Restrained whole body plethysmography for measure of strain-specific and
allergen-induced airway responsiveness in conscious mice

Jennifer L.S. Lofgren¹, Melissa R. Mazan¹, Edward P. Ingenito², Kara Lascola¹,
Molly Seavey¹, Ashley Walsh¹, and Andrew M. Hoffman¹, Cummings School of
Veterinary Medicine at Tufts University, North Grafton, MA, and ²Brigham and
Woman’s Hospital, Boston, MA.

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Corresponding author:
Andrew M. Hoffman
Tufts University’s Cummings School of Veterinary Medicine
200 Westboro Road
North Grafton, MA  01536
andrew.hoffman@tufts.edu
Abstract

The mouse is the most extensively studied animal species in respiratory research, yet the technologies available to assess airway function in conscious mice are not universally accepted. We hypothesized that whole body plethysmography employing non-invasive restraint (RWBP) could be used to quantify specific airway resistance (sRaw-RWBP) and airway responsiveness in conscious mice. Methacholine (Mch) responses were compared using sRaw-RWBP versus airway resistance by the forced oscillation technique (Raw-FOT) in groups of C57, A/J, and BALBc mice. SRaw-RWBP was also compared to sRaw derived from double chamber plethysmography (sRaw-DCP) in BALBc. Finally, airway responsiveness following allergen challenge in BALBc was measured using RWBP. SRaw-RWBP in C57, A/J, and BALBc mice was 0.51±0.03, 0.68±0.03, and 0.63±0.05 cm*sec, respectively. SRaw derived from Raw-FOT and FRC (Raw*FRC) was 0.095cm*sec, approximately 1/5th of sRaw-RWBP in C57 mice. The intra- and inter-animal coefficients of variations were similar between sRaw-RWBP (6.8 and 20.1%) and Raw-FOT (3.4 and 20.1%, respectively). The order of airway responsiveness employing sRaw-RWBP was AJ>BALBc>C57 and for Raw-FOT was AJ>BALBc=C57. There was no difference between the airway responsiveness assessed by RWBP vs. DCP; however baseline sRaw-RWBP was significantly lower than sRaw-DCP. Allergen challenge caused a progressive decrease in ED175 based on sRaw-RWBP. In conclusion, the technique of RWBP was rapid, reproducible, and easy to perform. Airway responsiveness measured using RWBP, DCP, and FOT was equivalent. Allergen responses could be followed longitudinally which may provide greater insight into the pathogenesis of chronic airway disease.
Background

The use of mice in respiratory research is growing, in part due to the rapid development of new transgenic strains. Precise measurements of airway function may be obtained using invasive technologies that control for the confounding influences of lung volume (i.e. volume history, lung volume during measurement) and respiratory frequency (6). However, an important place remains for the in vivo study of conscious mice, where the influences and risks of anesthesia are absent, and longitudinal studies are desired. Currently, a problem with the study of conscious mice is the lack of widely accepted techniques for pulmonary function testing. The available methods each have advantages and disadvantages that relate to: (1) ease of the procedure, (2) animal tolerance, (3) precision and validation, and (4) their basis in known physical determinants of airway function (i.e., pressure and flow). Recently, concern has been expressed over the widespread use of unrestrained technology to characterize ‘airway function’ per se (5, 6, 28, 41, 50). These opinions were formed after several studies failed to corroborate data derived from unrestrained whole body plethysmography (UWBP) with more rigorous invasive techniques (1, 40, 43, 47). Since UWBP is a commonplace application, this has left many users searching for practical alternatives (33). Several alternative methods been studied which do provide more direct measures airway mechanics. Mid-tidal expiratory flow (EF50) has been evaluated extensively for the ability to characterize bronchoconstriction in conscious mice (21, 22, 24, 55). Outcomes of EF50 have correlated well with simultaneous invasive measures of pulmonary resistance and dynamic compliance during allergen, cholinergic agonist, and hyperoxia
challenges (21, 23, 24). While EF50 permits the monitoring of tidal breathing flow limitation, one can only infer airway resistance from plethysmographically derived flow. Specific airway resistance (sRaw), the product of airway resistance (Raw) and lung volume could provide greater insight into airway mechanics (18, 20). This variable can be measured using double chamber plethysmography (42), although there are practical limitations such as the use a neck seal and complex restrainer (20). The reproducibility of airway responsiveness derived from double chamber plethysmography (DCP) has also been challenged (12), and strain-specific responses to methacholine were discordant with more invasive methods (16). For these reasons, the current method to measure sRaw using DCP is not widely cited. Transfer impedance (Ztr) is yet another conscious method, which is used to probe central (airway) vs. peripheral (tissue) contributions to bronchoconstriction and permit serial measurements over time (30). One limitation of Ztr is the need for acclimation to obtain acceptable coefficients of variation, although this can be obtained after one day of conditioning the mice to the instrument. In sum, each available system provides a different level of user satisfaction and certainty regarding the status of the airways (6), and therefore the development of novel systems that improve upon this spectrum of technologies should continue.

We investigated the use of restrained whole body plethysmography (RWBP), an adaptation of the original method of body plethysmography (pressure plethysmography) in humans (15) that was later used in guinea pigs (3). Mice are fast breathers (3-6 Hz) so special considerations concerning plethysmographic design and validation were addressed. The intent of this study was to provide an initial proof of concept for RWBP in conscious mice and thus stimulate future applications and comparisons with alternative
systems. We hypothesized that 1) baseline sRaw-RWBP and associated methacholine responses would be similarly reproducible to Raw-FOT, 2) values for sRaw measured with RWBP (sRaw-RWBP) would be comparable to sRaw derived from a combination of airway resistance from the forced oscillation technique (Raw-FOT) and Boyles Law plethysmography to obtain functional residual capacity (FRC), or double chamber plethysmography to obtain an alternative conscious sRaw (sRaw-DCP), 3) strain-specific airway responsiveness would be accurately characterized by RWBP and lastly, 4) RWBP could be used to characterize longitudinally the increase in airway responsiveness associated with chronic (10 wk) allergen challenge in a single group of BALBc mice.

Methods

The experimental protocol followed NIH guidelines and was approved by the Institutional Animal Care and Use Committee at Tufts University Cummings School of Veterinary Medicine (IACUC Protocol G670-04).

