Use of the Allay™Restraint Collar to Facilitate the Measurement of Ventilation in Conscious Rats

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Introduction

Buxco Research Systems has several products that involve rodent restraint. These include 1) nose-only and head-out plethysmographs for the measurement of ventilation in conscious animals 2) double-chamber plethysmograph for the assessment of lung mechanics and airway reactivity and 3) nose-only inhalation towers for the delivery of inhaled drugs. Conventional methods for rodent restraint involve the use of a push bar or barrier placed at the rear of the animal (1-4) and while this method has been used for many years, rear-end restraint often causes compression of the animal, particularly in systems that require nose or head seals or precise positioning of the animal in the holding chambers. Buxco Research Systems have recently designed a new and improved restraint system for use in rodents that involves the use of Allay™ restraint collars and totally avoids the problems associated with rear-end restraint.

To assess the utility of the Allay™ restraint collar for the measurement of ventilation in rats, experiments were performed with a Buxco Research Systems nose-only plethysmograph that was adapted to accommodate the use of the Allay™ restraint collar. In these experiments, tidal volume, respiratory rate and minute volume were measured in female Wistar rats while breathing room air for 30 min per day for 3 consecutive days, while breathing room air for 60 min per day on days 1, 7, 14 and 21 of a 3 week observation period and while breathing air at different flow rates (250 and 500 ml/min) that was circulated through a nose-only inhalation tower (Buxco Research Systems). The data generated in this report was kindly provided by Aileen House and Peter Mauser from the Merck Research Laboratories (Kenilworth, NJ, USA).

Methods

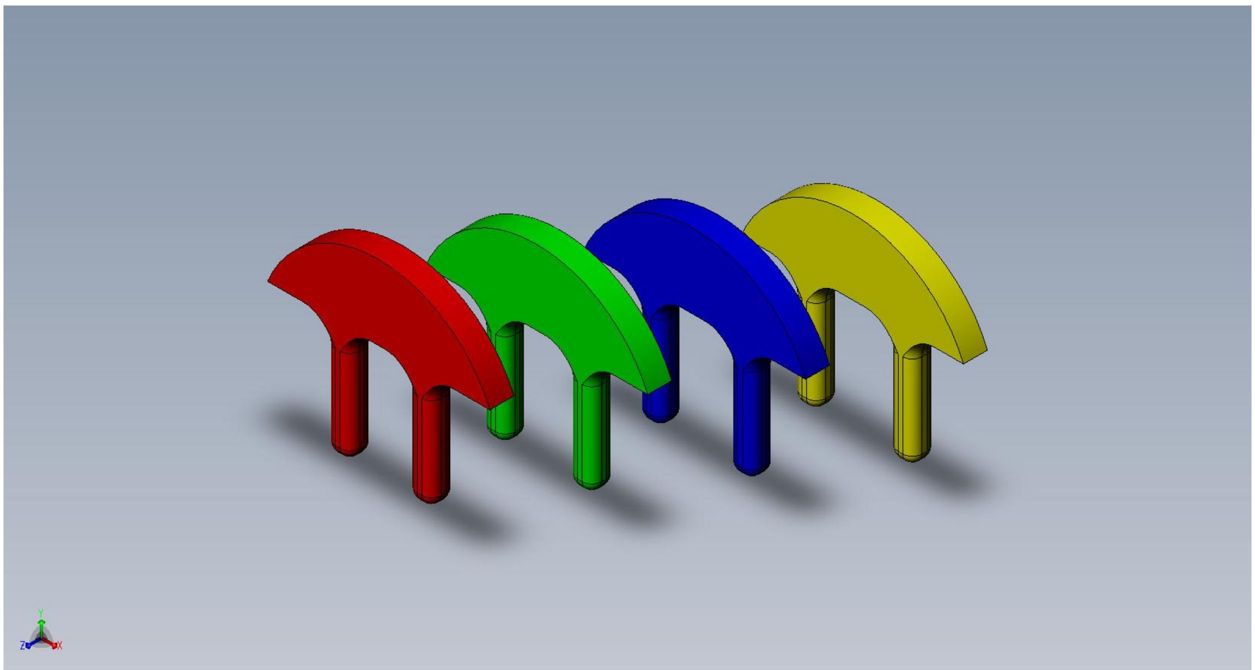
Animals

Experiments were performed on female Wistar rats ranging in weight from 175-250 g.

