A Good Death?
Are our methods of killing animals humane?

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Aims

Examine the current state of knowledge regarding animal welfare aspects of the humane killing of lab animals – especially rodents

Pay particular attention to those methods permitted in the new EU directive (2010/63/EU)

Look especially at the problems with CO_2 and whether any alternative inhalation method is better

Why is the issue important?

We use large numbers of animals in the name of scientific research.

Almost all of these will have to be killed.

For instance; >20,000 rodents per year in my medium sized university alone.

Euthanasia

Greek: euthanasi^{ā} , *a good death* :

eu-, *good* + thanatos, *death*.

"A good death would be one that occurs with minimal pain and distress."

2007 AVMA Guidelines on Euthanasia

The term Humane Killing is perhaps more appropriate for laboratory animals

Directive 2010/63/EU

"Member States shall ensure that animals are **killed** with **minimum** pain, suffering and distress."

Directive 2010/63 (Annex IV) Permitted Killing Methods for Rodents

- Anaesthetic Overdose
- Carbon Dioxide (gradual fill only)
- Physical methods: cervical dislocation, concussion, or decapitation
- Inert Gases (Argon, Nitrogen)

Other methods acceptable if animal anesthetised first

But also,

Competent authorities can allow other methods;

If they're shown to be at least as humane

or, if they're scientifically justified

or, in an emergency.

Physical Methods

- Cervical dislocation: fast, requires skill.

  sedate >150g

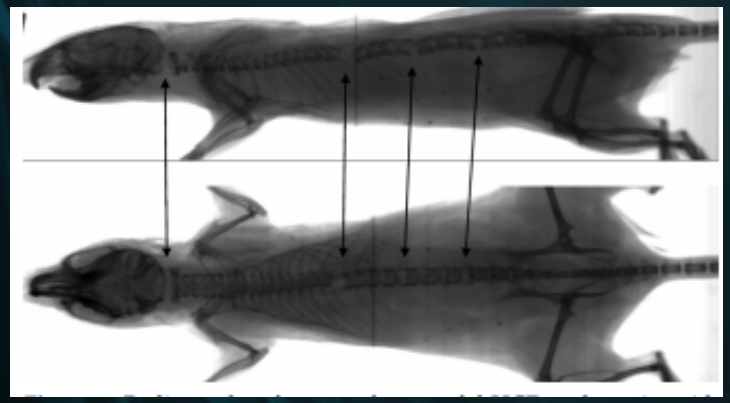
- Concussion: fast, requires skill

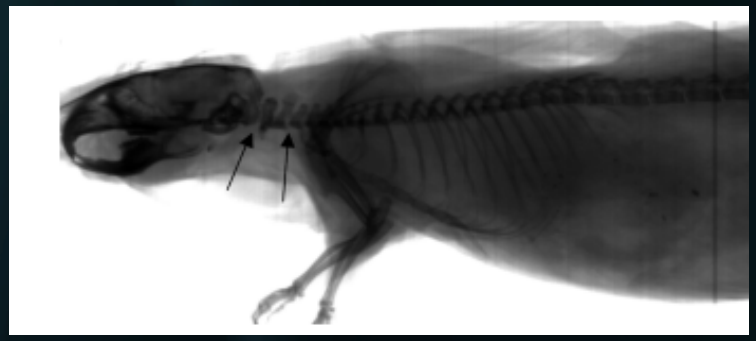
  Animals<1kg

- Decapitation: only if no other method possible.

"…The level of competence of the person carrying out this operation is equally important. Animals should therefore be killed only by a competent person….."

Problems with Physical Methods





Failures – in one study 21% of mice undergoing cervical dislocation under anaesthesia kept breathing.

Incomplete dislocation, wrong vertebra (thoracic not cervical etc.)

Carbone, L et al. . "Assessing Cervical Dislocation as a Humane Euthanasia Method in Mice." *Journal of the American Association for Laboratory Animal Science* 51, 352–356.

Parenteral Anaesthetic Overdose

Typically Pentobarbitone Sodium by the intraperitoneal route

I.P. Pentobarbitone causes abdominal writhing and possibly pain due to its alkalinity.

Svendsen et al. (2007),. Lab Anim. 41 197-203

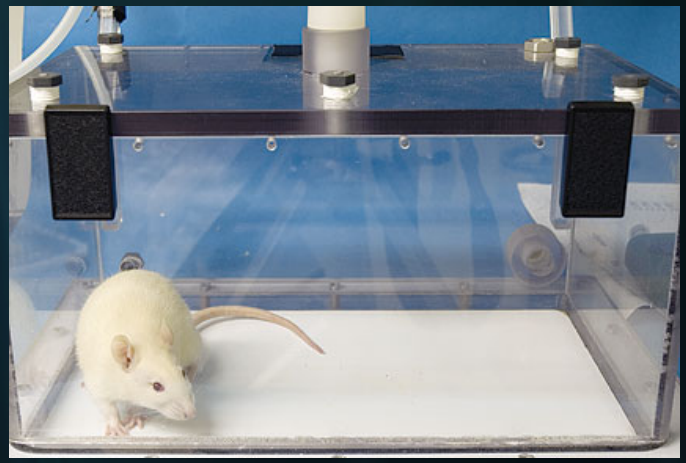
Improvements to Pentobarbitone

Adding lidocaine to the mixture significantly ameliorates nociceptive responses to pentobarbitone injection.

sodium pentobarbitone 0.4 ml/kg of (100 mg/ml) + lidocaine 10 mg/ml.

Svendsen et al. (**2007**),. Lab Anim. **41 197-20** 3

Inhalation Methods



Inhalation methods may not be the most humane for individual animals.

However, they're the only practical method for large numbers of animals

Most animals will be killed this way, and I will concentrate on these methods throughout the remainder of this talk

Carbon Dioxide

Most widely used method to kill lab rodents. Also widely used for poultry and pigs.

Simple, safe, effective, cheap, doesn't contaminate tissues, relatively un-distressing for operator.

REVIEW ARTICLE

Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats

K M Conlee[1], M L Stephens[1], A N Rowan[1] and L A King[2]

[1]The Humane Society of the United States, Animal Research Issues, 2100 L Street NW, Washington, DC 20037, USA; [2]Linacre College, Oxford University, St Cross Road, Oxford OX1 3PS, UK

Three major questions;

- Does CO_2 cause pain?
- Does CO_2 cause distress?
- Is anything else better?

Conlee, K M, M L Stephens, A N Rowan, and L A King. "Carbon Dioxide for Euthanasia: Concerns Regarding Pain and Distress, with Special Reference to Mice and Rats." *Laboratory Animals* , 39, 137 –161.

CO_2-Induced Pain

Circumstantial Evidence for CO_2 - Induced Pain

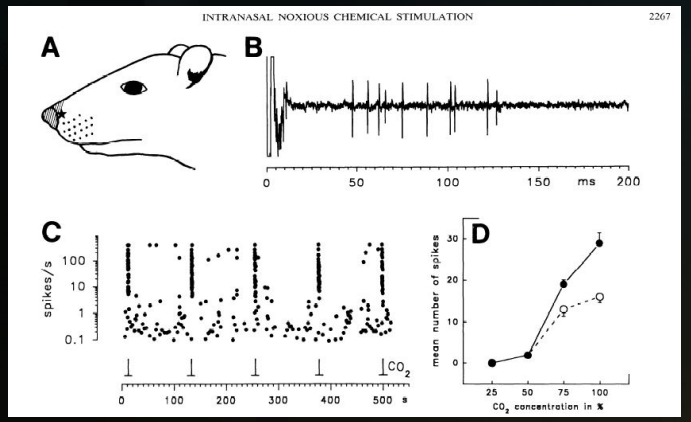
Humans find CO_2 painful at concentrations > ~50%

(Danneman, P J, et al. 1997. *Lab. Anim. Sci.* 47, 376–385.)

Rodents have similar nociceptors in a similar density in their nasal and ocular epithelia.

