Effects of prenatal nicotine exposure on the age-related ventilatory response to hypercapnia in neonatal mice

By
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I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

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Pledge

Approval by the Senior Thesis Committee:

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Table of Contents

Acknowledgements 4

Abstract 5

I. Introduction 6

II. Methods 14

a. Animals 14
b. Drug Administration 14
c. Ventilatory Measurements 15
d. Protocol 15
e. Data Analysis 17

III. Results 19

a. Mouse Data: Water Consumption and Weight 19
b. Acclimation and Baseline Ventilation 19
c. Control Mice: Breathing Frequency 20
d. Nicotine Mice: Breathing Frequency 21
e. Frequency vs. % CO_2 22
f. Control Mice: Tidal Volume 23
g. Nicotine Mice: Tidal Volume 23
h. Tidal Volume vs. % CO_2 24
i. Control Mice: Minute Ventilation 24
j. Nicotine Mice: Minute Ventilation 25
k. Minute Ventilation vs. % CO_2 25

IV. Discussion 37

V. References 48

VI. Appendix 52

Table of Figures

Figure 1. Triple risk model of SIDS 7
Figure 2. Schematic representation of pressure plethysmography 15
Figure 3. Photograph of a mouse pup (day 1) within the plethysmograph chamber 16
Figure 4. Experimental protocol 17
Figure 5. Schematic representation of data analysis 18
Figure 6. Example of a ventilatory tracing 18
Figure 7. Ventilatory tracing of apneas 26
Figure 8. Age-related frequency of control mice 27
Figure 9. Age-related frequency of mice exposed to nicotine 28
Figure 10. Frequency response for control mice on day 1 29
Figure 11. Age-related sensitivity to CO_2 assessed by the slope of *f* vs. % CO_2 30
Figure 12. Age-related tidal volume of control mice 31
Figure 13. Age-related tidal volume of mice exposed to nicotine 32
Figure 14. Age-related sensitivity to CO_2 assessed by the slope of V_T vs. % CO_2 33
Figure 15. Age-related minute ventilation of control mice 34
Figure 16. Age-related minute ventilation of mice exposed to nicotine 35
Figure 17. Age-related sensitivity to CO_2 assessed by the slope of V_E vs. % CO_2 36

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Abstract

Sudden Infant Death Syndrome (SIDS) is thought to result from an abnormality in ventilatory control that prevents an infant from regulating ventilation in the presence of an exogenous stressor during a critical period in development. Prenatal exposure to cigarette smoke, and particularly nicotine, is thought to impair development of peripheral and central chemoreceptors in the brainstem, resulting in the inability to properly regulate breathing. This study sought to determine the effects of prenatal nicotine exposure on the ventilatory response to prolonged, sustained increases in hypercapnia in neonatal mice. Ten mice pups served as controls and seven mice pups were prenatally exposed to 3 mg/kg of nicotine, which was administered to maternal mice through a drinking solution throughout gestation and the testing period. Pressure plethysmography was used to measure the ventilatory responses of the control and nicotine litters in response to 3 min exposures to 2 % step-wise increases in hypercapnia from 0 to 6 % CO_2 on postnatal days 1, 2, 3, 5, 8, and 11. Ventilatory parameters of frequency, tidal volume, and minute ventilation were analyzed and represented as functions of % CO_2 to determine the sensitivity of the mice to CO_2. The results of this study found that mice prenatally exposed to nicotine exhibited apneas and a blunted ventilatory response to hypercapnia; however, they were found to demonstrate both lower or higher sensitivities to CO_2 than controls. These results suggest that even a low dose of nicotine can elicit impairments in ventilation.

Introduction

Sudden infant death syndrome (SIDS) is one of the leading causes of post-neonatal infant death in developed countries, in addition to respiratory distress syndrome and congenital or developmental abnormalities, occurring at a rate of 1.3 per 1000 live births in the United States alone (Gibson et al., 2000; Kinney & Thach, 2009). SIDS is typically defined as the unexpected death of an infant less than one year of age whose cause of death remains unknown following a complete autopsy and review of medical history (Adgent et al., 2006). Its incidence is highest between two and six months of age, though cases have been reported up to one year after birth (Blood-Seigfried, 2008). Many factors have been proposed to induce SIDS, which led to the development of the Triple Risk Model to speculate the onset of SIDS (Figure 1) (Filiano & Kinney, 1994). The model contends that the onset of SIDS is the result of three culminating factors: an exogenous stressor, a critical period of development (1 to 6 months of age), and abnormalities in the ventilatory control centers of the brain (Filiano & Kinney, 1994). According to the model, SIDS causes death only in vulnerable infants, that is, infants who have not developed the mechanisms of cardiorespiratory control or sleep-wake arousal to maintain homeostasis in the presence of a stressor (Filiano & Kinney, 1994). Infants susceptible to SIDS are thought to elicit an ineffectual gasping response to restore regular ventilation in the presence of a stressor, such as heat loss, diminished O_2, or increased CO_2 that can result from sleeping face down, in the prone position (Kinney & Thach, 2009). Other predisposing factors can include smoke exposure in utero, diet, lifestyle, and socioeconomic status (Gibson et al., 2000; Bamford et al., 1996).

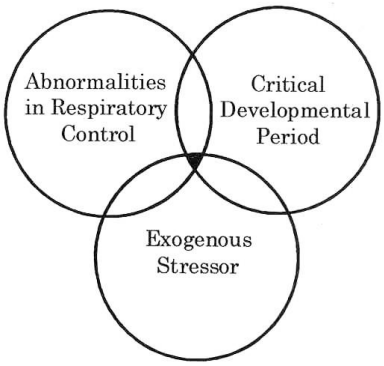


Figure 1. Triple risk model of SIDS. The overlap of the three proposed factors predisposes an infant to SIDS (derived from Filiano & Kinney, 1994).

The major risk factor for SIDS was thought to be a result of infants sleeping in the prone position, which was speculated to cause suffocation due to re-inspiration of expired gases, such as CO_2 (Kinney & Thach, 2009). The introduction of the "Back to Sleep" campaign in the United States in 1994 subsequently decreased the incidence of SIDS to 0.9 per 1000 live births in 1995 from 1.16 per 1000 live births in 1993, demonstrating a 30 % reduction (Gibson et al., 2000). Since 72 % of infants are now positioned to sleep in the supine position, on their backs, other factors have been implicated to increase the risk of inducing SIDS (Gibson et al., 2000). Socioeconomic factors are thought to contribute to the high incidence, taking into account diet, lifestyle, form of employment, and housing. One study observed that 42 % of mothers of SIDS infants in lower socioeconomic strata were reported to smoke cigarettes compared to 27 % of control mothers of surviving infants (Fleming & Blair, 2007). The risks associated with smoking during pregnancy could be associated with socioeconomic status, accounting for the variation in reports of incidence of maternal smoking ranging from 7 to 33 % (Blood-Siegfried & Rende, 2010). Low birth weight, shorter gestation periods, and brainstem abnormalities have also been implicated in the cause of SIDS; however, the cause of the syndrome remains unknown, despite knowledge of risk factors (Fleming & Blair, 2007; Adgent, 2006).

Cigarette smoke has been proposed to be one of the most significant risk factors in the onset of SIDS. There is a 2-3 fold risk associated with SIDS and exposure to cigarette smoke in utero, and 9 out of 10 observational studies investigating smoking during pregnancy observed a dose-dependent increase in risk with the number of cigarettes (Fleming & Blair, 2007; Adgent, 2006). More than 50% of SIDS mortalities in the United States are thought to be a result of smoking during pregnancy (Blood-Seigfried & Rende, 2006). The potentially detrimental effects of cigarette smoke can be attributed to the presence of over 4000 harmful compounds in the smoke, including nicotine, CO, and aldehydes, all of which can cause fetal brain damage (Blood-Siegfried & Rende, 2006). Despite the presence of numerous harmful agents, nicotine is thought to be the most significant contributor to SIDS onset because it can cross the placental and blood brain barriers to directly enter the bloodstream and cerebral spinal fluid of the fetus (Blood-Siegfried & Rende, 2006; Bamford et al., 2006). Nicotine acts by targeting nicotinic cholinergic receptors in the brain. When it binds to these receptors in infants, abnormalities in cell growth and neural development can occur (Slotkin, 1998). Nicotine also makes the fetal brain susceptible to hypoxic damage resulting from a reduction in uterine blood flow (Slotkin, 1998; Bamford et al., 1996). Damage induced by nicotine exposure is thought to disrupt fetal development, making the infant vulnerable to SIDS. Many studies have examined ventilation in response to hypoxic, diminished O_2, and hypercapnic, diminished CO_2, breathing challenges following prenatal exposure to nicotine to attempt to define the link between SIDS and cigarette smoke. Animal models have traditionally been employed for these studies because they enable manipulation of only one variable, smoking, eliminating the confounding factors of socioeconomic status (Blood-Siegfried & Shelton, 2004).

Cigarette smoking induces intrauterine hypoxia, which presents a developmental challenge for infants predisposed to SIDS and can cause decreased birth weight or retardation in brain development (Adgent, 2006). Chronic prenatal exposure to nicotine has been found to reduce the release of norepinephrine and serotonin in the brain, which can impair the infant's ability to arouse itself in response to hypoxia or hypercapnia (Adgent, 2006; Fleming & Blair, 2007). Smoking is thought to exacerbate the infant's inability to signal the brain to adjust ventilation in response to breathing challenges (Fleming & Blair, 2007). Hypoxia is an environment of less than 20 % O_2, the amount typically inspired when breathing normal air. SIDS may have implications in both hypoxic and hypercapnic environments since changes in O_2 and CO_2 are sensed in a similar manner by the body. Carotid body receptors in the carotid artery sense changes in O_2 levels while changes in CO_2 levels are mostly sense by CO_2 receptors in the brainstem. Both receptors send signals to the medulla oblongata, which subsequently initiates compensatory changes in respiration. In normal rats, a biphasic response has been observed in response to 10 min of 5 % CO_2, in which ventilation decreased from postnatal days 0 to 8 then increased to a stable ventilatory response by day 21 (Stunden et al., 2001). It has been speculated that the development of the cardiorespiratory system is completed by postnatal day 7; thus, if an abnormality in development has occurred, it is expected to elicit itself in response to a breathing challenge after day 7 (Pendlebury et al., 2008).

Nicotine has been experimentally investigated to determine its effects on respiratory development in utero. Pendlebury et al. (2008) observed that in 8 pregnant rats inhaling 10 puffs of smoke from 1 cigarette 10 times a day (20-60 ng/mL nicotine), pups elicited gasping and an inability to recover eupneic (regular) breathing within 1 to 2 min of 5 min of hypoxia (10 % O_2). Prenatal nicotine exposure in rats has typically been found to induce the loss of hypoxic

tolerance (Pendlebury et al., 2008; Slotkin et al., 1997). Blood-Seigfried & Rende (2010) proposed that the loss of hypoxic tolerance was due to a decrease in the secretion of norepinephrine and epinephrine from the adrenal medulla as a result of nicotine exposure. The reduced levels of these two neurotransmitters are thought to also affect normal cardiac function; thus hypoxic intolerance could be a result of impairment in cardiorespiratory function (Slotkin et al., 1997). In contrast, rats prenatally exposed to nicotine (6 mg/kg/day) demonstrated no difference in ventilation in response to 10 % and 15 % O_2 for 10 min on postnatal days 3, 8, 18, and 34 in comparison to control rats; consequently, nicotine exposure was not found to elicit a loss in respiratory control in response to hypoxia (Bamford et al., 1996).

In a follow-up study, Bamford & Carrol (1999) tested the effects of an even shorter duration of hypoxia, recording minute ventilation in rat pups following prenatal nicotine exposure on postnatal days 3, 8, and 18 in response to a 5 sec inhalation of 100 % O_2 and 5 % O_2 + 5 % CO_2 mixture. The nicotine-exposed rats did not demonstrate a significant reduction in ventilation in response to 100 % O_2, as was observed in control animals, and neither the control nor the experimental rats elicited a change in ventilation in response to the 5 % O_2 + 5 % CO_2 mixture; thus the effects of nicotine exposure could not be conclusively determined (Bamford & Carrol, 1999). In lieu of the previous studies, St-John & Leiter (1999) prenatally exposed rats to nicotine (6 mg/kg/day) and observed that rats demonstrated significantly lower ventilatory responses when breathing control air and hypoxic gas mixtures of 8 % O_2 and 0 % O_2 for 5 min on postnatal days 0 to 4. A biphasic response was observed in control animals in response to hypoxia, in which ventilation increased within 5 to 35 sec of exposure but subsequently declined as duration prolonged to 5 min, but the same response was not observed in nicotine-exposed rats (St-John & Leiter, 1999). Given the previous studies, nicotine cannot be conclusively identified

as a cause of SIDS since ventilatory responses of nicotine-exposed animals closely resembled those observed in control animals (Bamford et al., 1996; Bamford & Carrol, 1999; St-John & Leiter, 1999).

The effects of prenatal nicotine exposure in response to hypercapnia have also been investigated. Bamford et al. (1996) studied the ventilatory response of rats prenatally exposed to nicotine (6 mg/kg/day) in response to a 10 min exposure of 5 % CO_2 on postnatal days 3, 8, 18, and 34; no impairment in the ventilatory response of the rat was observed in comparison to controls. More recently, Eugenin et al. (2008) observed that mice prenatally exposed to nicotine (60 mg/kg/day) exhibited a decrease in ventilation in response to a 20 min exposure of 10 % CO_2 on postnatal days 0, 1, and 3 in comparison to controls. The decrease was speculated to be attributed to a decrease in chemoreception, since CO_2 chemoreceptors in the brain are thought to be underdeveloped, and therefore less sensitive to CO_2, early in development (Eugenin et al., 2008). Rats prenatally exposed to nicotine (6 mg/kg/day) were exposed to 3, 6, and 9 % CO_2 for 3 min, with a 30 minute recovery period between conditions, on postnatal days 1, 3, 6, 9, 12, and 18 (Huang et al., 2010). The ventilatory response was blunted at all levels of CO_2 exposure at all ages, with the nicotine-exposed rats eliciting greater decreases in ventilation in response to hypercapnia than the controls (Huang et al., 2010). Stunden et al. (2001), however, observed that rats exposed to 10 % CO_2 elicited gasping as a result of CO_2 toxicity. Since the results of these studies differ, it is possible that the variation in duration of exposure to hypercapnia may evoke differing results; a shorter duration would stimulate only the peripheral chemoreceptors in the carotid arteries while a longer duration would stimulate central chemoreceptors throughout the medulla (Blood-Siegfried & Rende, 2010). The culmination of results from the described studies does not provide conclusive results for the contribution of nicotine in SIDS.

Hypoxia is more commonly investigated in animal models of SIDS because of nicotine's ability to induce hypoxia in utero (Bamford et al., 1996). Fetuses, however, have a much higher affinity for oxygen than adults; thus, the fetus is likely to be able to attain an adequate amount of oxygen during hypoxia, especially if the hypoxia induced is only mild. Since postnatal exposure to hypercapnia is less studied and has elicited a variety of unclear results, further investigation of the effects of prenatal exposure of nicotine on the ventilatory responses in neonatal mice in response to hypercapnia is necessary. No studies to date have investigated the effects of hypercapnic conditions on the ventilatory responses of nicotine-exposed animals using step-wise increments of CO_2. Stunden et al. (2001) implemented a step-wise protocol in normal rats in which the rat was exposed to 1 % increments in CO_2 from 0 % to 5 % for durations of 10 min. A step-wise protocol models an environment of a sustained inspiration of hypercapnia, that could occur in sleep, and measures the animal's ability to sense increases in CO_2 (Stunden et al., 2001).

Despite the implementation of stepwise protocols, many studies have also implemented the use of osmotic mini-pumps to administer nicotine to the animals (Bamford et al., 1996; Bamford & Carrol, 1999; St-John & Leiter, 1999; Eugenin et al., 2008; Huang et al., 2010). Consumption through drinking water, however, is thought to more accurately model nicotine ingestion by humans because it is ingested as desired (Schneider et al., 2010). An osmotic mini-pump is not a very accurate model of smoking in the human because it delivers a constant rate of nicotine; it serves as a better model of the nicotine transdermal patch. With nicotine constantly present in the blood stream, the nicotinic acetylcholine receptors in the brain can become desensitized, no longer stimulating the brain when ingested (Schneider et al., 2010). Krous et al. (1981) and Schneider et al. (2010) administered nicotine to maternal rats through a solution of 0.02 mg/mL and 0.03 mg/mL, respectively, nicotine in water. Injection was not used because

injection of nicotine into a pregnant rat can result in necrosis of the brain stem tissue in the fetuses (Krous et al., 1981). Since nicotine is thought to make the drinking water less palatable, a 2 % saccharin drinking solution has been implemented to mask the taste without increasing H_2O consumption (Wang et al., 2008; Schneider et al., 2010).

Few studies have also investigated a variation in the dose of nicotine received. To model smoke exposure in utero, animal models are exposed to nicotine doses that approximate doses typically ingested by a smoking pregnant mother (Adgent, 2006). Plasma levels between 10 and 50 ng/mL nicotine model plasma concentrations of transdermal nicotine patch users and have been found to affect fetal development as they deliver 2.5 times more nicotine than typically found in the blood of smokers (Blood-Siegfried & Rende, 2010; Schneider et al., 2010; Simakajornboon et al., 2004; Bamford et al., 1996). Nicotine consumption between 6 and 8 mg/kg/day has been found to correlate to the nicotine consumption of smoking approximately 20 cigarettes a day, the equivalent of one pack of cigarettes (Krous et al., 1981; Schneider et al., 2010). Bamford et al. (1996) reported that a dose of 12 mg/kg/day of nicotine caused obvious somatic restriction and elicited no effect of nicotine on the gasping response to anoxia. Higher dosages are associated with very heavy smoking, more than 20 cigarettes per day, which is somewhat uncommon during pregnancy and has been observed to evoke death (Krous et al., 1981). Schneider at al. (2010) observed that a dose of 0.12 mg/mL (~9 mg/kg) of nicotine in drinking water evoked a significant drop in fluid consumption by rats, a decrease in weight, and eventual death of the rat. Since most smokers tend to wean themselves off of nicotine during pregnancy, moderate dosages of 3 mg/kg and 6 mg/kg most closely model a smoking mother (Fleming & Blair, 2007; Krous et al., 1981). In the present study, prenatal nicotine exposure in neonatal mice was hypothesized to cause age-related developmental impairments in ventilatory

responses to hypercapnia at 0, 2, 4, and 6 % CO_2. The nicotine-exposed mice were expected to exhibit the greatest impairment in ventilation at 6% CO_2 and at a higher dose of nicotine exposure.

Methods

Animals

Five adult female and five adult male C57BL/6J mice (Jackson Labs) were used in these experiments. Mice were housed in a temperature controlled room (21-23ºC) with a 12 hour light and 12 hour dark schedule. The mice had access to food and water *ad libitum,* and the amount of water consumed was monitored bi-weekly. Female mice were housed with their male mates throughout experimentation. Three breeding pairs were used as controls and two breeding pairs were used as experimental nicotine groups.

Drug Administration

Nicotine hydrogen tartrate (Sigma) was administered to two breeding pairs of mice through the drinking water. In a pilot study prior to administration of nicotine, daily water consumption of three female mice was determined. From the pilot experiment, an average daily consumption of ~ 8 mL H_2O per day per mouse was observed and used for calculating nicotine dosing. The average weight of a mouse was assumed to be 25 g, in which case the dose of nicotine (3 mg/kg) was multiplied by the weight of the mouse and divided by the determined daily consumption to obtain the dilution for the nicotine solution. (See Appendix B for actual dosages). To make the consumption of nicotine more palatable, sucrose was dissolved in the drinking water of nicotine and control groups to create a 2% sucrose solution (Wang et al., 2008). Two breeding pairs were administered a nicotine drinking solution (0.009375 mg/mL) at the start of breeding and throughout gestation. The nicotine solution was also administered to the

mice throughout testing to prevent the pups from experiencing withdrawal. The weight of the water was measured to monitor the amount of consumption, to prevent over-hydration if the water was appetizing to the mice and dehydration if the water was too bitter.

Ventilatory Measurements

Ventilatory measurements were recorded using pressure plethysmography (Figure 2). A known volume of air (0.1 cc) was injected into the plethysmograph with a syringe to calibrate the tidal volume (Coates & Silvis, 1999). Carbon dioxide was delivered to the mice pups by placing the pups in the plethysmograph and placing a small plastic chamber over the head (Figure 2). A CO_2 analyzer was used to record the concentration delivered and the plethysmograph recorded the changes in pressure of the apparatus (Figure 3). The ventilatory responses to CO_2 were digitized and stored on a computer for data analysis.

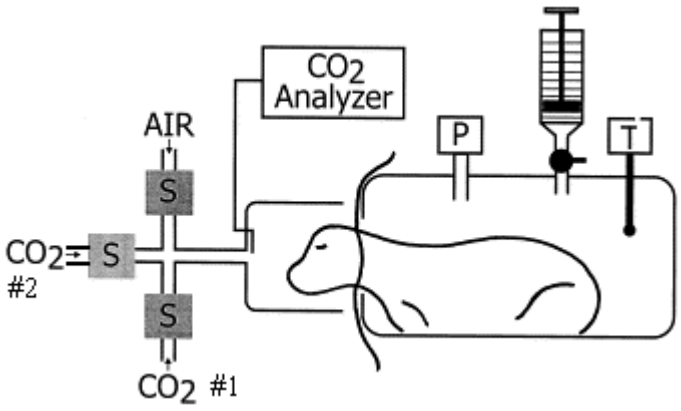


Figure 2. Schematic representation of pressure plethysmography. Adapted from Coates & Silvis (1999).Syringe calibrates tidal volume, T is the thermometer, P is the pressure transducer, and S are the solenoid switches.

Protocol

The mice pups were weighed prior to testing and tattooed using a sequence of dots on the paw for identification. To maintain a constant temperature (33 ºC) in the plethysmograph, a heating pad was placed under the chamber (Coates & Silvis, 1999). The day of birth was taken to represent postnatal day 0, and the ventilatory responses of the pups were recorded on postnatal

days 1, 2, 3, 5, 8, and 11. For testing, the mouse was placed in a plethysmograph chamber and a circular piece of rubber from a latex examination glove with a hole in it was placed around the neck of the mouse and attached to the plethysmograph with a rubber band to create an airtight seal (Figure 3) (Coates & Silvis, 1999).



Figure 3. Photograph of a mouse pup (day 1) within the plethysmograph chamber.

A chamber was then placed over the head of the mouse and connected to a solenoid controller to deliver combinations of humidified air and CO_2.

The protocol for testing ventilatory responses to different levels of CO_2 was modeled after Stunden et al. (2001). Each mouse was exposed to humidified air (control air) (0 % CO_2, 21 % O_2, and balance N_2) to determine a baseline breathing response. The mouse was exposed to control air for a period of up to 10 min to allow the mouse to acclimate to the plethysmograph to reduce the occurrence of movements in the breathing record (Figure 4). If movement artifacts were absent after five minutes of acclimation, testing was begun. To determine if the ventilatory response was due to airflow, two different solenoids were used to deliver control air and a second source of air containing 0 % CO_2 (Figure 2). The protocol for testing ventilation was the same for the control and nicotine litters. Experiments were conducted on the mice pups to

evaluate their ventilatory responses to 2 % step-wise increases in CO_2 levels from 0 %, normocapnia, to 6 %, hypercapnia, for periods of 3 min (Figure 4). The mice were not exposed to control air between conditions to allow for re-acclimation. Ventilatory responses were recorded for the same mice on each day of testing. Ten pups were randomly selected from 3 control litters and 7 pups were tested from 1 nicotine litter. After testing, the mouse was immediately returned to the mother to prevent additional stress.

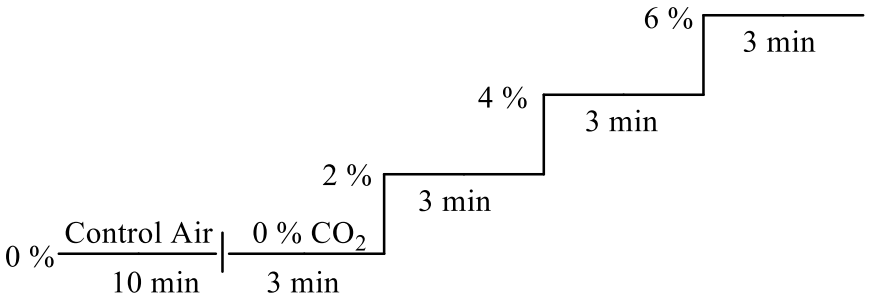


Figure 4. Experimental protocol. Mice acclimated to control air for up to 10 min. Ventilatory response was recorded for 3 min after acclimation using 0 % CO_2 to determine a baseline response. Carbon dioxide levels were increased step-wise by 2 % intervals and ventilation was recorded for 3 min at 2, 4, and 6 % CO_2 on postnatal days 1, 2, 3, 5, 8, and 11 for control and nicotine groups.

Data Analysis

Four different periods of the ventilatory responses during hypercapnic conditions were analyzed: 10 sec pre-CO_2, the first 10 sec of CO_2 exposure, the middle 10 sec of CO_2 exposure (after 90 sec of exposure), and the last 10 sec of CO_2 exposure at each concentration of CO_2 (0, 2, 4, and 6 %) on each day (Figure 5). AcqKnowledge 3.8.2 was used to record and analyze ventilation in the mice. Figure 6 depicts a typical tracing of a mouse's respiratory response to illustrate the parameters investigated. Frequency (*f*) was calculated by counting the number of breaths in a 10 sec period and multiplying by 6 to obtain a value for the number of breaths per min. To calculate tidal volume (V_T), the integral of the inspiratory response was measured over a 10 sec period and represented as the average volume/breath. It was then multiplied by a known

calibration factor and divided by the weight of the mouse to obtain average volume/breath/g. Minute ventilation (V_E) was calculated as the product of V_T and *f* and divided by the weight of the mouse to obtain mL/min/g. A repeated measures one-way ANOVA was used to analyze *f*, V_T, and V_E to compare the first 10 sec, middle 10 sec, and last 10 sec of CO_2 exposure to the 10 sec pre-CO_2. Fischer's Post-hoc Least Significant Difference test was used to determine the significance between the periods. A one-way ANCOVA was used to analyze linear regressions and determine the homogeneity between slopes (VassarStats: Website for Statistical Computation). A probability of $p < 0.05$ was considered significant.

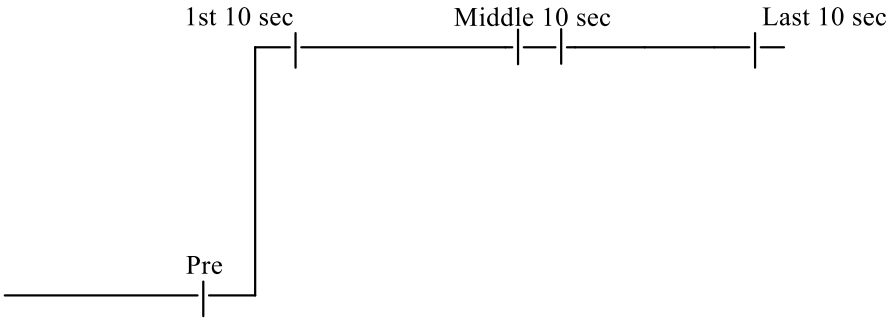


Figure 5. Schematic representation of data analysis. V_T, V_E, and f was calculated for 10 sec pre-CO_2 inspiration, first 10 sec of CO_2 inspiration, middle 10 sec of CO_2 inspiration (90 sec) and last 10 sec at 0, 2, 4, and 6 % CO_2 for each day and treatment.

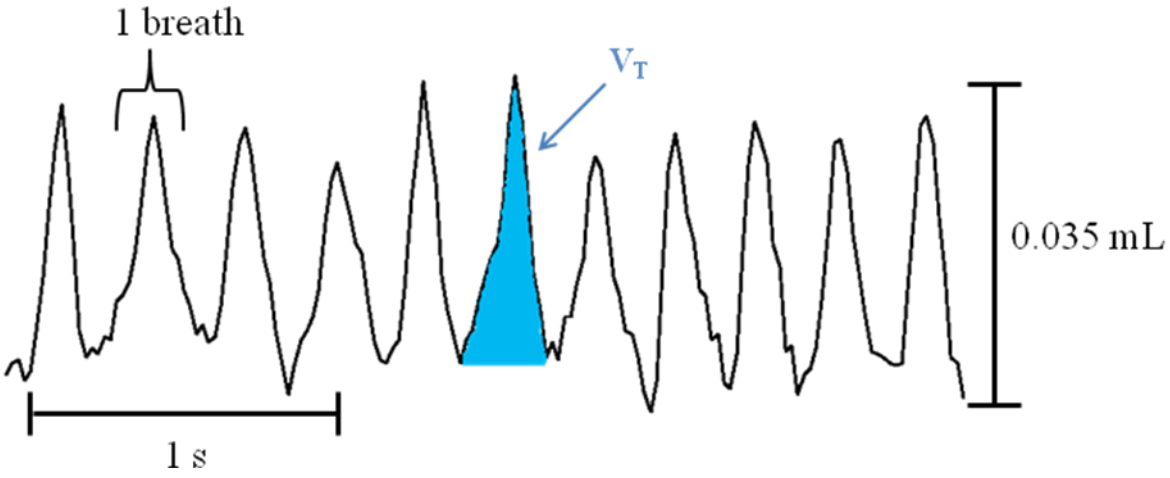


Figure 6. Example of a ventilatory tracing. The tracing is a 2 s snapshot of ventilation in a control pup in response to control air on day 5. *F* was determined by counting the number of breaths; V_T was calculated by averaging the area under the curve per breath.

Results

Mouse Data: Water Consumption and Weight

Control mice were observed to consume an average of 7.71 ± 0.31 g of water per day during gestation, which is in accordance with mean consumption of C57BL/6J mice determined from a pilot experiment (7.64 ± 3.0 g) (Appendix A and B). Mice exposed to nicotine through drinking water demonstrated an average daily water consumption of 11.01 ± 5.29 g. The nicotine exposed mice demonstrated a much greater variability in the amount of water consumed, ranging from 5.36 g to 18.19 g during gestation. The average dosing of nicotine consumed by the female mice was 3.91 ± 1.72 mg/kg/day, with actual nicotine doses ranging from 1.86 mg/kg/day to 6.44 mg/kg/day (Appendix C).

