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Unrestrained Acoustic Plethysmograph for Measuring Tidal Volume in Mice

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Abstract—The traditional method for measurement of tidal volume in unrestrained mice relies on pressure changes induced by a freely respiring animal in a whole body plethysmograph. These changes have been assumed to be the result of thermo-hygrometric differences between respired air and gas within the chamber. It is known, however, that gas compression in the lung can also contribute significantly to changes in plethysmograph pressure. This study describes an acoustic plethysmograph for mice that is capable of measuring the tidal volume time series without the errors associated with the traditional method.

The plethysmograph was designed as a resonating cavity at a fixed frequency. It had a sharp resonant peak and was tuned so that changes in body volume produced nearly linear changes in sound amplitude. The plethysmograph was tested with a water filled balloon connected to a syringe pump. The volume of the balloon was varied as a triangle wave with an amplitude of 250 μL. The RMS error between measured and delivered volume was 4.43 μL. A volume step test, performed to assess the response time of the system, showed that the plethysmograph responded in less than one millisecond.

Keywords—Helmholtz resonator, Whole-body plethysmography, Barometric plethysmography, Gas compression.

INTRODUCTION

Measurement of tidal volume in conscious, unrestrained mice has traditionally been performed using a whole-body plethysmograph (WBP). An animal is placed in a chamber where pressure changes due to respiration are observed, which are then related to tidal volume. The advantages of this type of system over a restrained plethysmograph are the extreme ease of use, reduced stress on the animal, and the ability for repeated and prolonged measurements.

Drorbaugh and Fenn[5] related tidal volume to the pressure changes measured in a closed chamber due to thermo-hygrometric differences between respired air and gas within the chamber. Epstein and Epstein[7] later pointed out a systematic error when only inspiratory events are used to calculate tidal volume. Epstein *et al.*[8] proposed a method to account for these systematic errors. At nearly the same time, Jacky[13] also proposed an improved method of analysis that permitted long term measurements of tidal volume. All barometric plethysmograph techniques assume that changes in plethysmograph pressure can be accounted for solely by changes in temperature and humidity.

It is known, however, that gas compression in the lung can also contribute significantly to the pressure measured in an unrestrained plethysmograph. Frazer *et al.*[10] demonstrated the effects of compression on the WBP signal with a model validated by simultaneously measuring plethysmograph pressure and chest wall motion of guinea pigs with a laser displacement sensor. Enhorning *et al.*[6] used a mechanical model of the chest to show that plethysmographic pressure was not only affected by breathing pattern, but also by airway resistance. Although there is some controversy as to the extent and conditions under which gas compression becomes a significant portion of the WBP signal measured in mice,[4,12,15,16,18] it is clear that tidal volume measurements of mice with increased airway resistance or breathing rate are likely to contain a significant component related to gas compression.

In this study, we present a novel acoustic whole-body plethysmograph (AWBP) capable of measuring spontaneous changes in lung volume of mice without the errors associated with traditional unrestrained plethysmography. The system uses an acoustic excitation of a resonant chamber and open nozzle. The system has a sharp resonant peak that is extremely sensitive to changes in volume. The use of a resonating cavity to measure volume has been used in the past to design an infant plethysmograph for static body volume measurements.[2,3,19] By manually changing the frequency to measure the resonance of the plethysmograph with a subject inside, and comparing this to the resonance of an empty chamber, they were able to measure average body volume. Jimenez *et al.*[14] later designed a modified and improved acoustic plethysmograph. No attempt, however, was made to track the resonate frequency as a function of time to measure tidal volume in either system. In contrast,

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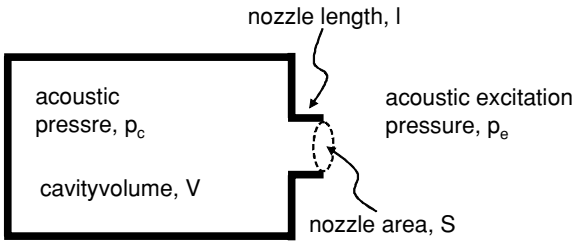

FIGURE 1. Helmholtz resonator.

we present a system that operates at a fixed frequency, and automatically tracks the change in sound amplitude as a function of time due to change in animal volume.