Nociceptors respond to CO_2 at similar concentrations

(e.g. Peppel P, Anton F (1993). J. Neurophysiol, **70**, 2260–75)

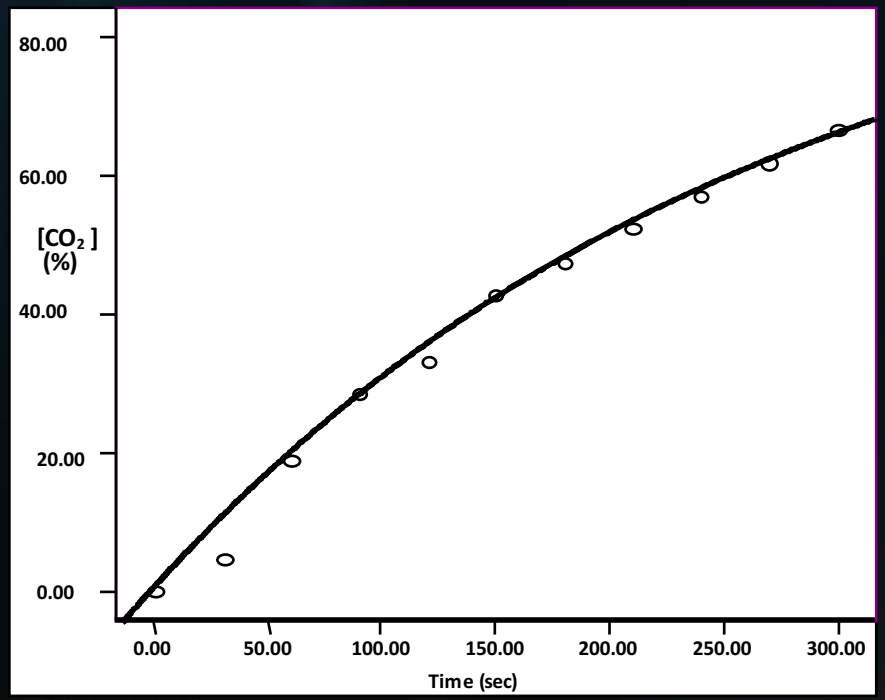


BUT, Using "Gradual Fill" probably avoids causing pain

Mandated by 2010/63/EU

Animal enters empty chamber

Typically, 20% of chamber volume per minute CO_2 enters



Time Course of Gradual-Fill

CO_2 is a general anaesthetic

During slow-fill animals become anaesthetised before CO_2 reaches potentially painful levels

Event	Time (Seconds)	CO_2 Concentration (%)
Recumbency	110±6	29
Loss of Consciousness	156±5	39

During pre-fill or rapid-fill animals may be exposed to CO_2 concentrations 50% before losing consciousness

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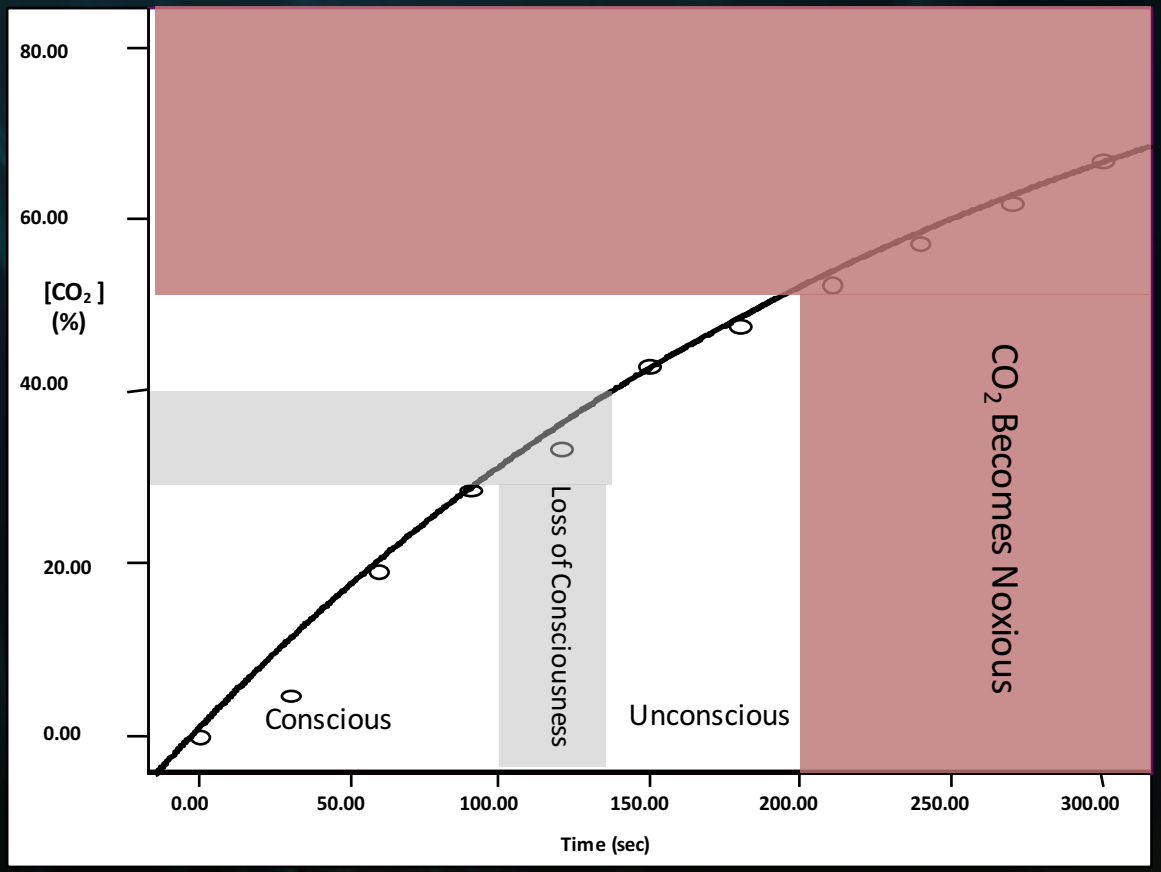
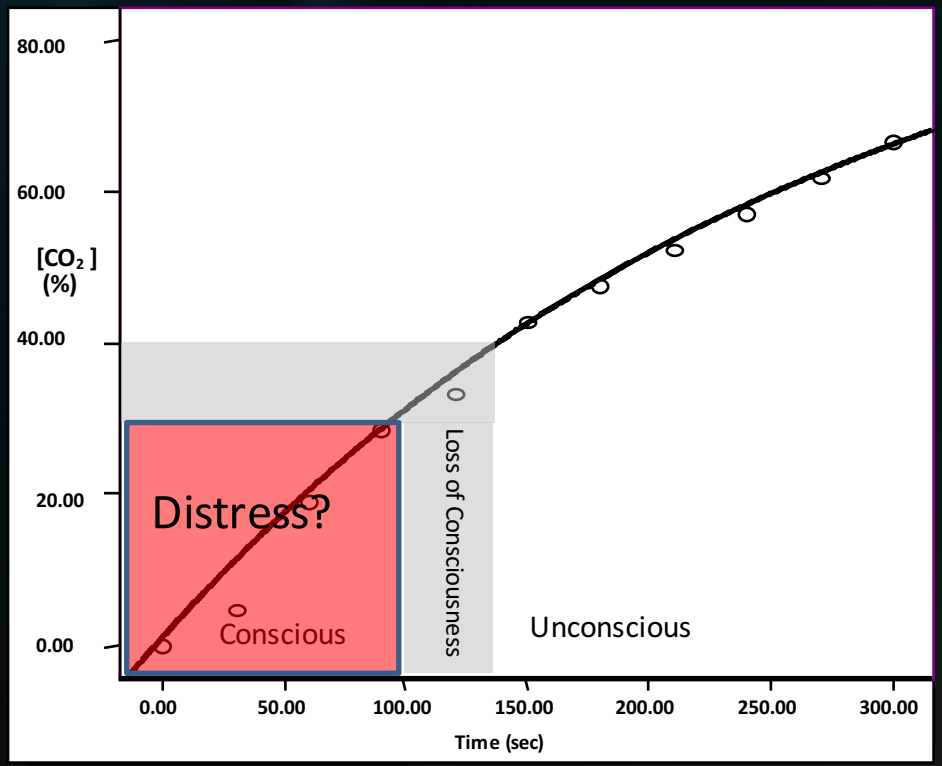
Gradual-Fill



So, why worry about slow fill?

Several studies have examined whether rodents show aversion to CO_2.