Three control breeding pairs gave birth to three litters of 10, 8, and 6 pups. Of the 10 pups randomly selected for testing, 9 survived to postnatal day 11 (90%). Two breeding pairs administered a low dose of nicotine produced only 1 litter of 7 pups, all of which survived to postnatal day 8 of testing (100%). There was no significant difference found between the mean weights of the control and nicotine mice on any day of testing, although comparisons on day 11 were not available. Both groups of mice were observed to demonstrate weight gain with age (Appendix C).

Acclimation and Baseline Ventilation

Both control and nicotine mice pups were observed to demonstrate age-related changes in the time required to acclimate to the breathing chamber. Pups required a period of approximately 10 minutes on postnatal day 1 until movements within the chamber were reduced and a period of approximately 6 minutes on day 5. Mice were considered agitated if after ten minutes, movement artifacts were still observed in the record. The most movement and agitation within the chamber was observed on day 8 for the nicotine mice pups, and days 8 and 11 for control pups. Nicotine

was not observed to increase the amount of time required for acclimation on any of the days of testing compared to controls. Nicotine mice were observed to elicit irregular breathing, in which breath size and rate fluctuated, rarely attaining a stable baseline on days 1-3. Apneas were observed in the presence of control air and 0 % CO_2 on days 1, 2 and 3 in nicotine mice but were not observed in control mice. The size of the apnea ranged from 1 sec to as long as 5 sec and occurred randomly during testing (Figure 7A & B).

Frequency (*f*) obtained during the 10 sec prior to CO_2 exposure was taken to represent baseline ventilation, once a relatively stable respiratory rate was produced and movements had ceased. The control pups were found to demonstrate the lowest baseline *f* on day 1 (104.1 ± 4.2 breaths/min) and the highest baseline *f* on day 5 (226.5 ±8.7 breaths/min), showing an upward trend in *f* to day 5 followed by a slight downward trend to day 11 (Figure 8A). Nicotine-treated pups also demonstrated an upward trend in *f*, eliciting the lowest *f* on day 1 (100.3 ± 3.4 breaths/min) and the highest *f* on day 8 (208.7 ± 8.6 breaths/min) (Figure 9A).

Control Mice: Breathing frequency (f)

Control mice pups were not observed to increase *f* in response to 0 % CO_2 on any of the days tested (Figure 8A). ANOVA revealed no significant differences from baseline *f* during the first, middle, or last 10 sec of exposure on any of the days of testing (Figure 8A). As stated previously, *f* increased to a maximum on day 5, falling off to day 11.

In response to 2 % CO_2, the mice pups were observed to increase *f* with prolonged exposure on each of the days of testing (Figure 8B). Days 1, 2 and 3 demonstrated significant increases in *f* from baseline by the middle or last 10 sec of exposure, while day 11 demonstrated increases within the first, middle, and last 10 sec of exposure. Increases were also observed on days 5 and 8, but were not found to be significant (Figure 8B).

Increases in *f* from baseline were also observed on each of the days in response to 4 % CO_2; however significant increases were only observed within the first and last 10 sec on day 2 and the first 10 sec on day 5 (Figure 8C). Differences from baseline on days 1, 2, 3, and 5 were not found to be significant.

Exposure to 6 % CO_2 revealed significant increases from baseline during the middle and last 10 sec on days 1, 2, and 3 and during the first and last 10 sec on day 5 (Figure 8D). Mice on days 8 and 11 also demonstrated increases in *f*, though they were not observed to be significant. While *f* demonstrated some variability with time and age, *f* tended to increase in response to 2, 4, and 6 % CO_2.

Nicotine Mice: Breathing Frequency

In response to 0 % CO_2, nicotine mice pups demonstrated significant declines from baseline within the first 10 sec of exposure on days 1 and 3, but significant differences in *f* were not observed on any other days (Figure 9A). As noted previously, *f* was observed to increase with age.

Mice were not observed to elicit significant differences in *f* on any of the days of testing, except on day 2, in response to 2 % CO_2 (Figure 9B). While decreases in *f* within the first 10 sec were observed on days 1, 2, 3 and 8, no significance from baseline was found since variability was high. Frequency was typically observed to increase over time, but days 5 and 8 demonstrated decreases with time (Figure 9B).

In contrast to control mice, nicotine mice did not demonstrate significant differences in *f* in response to 4 % CO_2 on any of the days of testing (Figure 9C). No obvious trends could be detected in response to exposure as both increases and decreases in *f* were observed with time and age (Figure 9C).

Exposure to 6 % CO_2 elicited significant increases in *f* in nicotine mice on days 1 and 2 while a significant decrease in *f* was observed on day 5 (Figure 9D). Much like controls, increases from baseline were typically observed with time on days 1, 2, 3, and 8, but on day 5 nicotine mice demonstrated decreases with time, reaching significance by the last 10 sec of exposure (Figure 9D).

Frequency vs. % CO_2

Overall, nicotine pups were observed to elicit fewer significant differences in *f* with age than control pups in response to increases in CO_2 (Figure 9A-D; Figure 8A-D). To better illustrate the responsiveness and sensitivity to CO_2 of the control and nicotine mice, *f* within the last 10 sec of exposure at each level of CO_2 was plotted for both groups on each day. Linear increases were typically observed in response to increases in CO_2, and a linear regression generated a slope of the line for *f* vs. % CO_2 which served as an indicator of sensitivity to CO_2. Figure 10 depicts the linear increase in *f* to increasing concentrations of CO_2 in control mice on day 1.

The slope of *f* vs. % CO_2 as a function of age was found to be variable in control and nicotine mice as mice demonstrated both positive and negative linear trends in sensitivity (Figure 11A & B). High amounts of variability were observed during early development with days 3 and 5 demonstrating the most variability, but variability was found to decrease in later development in control mice (Figure 11A). Nicotine mice demonstrated a high amount of variability throughout development (Figure 11B). Linear decreases in sensitivity to CO_2 with age were observed in 7 of the control mice with the other 3 mice demonstrating increases, whereas all 7 seven of the mice exposed to nicotine demonstrated decreases in sensitivity with age (Figure 11A & B). As a result, mean sensitivity to CO_2 was demonstrated to decrease with age in control (m =

-0.25 breaths/min/%CO_2) and nicotine mice (m = -1.41 breaths/min/%CO_2); however, control mice demonstrated positive sensitivities at all ages while nicotine mice demonstrated positive sensitivities only on days 1-3 (Figure 11C). A more linear decline in sensitivity was observed in nicotine mice ($r^2 = 0.79$) than in control mice ($r^2 = 0.41$) since a somewhat biphasic trend was observed in controls in which sensitivity increased initially, decreased, and then somewhat stabilized by day 11 (Figure 11C). A one-way ANCOVA revealed that the difference in mean slopes of the CO_2 sensitivity functions were significant ($F_{crit\ (1,7)} = 5.59$, $p = 0.022$).

Control Mice: Tidal Volume (V_T)

Tidal volume (V_T) was observed to vary with age and time of exposure to all concentrations of CO_2. Mice pups were observed to demonstrate the lowest V_T on days 5, 8, and 11 at each level of CO_2 exposure (Figure 12A-D). Significant differences from baseline were observed within the first 10 sec of exposure to 0 % CO_2 on days 1 and 11 (Figure 12A). Overall, an increase in V_T was observed in response to increases in CO_2 as more significant increases in V_T were observed in the middle and last 10 sec at 2, 4 and 6 % CO_2 during early and later development (days 1, 5, and 11) (Figure 12B-D). No obvious trends were revealed as both increases and decreases in response to CO_2 were observed (Figure 12A-D).

Nicotine Mice: Tidal Volume

Much like control mice, nicotine mice exhibited high variability in V_T at all ages and concentrations of CO_2. There did not appear to be an age-related trend in response to increases in CO_2 exposure in mice exposed to nicotine (Figure 13A-D). Except for the significant increase in V_T elicited on day 3 during the first 10 sec of exposure in response to 0 % O_2, no significant differences were observed in V_T with age, time, or concentration of CO_2 (Figure 13A-D).

Tidal Volume vs. % CO_2

The slope of V_T vs. % CO_2 as a function of age was found to vary amongst the individual mice of both the control and nicotine litters (Figure 14A&B). The control mice demonstrated the most variability in sensitivity to CO_2 on days 1, 2, and 3 and the least variability on days 5, 8 and 11 (Figure 14A). In contrast to sensitivity measured by *f* vs. % CO_2, the slopes of V_T vs. % CO_2 revealed that only 2 control mice demonstrated linear decreases in sensitivity while the other 7 demonstrated linear increases (Figure 14A). Four nicotine mice demonstrated linear increases in sensitivity, 1 demonstrated no change in sensitivity, and 2 demonstrated linear decreases in sensitivity; the same amount of variability was observed on each day (Figure 14B). Mean sensitivity to CO_2 in both control and nicotine mice was observed to increase with age (m_{con} = 1E-5 average volume/breath/g/%CO_2; m_{nic} = 5E-5 average volume/breath/g/%CO_2) (Figure 14C). Nicotine mice demonstrated a higher and more variable sensitivity than controls at each age. Both trends were not observed to fit a linear model well (r^2_{con} = 0.21; r^2_{nic} = 0.31). A one-way ANCOVA revealed that the mean slopes of the lines in nicotine and control mice were not statistically significant ($F_{crit(1,7)}$ = 5.59, p = 0.27) (Figure 14C).

Control Mice: Minute Ventilation (V_E)

Much like tidal volume, minute ventilation (V_E) of control mice did not exhibit an obvious trend, as it varied with time and age at each concentration of CO_2. A significant increase in V_E was observed during the first 10 sec on day 11 and during the last 10 sec on day 5 in response to 0 % and 2 % CO_2, respectively (Figure 15B & C). On days 1 and 5 significant differences were observed during the first, middle, and last 10 sec of exposure to 4 % CO_2, while significant differences were observed during the middle and last 10 sec of exposure to 6 % CO_2 on days 1, 3, and 11 (Figure 15C & D). Minute ventilation was found to fluctuate with time at all

ages, and exposure to 6 % CO_2 elicited the largest V_E responses and the most significant differences from baseline.

Nicotine Mice: Minute Ventilation

Minute ventilation in the nicotine mice was lowest in response to 0 % CO_2 and highest in response to 4 and 6 % CO_2 exposures. The only significant difference observed occurred during the first 10 sec of exposure to 0 % CO_2 on day 3 (Figure 16A). Much like V_T, no significant differences from baseline were observed in response to 2, 4, and 6 % CO_2 (Figure 16B, C & D). Although fluctuations in V_E were evident, the mice generally did not demonstrate differences from baseline V_E with age and concentration of CO_2 (Figure 16A-D).

Minute Ventilation vs. % CO_2

As the product of V_T and *f*, the slopes of V_E vs. % CO_2 were observed to elicit similar trends in sensitivity with age as those elicited by the *f* and V_T sensitivity functions. Individual control mice demonstrated the most variability in sensitivity on days 2 and 3 (Figure 17A). Five mice demonstrated linear decreases in sensitivity, 2 demonstrated no change, and 3 demonstrated decreases with age (Figure 17A). Individual nicotine mice exhibited equal amounts of variability in sensitivity with age, with 4 mice demonstrating increases, 1 demonstrating no change, and 2 demonstrating decreases (Figure 17B). Sensitivity to CO_2 in control mice was not observed to change with age or follow a linear trend (m = -6 E-5 mL/min/g/% CO_2; $r^2 = 0$) (Figure 17C). Nicotine mice were observed to exhibit more variability and greater sensitivities than controls; a slight linear increase in mean sensitivity to CO_2 with age was observed (m = 0.0048 mL/min/g/% CO_2; $r^2 = 0.43$). A slight biphasic response in sensitivity was observed in both control and nicotine mice (Figure 17C). ANCOVA revealed that there was no significant difference between slopes of V_E vs. % CO_2 in control and nicotine mice ($F_{crit(1,7)} = 5.59$, p = 0.29) (Figure 17C).

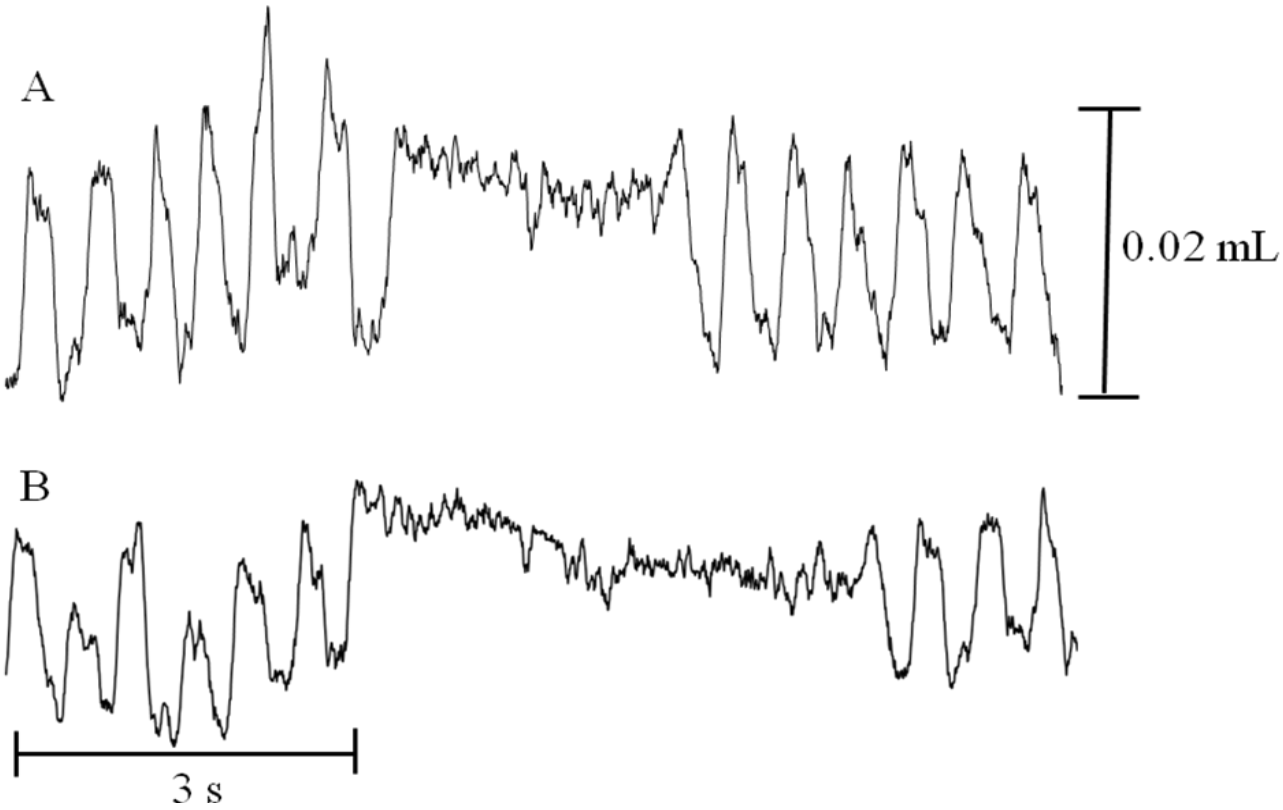


Figure 7. Ventilatory tracing of a (A) short apnea (2 sec) and (B) long apnea (5 sec) in the presence of control air. Tracing represents a 9 sec snapshot of a nicotine mouse on day 1.

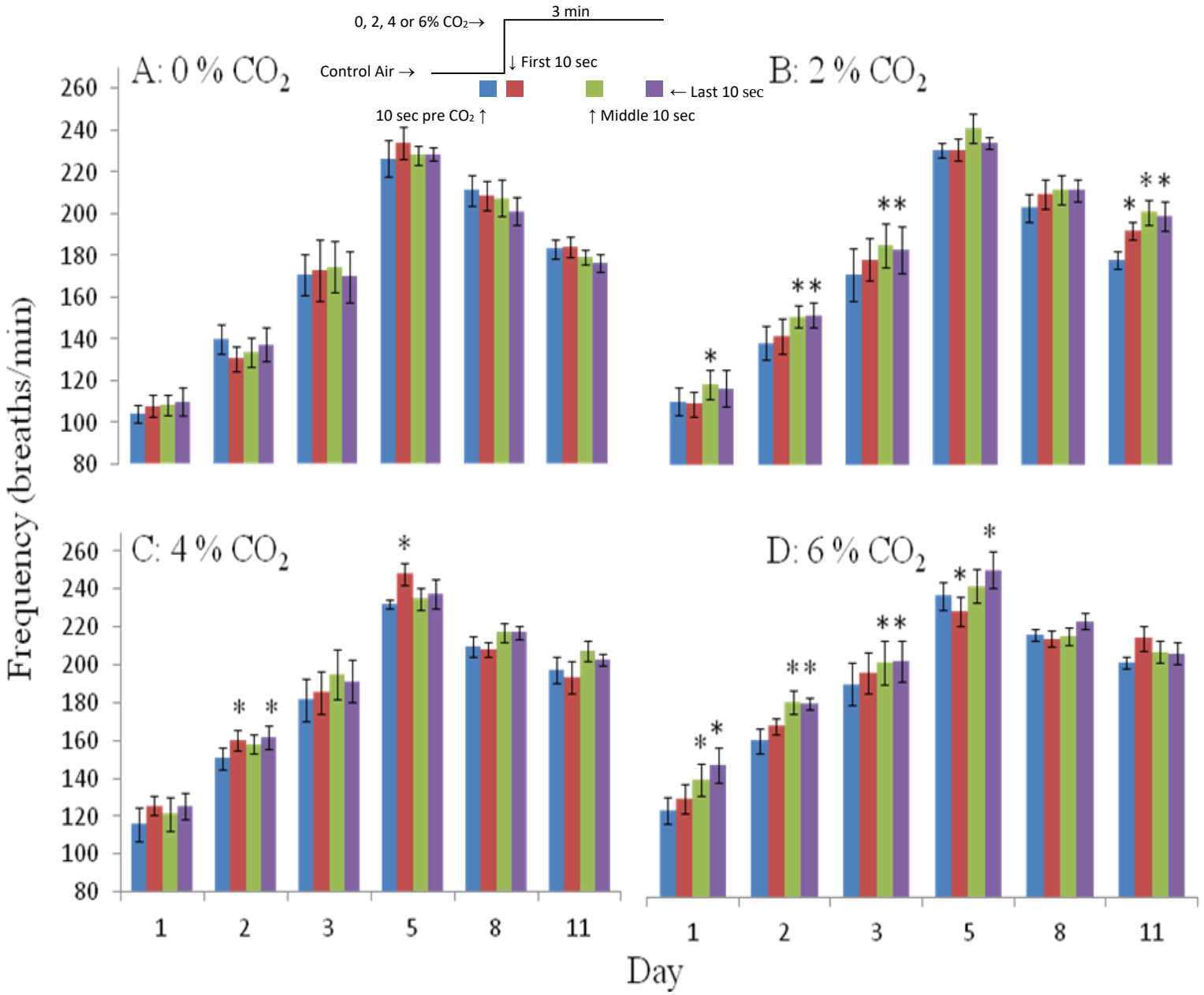


Figure 8. Age-related frequency of control mice in response to hypercapnia, (A) 0 %, (B) 2 %, (C) 4 %, and (D) 6% CO_2, on postnatal days 1, 2, 3, 5, 8, and 11. The first bar (pre) represents baseline *f* prior to CO_2 exposure; the second, third, and fourth respresent the first, middle, and last 10 sec of a 3 min exposure, respectively. N = 10 on all days except day 11, on which n = 9. Bars depict average *f*, and error bars represent SEM. Astericks indicate significance ($p < 0.05$) from baseline.

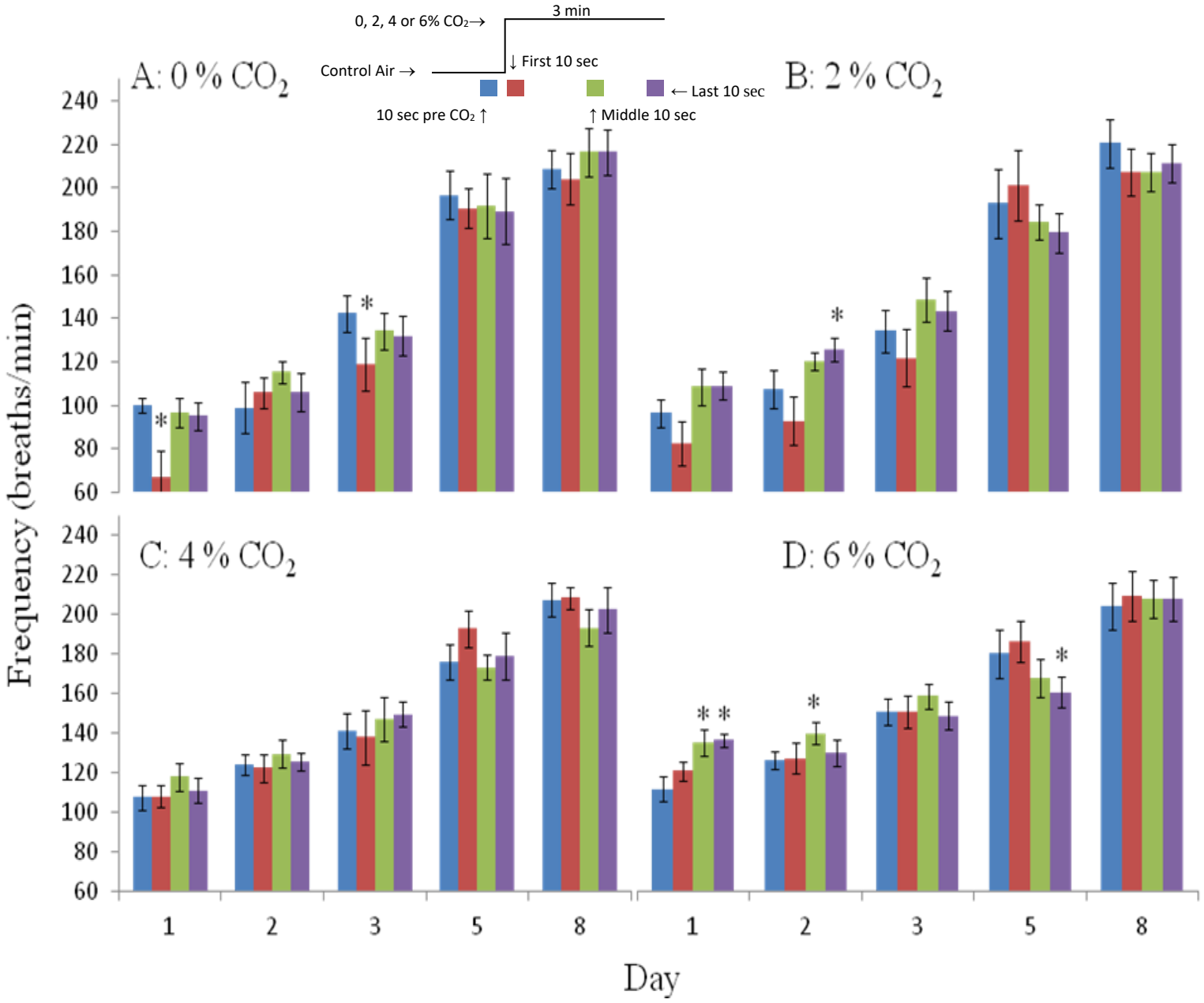


Figure 9. Age-related frequency of mice exposed to nicotine in response to hypercapnia, (A) 0%, (B) 2%, (C) 4 %, and (D) 6% CO_2, on postnatal days 1, 2, 3, 5, and 8. The first bar (pre) represents baseline *f* prior to CO_2 exposure; the second, third, and fourth respresent the first, middle, and last 10 sec of a 3 min exposure, respectively. N = 7; bars depict average *f*, and error bars represent SEM. Astericks indicate significance ($p < 0.05$) from baseline.

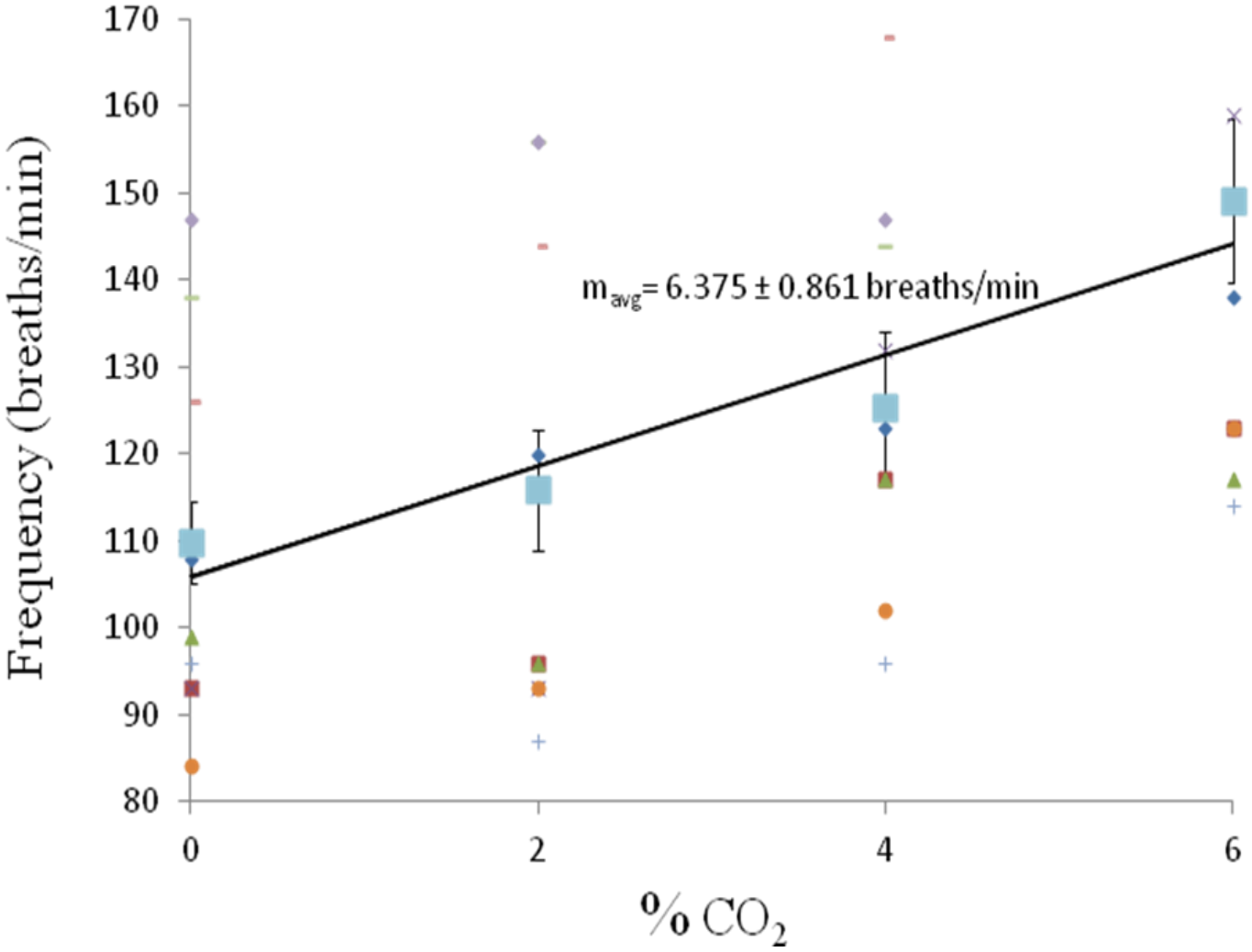


Figure 10. Frequency response for control mice on day 1 in response to 2 % step-wise increases in hypercapnia. Each shape represents an individual mouse's *f* during the last 10 sec of CO_2 exposure; mean *f* (■) demonstrated an increasing linear trend (n = 10; error bars represent SEM). Slope of a linear fit of the average response was taken to represent sensitivity to CO_2 on that day (m = 6.375 ± 0.861 breaths/min; r^2 = 0.9065).

