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Instrumentation for Small-Animal Capnometry

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Abstract—**Monitoring of human vital signs – heart rate, respiratory rate, hemoglobin oxygen saturation and others – has become an indispensable part of the standard of care in a hospital setting. For example, vital sign monitoring during administration of general anesthesia is essential, given the role that the anesthesiologist plays in assuming physiologic control. In veterinary settings, however, vital sign monitoring under anesthesia is less common, and may consist simply of a visual assessment of respiratory rate. Vital sign monitoring is especially challenging in small animals, given the high metabolic rates and small volumes under consideration. In this paper, we present a unique nose-cone design and associated instrumentation which allows for measurement of respiratory parameters – e.g., anesthesia gas concentration, inspiratory and expiratory O_2, and inspiratory and expiratory CO_2 (capnometry). Such instrumentation facilitates a physiologic assessment of small animals undergoing general anesthesia, an increasingly important consideration as small animals play a greater role in *in vivo* biomedical studies. In addition, the techniques proposed herein are suitable for measurement on small respiratory volumes associated with neonatal monitoring.**

I. Introduction

THE IMPORTANCE of physiologic monitoring in a hospital setting is obvious; no hospital patient undergoing surgery would argue with the need for the anesthesiologist to closely monitor numerous vital sign parameters during administration of general anesthesia. Likewise, the need for vital sign monitoring of patients in an intensive-care unit is also clear, given the fact that the hospital intensivist may be assuming full control of respiratory rates, volumes, pressures and inhaled gas concentrations for ventilator-dependent patients.

In veterinary settings, however, electronic physiologic monitoring typically plays less prominent a role. For simple procedures under general anesthesia, a veterinarian may simply use a visual observation of respiratory rate as an indicator of depth of anesthesia. While the historic reasons for the relative absence of veterinary physiologic monitoring instrumentation may partially relate to cost, the technical difficulties associated with measurement of small quantities and volumes are also an important factor. For example, the tidal volumes associated with respiration in a rat may be as low as 600 microliters [1], almost three orders of magnitude smaller than the typical 500 milliliter respiratory tidal volume for a young man [2]. In addition, the elevated metabolic rates in small animals – heart rates as high as 450 beats per minute and respiratory rates as high as 115 breaths per minute [1] – also place demands on the sampling instrumentation which is required to convert time-varying physiologic variables into useful electronic signals. For this reason, scaling of physiologic monitoring instrumentation designed for humans to the small-animal veterinary domain is not a trivial undertaking.

In this paper, we describe the design of instrumentation intended for real-time measurement of physiologic respiratory parameters in rodents and other small animals undergoing general anesthesia, focusing attention on inspiratory and expiratory gas concentrations (anesthetic agent as well as O_2 and CO_2 concentrations). Small animals play an increasingly vital role in assisting biomedical researchers with validation of new medical advances and technologies, such as novel experimental imaging modalities. As an example, full physiologic monitoring is not uncommon for rodents undergoing functional magnetic resonance imaging (fMRI) in high-field MR scanners. Such animals are typically sedated or anesthetized during an imaging session, and physiologic monitoring may be used for confirmation of animal safety and well-being, as well as for synchronization and gating of imaging acquisition signals. In addition, our approach to physiologic measurement on small respiratory volumes may also be directly adapted to neonatal monitoring. Finally, we note that several manufacturers (e.g., Kent Scientific, Harvard Apparatus) do make highly specialized instrumentation specifically for small-animal respiratory measurements; however, the approach described herein allows instrumentation designed for humans to be easily adapted to small animal measurements, with the simple addition of a novel, low-cost nose cone.

II. Instrumentation Design

The present work addresses the low volumes associated with small animal respiration, for which pulmonary tidal volumes fall in the range 0.6-2.0 milliliters. A similar approach may also be used in neonatal applications, where respiratory volumes are also low [3]. Tidal volume is

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defined as the magnitude of the difference between lung volume at the end of inspiration and lung volume at end of expiration, for "normal" unforced breathing:

$$V_T = V_{i,end} - V_{e,end}\,, \qquad (1)$$

where V_T is the tidal volume, $V_{i,end}$ is the end-inspiratory volume, and $V_{e,end}$ is the end-expiratory volume [2]. Simple scaling of respiratory instrumentation and face masks used in humans will not suffice for small animals, since the gas flows involved are much larger in humans when compared to respiratory gas flows encountered in small animals.

