Refining intratracheal administration

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Introduction

This poster will provide an overview of intratracheal administration in mice with a focus on the refinements made by Christopher Moore from the Francis Crick Institute. It will explore the equipment that has been developed to increase Animal Welfare and maximise scientific outcomes when performing this procedure. The refined process enables easier training and demonstration of competence whilst minimising potential impact to the animal.

Intratracheal administration is one of the primary methods for introducing substances into the lungs of experimental mice. In order for all lobes of the lungs to be targeted intubation under anaesthesia is the ideal method. This procedure has been amended in multiple stages over time with Chris Moore's approach designed to refine the intubation process into a safer and more humane procedure for both animals and technicians. This poster will explore the ways in which this method is a refinement of intratracheal intubation.

The problems and the solutions

Problem: Injectable anaesthesia

- Increased mortality rate due to non-recovery of consciousness.
- Length of procedure was longer so inhalation anaesthesia would result in animal regaining consciousness mid-procedure.
- Increased training time, affecting motivation and confidence of trainee.

Solution: Inhalation anaesthesia

- Overall refinements mean the procedure can be competently completed in a fraction of the time allowing for inhalation anaesthesia.
- Mice are anaesthetised for a much shorter period of time, resulting in faster recovery.
- Does not need heat mats/lamps for recovery – mice can instead recover in their home cage, reducing stress levels.

Problem: Tongue damage

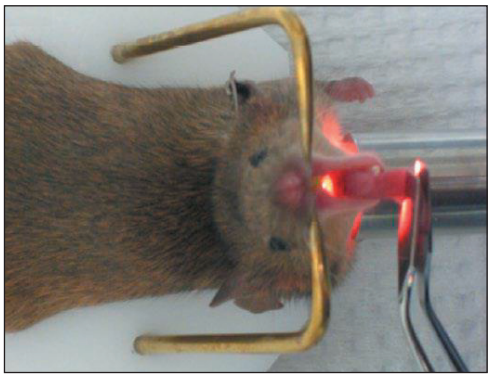

Figure 1. Preparing for intubation by holding tongue with forceps.

- Continued concentration by trainee meant increased pressure on forceps, damaging tongue and resulted in humane culling.
- Required additional light source to visualise trachea.
- Increased procedure time.

Solution: The tongue

- The tongue does not need to be pulled out of the mouth to guide the cannula into the trachea.
- Hooking the forceps on the bottom teeth and gently pushing down to open the mouth provides clear access.
- Allows for very swift and easy intubation, without the risk of causing injury to the tongue or the trachea itself.

Figure 2. Hooking the forceps onto the incisors to smoothly intubate mouse.

Problem: Intubation needle

Figure 3. Holding the cannula so that it covers the tip of the intubation needle.

- Damage to trachea by intubation needle being longer than overlaid plastic canula.
- Canula is too soft and flexible to be used without the needle.
- Needle and cannula need to be held manually at different lengths to keep pointed metal tip covered.
- Increases training time and mortality rate, which also affects trainee confidence and number of animals used in the experiment.

Solution: The collar

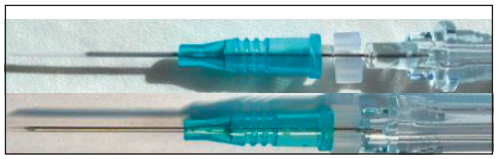

Figure 4. The collar inserted on an intubation needle.

- Chris Moore created a 3D printed spacer, referred to as a 'collar' to fit onto the intubation needle.
- This collar lengthens the cannula so that it covers the entirety of the intravenous (IV) needle, thus negating the risk of puncturing the trachea during insertion.
- Using the collar preserves the rigidity of the needle that is necessary to accurately target the trachea, without compromising Animal Welfare.