Animals. Pathogen- free female C57BL6 (n=18) (Charles River Laboratories, Wilmington, MA), A/J (n=18) and BALBc (n=59) (Jackson Laboratories, Bar Harbor, Maine), purchased at 8-10 weeks of age (19-22 g) were used for this experiment. Each mouse was individually identified; they were housed in cages in groups of 4-5 in an AAALAC accredited facility that provided only Hepa filtered air. Food and water free of ovalbumin was provided ad libitum. A sentinel program ruled out the presence of any of the following infectious agents in the housing area of the mice during the study:
Parvoviruses (MPV-1, MPV-2, MVM, NS-1), Sendai virus (SEND), Pneumonia Virus of Mice (PVM), Mouse Hepatitis virus (MHV), Theiler’s Murine Encephalitis Virus (TMEV), Reovirus (REO), Mycoplasma pulmonis (MPUL), and Mouse rotavirus (EDIM).

Study design

In A/J, C57, and BALBc mice, the responses to methacholine (Mch) aerosol were measured using restrained whole body plethysmography (RWBP) and the forced oscillation technique (FOT). In BALBc mice, we additionally used double chamber plethysmography (DCP). Finally, we measured Mch responses in the BALBc mice after allergen sensitization (intra-peritoneal) and aerosol challenges.

Physical properties of the restrained whole body plethysmography (RWBP)

The custom-built whole body pressure plethysmograph (RWBP) consisted of clear Lucite outer chamber walls (5.8 height x 7.7 cm width x 20.2 cm length = 902 mL, thickness 12 mm). The outer chamber held an inner restraint chamber (e.g. Nose Cone Restrainer, Kent Scientific, Torrington, CT) (Fig 1). The restraint chamber was modified by drilling several holes (2-3 mm) into the wall, overlying the region where the thorax and abdomen of the mice lie.

The dynamic properties of the plethysmograph were studied by varying pressures in the chamber (32). A leak (3.2 sec to 36% peak pressure) was created by a Luer connector mounted in the wall of the box, which was attached to a length of tubing, a stopcock and needle (22 g, 2 cm). The leak resistance was measured to be 5.37 cm
H,O/mL/sec and inertance 0.029 s²/mL. A step pressure created by (a) hammering gently the plunger of a syringe loaded to an expected volume (0.02 mL), and (b) balloon burst both peaked between 19-21 msec, with a thermal time constant (described by a single compartment) of 0.95 sec. Amplitude of box volume was examined as a function of input frequency using digitally controlled square and sinusoidal flows delivered with a piston-driven mouse ventilator (flexiVent, Scireq Corp, Montreal, Canada). Box volume amplitude remained within 3% of the delivered volume (0.1 mL) from 1-10 Hz, and within 0.075% between 2 and 5 Hz, the range most relevant to breathing in conscious mice. Therefore the conditions were adiabatic across these frequencies. Time shifts between flow, delivered via the pneumotachograph within the box, and peak box pressure averaged less than 3 msec (range 1-6 msec) from 0.5-20.5 Hz generated using a broadband input generator (flexiVent, Scireq Corp). Pressure in the plethysmographic chamber was sampled using a low-range (+/- 10 cm H₂O) differential pressure transducer (TRD 5700, Buxco Electronics, Wilmington, NC) referenced to a chamber open to atmosphere (τ = 6 sec). A pneumotachograph (8431 Series, Hans Rudolph, Kansas City, MO) with dead space 0.3 mL, heated to 38-39°C was custom fitted via O-ring assembly to the proximal port of the nose cone for measurement of flow and flow-derived parameters. Data was sampled at 2500 Hz per channel using commercial hardware (Max1420 Buxco Electronics, PCI 6024E, National Instruments) and software (Biosystem XA version 2.7.4, Buxco Electronics, Inc). The pneumotachograph was calibrated by integration of the flow, injected as a known volume (0.5 mL). Box volume (Vbox) derived from box pressure, was calibrated by rapid injection of a known volume (0.1 mL) into the chamber, between each mouse experiment, and checked
repeatedly using injections of varying volumes and quasi-sinusoidal inputs. Bias flow (0.5 L/min) was employed between recordings, and the box vented fully between Mch challenges.

Measurement of sRaw using restrained whole body plethysmography (RWBP)

For measurements using RWBP, mice were loaded into the restrainer from the rear and using the mobile back plate, were pushed forward until their heads were straight and muzzles in contact with the inner wall of a rubber O-ring (i.e. ‘cuff’) that was used to prevent leak.

The amount of leak around the muzzle was investigated in a pilot study involving BALBc female mice (n=11, 20-26 gm). A steady airflow (30 mL/sec) was delivered simultaneously via Y piece to (1) the mouse assembly (i.e. pneumotachograph, nosecone, and mouse), and (2) a parallel ‘shunt’ pathway opened to atmosphere. Flow through the shunt pathway was measured using a separate calibrated pneumotachograph. After total flow was measured through the shunt pathway, the other pathway was attached to the mouse assembly. The proportion of flow lost to the mouse pathway was considered the percentage leak.

The mean (± SD) reduction in shunt flow was 0.93 ± 0.97% (range 0 to 2.6%), indicating that the flow resistance through the cuff area was on average >100 times greater than the shunt pathway. Thus the leak in the cuff contributed to <1% error in the measurement of flow or specific airway resistance.
The box volume-flow (X-Y) plots were constructed post-hoc from the primary signals using commercial software (Acknowledge v. 3.7.3, Goleta, CA). Primary waveforms (box volume, flow) were reviewed and the time-period indicative of peak responses to Mch was identified by repeated sampling. Peak responses to Mch were found in pilot studies to occur between 1.5-2.5 min after the initiation of exposure to Mch. The slope (θ) of the box volume-flow plot was measured manually using a protractor (accurate to 0.5 degrees) on the straightest possible segment between 1 to -1 mL/sec (i.e. the left side of the loop). Examples of a complete bronchoprovocation and the derivation of sRaw ("sRaw-RWBP") from primary signals are shown in Fig 2a.

The straight segment corresponded to the rapid (10-12 msec) transition from expiration to inspiration where volume shift was negligible. This technique has been shown to minimize the contribution of heating and humidification to the box pressure (3, 4), and sRaw derived from this period was equivalent in panting and non-panting human subjects (35). Gains were set to produce angles (tangents) between 40 and 75 degrees. Breaths with evidence of laryngeal braking were avoided (56). Specific airway resistance-RWBP was computed from the tangents as follows:

\[
s_{\text{Raw-RWBP}} = \frac{1}{\tan \theta} \times (P_{\text{atm}} - P_{\text{H2O}}) \times C_f \times \frac{V_{\text{box}} - bwt \text{ mouse}}{V_{\text{box}}}, (2),
\]

where Patm was atmospheric pressure, P_{H2O} was water vapor pressure, C_f was a scaling factor for the X and Y axes, Vbox was total volume of the outer chamber, and bwt of mouse was body weight (gm).