MATERIALS AND METHODS

Theory

The plethysmograph operates as a resonant cavity, or Helmholtz resonator consisting of a cavity with an open neck, or nozzle, such as the one show in Fig. 1. This system has an acoustic resonance at a frequency determined by the nozzle dimensions and the cavity volume. When considering acoustic wavelengths much larger than cavity dimensions, a sinusoidal source with amplitude p_e will produce a pressure signal amplitude inside the cavity given by:

$$p_c = \frac{p_e}{[(1-\omega^2/\omega_o{}^2)^2 + \omega^2 R^2 C^2]^{1/2}} \tag{1}$$

where ω is the excitation frequency, ω_0 is the resonant frequency, R is the acoustic resistance of the nozzle, and C is the compliance of the air in the chamber. Both ω_0 and C are dependent on the volume, V, of the cavity and can be described by the following equations:

$$\omega_0 = \sqrt{\frac{c_0^2 S}{lV}} \tag{2}$$

$$C = \frac{V}{\rho_0 c_0^2} \tag{3}$$

In these expressions, c_0 is the speed of sound, S is the nozzle cross sectional area,l is the effective length [1] of the nozzle, and ρ_0 is the density of air. Fig. 2 shows the magnitude of p_c/p_e versus volume for a chamber with nozzle dimensions $l = 4$ cm, $S = 0.785\,\text{cm}^2$, and a resistance of $R = 0.0004$ cm H_2O s cm^{-3}. The peak output occurs at approximately 77 mL. To the left of the peak, there is a region on the curve that is nearly linear. The midpoint of this region is denoted V_x on the graph, and represents the operating point of the plethysmograph. When the acoustic excitation (p_e) is held at a constant frequency and amplitude,

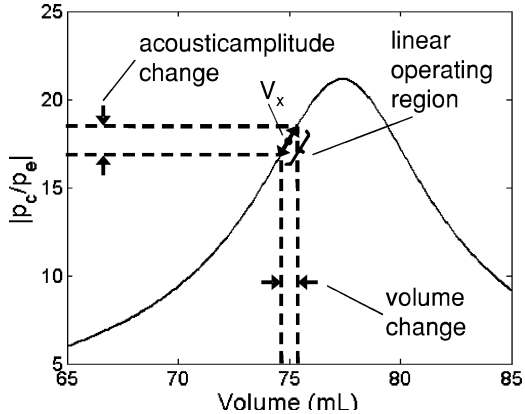

FIGURE 2. Magnitude of the excitation to chamber acoustic pressure ratio versus cavity volume. Small perturbations about the operating volume, *Vx*, produce linear changes in the chamber acoustic pressure amplitude.

small perturbations about V_x will produce essentially linear changes in the output acoustic pressure amplitude (p_c).

The concept of the AWBP is to place an animal in the plethysmograph and adjust the chamber volume such that the volume of air surrounding the animal (the deadspace) is V_x. As the animal respires and its chestwall expands and contracts, the deadspace volume changes which modulates the amplitude of the acoustic pressure in the chamber. Inspiration corresponds to a decrease in output amplitude while expiration corresponds to an increase in output amplitude. Note that the acoustic input impedance of the mouse respiratory system, beginning with the large change in area from the chamber to the nasal opening, is very large. For this reason, the volume of air inside the mouse lungs has little or no effect on the acoustic pressure in the plethysmograph.

There is a direct correlation between the signal to noise ratio of volume measurements and the sound pressure level (SPL) inside the chamber. For this reason, the sensitivity of the system is increased with an increased SPL. Since the excitation frequency is near 300 Hz and the hearing range of the mouse does not extend below 2000 Hz,[9,11] a higher SPL can be tolerated than has been used in systems designed for humans.

System

A diagram of the mouse plethysmograph used in this study is shown in Fig. 3. A photograph of the prototype system is shown in Fig. 4. The plethysmograph consisted of a main chamber, nozzle, speaker, and end stop assembly. The main chamber was constructed from a plexiglass tube with a 3.68 cm internal diameter. A microphone was mounted in a fixed plate which sealed one end of the chamber. The opposite end of the chamber was sealed with an adjustable end stop that was used to set the chamber volume for each mouse. The end stop was connected to a micrometer (The

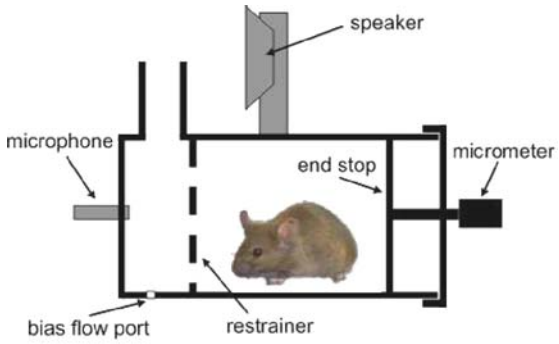

FIGURE 3. Schematic of the acoustic plethysmograph.