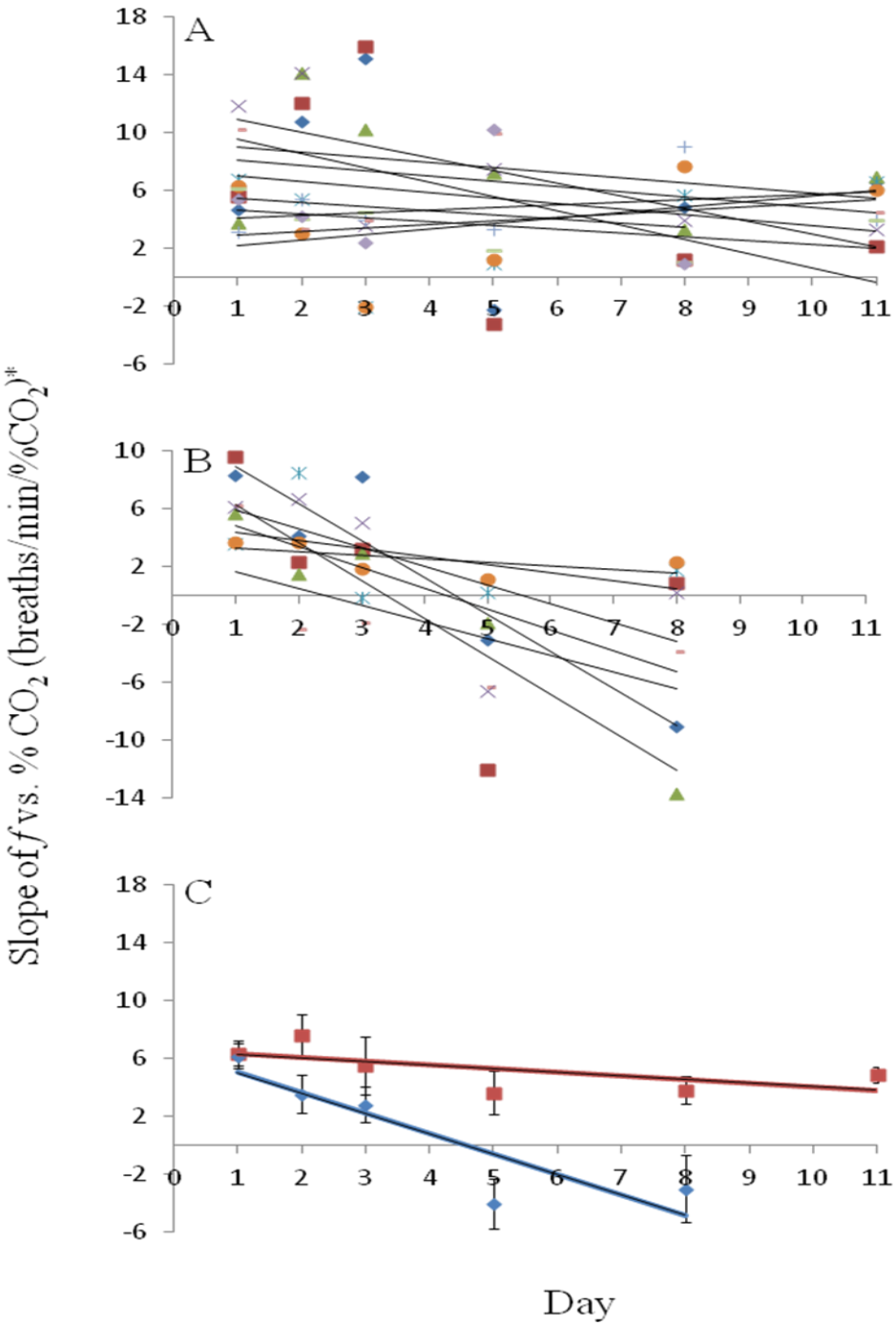


Figure 11. Age-related sensitivity to CO_2 assessed by the slope of *f* vs. % CO_2. Slopes of *f* vs. % CO_2 are represented by a linear trend with age. (A) Individual variability in sensitivity in control mice, represented by different shapes (n = 10), with age. (B) Individual variability in sensitivity in nicotine mice, represented by different shapes (n = 7), with age. (C) Linear relationship between sensitivity to CO_2 and age in control mice (blue line; m = -0.25 breaths/min/%CO_2; r^2 = 0.41) and nicotine mice (red line; m = -1.41 breaths/min/% CO_2; r^2 = 0.79). Points represent mean slope of *f* vs. % CO_2, and error bars represent SEM. Sensitivity was observed to decrease with age in controls and nicotine mice. ANCOVA revealed that the difference in slopes of the lines were significant ($F_{crit\,(1,7)}$ = 5.59, p = 0.022). * Scaling of slope of *f* vs. % CO_2 varies in A, B, and C due to large variability between control and nicotine groups.

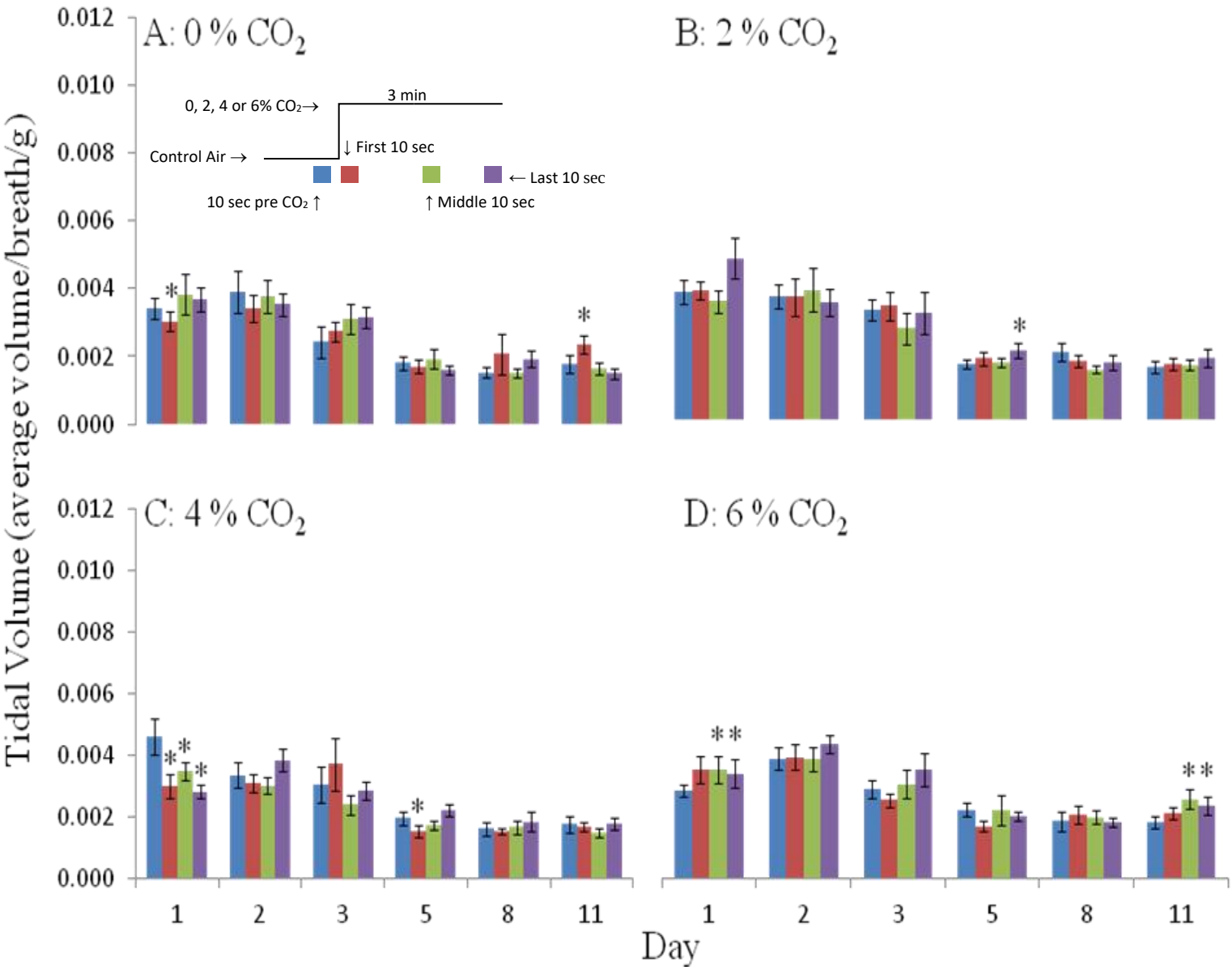


Figure 12. Age-related tidal volume of control mice in response to hypercapnia, (A) 0%, (B) 2%, (C) 4 %, and (D) 6% CO_2, on postnatal days 1, 2, 3, 5, 8, and 11. The first bar (pre) represents baseline V_T prior to CO_2 exposure; the second, third, and fourth respresent the first, middle, and last 10 sec of a 3 min exposure, respectively. N = 10 on all days except day 11, on which n = 9; bars depict average V_T, and error bars represent SEM. Astericks indicate significance ($p < 0.05$) from baseline.

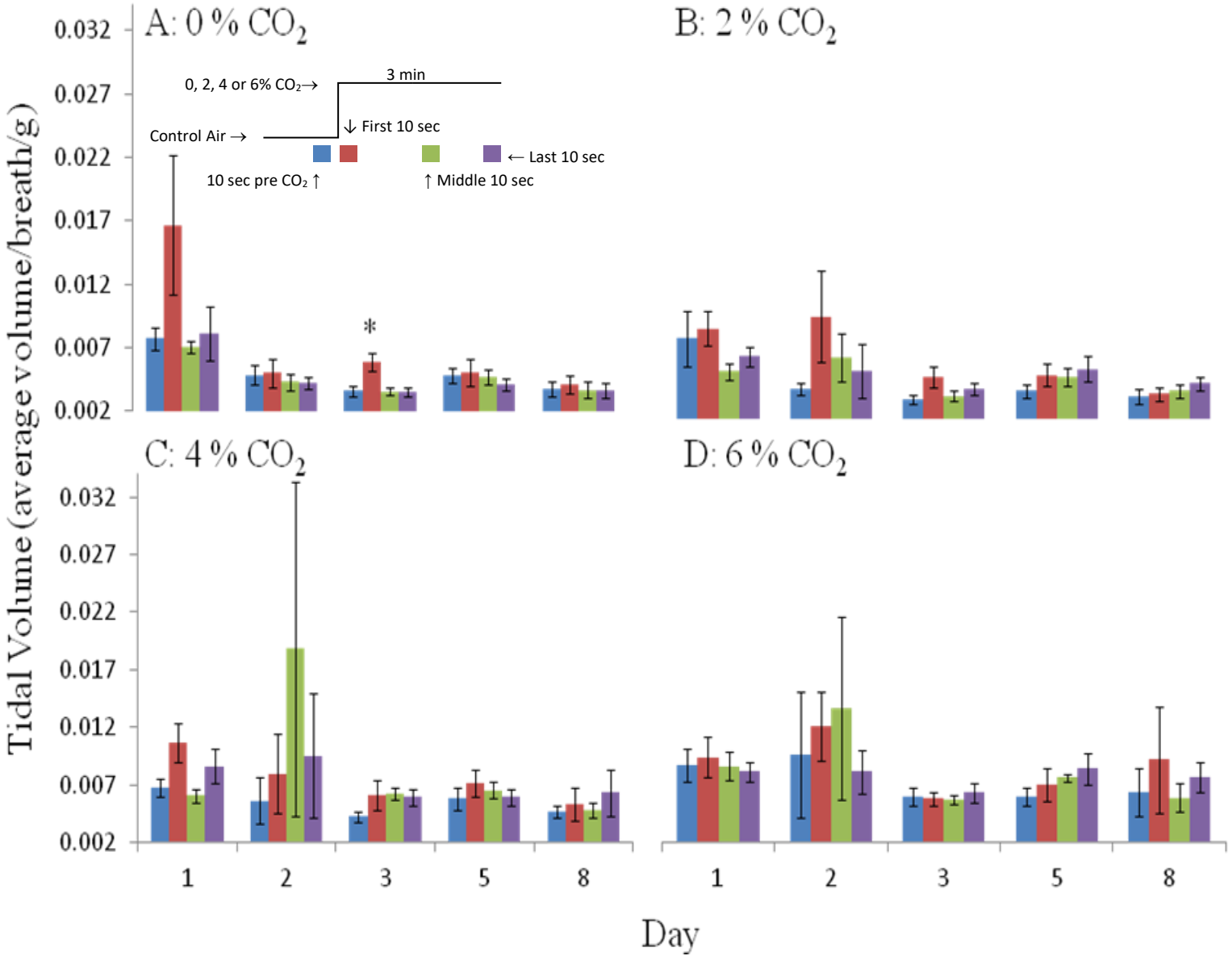


Figure 13. Age-related tidal volume of mice exposed to nicotine in response to hypercapnia, (A) 0%, (B), 2%, (C) 4%, and (D) 6% CO2, on postnatal days 1, 2, 3, 5, and 8. The first bar (pre) represents baseline V_T prior to CO_2 exposure; the second, third, and fourth represent the first, middle, and last 10 sec of a 3 min exposure, respectively. N = 7; bars depict average V_T, and error bars represent SEM. Astericks indicate significance ($p < 0.05$) from baseline.

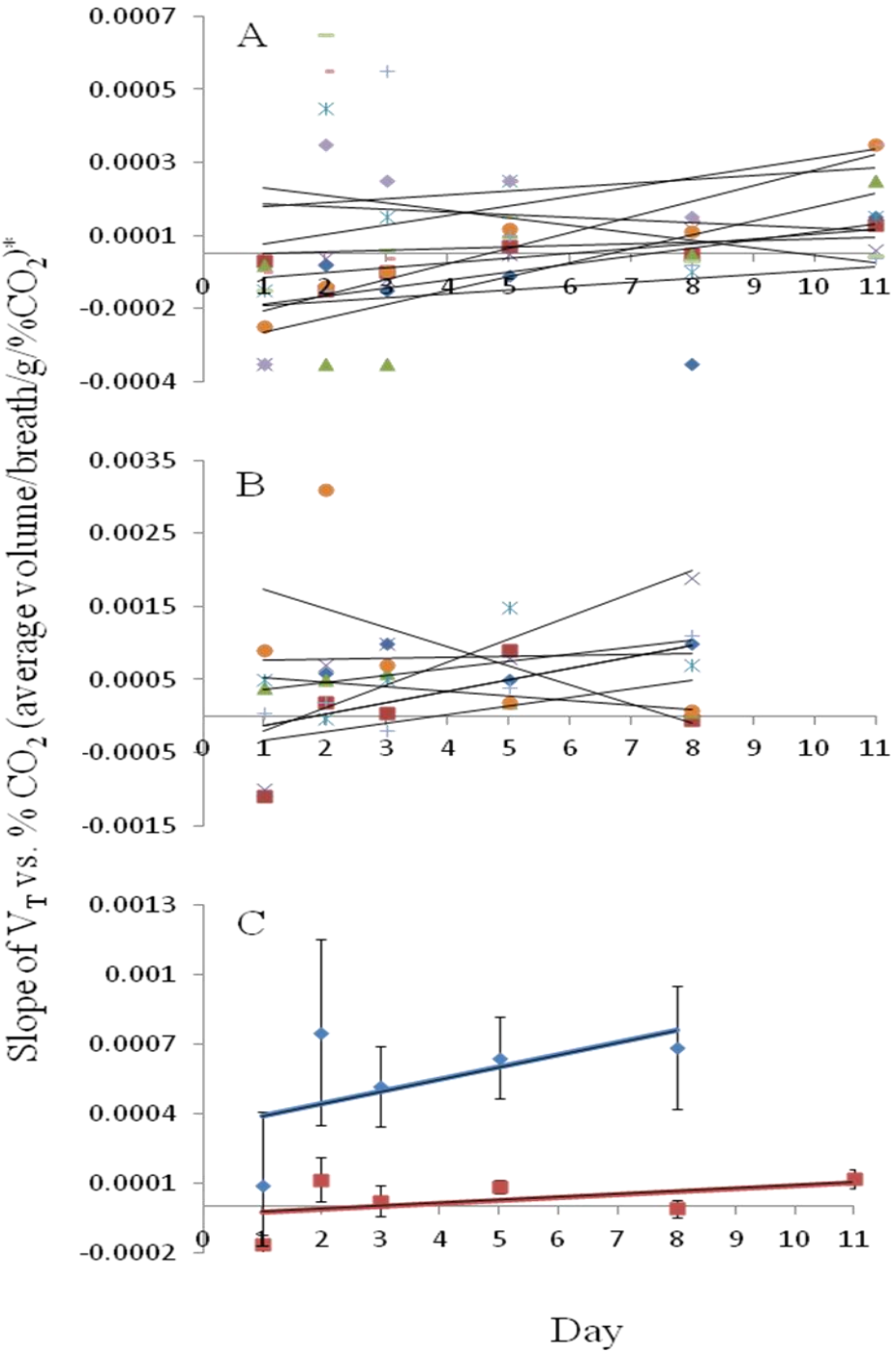


Figure 14. Age-related sensitivity to CO_2 assessed by the slope of V_T vs. % CO_2. Slopes of V_T vs. % CO_2 are represented by a linear trend with age. (A) Individual variability in sensitivity in control mice with age; shapes represent individual mice (n = 10). (B) Individual variability in sensitivity in nicotine mice with age; shapes represent individual mice (n = 7). (C) Linear relationship between sensitivity to CO_2 and age in control mice (red line; m = 1E-5 avg. vol/breath/%CO_2; r^2 = 0.21) and nicotine mice (blue line; m = 5E-5 avg. vol/breath/% CO_2; r^2 = 0.31). Points represent mean slope of V_T vs. % CO_2, and error bars represent SEM. Sensitivity was observed to increase with age in controls and nicotine mice. ANCOVA revealed that the difference in slopes of the lines was not significant ($F_{crit(1,7)}$ = 5.59, p = 0.27). * Scaling of slope of V_T vs. % CO_2 varies in A, B, and C due to large variability between control and nicotine groups.

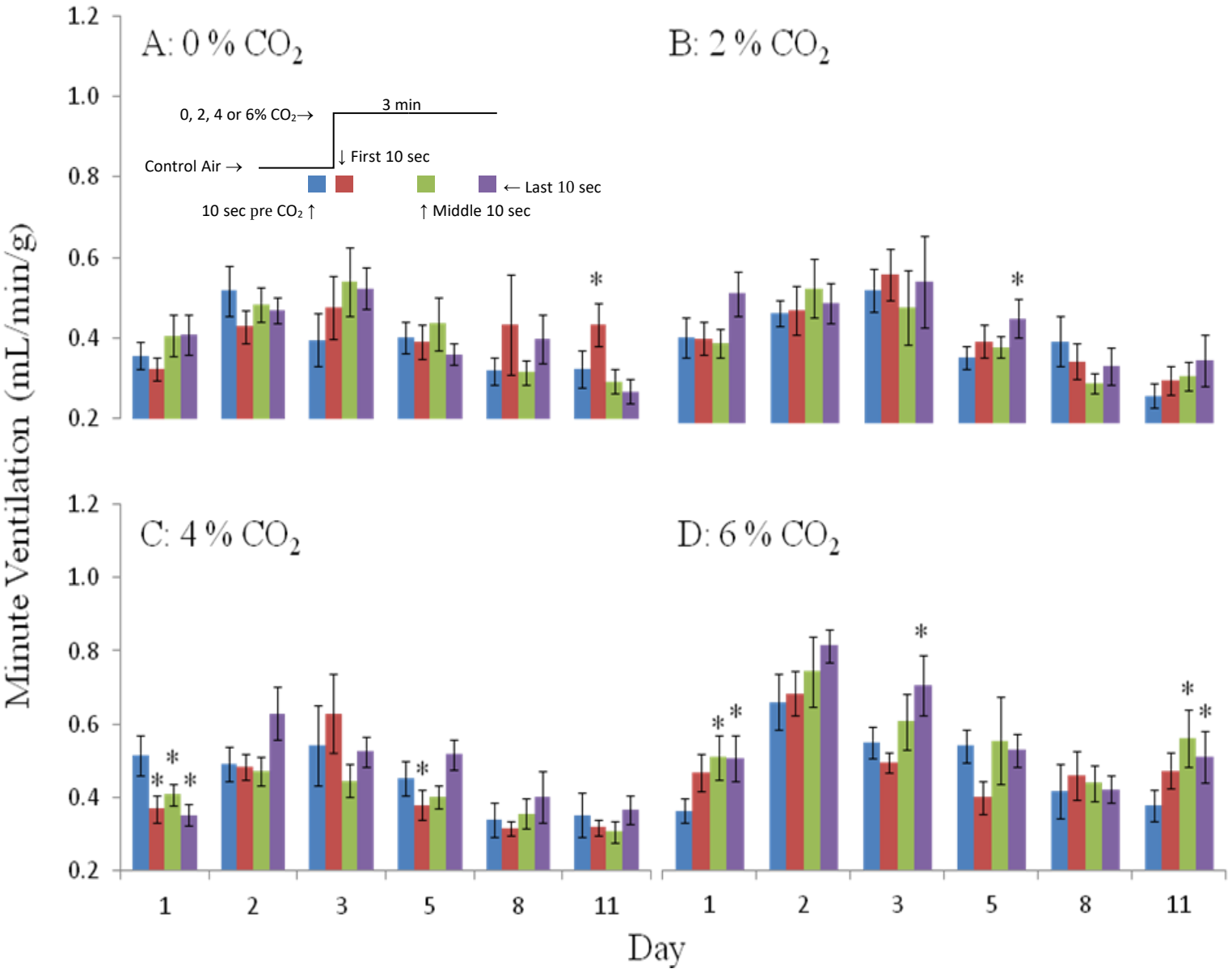


Figure 15. Age-related minute ventilation of control mice in response to hypercapnia, (A) 0%, (B) 2%, (C) 4 %, and (D) 6% CO_2, on postnatal days 1, 2, 3, 5, 8, and 11. The first bar (pre) represents baseline V_E prior to CO_2 exposure; the second, third, and fourth respresent the first, middle, and last 10 sec of a 3 min exposure, respectively. N = 10 on all days except day 11, on which n = 9; bars depict average V_E, and error bars represent SEM. Astericks indicate significance ($p < 0.05$) from baseline.

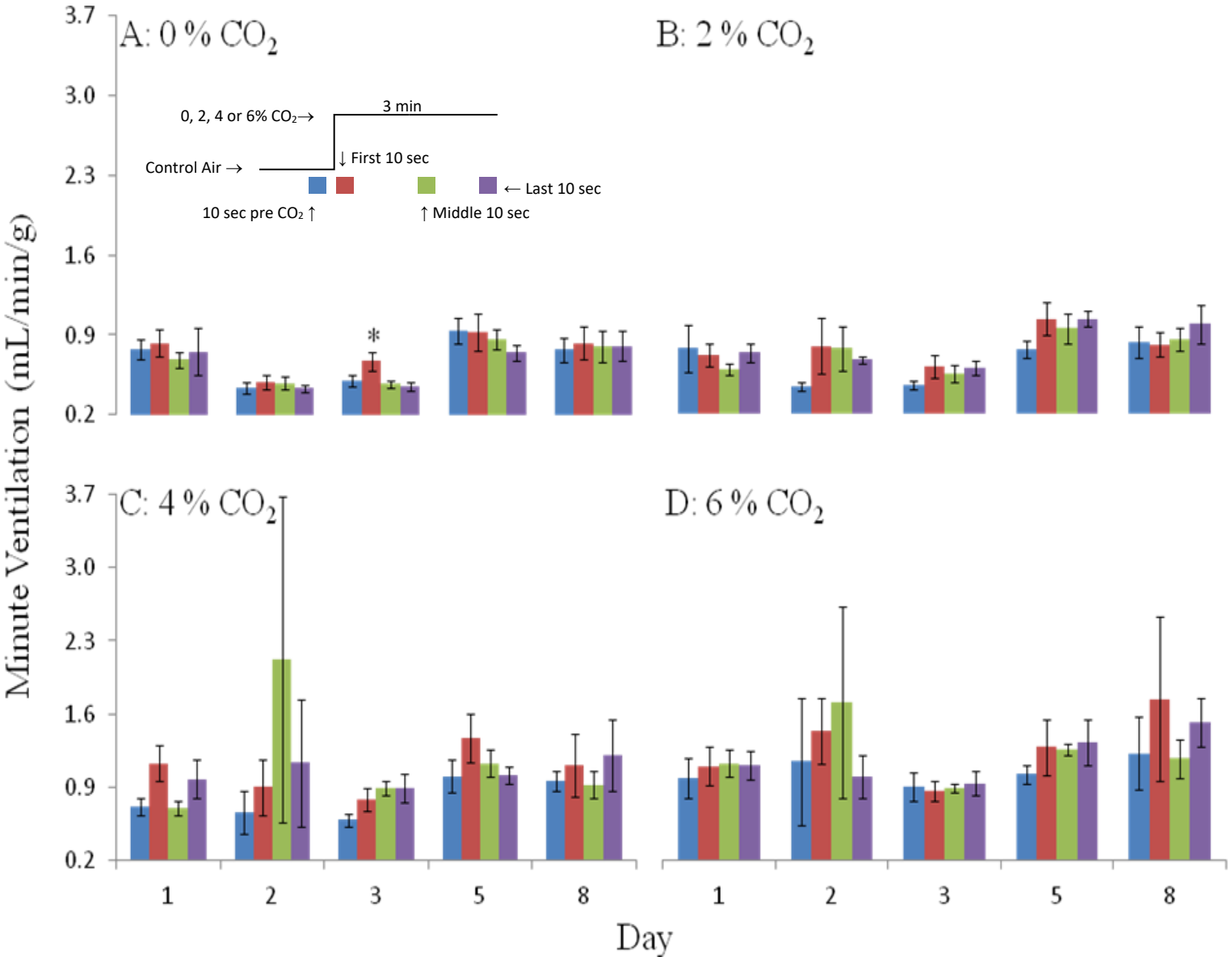


Figure 16. Age-related minute ventilation of mice exposed to nicotine in response to hypercapnia, (A) 0%, (B) 2%, (C) 4 %, and (D) 6% CO_2, on postnatal days 1, 2, 3, 5, and 8. The first bar (pre) represents baseline V_E prior to CO_2 exposure; the second, third, and fourth respresent the first, middle, and last 10 sec of a 3 min exposure, respectively. N = 7; bars depict average V_E, and error bars represent SEM. Astericks indicate significance ($p < 0.05$) from baseline.

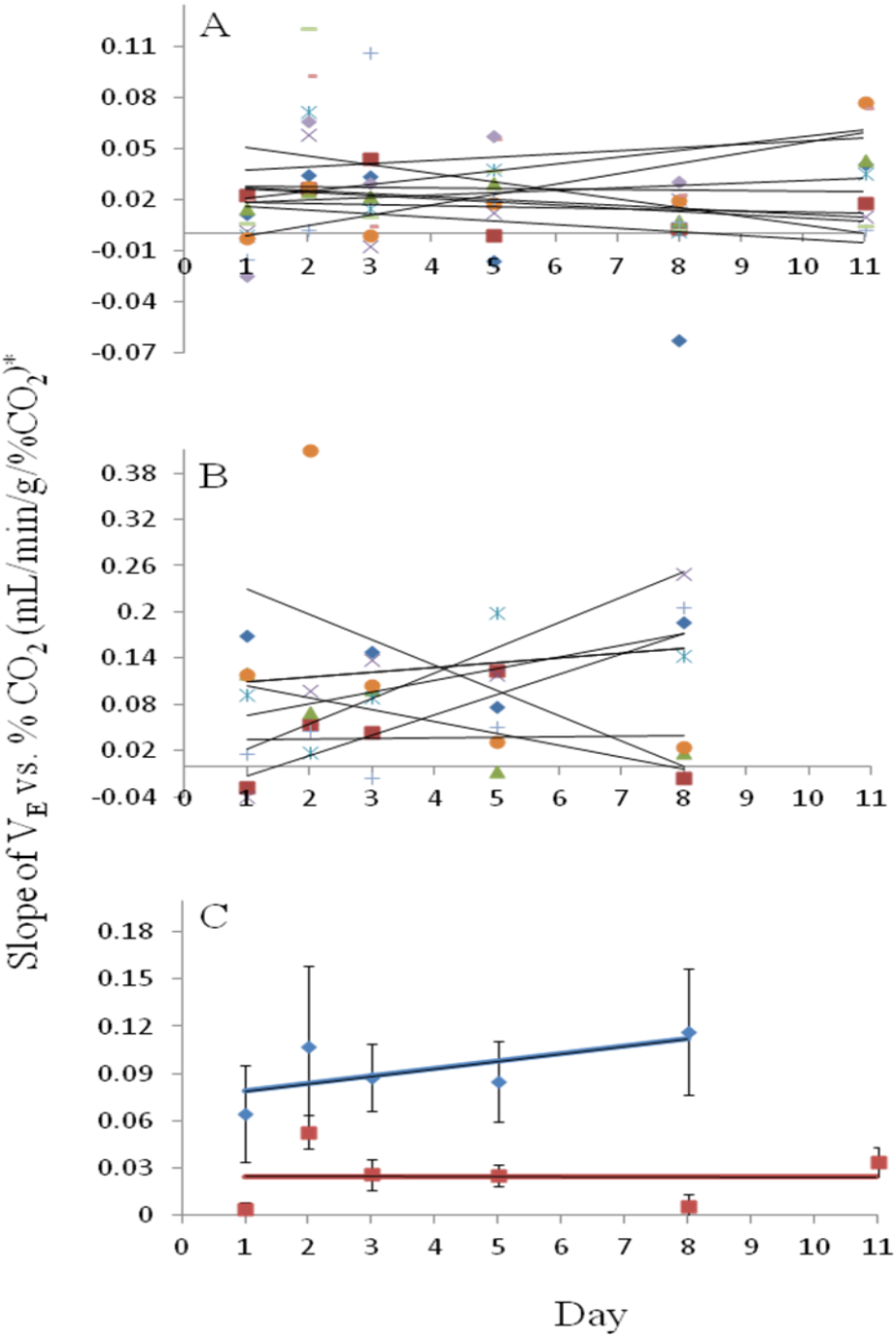


Figure 17. Age-related sensitivity to CO_2 assessed by the slope of V_E vs. % CO_2. Slopes of V_E vs. % CO_2 are represented by a linear trend with age. (A) Individual variability in sensitivity in control mice with age; shapes represent individual mice (n = 10). (B) Individual variability in sensitivity in nicotine mice; shapes represent individual mice (n = 7). (C) Linear relationship between sensitivity to CO_2 and age in control mice (red line; m = -6E-5 mL/min/g/%CO_2; r^2 = 0.001) and nicotine mice (blue line; m = 0.0048 mL/min/g/% CO_2; r^2 = 0.43). Points represent mean slope of V_T vs. % CO_2, and error bars represent SEM. Sensitivity was observed to increase with age in nicotine mice while controls demonstrated no change. ANCOVA revealed that the difference in slopes of the lines was not significant ($F_{crit(1,7)}$ = 5.59, p = 0.29). * Scaling of slope of V_T vs. % CO_2 varies in A, B, and C due to large variability between control and nicotine groups.

Discussion

The results of this experiment were found to support the hypothesis that prenatal nicotine exposure would elicit age-related impairments in ventilatory responses to hypercapnia at 0, 2, 4, and 6 % CO_2. Although an original objective of this experiment was to test a low dose and a high dose of nicotine, only a low dose of nicotine (3.91 ± 1.72 mg/kg/day) was able to be tested. Comparisons between ventilatory measurements with controls revealed that nicotine mice elicited a blunted response (Figure 9A-D, 13A-D & 16A-D). Ventilatory measurements, however, revealed contradictory results for comparisons between sensitivity to CO_2 in the control and nicotine groups, as nicotine mice were observed to be generally less sensitive to CO_2 than controls (Figure 11C, 14C & 17C).