One major concern with the use of an unheated plethysmograph was the potential for errors to arise in the computation of sRaw, from heating and humidification of
inspired gas which has been shown to cause the box pressure amplitude to nearly double (39, 40, 57). We explored this issue by performing a pilot experiment with conscious mice (BALBc female, n=5, 21.6-27.1 g) whereby sRaw was determined in the heated and humidified (37°C, 90% humidity) versus unheated (26°C, 66% humidity) box. Calibrations were performed before each measurement and mice were removed from the restrainers between measurements. In the unheated box, the amplitude of Vbox was only 10% greater, suggesting that there is a significant amount of gas conditioning (heating and/or humidification) while under restraint for RWBP (Table 1). sRaw did not change with heating of the box, although the loops were tighter with heating of the plethysmograph (Fig 2b). Hence, the measurement of the sRaw based on the tangent as described was repeatable despite slight differences in gas conditioning between heated and unheated conditions. In contrast to RWBP, we found in a separate pilot study in BALBc mice (n=10, 18.6-25.3 g) similar to Lundblad et al (40) that Vbox doubled when mice were left unrestrained in the plethysmograph (Table 1). Hence, the use of RWBP to obtain a tangent for computation of sRaw appeared to be relatively free from errors associated with gas conditioning at least to the extent that could be measured at baseline prior to any provocations.

**Double chamber plethysmography**

The double chamber plethysmograph was purchased from a commercial source (PLY3351, Buxco Electronics Inc, Wilmington, NC). The techniques for measurement with this technique have been described previously (12, 20). Briefly the difference between RWBP and DCP is that RWBP isolates nasal flow, and DCP isolates both nasal and thoracic flow (i.e. they are separated). Hence, the measurement of DCP relies on the
comparison of phase and magnitude of the nasal and thoracic flows, whereby RWBP relies on comparison between nasal flow and box pressure. For DCP, the flow for each chamber (nasal and thoracic) of the DCP was calibrated separately by rapid injection of a known volume (0.5mL) into the chamber; volume was matched by the integration of flow. The accuracy of calibration was checked volumetrically before each mouse was placed in the chamber. An AC offset was used to condition each signal (nasal, thoracic) in order to compensate for the bias flow in the nasal chamber. The phase lag between the chambers in the absence of a mouse was negligible (<0.01 msec up to 10 Hz). For DCP, mice were loaded into the rear of the thoracic chamber and pushed forward until the head protruded through a hole in a latex neck seal provided with the equipment. Four different sized neck seal openings were used (0.6-1.0 mm), with the smallest size that did not diminish peak flow or minute ventilation (20) employed for measurements. Once the mouse was secured within the thoracic chamber with the head protruding through an appropriate neck seal, the nasal chamber was attached and bias flow (0.5 L/min) initiated. For measurements, the bias flow was turned off temporarily to maximize signal to noise. Computation of sRaw measured with DCP (herein “sRaw-DCP”) followed protocols established by Pennock (42) and later applied to mice by Flandres et al (20), whereby the time lag (dT) between the thoracic and nasal flow at zero crossing (during transition between inspiration and expiration) was utilized as follows:

\[
s_{\text{Raw-DCP}} = \frac{(T_i+T_e)}{2\pi} \times (P_{\text{atm}}-47) \times 1.36 \times \pi \times \frac{dT}{(T_i+T_e)}
\]
where $T_i$ and $T_e$ equal inspiratory and expiratory time (sec) and $P_{atm}$ atmospheric pressure (cm H$_2$O). The peak $dT$ was identified and 10 sequential breaths free from movement artifacts were measured for that period.

**Measurement of respiratory system impedance using the forced oscillation technique**

Methods for the forced oscillation technique (FOT) including calibration techniques followed previous publications (25, 54). Briefly, mice were anesthetized with ketamine (50-75 mg/kg) and xylazine (5mg/kg) (Butler Co, Dublin, OH) intra-peritoneally, and a tracheostomy performed with a 19 g cannula (Becton Dickinson and Company, Franklin Lakes, NY). Once anesthesia was confirmed by lack of response to toe pinch, mice were paralyzed with pancuronium (1mg/ml, Baxter Healthcare Corp. Irvine, CA). Supplemental ketamine (25 mg/kg IP) was provided every $\frac{1}{2}$ hr. Ventilation was set at frequency of 200 BPM, $V_T$ 0.3ml, PEEP 3.0cm H$_2$O, and inspired oxygen was supplemented at all times. Repeated measurements were performed using a commercial data acquisition system for input impedance between 1-19 Hz (Quick Prime 3 analyzer, FlexiVent System, SCIREQ Corp, Montreal, Quebec). A constant phase model (25) was employed to compute airway resistance (“Raw-FOT”), in addition to tissue resistance ($G_{ti}$) and elastance ($H_{ti}$) coefficients. Baseline Raw, $G_{ti}$, and $H_{ti}$ were tabulated but the Mch responses using $G_{ti}$ and $H_{ti}$ were not used for comparisons with conscious measurements; only Raw was used for this purpose. In order to derive a value of $s$Raw (i.e. $\text{Raw} \times \text{FRC}$) from FOT for comparison with conscious $s$Raw-RWBP, we measured functional residual capacity (FRC) using the Boyles law method in a separate group of similarly anesthetized-tracheostomized female C57 mice ($n=30$, 19-22 g) using a
commercial plethysmograph (PLY 3111, Buxco Electronics), data acquisition system (XA Biosystem 2.7.4, Buxco Electronics), and standard calibration routines.

**Reproducibility of baseline (unprovoked) measurements**

A subset of C57BL/6 mice (n=12) used for RWBP and FOT were initially used to examine reproducibility (within-animal, within-group) of sRaw-RWBP. Several recordings were made over 45 minutes, on a separate day in the AM and PM (10 min each), and on a third day for 10 min. For each time-point an average of 10 breaths selected at random was used for analysis. The results were expressed as coefficient of variation and 95% confidence intervals (see Statistical analyses). The within-group CV was also compared between strains of mice.

