Laboratory mouse euthanasia: aversion and refinement

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Introduction

Two common methods of euthanasia for laboratory rodents are exposure to gradual-fill carbon dioxide (CO_2) and exposure to isoflurane

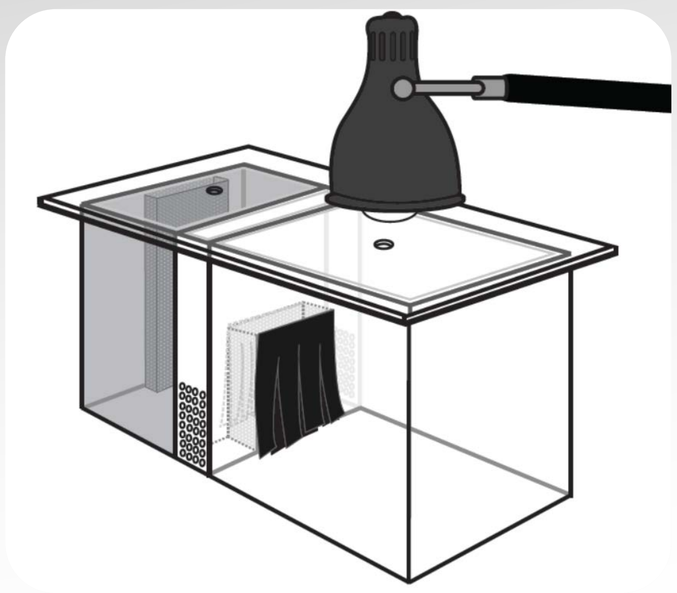
Regardless, once animals are anesthetised they can be exposed to otherwise painfully high concentrations of CO_2

Our objectives were to determine 1) which method is more humane to anesthetise mice; and 2) at what point mice are sufficiently insensible that it is safe to expose them to a high concentration of CO_2

Exp. 1: aversion to CO_2 vs. isoflurane

Methods

- Male C57BL/6J mice were tested in a light-dark box
  (light side: > 700 lux; dark side: <3 lux)
- Mice chose whether to remain on the normally preferred dark side when exposed to a rising concentration of:
  - CO_2 at flow rate of 20% compartment vol/min (*n*=8)
  - 5% liquid isoflurane dropped on gauze (*n*=9)
  - 5% isoflurane in O_2 at 4L/min from a vaporizer (*n*=9; initial and repeat exposure)

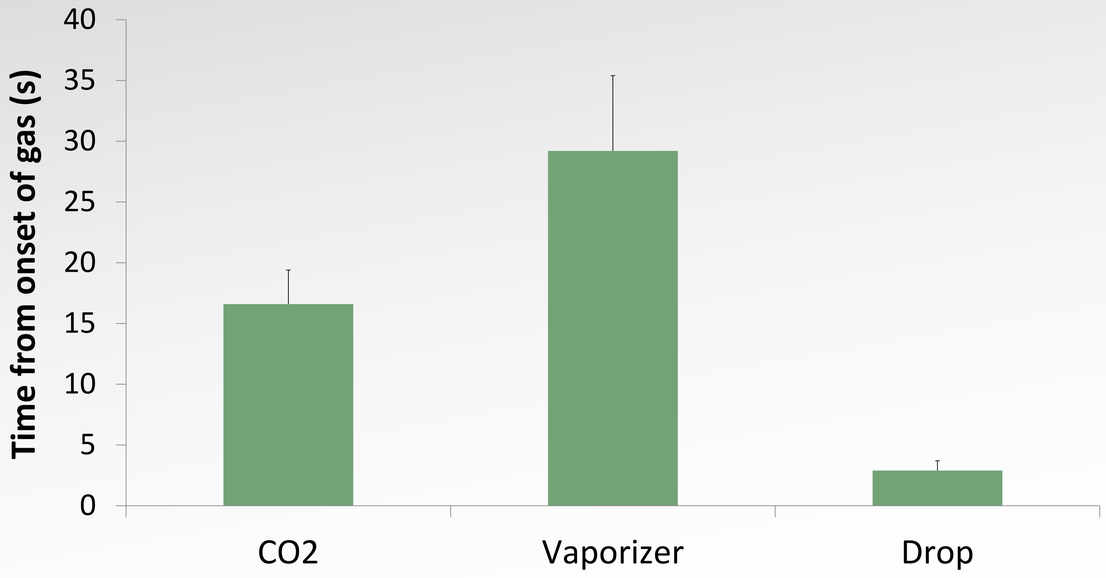


Results

Proportion of mice that became recumbent, and mean (± S.E.) number of re-entries into the dark compartment

	Recumbent	Re-entries
CO_2	0/8	1.0 ± 0.3
Isoflurane drop	2/9	1.2 ± 0.4
Isoflurane vaporizer – initial exposure	5/9	3.6 ± 1.0
Isoflurane vaporizer – re-exposure	2/9	1.0 ± 0.4