<u>Allay™ Restraint Collars</u>

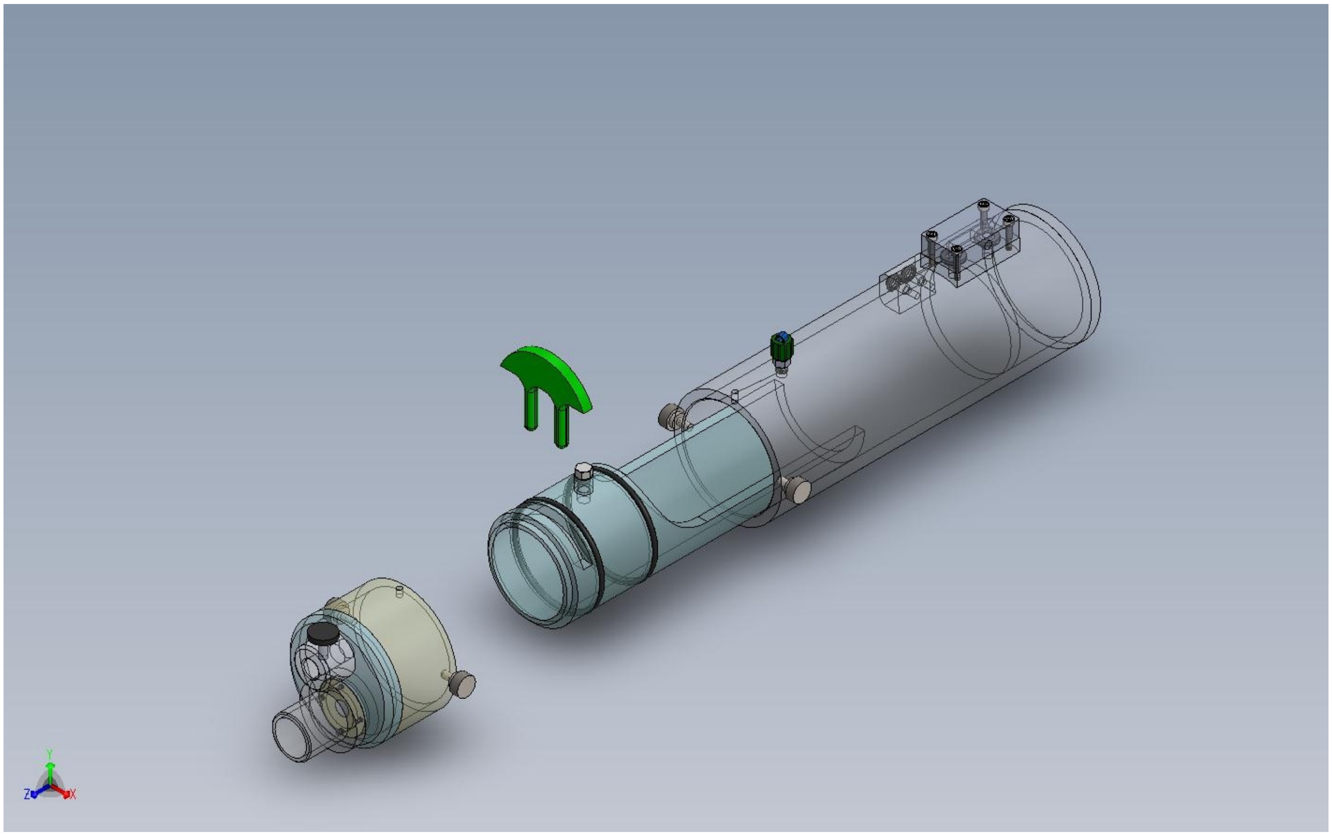
Step 1: The Allay™ restraint collars are color coded to fit different size rats (Fig 1). The appropriate size collar was selected to fit snugly over the rat's neck, just behind the ears and in front of the shoulders. The size of the collar was occasionally increased in 3 week studies as the weight and size of the rat increased over time.

Figure 1: Allay™ restraint collars for use in rats. Yellow is the largest, blue is intermediate, green is smaller and red is the smallest.