Most find evidence of aversion to relatively low levels of CO_2



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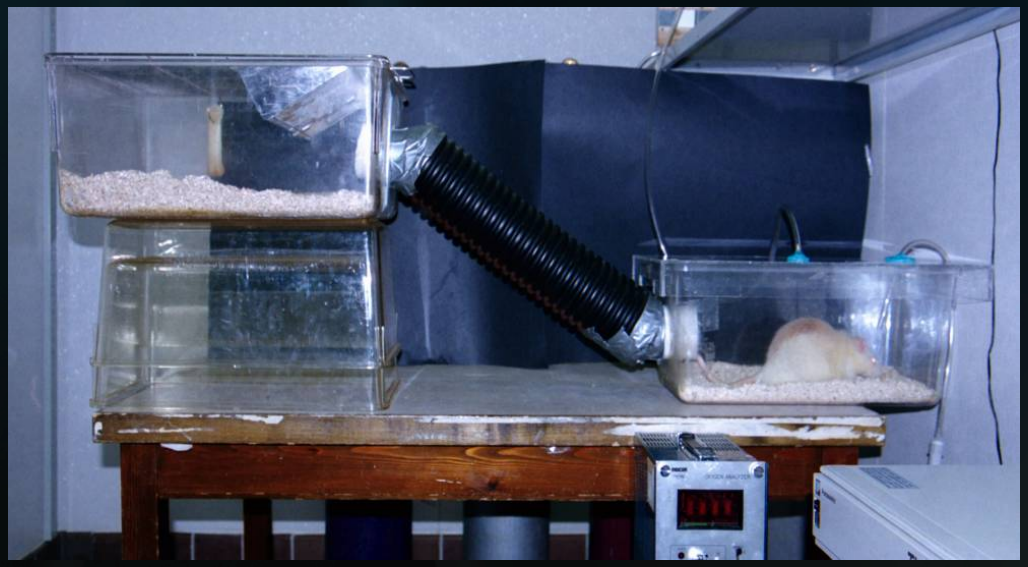
Approach-Avoidance

How strong is the aversion to CO_2?

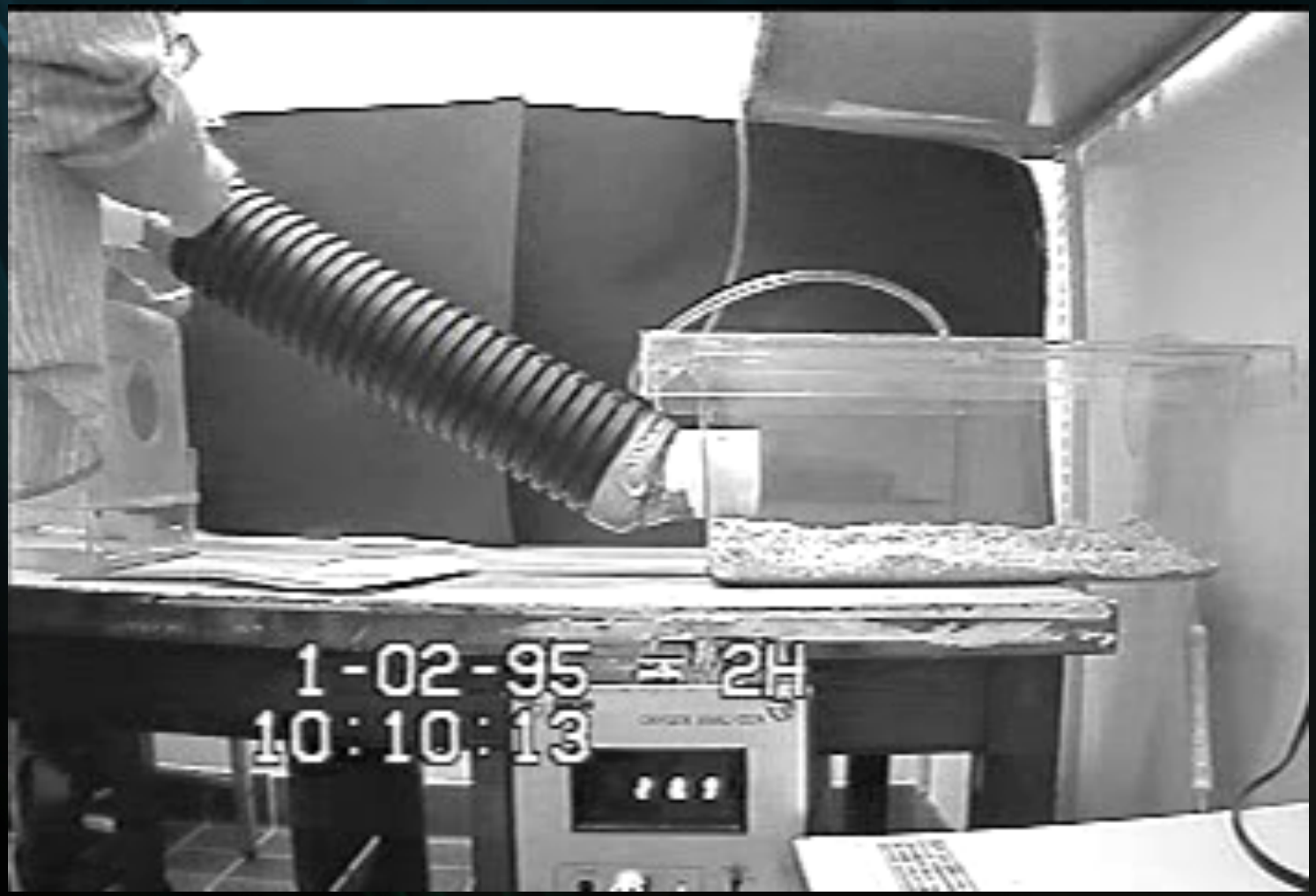
Lee Niel, Richard Kirkden, Joanna Makowska and Dan Weary,
Animal Welfare Program, University of British Columbia

- Rats trained to enter a chamber for a food reward – Honey Nut Cheerios.
- Chamber then filled with CO_2
- Time spent in chamber and number of cheerios eaten before leaving measured.

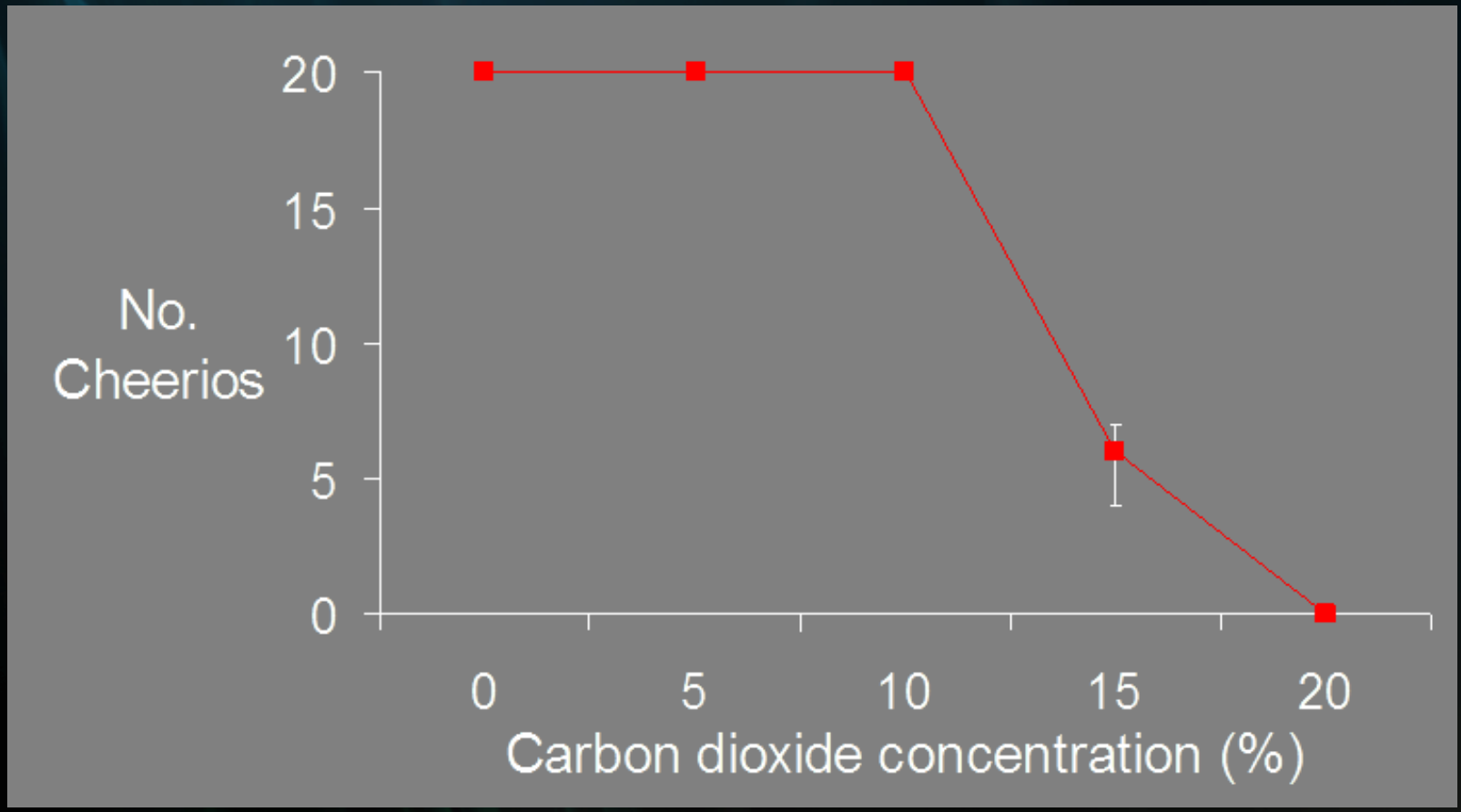


Kirkden RD, Niel L, et al. (2008) *Applied Animal Behaviour Science,* **114,** 216-234.
Kirkden RD, Niel L, et al. (2005), *Lab Anim,* **39,** 453-5.
Niel L, Weary D (2006), *Applied Animal Behaviour Science* **100,** 295-308.
Niel L, Weary DM (2007), *Applied Animal Behaviour Science,* **107,** 100-109.

