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Use of an integrated low-flow anesthetic vaporizer, ventilator, and physiological monitoring system for rodents.

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TITLE:

Use of an Integrated Low-Flow Anesthetic Vaporizer, Ventilator, and Physiological Monitoring System for Rodents

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KEYWORDS:

Anesthesia, isoflurane, surgery, mouse, rat, monitoring

SUMMARY:

Here, we present a protocol to safely and effectively administer anesthetic gas to mice using a digital, low flow anesthesia system with integrated ventilator and physiological monitoring modules.

ABSTRACT:

Low-flow digital vaporizers commonly utilize a syringe pump to directly administer volatile anesthetics into a stream of carrier gas. Per animal welfare recommendations, animals are warmed and monitored during procedures requiring anesthesia. Common anesthesia and physiological monitoring equipment include gas tanks, anesthetic vaporizers and stands, warming controllers and pads, mechanical ventilators, and pulse oximeters. A computer is also necessary for data collection and to run equipment software. In smaller spaces or when performing field work, it can be challenging to configure all this equipment in limited space.

The goal of this protocol is to demonstrate best practices for use of a low-flow digital vaporizer using both compressed oxygen and room air, along with an integrated mechanical ventilator, pulse oximeter, and far infrared warming as an all-inclusive anesthesia and physiological monitoring suite ideal for rodents.

INTRODUCTION:

Research involving animal models often requires specialized data collection equipment. There

are two common types of anesthetic vaporizer commonly used for small animal surgery. Traditional anesthetic vaporizers rely on the passive vaporization of volatile anesthetics based on atmospheric pressure and gas flow[1-10]. They are designed to operate at flow rates of 0.5 L/min to 10 L/min, making them ideal for large animal models[11].

We recently demonstrated the effects of a low-flow digital vaporizer compared to a traditional vaporizer[12,13]. The low-flow digital anesthesia system can be used to maintain an animal on a nose cone at very low flow rates of 1.5-2.2 times the animal's minute volume[14-16].

There are numerous benefits to using a digital anesthesia system. It incorporates a built-in pump, which draws in ambient air to use as a carrier gas. This allows the user to administer anesthesia without the use of compressed gas. Recent studies[17,18] have suggested that using air instead of oxygen as a carrier gas may be beneficial for many procedures.

Physiological monitoring and warming capabilities can also be installed into the digital low-flow anesthesia system. In most institutions, animal warming and physiological monitoring are required by Institutional Animal Care and Use Committees[19-22]. Studies comparing the physiological effects of anesthetic agents have shown a drastic depression of body temperature, cardiac function, and respiratory function[23-25]. Placing the animal on a warming pad to monitor and maintain a normal body temperature is often required. There are many methods of animal warming available, such as warm water heaters, electric heating pads, and heat lamps, but each of these have significant drawbacks. In studies comparing different methods of animal warming, far infrared warming has been found to be the most beneficial[26]. The digital vaporizer includes built in homeothermic far infrared warming to maintain a specific animal body temperature. This eliminates the needs for any additional warming pad controllers.

In addition to monitoring body temperature, pulse oximetry is a popular method of monitoring the animal's heart rate and oxygen saturation. This noninvasive method is simple, accurate, and provides an overall assessment of the animal's ability to regulate blood oxygenation levels. A paw sensor for pulse oximetry can be connected to the anesthesia system, as we have previously demonstrated[2].

Mechanical ventilation is often required when the animal is under longer periods of anesthesia, or whenever the animal's respiration pattern needs to be controlled. The low-flow digital vaporizer has the capability to deliver controlled breaths in either pressure- or volume-control. An integrated ventilator eliminates the need for an external ventilator and excess tubing setup requirements.

Because all these common monitors and features are combined into a single piece of equipment, the tubing setup is substantially simplified. The purpose of this protocol is to demonstrate the setup and use of an all-in-one digital anesthesia system.

PROTOCOL:

All animal studies were approved by the Purdue Animal Care and Use Committee.

1. Setup of the low-flow vaporizer

1.1. Isoflurane or sevoflurane delivery

1.1.1. Select a carrier gas source. To utilize the internal air pump, remove the red cap from the Inlet port on the back of the system, allowing the system to intake room air. To use compressed gas, use a pressure regulator or pressure reducer set to 15 PSI, and connect to the Compressed Gas port on the back of the system.

1.1.2. Connect the charcoal canister to the exhaust port.

1.1.3. Connect the Accessory Connector to the Inspiratory and Expiratory ports on the front of the system. Connect the induction chamber to branches with blue clips and the nose cone to branches with white clips (**Figure 1**).

1.2. For mechanical ventilation

1.2.1. Calibrate the ventilator by performing a deadspace calibration. From the **Vent Run Screen**, touch **Setup**, and then **Calib & Tests**. Select **Deadspace Calibration** and press **Dial B**.

1.2.2. Connect the intubation connector tubing to the yellow coded clips (**Figure 2**).

1.3. For pulse oximetry

1.3.1. Connect the sensor to the port on the back of the system, labeled MouseSTAT.

1.4. For warming

1.4.1. Connect the warming pad to the 'Pad Power' port on the front of the system.

1.4.2. Connect one sensor to the "Body Sensor' port, and the other to the 'Pad Sensor' port. Secure the Pad Sensor to the warming pad.

2. Configure the settings

2.1. For anesthesia

2.1.1. Power on the anesthesia system. From the **Anest Run Screen**, touch **Set Up**.

2.1.2. Choose the anesthetic agent. Touch **Type Anest**, and then turn Dial B to select **Isoflurane** or **Sevoflurane**.

2.1.3. Set the syringe size. Touch **Syringe Size**, and then turn Dial B to select a size.

2.1.4. Touch Back to return to the **Anest Run Screen**.

2.1.5. Using the bottle top adapter, fill the syringe with anesthetic.

2.1.6. Connect the syringe to the anesthesia system. Touch **Remove** to move the pusher block backwards if needed.

2.1.7. Prime the syringe. Touch and hold **Prime** to move the pusher block forward until the pusher block touches the top of the syringe plunger. Turning B while holding the **Prime** button regulates the pusher block speed.

