Validation of the MouseOx® Arterial Oxygen Saturation Measurements

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Summary

An oximeter (MouseOx) has been developed for use on small laboratory rodents. The device was validated against an Instrumentation Laboratory IL-682 CO-Oximeter on blood samples (N=83) from five rats. The goal was to assess accuracy of measured S_pO_2 values, particularly at very low saturation values. There is a linear relationship ($y = 1.01 x + 0.11$; $R^2 = 0.98$) between S_pO_2 values measured from the MouseOx and those measured from the IL-682.

Introduction

The MouseOx small animal pulse oximeter was originally validated using a Radiometer ABL5 blood gas analyzer, but because blood gas analyzers only provide an indirect calculation for arterial blood oxygen saturation, a validation of this calibration for arterial oxygen saturation measurements was conducted separately, by comparing MouseOx saturation values against a co-oximeter. The results of this validation are presented here.

Methods and Materials

Five white, male, Sprague-Dawley rats were obtained from Zivic Laboratories, Inc. (Pittsburgh, PA). All animals were purchased with pre-embedded carotid artery cannulas, tunneled to a head cap, for sampling of arterial blood. The rats were provided food and water *ad libitum*. During the experiments, the animals were anesthetized using isoflurane, and the level of anesthesia was adjusted by monitoring the response to tail pinch and physical stimulation. The animals were allowed to breathe spontaneously during all experiments.

During the experiments, the animals were oriented in the prone position on a warm surface to allow access to the head plug containing the termination of the carotid cannula. The MouseOx sensor was placed on either back foot along the foot axis, with the photodiode located on the ventral side of the foot, as recommended by the STARR Life Sciences Corp. user manual. For each animal, a MouseOx data file was saved in a format that could be used to identify the values for S_pO_2 pertaining to each blood sample.

A gas-blending system developed by STARR Life Sciences Corp. allows the user to mechanically blend nitrogen with oxygen to create inspired oxygen concentrations between 0% and 21% oxygen. During the experiments, a given oxygen concentration was randomly chosen, with inspired oxygen (F_IO_2) values ranging from 5% to 21%. At each F_IO_2 level, physiologic parameters (S_pO_2 and pulse rate from the MouseOx) were followed until the values stabilized, after which time the blood sample was collected. The reason for using the MouseOx S_pO_2 to determine the stability of the animal is because it has a time-based trace that allowed visual feedback to determine when the S_pO_2 and heart rate became the most stable in response to a change in F_IO_2. In this application, only the shape of the trace was used to determine stability, and not the values calculated by the device.

The device used to measure the S_aO_2 of the blood sample was an Instrumentation Laboratory IL-682 CO-Oximeter (Orangeburg, NY). This particular co-oximeter was chosen because of its established record in co-oximetry, and also because it has calibration values designated specifically for rat blood. These rat calibration parameters were activated within the device software during all experiments described here. The IL-

682 was regularly calibrated before and after each session, in accordance with the manufacturer's recommendations, using standard Instrumentation Laboratory calibration dyes.

Collection of a blood sample consisted first of a pre-draw to clear heparinized saline from the cannula tubing and stopcock. The amount of fluid drawn was approximately 1½ times the calculated volume of the arterial line (~600 μL). Immediately after clearing the line, an electronic file marker was placed into the MouseOx data file being saved to indicate the temporal location in the dataset at which that blood draw occurred. Simultaneously, the actual blood sample (~200 μL) was drawn over a 5-10 second period of time, using a 1 mL syringe. Care was taken to determine that no bubbles formed within the blood sample inside the syringe. After each draw, the pre-draw blood was re-infused into the animal, followed by a flush of the arterial line with heparinized saline. An attempt was made to balance the total fluid entering and leaving the animal during the course of the experiment.

Once the blood sample was drawn, it was immediately inserted into the IL-682 co-oximeter, and the measured value of HbO_2 was recorded. Blood sample tickets from the IL-682 were also saved for off-line analysis. After every grouping of three blood samples was collected, a flush cleaning cycle was conducted on the IL-682 to prevent fibrin clotting in the tubing and to prevent contamination of the cuvette. To obtain the average values of S_pO_2 from the MouseOx, the data file for each animal was evaluated, and at each file marker, the raw S_pO_2 value was averaged over a 5-10 second period just prior to the file marker (the blood to be sampled was already in the arterial line when the file marker was pressed).

The raw S_pO_2 values over this period (at 15 Hz temporal resolution) were then inserted into the S_pO_2 calibration equation for the MouseOx to obtain the MouseOx value of S_pO_2 that was to be compared against the value from the IL-682.

Some additional data points (n=19) were collected but were rejected based on a blood sample being too dilute (total hemoglobin < 10 g/dL) (n=5), air bubbles or a bad sample drawn into the co-oximeter (total dysfunctional hemoglobin > 4%) (n=4), or the MouseOx was unable to establish a valid S_pO_2 calculation based on its internal algorithms (n=10).

Hemoglobin Saturation Definition

Before reviewing the results, it is important to understand the precise saturation parameter from the IL-682 co-oximeter that was used for comparison with MouseOx S_pO_2. Because the IL-682 is a co-oximeter, it has the ability to measure concentrations of the functional hemoglobins, oxyhemoglobin (HbO_2) and reduced hemoglobin (Hb), as well as the common non-functional hemoglobins, methemoglobin (metHb) and carboxyhemoglobin (COHb). These non-functional forms of hemoglobin are always present in normal, healthy humans and animals. They normally combine to represent about 2% - 2.5% of total hemoglobin[1].