**Methacholine challenges in conscious mice**

Challenges were conducted at the same time of day (morning or afternoon) for each mouse. Ambient temperatures in the laboratory ranged 21-23°C and humidity 25-45%. Baseline recordings were obtained after 2 min acclimation to the chamber. For aerosol exposures, the mice within their restraint chambers were detached from the pneumotachograph, and attached to an adjacent enclosure set up for nose-only exposures. For RWBP, aerosols were generated with an ultrasonic nebulizer (Aerogen, Aeroneb, Dangan, Galway, Ireland), and directed through the T piece using a low-flow (470 mL/min) regulator, thus permitting the mice to breath a standardized aerosol concentration (reported mass median diameter = 3.1 microns). Following each exposure, mice were returned within 20 sec to the RWBP, and the recording of data resumed for 5
min. For DCP, aerosols were delivered from the Aerogen nebulizer directed via stopcock into the nasal chamber. Bias flow caused the aerosol to traverse the nasal chamber at the level of the nares, assuring exposure to the aerosol. Doses of Mch were chosen that induced on average an increase in sRaw-RWBP to 300% baseline. Specifically, Mch (Provocholine, Methapharm, Brantford, Ontario) was nebulized for 60 sec to C57 at concentrations of 0 (i.e., saline), 10, 50, and 100 mg/mL, in A/J mice at 0, 1, 5, and 10 mg/mL, and in BALBc mice at 0, 6.25, 12.5, and 25 mg/mL (pre and post OVA exposure). During the comparison of mouse strains (A/J, C57, and BALBc) using RWBP the above doses were delivered without stopping criteria. However, during the comparison of techniques (RWBP, DCP, and FOT) and following allergen challenge in BALBc mice, we stopped Mch challenge when mid-tidal expiratory flow to \(<1\) mL/sec since these mice were expected to become more responsive over time, and it was therefore deemed unsafe to give all doses of Mch. The provocative concentration that caused sRaw to increase to \(>175\)% post-saline value was computed by log-linear interpolation across the final two concentrations of Mch employed for each mouse, according to Sterk et al (51). The provocative concentration of Mch that increased sRaw to 175% post-saline values (‘ED175’) was used as an index of airway responsiveness.

**Methacholine challenges using the forced oscillation technique (FOT)**

Aerosols were delivered directly into the tracheal cannula during lung inflation during mechanical ventilation (flexiVent, Scireq Corp, Montreal, Quebec). Ten second nebulization periods were used, followed immediately by a series (8-15) of measurements. The lung was inflated to TLC (30 cm H\(_2\)O airway pressure) once after
each aerosol delivery. Forced oscillations during apnea (3 seconds in duration) were applied every 17 seconds for 5 minutes. Dosage ranges were pre-determined in pilot studies to evoke between 10 and >75% increase in airway resistance (Raw). Methacholine in C57 mice was delivered at 0 (saline), 4, 8, and 16 mg/mL, and in AJ we used 0, 2, 4, and 8 mg/mL. The concentration of Mch that provoked an increase in Raw-FOT to 175% baseline (ED175) was determined by log-linear interpolation as described above.

**Allergen sensitization and aerosol exposure of BALBc mice**

A subset of the BALBc mice (n=9) were immunized and sensitized to ovalbumin using a procedure modified from past studies (10, 36, 37, 53). Briefly, mice received an intraperitoneal injection of 50 ug ovalbumin (grade V, 98% pure, Sigma Aldrich, St Louis, MO) precipitated in aluminum hydroxide and magnesium hydroxide (Imject Alum, Pierce, Rockford, IL) 14 and 7 days before inhalational exposure. Mice were then exposed to 2.5% aerosolized ovalbumin for 30 minutes, three times a week for a total of 10 weeks, and on the 5th and 10th week, methacholine challenges were performed 48 hrs after ovalbumin exposure. Inhalation exposures were performed using a custom-built whole body exposure system during which air was drawn through a 0.4 cubic meter chamber at a flow rate of 80 l/min. The ovalbumin solution was delivered into the chamber using a Pari LC JET fine particle nebulizer (Pari Corp, Paris, France) delivering particles with reported mean median diameter of 1.6 um, and compressor (Model NE-CO8, Omron Healthcare, Inc, Vernon Hills, Illinois, USA). The mass concentration of
the particles within the breathing zone of the mice was continuously monitored using a laser photometer (SidePak, AM510, TSI Inc, Shoreview, MN). Flow from the compressor was regularly adjusted throughout the exposure period in order to keep the ovalbumin concentration between 15 and 20 mg/m³.

Statistical Analyses

The intra-animal and within-group reproducibility of non-invasive sRaw versus invasive Raw were expressed as coefficients of variation (CV) and 95% confidence limits, and time effects tested using repeated measures ANOVA. The Mch responses were analyzed using repeated measures ANOVA. The effect of mouse strains on sRaw-RWBP, sRaw-DCP, Raw-FOT, or indices of airway reactivity (ED175) was analyzed using ANOVA. Pairwise comparison between strains was performed using Student’s T-tests. Paired tests were employed to test the effect of allergen on ED175 in BALBc mice. Significance was attributed to data when $P<0.05$. All values are expressed as mean ± SEM except where indicated.

Results

Reproducibility of sRaw using RWBP

In the group of C57 mice used to study repeatability, there were no significant changes in sRaw-RWBP within the 45 minute measurement periods, between AM and PM measurements, or between different experimental days (Table 2). The mean intra-animal CV over the short term (i.e., 3 measurements evenly spaced over 45 min) was 6.8% for sRaw-RWBP and for Raw-FOT it was 3.4% (for 3 successive measurements).
The mean intra-animal CV for Raw-FOT was significantly lower \((P<0.05)\). The mean intra-animal CV in the longer term (across days 1, 2, and 3) was 22.8\% for sRaw-RWBP. The *inter-animal* CV for sRaw-RWBP for this set of C57 mice on days 1, 2, and 3, were 26\%, 27\%, and 14\%, respectively (mean 22.3\%). For the subsequent groups used in Mch challenges, the *inter-animal* group CV for sRaw-RWBP was 26.5\% for C57, 17.2\% for AJ, and 30.2\% in BALBc mice (pre-OVA). In comparison, *inter-animal* CV for Raw-FOT was 19.5\% for C57 and 20.7\% for A/J. Therefore the reproducibility of these techniques was very similar.