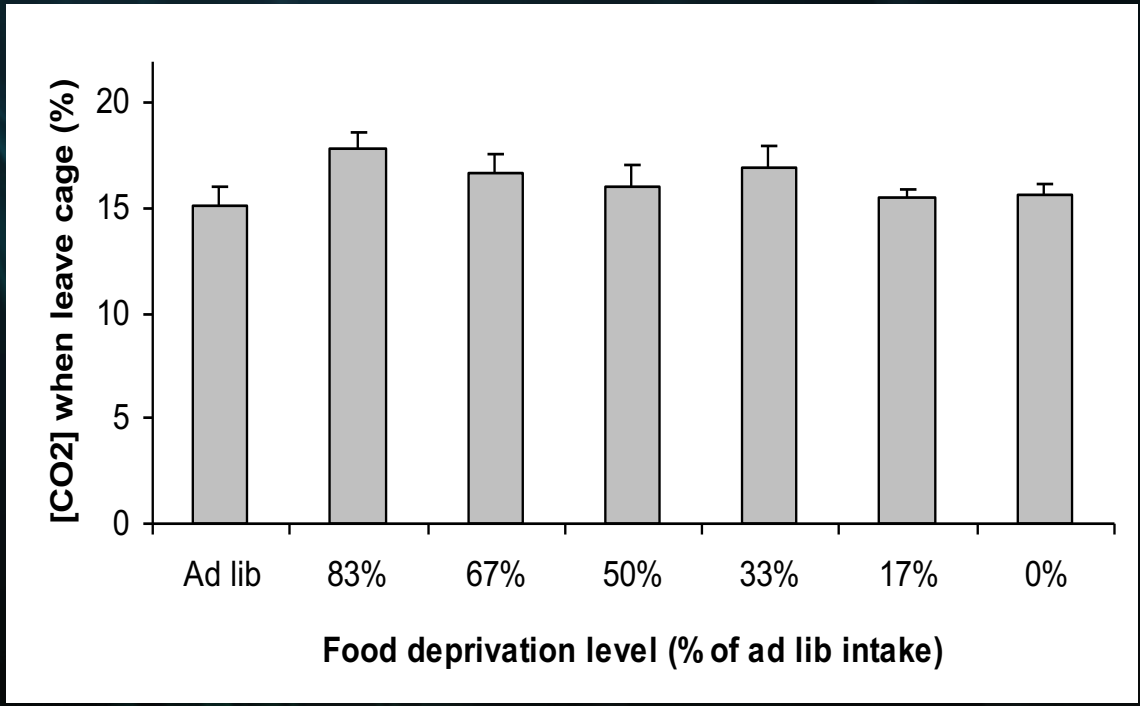
Approach-Avoidance in CO_2



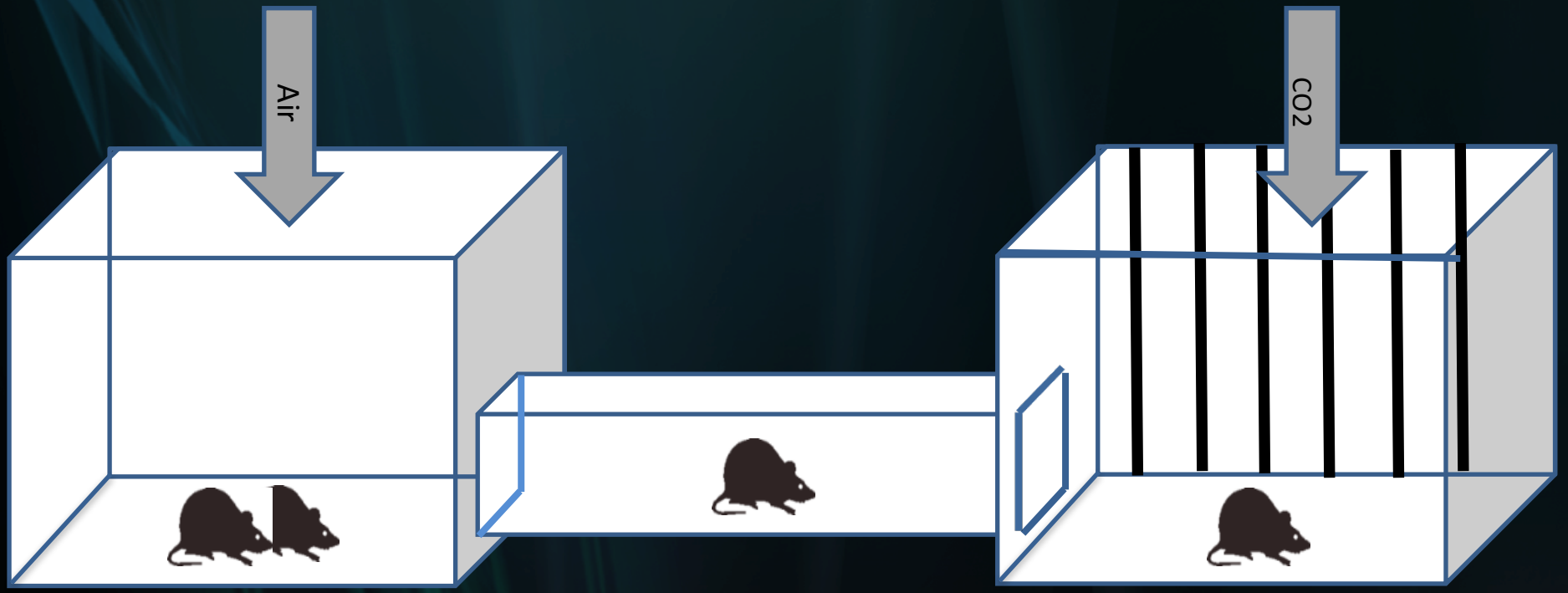
Rats are Averse to CO_2 at Concentrations > ~15%



Even Hungry Rats won't Tolerate 15% CO_2



CO_2-Conditioned Place Aversion



Rats remember that CO_2 is aversive and avoid CO_2-paired chamber.
Spend 10% less time on CO_2 side after training
This learning persists for many days post-exposure.

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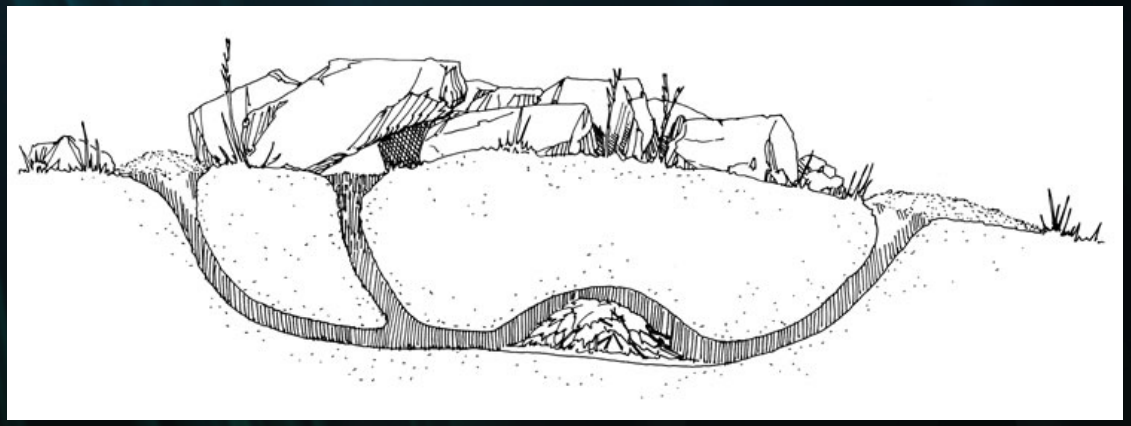
NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

If it's not pain, what causes aversion?

Adaptation to burrowing?



Mice can smell CO_2 at near atmospheric concentrations[2]

– avoid 0.2%!