Step 2: With the front and the back of the plethysmograph open, the rat was allowed to crawl into the rear of the restraint tube. When the rat's head appeared at the front end of the tube, the Allay™ restraint collar was inserted through the slot at the top of the tube and positioned over the neck of the rat just behind the ears (Fig 2). The Allay™ restraint collar was held firm until the rat equilibrated to the restraint which usually took between 1-2 minutes.

Figure 2: Nose-only plethysmograph adapted for use with the Allay™ restraint collar.



Step 3: The nose-cap was attached to the front end of the restraint tube until the O-rings made a seal. Care was taken to ensure that the nose of the rat passed through the latex nose-seal to a position just beyond the whiskers (Fig 2). The rear bell of the plethysmograph was then added until the O-rings made a seal. The rats were allowed to equilibrate to the restraint for 15 min before measurements were obtained.

Measurement of Ventilation

Calibration and set-up of the rat nose-only plethysmograph was performed according to the directions given in the Buxco Research Systems operating manual. Airflow was measured as the pressure drop across a Halcyon™ wire mesh screen pneumotachograph placed in the wall of the plethysmograph using a differential pressure transducer (TRD5700) and volume signals were derived by integration of this airflow signal. Volume calibrations were performed before each experiment with a 3 ml syringe. With the rat in the plethysmograph, tidal volume (Vt in ml), respiratory rate (f in breaths/min) and minute volume (MV in ml/min) were measured for each breath and the average of this data displayed every 2 min. All data was generated using Buxco's FinePointe™ software.

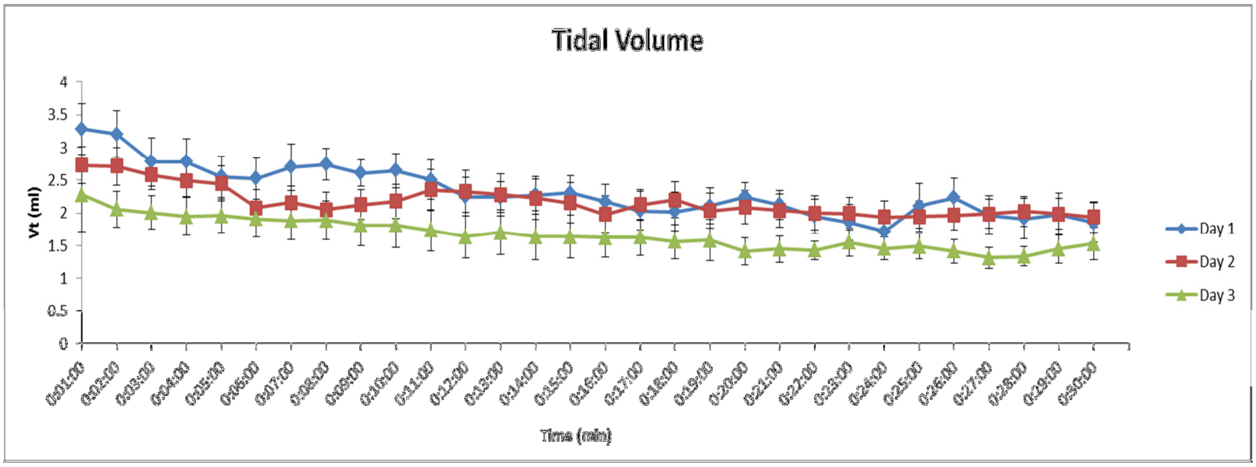
Three different experiments were performed to assess the quality of the ventilation data with this system: 1) ventilation measured for 30 min per day on 3 consecutive days while breathing room air. 2) ventilation measured for 60 min per day on days 1, 7, 14 and 21 while breathing room air and 3) ventilation measured over a 60 min period while breathing air from a nose-only inhalation tower at 2 different flow rates of 250 and 500 ml/min. These flow rates are approximately 1.5 X and 2.5 X the average MV of a 200 g rat, respectively. The set-up and calibration of the nose-only inhalation tower was performed according to the directions given in Buxco Research Systems operating manual.