Water consumption in control mice did not appear to be affected by the addition of sucrose; however, consumption by the nicotine mice was contrastingly different than expected. Water consumption by controls fell within the range of 4-8 mL found by Bachmanov et al. (2002) from 28 strains of mice, with C57BL/6J in particular consuming approximately 6 mL, thus indicating that the increase in consumption by nicotine mice was probably not a result of the addition of sucrose. While the nicotine mice consumed a higher mean amount of water than controls, there was much more variability in consumption, which could have been the result of the low sample size (n = 2) or a difference in weight of the animals in the two groups. There is also some inaccuracy in the reported values of water consumption since daily intake was calculated by dividing the measured difference in water over the period of days and the number of mice who shared the source; it is possible that some leakage of water could have occurred, but the bottles were tested prior to experimentation for leakage. The large amounts of water consumed could also be an indication that the nicotine water was appealing, which contrasts the reductions in consumption observed by Murrin et al. (1987) and Schneider et al. (2010) to doses

of ~ 2 mg/kg nicotine in drinking water. Since these studies did not include the addition of sucrose, it is possible that the sucrose was able to mask any bitterness in the nicotine.

Although nicotine has been typically observed to decrease birth weight and weight gain, no differences in weight or litter size were observed between nicotine mice and controls. Contradictory results have been previously observed in growth of mice prenatally exposed to nicotine. Mice exposed to nicotine as low as 2.79 ± 0.32 mg/kg and as high as 6 mg/kg exhibited lower weights during early development (Schneider et al., 2010; Bamford & Carrol, 1999). The results of this study, however, are in accordance with Eugenin et al. (2008), who observed that a dosage as high as 60 mg/kg did not affect birth weight and gain. The discrepancy could be due in part to the method of nicotine administration: drinking water vs. osmotic minipump (Schneider et al., 2010; Eugenin et al., 2008). While the toxicity of nicotine typically affects the rate of maturation, the lack of significance in weights between nicotine and control mice suggests that the nicotine mice were developing at the same rate as the controls (Schneider et al., 2010). It is possible that the dose of nicotine used in this experiment may not have been high enough to elicit the changes observed by other doses of nicotine in drinking solution (Krous et al., 1981; Schneider et al., 2010).

Nicotine was not observed to affect baseline *f* in the nicotine mice; however, it did appear to elicit the onset of apneas. Nicotine mice elicited an increasing trend in baseline *f* from day 1 to day 8, much like the increasing trend observed in control baseline *f* (Figure 9A & 10A). Although nicotine mice have been observed to demonstrate lower ventilatory responses than controls, the relative consistency in *f* between the two groups observed here could be a result of the relatively low dose of nicotine used (Eugenin et al., 2008). Despite this similarity, the presence of apneas in the nicotine mice evidenced the lack of regularity in *f* demonstrated by

control mice. Krous et al. (1981) similarly observed irregular breathing and increases in apneas in rats administered a nicotine dose of 2.6 ± 0.3 mg/kg in drinking water. As no apneas were elicited by control mice, the presence of apneas in the nicotine mice may be an indication of a developmental impairment in respiratory control elicited during early development (Schneider et al., 2008). Nicotine has been observed to increase the prevalence of necrosis in cells of the brain stem, which can affect respiratory control and induce the occurrence of apneas (Krous et al., 1981). The significant decreases observed during the first 10 sec of exposure to 0 % CO_2 on days 1 and 3 in nicotine mice are thought to be attributed to the presence of apneas within that time period since similar decreases were not observed in control mice (Figure 10A). The decreases observed on the first 3 days in response to 2 % CO_2 can also be attributed to apneas; however, the large variance in *f* within this period prevents statistical significance from being attained (Figure 10B). It is possible that nicotine exposure led to impairments in the sensitivity of upper airway chemoreceptors to CO_2, which would have impaired responsiveness within the first 10 sec of CO_2 exposure (Coates & Silvis, 1999). It is also possible that the apneas were a result of a developmental impairment in the central chemoreceptors in the brainstem, which can sense increases in CO_2 and provide feedback to the brainstem to consequently increase breathing (Brundage & Taylor, 2009). The occurrence of apneas may also have implications in SIDS, as the frequency of apneas has been observed to be higher during active sleep, when infants have been speculated to re-inspire accumulated levels of CO_2 (Brundage & Taylor, 2009).

In response to CO_2, control mice were observed to demonstrate age-related increases in *f*. As expected, control mice were not observed to elicit significant differences in *f* during the first, middle, or last 10 sec from baseline in response to 0 % CO_2 on all days of testing (Figure 9A). An age-related increase in *f* was observed during the early days of development, which could be

an indication of continued development of the ventilatory control system in the brainstem (St.-John & Leiter, 1999; Brundage & Taylor, 2009; Pendlebury et al., 2008). While a decrease in *f* was observed from day 5 to 11, Smith (2009) observed that after the maximum response on day 5, *f* appeared to stabilize. Increases in *f* in early and later development in response to increases in CO_2 suggest that both the upper airways and central chemoreceptors were fully developed by day 1 (Figure 9B-D). Although days 8 and 11 did not demonstrate significant increases in *f* in response to high levels of CO_2 (4 and 6 %), increases have been observed in other experiments in response to prolonged (5 min) exposures to CO_2, indicating that the CO_2 chemoreceptors are probably fully developed by this age (Smith, 2009). An overall increase in *f* in response to hypercapnia confirms that the respiratory control regions in the brainstem functioned properly, signaling increases in ventilation in response to the sensation of hypercapnia (Brundage & Taylor, 2009).

In contrast to controls, nicotine mice demonstrated a blunted ventilatory response in response to increases in CO_2 (Figure 10A-D). Although significant decreases in *f* were observed on days 1 and 3 in nicotine mice in response to 0 % CO_2, as stated previously, the depressions can be attributed to the high frequency of apneas. The results of this study support the blunted ventilatory response observed by Huang et al.(2010) in mice prenatally exposed to 6 mg/kg nicotine. The decrease could also have been a result of inhibition of the upper airway (carotid) chemoreceptors, which could also have led to the blunted frequencies observed in later development if the central chemoreceptors could not override this inhibition (Coates & Silvis, 1999). Aside from day 2, no significant increases in response to CO_2 were observed in nicotine mice until 6 % CO_2 was reached, which could be an indication that central chemosensitivity was impaired in response to low concentrations of CO_2 or that respiratory control had not fully

developed in the mice (Figure 10B-D) (St.-John & Leiter, 1999). Although a significant increase was observed on day 2 in response to 2 % CO_2, the lack of significance on day 2 in response to 6 % CO_2 suggests that the significance may have been a result of fast irregular breathing rather than sensitivity to CO_2 (Krous et al., 1981). Increases in *f* observed in response to 6 % CO_2 suggest that hypercapnia was high enough to overcome potential impairments in chemosensitivity.

An assessment of the sensitivities of the mice to increases in CO_2 revealed that the control mice demonstrated a slight decrease in mean sensitivity with age (Figure 11C). A similar age-related decrease in CO_2 sensitivity has been previously observed in neonatal control mice (Smith, 2009). While a mean decrease in sensitivity was observed overall, the *f* vs. % CO_2 response function was not observed to fit a linear model well ($r^2 = 0.41$) (Figure 11C). As such, the mean slopes of *f* vs. % CO_2 probably demonstrated more of a biphasic response than a linear one. Stunden et al. (2001) observed a biphasic CO_2 response in control mice from day 1 to day 16, in which mice decreased ventilation from day 1 to day 8 and increased from day 8 to day 21, at which a stable response was observed. This experiment revealed an increase in sensitivity from day 1 to day 2, and the second phase of the trend reported by Stunden et al. (2001) was observed here, supporting the notion that the CO_2 ventilatory pathway is still being developed during the first week of development (Stunden et al., 2001; Pendlebury et al., 2008). Individual responsiveness of the control mice also demonstrated this notion, as the variability in sensitivity observed in early development was seen to diminish by day 11 (Figure 11A). As a result, the period from day 1 to day 5 (or day 8) could be considered the critical period of development for CO_2 sensitivity that factors into the onset of SIDS (Stunden et al., 2001; Filiano & Kinney, 1994; Brundage & Taylor, 2009; Pendlebury et al., 2008).

Although no basis for comparison exists for the sensitivity of the nicotine mice to CO_2, the nicotine mice demonstrated a significantly greater decreased mean sensitivity with age than controls (Figure 11C). Mice were not observed to elicit the biphasic trend observed by controls, but rather demonstrated a fairly linear ($r^2 = 0.79$) decrease in mean sensitivity to CO_2 (Figure 11C). Unlike the controls, the nicotine mice did not elicit a recovery from depressed sensitivity on day 5, suggesting that nicotine impaired the development of the central chemoreceptors, thus impairing their ability to sense prolonged increases in CO_2 (Bamford et al., 1996; Stunden et al., 2001). The mice were not tested on day 11, but it is likely that they would have exhibited a higher sensitivity at this age, since they would not be able to sustain life if the decreasing trend continued. It is important to note, though, that a large amount of variance was observed in sensitivity, thus weakening the linear trend (Figure 11B & C). While mice exposed to nicotine (6 mg/kg) did not demonstrate a significant response in ventilation in response to a 10 min exposure to 5 % CO_2, mice were tested during later development when an impairment in chemosensitivity could have been overcome (Bamford et al., 1996). It has been speculated that nicotine affects neural development of the regions of the brainstem that regulate ventilatory control, and the lack of sensitivity of the mice on day 8 can be interpreted as an indication that nicotine impaired ventilatory control (Pendlebury et al., 2008). Although previous studies have not exposed nicotine mice to prolonged, step-wise increases in CO_2, the slope of the *f* vs. % CO_2 function suggests that nicotine causes a developmental impairment in sensitivity to CO_2, such that mean sensitivity to increases in hypercapnia is significantly blunted in comparison to controls ($p = 0.022$) (Figure 11C).

Tidal volume in control mice was observed to vary throughout the exposure periods, and did not evidence a definite responsiveness of V_T to CO_2 (Figure 12A-D). Control mice have been

observed to demonstrate relatively no change in V_T in response to 4, 5 and 8 % CO_2, though V_T is typically higher at higher levels of CO_2 (Bamford et al., 1996; Longo, 2011). This increasing trend was also observed in this experiment; however, V_T during the different periods of exposure at each concentration of CO_2 was observed to be highly variable, making it difficult to ascertain conclusive effects (Figure 12A-D). It is likely that the irritability and arousal of the mice contributed to the variability in V_T, especially at 6 % CO_2. Since later development elicited lower V_T's, it is possible that regions of the brainstem responsible for ventilatory control of V_T were still developing early on; however, the presence of significant differences in V_T in early and later development suggest that respiratory control of V_T was intact from birth (Simakajornboon et al., 2004).

Much like the controls, the nicotine mice demonstrated variability in V_T throughout the different periods of exposure and did not elicit an obvious trend. Tidal volume was typically observed to be more variable in nicotine mice at all concentrations of CO_2 than in control mice, and some of this variability can be attributed to movements of the mice within the breathing chamber (Figure 13A-D; Figure 12A-D). A trend similar to controls was also observed in which values of V_T were generally lower in response to 0 % CO_2 and higher in response to 6 % CO_2, indicating that the compensatory behavior to increase breath size in response to hypercapnia may still have been intact, but the lack of significance over age and duration does not support this idea (Simakajornboon et al., 2004). Mice on day 3 were observed to elicit a significant increase in V_T in response to 0 % CO_2, but this increase was probably a result of the presence of an apnea or shallow breathing during baseline (Figure 13A). Overall, nicotine was not observed to affect V_T, supporting that the ventilatory response of the nicotine mice was blunted (Figure 13A-D).

Using V_T as the ventilatory parameter, mean sensitivity to CO_2 was not observed to be different between control and nicotine mice. Nicotine and control mice demonstrated linear increases in sensitivity with age, with nicotine mice demonstrating higher sensitivities on all days except day 1; however, a linear trend was not observed to fit the models well (Figure 14C). The large variability in mean sensitivity observed in nicotine mice makes conclusions difficult, but the low variability in mean sensitivity observed in control mice suggests that sensitivity to CO_2 was relatively stable throughout development (Figure 14C). As stated previously, the region of the brainstem responsible for modulation of V_T was probably fully developed at birth in control and in nicotine mice, since no significant difference in mean sensitivity was observed between the two (Simakajorboon et al., 2004). Tidal volume has been previously observed to be blunted in response to 3, 6, and 9 % CO_2 in mice prenatally exposed to nicotine (6 mg/kg), but these results suggest that nicotine exposure increases sensitivity to CO_2 with age, which also contrasts the results observed here in sensitivity assessed by *f* (Huang et al., 2010). It is possible that nicotine had a neurological effect on the brain, such that chemoreceptors were made more sensitive to CO_2 than controls; however, there is no evidence of such a stimulatory effect (Blood-Siegfried & Rende, 2010).

Minute ventilation (V_E) was observed to elicit trends similar to those observed by its two component parameters. The significant differences observed in V_E in response to all levels of CO_2 appear to be equally attributed to *f* and V_T, though V_T has been typically observed to be the large contributor to V_E (Figure 15A & B) (Stunden et al., 2001). This is probably due to the relatively high *f*'s and relatively low V_T's observed in this experiment. Since both *f* and V_T were observed to increase in response to CO_2, the increases observed in V_E with time at all ages in response to CO_2 were expected and indicative that development of central chemoreception was

completed as early as day 1, which opposes the idea that ventilation is still developing in young mice (Figure 15D) (Coates & Silvis, 1999; Stunden et al., 2001; Pendlebury et al., 2008).

The lack of statistical significance typically observed in V_E in the nicotine mice at all concentrations of CO_2 further confirms that nicotine elicited a blunted ventilatory (Figure 16A-D).The significant increase in V_E on day 3 in response to 0% CO_2 can be attributed to a large V_T observed during this period, thus 0% CO_2 probably did not elicit a change in ventilation (Figure 16A). The fluctuations in V_E observed with time in response to CO_2 were not observed to be significantly different from baseline, demonstrating that the mice were not responsive to any exposure to CO_2, which is in accordance with the results presented of *f* and V_T. While this study did not elicit changes in V_E to CO_2, Eugenin et al. (2008) found that mice exposed to nicotine (60 mg/kg) demonstrated decreases in ventilation in response to 10 % CO_2. Since this present study exposed the mice to a much lower dosage of nicotine, it is possible that increases in dosages could elicit decreases in V_E. Even a low dose of nicotine, though, appeared to interfere with the development of the chemosensory response (Blood-Siegfriend & Rende, 2010).

As observed with the slopes of the mean V_T sensitivity functions, the slopes of the mean V_E sensitivity functions in control and nicotine mice were not found to be statistically significant, and nicotine mice were observed to be more sensitive than controls (Figure 17C). It appeared that V_E sensitivity function was more influenced by V_T than *f*, since the shapes of the functions are similar. The nicotine mice were observed to be more sensitive to CO_2 with age than the controls, suggesting that nicotine may have a stimulatory effect. Although linear regressions were calculated for the mean slopes of V_E vs. % CO_2, the slopes of the control mice did not demonstrate a linear fit ($r^2 = 0$), suggesting that sensitivity to CO_2 does not linearly increase with age. While the slopes of the nicotine mice exhibited a fairly linear fit ($r^2 = 0.42$), there was a

great amount of variation in the individual slopes of V_E vs. % CO_2, weakening the strength of the regression. Like the slopes of *f* vs. % CO_2, the slopes of V_E vs. % CO_2 in both the nicotine and control mice demonstrated biphasic trends, similar to those previously described, observed by Stunden et al. (2001) (Figure 17C). The period of decline in sensitivity to CO_2 is thought to be a result of a period of development in which the central chemoreceptors in the brain mature so that they can properly send signals to the medulla to regulate respiratory control (Stunden et al., 2001; Pendlebury et al., 2008). In conclusion, ventilatory parameters evidenced that nicotine mostly blunted the ventilatory response in neonatal mice; however, it was also observed to enhance it.

Further experimentation is necessary to better elucidate the effects of nicotine on development and to overcome the limitations of this study. While one of the main objectives was to study CO_2 sensitivity in mice prenatally exposed to high and low doses of nicotine, only one mouse administered a low dose of nicotine produced a litter. The nicotine dosing of this study modeled a moderate smoker, who consumed the nicotine equivalent to approximately 10 cigarettes a day, and since even this low dose of nicotine evoked a blunted ventilatory response, it would be interesting to test a higher concentration to determine if impairments were more pronounced and a lower concentration to determine if impairments were still observable (Krous et al., 1981). Comparisons in effects between administration methods of nicotine could also be tested to determine if osmotic mini-pumps or drinking solutions are more effective at administering nicotine since water consumption is so variable. Measuring the recovery period subsequent to the step-wise increases to hypercapnia in addition to the four periods of measurement used in this study would be helpful in determining if nicotine impairs recovery, as has been observed in response to hypoxic challenges (Schneider et al., 2008; St.-John & Leiter,

1999). Multiple litters prenatally exposed to nicotine should be tested to increase the validity of the study and provide further support of the results presented here. Additionally, testing should be completed up to day 21 to determine if CO_2 sensitivity does stabilize and reach an adult response in both controls and nicotine mice (Stunden et al., 2001). A linear function was also not observed to be the most effective way to illustrate CO_2 sensitivity, as the slopes of the ventilatory parameters might not be linear. More data would be needed to better demonstrate the trends in CO_2 sensitivity elicited by both control and nicotine mice.

While previous results have contradicted each other, observing no effect of nicotine (6 mg/kg) on ventilation and a blunted ventilatory response of nicotine (60 mg/kg), these results support that even a low dose of nicotine can impair the ventilatory response of neonatal mice to prolonged exposure to hypercapnia (Bamford et al., 1996; Eugenin et al., 2008). Stunden et al. (2001) proposed that the minimal sensitivity to CO_2 observed on day 8, for each of the ventilatory parameters, represents a critical period of development for the mice, in which mice must develop an adult chemosensory pathway. It is speculated that an impairment in development during this time period can induce the onset of SIDS. The blunting of the ventilatory response observed in this study suggests that nicotine may play a role in the onset of SIDS by inducing a developmental impairment in respiratory control during the critical period of development. Although the results presented here observed higher and lower sensitivities of nicotine mice to CO_2 than control mice, the individual measures of ventilation generally demonstrated an impairment in responsiveness to CO_2, as elicited by a blunted f, V_T, and V_E response at all ages in response to all concentrations of CO_2; thus, the results of this experiment were observed to support the original hypothesis.

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Appendix

Appendix A. Average water consumption of adult female mice in g/day.

	Female #1	Female #2	Female #3	Female #4
	14.34	8.19	4.61	8.03
	9.61	7.19	5.33	6.64
	8.08	6.12	5.47	5.57
	7.96	16.2	6.16	16.1
	13.41	5.51	5.30	10.36
	5.53		5.79	8.31
	6.53		5.17	10.75
			6.57	6.38
			5.80	4.62
			6.47	7.15
			5.74	7.46
			6.36	8.11
Average	9.35	8.20	5.73	8.29
Std	3.11	3.69	0.59	3.03

Total Average	7.64
Total Std	3.00

Appendix B. Average water consumption of adult mice in g/day for each litter. Values represent average intake for each mouse and are not normalized by weight.

Litter	Control #1	Control #2	Control #3	Nicotine #1	Nicotine #2
	7.77	9.14	9.61	3.83	5.36
	7.12	7.51	8.62	4.03	5.42
	6.14	8.07	8.56	4.65	11.01
	5.78	8.18	7.42	4.49	8.67
	5.76	8.82	7.14	4.75	17.37
				4.91	15.23
				5.33	6.84
				4.44	18.19
Average	6.51	8.34	8.27	4.55	11.01
Std	0.89	0.64	1.00	0.48	5.29

	Tot Avg.	Std
Control	7.71	1.18
Nicotine*	11.01	5.29

*Does not include values from Nicotine #1 since no pups were produced for testing.

Appendix C. Nicotine dosing of female mice based on weight and daily consumption of nicotine water (0.00975M). Dosing was only calculated for the adult female from Nicotine #2 which produced a litter.

	Daily Intake of H_2O (g)	Weight (kg)	Nicotine (mg/kg)	Nicotine (mg/kg/g H_2O)
	5.36	0.02064	0.5025	2.43
	5.42	0.02094	0.0508	2.43
	11.01	0.02263	0.1032	4.56
	8.67	0.02626	0.0813	3.10
	17.37	0.02733	0.1628	5.96
	15.26	0.03174	0.1430	4.51
	6.84	0.03448	0.0641	1.86
	18.19	0.02648	0.1705	6.44
Average	11.01	0.02631	0.1033	3.91
Std	5.29	0.00495	0.0496	1.72

Appendix D. Weights of (A) control and (B) nicotine mice on postnatal days 1, 2, 3, 5, 8 and 11. (C) Results of a two-sample two-tailed independent samplet-test for weights on postnatal days 1, 2, 3, 5, and 8. Calculations were completed using VassarStats (http://www.vassarstats.net). $t_{crit(0.1,15)} = 1.753$; $p < 0.05$ was significant.

A. Control mice

Mouse	Day 1	Day 2	Day 3	Day 5	Day 8	Day 11
1	1.42	1.59	1.82	2.72	3.99	4.09
2	1.22	1.45	1.7	2.56	4.05	4.26
3	1.21	1.34	1.61	2.3	3.63	4.01
4	1.24	1.44	1.69	2.46	3.69	X*
5	1.51	1.73	1.95	2.75	3.78	4.72
6	1.75	1.93	2.21	3.2	4.07	4.91
7	1.19	1.39	1.64	2.26	3.11	3.93
8	1.72	1.95	2.15	3.16	3.14	4.81
9	1.53	1.76	2.03	2.96	3.51	4.65
10	1.48	1.65	1.87	2.61	3.63	4.19
Avg	1.427	1.623	1.867	2.698	3.66	4.39667
Std	0.19789	0.20761	0.20254	0.3116	0.32274	0.37513
SEM	0.06258	0.06565	0.06405	0.09854	0.10206	0.12504

*X indicates that the mouse was deceased.

B. Nicotine Mice

Mouse	Day 1	Day 2	Day 3	Day 5	Day 8
1	1.47	1.67	2.17	2.87	3.94
2	1.25	1.43	1.92	2.58	3.5
3	1.32	1.5	1.97	2.78	3.91
4	1.37	1.65	2.04	2.86	4.32
5	1.47	1.64	1.97	2.62	3.73
6	1.38	1.58	2.01	2.78	3.88
7	1.36	1.54	1.84	2.67	3.58
Avg	1.37429	1.57286	1.98857	2.73714	3.83714
Std	0.0785	0.08826	0.10286	0.11499	0.27146
SEM	0.02967	0.03336	0.03888	0.04346	0.1026

C.

	Day1	Day 2	Day 3	Day 5	Day 8
t	0.63	0.57	-1.39	-0.30	-1.27
p	0.54	0.57	0.18	0.77	0.24

Appendix E. Control mice data categorized by day.

Day 1

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Last**
Freq (10s)	L1M1	16.5	16.0	18.5	18.0	19.0	18.0	20.0	21.0	18.5	20.5	22.5	23.0	23.0
	L1M2	14.5	17.0	15.5	15.5	15.0	20.5	16.0	19.0	17.5	19.5	18.0	21.0	20.5
	L1M3	17.0	17.0	17.0	16.5	16.5	19.5	16.0	18.5	16.5	19.5	21.0	21.0	19.5
	L1M4	16.5	15.0	17.0	15.5	15.0	17.5	15.5	21.0	23.0	22.0	20.0	23.0	26.5
	L2M1	18.0	19.0	16.0	19.0	17.5	19.0	19.5	19.5	17.5	18.0	20.0	21.5	27.0
	L2M2	16.0	16.5	16.0	14.0	15.0	15.0	15.5	18.5	15.5	17.0	17.0	20.0	20.5
	L2M3	15.5	15.0	16.5	16.0	16.5	15.0	14.5	18.0	15.5	16.0	19.0	17.5	19.0
	L3M1	18.0	22.0	20.0	21.0	20.0	21.0	24.0	25.0	27.0	28.0	29.5	30.0	31.0
	L3M2	19.0	19.5	22.0	23.0	23.0	25.0	26.0	24.0	25.0	24.0	26.5	30.5	30.5
	L3M3	22.5	23.0	22.0	24.5	23.5	26.0	26.0	25.0	26.0	24.5	26.0	28.5	31.0
Freq (60s)	L1M1	99.0	96.0	111.0	108.0	114.0	108.0	120.0	126.0	111.0	123.0	135.0	138.0	138.0
	L1M2	87.0	102.0	93.0	93.0	90.0	123.0	96.0	114.0	105.0	117.0	108.0	126.0	123.0
	L1M3	102.0	102.0	102.0	99.0	99.0	117.0	96.0	111.0	99.0	117.0	126.0	126.0	117.0
	L1M4	99.0	90.0	102.0	93.0	90.0	105.0	93.0	126.0	138.0	132.0	120.0	138.0	159.0
	L2M1	108.0	114.0	96.0	114.0	105.0	114.0	117.0	117.0	105.0	108.0	120.0	129.0	162.0
	L2M2	96.0	99.0	96.0	84.0	90.0	90.0	93.0	111.0	93.0	102.0	102.0	120.0	123.0
	L2M3	93.0	90.0	99.0	96.0	99.0	90.0	87.0	108.0	93.0	96.0	114.0	105.0	114.0
	L3M1	108.0	132.0	120.0	126.0	120.0	126.0	144.0	150.0	162.0	168.0	177.0	180.0	186.0
	L3M2	114.0	117.0	132.0	138.0	138.0	150.0	156.0	144.0	150.0	144.0	159.0	183.0	183.0
	L3M3	135.0	138.0	132.0	147.0	141.0	156.0	156.0	150.0	156.0	147.0	156.0	171.0	186.0
	Avg	**104.1**	**108.0**	**108.3**	**109.8**	**108.6**	**117.9**	**115.8**	**125.7**	**121.2**	**125.4**	**131.7**	**141.6**	**149.1**
	Std	**13.4**	**16.8**	**14.8**	**21.1**	**19.2**	**22.1**	**27.3**	**16.6**	**27.3**	**22.4**	**24.6**	**26.9**	**29.6**
	SEM	**4.2**	**5.3**	**4.7**	**6.7**	**6.1**	**7.0**	**8.6**	**5.2**	**8.6**	**7.1**	**7.8**	**8.5**	**9.4**
Vt (Total)	L1M1	0.01498	0.01747	0.02614	0.02944	0.02341	0.01958	0.03666	0.01836	0.01486	0.02362	0.02634	0.02543	0.03746
	L1M2	0.01788	0.01908	0.03139	0.01606	0.01361	0.02209	0.01875	0.02322	0.01567	0.01540	0.02722	0.02224	0.02325
	L1M3	0.00938	0.01168	0.01601	0.01490	0.01624	0.02089	0.03065	0.02261	0.02275	0.01392	0.02456	0.02054	0.02441
	L1M4	0.00882	0.01015	0.01222	0.00973	0.00826	0.01267	0.02311	0.01219	0.01933	0.01155	0.01405	0.03504	0.01407
	L2M1	0.01971	0.01004	0.01206	0.01610	0.01536	0.01717	0.02088	0.01164	0.01674	0.01410	0.01693	0.01976	0.01818
	L2M2	0.01425	0.00993	0.01414	0.01150	0.02187	0.01146	0.01551	0.01466	0.01814	0.01015	0.01081	0.01082	0.01254
	L2M3	0.01197	0.01391	0.01487	0.01493	0.01425	0.01750	0.01480	0.01586	0.01372	0.01137	0.01846	0.01248	0.01224
	L3M1	0.02323	0.02220	0.02462	0.01842	0.02255	0.01899	0.02815	0.01810	0.02141	0.02195	0.02873	0.03450	0.02797
	L3M2	0.02716	0.01694	0.01795	0.02690	0.03489	0.02192	0.03021	0.01481	0.01941	0.02018	0.02099	0.02123	0.03239
	L3M3	0.02028	0.01754	0.01549	0.03117	0.02116	0.01636	0.01693	0.01523	0.02645	0.02116	0.01688	0.02131	0.02188
Vt (avg vol/breath)	L1M1	0.0009079	0.0010919	0.0014130	0.0016356	0.0012321	0.0010878	0.0018330	0.0008743	0.0008032	0.0011522	0.0011707	0.0011057	0.0016287
(raw)	L1M2	0.0012331	0.0011224	0.0020252	0.0010361	0.0009073	0.0010776	0.0011719	0.0012221	0.0008954	0.0007897	0.0015122	0.0010590	0.0011341
	L1M3	0.0005518	0.0006871	0.0009418	0.0009030	0.0009842	0.0010713	0.0019156	0.0012222	0.0013788	0.0007138	0.0011695	0.0009781	0.0012518
	L1M4	0.0005345	0.0006767	0.0007188	0.0006277	0.0005507	0.0007240	0.0014910	0.0005805	0.0008404	0.0005250	0.0007025	0.0015235	0.0005309
	L2M1	0.0010950	0.0005284	0.0007538	0.0008474	0.0008777	0.0009037	0.0010708	0.0005969	0.0009566	0.0007833	0.0008465	0.0009191	0.0006733
	L2M2	0.0008906	0.0006018	0.0008838	0.0008214	0.0014580	0.0007640	0.0010006	0.0007924	0.0011703	0.0005971	0.0006359	0.0005410	0.0006117
	L2M3	0.0007723	0.0009273	0.0009012	0.0009331	0.0008636	0.0011667	0.0010207	0.0008811	0.0008852	0.0007106	0.0009716	0.0007131	0.0006442
	L3M1	0.0012906	0.0010091	0.0012310	0.0008771	0.0011275	0.0009043	0.0011729	0.0007240	0.0007930	0.0007839	0.0009739	0.0011500	0.0009023
	L3M2	0.0014295	0.0008687	0.0008159	0.0011696	0.0015170	0.0008768	0.0011619	0.0006171	0.0007764	0.0008408	0.0007921	0.0006961	0.0010620
	L3M3	0.0009013	0.0007626	0.0007041	0.0012722	0.0009004	0.0006292	0.0006512	0.0006092	0.0010173	0.0008637	0.0006492	0.0007477	0.0007058
Vt (avg vol/breath)	L1M1	0.00464	0.00558	0.00722	0.00836	0.00630	0.00556	0.00937	0.00447	0.00410	0.00589	0.00598	0.00565	0.00832
	L1M2	0.00630	0.00574	0.01035	0.00529	0.00464	0.00551	0.00599	0.00624	0.00458	0.00404	0.00773	0.00541	0.00580
	L1M3	0.00282	0.00351	0.00481	0.00461	0.00503	0.00547	0.00979	0.00625	0.00705	0.00365	0.00598	0.00500	0.00640
	L1M4	0.00273	0.00346	0.00367	0.00321	0.00281	0.00370	0.00762	0.00297	0.00429	0.00268	0.00359	0.00778	0.00271
	L2M1	0.00560	0.00270	0.00385	0.00433	0.00449	0.00462	0.00547	0.00305	0.00489	0.00400	0.00433	0.00470	0.00344
	L2M2	0.00455	0.00308	0.00452	0.00420	0.00745	0.00390	0.00511	0.00405	0.00598	0.00305	0.00325	0.00276	0.00313
	L2M3	0.00395	0.00474	0.00461	0.00477	0.00441	0.00596	0.00522	0.00450	0.00452	0.00363	0.00496	0.00364	0.00329
	L3M1	0.00659	0.00516	0.00629	0.00448	0.00576	0.00462	0.00599	0.00370	0.00405	0.00401	0.00498	0.00588	0.00461
	L3M2	0.00730	0.00444	0.00417	0.00598	0.00775	0.00448	0.00594	0.00315	0.00397	0.00430	0.00405	0.00356	0.00543
	L3M3	0.00461	0.00390	0.00360	0.00650	0.00460	0.00322	0.00333	0.00311	0.00520	0.00441	0.00332	0.00382	0.00361
Vt (avg vol/breath/g)	L1M1	0.003267	0.003929	0.005085	0.005886	0.004434	0.003914	0.006596	0.003146	0.002891	0.004146	0.004213	0.003979	0.005861
	L1M2	0.005165	0.004701	0.008482	0.004340	0.003800	0.004513	0.004908	0.005119	0.003751	0.003308	0.006334	0.004436	0.004750
	L1M3	0.002330	0.002902	0.003977	0.003814	0.004157	0.004524	0.008090	0.005161	0.005823	0.003015	0.004939	0.004131	0.005287
	L1M4	0.002203	0.002789	0.002962	0.002587	0.002269	0.002984	0.006144	0.002392	0.003463	0.002164	0.002895	0.006278	0.002188
	L2M1	0.003706	0.001788	0.002551	0.002868	0.002970	0.003058	0.003624	0.002020	0.003237	0.002651	0.002865	0.003110	0.002279
	L2M2	0.002601	0.001757	0.002581	0.002399	0.004257	0.002231	0.002922	0.002314	0.003417	0.001743	0.001857	0.001580	0.001786
	L2M3	0.003316	0.003982	0.003870	0.004007	0.003709	0.005010	0.004383	0.003784	0.003801	0.003052	0.004172	0.003062	0.002766
	L3M1	0.003834	0.002998	0.003657	0.002606	0.003350	0.002687	0.003485	0.002151	0.002356	0.002329	0.002893	0.003417	0.002681
	L3M2	0.004774	0.002901	0.002725	0.003906	0.005066	0.002928	0.003881	0.002061	0.002593	0.002808	0.002645	0.002325	0.003547
	L3M3	0.003112	0.002633	0.002431	0.004393	0.003109	0.002173	0.002248	0.002103	0.003512	0.002982	0.002242	0.002582	0.002437
	Avg	**0.003431**	**0.003038**	**0.003832**	**0.003680**	**0.003712**	**0.003402**	**0.004628**	**0.003025**	**0.003484**	**0.002820**	**0.003505**	**0.003490**	**0.003358**
	Std	**0.000978**	**0.000938**	**0.001838**	**0.001087**	**0.000815**	**0.001014**	**0.001818**	**0.001247**	**0.000950**	**0.000665**	**0.001383**	**0.001312**	**0.001438**
	SEM	**0.000309**	**0.000297**	**0.000581**	**0.000344**	**0.000258**	**0.000321**	**0.000575**	**0.000394**	**0.000300**	**0.000210**	**0.000437**	**0.000415**	**0.000455**
Minute Ventilation	L1M1	0.3234	0.3772	0.5644	0.6357	0.5055	0.4228	0.7915	0.3964	0.3209	0.5100	0.5687	0.5491	0.8088
(mL/min/g)	L1M2	0.4493	0.4795	0.7889	0.4036	0.3420	0.5551	0.4712	0.5835	0.3938	0.3870	0.6841	0.5589	0.5843
	L1M3	0.2377	0.2960	0.4057	0.3775	0.4115	0.5293	0.7766	0.5729	0.5765	0.3527	0.6223	0.5205	0.6185
	L1M4	0.2181	0.2510	0.3021	0.2406	0.2042	0.3133	0.5714	0.3014	0.4779	0.2856	0.3474	0.8664	0.3479
	L2M1	0.4002	0.2039	0.2449	0.3269	0.3119	0.3486	0.4240	0.2363	0.3399	0.2863	0.3438	0.4012	0.3691
	L2M2	0.2497	0.1740	0.2477	0.2015	0.3832	0.2008	0.2717	0.2568	0.3178	0.1778	0.1894	0.1896	0.2197
	L2M3	0.3084	0.3584	0.3831	0.3847	0.3671	0.4509	0.3813	0.4086	0.3535	0.2929	0.4756	0.3215	0.3154
	L3M1	0.4141	0.3957	0.4389	0.3283	0.4020	0.3385	0.5018	0.3226	0.3816	0.3913	0.5121	0.6150	0.4986
	L3M2	0.5443	0.3395	0.3597	0.5391	0.6992	0.4393	0.6054	0.2968	0.3890	0.4044	0.4206	0.4254	0.6491
	L3M3	0.4201	0.3634	0.3209	0.6457	0.4384	0.3389	0.3507	0.3155	0.5479	0.4384	0.3497	0.4415	0.4533
	Avg	**0.356529**	**0.323841**	**0.405629**	**0.408357**	**0.406491**	**0.393749**	**0.514572**	**0.369103**	**0.409882**	**0.352640**	**0.451372**	**0.488905**	**0.486465**
	Std	**0.106066**	**0.093208**	**0.164810**	**0.152972**	**0.130222**	**0.106675**	**0.173428**	**0.122430**	**0.092746**	**0.094564**	**0.150456**	**0.182419**	**0.180130**
	SEM	**0.033541**	**0.029475**	**0.052118**	**0.048374**	**0.041180**	**0.033733**	**0.054843**	**0.038716**	**0.029329**	**0.029904**	**0.047578**	**0.057686**	**0.056962**