[1] Krohn TC, Hansen AK, et al. (2003), *Lab Anim,* 37, 94-9.
[2] Hu J, Zhong C, et al. (2007) *Science,* 317, 953-7

Dyspnoea

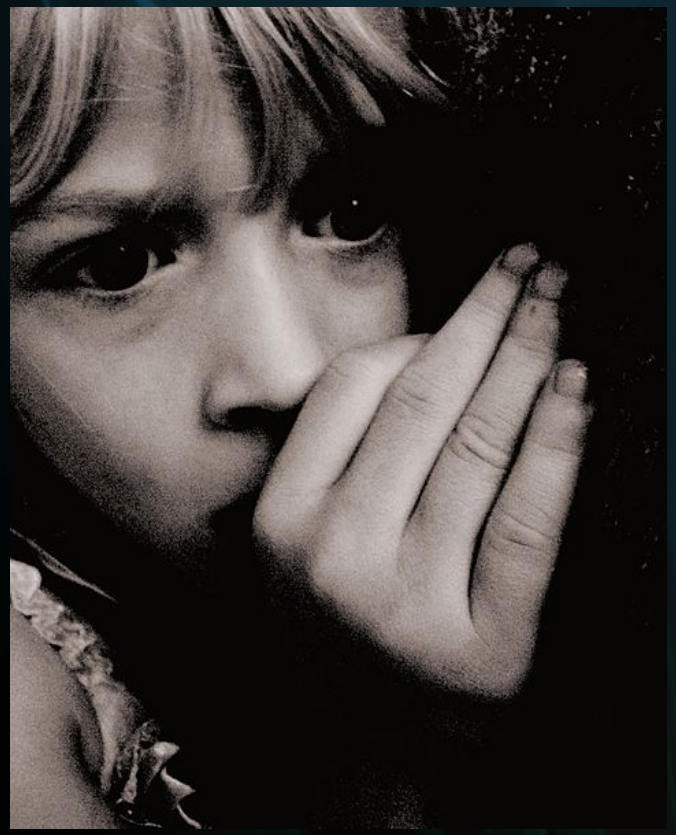
Dyspnoea - air hunger (uncomfortable urge to breathe/air starvation).

Breathing 7% CO_2 causes dyspnoea in humans.

May also occur in rodents

Banzett RB and SH Moosavi. (2001), *American Pain Society Bulletin* 11(2):1(6-8)

CO_2-induced fear

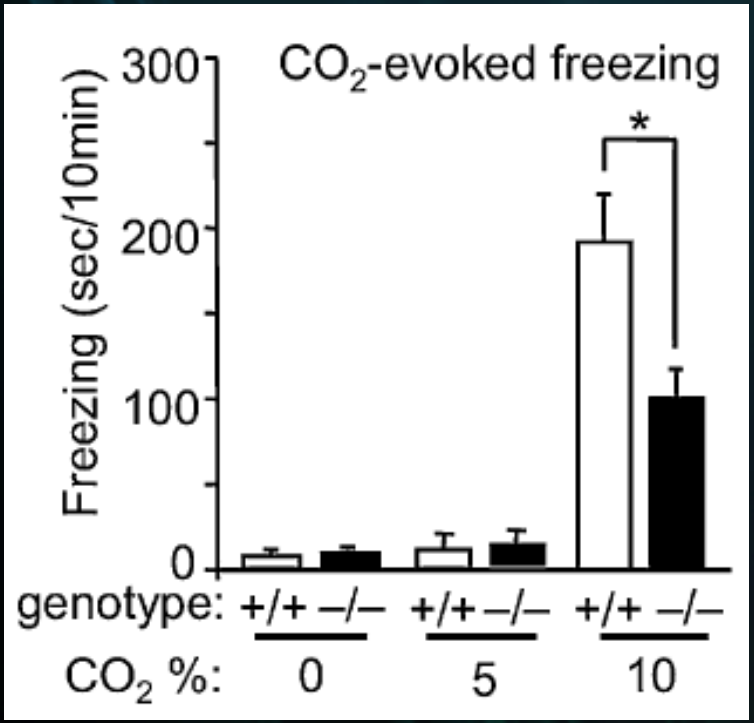


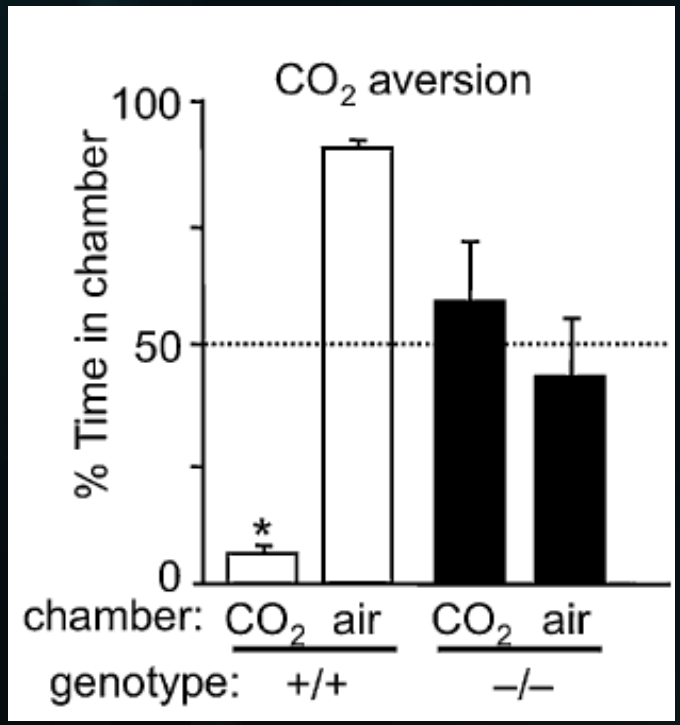
Breathing CO_2 can cause panic in humans.

Brief exposure to 20% CO_2 activates 'panic/defence' related brain circuits in rats (HPA axis etc).

Johnson, P et al.. "Induction of C-Fos in 'panic/defence'-Related Brain Circuits Following Brief Hypercarbic Gas Exposure." *Journal of Psychopharmacology* 25, (2011): 26–36.

CO_2-induced acidosis evokes fear responses in mice





Depends on sensing of acidosis by the ASIC1a channel – ASIC1a knockout mice have much less response

Ziemann et al. (2009). "The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior." *Cell* 139, 5: 1012–1021.

The case for CO_2

The previous studies don't model the euthanasia process.

Some studies find no behavioural signs of distress when exposing rats or mice to CO_2 for humane killing [1]

Others find no physiological signs of stress either[2]

Maybe the response to CO_2 induces stress but not *distress?*

1 Hackbarth, H-J et al. Euthanasia of rats with carbon dioxide--animal welfare aspects. .Lab Anim. 2000, 34:91-6.
2 Valentine, H et al. "Sedation or Inhalant Anesthesia Before Euthanasia with CO2 Does Not Reduce Behavioral or Physiologic Signs of Pain and Stress in Mice." *Journal of the American Association for Laboratory Animal Science* 51, no. 1 (2012): 50–57.
But see also: Makowska J, Golledge H, Marquardt N, Weary D. Comment on: Sedation or inhalant anesthesia before euthanasia with CO2 does not reduce behavioral or physiologic signs of pain and stress in mice. JAALAS 2013, 51, 396-397

Conclusions

Aversion to CO_2 is significant, outweighing the motivation to eat even when food-deprived.

Aversion is long-lasting.

Aversion occurs at levels where the rat would remain conscious during slow-fill, implying that it may be a serious welfare issue.

What are the Alternatives?

Inert Gases – Nitrogen/Argon etc

Volatile anaesthetics- Isoflurane, Halothane etc.