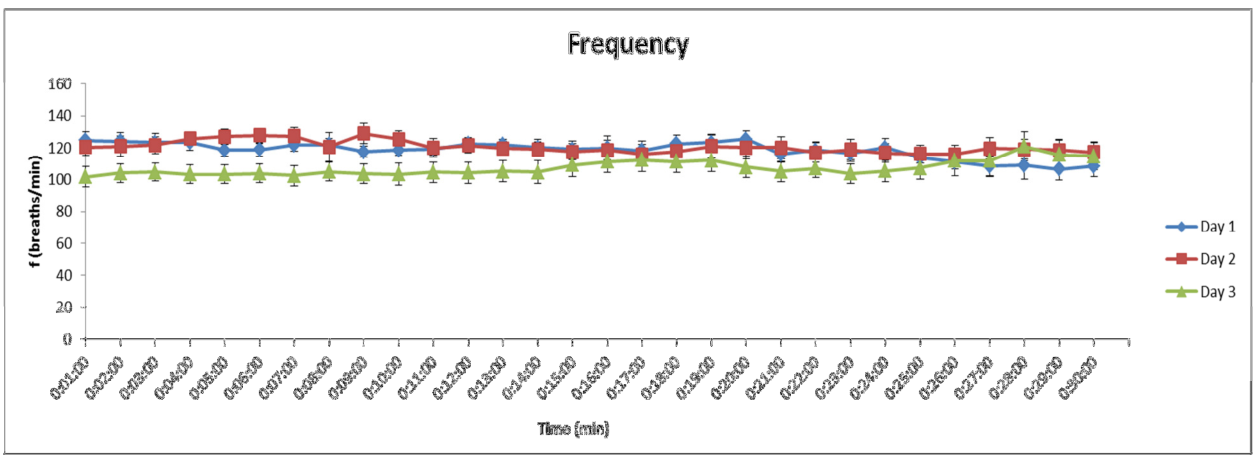
Results

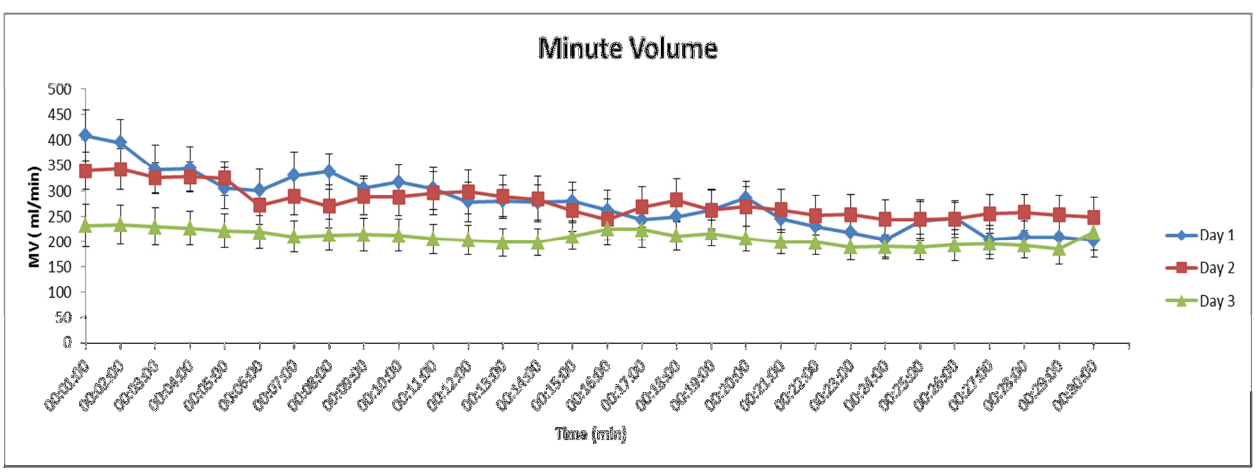
Ventilation measured on 3 consecutive days of breathing room air

The rats adapted quickly to restraint with the Allay™ restraint collar. Overall, there was excellent stability in the ventilation data over a 30 min observation period, although MV and Vt decreased slightly over time on days 1 and 2. By day 3, MV did not change over the 30 min observation period (Fig 3). Values for Vt, f and MV were virtually identical to each other at the end of the 30 min period on days 1, 2 and 3.

Figure 3: Tidal volume (Vt), respiratory rate (f) and minute volume (MV) in 8 rats breathing room air for 30 min per day on 3 consecutive days. Values are mean ± SEM.







Ventilation measured on days 1, 7, 14 and 21 while breathing room air

There was an increase in Vt and MV over the 21-day observation period with the rats breathing room air (Table 1). Respiratory rate did not change over this time period. Body weight of the rats also progressively increased over time from values of 215 ± 3 g on day 1 to 239 ± 4 g on day 21.