2.2. For mechanical ventilation

2.2.1. Touch the **Vent Run Screen** tab, and then **Setup**.

2.2.2. Touch **Body Weight** and enter the weight of the animal.

2.2.3. Touch **Priority** to choose volume or pressure-controlled ventilation. The Body Weight setting automatically sets appropriate respiratory rate and tidal volumes.

2.3. For pulse oximetry

2.3.1. Touch the **Oxi Run Screen** tab, and then **Setup**.

2.3.2. Touch **HR** and turn Dial B to set the minimum allowed heart rate reading. Presets are available.

2.4. For warming

2.4.1. From the **Warm Run Screen**, touch **Setup**. Choose a warming method and target temperature setting.

3. **Begin anesthesia delivery**

3.1. Anesthetize the mouse

3.1.1. From the **Anest Run Screen**, touch **Start Induction** to begin airflow. The default Induction flow rate is 500 mL/min. Turning Dial A adjusts the flow rate as needed.

3.1.2. Place the mouse in the induction chamber, closing the lid tightly. Adjust the **Anesthetic Agent Concentration** dial to 3% for isoflurane.

3.1.3. Monitor until the mouse has reached the desired anesthetic plane, determined by a decrease in respiration rate and a loss of righting reflex when the chamber is tipped. Adjust the **Anesthetic Agent Concentration** dial as necessary.

3.1.4. Once the animal has lost righting reflex and is sufficiently anesthetized, touch **Stop Induction**.

3.1.5. If desired, touch **Flush Chamber** to empty the chamber of residual anesthetic gas.

3.1.6. Open the clamps leading to the nose cone, and close the clamps leading to the chamber.

3.1.7. Touch **Start Nose Cone**. The Body Weight setting determines the nose cone flow rate, though it can be manually adjusted by turning Dial A.

3.1.8. Immediately fit the nose cone, and center the animal on the infrared warming pad (**Figure 3**).

3.1.9. Insert the Animal Sensor as a rectal probe.

4. **Begin mechanical ventilation**

4.1. Intubate the animal.

4.1.1. Transfer the animal to the intubation stage while keeping the animal anesthetized.

4.1.2. Suspend the animal from its upper incisors using a thread fixed onto the vertical intubation stage.

4.1.3. Gently displace the animal's tongue to the side and visualize the trachea using the lights provided in the intubation kit.

4.1.4. Carefully insert tracheal tube and verify correct placement by connecting small air bladder to the tube and checking if the lungs inflate.

4.2. Connect the endotracheal tube to the ventilation tubing.

4.3. Touch **Stop Nose Cone**, and then touch **Start Ventilator**.

NOTE: The Body Weight setting automatically determines proper respiration rate and tidal volumes. To perform pressure-controlled ventilation, set the target inspiratory pressure between 15-18 cm H_2O. Make adjustments to the ventilator settings as needed per surgical protocols.

5. **Begin physiological monitoring**

5.1. Place the sensor over the hind paw of the animal (**Figure 4**). The Pulse Oximeter will begin reading HR and SpO_2 automatically. Touch the **Oxi Run Screen Tab** to view pulse oximetry data.

REPRESENTATIVE RESULTS:

Ten week old, male, wild type C57Bl6j mice weighing 25.41 ± 0.8 g were used for this study. The mice were anesthetized and maintained on a nose cone or intubated and maintained on an integrated mechanical ventilator with 1.5-2.5% isoflurane while heart rate and oxygen saturation were monitored. The animals were group-housed in microisolation caging and provided free-access to standard rodent chow and water by bottle.

Heart rate and SpO_2 were monitored during maintenance via pulse oximetry (**Figures 5-7**). Body temperature was maintained at 36.5-37.5 °C via an infrared heating pad and heat lamp. Ventilated animals received continuous delivery of isoflurane during the intubation procedure via intubation stand with integrated nose cone. Each mouse was successfully ventilated or maintained on a nose cone at low flow rates not exceeding 141 mL/min of room air (RA) or oxygen (O_2) for 15 minutes. The animals' heart rates and blood oxygen saturation remained stable with few significant changes in either measurement for all groups. SpO_2 remained between 82-99% for all groups, while body temperature was maintained between 36-38 °C. We observed that both position of the pulse-oximeter and body temperature influenced SpO_2 measurements. If we observed an invalid reading from the pulse-oximeter, we adjusted the placement of the sensor and heating level to keep core body temperature stable.

A two-way ANOVA with a Bonferroni correction was performed to determine significance of data in **Figures 2-4**. A *p*-value less than 0.05 was considered significant.

FIGURE AND TABLE LEGENDS:

Figure 1: Diagram of tubing setup for anesthetic induction and nose cone maintenance.

Figure 2: Diagram of tubing setup for anesthetic induction, intubation, and ventilation.

Figure 3: Mice received continuous delivery of isoflurane during the intubation procedure via an intubation stand with an integrated nose cone.

Figure 4: Integrated pulse oximeter sensor placement over the hind paw.

Figure 5: Average heart rate over 15 minutes ± SD with room air (RA) or 100% oxygen (O_2) delivered through nose cone or ventilated through tracheal tube (n=5/group). No significant difference was observed between groups.

Figure 6: Heart rate values (bpm) recorded after initial anesthetic induction with the low flow anesthesia system. Average heart rate values calculated from 30-second time intervals over a

15-minute period. Each data point represents mean ± SD of all animals in each group (n=5). No significant changes in heart rate were observed over the 15-minute period in any group.

Figure 7: The tissue oxygen saturation levels (%) after initial anesthetic induction with the low flow anesthesia system. Average SpO_2 values calculated from 30-second time intervals over a 15-minute period. Each data point represents mean ± SD of all animals in each group (n=5). No significant changes in SpO_2 were observed over the 15-minute period in any group.

DISCUSSION:

This digital low-flow anesthesia system integrates anesthesia, ventilation, warming, and physiological monitoring systems into a single piece of equipment. Additionally, the system contains an internal pump, allowing it to draw in ambient air for use as a carrier gas, eliminating the need for a source of compressed gas.