*Comparison between sRaw-RWBP and sRaw-FOT*

The measurement of sRaw by RWBP at baseline in C57 mice was compared to sRaw derived from (1) Raw-FOT and (2) plethysmographically derived FRC also in anesthetized intubated mice. We measured an average FRC of 0.27 mL, which is close to published values in C57 (0.25 mL) (38). Hence, sRaw-FOT was computed as follows: Raw-FOT*FRC, or 0.353 cm H\(_2\)O/mL/sec*0.27mL = 0.09531 cm*sec. Therefore sRaw-FOT was approximately 1/5\(^{\text{th}}\) of sRaw-RWBP.

*Strain-specific airway responsiveness*

Measurement of sRaw-RWBP took between 3-6 min including loading, and Mch challenges took between 16-30 min depending on airway responsiveness. Baseline sRaw-RWBP was significantly lower \((P<0.001)\) in C57 compared to AJ or BALBc (Table 3). Baseline Raw-FOT was not different between AJ \((0.35 \pm 0.12 \text{ cm/mL/sec})\), C57 \((0.35 \pm 0.16 \text{ cm/mL/sec})\), and BALBc \((0.30 \pm 0.06 \text{ cm/mL/sec})\) mice. The mean ±
SEM FRC determined in a separate group of C57 mice was 0.265 ± 0.009 mL. These values were used to construct sRaw-FOT. Methacholine caused a significant dose-dependent increase in sRaw-RWBP (Fig 3a). Accompanying the change in sRaw-RWBP was a significant decrease in respiratory frequency but no significant change in tidal volume for any strain of mice (Fig 3b and 3c). Methacholine caused a dose-dependent increase in Raw in all 3 strains of mice (Fig 4). Airway responsiveness was significantly different between AJ, C57, and BALBc mice for sRaw-RWBP and the descending order of reactivity was AJ>BALBc>C57 (Table 4). Using Raw-FOT, there was no difference between C57 and BALBc ED175, but both strains had a significantly higher ED175 than A/J. Therefore airway reactivity in descending order by FOT was A/J>BALBc>C57. A comparison of Mch responses between methods using a single strain of mouse (BALBc) and standard doses for Mch is shown in Fig 5. There was no difference in ED175, derived for RWBP, DCP, and FOT. However, the percentage change in sRaw-RWBP or sRaw-DCP was significantly higher than the percentage change in Raw-FOT. Baseline sRaw-RWBP (0.63 ± 0.05 cm*sec) was significantly lower (P<0.001) than mean sRaw-DCP (1.82 ± 0.11 cm*sec).

Allergen responses

In BALBc mice exposed to ovalbumin for 5 or 10 weeks, there was no significant change (P>0.1) in baseline sRaw (mean±SD: pre-OVA 0.75 ± 0.12, 5 wks OVA: 0.66±0.16), 10 wks OVA0.79 ± 0.26 cm*sec). There was a significant (P=0.001)
Discussion

In this study we demonstrate for the first time, the application of whole body plethysmography in conscious mice, as originally described by Dubois, Botelho, and Comroe (15). Whole body plethysmography akin to the technique performed in humans for measurement of sRaw was first adapted for animals by Agrawal (3) who used conscious guinea pigs (3, 19, 26). More recently WBP for direct determination of sRaw has been employed in dogs (8) and sheep (7) using a head-out configuration. These studies demonstrate that WBP holds promise for characterization and longitudinal studies of airway mechanics and airway responsiveness in conscious animals. Measurement of sRaw appears to be an appropriate endpoint for methacholine responses since it combines information related to airway resistance and lung volume, both of which can be modified during tidal breathing after bronchoconstriction.

Advantages of RWBP may include the ease of loading, nose-only exposure, direct non-plethysmographic measurement of flow, and the lack of a neck seal that may constrict the airway or impair loading. For use of RWBP, none of the mice required acclimation in order to complete several sets of baseline measurements and one or more bronchoprovocations, which may be an additional advantage. While we did not
specifically measure indices of stress directly, and therefore it is not possible to comment on their physiologic responses to RWBP, the mice were active, grooming, and appeared unharmed each time they were removed from the chamber.

_Critique of the plethysmographic device (RWBP)_

The size of the box (902 mL, or 45 mL/g) was relatively large in comparison to past studies employing pressure plethysmographs in guinea pigs or mice (49, 56). This contributed to consistent adiabatic conditions, a acceptable feature of pressure plethysmography (32, 49). One problem was frequent drift in the baseline of the pressure signal due to warming of the chamber by reloading of the mouse. This could be avoided in future experiments by conducting the entire study without opening the chamber, i.e. aerosolizing agonists to the mice within the chamber using special delivery systems (3, 19). Incorporation of heating elements or warmed water (39, 40, 42) or decreasing the size or thickness of the chamber walls, may also serve to stabilize the thermal conditions of the box interior. Alternatively, flow-type plethysmography has been described (49) which would minimize the effects of baseline thermal drift, although pose additional challenges, e.g. maintaining calibration. A significant challenge in the use of any plethysmograph is to understand the confounding influence of heating and humidification on the box pressure signal. Heating and humidification of inspired gas likely created some looping in the XY plots (31) based on our pilot studies (Fig 2E). However, this source of looping appeared not to confound the measurement of sRaw (Table 1). Furthermore, heating of the plethysmograph caused a decrease in box pressure
amplitude (ie Vbox) by only 10%, suggesting that gas conditioning within the dead space in front of the mouse is significant when compared to the absence of conditioned gas as in the unrestrained setting. In sum, there are several improvements that could be made to the current design to improve stability and convenience, but none of these would improve fundamental accuracy of sRaw derived by RWBP.

Reproducibility of sRaw

An understanding of test reproducibility is paramount to the application of any device used to measure pulmonary function. The intra-animal CV for sRaw-RWBP (measurements made every 15 min) in mice (6.6%) was similar to guinea pigs (26), and very similar to Raw-FOT (3.4%) as measured. Similarly, the CV for respiratory system resistance in conscious mice using transfer impedance was 6%(30), although this was obtained after 2 previous acclimation periods of measurement, each 2 hrs apart. The within-group CV's were equivalent for sRaw-RWBP and Raw-FOT in the multiple strains of mice in this study. In sum, sRaw-RWBP was no less reproducible at baseline or after Mch than Raw, despite the fact that the measurement was made without control of several important factors such as tidal volume and PEEP.