Table 1. Ventilation over a 21-day observation period in female Wistar rats

DAY	Vt (ml)	f (breaths/min)	MV (ml/min)
1	1.60 ± 0.10	114 ± 6	178 ± 13
7	1.56 ± 0.12	117 ± 3	183 ± 18
14	1.88 ± 0.18	113 ± 6	214 ± 25
21	2.07 ± 0.18*	118 ± 6	238 ± 17*

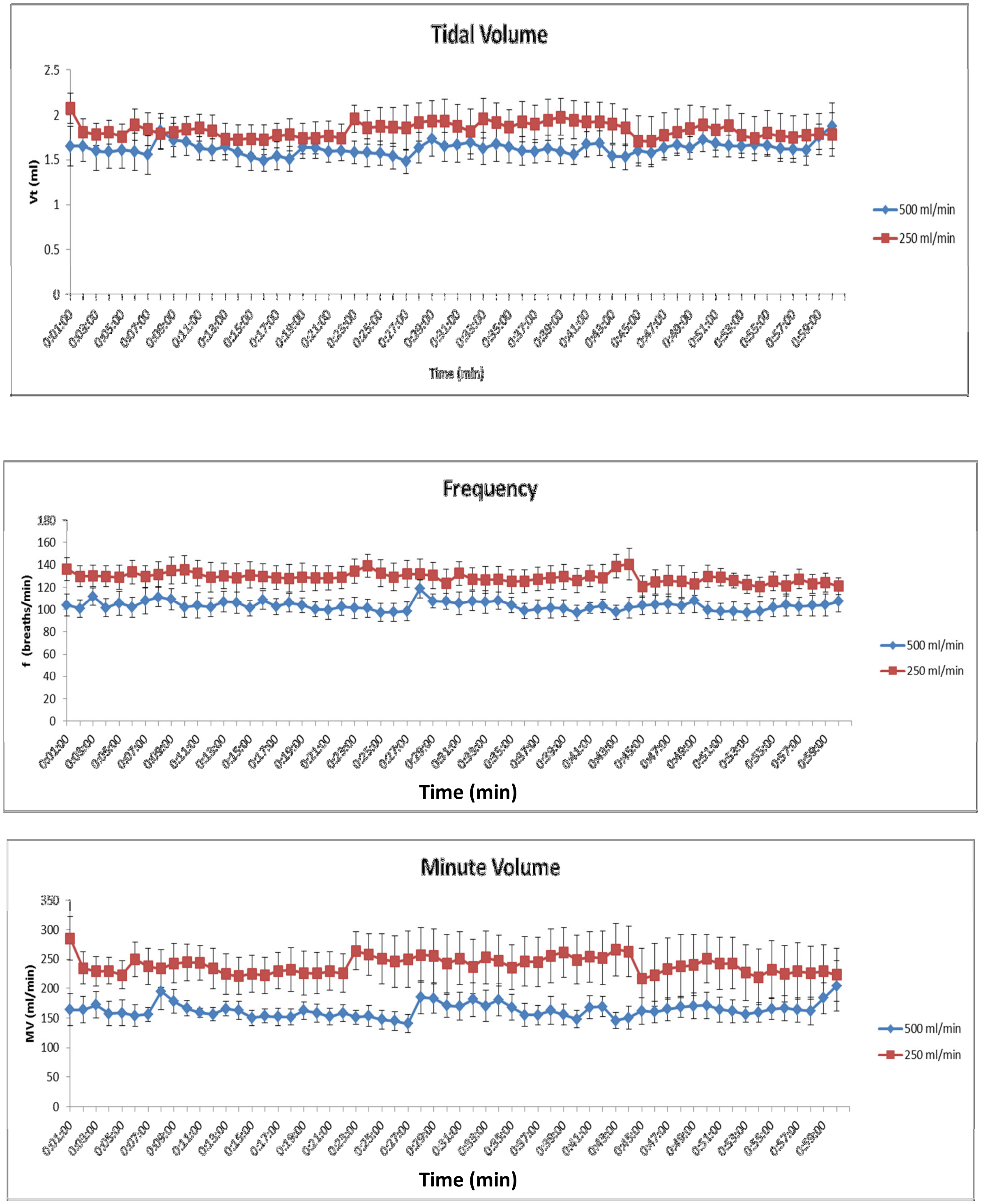
Values represent the mean ± SEM (n=11) ventilation data measured over a 60 min observation period.