In this procedure, the system is used as a sole piece of equipment to replace an anesthetic vaporizer, mechanical ventilator, pulse oximeter, and warming pad. We previously demonstrated anesthetic delivery at a flow rate of 100mL/min[2]. The flow rate settings are critical for this anesthetic delivery technique, as the flow rate directly controls the volume of liquid anesthetic used. We also previously demonstrated how using low flow rates save anesthetic liquid[1,2]. When a traditional vaporizer is connected to a mechanical ventilator, the vaporizer must run continuously while the ventilator inlet samples from the gas stream. In the case of the digital vaporizer with integrated ventilator, only the gas necessary for ventilation is output by the ventilator. This reduces the costs associated with anesthetic liquid, carrier gases, and charcoal filters.

Though there are many advantages to using a low-flow digital vaporizer, there are limitations as well. This system is designed to operate at low flow rates ideal for rodents and other small mammals, but does not deliver anesthesia above flow rates of 1000 mL/min. This particular system is therefore only suitable for small animal species. The integrated pulse oximeter includes a sensor for paw use only. The sensor is not recommended for use on the tail, which may be a limitation for certain surgical procedures. Further, while respiration rate can be monitored through this system via the paw sensor, it can be difficult to obtaining consistent respiratory recordings over an extended period of time. Finally, unlike a traditional vaporizer, this digital system requires electricity. Batteries are available for use in instances where electrical power is unavailable or in the event of a power outage, and can power the system through several hours of usage.

This setup and protocol demonstrate safe and effective use of a digital, low flow anesthesia system with integrated ventilator and physiological monitoring modules. This setup will be useful for any laboratories with limited bench spaces, or where it is not feasible to house multiple pieces of equipment and tubing near a surgical field. There are numerous benefits to an all-in-one system, including the elimination of compressed gas tanks and separate physiological monitoring equipment. Overall, this integrated system could be considered by groups where use of a traditional vaporizer is not ideal.

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DISCLOSURES:
This project was supported with equipment and funding by Kent Scientific Corporation. The authors Krista Bigiarelli and Dave FitzMiller are employees of Kent Scientific Corporation that manufactures equipment used in this article. Open access publication of this article is sponsored by Kent Scientific Corporation.

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Figure 1

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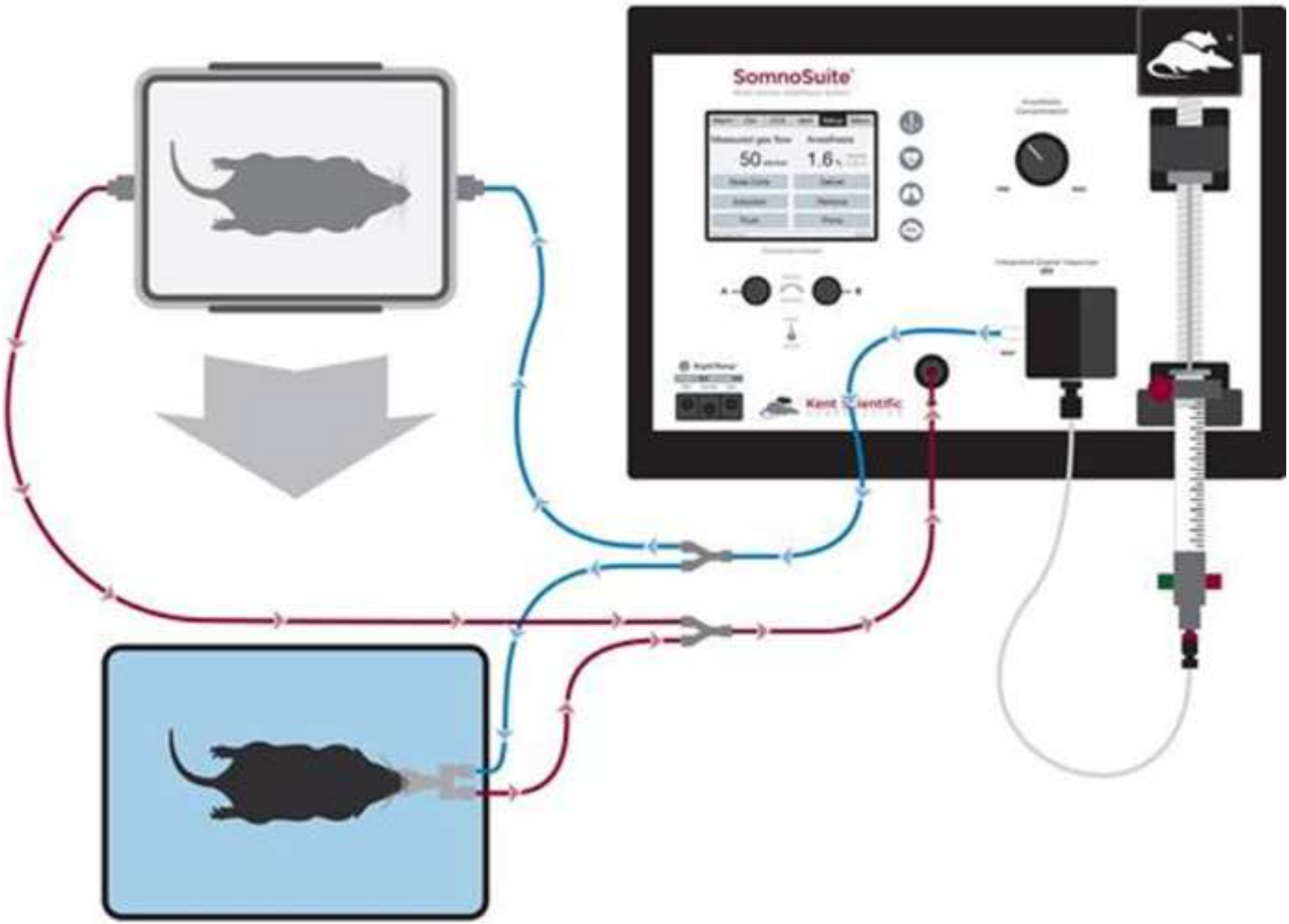