Comparison between sRaw-RWBP, sRaw-FOT, and sRaw-DCP

The measurements using FOT and FRC were employed in part to understand the validity of RWBP for measurement of airway mechanics, specifically sRaw. The baseline values for Raw in both C57 and AJ strains were comparable to previously
published values (9, 25, 44) and therefore served as a reasonable gold standard. The mean value of sRaw-RWBP in C57 (0.51 cm*sec) was about 5 times the value obtained for sRaw-FOT (0.095 cm*sec), commensurate with the contribution from the upper airways. In support of this notion, the upper airways contributed to about 4/5th of total Raw in rats (13). The exact contribution of the upper airways to the baseline and post-provocation measurements of sRaw-RWBP were not disclosed by this study but require further study in conscious mice.

An additional approach that was employed to better understand the validity of sRaw-RWBP was the comparison with sRaw-DCP. In BALBc, mice where both techniques were performed, sRaw-RWBP (0.63 ± 0.05 cm*sec) was significantly lower than sRaw-DCP in our study (1.82 ± 0.11 cm*sec), and lower than past measures of sRaw-DCP in BALBc mice, i.e. 1.02 (20), 1.18 ± 0.4 (18) and 1.50 (12) cm H2O*sec. Our values for sRaw-RWBP were also lower than sRaw-DCP in other strains of mice, i.e. 1.2 (20) or 1.44 ± 0.5 (18) cmH2O*s in C57, and 1.68 cm H2O*sec in AJ mice (20). The basis for the large discrepancy between sRaw-RWBP and sRaw-DCP was not disclosed by our study. The higher values across the board for sRaw-DCP when compared to sRaw-RWBP may relate to differences in the computation methods, and the constrictive effect of the neck seal used for DCP. The computation for DCP is based entirely on nasal vs. thoraco-abdominal phase lag, which may be influenced by several instrument and host factors previously reviewed by Pennock et al (42). Another factor that may have confounded our measurements of sRaw-DCP was the tension on the neck seal. The seal must be tight enough to restrain the mouse while avoiding leak, yet loose enough to avoid constriction (20). As it is difficult to standardize the tightness of neck seals, this
may introduce some additional variability in the measurements, and heighten baseline values.

The use of the restraint system used for RWBP may also confound measurements of sRaw by depressing lung volumes (FRC, TLC). Although the position of the rear plate was adjusted according to the size of the mouse, lung volumes may be reduced in this position. Further study employing imaging (e.g. CT) hold promise to explore the effects of tube restraint.

Strain specific airway responsiveness

The C57 mice exhibited significantly higher breathing frequency, minute volume, and peak flows than A/J or BALBc mice, and higher tidal volumes than A/J mice. The C57 strain was previously found to have higher minute ventilation than BALBc (20), and this was thought to reflect their greater basal metabolism, body temperature, and lower hematocrit. Breathing frequency, \( V_T \), and \( V_E \) were slightly higher in this study than several past studies employing double chamber plethysmography (DCP) (18, 20), than head-out plethysmography (17, 21, 55), or unrestrained whole body plethysmography (48), although Delorme et al (12) using DCP and Hamelmann and coworkers (27) using unrestrained plethysmography found similar values for frequency. We speculate that the higher ventilatory rates in this study relate to the presence of excessive dead space (0.3 mL), which cause the mice to compensate appropriately. This did not appear to complicate the process of data collection or cause harm to the mice. However, the presence of hyperpnea may have contributed to differences in Mch delivery or response to Mch due to altered lung volumes.
Airway responsiveness in the 3 strains of mice differed significantly (Table 3). Concerning our gold standard (FOT), Mch responsiveness paralleled past data on this subject using BALBc mice (54). The descending order of airway responsiveness differed between RWBP (A/J>BALBc>C57) and FOT (A/J>BALBc=C57) largely due to relatively lower responsiveness of conscious C57 mice. The relative airway responsiveness found in this study using RWBP (A/J>BALBc>C57) mirrored past studies that have employed invasive technologies in 2 or 3 of the same strains (11, 16, 52) as well as published data on airway reactivity (using UWBP) from the vendor for C57 and A/J mice (46). One study that compared double chamber (sRaw) to single chamber (Penh) measures of airway responsiveness disclosed rank orders BALBc>A/J>C57 for DCP, and A/J>BALBc>C57 as in our study for UWBP (12). The inability to characterize A/J mice as hyper-responsive, and the poor reproducibility of airway responsiveness indices (e.g. PCR1200) were cited as major weaknesses of DCP by that study. In another study using UWBP (58), the order was AJ>BALBc=C57 as seen in our invasive measurements. Hence, there are inconsistencies in the literature with regard to the airway responsiveness of BALBc vs. C57 mice, but most studies show that A/J mice are considerably more responsive to Mch than other strains prior to any sensitization or exposure to allergen. We do not have an explanation for the differences between conscious and invasive measures of airway responsiveness in our study. It is possible that conscious measurements of airway responsiveness in C57 mice, which are typically hypo-responsive, were influenced by their uniquely exaggerated minute ventilation, or another conscious factor that altered drug delivery.
To further compare RWBP to DCP and FOT for measurement of airway responsiveness, we employed all three techniques in a single strain (BALBc) of mice. Despite different modes of delivery and concentrations of Mch employed to construct a dose-response curve, the three methods produced equivalent ED175. Rather than imply that these methods are equally sensitive, the data would suggest that Mch delivery methods and doses have been adjusted over time by investigators to obtain a dose-response curve in non-sensitized BALBc mice between 0 and 30 mg/mL. If we had employed the same method of Mch delivery (e.g. intravenous route) for the same time, we might have obtained different results. For example, it would be expected that the delivery of Mch for 60 sec into the lower respiratory tract of intubated mice would likely have produced greater responses, and therefore our conclusion concerning airway responsiveness as a function of technique would have been different (i.e. FOT would have detected greater airway responsiveness). Because intravenous responses to Mch are poorer than aerosolized responses, we chose the aerosol route.

Furthermore, it is impossible to determine the contribution of the nose or glottis versus lower airways in the response to Mch. This could be a significant limitation to these methods during pharmacologic testing. The higher percentage responses in the conscious animals (RWBP, DCP) vs. anesthetized intubated mice (FOT) (Fig 5) may have resulted from upper airway constriction, either by direct stimulation of cholinergic receptors in the nasal mucosa (34, 45) or cholinergic reflexes involving the glottis, nasal cavities or other structures following lower airway deposition of Mch (13, 14) as observed previously in rats. In one study in rats (14), frequent glottic closure was noted, and this explained the observed marked increase in expiratory airway resistance with lower
airway deposition of Mch. We too noted frequency glottic closures (periods of zero flow at the initiation of expiration) but breaths with glottic closures, while excluded for analysis in this study, could be analyzed on the basis of the tangent immediately after glottic opening. Thus it is tempting to speculate that RWBP permitted a qualitative analysis of the ‘source’ of expiratory resistance, and therefore exclusion of resistance caused purely by glottic closure. Further studies are warranted to disclose the contribution of the upper airways to the responses measured using RWBP, particularly in mice where there is a paucity of such data.

