, the percentages of respondents for each demographic characteristic are reported in Table 1.

Order of videorecordings. Analysis by video order is not reported because the sample sizes for each variation of video order were too small for meaningful comparisons.
Comments. Few written comments were provided by the respondents in this study, with the majority provided by participants observing rats and told that they were observing euthanasia. For the participants told they were observing euthanasia, 4 who observed mice provided feedback, and only the isoflurane treatment triggered comments. Although all commented that this treatment took the longest time, 2 of the respondents indicated that they felt the animal was distressed, whereas the other 2 indicated they felt the mouse was engaged in the exploratory behavior. Among the volunteers observing rats and told they were watching euthanasia, 8 participants provided feedback: 2 indicated that they felt that the pentobarbital was the most humane because it was relatively quick with minimal stress; 3 commented that isoflurane took a long time with much distress to the animal, with 1 indicating early defecation as an indicator of distress for this rat. The remaining 3 respondents commented on the 30% VDR of CO2; 2 indicated that this rat was much more distressed, with 1 respondent stating that this recording made him or her want to cry. The third participant indicated that although the procedure took a long time, the rat did not seem overly distressed. One participant commented on 70%/min VDR of CO2, indicating that it was rapid and appeared to be good for the rat. One participant provided a general comment stating that it was difficult to draw a line between normal exploratory behavior and escape behaviors, because the distinction implies intent, which cannot be assessed.

For the volunteers who were told they were observing anesthesia, 2 participants observing mice provided feedback. One of these indicated that “Faster is better—less distressful to watch and less distress for the animal.” The other reported that isoflurane took much longer than the other 3 methods. Of those observing rats and told they were watching anesthesia, 2 participants provided feedback, both of whom indicated that isoflurane took a very long time, with one noting “animal in distress with lots of rearing, then a long period with labored breathing and staggering.” One noted that 70%/min VDR CO2 was his or her least favored method because the “induction was quick, but the animal seemed more stressed” and that pentobarbital was his or her preferred method because “the animal seemed minimally stressed, and the induction was quick.”

Discussion

The questions and scales provided to the participants were intentionally left nebulous and ill-defined to allow identification of the biases that were brought to the study by each participant. In the continuing discussion concerning appropriate methods of euthanasia, all participants bring a level of experience, education, and comfort to their interpretation of the behaviors that they are observing. The perception of the distress experienced by the animal during euthanasia by the human observer may not match the reality from the perspective of the rodent. This disconnect is why the scientific community continues to pur-
Perception bias in the interpretation of distress

The results of the current study suggest that a perception bias was present in the persons sampled. It is noteworthy that, overall, participants provided the 4 mouse videos rated the mouse's experience more negatively when they were told that the mouse was being euthanized as compared with being anesthetized. This finding is consistent with increased concern regarding the wellbeing of a rodent being euthanized as compared with being anesthetized for a procedure. A similar trend was present in the responses of the participants provided the 4 rat videos, although the overall mean scores did not differ significantly. Observers reported less potential distress for the rats might be due to a variety of reasons, including their larger size, which made it easier to evaluate the animal on the recording. In addition, species bias might be present, if the participants were more comfortable with the behaviors of mice and were less confident in their assessment of the behaviors of rats. Although we did not collect information regarding past experience and comfort with rats and mice, it should not be dismissed that bias (albeit statistically nonsignificant) was present.

When considering the results of this study, it is critical to recall that the wellbeing of the rat or the mouse was not evaluated, only the perception of the welfare of the rodents in these representative recordings. Each rat and mouse likely experienced some distress with the procedure, whether from the placement in a novel environment, restraint for an injection, or the inhalation of a potentially aversive substance. However, an evaluation of the distress that was experienced by these animals is beyond the scope of this study. Some of the participants might have correctly identified behavioral signs consistent with potential pain or distress, but the study assessed their interpretation of these signs rather than the actual wellbeing of the rodents being euthanized. Evaluation
Figure 13. Distribution of scores for other distress by number of respondents observing a rat anesthetized with the various anesthetic methods. The box indicates responses where the participant reported that the animal exhibited no other distress. (A) Pentobarbital. (B) Isoflurane. (C) 30%/min VDR CO₂. (D) 70%/min VDR CO₂.