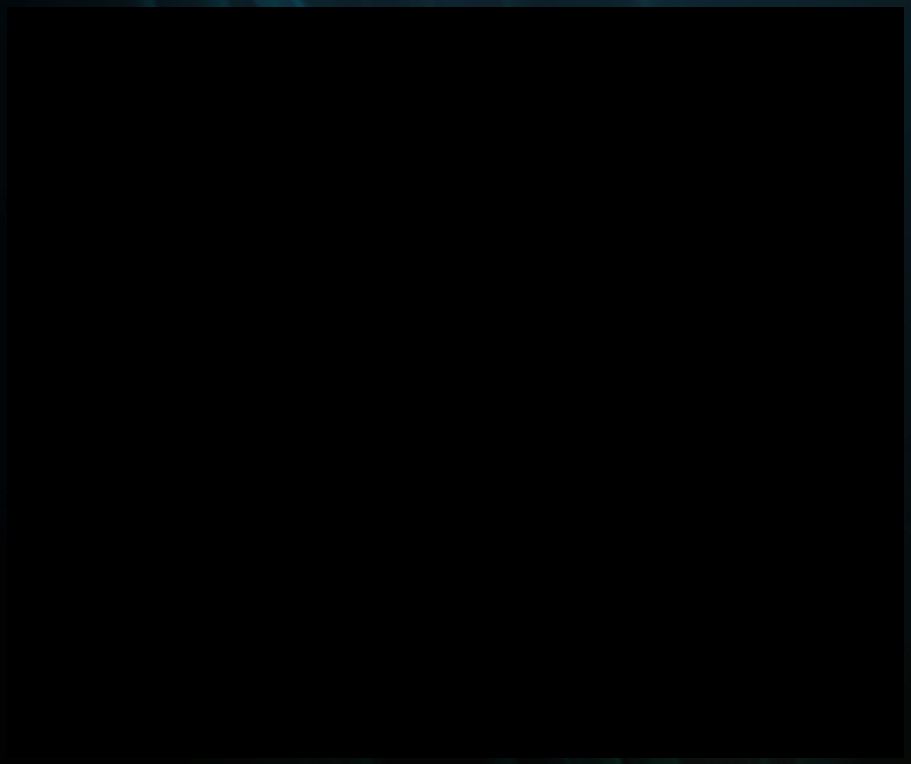
‘Inert’ Gases

Animals-remarks/ methods	Fish	Amphibians	Reptiles	Birds	Rodents	Rabbits	Dogs, cats, ferrets and foxes	Large mammals	Non-human primates
Inert gases (Ar, N_2)	X	X	X			X	X	(14)	X

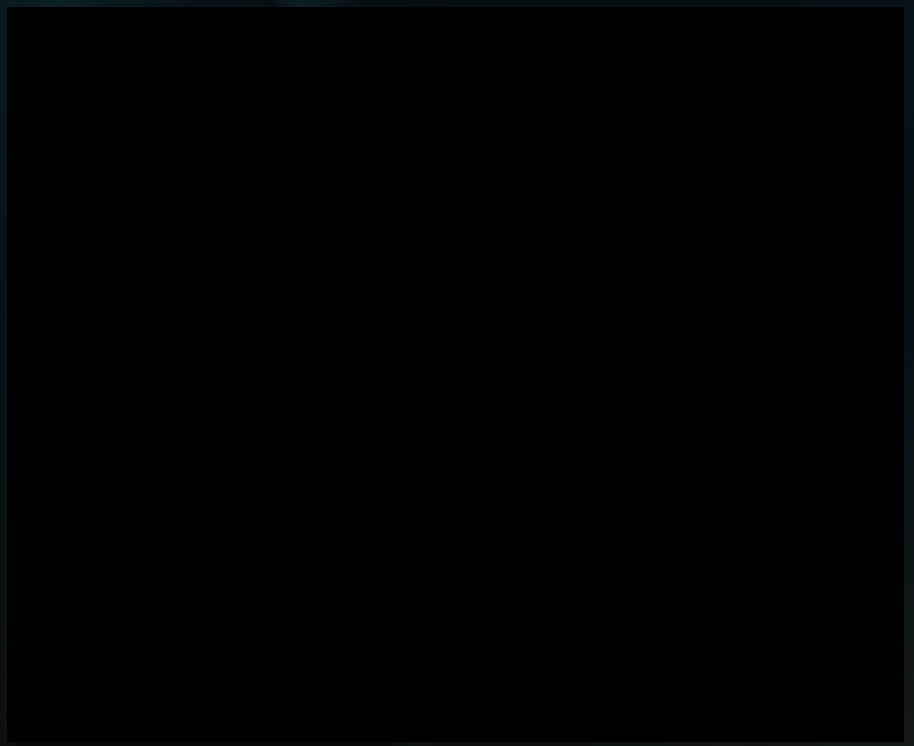
Argon, Nitrogen etc - induce anoxia

Pigs Tolerate Argon for Food Reward

Mohan Raj
University of Bristol



CO_2 – Video Clip



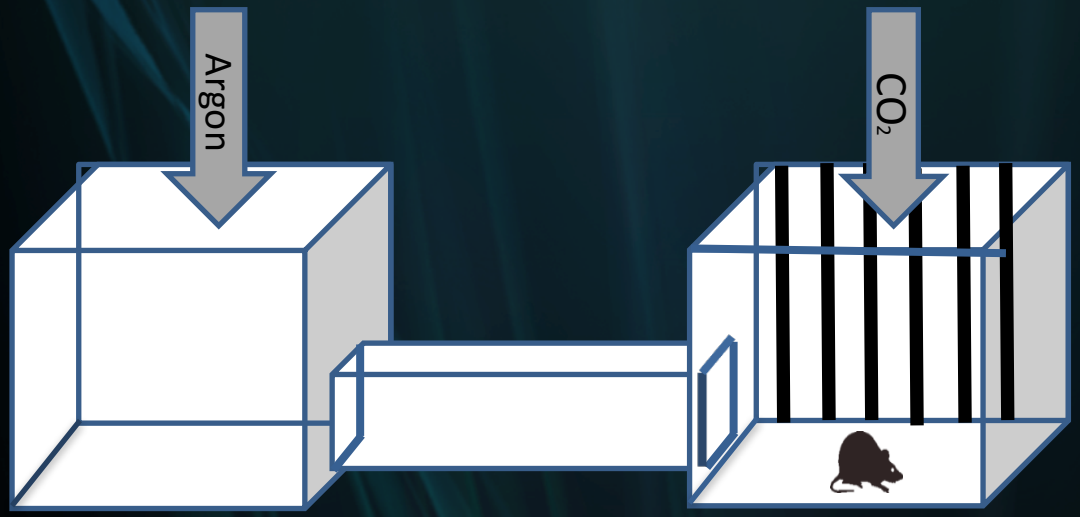
Argon – Video Clip

Raj, A.B.M. and Gregory, N.G. 1995. Welfare implications of gas stunning pigs 1.
Determination of aversion to the initial inhalation of carbon dioxide or argon. Animal Welfare, 4: 273-280.

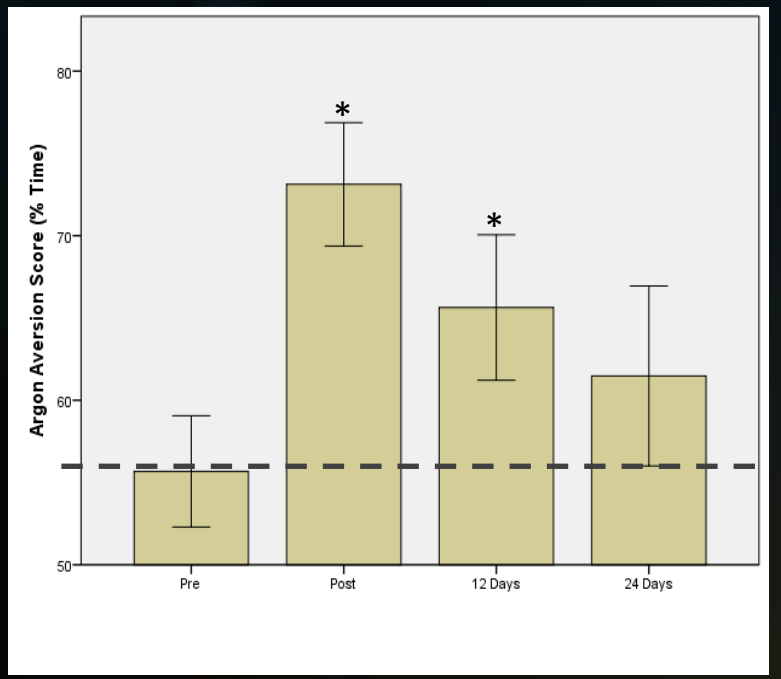
But, Rats are Averse to Argon

Niel, L and Weary, D.M.. "Rats Avoid Exposure to Carbon Dioxide and Argon." *Applied Animal Behaviour Science* 107, (2007) 100–109.
Makowska, I. J., et al.. "Rats Show Aversion to Argon-induced Hypoxia." *Applied Animal Behaviour Science* 114, (2008): 572–581.