Day 2

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**First**	**Middle**	**Last**
Freq (10s)	L1M1	20.5	17.5	20.0	20.0	20.0	22.0	23.0	25.5	24.5	26.0	29.0	33.0	31.0
	L1M2	19.0	19.0	18.0	19.0	19.0	22.0	23.0	24.0	25.0	27.0	27.0	32.0	31.0
	L1M3	19.0	17.5	19.0	17.0	17.0	21.0	23.0	26.5	24.0	25.0	28.0	33.0	32.0
	L1M4	19.0	20.0	18.0	17.0	18.5	20.0	23.0	24.0	27.0	28.0	27.0	29.0	31.0
	L2M1	28.0	23.0	21.0	26.0	25.0	27.0	24.0	26.0	24.0	22.0	25.0	27.0	27.0
	L2M2	29.0	22.0	23.0	24.0	25.0	27.0	24.0	26.0	24.0	22.0	26.0	26.0	28.0
	L2M3	23.5	22.0	24.0	25.0	24.0	21.0	24.0	24.0	27.0	27.0	30.0	25.0	30.0
	L3M1	26.0	26.5	28.0	27.0	29.0	30.0	31.0	32.0	31.0	30.0	31.5	34.0	31.0
	L3M2	24.0	26.0	26.0	27.0	28.0	30.0	31.0	32.0	31.0	32.5	31.0	33.0	31.0
	L3M3	25.0	24.0	26.0	27.0	29.0	30.0	25.0	27.0	26.0	30.0	28.0	31.0	30.0
Freq (60s)	L1M1	123.0	105.0	120.0	120.0	120.0	132.0	138.0	153.0	147.0	156.0	174.0	198.0	186.0
	L1M2	114.0	114.0	108.0	114.0	114.0	132.0	138.0	144.0	150.0	162.0	162.0	192.0	186.0
	L1M3	114.0	105.0	114.0	102.0	102.0	126.0	138.0	159.0	144.0	150.0	168.0	198.0	192.0
	L1M4	114.0	120.0	108.0	102.0	111.0	120.0	138.0	144.0	162.0	168.0	162.0	174.0	186.0
	L2M1	168.0	138.0	126.0	156.0	150.0	162.0	144.0	156.0	144.0	132.0	150.0	162.0	162.0
	L2M2	174.0	132.0	138.0	144.0	150.0	162.0	144.0	156.0	144.0	132.0	156.0	156.0	168.0
	L2M3	141.0	132.0	144.0	150.0	144.0	126.0	144.0	144.0	162.0	162.0	180.0	150.0	180.0
	L3M1	156.0	159.0	168.0	162.0	174.0	180.0	186.0	192.0	186.0	180.0	189.0	204.0	186.0
	L3M2	144.0	156.0	156.0	162.0	168.0	180.0	186.0	192.0	186.0	195.0	186.0	198.0	186.0
	L3M3	150.0	144.0	156.0	162.0	174.0	180.0	150.0	162.0	156.0	180.0	168.0	186.0	180.0
	Avg	**139.8**	**130.5**	**133.8**	**137.4**	**140.7**	**150.0**	**150.6**	**160.2**	**158.1**	**161.7**	**169.5**	**181.8**	**181.2**
	Std	**22.7**	**19.4**	**21.7**	**25.2**	**27.2**	**25.1**	**19.1**	**18.0**	**16.3**	**20.4**	**12.7**	**19.8**	**9.3**
	SEM	**7.2**	**6.1**	**6.9**	**8.0**	**8.6**	**7.9**	**6.0**	**5.7**	**5.1**	**6.5**	**4.0**	**6.3**	**2.9**
Vt (Total)	L1M1	0.02222	0.02473	0.03382	0.02206	0.01621	0.02200	0.03232	0.02579	0.02061	0.03026	0.03315	0.03695	0.03473
	L1M2	0.02083	0.02447	0.02452	0.02555	0.02092	0.02548	0.02278	0.02280	0.02408	0.04385	0.02770	0.03310	0.02707
	L1M3	0.04457	0.01842	0.03222	0.02223	0.03157	0.03188	0.03632	0.02611	0.02665	0.02567	0.04010	0.05308	0.03322
	L1M4	0.02230	0.02728	0.02491	0.01974	0.01593	0.02551	0.02367	0.02280	0.03151	0.04293	0.02053	0.02281	0.03151
	L2M1	0.02073	0.01221	0.01586	0.02445	0.01557	0.01700	0.01913	0.01909	0.02226	0.01735	0.05009	0.02405	0.04886
	L2M2	0.01959	0.01703	0.01945	0.03155	0.01629	0.01566	0.02378	0.02850	0.01257	0.02054	0.03636	0.02467	0.04291
	L2M3	0.01959	0.02571	0.02061	0.02529	0.03376	0.04027	0.02145	0.02518	0.01882	0.02292	0.02846	0.02488	0.02552
	L3M1	0.02020	0.01687	0.01964	0.01791	0.02420	0.03722	0.01661	0.01701	0.02269	0.03141	0.02847	0.06769	0.04797
	L3M2	0.03139	0.01712	0.02292	0.01628	0.02572	0.01978	0.01849	0.01753	0.02367	0.04853	0.02880	0.02734	0.04707
	L3M3	0.02733	0.02199	0.01887	0.02141	0.02610	0.01700	0.02203	0.02867	0.02514	0.02246	0.02363	0.03229	0.04231
Vt (avg vol/breath)	L1M1	0.0010839	0.0014131	0.0016910	0.0011030	0.0008105	0.0010000	0.0014052	0.0010114	0.0008412	0.0011638	0.0011431	0.0011197	0.0011203
(raw)	L1M2	0.0010963	0.0012879	0.0013622	0.0013447	0.0011011	0.0011582	0.0009904	0.0009500	0.0009632	0.0016241	0.0010259	0.0010344	0.0008732
	L1M3	0.0023458	0.0010526	0.0016958	0.0013076	0.0018571	0.0015181	0.0015791	0.0009853	0.0011104	0.0010268	0.0014321	0.0016085	0.0010381
	L1M4	0.0011737	0.0013640	0.0013839	0.0011612	0.0008611	0.0012755	0.0010291	0.0009500	0.0011670	0.0015332	0.0007604	0.0007866	0.0010165
	L2M1	0.0007404	0.0005309	0.0007552	0.0009404	0.0006228	0.0006296	0.0007971	0.0007342	0.0009275	0.0007886	0.0020036	0.0008907	0.0018096
	L2M2	0.0006755	0.0007741	0.0008457	0.0013146	0.0006516	0.0005800	0.0009908	0.0010962	0.0005238	0.0009336	0.0013985	0.0009488	0.0015325
	L2M3	0.0008336	0.0011686	0.0008588	0.0010116	0.0014067	0.0019176	0.0008938	0.0010492	0.0006970	0.0008489	0.0009487	0.0009952	0.0008507
	L3M1	0.0007769	0.0006366	0.0007014	0.0006633	0.0008345	0.0012407	0.0005358	0.0005316	0.0007319	0.0010470	0.0009038	0.0019909	0.0015474
	L3M2	0.0013079	0.0006585	0.0008815	0.0006030	0.0009186	0.0006593	0.0005965	0.0005478	0.0007635	0.0014932	0.0009290	0.0008285	0.0015184
	L3M3	0.0010932	0.0009163	0.0007258	0.0007930	0.0009000	0.0005667	0.0008812	0.0010619	0.0009669	0.0007487	0.0008439	0.0010416	0.0014103
Vt (avg vol/breath)	L1M1	0.00554	0.00722	0.00864	0.00564	0.00414	0.00511	0.00718	0.00517	0.00430	0.00595	0.00584	0.00572	0.00572
	L1M2	0.00560	0.00658	0.00696	0.00687	0.00563	0.00592	0.00506	0.00485	0.00492	0.00830	0.00524	0.00529	0.00446
	L1M3	0.01199	0.00538	0.00867	0.00668	0.00949	0.00776	0.00807	0.00503	0.00567	0.00525	0.00732	0.00822	0.00530
	L1M4	0.00600	0.00697	0.00707	0.00593	0.00440	0.00652	0.00526	0.00485	0.00596	0.00783	0.00389	0.00402	0.00519
	L2M1	0.00429	0.00307	0.00437	0.00544	0.00361	0.00365	0.00462	0.00425	0.00537	0.00457	0.01160	0.00516	0.01048
	L2M2	0.00391	0.00448	0.00490	0.00761	0.00377	0.00336	0.00574	0.00635	0.00303	0.00541	0.00810	0.00549	0.00887
	L2M3	0.00483	0.00677	0.00497	0.00586	0.00814	0.01110	0.00517	0.00607	0.00404	0.00492	0.00549	0.00576	0.00493
	L3M1	0.00450	0.00369	0.00406	0.00384	0.00483	0.00718	0.00310	0.00308	0.00424	0.00606	0.00523	0.01153	0.00896
	L3M2	0.00757	0.00381	0.00510	0.00349	0.00532	0.00382	0.00345	0.00317	0.00442	0.00865	0.00538	0.00480	0.00879
	L3M3	0.00633	0.00531	0.00420	0.00459	0.00521	0.00328	0.00510	0.00615	0.00560	0.00433	0.00489	0.00603	0.00817
Vt (avg vol/breath/g)	L1M1	0.003483	0.004542	0.005435	0.003545	0.002605	0.003214	0.004516	0.003250	0.002704	0.003740	0.003674	0.003599	0.003601
	L1M2	0.003864	0.004539	0.004801	0.004739	0.003880	0.004082	0.003490	0.003348	0.003394	0.005723	0.003616	0.003645	0.003077
	L1M3	0.008946	0.004014	0.006467	0.004987	0.007082	0.005789	0.006022	0.003757	0.004234	0.003916	0.005461	0.006134	0.003959
	L1M4	0.004165	0.004840	0.004911	0.004121	0.003056	0.004526	0.003652	0.003371	0.004141	0.005441	0.002698	0.002791	0.003607
	L2M1	0.002478	0.001777	0.002528	0.003147	0.002084	0.002107	0.002668	0.002457	0.003104	0.002639	0.006706	0.002981	0.006057
	L2M2	0.002027	0.002322	0.002537	0.003944	0.001955	0.001740	0.002973	0.003288	0.001571	0.002801	0.004195	0.002847	0.004598
	L2M3	0.003472	0.004868	0.003577	0.004214	0.005859	0.007988	0.003723	0.004370	0.002903	0.003536	0.003952	0.004145	0.003543
	L3M1	0.002307	0.001890	0.002083	0.001970	0.002478	0.003684	0.001591	0.001578	0.002173	0.003109	0.002684	0.005911	0.004595
	L3M2	0.004303	0.002166	0.002900	0.001984	0.003022	0.002169	0.001962	0.001802	0.002512	0.004912	0.003056	0.002726	0.004995
	L3M3	0.003836	0.003215	0.002547	0.002783	0.003158	0.001988	0.003092	0.003726	0.003393	0.002627	0.002961	0.003655	0.004949
	Avg	**0.003888**	**0.003417**	**0.003778**	**0.003543**	**0.003518**	**0.003729**	**0.003369**	**0.003095**	**0.003013**	**0.003844**	**0.003900**	**0.003843**	**0.004298**
	Std	**0.001947**	**0.001284**	**0.001513**	**0.001059**	**0.001677**	**0.001985**	**0.001267**	**0.000885**	**0.000830**	**0.001150**	**0.001293**	**0.001240**	**0.000902**
	SEM	**0.000616**	**0.000406**	**0.000479**	**0.000335**	**0.000530**	**0.000628**	**0.000401**	**0.000280**	**0.000263**	**0.000364**	**0.000409**	**0.000392**	**0.000285**
Minute Ventilation	L1M1	0.4285	0.4769	0.6522	0.4254	0.3126	0.4242	0.6232	0.4973	0.3974	0.5835	0.6392	0.7125	0.6697
(mL/min/g)	L1M2	0.4404	0.5174	0.5185	0.5403	0.4423	0.5388	0.4817	0.4821	0.5092	0.9272	0.5857	0.6999	0.5724
	L1M3	1.0198	0.4215	0.7372	0.5086	0.7223	0.7294	0.8310	0.5974	0.6098	0.5873	0.9175	1.2145	0.7601
	L1M4	0.4748	0.5808	0.5304	0.4203	0.3392	0.5432	0.5040	0.4855	0.6709	0.9141	0.4371	0.4857	0.6709
	L2M1	0.4163	0.2452	0.3185	0.4910	0.3127	0.3414	0.3841	0.3833	0.4470	0.3484	1.0059	0.4829	0.9812
	L2M2	0.3526	0.3065	0.3501	0.5679	0.2932	0.2819	0.4280	0.5130	0.2263	0.3697	0.6545	0.4441	0.7724
	L2M3	0.4896	0.6426	0.5151	0.6321	0.8438	1.0065	0.5361	0.6293	0.4704	0.5728	0.7113	0.6218	0.6378
	L3M1	0.3599	0.3005	0.3499	0.3191	0.4311	0.6631	0.2959	0.3030	0.4042	0.5596	0.5072	1.2059	0.8546
	L3M2	0.6196	0.3379	0.4524	0.3213	0.5077	0.3904	0.3650	0.3460	0.4672	0.9579	0.5685	0.5397	0.9291
	L3M3	0.5754	0.4630	0.3973	0.4508	0.5495	0.3579	0.4638	0.6036	0.5293	0.4729	0.4975	0.6799	0.8908
	Avg	**0.517690**	**0.429233**	**0.482150**	**0.467671**	**0.475442**	**0.527674**	**0.491290**	**0.484063**	**0.473164**	**0.629344**	**0.652440**	**0.708682**	**0.773895**
	Std	**0.195736**	**0.130624**	**0.136824**	**0.101309**	**0.185890**	**0.221639**	**0.151089**	**0.111172**	**0.122196**	**0.226194**	**0.183111**	**0.281042**	**0.136629**
	SEM	**0.061897**	**0.041307**	**0.043268**	**0.032037**	**0.058784**	**0.070089**	**0.047778**	**0.035156**	**0.038642**	**0.071529**	**0.057905**	**0.088873**	**0.043206**

Day 3

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Last**
Freq (10s)	L1M1	25.0	23.5	21.0	21.0	23.0	25.0	26.5	27.0	29.0	32.0	32.0	33.0	36.0
	L1M2	23.5	23.5	24.0	19.0	29.0	27.0	25.0	31.0	36.0	33.0	33.0	34.0	34.0
	L1M3	25.0	20.0	25.0	26.0	28.0	28.0	27.0	33.0	33.0	34.0	32.5	36.0	35.0
	L1M4	33.0	42.0	41.0	38.0	39.0	38.0	39.0	39.0	44.0	42.0	39.0	42.0	41.0
	L2M1	30.0	29.0	31.0	30.0	30.0	32.0	31.0	30.0	30.0	27.0	29.0	29.0	29.0
	L2M2	27.0	27.0	29.0	30.0	30.0	32.0	31.0	29.0	29.5	27.0	28.5	29.0	29.0
	L2M3	25.0	25.0	27.0	27.0	28.0	30.0	26.0	25.0	27.0	30.0	29.0	31.0	30.0
	L3M1	36.0	38.0	35.0	35.0	33.0	36.0	38.0	37.0	39.0	36.0	41.0	41.0	40.0
	L3M2	37.0	38.0	35.0	35.0	34.0	37.0	37.0	37.0	38.0	37.0	41.0	40.0	40.0
	L3M3	23.0	22.0	23.0	22.0	21.0	21.0	22.0	21.0	20.0	21.0	24.0	23.0	25.0
Freq (60s)	L1M1	150.0	141.0	126.0	126.0	138.0	150.0	159.0	162.0	174.0	192.0	192.0	198.0	216.0
	L1M2	141.0	141.0	144.0	114.0	174.0	162.0	150.0	186.0	216.0	198.0	198.0	204.0	204.0
	L1M3	150.0	120.0	150.0	156.0	168.0	168.0	162.0	198.0	198.0	204.0	195.0	216.0	210.0
	L1M4	198.0	252.0	246.0	228.0	234.0	228.0	234.0	234.0	264.0	252.0	234.0	252.0	246.0
	L2M1	180.0	174.0	186.0	180.0	180.0	192.0	186.0	180.0	180.0	162.0	174.0	174.0	174.0
	L2M2	162.0	162.0	174.0	180.0	180.0	192.0	186.0	174.0	177.0	162.0	171.0	174.0	174.0
	L2M3	150.0	150.0	162.0	162.0	168.0	180.0	156.0	150.0	162.0	180.0	174.0	186.0	180.0
	L3M1	216.0	228.0	210.0	210.0	198.0	216.0	228.0	222.0	234.0	216.0	246.0	246.0	240.0
	L3M2	222.0	228.0	210.0	210.0	204.0	222.0	222.0	222.0	228.0	222.0	246.0	240.0	240.0
	L3M3	138.0	132.0	138.0	132.0	126.0	126.0	132.0	126.0	120.0	126.0	144.0	138.0	150.0
	Avg	**170.7**	**172.8**	**174.6**	**169.8**	**177.0**	**183.6**	**181.5**	**185.4**	**195.3**	**191.4**	**197.4**	**202.8**	**203.4**
	Std	**31.3**	**46.5**	**38.2**	**38.8**	**31.1**	**33.0**	**35.9**	**34.5**	**41.5**	**35.8**	**34.5**	**36.5**	**33.0**
	SEM	**9.9**	**14.7**	**12.1**	**12.3**	**9.8**	**10.4**	**11.3**	**10.9**	**13.1**	**11.3**	**10.9**	**11.6**	**10.4**
Vt (Total)	L1M1	0.04542	0.02039	0.02766	0.02928	0.04135	0.01495	0.01762	0.02712	0.02312	0.03501	0.02260	0.02264	0.03543
	L1M2	0.01574	0.01711	0.01179	0.01125	0.02308	0.01679	0.01956	0.01622	0.01483	0.02326	0.02170	0.01993	0.02441
	L1M3	0.01362	0.01189	0.01262	0.03341	0.02261	0.01696	0.01639	0.01622	0.01392	0.01886	0.02255	0.01731	0.02584
	L1M4	0.02757	0.05333	0.03941	0.03558	0.03870	0.03417	0.02299	0.04012	0.02682	0.02389	0.02015	0.04684	0.03288
	L2M1	0.01303	0.03630	0.05660	0.03870	0.04429	0.06546	0.06803	0.05589	0.03436	0.04035	0.03559	0.03597	0.05363
	L2M2	0.01429	0.03074	0.05622	0.03823	0.04518	0.04098	0.07386	0.04976	0.04589	0.04129	0.03611	0.04910	0.04865
	L2M3	0.02691	0.02477	0.02135	0.02042	0.02262	0.02298	0.02635	0.02493	0.01879	0.02019	0.02566	0.03545	0.05606
	L3M1	0.01482	0.02202	0.02325	0.02636	0.02144	0.01348	0.01639	0.02178	0.02049	0.02254	0.02692	0.02152	0.02634
	L3M2	0.01746	0.02071	0.02143	0.02402	0.01601	0.01636	0.01848	0.01735	0.02129	0.03682	0.02104	0.02104	0.02196
	L3M3	0.01820	0.01611	0.02505	0.02340	0.02464	0.01711	0.01921	0.07051	0.02367	0.02300	0.02348	0.04068	0.03302
Vt (avg vol/breath)	L1M1	0.0018168	0.0008677	0.0013171	0.0013943	0.0017978	0.0005980	0.0006649	0.0010044	0.0007972	0.0010941	0.0007063	0.0006861	0.0009842
(raw)	L1M2	0.0006698	0.0007281	0.0004913	0.0005921	0.0007959	0.0006219	0.0007824	0.0005232	0.0004119	0.0007048	0.0006576	0.0005862	0.0007179
	L1M3	0.0005448	0.0005945	0.0005048	0.0012850	0.0008075	0.0006057	0.0006070	0.0004915	0.0004218	0.0005547	0.0006938	0.0004808	0.0007383
	L1M4	0.0008355	0.0012698	0.0009612	0.0009363	0.0009923	0.0008992	0.0005895	0.0010287	0.0006095	0.0005688	0.0005167	0.0011152	0.0008020
	L2M1	0.0004343	0.0012517	0.0018258	0.0012900	0.0014763	0.0020456	0.0021945	0.0018630	0.0011453	0.0014944	0.0012272	0.0012403	0.0018493
	L2M2	0.0005293	0.0011385	0.0019386	0.0012743	0.0015060	0.0012806	0.0023826	0.0017159	0.0015556	0.0015293	0.0012670	0.0016931	0.0016776
	L2M3	0.0010764	0.0009908	0.0007907	0.0007563	0.0008079	0.0007660	0.0010135	0.0009972	0.0006959	0.0006730	0.0008848	0.0011435	0.0018687
	L3M1	0.0004117	0.0005795	0.0006643	0.0007531	0.0006497	0.0003744	0.0004313	0.0005886	0.0005254	0.0006261	0.0006566	0.0005249	0.0006585
	L3M2	0.0004719	0.0005450	0.0006123	0.0006863	0.0004709	0.0004422	0.0004995	0.0004689	0.0005603	0.0009951	0.0005132	0.0005260	0.0005490
	L3M3	0.0007913	0.0007323	0.0010891	0.0010636	0.0011733	0.0008148	0.0008732	0.0033576	0.0011835	0.0010952	0.0009783	0.0017687	0.0013208
Vt (avg vol/breath)	L1M1	0.01052	0.00502	0.00763	0.00807	0.01041	0.00346	0.00385	0.00582	0.00462	0.00633	0.00409	0.00397	0.00570
	L1M2	0.00388	0.00422	0.00284	0.00343	0.00461	0.00360	0.00453	0.00303	0.00239	0.00408	0.00381	0.00339	0.00416
	L1M3	0.00315	0.00344	0.00292	0.00744	0.00468	0.00351	0.00351	0.00285	0.00244	0.00321	0.00402	0.00278	0.00427
	L1M4	0.00484	0.00735	0.00557	0.00542	0.00575	0.00521	0.00341	0.00596	0.00353	0.00329	0.00299	0.00646	0.00464
	L2M1	0.00251	0.00725	0.01057	0.00747	0.00855	0.01184	0.01271	0.01079	0.00663	0.00865	0.00711	0.00718	0.01071
	L2M2	0.00306	0.00659	0.01122	0.00738	0.00872	0.00741	0.01380	0.00993	0.00901	0.00885	0.00734	0.00980	0.00971
	L2M3	0.00623	0.00574	0.00458	0.00438	0.00468	0.00444	0.00587	0.00577	0.00403	0.00390	0.00512	0.00662	0.01082
	L3M1	0.00238	0.00336	0.00385	0.00436	0.00376	0.00217	0.00250	0.00341	0.00304	0.00363	0.00380	0.00304	0.00381
	L3M2	0.00273	0.00316	0.00355	0.00397	0.00273	0.00256	0.00289	0.00272	0.00324	0.00576	0.00297	0.00305	0.00318
	L3M3	0.00458	0.00424	0.00631	0.00616	0.00679	0.00472	0.00506	0.01944	0.00685	0.00634	0.00566	0.01024	0.00765
Vt (avg vol/breath/g)	L1M1	0.005780	0.002760	0.004190	0.004436	0.005719	0.001902	0.002115	0.003195	0.002536	0.003481	0.002247	0.002183	0.003131
	L1M2	0.002281	0.002480	0.001673	0.002017	0.002711	0.002118	0.002665	0.001782	0.001403	0.002401	0.002240	0.001996	0.002445
	L1M3	0.001959	0.002138	0.001815	0.004621	0.002904	0.002178	0.002183	0.001768	0.001517	0.001995	0.002495	0.001729	0.002655
	L1M4	0.002862	0.004350	0.003293	0.003208	0.003400	0.003081	0.002020	0.003524	0.002088	0.001949	0.001770	0.003821	0.002748
	L2M1	0.001290	0.003717	0.005421	0.003830	0.004384	0.006074	0.006516	0.005532	0.003401	0.004437	0.003644	0.003683	0.005491
	L2M2	0.001387	0.002983	0.005079	0.003339	0.003946	0.003355	0.006242	0.004495	0.004076	0.004007	0.003319	0.004436	0.004395
	L2M3	0.003800	0.003498	0.002792	0.002670	0.002852	0.002704	0.003578	0.003521	0.002457	0.002376	0.003124	0.004037	0.006597
	L3M1	0.001109	0.001561	0.001789	0.002028	0.001750	0.001008	0.001162	0.001585	0.001415	0.001686	0.001768	0.001414	0.001773
	L3M2	0.001346	0.001554	0.001746	0.001957	0.001343	0.001261	0.001425	0.001337	0.001598	0.002838	0.001464	0.001500	0.001566
	L3M3	0.002450	0.002267	0.003372	0.003293	0.003633	0.002523	0.002704	0.010396	0.003664	0.003391	0.003029	0.005476	0.004090
	Avg	**0.002426**	**0.002731**	**0.003117**	**0.003140**	**0.003264**	**0.002621**	**0.003061**	**0.003714**	**0.002416**	**0.002856**	**0.002510**	**0.003028**	**0.003489**
	Std	**0.001447**	**0.000921**	**0.001412**	**0.000974**	**0.001269**	**0.001418**	**0.001875**	**0.002720**	**0.000995**	**0.000935**	**0.000740**	**0.001432**	**0.001632**
	SEM	**0.000458**	**0.000291**	**0.000446**	**0.000308**	**0.000401**	**0.000449**	**0.000593**	**0.000860**	**0.000315**	**0.000296**	**0.000234**	**0.000453**	**0.000516**
Minute Ventilation	L1M1	0.8670	0.3892	0.5280	0.5589	0.7893	0.2854	0.3363	0.5177	0.4413	0.6683	0.4314	0.4322	0.6763
(mL/min/g)	L1M2	0.3217	0.3496	0.2409	0.2299	0.4716	0.3431	0.3997	0.3315	0.3031	0.4753	0.4434	0.4073	0.4988
	L1M3	0.2939	0.2566	0.2723	0.7209	0.4879	0.3660	0.3537	0.3500	0.3004	0.4070	0.4866	0.3735	0.5576
	L1M4	0.5667	1.0963	0.8101	0.7314	0.7955	0.7024	0.4726	0.8247	0.5513	0.4911	0.4142	0.9629	0.6759
	L2M1	0.2321	0.6467	1.0084	0.6895	0.7890	1.1662	1.2120	0.9957	0.6121	0.7189	0.6340	0.6408	0.9554
	L2M2	0.2246	0.4832	0.8837	0.6010	0.7102	0.6442	1.1610	0.7822	0.7214	0.6491	0.5676	0.7718	0.7648
	L2M3	0.5700	0.5247	0.4523	0.4326	0.4792	0.4868	0.5582	0.5281	0.3980	0.4277	0.5436	0.7509	1.1875
	L3M1	0.2395	0.3558	0.3757	0.4259	0.3464	0.2178	0.2648	0.3519	0.3311	0.3642	0.4350	0.3477	0.4256
	L3M2	0.2988	0.3544	0.3667	0.4111	0.2740	0.2800	0.3163	0.2969	0.3643	0.6301	0.3601	0.3601	0.3758
	L3M3	0.3381	0.2993	0.4654	0.4347	0.4578	0.3179	0.3569	1.3099	0.4397	0.4273	0.4362	0.7557	0.6134
	Avg	**0.395242**	**0.475579**	**0.540347**	**0.523576**	**0.560090**	**0.480964**	**0.543144**	**0.628856**	**0.446273**	**0.525883**	**0.475209**	**0.580289**	**0.673111**
	Std	**0.208360**	**0.246846**	**0.267086**	**0.163809**	**0.194503**	**0.289058**	**0.349161**	**0.339260**	**0.140791**	**0.127785**	**0.082745**	**0.222068**	**0.247133**
	SEM	**0.065889**	**0.078060**	**0.084460**	**0.051801**	**0.061507**	**0.091408**	**0.110415**	**0.107284**	**0.044522**	**0.040409**	**0.026166**	**0.070224**	**0.078150**

