Webinar Q&A Report:
Respiration in Anesthetized Mice: Evidence-Based Recommendations for Improved Monitoring & Supportive Care

1. Intubation and oxygen supplementation:

There were several questions concerning the value of intubating mice during anesthetic procedures. There are several values to this including ensuring that the upper airway is open and not obstructing airflow, the ability to deliver PEEP or a sigh to prevent atelectasis, the ability to ventilate the mouse, and it will enhance ETCO2 monitoring (I suspect). The disadvantages are that it is not the easiest thing in the world to do, you'll need to get adaptors to connect the anesthesia unit to the tube (usually a small gauge intravenous catheter works well for this), and it will require enhanced monitoring at the end of the procedure to pull the tube (there are a few additional notes below in the paragraph about the facemask). None of my work has included intubating the mice as most of my research is based on what is routinely done in mouse anesthesia. Therefore, I don't have specific recommendations for the pressure which can be applied without damaging the lungs, although I suspect it is roughly 10-20 cm water pressure, as that is what most species use. Delivering this accurately will likely be challenging due to the small lung volume, necessitating the use of mouse ventilator, which can easily be purchased.

As far as the value of the benefits, I would prioritize (1) supplementing 100% oxygen as the change which will provide the most benefit, (2) then monitoring every 5 minutes, and (3) including pulse oximetry (assuming we are pretty good as a field of thermal support and monitoring of mice). I think the value of intubation would be behind these, but I may be wrong, as I said, I have little experience with this.

If we aren't intubating mice, then a nicely fitting face mask is the method for oxygen delivery. The fit is critical for minimizing anesthetic loss to the environment (if not working in a hood) and to ensure the mouse is truly getting all of the oxygen supplementation and that it is not escaping into the environment. The problems with a facemask are that the mouse can easily be jostled out of the mask during surgical procedures and if the mouse arrests or needs support, having them intubated can allow directly supplementing oxygen to the lungs. With a facemask, we are relying on a mass flow diffusion of oxygen into the lungs, which is not as good. Doing small chest compressions with the recoil of the elastic elements of the thorax will provide suction of the air/oxygen into the lungs, which is OK but likely not as good as actively pushing it in with an endotracheal tube. I've no data on these differences.

2. Choice of gases:

As you can tell, my preference is supplementation with 100% oxygen for injectable protocols and as the carrier gas for inhalant anesthesia. And yes, there are places using compressed air as the carrier gas for mouse inhalant procedures. At the end I briefly discussed the use of 50% oxygen versus 100% oxygen as the carrier gas. In veterinary medicine, we typically use the 100% oxygen, but in human medicine, I understand that 50% oxygen is more commonly used. I suspect this is because it helps prevent atelectasis, prevent oxygen toxicity and adequately maintains the arterial oxygen in patients. Additionally, apparently 100% has some negative effects on local blood flow to the heart and brain. One of the big things I think is that human procedures are more likely to go many hours than our veterinary/mouse procedures (and anesthesia in people may last days, which would be very rare in veterinary medicine) making oxygen toxicity a very real concern. I'll let the veterinary anesthesiologists guide us on the topic of 50% vs 100% oxygen.

Nitrous oxide can be used to replace some of the oxygen to minimize the use of the other anesthetics. By itself it can't adequately achieve a surgical plane of anesthesia, but it can decrease the use of the other drugs. I haven't used this or seen much in its benefits in mice, but it could be advantageous in the future. Sevoflurane has some physiological benefits over isoflurane, but generally the differences aren't huge, as far as I know. And there is very real advantage to using an anesthetic protocol which you are very familiar with. If you've been using isoflurane and know what is normal vs. what is worrisome, that has a very real value which should not be discounted.

Oxygen flow rates are another interesting topic. The Kent SomnoSuite uses very low flow rates, which can be set to deliver adequate gas to the mouse, without providing huge volumes of gas, wasting anesthetic, pumping the waste anesthetic gases into the environment (or filling scavenging devices) and potentially cooling the animals. As most people currently use traditional anesthetic machines, we use traditional anesthetic machines for our studies. I don't believe ours is reasonably accurate below 300-500 ml of oxygen per minute, so that is what we set for our typical flow rates. This is probably an order of magnitude more than most mice require. One additional note is that the SomnoSuite can use 100% oxygen from a gas cylinder, the only thing to be aware of is that the pressure must be greatly reduced from the tank and regulator valve before it goes into the unit, to about 15 psi, I believe.

