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Mouse aversion to isoflurane versus carbon dioxide gas

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ABSTRACT

Isoflurane and carbon dioxide (CO_2) gas are used for rodent euthanasia. This study compared mouse aversion to isoflurane versus gradual-fill CO_2 gas, and compared two methods of isoflurane delivery: vaporizer and drop. Mouse acclimation to a light–dark apparatus was used to create a light aversion test based on an unconditioned preference for dark versus light areas. Mice chose between remaining in a dark compartment with rising concentration of one of three treatments (20% gradual-fill chamber vol/min of CO_2, $n=8$; 5% isoflurane administered using a vaporizer set at 4 L/min oxygen flow, $n=9$; or 5% liquid isoflurane dropped on gauze, $n=9$), or escaping to a brightly lit compartment. On average (±S.E.) mice left the dark compartment after 29.2 ± 6.1 s in the isoflurane vaporizer treatment. Initial withdrawal time was lower for the CO_2 treatment ($P=0.04$), averaging 16.6 ± 2.8 s, and lower still for the isoflurane drop treatment ($P<0.001$), averaging 2.9 ± 0.79 s. Five of nine mice became recumbent in the dark compartment when exposed to the isoflurane vaporizer treatment compared to only two of nine mice during the drop treatment ($P=0.3$) and zero of eight mice during the CO_2 treatment ($P=0.03$). The isoflurane concentrations rose more quickly using the drop versus the vaporizer method, likely explaining the increased willingness of mice to be exposed to isoflurane administered via a vaporizer machine. Re-exposure to isoflurane with the vaporizer was more aversive than initial exposure; only two of nine mice stayed in the dark compartment until recumbency. These results support the recommendation that mice with no previous exposure to isoflurane should be euthanised using isoflurane administered by a vaporizer rather than CO_2 gas, and suggest that the drop method (as applied in the current study) is not a suitable alternative.

1. Introduction

Current laboratory rodent euthanasia guidelines recommend using an inhalant anesthetic over carbon dioxide gas (CO_2) for rodent euthanasia (American Veterinary Medical Association, 2013; Canadian Council on Animal Care, 2010). Evidence suggests that isoflurane is less aversive to mice and rats than CO_2 (Leach et al., 2002b, 2004; Makowska and Weary, 2009; Wong et al., 2013) and other inhalant anesthetics (Makowska et al., 2009). Isoflurane is a volatile liquid halogenated hydrocarbon. Generally, one of two methods can be used to administer isoflurane for euthanasia: a vaporizer machine or the drop method. When administering isoflurane using a vaporizer machine, a carrier gas and an anesthetic waste gas scavenging system is required. Some animal users argue that the use of a vaporizer is unnecessary for rodent euthanasia. Vaporizers are intended to control the amount administered to reduce the risk of anesthetic overdose, a feature of little value when the intention is to kill the animal. In addition, vaporizer machines can be costly to purchase and maintain,

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reducing accessibility for some users. The drop method involves placing liquid isoflurane on an absorbent material such as gauze, and placing this in a closed compartment. To the best of our knowledge, no studies have compared aversion to the drop versus vaporizer methods of isoflurane administration. In addition, many laboratories still use gradual-fill CO_2 for euthanasia. Thus we tested mouse aversion to isoflurane administered by a vaporizer, isoflurane administered via the drop method, and gradual-fill CO_2.

The light–dark paradigm is a conflict-based anxiety test originally developed by Crawley and Goodwin (1980) to test anti-anxiety medications on mice. This paradigm uses the innate unconditioned preference for dark versus light areas and fear of open spaces in mice. The light–dark apparatus is composed of three compartments: a large light compartment, a small dark compartment and a middle compartment separating the light and dark areas. Acclimation to the apparatus changes this novel environment into a familiar one, therefore producing a light aversion test instead of testing anxiety (Matynia et al., 2012). This paradigm has been used to test aversion to CO_2 versus isoflurane in rats (Wong et al., 2013).