Allergen responses

The effect of OVA exposure by aerosol in sensitized mice did not evoke a change in baseline sRaw (Pre-OVA: 0.711±0.038, 5 weeks: 0.66±0.02; 10 weeks: 0.785±0.084 cm*sec). Similarly, no effect was seen in baseline airway or tissue mechanics in a past study using a similar protocol comparing OVA-/OVA- to OVA+/OVA+ BALBc mice (54). Using RWBP there was a significant decrease in ED175 at 5 and 10 weeks, demonstrating that it was feasible to track significant changes in airway responsiveness longitudinally. The site of antigen effects was not established in this study. While OVA challenge in sensitized mice is thought to induce airway hyper-responsiveness primarily due to lower airway inflammation and remodeling, similar responses to antigen have been found in the nasal passages of mice (14, 29). Further studies are warranted to relate structural to functional responses to allergen challenge. However, it is evident from our data that that RWBP offers an opportunity to perform longitudinal studies of chronic airway disease.
Conclusions

This study demonstrates for the first time the measurement of specific airway resistance (sRaw) using restrained whole body plethysmography (RWBP) in conscious mice. The non-invasive method of RWBP permitted highly reproducible measurements of sRaw, without acclimation to the instrument. Values for sRaw were very similar to those derived from the invasive forced oscillation technique. Strain specific and allergen-induced effects on airway reactivity were demonstrated using RWBP. The technique of RWBP holds promise for acute or longitudinal studies of airway disease in conscious mice.

Acknowledgments

We would like to thank Drs. Daniela Bedenice, Elizabeth Rozanski, Rose Nolen-Walston, Nate Derr, and Larry Tsai for their support and insight. We also gratefully acknowledge the diligent technical assistance of Kellie Cascanet, and Cathryn Bedard. Dr. Louis Maranda generously provided statistical support.
References


Table 1. Effects of heating the plethysmograph to body temperature (37°C) on flow derived parameters, amplitude of box pressure signal, and sRaw. Additionally shown are the effects of removing the mice from the restrainer and placing them in the open plethysmograph on respiratory frequency and box pressure. Box pressure (Vbox, in mL) and flow (mL/sec) were recalibrated before each perturbation. Shown are mean and SD; NS = not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>units</th>
<th>Unheated Restrained (n=5)</th>
<th>Heated Restrained (n=5)</th>
<th>P</th>
<th>Unheated Restrained (n=10)</th>
<th>Unheated Unrestrained (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>bpm</td>
<td>298 (20.1)</td>
<td>316 (33.4)</td>
<td>0.08</td>
<td>304</td>
<td>367</td>
<td>0.001</td>
</tr>
<tr>
<td>VT</td>
<td>mL</td>
<td>0.304 (0.025)</td>
<td>0.352 (0.018)</td>
<td>0.03</td>
<td>27</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>VE</td>
<td>mL/min</td>
<td>91 (6.7)</td>
<td>109 (10.8)</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIF</td>
<td>mL/sec</td>
<td>4.03 (0.315)</td>
<td>4.75 (0.53)</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEF</td>
<td>mL/sec</td>
<td>4.46 (0.23)</td>
<td>4.78 (0.72)</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sRaw</td>
<td>cm*sec</td>
<td>0.576 (0.112)</td>
<td>0.592 (0.089)</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δVbox</td>
<td>mL</td>
<td>0.0098 (0.0019)</td>
<td>0.0089 (0.0012)</td>
<td>NS</td>
<td>0.0135</td>
<td>0.0278</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 2. Specific airway resistance (sRaw) measured repeatedly in a group of conscious C57 mice (n=12) using restrained whole body plethysmography (RWBP).

<table>
<thead>
<tr>
<th>Time</th>
<th>0 min</th>
<th>10 min</th>
<th>30 min</th>
<th>45 min</th>
<th>Day 1 AM</th>
<th>Day 1 PM</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean—sRaw (cmH₂0*s)</td>
<td>0.573</td>
<td>0.609</td>
<td>0.544</td>
<td>0.564</td>
<td>0.619</td>
<td>0.556</td>
<td>0.602</td>
<td>0.645</td>
</tr>
<tr>
<td>SD—sRaw (cmH₂0*s)</td>
<td>0.101</td>
<td>0.100</td>
<td>0.132</td>
<td>0.128</td>
<td>0.095</td>
<td>0.127</td>
<td>0.088</td>
<td>0.052</td>
</tr>
<tr>
<td>Coefficient of Variation (%)*</td>
<td>17.6</td>
<td>16.5</td>
<td>24.3</td>
<td>22.7</td>
<td>15.4</td>
<td>22.8</td>
<td>14.6</td>
<td>8.1</td>
</tr>
<tr>
<td>95% C.I. cmH₂0*s</td>
<td>0.057</td>
<td>0.057</td>
<td>0.075</td>
<td>0.072</td>
<td>0.054</td>
<td>0.072</td>
<td>0.05</td>
<td>0.030</td>
</tr>
</tbody>
</table>

\*Within-group (within time period).
Table 3: Baseline measurements of conscious and invasive physiology by strain of mice. Values shown were derived from C57 (n=18), A/J (n=18), and BALBc (n=18) mice. Methods used were restrained whole body plethysmography (RWBP) and the forced oscillation technique (FOT).

<table>
<thead>
<tr>
<th>Strain</th>
<th>sRaw</th>
<th>f</th>
<th>VT</th>
<th>VE</th>
<th>PEF</th>
<th>PIF</th>
<th>Ti</th>
<th>Te</th>
<th>Raw</th>
<th>Gti</th>
<th>Hti</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>0.509 ± 0.033</td>
<td>357.7</td>
<td>0.281</td>
<td>100.3</td>
<td>4.90</td>
<td>4.36</td>
<td>0.089</td>
<td>0.082</td>
<td>0.35</td>
<td>5.28</td>
<td>28.84</td>
</tr>
<tr>
<td>A/J</td>
<td>0.676a ± 0.027</td>
<td>277.9a</td>
<td>0.245a</td>
<td>67.7a</td>
<td>3.27a</td>
<td>2.92a</td>
<td>0.109a</td>
<td>0.108a</td>
<td>0.35</td>
<td>4.95</td>
<td>21.94a</td>
</tr>
<tr>
<td>BALBc</td>
<td>0.633a ± 0.045</td>
<td>327</td>
<td>0.210a</td>
<td>67.1a</td>
<td>3.25a</td>
<td>3.04a</td>
<td>0.0964</td>
<td>0.109a</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

Values: Mean ± SEM.