Argon causes even stronger place aversion than CO_2



10/10 rats preferred CO_2 to Argon
Average preference shift to CO_2=17.4±3.4%),



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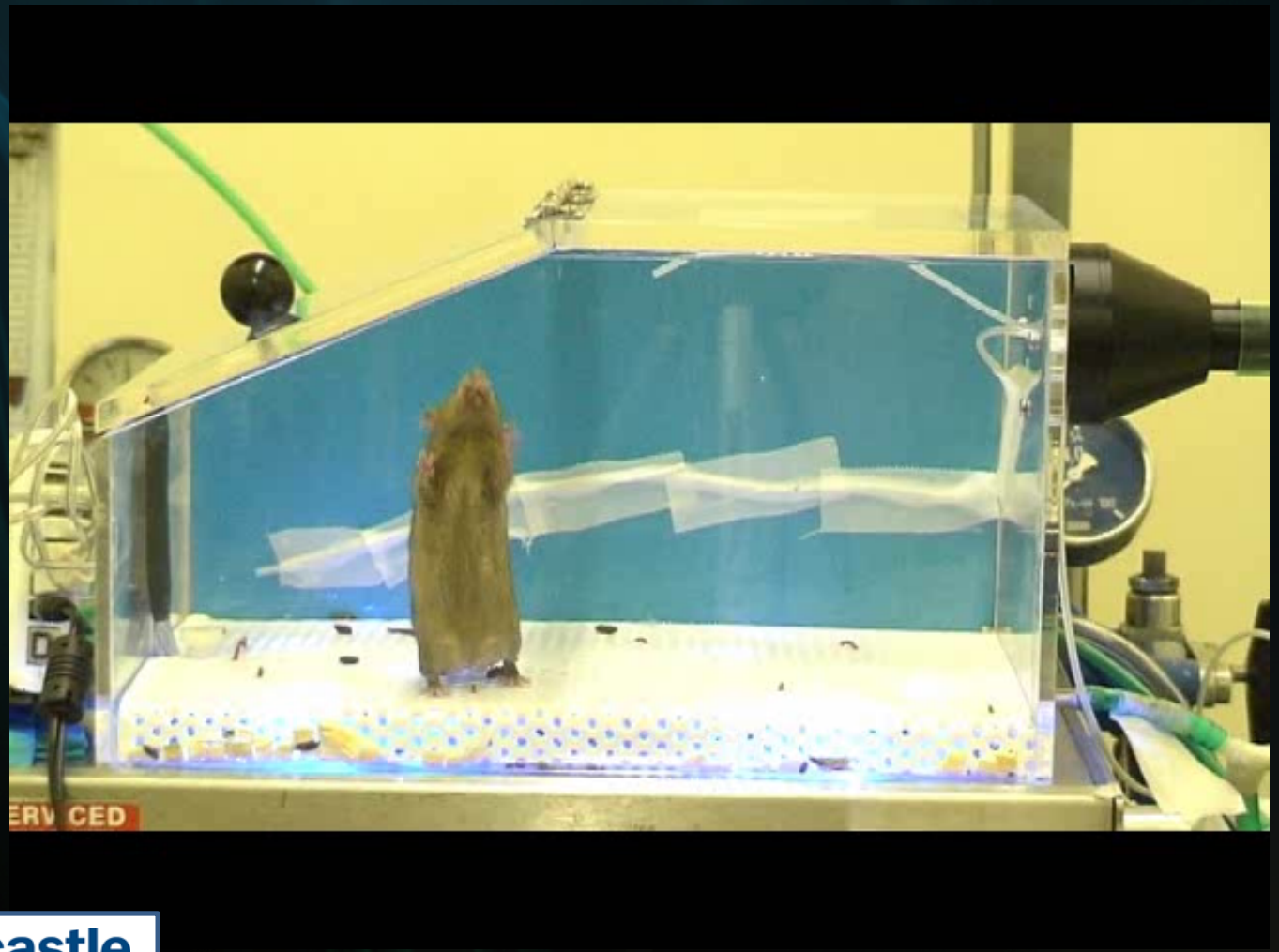
‘Escape’ reactions of mice to Nitrogen



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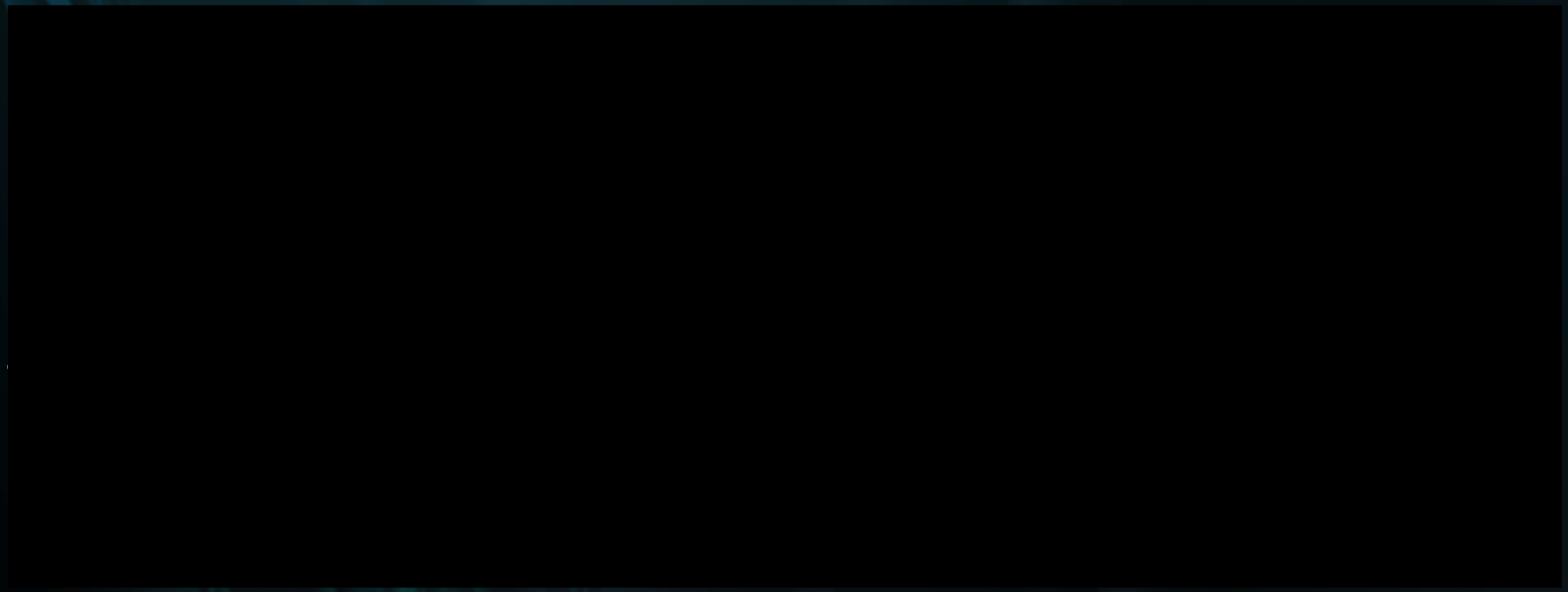
Volatile Anaesthetics

+ Possibly less aversive than CO_2

- Health and safety issues
- Normally don't actually kill the animal - need to kill with CO_2, physical method etc.

Golledge, Huw D. R. (2012) *Laboratory Animals* 46, no. 4, 358–359.



1

2

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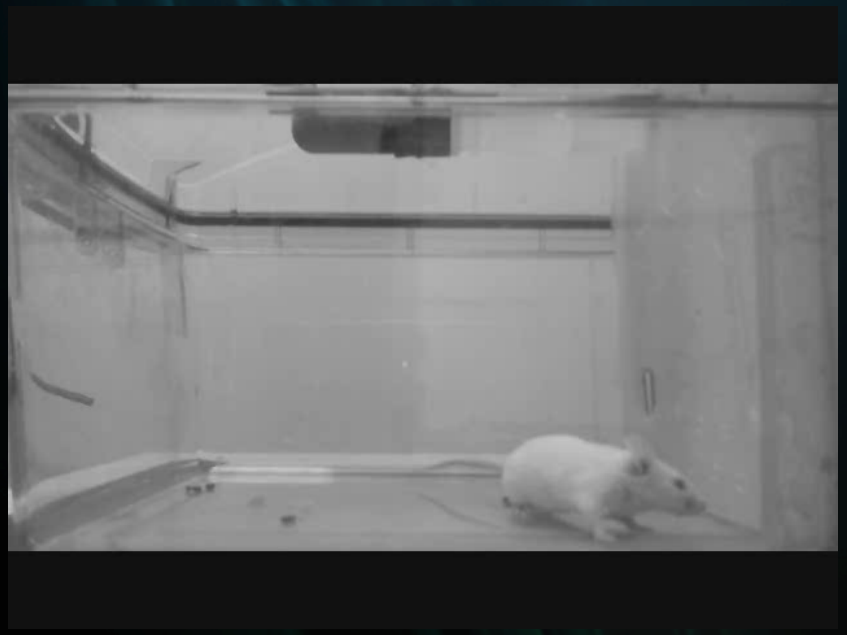