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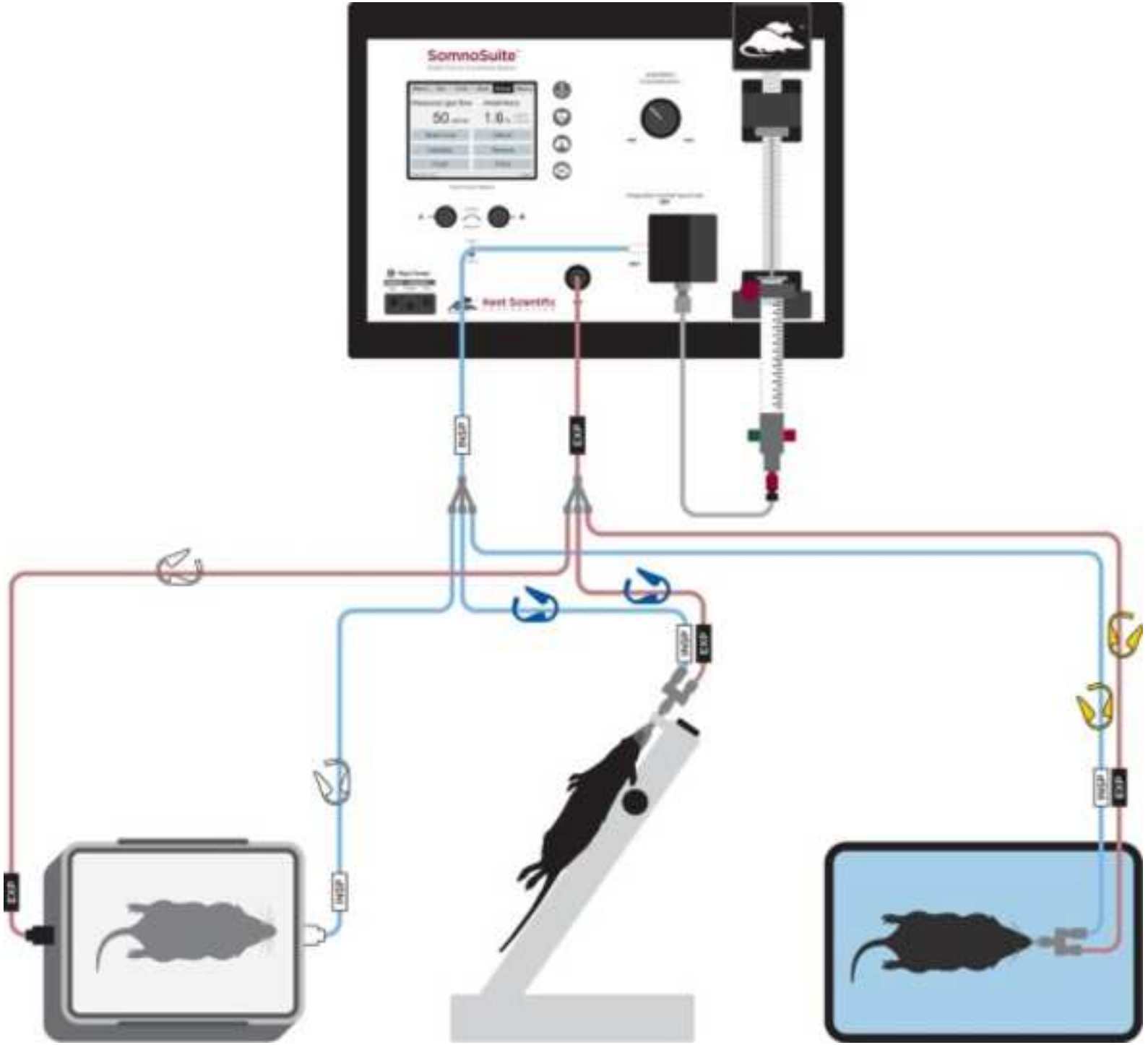

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Figure 4

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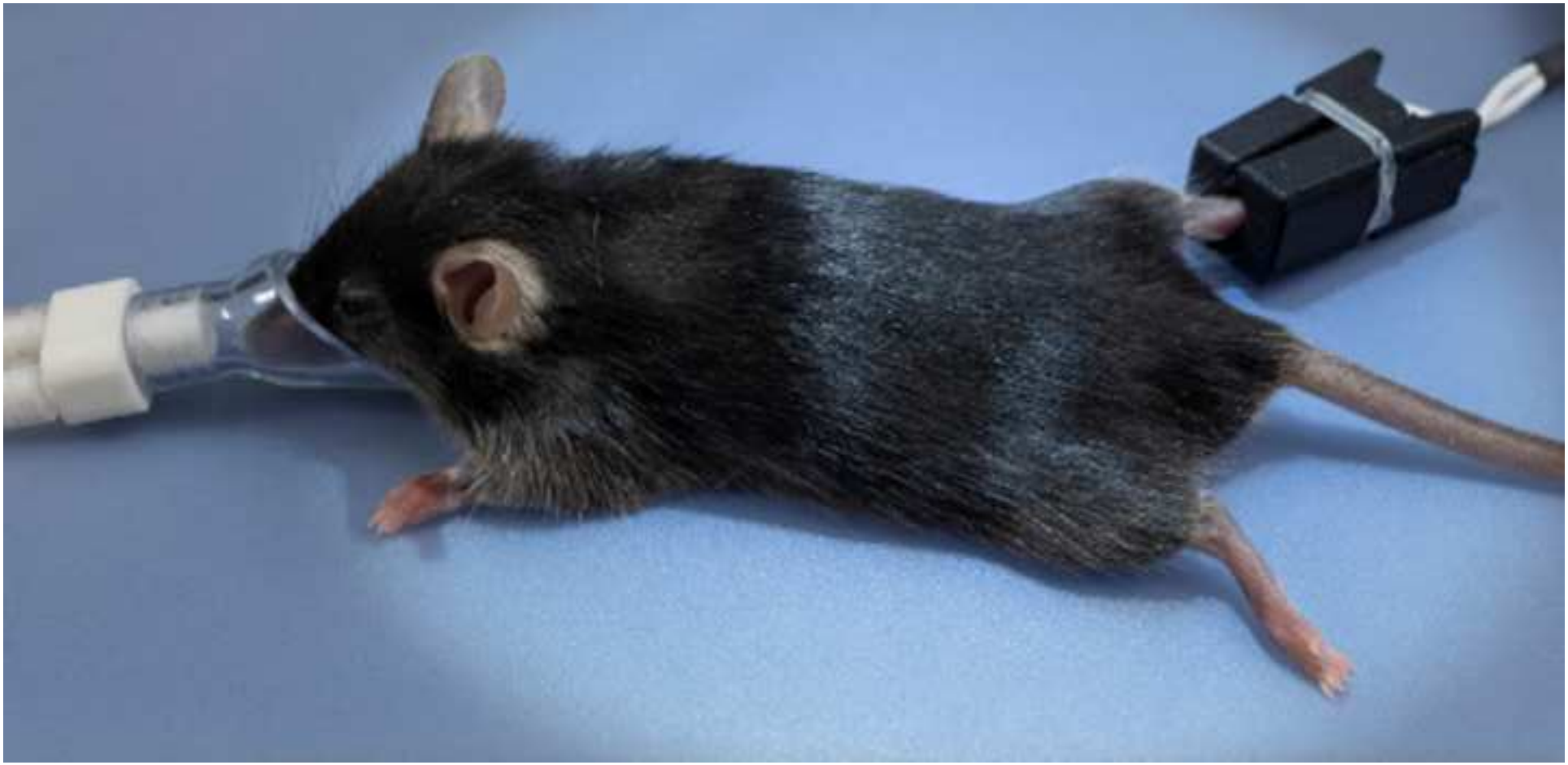