The refined procedure

1. Anaesthetise the mouse using inhalatory anaesthesia.
2. Once unconscious, hook top teeth onto wire frame.
3. Gently open the mouth by hooking the forceps around the bottom incisors to expose the throat and tongue.
4. Guide the intubation needle, with the collar carefully into the throat. Follow the curve of the tongue to allow for smooth insertion and to avoid the oesophagus.
5. When the needle has been fed all the way down, remove the metal I.V. needle so that only the plastic cannula remains in the trachea.
6. Take the pipette and place the tip at the end of the needle hub where it joins the plastic cannula. Insert the substance into the trachea.
7. If successful, the mouse should begin to breathe while a wet clicking sound can be heard. Gently remove the cannula from the trachea and place mouse back.

Benefits

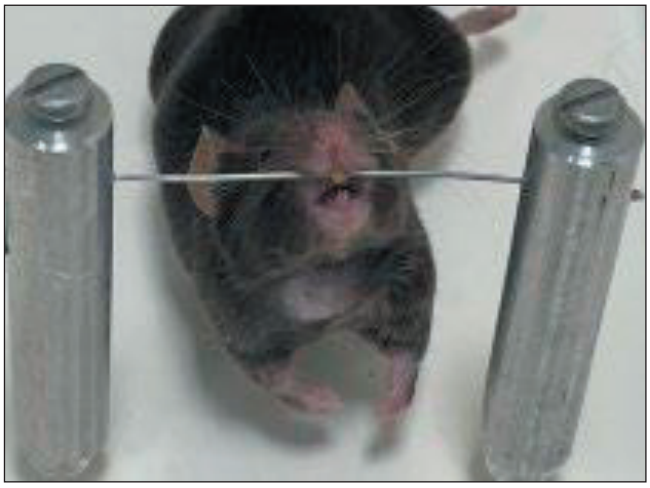

Figure 5. Mouse hooked onto the intubation frame.

Figure 6. Mouse receiving an intratracheal injection.

Welfare

- Lower mortality rate means less mice are used.
- Avoids prolonged anaesthesia.
- Able to safely intubate younger mice with no risk.
- Avoids potential mistakes that could cause injury or death to the animal.

Training

- Much safer to train – eliminates the mortality rate associated with puncturing the trachea.
- Quicker to achieve full competency.
- Increases trainee confidence – adopts a positive approach to the procedure, reducing stress and improving mental wellbeing.

Experimental

- Provides reliable experimental data –animals recover quickly without any adverse affects.
- Useful for long-term studies with multiple intubation periods, as there is no risk.
- Useful for studies that use younger mice.
- Able to accurately target the lungs.

Positive feedback

"Since being trained in using the collar and stand method, I now very rarely have failed intratracheal injections, resulting in better experiments, using less mice and shorter anaesthesia."

– Clare Weedan, Postdoctoral Project Research Scientist

"I can now accurately target all areas of the lungs in my experiments and have been able to confidently train others to reproduce the same results."

– William Hill, Postdoctoral Fellow

"At first, I was nervous that using the inhalatory anaesthesia would mean I had to be quicker with the procedure but it has become one of the easiest procedures for me to do and train others in."

– Michael Nagliati, BRF Research Scientist

"I did not feel nervous learning this procedure as the refinements meant that I didn't have to worry about how to hold the cannula or the tongue."

– Ryan Hoskins, BRF Research Officer

Potential future refinements

Alternatively, the use of extended pipette tips could be used instead of the needle/cannula combination. This would allow substances to be directly inserted but as the tips are considerably shorter than the cannula, full proliferation throughout the lungs may not be fully achieved.

Intranasal administration can also be used either instead of, or in conjunction with intratracheal intubation. While this is far less invasive for the mice, it is less accurate and can cause unwanted side effects such as tumour growth in the nasal passage which is dependent on the substance used.

Lowering the inhalator anaesthesia induction level is potentially a smoother recovery but needs care to ensure the animal does not wake up too soon.

Acknowledgements

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References

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