L.S. Starrett Company, Model 762) through a removable end cap connected to the main chamber. The micrometer head was used to adjust the chamber volume which could be varied from approximately 60–120 mL.

The nozzle was 3.5 cm long with an internal diameter of 1.1 cm. The nozzle was located near the front of the main chamber between the microphone and a small stainless steel restrainer. The restrainer confined the mouse to the back of the plethysmograph such that the nozzle could not be occluded. A small port in the chamber could be used to introduce a bias flow of air to flush out carbon dioxide and keep the animal cool. The impedance of this port and associated tubing must be large so as not to affect the resonance of the system.

The speaker was driven by a function generator (Medi Cal Instruments, Inc., Model 220) with a sine wave output. Acoustic pressure inside the chamber was measured with a Larson Davis microphone (Model 2530 microphone, Model 910B pre-amp, Model 2200C power supply). Since the plethysmograph was tested in a very noisy laboratory environment, the system was shielded by placing it in a box lined with acoustic foam. All data were digitized at a rate of10 kHz with a 16-bit data acquisition board and custom program (National Instruments, Model 6063E and LabView) after passing through a 1 kHz anti-aliasing filter (National Instruments, Model SC-2345 and SCC-LP04).

FIGURE 4. Photograph of the prototype acoustic plethysmograph.

Signal Processing

The signal processing in this application was performed using Matlab (The Mathworks). The system is designed to be excited by a single frequency (f_o) constant amplitude sine wave. The acoustic pressure signal measured inside the chamber was first passed through a band pass filter with corner frequencies at f_o −15 Hz and f_o + 15 Hz. The amplitude was demodulated by calculating the magnitude of the Hilbert transform.[17] This voltage was then related to tidal volume using the calibration described below.

Calibration

The excitation frequency of the system was selected by setting the chamber volume to 75 mL. The frequency of the function generator was adjusted in the 250–350 Hz range until the maximum output signal was obtained. Next, a mouse was placed in the chamber, which decreased the plethysmograph dead space. Note that the output signal varied as the animal's volume changed during respiration. The DC component of this signal represents the output at the average dead space volume. The plethysmograph volume was adjusted until the DC output was maximized. This represents the peak output amplitude at this excitation frequency and chamber dead space volume. The plethysmograph volume was then decreased by 2 mL in order to move from the peak of the output curve to the linear operating region (i.e. V_x in Fig. 2). This designated the operating point of the plethysmograph. The excitation amplitude was next adjusted until the mean SPL in the chamber was 110 dB. A three-point calibration was obtained by measuring the DC voltage output at three volumes: at the operating point, at the operating point minus 400 μL, and at the operating point plus 400 μL. These calibration volumes were achieved by adjusting the digital caliper on the end stop assembly. The slope of the best straight line fit of the calibration data is the ratio of voltage to volume.

Testing

The accuracy of the plethysmograph system was tested by measuring the change in volume of a water-filled balloon inside the chamber connected to an external syringe pump. A 250 μL syringe was incorporated in the pump and connected to the balloon through the plethysmograph bias flow port. The initial balloon volume was aproximately 20 mL, representing the volume of a mouse. All connections were made with stiff-walled Teflon tubing. A three way valve was connected inline for initial filling of the balloon and purging of air from the system. A linear potentiometer attached to

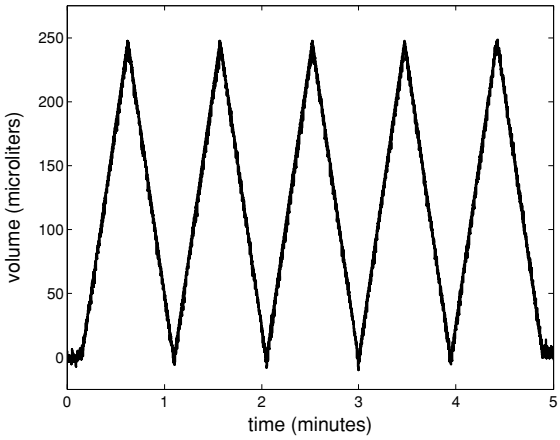

FIGURE 5. Accuracy test: syringe pump volume and plethysmograph output volume versus time.