The value of $\left[HbO_2\right]$ displayed on the co-oximeter after submission of each blood sample is given as

$$S_aO_2\big|_{fractional} = \frac{\left[HbO_2\right]}{\left[HbO_2\right]+\left[Hb\right]+\left[metHb\right]+\left[COHb\right]}.$$

This form of S_aO_2 is known as fractional hemoglobin because it represents oxyhemoglobin as a percentage of total hemoglobin, which includes both functional and non-functional.

The MouseOx device has not been calibrated to fractional hemoglobin however, but to functional hemoglobin. Functional hemoglobin[2] is given directly by the IL-682 using the parameter name $\%sO_2$, and is calculated by them as

[1] JG Webster (ed), Design of Pulse Oximeters, Inst. of Physics Publ., 159-161, 1997.
[2] Insturmentation Laboratory 682 CO-Oximeter System User Manual.

$$\%sO_2 = \frac{[HbO_2]}{100 - (\%COHb + \%MetHb)}.$$

Since the IL-682 measures only four types of hemoglobin (oxygenated, de-oxygenated, met and carboxy), and it is additionally assumed that there are no other types of hemoglobin present, or that they are inconsequentially small, this expression can be re-written in the standard form for functional hemoglobin, which is give as

$$S_aO_2\big|_{functional} = \frac{[HbO_2]}{[HbO_2]+[Hb]}.$$

Functional hemoglobin, represented by the equation above as $\%sO_2$, is the form that was recorded as the IL-682 value for S_aO_2 in the experiments described here. This parameter was listed on each blood sample ticket from the IL-682.

Results

Results of these experiments are plotted in Figure 1 below. The graph in Figure 1 shows MouseOx S_pO_2 data plotted against S_aO_2 derived from the IL-682 co-oximeter. A linear curve-fit of the data also appears on the graph as the solid black line that overlies a simple y = x line of identity (dashed line).

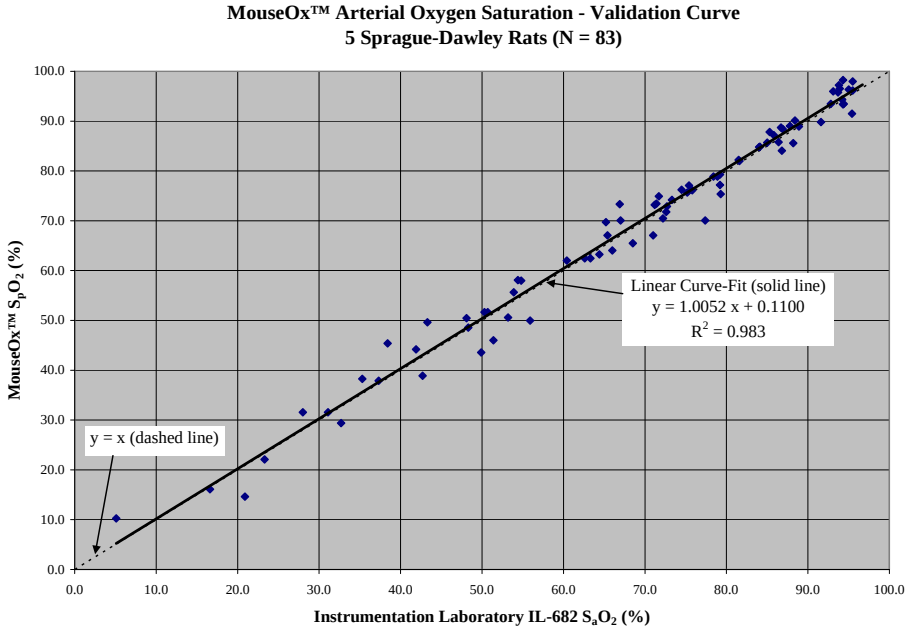

Figure 1: Calibrated MouseOx S_pO_2 versus IL-682 co-oximeter S_aO_2 ($\%sO_2$) for 5 rats.

All data from the 5 animals are presented in the plot. The number of data points from a given animal was a minimum of 13, and a maximum of 20.

Discussion

Figure 1 illustrates a comparison of rat S_aO_2 obtained from the co-oximeter and S_pO_2 data obtained from the calibrated MouseOx. The data in Figure 1 were not used in the development of the MouseOx algorithm for the estimation of arterial saturation. The validity of the calibration is demonstrated by the fact that the

MouseOx S_pO_2 and the IL-682 S_aO_2 illustrate a reflexive linear relationship (y = x). The curve-fit line is described by the equation $y = 1.0052x + 0.1100$, which indicates that the MouseOx arterial oxygen saturation values match almost identically, within a range of experimental error, with results from the IL-682, and since the offset term is nearly 0, there is essentially no bias in the MouseOx values. These data suggest that the MouseOx can provide non-invasive, functional hemoglobin S_pO_2 measurements in the anesthetized, non-ventilated rat that are accurate in a broad range of arterial oxygen saturations, from 100% to less than 20%.