Key to abbreviations: sRaw, specific airway resistance, cmH2O*s; f, respiratory rate in breaths/min; VT, tidal volume in ml; VE, minute ventilation in ml/min; PEF, peak expiratory flow in ml/s; PIF, peak inspiratory flow in ml/s; Ti, duration of inspiration in sec; Te, duration of expiration in sec; Raw, resistance of airways in cm H2O/mL/sec; Gti, tissue resistance in cmH2O*ml-1; Hti, elastance in cmH2O*ml-1

a Significant difference from C57 mice (P < 0.05).
Table 4. Measures of airway responsiveness in three strains of mice. Airway responsiveness was measured using forced oscillation technique (Raw), restrained whole body plethysmography (sRaw-RWBP), and double chamber plethysmography (sRaw-DCP). The provocative concentration that caused an increase in Raw or sRaw to 175% baseline (ED175) is expressed as the mean and 95% confidence interval.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Raw ED175 (mg/mL)</th>
<th>sRaw-RWBP ED175 (mg/mL)</th>
<th>sRaw-DCP ED175 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>18</td>
<td>8.3</td>
<td>40.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.5-10.2)</td>
<td>(26.9-54.2)</td>
<td></td>
</tr>
<tr>
<td>AJ</td>
<td>18</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.49&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.5-3.1)</td>
<td>(3.1-5.9)</td>
<td></td>
</tr>
<tr>
<td>BALBc</td>
<td>18</td>
<td></td>
<td>10.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>(OVA-/OVA-)</td>
<td></td>
<td></td>
<td>(7.8-13.9)</td>
<td></td>
</tr>
<tr>
<td>BALBc</td>
<td>9</td>
<td>9.48</td>
<td>10.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(OVA+/OVA-)</td>
<td></td>
<td>(6.2-12.8)</td>
<td>(7.3-13.1)</td>
<td>(4.9-9.3)</td>
</tr>
</tbody>
</table>

**Key:** Raw– airway resistance measured using FOT; sRaw-RWBP: specific airway resistance measured with RWBP; sRaw-DCP: specific airway resistance measured using DCP; ED175- provocative concentration that evoked an increase in Raw or sRaw to 175% post-saline value; OVA- = no sensitization or exposure to OVA; OVA+/OVA- = sensitization intraperitoneally without aerosol exposure.;

<sup>a</sup> Significantly different from C57 mice (P < 0.01).

<sup>b</sup> Significantly different from ED175 for Raw within strain (P < 0.01).
Fig 1. Schematic of restrained whole body plethysmography (RWBP). The RWBP consisted of a clear Lucite restrainer and plethysmographic chambers with assorted ports for bias flow, calibration, and leak testing. Mice entered the rear of the mouse restrainer and were then pushed forward using the adjustable backplate until the nose engaged the inner surface of the cuff (see Methods for leak testing data). The nose-cone was attached to a heated fiber screen pneumotachograph. The lid was closed and the box periodically vented during the first 30-60 sec of data collection to counteract thermal drift if necessary. Bias flow (0.5 L/min) was introduced between data recordings. The mouse and mouse restrainer were removed only for aerosol challenges.
Fig 2A. The derivation of specific airway resistance (sRaw) from primary data in a C57BL/6J mouse. Shown for each dose of methacholine are stripcharts for pneumotachograph flow and plethysmographic volume. Below each stripchart are the corresponding plots of flow (Y-axis) and box volume shift (X-axis). Units for X and Y axes are shown in the top left plot (‘post-saline’). Also shown are the average slopes obtained for each dose of Mch in this mouse. As described in the text, sRaw was computed as follows:

\[ sRaw = \frac{1}{\tan \theta} \times (PB - PH2O) \times Cf \times \left( \frac{Vpleth - bwt}{Vpleth} \right) \]

where \( \tan \theta \) is the tangent of angle shown in radians, PB is barometric pressure, PH2O is water vapor pressure, Cf is the scale factor of printout used for measurement of angle (2.0 sec), Vpleth is the total volume of plethysmograph (900 mL), and bwt is the body weight of mouse in gm.
Fig 2B. Effect of heating and humidification of the plethysmograph (RWBP) on the appearance of XY plots in a BALBc mouse. Measurements can be found in Table 1. Heating caused significant increases in VT, VE (P<0.05) and PIF (P=0.06). There was less evidence of XY looping when the box was heated, but these changes did not affect the values of sRaw.
Fig 3. Log-linear dose response curves in response to methacholine (Mch) in awake C57 (n=18, closed diamonds •), AJ (n=18, open squares □), and BALBc (n=18, open triangles △) mice. Statistical significance between C57 and A/J at equivalent doses of Mch indicated by ‘a’. 3a) Specific airway resistance (sRaw), 3b) Breathing frequency, 3c) Tidal volume.
Fig. 3b

frequency (breaths/min)

Methacholine (mg/mL)

0 (Saline) 1 10 100
Fig 3c

Tidal volume (mL)

Methacholine (mg/mL)
Fig. 4. Forced oscillation technique performed in anesthetized, tracheotomized C57 (n=18, closed diamonds •), AJ (n=18, open squares □), and BALBc (n=10, open triangles +) mice. Shown are saline (0 mg/mL Mch) followed by Mch concentrations which depended on strain. Significant difference between strains at equivalent doses is signified by ‘a’.

Methacholine (mg/mL)

Fig. 4.
Fig 5. Comparison of Mch responses between measurement methods. Conscious methods included restrained whole body plethysmography (sRaw-RWBP, n=10) and double chamber plethysmography (sRaw-DCP, n=10). Invasive method was the forced oscillation technique, which produced Raw-FOT (n=9). Significant difference (P<0.001) between RWBP or DCP versus FOT is signified by the letter ‘a’
Fig 6. Longitudinal measurements of airway responsiveness (ED175) in immunized BALBc mice (n=9) before exposure to OVA aerosol and 5 and 10 weeks after OVA aerosol exposures three times per week (OVA+/OVA+). Compared to baseline, there was a significant (P<0.001) reduction in ED175 at 5 and 10 weeks.