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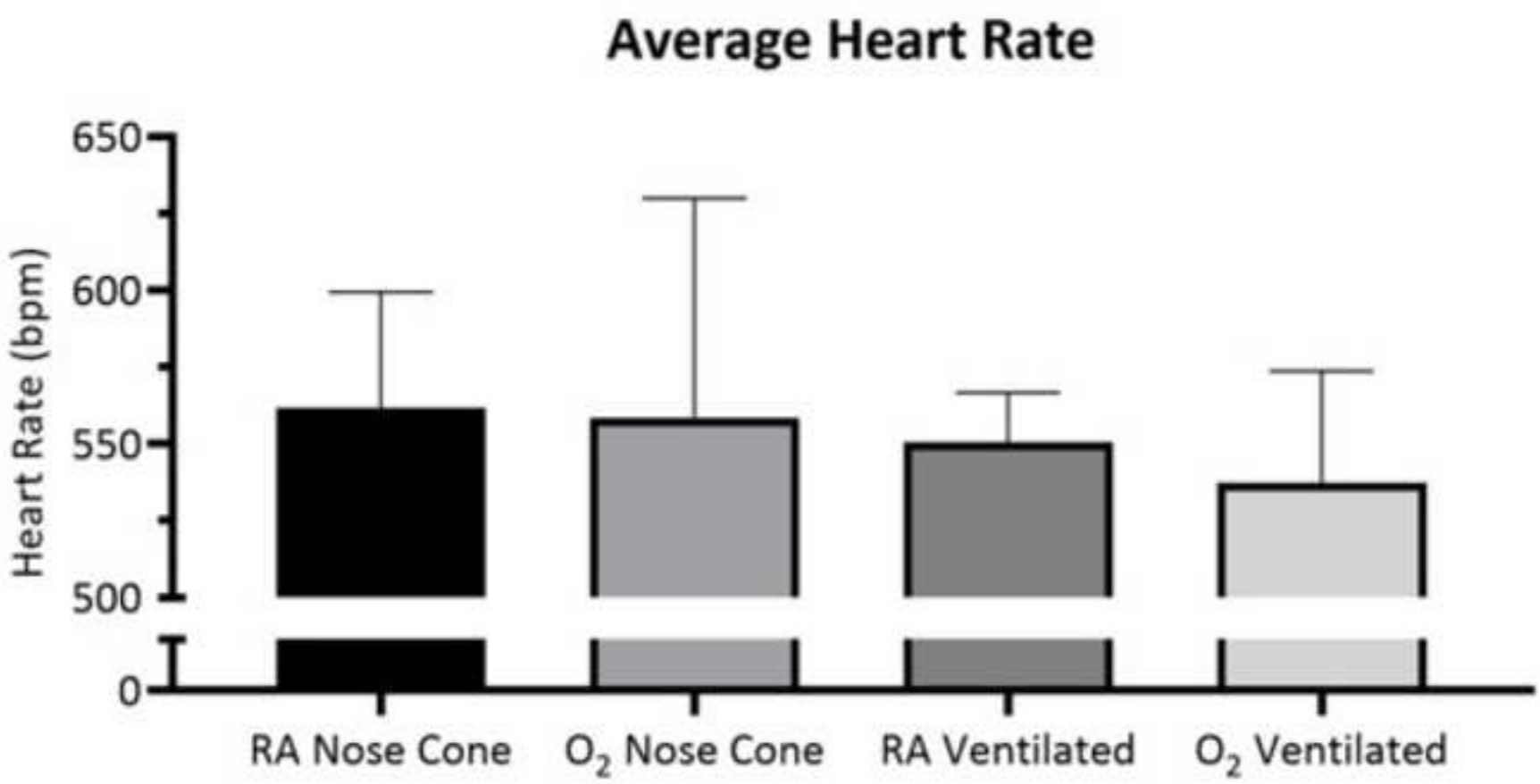