Day 5

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Last**
Freq (10s)	L1M1	33.0	36.5	35.0	37.0	34.0	38.0	37.0	35.5	35.0	35.5	32.0	33.0	35.0
	L1M2	33.0	36.0	35.0	37.0	35.0	34.0	37.0	37.5	35.0	35.0	32.0	32.5	34.0
	L1M3	36.0	36.5	38.5	39.0	40.5	39.0	40.0	42.0	39.0	43.0	43.0	44.0	46.0
	L1M4	37.0	37.0	36.5	39.0	40.0	38.5	40.0	42.0	40.0	44.0	43.0	44.0	46.0
	L2M1	35.0	36.0	40.0	39.0	41.0	42.0	40.0	44.0	40.0	35.5	37.5	40.0	41.5
	L2M2	39.0	36.0	39.0	39.0	41.0	41.0	40.0	44.0	41.0	38.0	37.0	40.0	41.0
	L2M3	36.0	37.0	36.0	34.0	35.0	36.0	37.0	38.5	35.5	36.0	36.0	39.0	38.0
	L3M1	45.5	45.0	42.0	39.0	39.0	45.0	38.0	43.0	40.0	44.0	42.0	44.0	48.0
	L3M2	37.0	44.0	41.0	40.0	38.0	40.0	39.0	44.0	44.0	42.0	39.0	43.0	41.0
	L3M3	46.0	45.5	37.0	38.0	38.0	45.0	39.0	43.0	42.0	43.0	41.0	45.0	48.0
					0.0			2.0			4.0			6.0
Freq (60s)	L1M1	198.0	219.0	210.0	222.0	204.0	228.0	222.0	213.0	210.0	213.0	192.0	198.0	210.0
	L1M2	198.0	216.0	210.0	222.0	210.0	204.0	222.0	225.0	210.0	210.0	192.0	195.0	204.0
	L1M3	216.0	219.0	231.0	234.0	243.0	234.0	240.0	252.0	234.0	258.0	258.0	264.0	276.0
	L1M4	222.0	222.0	219.0	234.0	240.0	231.0	240.0	252.0	240.0	264.0	258.0	264.0	276.0
	L2M1	210.0	216.0	240.0	234.0	246.0	252.0	240.0	264.0	240.0	213.0	225.0	240.0	249.0
	L2M2	234.0	216.0	234.0	234.0	246.0	246.0	240.0	264.0	246.0	228.0	222.0	240.0	246.0
	L2M3	216.0	222.0	216.0	204.0	210.0	216.0	222.0	231.0	213.0	216.0	216.0	234.0	228.0
	L3M1	273.0	270.0	252.0	234.0	234.0	270.0	228.0	258.0	240.0	264.0	252.0	264.0	288.0
	L3M2	222.0	264.0	246.0	240.0	228.0	240.0	234.0	264.0	264.0	252.0	234.0	258.0	246.0
	L3M3	276.0	273.0	222.0	228.0	228.0	270.0	234.0	258.0	252.0	258.0	246.0	270.0	288.0
	Avg	**226.5**	**233.7**	**228.0**	**228.6**	**228.9**	**239.1**	**232.2**	**248.1**	**234.9**	**237.6**	**229.5**	**242.7**	**251.1**
	Std	**27.5**	**24.6**	**14.9**	**10.4**	**15.9**	**21.4**	**8.0**	**18.4**	**18.4**	**23.5**	**24.7**	**27.3**	**30.7**
	SEM	**8.7**	**7.8**	**4.7**	**3.3**	**5.0**	**6.8**	**2.5**	**5.8**	**5.8**	**7.4**	**7.8**	**8.6**	**9.7**
Vt (Total)	L1M1	0.02025	0.01591	0.01985	0.02471	0.02300	0.02197	0.03110	0.01524	0.02668	0.02593	0.01528	0.01627	0.01935
	L1M2	0.02185	0.01969	0.02113	0.01925	0.02182	0.02262	0.02304	0.01772	0.02197	0.02419	0.01466	0.02181	0.01850
	L1M3	0.02089	0.02537	0.03369	0.02227	0.01750	0.01947	0.02564	0.02473	0.01925	0.02776	0.02258	0.02429	0.03296
	L1M4	0.02809	0.02272	0.02560	0.02302	0.02939	0.02291	0.03016	0.02472	0.02577	0.01967	0.02121	0.02416	0.03170
	L2M1	0.04315	0.02939	0.03313	0.02791	0.03754	0.03425	0.02684	0.02724	0.03415	0.04616	0.03417	0.04888	0.03873
	L2M2	0.03151	0.02527	0.02494	0.02629	0.02678	0.02299	0.04369	0.03654	0.03183	0.04953	0.03648	0.04072	0.03383
	L2M3	0.03205	0.04030	0.05327	0.03111	0.03813	0.03059	0.04601	0.03865	0.03502	0.03939	0.03662	0.08233	0.04063
	L3M1	0.02690	0.03139	0.02169	0.01853	0.02449	0.02371	0.02879	0.01918	0.02336	0.04035	0.02482	0.03574	0.04405
	L3M2	0.01945	0.02111	0.02210	0.02505	0.02430	0.03136	0.02092	0.02785	0.02526	0.03612	0.02164	0.02700	0.03836
	L3M3	0.02232	0.02573	0.02598	0.01935	0.02090	0.02568	0.02427	0.01944	0.02322	0.03987	0.02885	0.02609	0.03917
Vt (avg vol/breath)	L1M1	0.0006136	0.0004359	0.0005671	0.0006678	0.0006765	0.0005782	0.0008405	0.0004293	0.0007623	0.0007304	0.0004775	0.0004930	0.0005529
(raw)	L1M2	0.0006621	0.0005469	0.0006037	0.0005203	0.0006234	0.0006653	0.0006227	0.0004725	0.0006277	0.0006911	0.0004581	0.0006711	0.0005441
	L1M3	0.0005803	0.0006951	0.0008751	0.0005710	0.0004321	0.0004992	0.0006410	0.0005888	0.0004936	0.0006456	0.0005251	0.0005520	0.0007165
	L1M4	0.0007592	0.0006141	0.0007014	0.0005903	0.0007348	0.0005951	0.0007540	0.0005886	0.0006443	0.0004470	0.0004933	0.0005491	0.0006891
	L2M1	0.0012329	0.0008164	0.0008283	0.0007156	0.0009156	0.0008155	0.0006710	0.0006191	0.0008538	0.0013003	0.0009112	0.0012220	0.0009333
	L2M2	0.0008079	0.0007019	0.0006395	0.0006741	0.0006532	0.0005607	0.0010923	0.0008305	0.0007763	0.0013034	0.0009859	0.0010180	0.0008251
	L2M3	0.0008903	0.0010892	0.0014797	0.0009150	0.0010894	0.0008497	0.0012435	0.0010039	0.0009865	0.0010942	0.0010172	0.0021110	0.0010692
	L3M1	0.0005912	0.0006976	0.0005164	0.0004751	0.0006279	0.0005269	0.0007576	0.0004460	0.0005840	0.0009170	0.0005910	0.0008123	0.0009177
	L3M2	0.0005257	0.0004798	0.0005390	0.0006263	0.0006395	0.0007840	0.0005364	0.0006330	0.0005741	0.0008600	0.0005549	0.0006279	0.0009356
	L3M3	0.0004852	0.0005655	0.0007022	0.0005092	0.0005500	0.0005707	0.0006223	0.0004521	0.0005529	0.0009272	0.0007037	0.0005798	0.0008160
Vt (avg vol/breath)	L1M1	0.00411	0.00292	0.00380	0.00447	0.00453	0.00387	0.00563	0.00288	0.00511	0.00489	0.00320	0.00330	0.00370
	L1M2	0.00444	0.00366	0.00404	0.00349	0.00418	0.00446	0.00417	0.00317	0.00421	0.00463	0.00307	0.00450	0.00365
	L1M3	0.00389	0.00466	0.00586	0.00383	0.00290	0.00334	0.00429	0.00395	0.00331	0.00433	0.00352	0.00370	0.00480
	L1M4	0.00509	0.00411	0.00470	0.00395	0.00492	0.00399	0.00505	0.00394	0.00432	0.00300	0.00330	0.00368	0.00462
	L2M1	0.00826	0.00547	0.00555	0.00479	0.00613	0.00546	0.00450	0.00415	0.00572	0.00871	0.00611	0.00819	0.00625
	L2M2	0.00541	0.00470	0.00428	0.00452	0.00438	0.00376	0.00732	0.00556	0.00520	0.00873	0.00661	0.00682	0.00553
	L2M3	0.00596	0.00730	0.00991	0.00613	0.00730	0.00569	0.00833	0.00673	0.00661	0.00733	0.00682	0.01414	0.00716
	L3M1	0.00396	0.00467	0.00346	0.00318	0.00421	0.00353	0.00508	0.00299	0.00391	0.00614	0.00396	0.00544	0.00615
	L3M2	0.00352	0.00321	0.00361	0.00420	0.00428	0.00525	0.00359	0.00424	0.00385	0.00576	0.00372	0.00421	0.00627
	L3M3	0.00325	0.00379	0.00470	0.00341	0.00369	0.00382	0.00417	0.00303	0.00370	0.00621	0.00471	0.00388	0.00547
Vt (avg vol/breath/g)	L1M1	0.001512	0.001074	0.001397	0.001645	0.001666	0.001424	0.002070	0.001057	0.001878	0.001799	0.001176	0.001214	0.001362
	L1M2	0.001733	0.001431	0.001580	0.001362	0.001632	0.001741	0.001630	0.001237	0.001643	0.001809	0.001199	0.001756	0.001424
	L1M3	0.001690	0.002025	0.002549	0.001663	0.001259	0.001454	0.001867	0.001715	0.001438	0.001881	0.001530	0.001608	0.002087
	L1M4	0.002068	0.001672	0.001910	0.001608	0.002001	0.001621	0.002054	0.001603	0.001755	0.001218	0.001343	0.001495	0.001877
	L2M1	0.003004	0.001989	0.002018	0.001744	0.002231	0.001987	0.001635	0.001508	0.002080	0.003168	0.002220	0.002977	0.002274
	L2M2	0.001692	0.001470	0.001339	0.001411	0.001368	0.001174	0.002287	0.001739	0.001625	0.002729	0.002064	0.002131	0.001728
	L2M3	0.002639	0.003229	0.004387	0.002713	0.003230	0.002519	0.003687	0.002976	0.002925	0.003244	0.003016	0.006258	0.003170
	L3M1	0.001254	0.001479	0.001095	0.001007	0.001331	0.001117	0.001606	0.000946	0.001238	0.001944	0.001253	0.001722	0.001946
	L3M2	0.001190	0.001086	0.001220	0.001418	0.001447	0.001775	0.001214	0.001433	0.001299	0.001947	0.001256	0.001421	0.002118
	L3M3	0.001246	0.001452	0.001802	0.001307	0.001412	0.001465	0.001597	0.001161	0.001419	0.002380	0.001806	0.001488	0.002095
	Avg	**0.001803**	**0.001691**	**0.001930**	**0.001588**	**0.001758**	**0.001628**	**0.001965**	**0.001537**	**0.001730**	**0.002212**	**0.001686**	**0.002207**	**0.002008**
	Std	**0.000607**	**0.000626**	**0.000966**	**0.000450**	**0.000603**	**0.000411**	**0.000679**	**0.000575**	**0.000494**	**0.000654**	**0.000598**	**0.001507**	**0.000506**
	SEM	**0.000192**	**0.000198**	**0.000306**	**0.000142**	**0.000191**	**0.000130**	**0.000215**	**0.000182**	**0.000156**	**0.000207**	**0.000189**	**0.000476**	**0.000160**
Minute Ventilation	L1M1	0.2993	0.2351	0.2934	0.3652	0.3399	0.3247	0.4596	0.2252	0.3943	0.3832	0.2258	0.2405	0.2860
(mL/min/g)	L1M2	0.3431	0.3092	0.3318	0.3023	0.3426	0.3552	0.3618	0.2783	0.3450	0.3799	0.2302	0.3425	0.2905
	L1M3	0.3651	0.4434	0.5888	0.3892	0.3059	0.3403	0.4481	0.4322	0.3365	0.4852	0.3947	0.4245	0.5761
	L1M4	0.4590	0.3713	0.4183	0.3762	0.4803	0.3744	0.4929	0.4040	0.4211	0.3214	0.3466	0.3948	0.5180
	L2M1	0.6308	0.4296	0.4843	0.4080	0.5488	0.5007	0.3924	0.3982	0.4992	0.6748	0.4995	0.7145	0.5662
	L2M2	0.3958	0.3175	0.3133	0.3303	0.3364	0.2888	0.5489	0.4590	0.3999	0.6222	0.4583	0.5115	0.4250
	L2M3	0.5701	0.7168	0.9475	0.5534	0.6782	0.5441	0.8184	0.6875	0.6229	0.7007	0.6514	1.4645	0.7227
	L3M1	0.3422	0.3993	0.2759	0.2357	0.3116	0.3016	0.3663	0.2440	0.2972	0.5133	0.3157	0.4547	0.5604
	L3M2	0.2642	0.2867	0.3001	0.3402	0.3300	0.4259	0.2841	0.3782	0.3431	0.4905	0.2939	0.3667	0.5210
	L3M3	0.3438	0.3963	0.4002	0.2980	0.3219	0.3955	0.3738	0.2994	0.3576	0.6141	0.4444	0.4018	0.6033
	Avg	**0.401340**	**0.390529**	**0.435374**	**0.359851**	**0.399562**	**0.385126**	**0.454624**	**0.380607**	**0.401676**	**0.518532**	**0.386046**	**0.531604**	**0.506912**
	Std	**0.117957**	**0.132568**	**0.205291**	**0.084807**	**0.126760**	**0.083879**	**0.148569**	**0.134875**	**0.095836**	**0.131558**	**0.132041**	**0.350354**	**0.137311**
	SEM	**0.037301**	**0.041922**	**0.064919**	**0.026818**	**0.040085**	**0.026525**	**0.046982**	**0.042651**	**0.030306**	**0.041602**	**0.041755**	**0.110792**	**0.043422**

Day 8

			0%			2%			4%			6%		
	Mouse	Pre	1st	Middle	Pre	1st	Middle	Pre	1st	Middle	Pre	1st	Middle	Last
Freq (10s)	L1M1	36.0	34.0	33.0	35.0	35.0	36.0	34.0	35.0	38.0	38.0	38.0	35.0	39.0
	L1M2	42.0	40.0	42.0	40.0	40.0	43.0	41.0	40.0	41.0	39.0	35.0	42.0	42.0
	L1M3	35.0	35.0	34.0	35.0	36.0	35.0	35.0	34.0	35.0	37.0	38.0	35.0	38.0
	L1M4	31.0	30.5	30.0	32.0	30.0	32.0	35.0	33.0	34.0	34.5	36.0	34.0	36.5
	L2M1	34.0	32.0	32.0	31.0	33.0	32.0	35.0	33.0	34.0	37.5	38.5	37.0	36.5
	L2M2	29.0	29.0	29.0	28.5	32.0	32.5	33.0	33.0	33.0	36.0	34.0	36.0	36.0
	L2M3	34.0	35.0	34.0	29.0	29.0	30.0	30.0	34.0	37.0	33.0	34.0	36.0	38.0
	L3M1	38.0	38.0	39.0	36.0	38.0	37.0	36.0	35.0	38.0	36.0	37.0	35.0	37.0
	L3M2	40.0	39.5	41.0	36.0	39.0	37.0	35.5	35.0	39.0	36.0	36.0	38.0	37.0
	L3M3	33.0	36.0	32.0	33.0	34.5	35.0	35.0	35.0	33.0	35.0	32.0	33.0	34.0
					0.0			2.0			4.0			6.0
Freq (60s)	L1M1	216.0	204.0	198.0	210.0	210.0	216.0	204.0	210.0	228.0	228.0	228.0	210.0	234.0
	L1M2	252.0	240.0	252.0	240.0	240.0	258.0	246.0	240.0	246.0	234.0	210.0	252.0	252.0
	L1M3	210.0	210.0	204.0	210.0	216.0	210.0	210.0	204.0	210.0	222.0	228.0	210.0	228.0
	L1M4	186.0	183.0	180.0	192.0	180.0	192.0	210.0	198.0	204.0	207.0	216.0	204.0	219.0
	L2M1	204.0	192.0	192.0	186.0	198.0	192.0	210.0	198.0	204.0	225.0	231.0	222.0	219.0
	L2M2	174.0	174.0	174.0	171.0	192.0	195.0	198.0	198.0	198.0	216.0	204.0	216.0	216.0
	L2M3	204.0	210.0	204.0	174.0	174.0	180.0	180.0	204.0	222.0	198.0	204.0	216.0	228.0
	L3M1	228.0	228.0	234.0	216.0	228.0	222.0	216.0	210.0	228.0	216.0	222.0	210.0	222.0
	L3M2	240.0	237.0	246.0	216.0	234.0	222.0	213.0	210.0	234.0	216.0	216.0	228.0	222.0
	L3M3	198.0	216.0	192.0	198.0	207.0	210.0	210.0	210.0	198.0	210.0	192.0	198.0	204.0
	Avg	**211.2**	**209.4**	**207.6**	**201.3**	**207.9**	**209.7**	**209.7**	**208.2**	**217.2**	**217.2**	**215.1**	**216.6**	**224.4**
	Std	**23.8**	**22.0**	**27.2**	**21.3**	**22.3**	**22.1**	**16.4**	**12.3**	**16.7**	**10.6**	**12.7**	**15.1**	**12.6**
	SEM	**7.5**	**7.0**	**8.6**	**6.7**	**7.0**	**7.0**	**5.2**	**3.9**	**5.3**	**3.4**	**4.0**	**4.8**	**4.0**
Vt (Total)	L1M1	0.04135	0.02685	0.03303	0.05893	0.02344	0.02725	0.03936	0.01817	0.02539	0.03347	0.03299	0.03371	0.02815
	L1M2	0.01972	0.02562	0.01948	0.04138	0.02377	0.02052	0.02115	0.02507	0.03059	0.02757	0.03298	0.03027	0.04085
	L1M3	0.02237	0.03064	0.02772	0.03298	0.02797	0.02602	0.04202	0.02342	0.02648	0.03626	0.03299	0.03496	0.03869
	L1M4	0.02357	0.02495	0.02739	0.02096	0.03070	0.01998	0.01785	0.02682	0.03959	0.02671	0.03348	0.03192	0.02803
	L2M1	0.01608	0.01560	0.01383	0.02328	0.01523	0.01513	0.01609	0.01701	0.01930	0.01643	0.02037	0.02015	0.02221
	L2M2	0.02260	0.01924	0.01340	0.01143	0.01393	0.01445	0.01609	0.02327	0.01789	0.01216	0.02747	0.02074	0.02339
	L2M3	0.02862	0.01720	0.01792	0.01666	0.01725	0.01736	0.01428	0.02153	0.01778	0.01865	0.01563	0.02372	0.01817
	L3M1	0.02202	0.01823	0.02121	0.03764	0.04076	0.02618	0.03162	0.02241	0.01854	0.05895	0.05924	0.04765	0.03789
	L3M2	0.01750	0.02316	0.03068	0.03225	0.02971	0.02629	0.02837	0.01952	0.01942	0.03105	0.03296	0.03114	0.03009
	L3M3	0.01946	0.01979	0.02680	0.01651	0.03199	0.02596	0.02166	0.03847	0.05503	0.02785	0.03490	0.03386	0.03296
Vt (avg vol/breath)	L1M1	0.0011486	0.0007897	0.0010009	0.0016837	0.0006697	0.0007569	0.0011576	0.0005191	0.0006682	0.0008808	0.0008682	0.0009631	0.0007218
(raw)	L1M2	0.0004695	0.0006405	0.0004638	0.0010345	0.0005943	0.0004772	0.0005159	0.0006268	0.0007461	0.0007069	0.0009423	0.0007207	0.0009726
	L1M3	0.0006391	0.0008754	0.0008153	0.0009423	0.0007769	0.0007434	0.0012006	0.0006888	0.0007566	0.0009800	0.0008682	0.0009989	0.0010182
	L1M4	0.0007603	0.0008180	0.0009130	0.0006550	0.0010233	0.0006244	0.0005100	0.0008127	0.0011644	0.0007742	0.0009300	0.0009388	0.0007679
	L2M1	0.0004729	0.0004875	0.0004322	0.0007510	0.0004615	0.0004728	0.0004597	0.0005155	0.0005676	0.0004381	0.0005291	0.0005446	0.0006085
	L2M2	0.0007793	0.0006634	0.0004621	0.0004011	0.0004353	0.0004446	0.0004876	0.0007052	0.0005421	0.0003378	0.0008079	0.0005761	0.0006497
	L2M3	0.0008418	0.0004914	0.0005271	0.0005745	0.0005948	0.0005787	0.0004760	0.0006332	0.0004805	0.0005652	0.0004597	0.0006589	0.0004782
	L3M1	0.0005795	0.0004797	0.0005438	0.0010456	0.0010726	0.0007076	0.0008783	0.0006403	0.0004879	0.0016375	0.0016011	0.0013614	0.0010241
	L3M2	0.0004375	0.0005863	0.0007483	0.0008958	0.0007618	0.0007105	0.0007992	0.0005577	0.0004979	0.0008625	0.0009156	0.0008195	0.0008132
	L3M3	0.0005897	0.0005497	0.0008375	0.0005003	0.0009272	0.0007417	0.0006189	0.0010991	0.0016676	0.0007957	0.0010906	0.0010261	0.0009694
Vt (avg vol/breath)	L1M1	0.00967	0.00665	0.00843	0.01418	0.00564	0.00637	0.00975	0.00437	0.00563	0.00742	0.00731	0.00811	0.00608
	L1M2	0.00395	0.00539	0.00391	0.00871	0.00500	0.00402	0.00434	0.00528	0.00628	0.00595	0.00793	0.00607	0.00819
	L1M3	0.00538	0.00737	0.00686	0.00793	0.00654	0.00626	0.01011	0.00580	0.00637	0.00825	0.00731	0.00841	0.00857
	L1M4	0.00640	0.00689	0.00769	0.00552	0.00862	0.00526	0.00429	0.00684	0.00980	0.00652	0.00783	0.00790	0.00647
	L2M1	0.00398	0.00410	0.00364	0.00632	0.00389	0.00398	0.00387	0.00434	0.00478	0.00369	0.00445	0.00459	0.00512
	L2M2	0.00656	0.00559	0.00389	0.00338	0.00367	0.00374	0.00411	0.00594	0.00456	0.00284	0.00680	0.00485	0.00547
	L2M3	0.00709	0.00414	0.00444	0.00484	0.00501	0.00487	0.00401	0.00533	0.00405	0.00476	0.00387	0.00555	0.00403
	L3M1	0.00488	0.00404	0.00458	0.00880	0.00903	0.00596	0.00740	0.00539	0.00411	0.01379	0.01348	0.01146	0.00862
	L3M2	0.00368	0.00494	0.00630	0.00754	0.00641	0.00598	0.00673	0.00470	0.00419	0.00726	0.00771	0.00690	0.00685
	L3M3	0.00395	0.00368	0.00561	0.00335	0.00621	0.00497	0.00415	0.00736	0.01117	0.00533	0.00731	0.00687	0.00650
Vt (avg vol/breath/g)	L1M1	0.002424	0.001666	0.002112	0.003553	0.001413	0.001597	0.002443	0.001096	0.001410	0.001859	0.001832	0.002032	0.001523
	L1M2	0.000976	0.001332	0.000964	0.002151	0.001235	0.000992	0.001072	0.001303	0.001551	0.001470	0.001959	0.001498	0.002022
	L1M3	0.001483	0.002031	0.001891	0.002186	0.001802	0.001724	0.002785	0.001598	0.001755	0.002273	0.002014	0.002317	0.002362
	L1M4	0.001735	0.001867	0.002083	0.001495	0.002335	0.001425	0.001164	0.001855	0.002657	0.001767	0.002122	0.002142	0.001752
	L2M1	0.001053	0.001086	0.000963	0.001673	0.001028	0.001053	0.001024	0.001148	0.001264	0.000976	0.001179	0.001213	0.001355
	L2M2	0.001612	0.001373	0.000956	0.000830	0.000901	0.000920	0.001009	0.001459	0.001122	0.000699	0.001671	0.001192	0.001344
	L2M3	0.002279	0.001330	0.001427	0.001555	0.001610	0.001567	0.001289	0.001714	0.001301	0.001530	0.001245	0.001784	0.001295
	L3M1	0.001554	0.001286	0.001458	0.002804	0.002876	0.001897	0.002355	0.001717	0.001308	0.004391	0.004293	0.003651	0.002746
	L3M2	0.001050	0.001407	0.001795	0.002149	0.001827	0.001704	0.001917	0.001338	0.001195	0.002069	0.002196	0.001966	0.001951
	L3M3	0.001088	0.001015	0.001546	0.000923	0.001711	0.001369	0.001142	0.002029	0.003078	0.001469	0.002013	0.001894	0.001789
	Avg	**0.001525**	**0.001439**	**0.001520**	**0.001932**	**0.001674**	**0.001425**	**0.001620**	**0.001526**	**0.001664**	**0.001850**	**0.002052**	**0.001969**	**0.001814**
	Std	**0.000513**	**0.000323**	**0.000451**	**0.000829**	**0.000597**	**0.000337**	**0.000686**	**0.000308**	**0.000667**	**0.001010**	**0.000861**	**0.000701**	**0.000474**
	SEM	**0.000162**	**0.000102**	**0.000143**	**0.000262**	**0.000189**	**0.000107**	**0.000217**	**0.000097**	**0.000211**	**0.000319**	**0.000272**	**0.000222**	**0.000150**
Minute Ventilation	L1M1	0.5236	0.3400	0.4182	0.7462	0.2968	0.3450	0.4984	0.2301	0.3215	0.4238	0.4177	0.4268	0.3564
(mL/min/g)	L1M2	0.2460	0.3196	0.2430	0.5162	0.2965	0.2560	0.2638	0.3127	0.3816	0.3439	0.4114	0.3776	0.5096
	L1M3	0.3113	0.4264	0.3858	0.4590	0.3893	0.3621	0.5848	0.3259	0.3685	0.5046	0.4591	0.4866	0.5385
	L1M4	0.3227	0.3416	0.3750	0.2870	0.4203	0.2735	0.2444	0.3672	0.5420	0.3657	0.4584	0.4370	0.3838
	L2M1	0.2149	0.2085	0.1848	0.3111	0.2036	0.2022	0.2150	0.2273	0.2579	0.2196	0.2722	0.2693	0.2968
	L2M2	0.2805	0.2388	0.1663	0.1419	0.1729	0.1794	0.1997	0.2888	0.2221	0.1509	0.3410	0.2574	0.2903
	L2M3	0.4649	0.2794	0.2911	0.2706	0.2802	0.2820	0.2320	0.3497	0.2888	0.3030	0.2539	0.3853	0.2952
	L3M1	0.3543	0.2933	0.3413	0.6056	0.6558	0.4212	0.5087	0.3606	0.2983	0.9485	0.9531	0.7666	0.6096
	L3M2	0.2519	0.3333	0.4416	0.4642	0.4276	0.3784	0.4083	0.2810	0.2795	0.4469	0.4744	0.4482	0.4331
	L3M3	0.2155	0.2192	0.2968	0.1828	0.3543	0.2875	0.2399	0.4260	0.6094	0.3084	0.3865	0.3750	0.3650
	Avg	**0.318560**	**0.300010**	**0.314389**	**0.398455**	**0.349724**	**0.298736**	**0.339506**	**0.316940**	**0.356969**	**0.401530**	**0.442776**	**0.422988**	**0.407827**
	Std	**0.103983**	**0.066605**	**0.094929**	**0.192746**	**0.137198**	**0.077420**	**0.145328**	**0.062507**	**0.125640**	**0.218887**	**0.194897**	**0.141343**	**0.111876**
	SEM	**0.032882**	**0.021062**	**0.030019**	**0.060952**	**0.043386**	**0.024482**	**0.045957**	**0.019766**	**0.039731**	**0.069218**	**0.061632**	**0.044696**	**0.035378**