Before addressing our approach for increasing the sensitivity of instrumentation to the small volumes encountered in rodents, it may be instructive to review the typical approach for measurement of respiratory gas concentrations [4]. Figure 1 illustrates the typical spectroscopic approach: an optical source is used to illuminate a gas being measured in a precisely-defined flow cell which is essentially transparent to specific wavelength(s) used for the measurement. A narrow bandpass filter (or filters) is used to select a specific wavelength, and the optical absorption of the gas is determined using an electronic photodetector. Optical absorption follows the Beer-Lambert Law [5]:

$$I_{det}(\lambda) = I_o(\lambda)\, e^{-k(\lambda)bc}, \qquad (2)$$

where $k(\lambda)$ is the extinction coefficient of the gas at a wavelength λ, b is the optical path length of the flow cell, and c is the concentration of the absorbing gas.

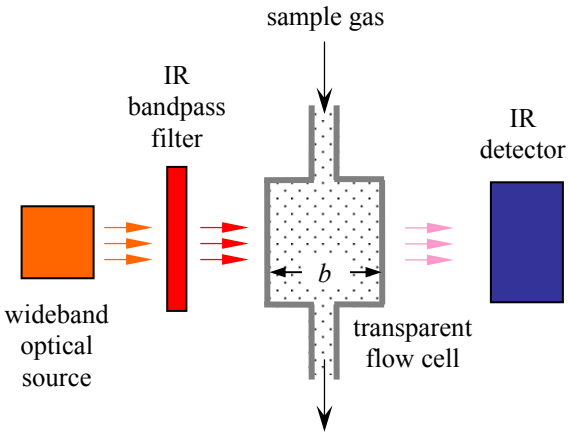

Fig. 1. Schematic illustration of a typical infrared absorbance cell

Thus, a measurement of I_{det}/I_o allows one to calculate the unknown concentration c, assuming *a priori* knowledge of b and k. In practice, sample respiratory gas is withdrawn from a closely-fitting face mask, preferably as close to the nares as possible, and wavelengths used for the measurement typically fall in the infrared region of the spectrum [4]. In small animals, respiratory gas volumes are quite small, and easily overwhelmed by the delivery of anesthetic agent and oxygen carrier gas.

To address this problem, we have created a novel nose cone design which permits concentration of expired gas in a small inner chamber, from which the sampled gas is withdrawn for measurement. Figure 2 illustrates the design of the cylindrical nose cone which consists of a larger outer shell enclosing a smaller (but relatively tight-fitting) inner cylinder which can be adjusted in longitudinal position within the outer cylinder. Figure 2 represents a longitudinal cross-section at the midplane of the nose cone.

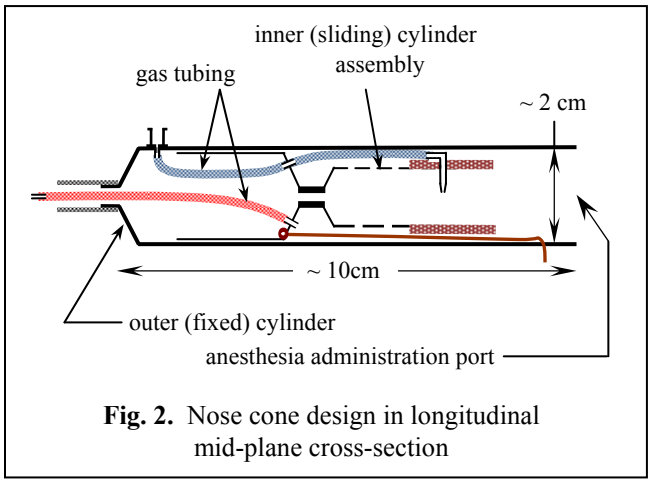

Fig. 2. Nose cone design in longitudinal mid-plane cross-section

Figure 3 illustrates the nose cone in use, as well as a schematic illustration of the components of the nose cone. Contact with the rodent face and nose is made with an open silicone cylinder terminating the administration end of the inner sliding cylinder. A drawstring is used to pull the inner cylinder into proximity with the rodent; the soft silicone ensures that there is no traumatic injury to the rodent nose.

Figure 4 illustrates the gas flow dynamics during use; an anesthesia gas/oxygen mixture is delivered to the anesthesia inlet port (port A), and the gas is directed through small tubing to an area just external to the inner sliding cylinder. Open ports allow the anesthesia gas to diffuse into the inner cylinder lumen for rodent inspiration. Expiratory gases and superfluous anesthesia gas are exhausted through a central bore, and drawn into a low (fumehood) vacuum through tubing connected at port B. Note that the inner cylinder is divided into a larger vacuum chamber (distal from the animal nose), and a smaller proximal sampling chamber; the two chambers are connected by a vacuum constriction, approximately 2-3mm in diameter (Fig 3). This arrangement allows for independent control of the pressure/flow rate of the applied anesthesia gases which reach the proximal sampling chamber, and the magnitude of

the exhausting vacuum which is applied to the inner distal vacuum chamber (discussed below).