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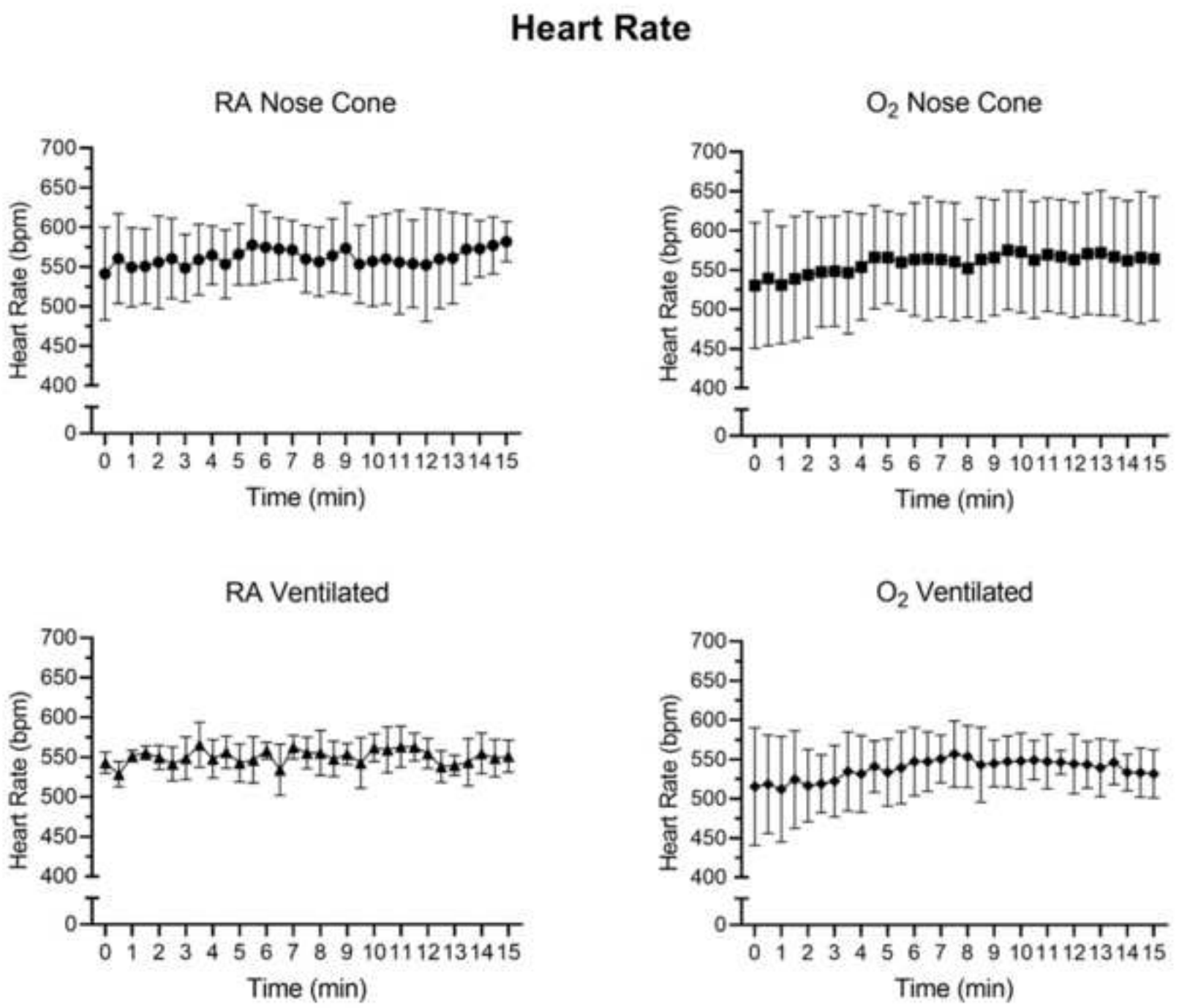

Figure 7

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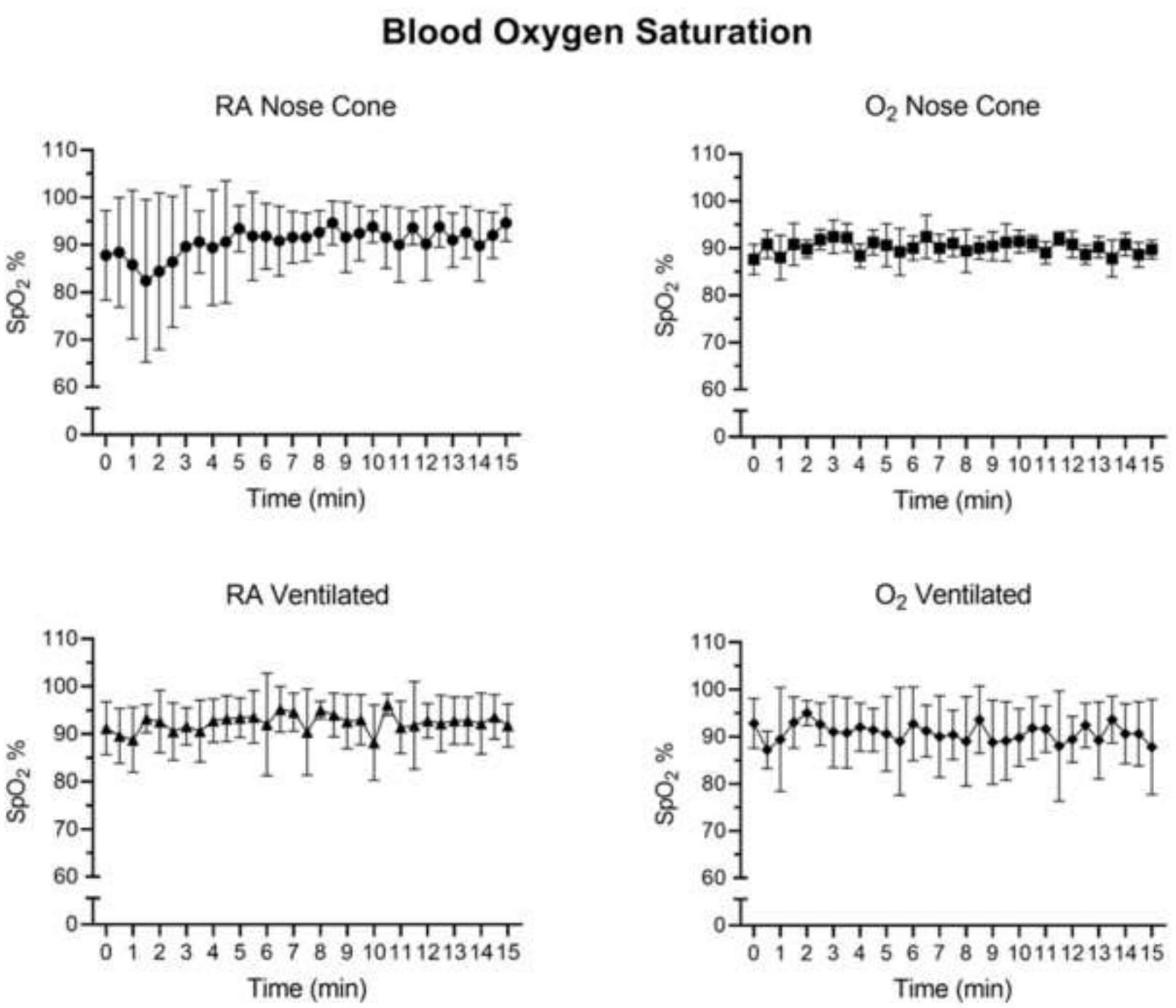