Latency to leave the dark compartment



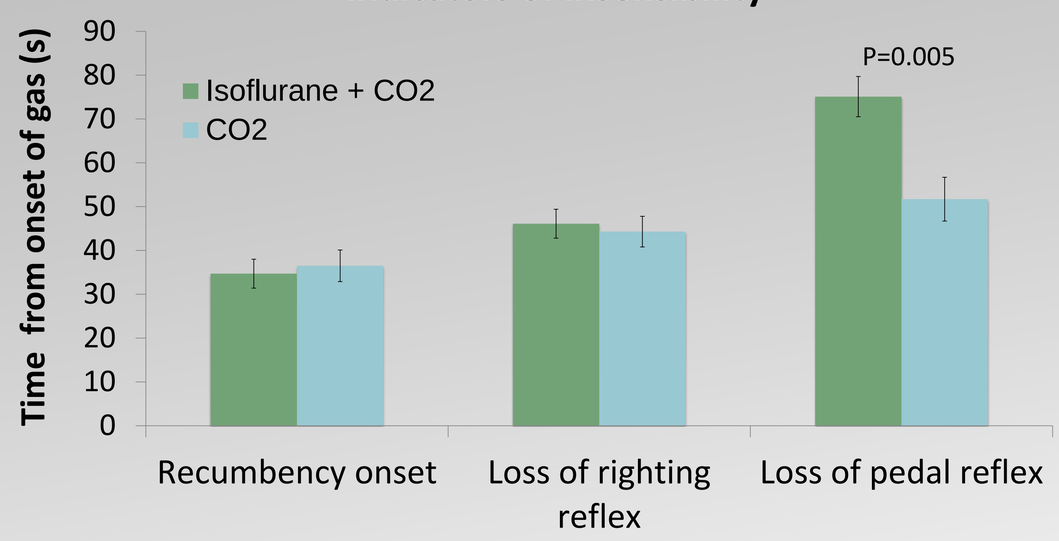
Exp. 2: when is it safe to switch to high CO_2?

Methods

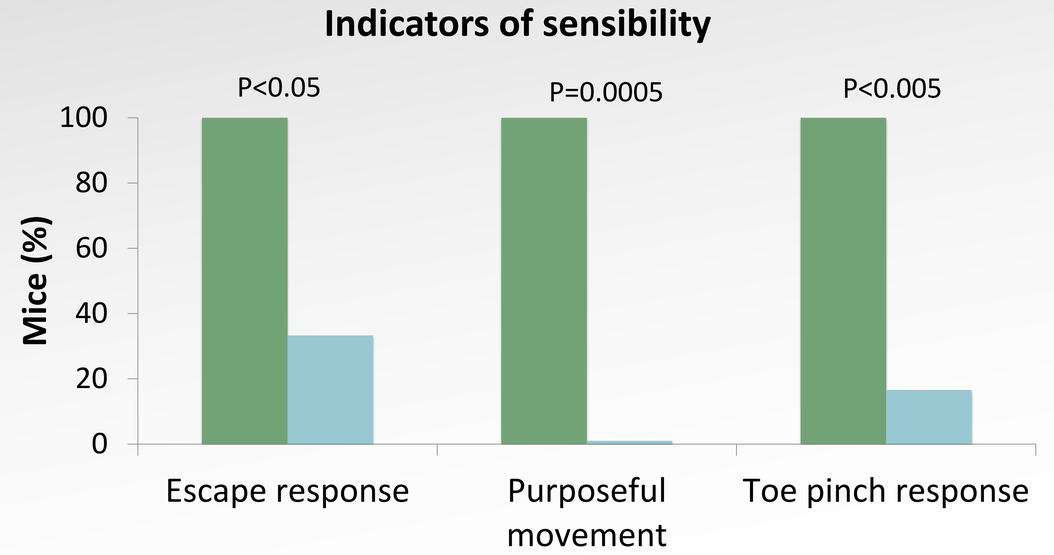
- Male C57BL/6J mice were exposed to:
  - 5% isoflurane in O_2 at 4L/min from a vaporizer (*n*=7)
  - CO_2 at flow rate of 20% cage vol/min (*n*=6)
- During exposure, we assessed indicators of insensibility (onset of recumbency, loss of righting, loss of pedal reflex). Upon recumbency we assessed signs of sensibility (escape movements, purposeful movements, response to toe pinch)

Results

Indicators of insensibility



Indicators of sensibility



Conclusions

Aversion to isoflurane by vaporizer was weaker than that to either the drop method or exposure to CO_2; these results support the use of isoflurane by vaporizer to anesthetise mice prior to killing with high concentrations of CO_2

Recumbency should not be used to infer insensibility, especially for mice anesthetised with isoflurane; we recommend users wait a minimum of 80 s following onset of recumbency before exposing mice to high CO_2

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