Day 11

			0%			2%			4%			6%		
	Mouse	Pre	1st	Middle	Pre	1st	Middle	Pre	1st	Middle	Pre	1st	Middle	Last
Freq (10s)	L1M1	30.0	29.0	31.0	30.0	31.0	37.0	37.0	37.0	37.0	35.0	36.0	37.0	38.0
	L1M2	32.0	34.0	33.0	32.0	33.0	35.0	34.0	38.0	36.0	35.0	35.0	38.0	34.0
	L1M3	29.0	29.0	28.0	30.0	29.0	33.0	36.0	35.0	37.0	35.0	39.0	34.0	38.0
	L2M1	27.0	27.0	30.0	31.0	33.0	38.0	38.0	36.0	39.0	34.0	35.0	39.0	36.0
	L2M2	33.0	33.0	29.0	29.0	31.0	32.0	30.0	28.0	32.0	34.0	38.0	34.0	35.0
	L2M3	33.0	33.0	28.0	30.0	34.0	32.0	30.0	28.0	32.0	35.0	39.0	34.0	35.0
	L3M1	29.0	29.0	29.0	26.0	30.0	30.0	30.0	30.0	33.0	31.0	31.0	30.0	30.0
	L3M2	33.0	33.0	32.0	31.0	35.0	32.0	31.0	28.0	32.0	34.0	39.0	34.0	35.0
	L3M3	29.0	29.0	29.0	26.0	29.5	30.0	30.0	30.0	33.5	31.0	31.0	32.0	30.0
					0.0			2.0			4.0			6.0
Freq (60s)	L1M1	180.0	174.0	186.0	180.0	186.0	222.0	222.0	222.0	222.0	210.0	216.0	222.0	228.0
	L1M2	192.0	204.0	198.0	192.0	198.0	210.0	204.0	228.0	216.0	210.0	210.0	228.0	204.0
	L1M3	174.0	174.0	168.0	180.0	174.0	198.0	216.0	210.0	222.0	210.0	234.0	204.0	228.0
	L2M1	162.0	162.0	180.0	186.0	198.0	228.0	228.0	216.0	234.0	204.0	210.0	234.0	216.0
	L2M2	198.0	198.0	174.0	174.0	186.0	192.0	180.0	168.0	192.0	204.0	228.0	204.0	210.0
	L2M3	198.0	198.0	168.0	180.0	204.0	192.0	180.0	168.0	192.0	210.0	234.0	204.0	210.0
	L3M1	174.0	174.0	174.0	156.0	180.0	180.0	180.0	180.0	198.0	186.0	186.0	180.0	180.0
	L3M2	198.0	198.0	192.0	186.0	210.0	192.0	186.0	168.0	192.0	204.0	234.0	204.0	210.0
	L3M3	174.0	174.0	174.0	156.0	177.0	180.0	180.0	180.0	201.0	186.0	186.0	192.0	180.0
	Avg	**183.3**	**184.0**	**179.3**	**176.7**	**190.3**	**199.3**	**197.3**	**193.3**	**207.7**	**202.7**	**215.3**	**208.0**	**207.3**
	Std	**13.5**	**15.3**	**10.6**	**12.8**	**12.6**	**17.2**	**20.2**	**25.2**	**16.0**	**9.8**	**19.3**	**17.2**	**17.5**
	SEM	**4.5**	**5.1**	**3.5**	**4.3**	**4.2**	**5.7**	**6.7**	**8.4**	**5.3**	**3.3**	**6.4**	**5.7**	**5.8**
Vt (Total)	L1M1	0.01495	0.03585	0.02289	0.01790	0.02118	0.02198	0.01789	0.02503	0.02133	0.02763	0.03342	0.05731	0.03699
	L1M2	0.02240	0.03169	0.01753	0.01988	0.02737	0.03290	0.03267	0.02979	0.03696	0.02786	0.03505	0.02836	0.03160
	L1M3	0.02130	0.02439	0.02648	0.01807	0.01579	0.01697	0.01768	0.02222	0.02366	0.02329	0.03447	0.05827	0.03934
	L2M1	0.05292	0.05140	0.03265	0.04512	0.03418	0.04306	0.07548	0.03137	0.03477	0.04046	0.06893	0.08552	0.06311
	L2M2	0.03581	0.04908	0.03460	0.02782	0.04398	0.03382	0.02492	0.03365	0.03107	0.03305	0.04957	0.05921	0.04790
	L2M3	0.03577	0.05573	0.03331	0.02507	0.03245	0.03154	0.03219	0.03286	0.02872	0.04833	0.04176	0.03782	0.05984
	L3M1	0.01538	0.01991	0.01869	0.01744	0.01709	0.01707	0.02302	0.02197	0.01587	0.01924	0.02518	0.02644	0.02002
	L3M2	0.03962	0.04923	0.02962	0.02328	0.03264	0.03352	0.03143	0.03394	0.02996	0.04226	0.04426	0.04053	0.06511
	L3M3	0.01729	0.02156	0.01337	0.01647	0.01529	0.01615	0.02387	0.01916	0.01761	0.02341	0.02518	0.02686	0.01911
Vt (avg vol/breath)	L1M1	0.0004983	0.0012362	0.0007384	0.0005967	0.0006832	0.0005941	0.0004835	0.0006765	0.0005765	0.0007894	0.0009283	0.0015489	0.0009734
(raw)	L1M2	0.0007000	0.0009321	0.0005312	0.0006213	0.0008294	0.0009400	0.0009609	0.0007839	0.0010267	0.0007960	0.0010014	0.0007463	0.0009294
	L1M3	0.0007345	0.0008410	0.0009457	0.0006023	0.0005445	0.0005142	0.0004911	0.0006349	0.0006395	0.0006654	0.0008838	0.0017138	0.0010353
	L2M1	0.0019600	0.0019037	0.0010883	0.0014555	0.0010358	0.0011332	0.0019863	0.0008714	0.0008915	0.0011900	0.0019694	0.0021928	0.0017531
	L2M2	0.0010852	0.0014873	0.0011931	0.0009593	0.0014187	0.0010569	0.0008307	0.0012018	0.0009709	0.0009721	0.0013045	0.0017415	0.0013686
	L2M3	0.0010839	0.0016888	0.0011896	0.0008357	0.0009544	0.0009856	0.0010730	0.0011736	0.0008975	0.0013809	0.0010708	0.0011124	0.0017097
	L3M1	0.0005303	0.0006866	0.0006445	0.0006708	0.0005697	0.0005690	0.0007673	0.0007323	0.0004809	0.0006206	0.0008123	0.0008813	0.0006673
	L3M2	0.0012006	0.0014918	0.0009256	0.0007510	0.0009326	0.0010475	0.0010139	0.0012121	0.0009363	0.0012429	0.0011349	0.0011921	0.0018603
	L3M3	0.0005962	0.0007434	0.0004610	0.0006335	0.0005183	0.0005383	0.0007957	0.0006387	0.0005257	0.0007552	0.0008123	0.0008394	0.0006370
Vt (avg vol/breath)	L1M1	0.00420	0.01041	0.00622	0.00502	0.00575	0.00500	0.00407	0.00570	0.00485	0.00665	0.00782	0.01304	0.00820
	L1M2	0.00589	0.00785	0.00447	0.00523	0.00698	0.00791	0.00809	0.00660	0.00864	0.00670	0.00843	0.00628	0.00783
	L1M3	0.00618	0.00708	0.00796	0.00507	0.00458	0.00433	0.00414	0.00535	0.00538	0.00560	0.00744	0.01443	0.00872
	L2M1	0.01650	0.01603	0.00916	0.01226	0.00872	0.00954	0.01672	0.00734	0.00751	0.01002	0.01658	0.01846	0.01476
	L2M2	0.00914	0.01252	0.01005	0.00808	0.01195	0.00890	0.00699	0.01012	0.00818	0.00818	0.01098	0.01466	0.01152
	L2M3	0.00913	0.01422	0.01002	0.00704	0.00804	0.00830	0.00903	0.00988	0.00756	0.01163	0.00902	0.00937	0.01440
	L3M1	0.00447	0.00578	0.00543	0.00565	0.00480	0.00479	0.00646	0.00617	0.00405	0.00523	0.00684	0.00742	0.00562
	L3M2	0.01011	0.01256	0.00779	0.00632	0.00785	0.00882	0.00854	0.01021	0.00788	0.01047	0.00956	0.01004	0.01566
	L3M3	0.00502	0.00626	0.00388	0.00533	0.00436	0.00453	0.00670	0.00538	0.00443	0.00636	0.00684	0.00707	0.00536
Vt (avg vol/breath/g)	L1M1	0.001026	0.002545	0.001520	0.001228	0.001407	0.001223	0.000995	0.001393	0.001187	0.001625	0.001911	0.003189	0.002004
	L1M2	0.001384	0.001842	0.001050	0.001228	0.001639	0.001858	0.001899	0.001549	0.002029	0.001573	0.001979	0.001475	0.001837
	L1M3	0.001542	0.001766	0.001986	0.001265	0.001143	0.001080	0.001031	0.001333	0.001343	0.001397	0.001856	0.003599	0.002174
	L2M1	0.003496	0.003396	0.001941	0.002596	0.001848	0.002021	0.003543	0.001554	0.001590	0.002123	0.003513	0.003912	0.003127
	L2M2	0.001861	0.002550	0.002046	0.001645	0.002433	0.001812	0.001424	0.002061	0.001665	0.001667	0.002237	0.002986	0.002347
	L2M3	0.002322	0.003618	0.002549	0.001790	0.002045	0.002112	0.002299	0.002514	0.001923	0.002958	0.002294	0.002383	0.003663
	L3M1	0.000928	0.001202	0.001128	0.001174	0.000997	0.000996	0.001343	0.001282	0.000842	0.001086	0.001422	0.001543	0.001168
	L3M2	0.002174	0.002701	0.001676	0.001360	0.001689	0.001897	0.001836	0.002195	0.001695	0.002251	0.002055	0.002159	0.003369
	L3M3	0.001198	0.001494	0.000926	0.001273	0.001042	0.001082	0.001599	0.001283	0.001056	0.001518	0.001632	0.001687	0.001280
	Avg	**0.001770**	**0.002346**	**0.001647**	**0.001507**	**0.001582**	**0.001565**	**0.001775**	**0.001685**	**0.001481**	**0.001800**	**0.002100**	**0.002548**	**0.002330**
	Std	**0.000810**	**0.000833**	**0.000540**	**0.000459**	**0.000485**	**0.000457**	**0.000784**	**0.000455**	**0.000400**	**0.000559**	**0.000596**	**0.000912**	**0.000889**
	SEM	**0.000270**	**0.000278**	**0.000180**	**0.000153**	**0.000162**	**0.000152**	**0.000261**	**0.000152**	**0.000133**	**0.000186**	**0.000199**	**0.000304**	**0.000296**
Minute Ventilation	L1M1	0.1847	0.4428	0.2827	0.2211	0.2616	0.2715	0.2210	0.3092	0.2635	0.3413	0.4128	0.7079	0.4569
(mL/min/g)	L1M2	0.2656	0.3758	0.2079	0.2358	0.3246	0.3902	0.3874	0.3533	0.4383	0.3304	0.4157	0.3363	0.3747
	L1M3	0.2683	0.3073	0.3336	0.2277	0.1989	0.2138	0.2227	0.2799	0.2981	0.2934	0.4343	0.7341	0.4956
	L2M1	0.5664	0.5502	0.3495	0.4829	0.3658	0.4609	0.8079	0.3358	0.3722	0.4331	0.7378	0.9154	0.6755
	L2M2	0.3685	0.5050	0.3560	0.2862	0.4525	0.3480	0.2564	0.3462	0.3197	0.3401	0.5100	0.6092	0.4929
	L2M3	0.4598	0.7164	0.4282	0.3223	0.4171	0.4054	0.4138	0.4224	0.3692	0.6213	0.5368	0.4862	0.7692
	L3M1	0.1615	0.2091	0.1963	0.1832	0.1795	0.1793	0.2418	0.2308	0.1667	0.2021	0.2645	0.2777	0.2103
	L3M2	0.4305	0.5349	0.3218	0.2529	0.3546	0.3642	0.3415	0.3687	0.3255	0.4591	0.4809	0.4403	0.7074
	L3M3	0.2085	0.2600	0.1612	0.1986	0.1844	0.1947	0.2878	0.2310	0.2123	0.2823	0.3036	0.3239	0.2304
	Avg	**0.323758**	**0.433489**	**0.293025**	**0.267851**	**0.304345**	**0.314219**	**0.353369**	**0.319702**	**0.307268**	**0.366997**	**0.455146**	**0.536777**	**0.490326**
	Std	**0.139724**	**0.161571**	**0.088023**	**0.091231**	**0.102665**	**0.102245**	**0.184488**	**0.063751**	**0.084028**	**0.122490**	**0.138447**	**0.218314**	**0.199903**
	SEM	**0.046575**	**0.053857**	**0.029341**	**0.030410**	**0.034222**	**0.034082**	**0.061496**	**0.021250**	**0.028009**	**0.040830**	**0.046149**	**0.072771**	**0.066634**

Appendix F. Nicotine mice data categorized by day.

Day 1

				Day 1										
			0%			2%			4%			6%		
	Mouse	Pre	1st	Middle	Pre	1st	Middle	Pre	1st	Middle	Pre	1st	Middle	Last
Freq (10s)	M1	18.0	18.5	20.0	19.0	22.0	21.0	17.0	22.0	25.0	23.0	21.0	25.0	24.0
	M2	16.0	5.0	17.0	12.0	10.0	16.0	17.0	14.0	17.0	18.0	17.0	20.0	21.0
	M3	14.0	6.0	13.0	12.0	14.0	15.0	15.0	17.0	16.0	14.0	20.0	17.0	23.0
	M4	18.0	11.0	18.0	17.0	13.0	14.0	15.5	19.0	19.0	19.5	20.0	22.0	22.0
	M5	17.0	15.0	15.0	18.0	9.5	15.0	18.0	16.5	18.5	17.5	21.0	24.5	25.0
	M6	16.0	16.0	12.0	16.5	16.0	21.0	23.0	18.0	22.0	18.5	23.0	23.0	22.0
	M7	18.0	6.5	18.0	16.5	11.0	23.0	20.0	19.5	20.0	19.0	18.0	25.0	21.0
Freq (60s)	1	108.0	111.0	120.0	114.0	132.0	126.0	102.0	132.0	150.0	138.0	126.0	150.0	144.0
	2	96.0	30.0	102.0	72.0	60.0	96.0	102.0	84.0	102.0	108.0	102.0	120.0	126.0
	3	84.0	36.0	78.0	72.0	84.0	90.0	90.0	102.0	96.0	84.0	120.0	102.0	138.0
	4	108.0	66.0	108.0	102.0	78.0	84.0	93.0	114.0	114.0	117.0	120.0	132.0	132.0
	5	102.0	90.0	90.0	108.0	57.0	90.0	108.0	99.0	111.0	105.0	126.0	147.0	150.0
	6	96.0	96.0	72.0	99.0	96.0	126.0	138.0	108.0	132.0	111.0	138.0	138.0	132.0
	7	108.0	39.0	108.0	99.0	66.0	138.0	120.0	117.0	120.0	114.0	108.0	150.0	126.0
	Avg	**100.3**	**66.9**	**96.9**	**95.1**	**81.9**	**107.1**	**107.6**	**108.0**	**117.9**	**111.0**	**120.0**	**134.1**	**135.4**
	Std	**9.0**	**32.7**	**17.5**	**16.7**	**26.1**	**22.0**	**16.7**	**15.2**	**18.4**	**16.1**	**12.0**	**17.9**	**9.1**
	SEM	**3.4**	**12.4**	**6.6**	**6.3**	**9.9**	**8.3**	**6.3**	**5.7**	**7.0**	**6.1**	**4.5**	**6.8**	**3.4**
Vt (Total)	1	0.03217	0.03885	0.02902	0.03098	0.05325	0.02772	0.03798	0.04214	0.03206	0.06371	0.05460	0.03823	0.07645
	2	0.02198	0.05893	0.03203	0.03950	0.03402	0.02647	0.01294	0.05368	0.02572	0.02035	0.02664	0.02870	0.02985
	3	0.03766	0.02829	0.02581	0.01754	0.03402	0.01777	0.03627	0.03613	0.02818	0.02196	0.04513	0.05646	0.05709
	4	0.05376	0.03985	0.03385	0.08376	0.01635	0.03332	0.03219	0.03486	0.04017	0.06818	0.05054	0.07741	0.06026
	5	0.04335	0.04341	0.03009	0.02019	0.02237	0.01942	0.02680	0.03207	0.02926	0.02211	0.03674	0.04957	0.05156
	6	0.02356	0.02532	0.01917	0.01823	0.02201	0.03067	0.03955	0.07610	0.04460	0.07016	0.03837	0.04884	0.04352
	7	0.03043	0.02322	0.04334	0.02506	0.03659	0.02642	0.04098	0.07785	0.02178	0.04339	0.09144	0.05147	0.02889
Vt (avg vol/breath)	1	0.0017872	0.0021000	0.0014510	0.0016305	0.0024205	0.0013200	0.0022341	0.0019155	0.0012824	0.0027700	0.0026000	0.0015292	0.0031854
(raw)	2	0.0013738	0.0117860	0.0018841	0.0032917	0.0034020	0.0016544	0.0007612	0.0038343	0.0015129	0.0011306	0.0015671	0.0014350	0.0014214
	3	0.0026900	0.0047150	0.0019854	0.0014617	0.0024300	0.0011847	0.0024180	0.0021253	0.0017613	0.0015686	0.0022565	0.0033212	0.0024822
	4	0.0029867	0.0036227	0.0018806	0.0049271	0.0012577	0.0023800	0.0020768	0.0018347	0.0021142	0.0034964	0.0025270	0.0035186	0.0027391
	5	0.0025500	0.0028940	0.0020060	0.0011217	0.0023547	0.0012947	0.0014889	0.0019436	0.0015816	0.0012634	0.0017495	0.0020233	0.0020624
	6	0.0014725	0.0015825	0.0015975	0.0011048	0.0013756	0.0014605	0.0017196	0.0042278	0.0020273	0.0037924	0.0016683	0.0021235	0.0019782
	7	0.0016906	0.0035723	0.0024078	0.0015188	0.0033264	0.0011487	0.0020490	0.0039923	0.0010890	0.0022837	0.0050800	0.0020588	0.0013757
Vt (avg vol/breath)	1	0.00913	0.01073	0.00741	0.00833	0.01237	0.00675	0.01142	0.00979	0.00655	0.01415	0.01329	0.00781	0.01628
	2	0.00702	0.06023	0.00963	0.01682	0.01738	0.00845	0.00389	0.01959	0.00773	0.00578	0.00801	0.00733	0.00726
	3	0.01375	0.02409	0.01015	0.00747	0.01242	0.00605	0.01236	0.01086	0.00900	0.00802	0.01153	0.01697	0.01268
	4	0.01526	0.01851	0.00961	0.02518	0.00643	0.01216	0.01061	0.00938	0.01080	0.01787	0.01291	0.01798	0.01400
	5	0.01303	0.01479	0.01025	0.00573	0.01203	0.00662	0.00761	0.00993	0.00808	0.00646	0.00894	0.01034	0.01054
	6	0.00752	0.00809	0.00816	0.00565	0.00703	0.00746	0.00879	0.02160	0.01036	0.01938	0.00852	0.01085	0.01011
	7	0.00864	0.01825	0.01230	0.00776	0.01700	0.00587	0.01047	0.02040	0.00556	0.01167	0.02596	0.01052	0.00703
Vt (avg vol/breath/g)	1	0.006213	0.007300	0.005044	0.005668	0.008414	0.004589	0.007766	0.006658	0.004458	0.009629	0.009038	0.005316	0.011073
	2	0.005616	0.048181	0.007702	0.013456	0.013907	0.006763	0.003112	0.015675	0.006185	0.004622	0.006406	0.005866	0.005811
	3	0.010414	0.018253	0.007686	0.005658	0.009407	0.004586	0.009361	0.008227	0.006818	0.006072	0.008735	0.012857	0.009609
	4	0.011140	0.013513	0.007014	0.018378	0.004691	0.008877	0.007746	0.006843	0.007886	0.013041	0.009426	0.013124	0.010217
	5	0.008864	0.010060	0.006973	0.003899	0.008186	0.004501	0.005176	0.006756	0.005498	0.004392	0.006082	0.007033	0.007169
	6	0.005453	0.005860	0.005915	0.004091	0.005094	0.005408	0.006367	0.015655	0.007507	0.014043	0.006177	0.007863	0.007325
	7	0.006352	0.013422	0.009047	0.005707	0.012498	0.004316	0.007699	0.015001	0.004092	0.008581	0.019087	0.007736	0.005169
	Avg	**0.007722**	**0.016656**	**0.007055**	**0.008122**	**0.008885**	**0.005577**	**0.006747**	**0.010688**	**0.006063**	**0.008626**	**0.009279**	**0.008542**	**0.008053**
	Std	**0.002380**	**0.014516**	**0.001300**	**0.005562**	**0.003445**	**0.001685**	**0.002066**	**0.004484**	**0.001459**	**0.003880**	**0.004558**	**0.003177**	**0.002269**
	SEM	**0.000899**	**0.005487**	**0.000491**	**0.002102**	**0.001302**	**0.000637**	**0.000781**	**0.001695**	**0.000551**	**0.001467**	**0.001723**	**0.001201**	**0.000857**
Minute Ventilation	1	0.6710	0.8103	0.6053	0.6462	1.1106	0.5782	0.7922	0.8789	0.6687	1.3288	1.1388	0.7974	1.5945
(mL/min/g)	2	0.5391	1.4454	0.7856	0.9689	0.8344	0.6493	0.3174	1.3167	0.6309	0.4991	0.6534	0.7040	0.7322
	3	0.8747	0.6571	0.5995	0.4074	0.7902	0.4127	0.8425	0.8392	0.6545	0.5101	1.0482	1.3114	1.3260
	4	1.2031	0.8918	0.7575	1.8745	0.3659	0.7457	0.7204	0.7802	0.8990	1.5258	1.1311	1.7324	1.3486
	5	0.9042	0.9054	0.6276	0.4211	0.4666	0.4050	0.5590	0.6689	0.6103	0.4612	0.7663	1.0339	1.0754
	6	0.5234	0.5625	0.4259	0.4050	0.4890	0.6814	0.8787	1.6907	0.9909	1.5588	0.8525	1.0851	0.9669
	7	0.6860	0.5235	0.9771	0.5650	0.8249	0.5956	0.9239	1.7551	0.4910	0.9782	2.0614	1.1603	0.6513
	Avg	**0.771654**	**0.828012**	**0.682644**	**0.755430**	**0.697379**	**0.581131**	**0.719132**	**1.132804**	**0.706465**	**0.980282**	**1.093106**	**1.117781**	**1.099274**
	Std	**0.240659**	**0.311987**	**0.175472**	**0.532572**	**0.264894**	**0.129969**	**0.214072**	**0.451395**	**0.174870**	**0.495958**	**0.465694**	**0.341342**	**0.344579**
	SEM	**0.090960**	**0.117920**	**0.066322**	**0.201293**	**0.100120**	**0.049124**	**0.080912**	**0.170611**	**0.066095**	**0.187455**	**0.176016**	**0.129015**	**0.130239**