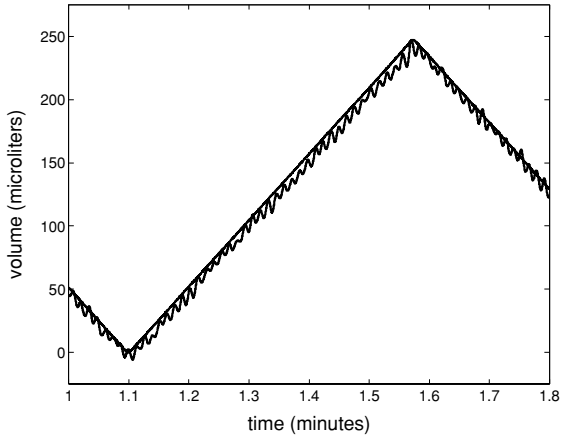

FIGURE 6. Zoomed view of syringe pump volume and plethysmograph output volume versus time.

the syringe pump was used to record syringe displacement. The system was calibrated as described above. The syringe pump was programmed to move between 0 and 250 μL at 527 μL/min (maximum speed). Because the syringe pump produced a slight vibration, the data for this test were post-processed with a 30 ms moving average filter.

A step test was used to assess the response time of the system. Since the syringe pump was not fast enough for this purpose, the test was administered manually. The syringe assembly was disengaged from the screw drive and the test was performed by manually pushing the syringe assembly as fast as possible.

Finally, the plethysmograph was used to measure the tidal volume of a 19 g specific pathogen-free female A/J mouse (Jackson Laboratory). The animal was housed in an AAALAC-accredited animal facility at 23°C and 50% humidity with a 12 h light/dark cycle, and was provided standard laboratory mouse chow and tap water *ad libitum*. The mouse was weighed and placed in the chamber, and tidal volume measured.

RESULTS

Results of the water-filled balloon test are shown in Figs. 5 and 6. Note that the time scale for this test is in minutes. This was due to the speed limitations of the syringe pump. Since it is difficult to see the syringe volume signal in Fig. 5, a zoomed view is shown in Fig. 6. The root-mean-square error over the five minute test was 4.43 μL. The standard deviation of the error was 4.09 μL.

The step test is shown in Fig. 7. Note that since the syringe step is generated manually, the final volume of the step is less than 250 μL. This was to avoid the noise generated by banging the end of the syringe. Also, the displacement of the syringe is not a true step. However, the results of this test demonstrate that the plethysmograph is able to track extremely fast changes in volume. The time for the input to go from 10% to 90% of the peak value was 7.91 ms. The 10% to 90% rise time of the output was 8.83 ms giving a difference of less than 1 ms.

Figure 8 displays the plethysmograph output for the A/J mouse. The average peak-to-peak tidal volume of each breath ($\pm$standard deviation) was 270 μL ($\pm$15.8 μL). The average rate of breathing ($\pm$standard deviation) was 3.95 Hz ($\pm$ 0.196 Hz).

DISCUSSION

The present study was motivated by the widespread use of the whole-body plethysmograph in assessing lung function of mice following exposure. The traditional WBP has been used to measure tidal volume assuming the pressure signal results solely from thermic differences between respired and ambient air.[5,7,8,13] Although the effects of gas

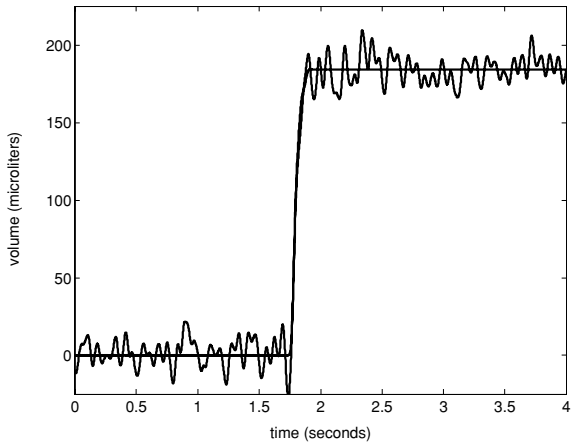

FIGURE 7. Response time test: syringe pump volume and plethysmograph output volume versus time.