Using the light–dark box paradigm, we tested mouse aversion to three euthanasia methods: (1) 20% gradual-fill chamber vol/min of CO_2, (2) 5% isoflurane administered using a vaporizer set at 4 L/min (40% chamber vol/min) oxygen (O_2) flow, and (3) 5% isoflurane administered using the drop method. Mice were able to choose between remaining in a small dark compartment with a rising concentration of one of three treatments or escaping to a larger brightly lit compartment. Remaining in the small dark compartment with a rising concentration of test gas indicates that mice find the larger bright compartment more aversive than the test gas. Alternatively, leaving the small dark compartment with the test gas indicates that the test gas is more aversive than the large bright compartment. Initial exposure aversion was examined for all treatments. In addition, re-exposure aversion was examined for the isoflurane vaporizer treatment; mice commonly undergo surgical procedures using an isoflurane vaporizer machine, and re-exposure may be more aversive than initial exposure (Wong et al., 2013).

2. Materials and methods

2.1. Pre-trial

During the pre-trial we measured the rate at which isoflurane concentration increased within a compartment when using the vaporizer and drop treatments (Fig. 1b); the results allowed us to estimate the isoflurane concentrations that mice would be exposed to during the experiment. Use of a theoretical 20% gradual-fill CO_2 curve (Fig. 1a), allowed us to estimate CO_2 exposure concentrations during this experiment.

An Innocage® disposable individually ventilated transparent mouse cage (Universal Euro Type II Long, Innovive Inc. San Diego, California, USA, 37.3 cm length × 23.4 cm width × 14.0 cm height) was used as the test cage. A Plexiglass lid with a centrally placed hole was placed on top of the cage during testing. A Capnomac Ultima™ (Datex Ohmeda Instrumentation Corporation, Helsinki, Finland) capnograph was used to measure the rising concentration of isoflurane in the cage, via a polyethylene/polyvinalchloride sampling line (Datex Ohmeda Instrument Corporation, Finland) inserted into a hole near the base of the anterior wall of the cage. Testing took place in Medical Block C at the University of British Columbia, Vancouver, Canada.

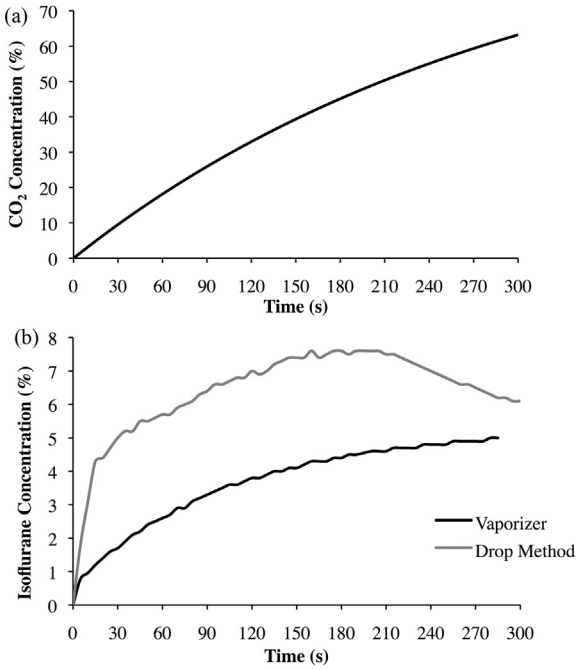

Fig. 1. Rising concentrations of test gases during (a) CO_2 administered at 20% chamber vol/min gradual-fill (based on gas fill equations), and (b) isoflurane administered either with a vaporizer (using a 5% concentration and 4 L/min O_2 as the carrier gas) or the drop method (measured using a capnograph).

For the isoflurane vaporizer treatment, 5% isoflurane (Baxter Corporation, Ontario, Canada) was administered via an Isotec 4 isoflurane vaporizer (Ohmeda, Steeton, West Yorkshire, England, UK) using 4 L/min (33% chamber vol/min) of room air as the carrier gas. The isoflurane drop treatment used wire mesh (Activ™-wire mesh, Activa Products Inc., Marshall, TX, USA) shaped to create a rectangular apparatus (7 cm length × 3 cm width × 11 cm height), that was closed on all sides except the top, to allow a piece of 5.1 cm × 5.1 cm gauze (Professional Preference, Rafter 8 Products, Calgary, AB, Canada) opened length-wise for vertical placement down into the wire rectangular apparatus. The wire apparatus was placed at the end of the rectangular test cage, standing up against the cage wall. The volume of isoflurane required to provide a 5% concentration in the compartment was determined to be 4.6 mL using the universal gas law ($PV = nRT$) and a room temperature of 22 °C. A glass syringe was used to distribute the liquid isoflurane down the piece of gauze within the wire mesh apparatus.