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Name of Material/Equipment	Company	Catalog Number	Comments/Description
Intubation Kit	Kent Scientific Corporation	ETM-MSE	Includes intubation stage, intubation tube, LED light
Isoflurane Liquid Inhalation 99.9%	Henry Schein, Inc.	1182097	Glass bottle 250mL
MouseSTAT Pulse Oximeter	Kent Scientific Corporation	SS-03	Integrated into SomnoSuite
Oxygen Tank	Indiana Oxygen Company	23-160246	Medical Grade O_2 99%
RoVent Automatic Ventilator	Kent Scientific Corporation	SS-04	Integrated into SomnoSuite
SomnoSuite Low Flow Digital Anesthesia System	Kent Scientific Corporation	SS-01	Includes RightTemp Homeothermic Warming control, pad, and temperature sensors
SomnoSuite Mouse Starter Kit	Kent Scientific Corporation	SOMNO-MSEKIT	Includes nose cone, syringes, induction chamber, and charcoal canister

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Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
We have made multiple minor revisions to correct spelling and grammar issues.

2. Authors and affiliations: Please provide the full postal address of each affiliation.
Affiliations, addresses, and contact information have been updated for each listed author.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials. You may use the generic term followed by "(Table of Materials)" to draw the readers' attention to specific commercial names. Examples of commercial sounding language in your manuscript are: SomnoSuite, Kent Scientific Corp., GraphPad Software, etc.
All commercial language and references have been removed from the body of the manuscript.

4. All methods that involve the use of human or vertebrate subjects and/or tissue sampling must include an ethics statement. Please provide an ethics statement at the beginning of the protocol section indicating that the protocol follows the guidelines of your institution.
We have added an ethics statement at the beginning of the protocol section as recommended.

5. 3.1.2: Please specify the age, gender and strain of mouse used.
We have added animal age, sex and strain under "Animals" in the Representative Results section.

6. 4.1: Please describe how to intubate the animal.
We have added further details regarding the intubation procedure as recommended.

7. Please reference Figure 1-4 in the protocol section. Please reference Figure 5 in the results section.
We have added Figure references through the protocol and results section as recommended.

8. Discussion: Please discuss any limitations of the equipment.
We have added statements to the Discussion to address limitations.

9. Please remain neutral in tone when discussing the commercial device. The accompanying video cannot become an advertisement.
All commercial language has been removed and replaced with generic terminology throughout the manuscript.

10. Please remove the embedded figure(s) from the manuscript.
Embedded figures have been removed.

11. Table of Materials: Please ensure that it has information on all relevant supplies, reagents, equipment and software used, especially those mentioned in the Protocol. Please sort the materials alphabetically by material name.
We have added missing items and sorted the Table alphabetically as recommended.

Reviewers' comments:

We would like to sincerely thank all three reviewers for their comments and chance to improve this manuscript. Below are details describing our changes to the text.

Reviewer #1:

Manuscript Summary:
In their work "Use of an integrated low-flow anesthetic vaporizer, ventilator, and physiological monitoring system for rodents", Bigiarelli et al. describe the use of a commercial device that provides anesthesia delivery, mechanical ventilation, controlled body temperature maintenance, and pulse oximetry for rodents. Such multi-purpose device may indeed be valuable in certain lab environments, where space is limited and/or equipment is required to be transferred quickly. The authors provide a guideline for basic use, and demonstrate physiological data obtained with the system from mice.

Major Concerns:
1. The authors refer multiple times to previous studies, but there are no references embedded in the text available for review process. Despite there is a bibliography, it is rather difficult for a reviewer to evaluate whether necessary amount of references is provided in the text.
We appreciate the need to reference previous studies and have embedded references throughout the introduction and discussion to better highlight previous work. These also include three additional references (24-27).

2. The statement that non-invasive measurement of blood oxygen saturation with pulse oximetry in small animals is accurate (row 75) can be questionable if there are no experiments where the measured PulseOx values obtained with the current equipment are compared with values obtained directly from blood samples. In current work, the authors show that there are no differences in blood oxygen saturation between animals breathing either room air or pure oxygen (Figure 7). According to common sense and empirical experience, there should be a clear difference between these groups, and this observation emphasizes the concern related to the reliability of the blood oxygen saturation measurements. This factor should be discussed by the authors. In any case, if blood samples (or references to previous work) are not available to confirm the accurate oxygen saturation, I suggest to remove the sentence emphasizing the accuracy.
We agree with this comment and have removed the statement on accuracy since no blood samples were obtained to truly determine to PulseOx values. The similarities in SpO_2 values between mice breathing room air and 100% O_2 could be due to the fact that normal blood O_2 saturation levels in a mouse are already close to 100%. Given that the purpose of this manuscript was to demonstrate how to operate a digital vaporizer, we have not made any claims on PulseOx accuracy, which can depend greatly on position of the paw sensor, skin pigmentation, and size of the hindlimb.

3. The authors state that the device provides all-in-one digital anesthesia system (e.g. row 88). Although the device has many important functions, one key function, namely breathing frequency measurement, is not mentioned. Breathing rate is one of the most essential parameters to provide an

estimation for the depth of the anesthesia, and changes in breathing rate provide the necessary information to adjust the concentration of anesthetic. If the device is missing the breathing rate measurement option, it should be noted as a limitation.
Respiration rate can be recorded using the all-in-one digital anesthesia system via the optical paw sensor, but it was difficult to obtain consistent recordings over the 15-minute period for the majority of animals in this study and therefore not reported. Although the system has the capability to noninvasively measure RR from the SpO_2 sensors, it can be difficult to consistently measure this over an extended period of time. These details have been added to line 330 in the discussion of the manuscript.