3. Monitoring devices:

There is an interesting question about the values of adding pulse oximetry and end tidal carbon dioxide (CO_2) monitoring to Respiratory Rate (RR) monitoring in anesthetized mice. RR is just a method to estimate how well the animal is ventilating. The goal of respiration is (simplified I'm sure) to deliver oxygen to the tissues and remove carbon dioxide. An animal can have a high RR but isn't achieving those goals, which can certainly happen. Pulse oximetry and end tidal CO_2 gives us a much better estimate of how well those goals are being achieved. I also think about the fact that oxygen sensing is lost at lower doses than CO_2 sensing in the blood. Importantly, pulse oximetry tells us about the Heart Rate (HR), which will be important as we continue to refine and understand the changes in mouse anesthesia. At this time, I think RR is a better predictor of an issue (at least with the injectable anesthetics), but the changes associated with impending arrest may be better assessed with HR in inhalant anesthetics. I just cannot provide strong evidence at this time.

I've been pleased with the Kent mouse pulse oximeter. We have two of them and in our initial studies, we used both at the same time and altered oxygen supplementation to see how responsive the units were and they very nicely moved in parallel as expected. It was rare to have greater than a 3% difference in reading and if the difference in oxygen saturation was greater than this, we didn't include the data points in any analysis. I forget the actual percentage of times this happened, but it was very low. It would be great to validate the accuracy with direct oxygen measurements with a more definitive proven testing system, but obtaining adequate volumes of arterial blood for our blood gas analysis systems is proving challenging. Knowing this limitation, I would be hesitant to say a reading of 94% isn't really 92% or 96%, but in mice, I'm typically not looking for that degree of accuracy. I am confident that 94% isn't really 74%, and this has real value in our mice. The accuracy becomes more relevant as we get into the low 90's and upper 80's where we are concerned about the transition from normoxia to hypoxia, but this is just something we'll need to deal with. The HR reported are spot on as we have compared these to ECG recordings from an ECGenie and there is a great correlation there. Also, when testing the two units at the same time, the HR reported were virtually 100% the same. Positioning the monitor on the paw and blocking out extra light have proven very helpful in getting consistent measures for us.

End tidal CO2 in mice is really challenging. The tidal volume is so small that getting accurate measures is difficult. You'll need to use a low-flow anesthetic system to get these values with mice, otherwise the massive extra air flow will dilute the expired CO2 too much. I don't have much direct experience with the Kent Scientific end tidal CO2 monitor (yet) but hope to see it in use in the near future.

As far as monitoring/support is concerned, we do pretty well with thermal support. It is important to actually monitor the temperature and not just assume that the thermal support provided is working. Or at least monitor a few mice and confirm that it is working and then recheck periodically if monitoring every mouse isn't possible. Cold mice under anesthesia have 4 real consequences: impaired coagulation, increased risk of infection, arrhythmias, and delayed recovery, which has real time issues for labs. In our studies, we aim for 36 degrees.

4. Animal specifics:

The strain sensitivity to isoflurane generally range between 1.3 and 1.6% for minimum alveolar concentration (MAC) values (Sonner and Eger have a really good paper about this circa 2000). This looks small, but can translate to a pretty significant range of isoflurane requirements between different strains of mice. Always wise to do a few pilots of your mice to see what they will specifically respond to. I would expect even greater variability in response to the injectables between strains. Old mice are definitely more sensitive to anesthetics and require lower dosing than young mice. That is across species, not just in mice.

The responses to injectable anesthetics are much more variable than the inhalants, probably because the inhalants work through many receptors and our injectable agents tend to act at just one. There are many papers looking at strain sensitivity in response to the opiates, but much less information exists about the response to ketamine/xylazine or acepromazine. The highly variable response plays a role in many ways, resulting in mice not reaching a surgical plane when they should, mice waking up

earlier than normal and some mice being too deep. We have a couple of papers addressing the redosing of anesthetics and extending the duration of anesthesia with an IP infusion of anesthetics (Jaber et al and Erickson et al. around 2016-18). We don't have much for bringing a mouse to a surgical plane of anesthesia if the initial dose doesn't work. I'd probably try to supplement with ketamine, but I don't have much experience with this, so it is tough. I am hoping people will find the alfaxalone/xylazine combination in mice to be more predictable than ketamine/xylazine/ace, but we'll have to get people to try it out first.

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Kent Scientific

1116 Litchfield Street, Torrington, Connecticut, United States, 6790

https://www.kentscientific.com/
Phone: 888-572-8887
Email: sales@kentscientific.com