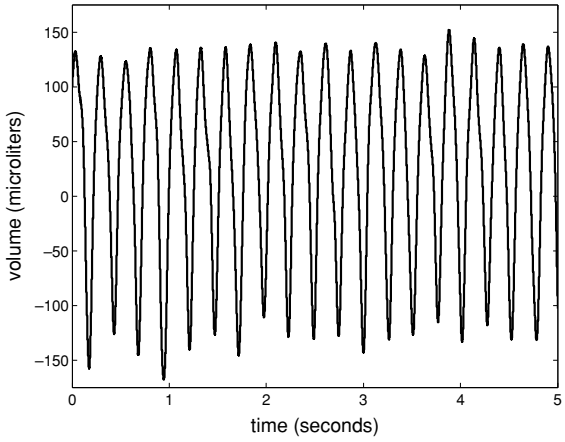

FIGURE 8. Tidal volume of an A/J mouse.

compression on the WBP signal of normal mice may be negligible, gas compression in mice with altered breathing pattern and/or increased airway resistance can produce significant errors in the measurement of tidal volume. The primary advantage of the acoustic plethysmograph is the ability to measure the tidal volume signal independent of gas compression. This is particularly useful when measuring mice that have been exposed to a respiratory irritant or toxin that increases either airway resistance or the frequency content of the breathing pattern. Note that the slope of the rising edge of an individual breath can increase even when the breathing rate goes down. Therefore, it is possible to get an increase in lung gas compression due to the increase frequency content even when breathing rate is reduced.

Another advantage of this system is that it provides the first step toward an extended system in which it will be possible to measure specific airway resistance in the mouse by simultaneously measuring tidal volume and the traditional WBP box flow signal. Previous attempts to measure specific airway resistance with a WBP have failed largely because changes in these measures can be explained not only by changes in airway resistance, but also by changes in the tidal volume breathing pattern. Lundblad *et al.*[16] showed that gas compression could be estimated by conditioning the chamber air to near alveolar conditions, but that estimates of airway resistance still require knowledge of the tidal breathing pattern. If tidal volume is known, however, then either gas conditioning or measurement of temperature and humidity in the chamber would allow estimation of specific airway resistance.

The acoustic plethysmograph could be transformed to measure the traditional WBP signal by adding a screen of sufficient resistance to the nozzle and measuring the pressure drop across the screen. By adding resistance to the nozzle, however, the sharpness of the resonant curve (the circuit Q) is reduced. Therefore, simultaneously measuring airflow and tidal volume via acoustic pressure is a trade-off between sensitivities. If the screen resistance is too small, it will be difficult to measure airflow. If the screen resistance is too large, the acoustic pressure amplitude will not be modulated sufficiently by changes in mouse volume.

The acoustic plethysmograph presented in this research has some limitations. First, the system is sensitive to noise generated at or near the excitation frequency in the room and by the animal in the chamber. The room noise can be abated by placing the plethysmograph inside a box lined with acoustic foam during testing. Band pass filtering the output signal prior to demodulation further reduces the effects of external noise and reduces noise generated by the animal.

The AWBP output voltage curve is highly sensitive to the excitation frequency. It is important to use a generator with a stable output over the length of the testing period. In our system, the function generator exhibited a slight frequency drift over a period of hours. This small drift produced a shift in the output curve. Since we calibrated daily, this did not present a problem, but if the shift had occurred during a test, a corresponding change in the DC level of the output voltage would have been observed. Although small deviations in frequency produced shifts in the output curve, the slope of the curve at the operation point remained nearly the same. Thus, the calibration is not changed, but a DC shift in output voltage is observed.

Motion of the animal in the chamber also produced some artifact in the output voltage. Small movements and animal grooming did not produce any observable distortions. Large animal movement tended to produce a shift in the DC level of the signal. One possible explanaton is that the placement of the animal affects the radiation resistance of the nozzle. Small changes in resistance would produce slights shifts in the output curve. As in the case with excitation frequency, it is unlikely the calibration would be affected, but a shift in the DC signal level would be observed. The animal restrainer is designed to keep the animal from affecting the nozzle resistance, but it may be too close in the prototype system. However, the acoustic plethysmograph appears to be much less sensitive to animal motion compared to the traditional whole body plethysmograph.

In summary, a novel acoustic whole body plethysmograph had been developed which allows measurement of tidal volume of mice independent of gas compression in the lung. This system provides particular advantages over the traditional whole body plethysmograph when measuring animals with increased gas compression due to increased airway resistance or increased acceleration in the breathing pattern.

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