4. The protocol description is occasionally rather general. For example, i) intubation protocol 4.1 should be described in more detail or reference should be provided as the task is not straightforward. ii) There are no instructions to what direction user should adjust ventilation settings (volume, rate) if needed, and when the adjustments are necessary (row 197). iii) There is no description how to estimate the validity of pulse-oximetry signal, as the authors state that invalid measures can be observed.
We have added additional details related to flow and ventilation settings. The system automatically calculates flow rate and ventilation settings, though these can be manually adjusted if needed. Adjustments will be dependent on the needs of individual protocols and has been noted.

5. No raw heart rate / pulse oximetry data is shown. It would be beneficial for the user to have a reference image/video for signal quality, which would allow the user to estimate when their signal is good or bad.
We will include video footage of signal quality, including the expected signal.

Minor Concerns:
6. It is unclear what are "T-circuit" or "modified bain circuit" (rows 52-53).
We have clarified this by simplifying the statement to nose cone maintenance, which is further in line with the protocol. The circuit will be demonstrated in both the figure diagrams and accompanying video.

7. In the protocol section 3.1.2 it would be beneficial to mention that the anesthetic concentration 3% refers to isoflurane. Even though it has been mentioned earlier that the setup is for isoflurane, some users may accidentally follow this guideline with sevoflurane, which is also supported by the device.
We have added this clarification.

8. Figure 4 shows the pulse-oximetry probe placed on the hindpaw of a mouse. Is the probe compatible with tail? In some cases the tail may provide stronger signal compared to the paw, which could potentially help to solve issues with weak signal.
Per manufacturer recommendations, this pulse oximetry sensor is for paw usage only. We have added a statement addressing this in the Discussion section.

9. Anesthetic agent is missing from the material list.
We have added isoflurane and alphabetized the list.

Reviewer #2:
This article shows the step-by-step how to use low flow SomnoSuite. It has sufficient information with some minor revisions needed.

Major Concerns:

N/A

Minor Concerns & questions:
Protocols
-How long will the battery last during power outage?
We have added a statement stating that batteries can last several hours in the Discussion section (lines 420-422).

-**During chamber induction, why do you not recommend to use high flow (normal flow) which lead to quicker induction?**
This system uses a higher flow for the induction phase compared to maintenance on the nose cone. We have added that the flow rates can be manually adjusted in the protocol.

-Is the software compatible with either Mac or PC computer?
This system does not have designated software, but is compatible with PC-based data collection software only. It is not compatible with Mac operating systems.

-During the use of room air, should medical grade air be used? If the regular room air is used (not medical grade air), what are possible complications that can occur?
The reviewer brings an important and valid point. We have used room air for the vast majority of our previous experiments and have not observed any complications that occurred due to the use of normal room air, as opposed to medical grade air. While medical grade air does have fewer particles, it is also extremely dry with a maximum allowable moisture concentration under 67 parts per million by volume. This makes its use in longer studies ill-advised as pulmonary tissue can quickly dry out. If medical grade air is required for a procedure, such as with immunocompromised mice or with animals that have reduced respiratory capacity, a humidifier can be combined as part of the circuit in order to increase the moisture concentration.

-Line 70, why was the use of "far infrared" found to be beneficial to other types of heating pads?
We have added a citation to this statement for further clarification.

-Line 123, was this a feedback system? Please specify.
The warming methods do include a feedback setting, which will be demonstrated in the accompanying video.

-Line 151, how can users choose pressure vs. volume control?
We have added an additional step in the protocol to choose pressure vs. volume control settings.

-Line 170, was the highest concentration 3%? Please discuss the advantages and disadvantages compared to regular vaporizers (5% for isoflurane or 8% for sevoflurane).
The system has the same concentration scales (0-5% isoflurane and 0-8% sevoflurane) as traditional vaporizers. This will be further demonstrated in the accompanying video. 3% isoflurane was used during this protocol as sufficient induction concentration.

-Line 176, please describe "sufficiently anesthetized".
We have added clarification on line 203-204 describing loss of righting reflex and a decrease in respiration rate as indications of sufficient anesthetization.

-Line 203, can the sensor be applied to other areas i.e. front paws, thighs ears, etc. Please explain.
Per manufacturer recommendation, the sensor is for use on the paws only. We have added a statement in the Discussion section to address this as a possible limitation.

-Line 204, what should SpO2 read at room air vs. 100%O2?
In general, SpO_2 readings above 95% are considered normal, with 98-99% being ideal values. That said, the reliability of the pulseox sensor was not ideal, leading to fluctuations in these readings.

-Line 222, SpO2 reading at 82-99% is wide range. When SpO2 is below than 90%, it's considered severe hypoxemia. Are you suggesting that SpO2 reading at 82-99% is fine?
Under physiologic parameters, we mentioned that the position of the pulseox paw sensors and the animal's body temperature may affect SpO_2 measurements. Readings indicating hypoxemia can likely be attributed to low body temperature, especially after animals were transferred from the intubation stage, or due to slight misplacement of the pulseox sensor.

-Line 228, because your animal numbers (n) per group were small, what was the power analysis? Please provide.
A power analysis to determine group sizes for this methods paper is not really appropriate as we had no anticipated means for the physiologic metrics that were quantified. In future studies aimed at quantifying differences in heart rate, respiration rate, or SpO_2 between groups, a power analysis would be warranted and helpful. A group size of 5 was chosen for practical reasons – it allowed us to calculate averages while also abiding by the reduction principle of animal research that aims to minimize the number of animals used per experiment.

-Figure 7, change "blood oxygen saturation" to "tissue oxygen saturation"
We have made this recommended revision.

Reviewer #3:
I have no ethical concerns with the paper. Note that there are other systems available that permit use of low flow anesthetic gas rates with room air (e.g., Vet Equip), so the emphasis on the 'somnosuite' in this paper and the images did feel like excessive marketing.
Thank you for your comment. We have reduced the number of references to the "SomnoSuite" and made additional edits to focus on the data and results from this study.