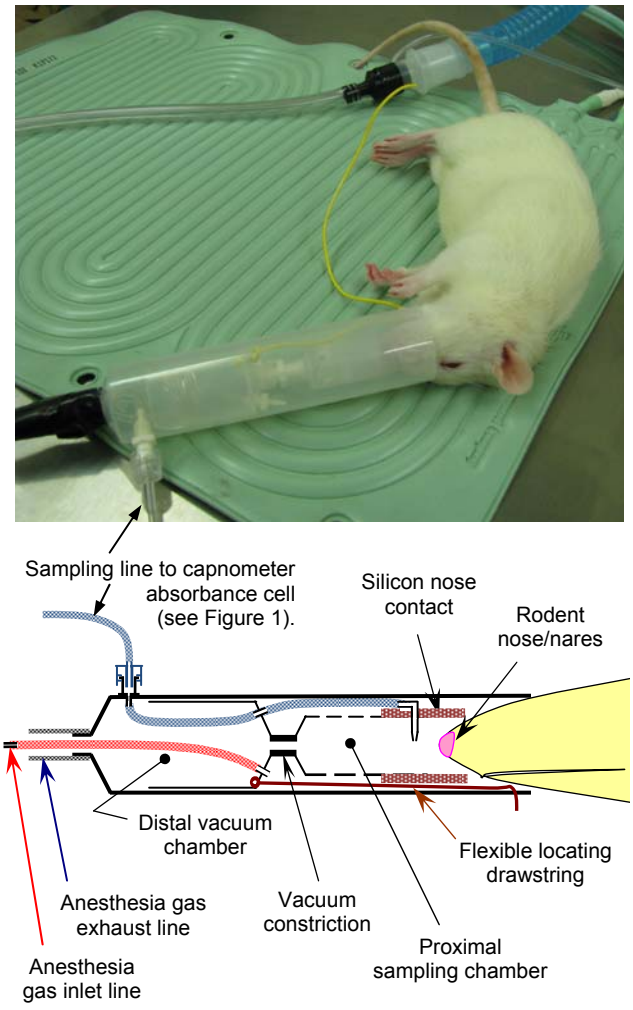

Fig. 3. Application of nose cone for anesthesia administration: photograph and schematic illustration

Another small section of tubing is connected to the capnometer, and — under capnometer-supplied vacuum — a sample of gas very close to the rodent nares is withdrawn and passed to the absorbance cell in a standard (human) physiologic monitoring system. The gas withdrawal rate is adjustable from 100 to 200 ml/minute; 100ml/min was used for the measurements in Figure 5. Given the small volume within the inner sampling chamber, this sample gas is quite representative of the rodent respiratory gases on inspiration and expiration. We note that mixing of inspiratory and expiratory gases may occur in the sampling chamber, but careful independent adjustment of applied anesthesia gas flow rate and exhaust vacuum can minimize this effect, determined experimentally.

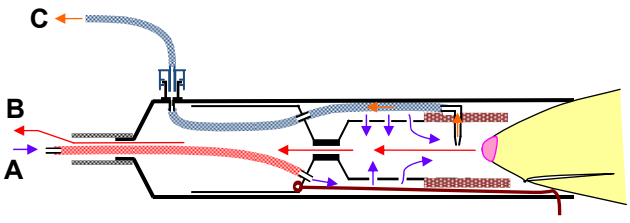

Fig. 4. Inlet, exhaust and sampled gas flows during anesthesia administration.
(A = inlet anesthesia gas (lavender); B = exhausted anesthesia + expiratory gas (red); C = sampled respiratory gas (orange)).

III. Measurements

Validation of the measurement instrumentation and nose cone was accomplished under rodent anesthesia using isoflurane anesthesia, under an animal protocol approved by the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee (IACUC Protocol 05-06 #33 [6]). A female Sprague-Dawley rat approximately eight weeks of age was used in the validation. Research personnel included an engineer responsible for instrument testing, and a researcher with sole responsibility for anesthesia administration, monitoring of anesthesia depth and animal safety. Capnometry and anesthesia gas concentrations were recorded by a flow (absorbance) cell interrogated by a specially-modified *Poet IQ2* Anesthesia Gas Monitor provided by Criticare Systems, Inc. Figure 5 illustrates results of a measurement for which isoflurane concentration was 1.5%, with oxygen administered at a rate of 2 liters/min.