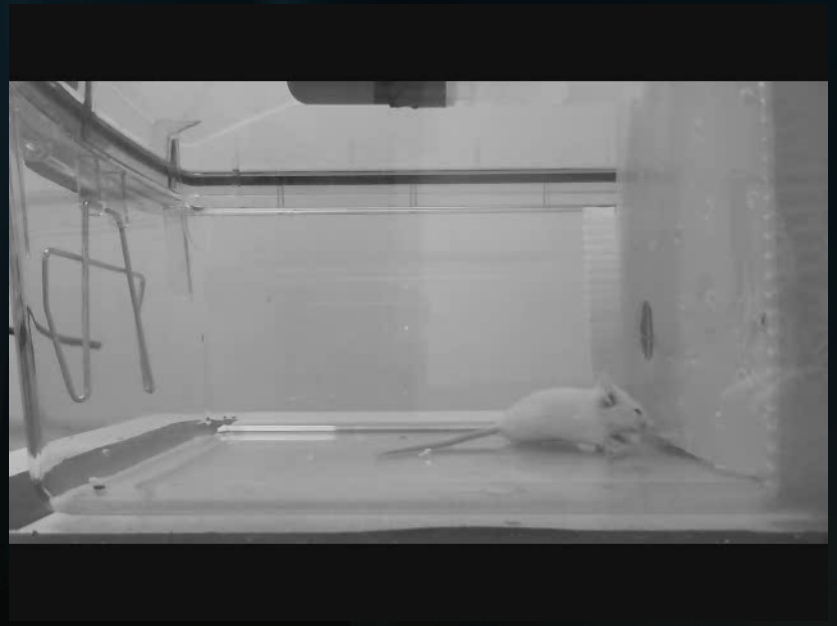

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“Involuntary Excitation”

“Given that multiple experiments have shown that isoflurane is less aversive than CO_2, we conclude that the behavioural differences are due to an excitatory phase during induction with isoflurane.”

I. J. Makowska and D. M. Weary

World Congress on Alternatives to Animal Experimentation 2011

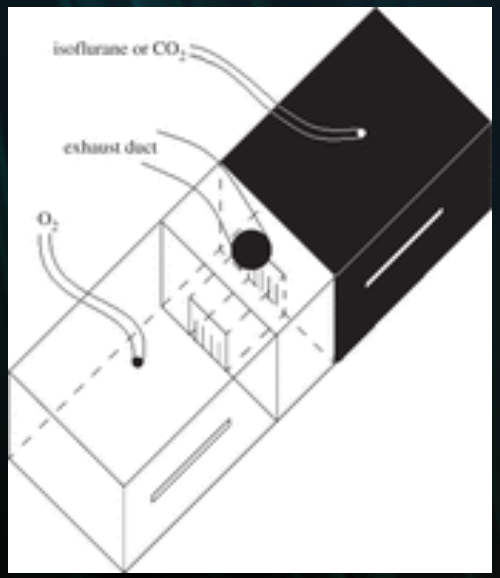
The question then becomes; is involuntary excitation an animal welfare issue?

Evidence of Stress during Isoflurane Induction

Behavioural signs of agitation[1,2]

Induction of brain *c-fos*[1]

After more than one exposure Iso becomes as aversive as CO_2[3]

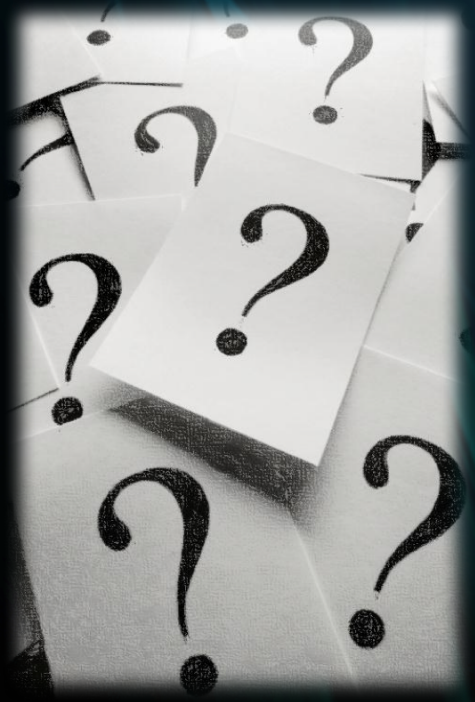


Exposure 1 - 4/8 remain in Iso

Exposure 2 - 0/8 remain in Iso

1. Valentine, H et al.. *Am. Assoc. Lab. Anim.Sci.*51, no. 1 (2012): 50–57.
2. Golledge, HDR, et al. (2011). Altex, **28,** 248
3. Wong, D et al. "Rat Aversion to Isoflurane Versus Carbon Dioxide." *Biology Letters* 9,1 **,1** 20121000

Summary – More Questions than Answers?



Carbon dioxide is not the ideal euthanasia agent but it's unclear if Isoflurane is significantly better.

Inert gases are almost certainly worse.

Some Practicalities

Carbon Dioxide or Isoflurane

CO_2: gradual fill – ~20-30% chamber volume per minute.

Home cage euthanasia if possible.

Improvements to CO_2 or Isoflurane

Carrying out the procedure in the home cage on relaxed (even sleeping) animals reduces "procedural stress" (handling, unfamiliar environment etc.).

May reduce stress responses during euthanasia itself.

Golledge, HDR, Lukic, J and Flecknell, PA. (2011). Altex, **28,** 248

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Lee Niel (Guelph, Canada).
Dan Weary and Joanna Makowska (UBC, Vancouver).

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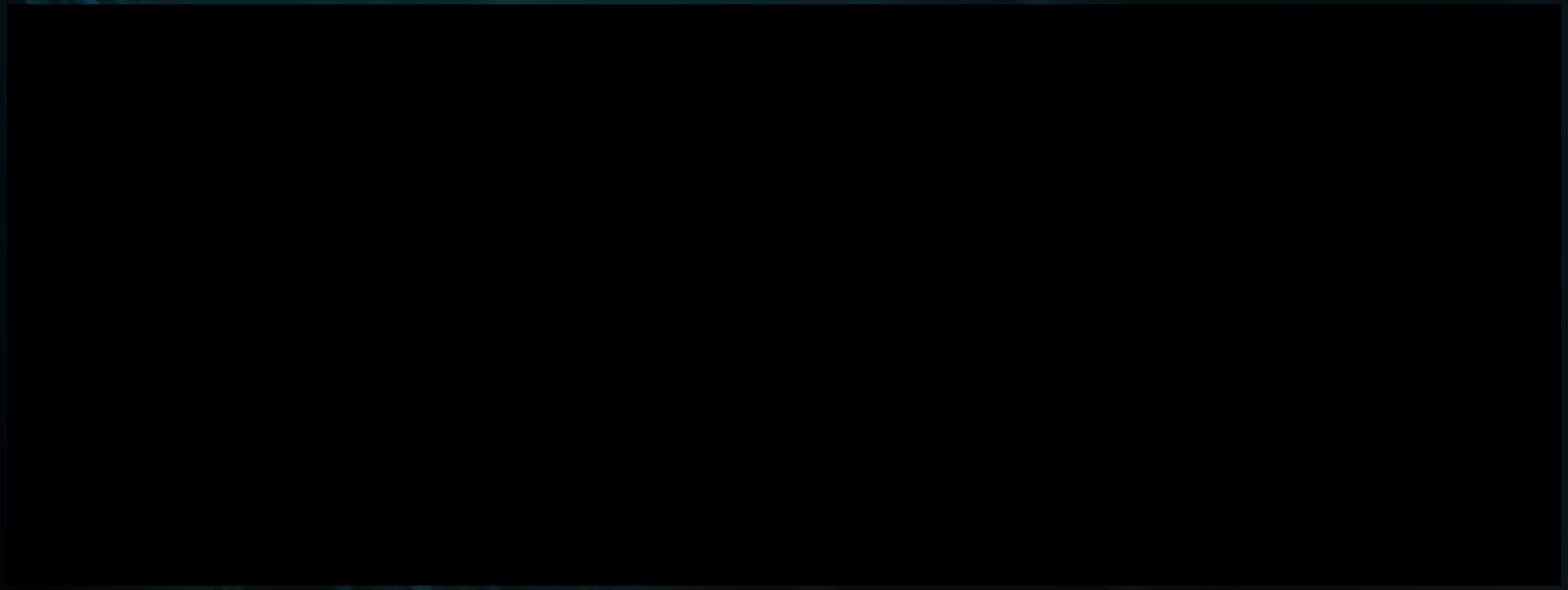
Is Isoflurane Really Better?



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