of parameters such as cardiovascular physiology and endocrine response can provide information about the wellbeing of animals during the euthanasia process. In addition, behavioral assessments are of critical importance when evaluating animal wellbeing. Subjective assessments can be made more reliable with training on well-characterized objective data. Ideally, these evaluations are performed by using objective data and defined scales, such as the mouse grimace score and other assays that take normal animal behavior into account.

The current findings strongly support why controlled studies that are objectively focused on the animal and a combination of its behavior, physiology, and affective state provide stronger evidence for evaluating potential pain and distress than studies that rely heavily on subjective—and potentially biased—interpretation of behaviors alone.

Acknowledgments

We thank Anish Patel for his assistance in data entry and Dr Ky Dehlinger for his thoughtful review.

References


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Figure 14. Distribution of scores of appropriateness by number of respondents observing a rat anesthetized with the various anesthetic methods. The box indicates responses of ‘appropriate.’ (A) Pentobarbital. (B) Isoflurane. (C) 30%/min VDR CO2. (D) 70%/min VDR CO2.


Table 1. Number of participants (% of total) responding in each demographic category

<table>
<thead>
<tr>
<th>What is your generation?</th>
<th>Mouse (n = 35)</th>
<th>Rat (n = 38)</th>
<th>Total (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Born 1945 or earlier</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Born 1946 through 1964</td>
<td>7 (20%)</td>
<td>2 (5%)</td>
<td>9 (12%)</td>
</tr>
<tr>
<td>Born 1965 through 1982</td>
<td>14 (40%)</td>
<td>22 (58%)</td>
<td>36 (49%)</td>
</tr>
<tr>
<td>Born 1983 or later</td>
<td>14 (40%)</td>
<td>14 (37%)</td>
<td>28 (38%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is your highest level of education?</th>
<th>Mouse (n = 35)</th>
<th>Rat (n = 38)</th>
<th>Total (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High school or GED</td>
<td>3 (9%)</td>
<td>4 (11%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>Associate degree (AA, AS)</td>
<td>4 (11%)</td>
<td>4 (11%)</td>
<td>8 (11%)</td>
</tr>
<tr>
<td>Bachelor degree (BA, BS)</td>
<td>16 (46%)</td>
<td>11 (29%)</td>
<td>27 (37%)</td>
</tr>
<tr>
<td>Master degree (MA, MBA, MS)</td>
<td>3 (9%)</td>
<td>2 (5%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Doctoral degree (PhD, DVM, VMD, MD)</td>
<td>9 (26%)</td>
<td>17 (45%)</td>
<td>26 (36%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Which best describes your position?</th>
<th>Mouse (n = 35)</th>
<th>Rat (n = 38)</th>
<th>Total (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal investigator (PI)</td>
<td>3 (9%)</td>
<td>4 (11%)</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>Research staff (nonPI)</td>
<td>17 (49%)</td>
<td>18 (47%)</td>
<td>35 (48%)</td>
</tr>
<tr>
<td>Veterinarian</td>
<td>5 (14%)</td>
<td>5 (13%)</td>
<td>10 (14%)</td>
</tr>
<tr>
<td>Veterinary technician</td>
<td>6 (17%)</td>
<td>6 (16%)</td>
<td>12 (16%)</td>
</tr>
<tr>
<td>Animal care technician</td>
<td>4 (11%)</td>
<td>5 (13%)</td>
<td>9 (12%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is your gender?</th>
<th>Mouse (n = 35)</th>
<th>Rat (n = 38)</th>
<th>Total (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>25 (71%)</td>
<td>24 (63%)</td>
<td>49 (67%)</td>
</tr>
<tr>
<td>Male</td>
<td>10 (29%)</td>
<td>14 (37%)</td>
<td>24 (33%)</td>
</tr>
</tbody>
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