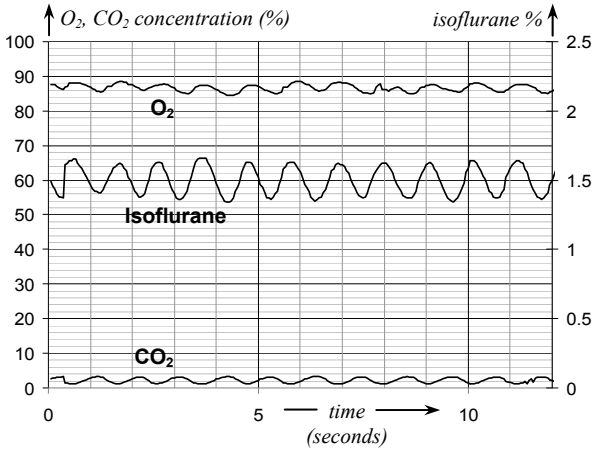

Fig. 5. Rodent respiratory waveforms under 1.5% isoflurane anesthesia.
Left axis: O_2 (top trace) and CO_2 (bottom trace) concentration.
Right axis: Isoflurane (center trace) concentration.

As expected, the oxygen (top) and CO_2 (bottom) waveforms are out of phase by 180 degrees, with O_2 reaching its peak at end-inspiration, and CO_2 reaching a peak at end-expiration. (By definition, end-expiration occurs when CO_2 reaches a maximum.) Likewise, peak values for isoflurane coincide with O_2 peaks at end-inspiration, and isoflurane minima occur at end-expiration. The difference between isoflurane peak and trough represents biologic uptake of isoflurane. In this measurement, respiratory rate was 52 breaths/minute; the monitor indication agreed with the visually-observed respiratory rate.

Expiratory CO_2 waveforms are shown over two time scales in Figure 6, with one unit on the horizontal axis representing one second; the upper graph in Figure 6 represnts the entire 30-minute anesthesia session. There are advantages associated with viewing data like this at various time scales. On a fast scale – the lower trace in Figure 6 – breath-to-breath variations can be seen, and anomalous breathing patterns (e.g., Cheyne-Stokes respiration [7]) can easily be recognized. On a long time axis – the upper trace – the trend in increasing levels of CO_2 is obvious, associated with an anesthestic-induced decrease in respiratory rate.

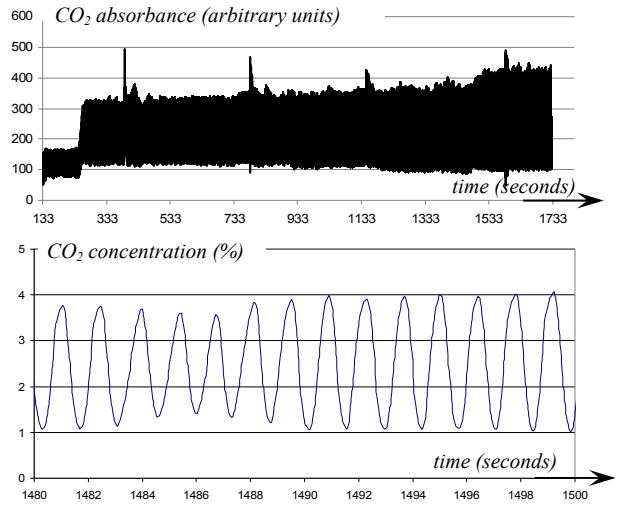

Fig. 6. CO_2 waveforms over two time scales.
(One unit = one second for both plots.)

Finally, as anesthesia depth increases, the capnographic waveform shows marked changes, seen in the Figure 7 graph recorded directly from the *nCompass* Vital Signs Monitor screen. At slow respiratory rates, a marked increase in end-expiratory CO_2 is evident, perhaps reflecting greater CO_2 concentrations associated with pulmonary/alveolar dead space, more noticeable at low respiratory rates.

IV. Discussion and Conclusions

It is clear that there is a wealth of information carried in respiratory capnometric waveforms: metabolic activity, depth of anesthesia, oxygen/carbon dioxide exchange and respiratory rate. Such information is not only useful for monitoring of animal health and well-being while under anesthesia, but can also indicate pathology or disease states – e.g., manifestations of impaired oxygen exchange during acute pulmonary edema. Because the rates and volumes are much smaller than those of humans, simple scaling of physiologic monitoring instrumentation to small-animal dimensions may be unsatisfactory. In this work we have shown that careful design of a small animal nose cone can allow for appropriate sampling of the small volumes which are associated with rodent respiration, even without significant modification of existing, commercially-available electronic sampling circuitry. Adaptation of this nose cone to conventional respiratory and anesthesia monitoring instrumentation can successfully permit the acquisition of useful physiologic waveforms.

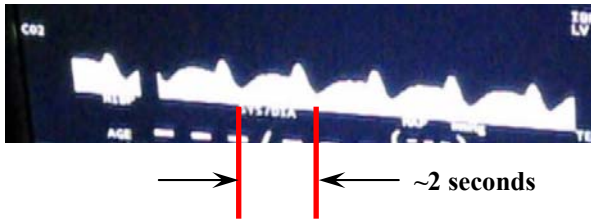

Fig. 7. A detailed view of the expiratory CO_2 waveform at a deep level of anesthesia.

V. Acknowledgment

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