*$P<0.05$ compared to Day 1 using a paired t-test.

Ventilation measured while breathing air circulated through an inhalation tower

There was also excellent stability in the ventilation data over a 60 min observation period with the rats were breathing air circulated through the inhalation tower. Values for MV, Vt and f measured at a flow rate of 500 ml/min through the inhalation tower were less than measured values at a circulated flow of 250 ml/min (Fig 4).

Figure 4: Tidal volume (Vt), respiratory rate (f) and minute volume (MV) measured over a 60 min period in rats breathing air circulated through a nose-only inhalation tower at flow rates of 250 and 500 ml/min. Values are mean ± SEM.



Discussion

A variety of techniques are available to measure ventilation in conscious rodents, many of which include the use of rodent restraint (1-4). It is essential that that the technique used to restrain the animal provide no limitation to the animal's breathing such as compression of the thorax and abdomen. Buxco Research Systems have designed a method for rodent restraint that involves the use of an Allay™ restraint collar place over the head of the animal that allows free mobility of the respiratory apparatus and can be used to restrain the animal for an extended period of time. In this report, the Allay™ restraint collar was used in conjunction with a nose-only plethysmograph to measure ventilation in female Wistar rats in a variety of experimental settings.

Allay™ Restraint Collars

Restraint of the rat with the Allay™ restraint collar was simple to perform and easily handled by a single investigator. The collars are conveniently color coordinated and fit over the neck of the rat just behind the ears and in front of the shoulders. Slots are placed at the top of the restraint tube to accommodate the Allay™ restraint collar which is placed over the neck of the animal when the animal moves to a forward position. Once the rat has accommodated to the Allay™ restraint collar, the nose-cap is placed over the head of the rat ensuring that the latex seal snugly fits over the nose beyond the whiskers. The rats adapted quickly to this restraint and quality data was obtained within a matter of minutes. Training studies were performed in a cohort of rats in which ventilation was measured for 30 min per day on 3 consecutive days. There was excellent reproducibility of this ventilation data between the three trials at the end of the 30 min period. MV and Vt did fall slightly over the 30 min period on days 1 and 2 and probably reflects adaptation of the rats to restraint in the plethysmograph. As is usually the case with most restraint systems, a training period is recommended to yield the best quality data and minimize stress-related factors from influencing the overall response. Narciso et al (2003) recommend an acclimatization period of at least 7 days in rats and mice restrained in nose-only inhalation holders (5). However, based upon data generated in the present study a 3-day training period appears to be adequate to yield high quality ventilation data with the Allay™ restraint collar used in conjunction with a nose-only plethysmograph.

It is important to note that the Allay™ restraint collar gives the animal free mobility of the thorax and abdomen. This is in sharp contrast to many of the rear-end restraint systems that we have used which often require the rear-end barrier or push bar be positioned so far forward that it often results in compression of the animal and limitation of their ventilatory performance. Rear-end restraint is particularly problematic in nose-out and head-out plethysmographs and double-chamber plethysmographs as precise positioning of the animal is required to ensure that the nose and head seals are not disrupted by animal movement.

Ventilation measurements

The values for respired minute volume found in the present studies are similar to values derived from algorithms based upon body weight (6, 7). In studies in which ventilation was measured over 21-days, MV progressively increased over time, most probably reflecting the increase in size of the rat. These results identify the Allay™ restraint collar/nose-only plethysmograph system is perfect for use in long-term ventilation studies. This system also generated high quality on-line ventilation data when measured from an inhalation tower. This extends the application of the Allay™ restraint collar for studies requiring the measurement of ventilation following exposure to inhaled drugs. Indeed, respiratory irritancy is an important assessment in most toxicological studies with inhaled drug substances (8-10). Minute ventilation measured at a circulated flow rate of 500 ml/min through the tower was lower than values measured at a flow rate of 250 ml/min. The most likely explanation for this result is that rebreathing of circulated air occurred at the lower flow rate. Indeed, previous studies have found that relatively high flow rates are required in inhalation towers to prevent rebreathing of exhaled air (1). Additional studies are required to determine the optimum circulated airflow rates through the inhalation tower used in the present studies.

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