Day 2

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Last**
Freq (10s)	M1	20.0	19.0	20.5	20.5	12.5	22.0	21.0	22.0	24.0	21.0	19.0	22.0	18.0
	M2	18.0	12.0	16.0	17.0	15.0	18.0	21.0	19.0	22.0	23.0	24.0	25.0	21.0
	M3	17.0	16.5	17.0	18.0	14.0	18.0	20.0	20.0	20.0	19.0	18.0	22.5	21.0
	M4	18.0	16.0	20.0	19.0	10.5	21.0	21.0	21.0	20.0	20.0	20.0	23.0	21.0
	M5	18.5	19.0	18.0	17.0	19.0	20.0	19.0	19.0	20.0	20.5	21.0	23.0	24.0
	M6	5.0	20.0	21.0	10.0	12.0	18.0	17.5	16.0	18.0	19.0	18.5	19.5	19.0
	M7	19.0	21.0	22.0	22.0	24.0	21.0	25.0	26.0	27.0	24.0	27.0	27.0	26.5
Freq (60s)	1	120.0	114.0	123.0	123.0	75.0	132.0	126.0	132.0	144.0	126.0	114.0	132.0	108.0
	2	108.0	72.0	96.0	102.0	90.0	108.0	126.0	114.0	132.0	138.0	144.0	150.0	126.0
	3	102.0	99.0	102.0	108.0	84.0	108.0	120.0	120.0	120.0	114.0	108.0	135.0	126.0
	4	108.0	96.0	120.0	114.0	63.0	126.0	126.0	126.0	120.0	120.0	120.0	138.0	126.0
	5	111.0	114.0	108.0	102.0	114.0	120.0	114.0	114.0	120.0	123.0	126.0	138.0	144.0
	6	30.0	120.0	126.0	60.0	72.0	108.0	105.0	96.0	108.0	114.0	111.0	117.0	114.0
	7	114.0	126.0	132.0	132.0	144.0	126.0	150.0	156.0	162.0	144.0	162.0	162.0	159.0
	Avg	**99.0**	**105.9**	**115.3**	**105.9**	**91.7**	**118.3**	**123.9**	**122.6**	**129.4**	**125.6**	**126.4**	**138.9**	**129.0**
	Std	**30.9**	**18.4**	**13.4**	**23.0**	**28.2**	**10.2**	**13.9**	**18.6**	**18.3**	**11.5**	**19.8**	**14.1**	**17.4**
	SEM	**11.7**	**7.0**	**5.1**	**8.7**	**10.7**	**3.9**	**5.3**	**7.0**	**6.9**	**4.4**	**7.5**	**5.3**	**6.6**
Vt (Total)	1	0.02163	0.02689	0.01726	0.02380	0.02037	0.01857	0.01980	0.02953	0.02494	0.02917	0.05856	0.04714	0.03802
	2	0.01628	0.03336	0.02561	0.02408	0.02032	0.03667	0.03111	0.02052	0.03061	0.02883	0.05477	0.04271	0.03969
	3	0.01953	0.01703	0.02023	0.02060	0.04303	0.03507	0.01989	0.06787	0.02176	0.01760	0.07160	0.03406	0.04171
	4	0.03139	0.02599	0.01801	0.01785	0.03338	0.03130	0.02459	0.02704	0.02325	0.02682	0.09262	0.05061	0.04842
	5	0.02435	0.01843	0.03431	0.01804	0.01511	0.01928	0.01233	0.01465	0.02332	0.01746	0.01898	0.02074	0.02228
	6	0.01178	0.01568	0.01974	0.01645	0.09491	0.08115	0.08383	0.09970	0.52069	0.21650	0.12870	0.32190	0.09651
	7	0.01380	0.01614	0.01613	0.01540	0.01755	0.01611	0.01599	0.02466	0.02867	0.02350	0.02519	0.02168	0.02628
Vt (avg vol/breath)	1	0.0010815	0.0014153	0.0008420	0.0011610	0.0016296	0.0008441	0.0009429	0.0013423	0.0010392	0.0013890	0.0030821	0.0021427	0.0021122
(raw)	2	0.0009044	0.0027800	0.0016006	0.0014165	0.0013547	0.0020372	0.0014814	0.0010800	0.0013914	0.0012535	0.0022821	0.0017084	0.0018900
	3	0.0011488	0.0010321	0.0011900	0.0011444	0.0030736	0.0019483	0.0009945	0.0033935	0.0010880	0.0009263	0.0039778	0.0015138	0.0019862
	4	0.0017439	0.0016244	0.0009005	0.0009395	0.0031790	0.0014905	0.0011710	0.0012876	0.0011625	0.0013410	0.0046310	0.0022004	0.0023057
	5	0.0013162	0.0009700	0.0019061	0.0010612	0.0007953	0.0009640	0.0006489	0.0007711	0.0011660	0.0008517	0.0009038	0.0009017	0.0009283
	6	0.0023560	0.0007840	0.0009400	0.0016450	0.0079092	0.0045083	0.0047903	0.0062313	0.0289272	0.0113947	0.0069568	0.0165077	0.0050795
	7	0.0007263	0.0007686	0.0007332	0.0007000	0.0007313	0.0007671	0.0006396	0.0009485	0.0010619	0.0009792	0.0009330	0.0008030	0.0009917
Vt (avg vol/breath)	1	0.00626	0.00819	0.00487	0.00672	0.00944	0.00489	0.00546	0.00777	0.00602	0.00804	0.01785	0.01241	0.01223
	2	0.00524	0.01610	0.00927	0.00820	0.00784	0.01180	0.00858	0.00625	0.00806	0.00726	0.01321	0.00989	0.01094
	3	0.00665	0.00598	0.00689	0.00663	0.01780	0.01128	0.00576	0.01965	0.00630	0.00536	0.02303	0.00876	0.01150
	4	0.01010	0.00941	0.00521	0.00544	0.01841	0.00863	0.00678	0.00746	0.00673	0.00776	0.02681	0.01274	0.01335
	5	0.00762	0.00562	0.01104	0.00614	0.00460	0.00558	0.00376	0.00446	0.00675	0.00493	0.00523	0.00522	0.00538
	6	0.01364	0.00454	0.00544	0.00952	0.04579	0.02610	0.02774	0.03608	0.16749	0.06598	0.04028	0.09558	0.02941
	7	0.00421	0.00445	0.00425	0.00405	0.00423	0.00444	0.00370	0.00549	0.00615	0.00567	0.00540	0.00465	0.00574
					0.00000			2.00000			4.00000			6.00000
Vt (avg vol/breath/g)	1	0.003750	0.004907	0.002919	0.004025	0.005650	0.002927	0.003269	0.004654	0.003603	0.004816	0.010686	0.007429	0.007323
	2	0.003662	0.011256	0.006481	0.005735	0.005485	0.008249	0.005998	0.004373	0.005634	0.005075	0.009240	0.006917	0.007653
	3	0.004434	0.003984	0.004593	0.004418	0.011864	0.007521	0.003839	0.013099	0.004200	0.003576	0.015354	0.005843	0.007667
	4	0.006119	0.005700	0.003160	0.003297	0.011156	0.005230	0.004109	0.004518	0.004079	0.004706	0.016251	0.007722	0.008091
	5	0.004647	0.003425	0.006730	0.003746	0.002808	0.003403	0.002291	0.002722	0.004117	0.003007	0.003191	0.003184	0.003277
	6	0.008634	0.002873	0.003445	0.006028	0.028984	0.016521	0.017554	0.022835	0.106005	0.041757	0.025493	0.060493	0.018614
	7	0.002731	0.002890	0.002757	0.002632	0.002749	0.002884	0.002405	0.003566	0.003992	0.003681	0.003508	0.003019	0.003729
	Avg	**0.004854**	**0.005005**	**0.004298**	**0.004269**	**0.009814**	**0.006676**	**0.005638**	**0.007967**	**0.018804**	**0.009517**	**0.011960**	**0.013515**	**0.008050**
	Std	**0.001969**	**0.002948**	**0.001686**	**0.001240**	**0.009206**	**0.004854**	**0.005400**	**0.007417**	**0.038457**	**0.014237**	**0.007858**	**0.020804**	**0.005064**
	SEM	**0.000744**	**0.001114**	**0.000637**	**0.000469**	**0.003480**	**0.001835**	**0.002041**	**0.002803**	**0.014536**	**0.005381**	**0.002970**	**0.007863**	**0.001914**
Minute Ventilation	1	0.4500	0.5594	0.3590	0.4951	0.4237	0.3863	0.4119	0.6143	0.5188	0.6068	1.2182	0.9806	0.7909
(mL/min/g)	2	0.3955	0.8104	0.6222	0.5850	0.4936	0.8909	0.7558	0.4985	0.7436	0.7004	1.3306	1.0376	0.9642
	3	0.4523	0.3944	0.4685	0.4771	0.9966	0.8122	0.4607	1.5719	0.5040	0.4076	1.6583	0.7888	0.9660
	4	0.6609	0.5472	0.3792	0.3758	0.7028	0.6590	0.5177	0.5693	0.4895	0.5647	1.9501	1.0656	1.0195
	5	0.5158	0.3904	0.7268	0.3821	0.3201	0.4084	0.2612	0.3103	0.4940	0.3699	0.4021	0.4393	0.4720
	6	0.2590	0.3448	0.4340	0.3617	2.0868	1.7843	1.8432	2.1921	11.4486	4.7603	2.8298	7.0777	2.1220
	7	0.3113	0.3641	0.3639	0.3474	0.3959	0.3634	0.3607	0.5563	0.6468	0.5301	0.5682	0.4891	0.5928
	Avg	**0.434971**	**0.487242**	**0.479088**	**0.432034**	**0.774223**	**0.757782**	**0.658735**	**0.901821**	**2.120750**	**1.134247**	**1.422450**	**1.696962**	**0.989626**
	Std	**0.132864**	**0.166691**	**0.142379**	**0.088660**	**0.622948**	**0.499949**	**0.544562**	**0.699884**	**4.114308**	**1.602917**	**0.830219**	**2.386229**	**0.539950**
	SEM	**0.050218**	**0.063003**	**0.053814**	**0.033510**	**0.235452**	**0.188963**	**0.205825**	**0.264531**	**1.555062**	**0.605846**	**0.313793**	**0.901910**	**0.204082**

Day 3

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Last**
Freq (10s)	M1	26.0	16.0	27.0	28.0	27.0	30.0	29.0	29.0	32.0	29.0	29.0	27.0	26.0
	M2	23.0	18.0	20.0	20.0	14.0	24.0	27.0	29.0	29.0	27.5	26.5	30.0	29.0
	M3	27.0	28.0	24.0	23.0	22.0	29.0	25.0	29.0	24.5	27.0	26.0	27.0	26.0
	M4	20.5	20.0	22.0	19.0	23.0	21.0	22.0	22.0	23.0	23.0	19.0	24.0	22.0
	M5	20.0	18.0	17.0	19.0	20.0	18.0	17.0	15.0	24.0	22.0	28.0	25.0	23.0
	M6	29.0	26.0	26.5	27.0	11.0	26.0	23.0	17.0	17.0	24.0	25.0	28.5	26.0
	M7	20.5	13.0	20.0	18.0	23.0	22.5	21.5	20.0	22.0	22.0	21.0	22.0	20.0
					0.0			2.0			4.0			6.0
Freq (60s)	1	156.0	96.0	162.0	168.0	162.0	180.0	174.0	174.0	192.0	174.0	174.0	162.0	156.0
	2	138.0	108.0	120.0	120.0	84.0	144.0	162.0	174.0	174.0	165.0	159.0	180.0	174.0
	3	162.0	168.0	144.0	138.0	132.0	174.0	150.0	174.0	147.0	162.0	156.0	162.0	156.0
	4	123.0	120.0	132.0	114.0	138.0	126.0	132.0	132.0	138.0	138.0	114.0	144.0	132.0
	5	120.0	108.0	102.0	114.0	120.0	108.0	102.0	90.0	144.0	132.0	168.0	150.0	138.0
	6	174.0	156.0	159.0	162.0	66.0	156.0	138.0	102.0	102.0	144.0	150.0	171.0	156.0
	7	123.0	78.0	120.0	108.0	138.0	135.0	129.0	120.0	132.0	132.0	126.0	132.0	120.0
	Avg	**142.3**	**119.1**	**134.1**	**132.0**	**120.0**	**146.1**	**141.0**	**138.0**	**147.0**	**149.6**	**149.6**	**157.3**	**147.4**
	Std	**21.8**	**32.2**	**22.1**	**24.5**	**33.6**	**25.8**	**23.7**	**36.2**	**29.1**	**17.2**	**21.9**	**16.4**	**18.3**
	SEM	**8.2**	**12.2**	**8.4**	**9.3**	**12.7**	**9.8**	**9.0**	**13.7**	**11.0**	**6.5**	**8.3**	**6.2**	**6.9**
Vt (Total)	1	0.02367	0.04626	0.02908	0.02553	0.03979	0.04654	0.04956	0.03965	0.06265	0.10400	0.08428	0.05469	0.06932
	2	0.02410	0.03478	0.02697	0.03134	0.03129	0.01947	0.03359	0.03743	0.04765	0.04600	0.04194	0.05077	0.04379
	3	0.03650	0.06103	0.02582	0.02564	0.02702	0.04075	0.02466	0.06561	0.05256	0.04917	0.04229	0.04720	0.05518
	4	0.04088	0.02857	0.02656	0.02438	0.03333	0.03077	0.04002	0.03522	0.03317	0.04773	0.05425	0.04493	0.07605
	5	0.02191	0.02319	0.01776	0.01643	0.06446	0.01968	0.02973	0.06987	0.06183	0.03516	0.05338	0.05570	0.04821
	6	0.02819	0.04003	0.03116	0.02719	0.01745	0.02183	0.01938	0.02677	0.04758	0.03163	0.03476	0.04452	0.06390
	7	0.02520	0.03561	0.03126	0.02754	0.02619	0.03775	0.03991	0.03749	0.05196	0.04978	0.03514	0.05458	0.01885
Vt (avg vol/breath)	1	0.0009104	0.0028913	0.0010770	0.0009118	0.0014737	0.0015513	0.0017090	0.0013672	0.0019578	0.0035862	0.0029062	0.0020256	0.0026662
(raw)	2	0.0010478	0.0019322	0.0013485	0.0015670	0.0022350	0.0008113	0.0012441	0.0012907	0.0016431	0.0016727	0.0015826	0.0016923	0.0015100
	3	0.0013519	0.0021796	0.0010758	0.0011148	0.0012282	0.0014052	0.0009864	0.0022624	0.0021453	0.0018211	0.0016265	0.0017481	0.0021223
	4	0.0019941	0.0014285	0.0012073	0.0012832	0.0014491	0.0014652	0.0018191	0.0016009	0.0014422	0.0020752	0.0028553	0.0018721	0.0034568
	5	0.0010955	0.0012883	0.0010447	0.0008647	0.0032230	0.0010933	0.0017488	0.0046580	0.0025763	0.0015982	0.0019064	0.0022280	0.0020961
	6	0.0009721	0.0015396	0.0011758	0.0010070	0.0015864	0.0008396	0.0008426	0.0015747	0.0027988	0.0013179	0.0013904	0.0015621	0.0024577
	7	0.0012293	0.0027392	0.0015630	0.0015300	0.0011387	0.0016778	0.0018563	0.0018745	0.0023618	0.0022627	0.0016733	0.0024809	0.0009425
Vt (avg vol/breath)	1	0.00527	0.01674	0.00624	0.00528	0.00853	0.00898	0.00989	0.00792	0.01134	0.02076	0.01683	0.01173	0.01544
	2	0.00607	0.01119	0.00781	0.00907	0.01294	0.00470	0.00720	0.00747	0.00951	0.00969	0.00916	0.00980	0.00874
	3	0.00783	0.01262	0.00623	0.00645	0.00711	0.00814	0.00571	0.01310	0.01242	0.01054	0.00942	0.01012	0.01229
	4	0.01155	0.00827	0.00699	0.00743	0.00839	0.00848	0.01053	0.00927	0.00835	0.01202	0.01653	0.01084	0.02001
	5	0.00634	0.00746	0.00605	0.00501	0.01866	0.00633	0.01013	0.02697	0.01492	0.00925	0.01104	0.01290	0.01214
	6	0.00563	0.00891	0.00681	0.00583	0.00919	0.00486	0.00488	0.00912	0.01621	0.00763	0.00805	0.00904	0.01423
	7	0.00712	0.01586	0.00905	0.00886	0.00659	0.00971	0.01075	0.01085	0.01367	0.01310	0.00969	0.01436	0.00546
Vt (avg vol/breath/g)	1	0.002429	0.007714	0.002874	0.002433	0.003932	0.004139	0.004560	0.003648	0.005224	0.009569	0.007754	0.005405	0.007114
	2	0.003160	0.005827	0.004067	0.004725	0.006740	0.002446	0.003752	0.003892	0.004955	0.005044	0.004773	0.005103	0.004554
	3	0.003973	0.006406	0.003162	0.003276	0.003610	0.004130	0.002899	0.006649	0.006305	0.005352	0.004781	0.005138	0.006238
	4	0.005660	0.004054	0.003427	0.003642	0.004113	0.004159	0.005163	0.004544	0.004093	0.005890	0.008104	0.005313	0.009811
	5	0.003220	0.003787	0.003070	0.002542	0.009473	0.003213	0.005140	0.013690	0.007572	0.004697	0.005603	0.006548	0.006161
	6	0.002800	0.004435	0.003387	0.002901	0.004570	0.002419	0.002427	0.004536	0.008062	0.003796	0.004005	0.004500	0.007080
	7	0.003868	0.008620	0.004918	0.004815	0.003583	0.005280	0.005841	0.005899	0.007432	0.007120	0.005266	0.007807	0.002966
	Avg	**0.003587**	**0.005835**	**0.003558**	**0.003476**	**0.005146**	**0.003684**	**0.004255**	**0.006123**	**0.006235**	**0.005924**	**0.005755**	**0.005688**	**0.006275**
	Std	**0.001065**	**0.001868**	**0.000709**	**0.000976**	**0.002196**	**0.001043**	**0.001268**	**0.003505**	**0.001517**	**0.001908**	**0.001568**	**0.001118**	**0.002152**
	SEM	**0.000402**	**0.000706**	**0.000268**	**0.000369**	**0.000830**	**0.000394**	**0.000479**	**0.001325**	**0.000573**	**0.000721**	**0.000593**	**0.000423**	**0.000813**
Minute Ventilation	1	0.3789	0.7406	0.4655	0.4087	0.6370	0.7451	0.7934	0.6348	1.0030	1.6650	1.3493	0.8755	1.1098
(mL/min/g)	2	0.4361	0.6293	0.4880	0.5671	0.5662	0.3523	0.6078	0.6772	0.8622	0.8323	0.7589	0.9186	0.7923
	3	0.6437	1.0762	0.4553	0.4521	0.4765	0.7186	0.4349	1.1570	0.9269	0.8671	0.7458	0.8323	0.9731
	4	0.6962	0.4865	0.4523	0.4152	0.5676	0.5240	0.6815	0.5998	0.5649	0.8128	0.9238	0.7651	1.2951
	5	0.3864	0.4089	0.3132	0.2897	1.1367	0.3470	0.5243	1.2321	1.0903	0.6200	0.9413	0.9822	0.8502
	6	0.4872	0.6919	0.5386	0.4699	0.3016	0.3773	0.3350	0.4627	0.8224	0.5467	0.6008	0.7695	1.1044
	7	0.4758	0.6723	0.5902	0.5200	0.4945	0.7127	0.7535	0.7078	0.9810	0.9399	0.6635	1.0305	0.3559
	Avg	**0.500600**	**0.672256**	**0.471873**	**0.446106**	**0.597148**	**0.539577**	**0.590045**	**0.781632**	**0.892943**	**0.897679**	**0.854755**	**0.881978**	**0.925817**
	Std	**0.123505**	**0.213668**	**0.086203**	**0.088972**	**0.260452**	**0.183932**	**0.168444**	**0.293416**	**0.170073**	**0.365877**	**0.251322**	**0.101874**	**0.303502**
	SEM	**0.046680**	**0.080759**	**0.032582**	**0.033628**	**0.098442**	**0.069520**	**0.063666**	**0.110901**	**0.064282**	**0.138289**	**0.094991**	**0.038505**	**0.114713**

Day 5

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Last**
Freq (10s)	M1	40.0	36.0	41.0	42.0	44.0	32.0	34.0	40.0	34.0	37.0	38.0	35.0	34.0
	M2	30.0	28.0	29.0	29.0	27.0	28.0	26.0	28.0	29.0	25.0	27.0	26.0	26.0
	M3	35.0	34.0	38.0	38.0	37.0	31.0	30.0	32.0	28.0	29.0	25.0	25.0	25.0
	M4	28.0	28.0	26.0	27.0	30.0	31.0	27.0	28.0	28.0	27.0	27.5	26.0	25.0
	M5	38.0	37.0	37.0	35.0	37.5	35.0	35.0	33.0	30.0	37.0	33.0	32.0	27.0
	M6	31.0	32.0	29.0	26.0	31.0	30.0	28.5	31.0	28.0	29.5	34.0	26.5	26.0
	M7	27.5	27.5	24.0	24.0	24.0	24.0	25.0	33.0	25.0	24.0	31.0	23.5	23.0
Freq (60s)	1	240.0	216.0	246.0	252.0	264.0	192.0	204.0	240.0	204.0	222.0	228.0	210.0	204.0
	2	180.0	168.0	174.0	174.0	162.0	168.0	156.0	168.0	174.0	150.0	162.0	156.0	156.0
	3	210.0	204.0	228.0	228.0	222.0	186.0	180.0	192.0	168.0	174.0	150.0	150.0	150.0
	4	168.0	168.0	156.0	162.0	180.0	186.0	162.0	168.0	168.0	162.0	165.0	156.0	150.0
	5	228.0	222.0	222.0	210.0	225.0	210.0	210.0	198.0	180.0	222.0	198.0	192.0	162.0
	6	186.0	192.0	174.0	156.0	186.0	180.0	171.0	186.0	168.0	177.0	204.0	159.0	156.0
	7	165.0	165.0	144.0	144.0	144.0	144.0	150.0	198.0	150.0	144.0	186.0	141.0	138.0
	Avg	**196.7**	**190.7**	**192.0**	**189.4**	**197.6**	**180.9**	**176.1**	**192.9**	**173.1**	**178.7**	**184.7**	**166.3**	**159.4**
	Std	**29.6**	**24.1**	**39.5**	**40.8**	**41.5**	**20.6**	**23.3**	**24.4**	**16.4**	**31.8**	**27.5**	**25.0**	**21.0**
	SEM	**11.2**	**9.1**	**14.9**	**15.4**	**15.7**	**7.8**	**8.8**	**9.2**	**6.2**	**12.0**	**10.4**	**9.4**	**7.9**
Vt (Total)	1	0.03430	0.03115	0.05002	0.04912	0.05910	0.04984	0.04806	0.06823	0.07619	0.07463	0.07500	0.09178	0.07674
	2	0.05584	0.03942	0.06392	0.02653	0.05496	0.04984	0.04879	0.06755	0.05580	0.07651	0.06823	0.07491	0.07061
	3	0.09813	0.06336	0.04017	0.05032	0.04642	0.03829	0.05346	0.05076	0.04672	0.05778	0.04699	0.07068	0.04559
	4	0.06706	0.13176	0.04825	0.05253	0.12597	0.07791	0.05751	0.07222	0.09677	0.09745	0.10847	0.08300	0.09582
	5	0.06451	0.05847	0.07244	0.06544	0.07011	0.06357	0.07717	0.09498	0.11431	0.04376	0.04748	0.09592	0.16283
	6	0.07887	0.06069	0.08124	0.05506	0.07477	0.10959	0.13035	0.13035	0.07772	0.07249	0.07979	0.09511	0.08890
	7	0.04790	0.05841	0.05470	0.05725	0.05075	0.05326	0.06446	0.16332	0.07079	0.06338	0.17946	0.08160	0.08042
Vt (avg vol/breath)	1	0.0008575	0.0008653	0.0012200	0.0011695	0.0013432	0.0015575	0.0014135	0.0017058	0.0022409	0.0020170	0.0019737	0.0026223	0.0022571
(raw)	2	0.0018613	0.0014079	0.0022041	0.0009148	0.0020356	0.0017800	0.0018765	0.0024125	0.0019241	0.0030604	0.0025270	0.0028812	0.0027158
	3	0.0028037	0.0018635	0.0010571	0.0013242	0.0012546	0.0012352	0.0017820	0.0015863	0.0016686	0.0019924	0.0018796	0.0028272	0.0018236
	4	0.0023950	0.0047057	0.0018558	0.0019456	0.0041990	0.0025132	0.0021300	0.0025793	0.0034561	0.0036093	0.0039444	0.0031923	0.0038328
	5	0.0016976	0.0015803	0.0019578	0.0018697	0.0018696	0.0018163	0.0022049	0.0028782	0.0038103	0.0011827	0.0014388	0.0029975	0.0060307
	6	0.0025442	0.0018966	0.0028014	0.0021177	0.0024119	0.0036530	0.0045737	0.0042048	0.0027757	0.0024573	0.0023468	0.0035891	0.0034192
	7	0.0017418	0.0021240	0.0022792	0.0023854	0.0021146	0.0022192	0.0025784	0.0049491	0.0028316	0.0026408	0.0057890	0.0034723	0.0034965
Vt (avg vol/breath)	1	0.00575	0.00580	0.00817	0.00784	0.00900	0.01044	0.00947	0.01143	0.01501	0.01351	0.01322	0.01757	0.01512
	2	0.01247	0.00943	0.01477	0.00613	0.01364	0.01193	0.01257	0.01616	0.01289	0.02050	0.01693	0.01930	0.01820
	3	0.01878	0.01249	0.00708	0.00887	0.00841	0.00828	0.01194	0.01063	0.01118	0.01335	0.01259	0.01894	0.01222
	4	0.01605	0.03153	0.01243	0.01304	0.02813	0.01684	0.01427	0.01728	0.02316	0.02418	0.02643	0.02139	0.02568
	5	0.01137	0.01059	0.01312	0.01253	0.01253	0.01217	0.01477	0.01928	0.02553	0.00792	0.00964	0.02008	0.04041
	6	0.01705	0.01271	0.01877	0.01419	0.01616	0.02448	0.03064	0.02817	0.01860	0.01646	0.01572	0.02405	0.02291
	7	0.01167	0.01423	0.01527	0.01598	0.01417	0.01487	0.01728	0.03316	0.01897	0.01769	0.03879	0.02326	0.02343
Vt (avg vol/breath/g)	1	0.002002	0.002020	0.002848	0.002730	0.003136	0.003636	0.003300	0.003982	0.005231	0.004709	0.004608	0.006122	0.005269
	2	0.004834	0.003656	0.005724	0.002376	0.005286	0.004622	0.004873	0.006265	0.004997	0.007948	0.006562	0.007482	0.007053
	3	0.006757	0.004491	0.002548	0.003191	0.003024	0.002977	0.004295	0.003823	0.004021	0.004802	0.004530	0.006814	0.004395
	4	0.005611	0.011024	0.004347	0.004558	0.009837	0.005888	0.004990	0.006042	0.008096	0.008455	0.009240	0.007478	0.008979
	5	0.004341	0.004041	0.005007	0.004781	0.004781	0.004645	0.005638	0.007360	0.009744	0.003024	0.003679	0.007665	0.015422
	6	0.006132	0.004571	0.006752	0.005104	0.005813	0.008804	0.011023	0.010134	0.006690	0.005922	0.005656	0.008650	0.008241
	7	0.004371	0.005330	0.005719	0.005986	0.005306	0.005569	0.006470	0.012419	0.007106	0.006627	0.014527	0.008713	0.008774
	Avg	**0.004864**	**0.005019**	**0.004706**	**0.004104**	**0.005312**	**0.005163**	**0.005798**	**0.007147**	**0.006555**	**0.005927**	**0.006972**	**0.007561**	**0.008305**
	Std	**0.001552**	**0.002841**	**0.001559**	**0.001349**	**0.002272**	**0.001898**	**0.002510**	**0.003158**	**0.001978**	**0.001921**	**0.003797**	**0.000928**	**0.003592**
	SEM	**0.000587**	**0.001074**	**0.000589**	**0.000510**	**0.000859**	**0.000717**	**0.000949**	**0.001193**	**0.000747**	**0.000726**	**0.001435**	**0.000351**	**0.001358**
Minute Ventilation	1	0.4804	0.4363	0.7006	0.6880	0.8278	0.6981	0.6732	0.9557	1.0672	1.0453	1.0505	1.2856	1.0749
(mL/min/g)	2	0.8701	0.6142	0.9960	0.4134	0.8564	0.7766	0.7602	1.0525	0.8694	1.1921	1.0631	1.1672	1.1002
	3	1.4190	0.9162	0.5809	0.7276	0.6713	0.5537	0.7731	0.7340	0.6756	0.8355	0.6795	1.0221	0.6593
	4	0.9426	1.8520	0.6782	0.7384	1.7706	1.0951	0.8084	1.0151	1.3602	1.3698	1.5246	1.1666	1.3468
	5	0.9898	0.8971	1.1115	1.0041	1.0757	0.9754	1.1841	1.4573	1.7539	0.6714	0.7285	1.4717	2.4984
	6	1.1405	0.8776	1.1748	0.7962	1.0812	1.5847	1.8849	1.8849	1.1239	1.0482	1.1538	1.3753	1.2855
	7	0.7212	0.8794	0.8236	0.8620	0.7641	0.8019	0.9705	2.4590	1.0658	0.9543	2.7020	1.2286	1.2108
	Avg	**0.937656**	**0.924704**	**0.866498**	**0.747092**	**1.006727**	**0.926496**	**1.007757**	**1.365510**	**1.130861**	**1.016668**	**1.271725**	**1.245305**	**1.310846**
	Std	**0.298902**	**0.447199**	**0.230290**	**0.181181**	**0.369659**	**0.339907**	**0.422285**	**0.613915**	**0.347547**	**0.228462**	**0.690625**	**0.148441**	**0.569621**
	SEM	**0.112974**	**0.169025**	**0.087041**	**0.068480**	**0.139718**	**0.128473**	**0.159609**	**0.232038**	**0.131361**	**0.086351**	**0.261032**	**0.056105**	**0.215296**

Day 8

			0%			2%			4%			6%		
	Mouse	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Pre**	**1st**	**Middle**	**Last**
Freq (10s)	M1	41.0	41.0	43.0	42.0	37.0	31.0	34.0	36.0	36.0	42.0	41.0	37.0	35.0
	M2	31.0	33.0	37.0	39.0	34.0	40.0	39.0	37.0	32.5	36.0	40.0	36.0	39.0
	M3	31.0	30.5	36.0	37.0	34.0	34.5	39.0	36.0	35.0	36.0	39.0	36.5	39.0
	M4	38.0	40.5	40.5	39.5	40.0	37.0	30.0	34.0	31.0	28.0	29.0	25.0	25.0
	M5	36.0	35.0	36.0	35.0	36.0	31.0	37.0	36.0	37.0	35.0	34.0	36.5	36.0
	M6	32.0	31.0	31.0	31.0	30.5	29.0	33.0	30.0	26.0	27.0	30.0	34.0	35.0
	M7	34.5	27.0	29.0	29.0	26.0	35.0	30.0	34.0	28.0	32.0	29.0	35.0	31.0
				38.0										
Freq (60s)	1	246.0	246.0	258.0	252.0	222.0	186.0	204.0	216.0	216.0	252.0	246.0	222.0	210.0
	2	186.0	198.0	222.0	234.0	204.0	240.0	234.0	222.0	195.0	216.0	240.0	216.0	234.0
	3	186.0	183.0	216.0	222.0	204.0	207.0	234.0	216.0	210.0	216.0	234.0	219.0	234.0
	4	228.0	243.0	243.0	237.0	240.0	222.0	180.0	204.0	186.0	168.0	174.0	150.0	150.0
	5	216.0	210.0	216.0	210.0	216.0	186.0	222.0	216.0	222.0	210.0	204.0	219.0	216.0
	6	192.0	186.0	186.0	186.0	183.0	174.0	198.0	180.0	156.0	162.0	180.0	204.0	210.0
	7	207.0	162.0	174.0	174.0	156.0	210.0	180.0	204.0	168.0	192.0	174.0	210.0	186.0
	Avg	**208.7**	**204.0**	**216.4**	**216.4**	**203.6**	**203.6**	**207.4**	**208.3**	**193.3**	**202.3**	**207.4**	**205.7**	**205.7**
	Std	**22.8**	**31.3**	**29.4**	**28.3**	**27.4**	**23.1**	**23.2**	**14.2**	**24.9**	**31.1**	**32.3**	**25.3**	**29.5**
	SEM	**8.6**	**11.8**	**11.1**	**10.7**	**10.4**	**8.7**	**8.8**	**5.4**	**9.4**	**11.8**	**12.2**	**9.6**	**11.2**
Vt (Total)	1	0.05737	0.05817	0.06716	0.07776	0.05058	0.04517	0.03766	0.03835	0.03790	0.04600	0.07013	0.10267	0.17234
	2	0.07684	0.08230	0.05450	0.07018	0.07095	0.07448	0.06627	0.06875	0.07097	0.05542	0.06793	0.05661	0.06690
	3	0.07216	0.07213	0.06450	0.05472	0.06647	0.06264	0.06672	0.07638	0.06176	0.07030	0.06433	0.07729	0.06326
	4	0.05760	0.03937	0.05863	0.05615	0.05597	0.07460	0.09497	0.23515	0.10183	0.24947	0.53727	0.14494	0.14737
	5	0.07577	0.10649	0.11410	0.10136	0.09172	0.09280	0.10055	0.10205	0.11039	0.14894	0.12430	0.14151	0.15555
	6	0.04875	0.04591	0.04377	0.04539	0.05466	0.05481	0.07136	0.05316	0.07011	0.04923	0.04626	0.05497	0.06530
	7	0.01997	0.03113	0.02243	0.02136	0.02708	0.03601	0.07265	0.03636	0.04140	0.04299	0.05645	0.04734	0.12889
Vt (avg vol/breath)	1	0.0013993	0.0014188	0.0015619	0.0018514	0.0013670	0.0014571	0.0011076	0.0010653	0.0010528	0.0010952	0.0017105	0.0027749	0.0049240
(raw)	2	0.0024787	0.0024939	0.0014730	0.0017995	0.0020868	0.0018620	0.0016992	0.0018581	0.0021837	0.0015394	0.0016983	0.0015725	0.0017154
	3	0.0023277	0.0023649	0.0017917	0.0014789	0.0019550	0.0018157	0.0017108	0.0021217	0.0017646	0.0019528	0.0016495	0.0021175	0.0016221
	4	0.0015158	0.0009721	0.0014477	0.0014215	0.0013993	0.0020162	0.0031657	0.0069162	0.0032848	0.0089096	0.0185266	0.0057976	0.0058948
	5	0.0021047	0.0030426	0.0031694	0.0028960	0.0025478	0.0029935	0.0027176	0.0028347	0.0029835	0.0042554	0.0036559	0.0038770	0.0043208
	6	0.0015234	0.0014810	0.0014119	0.0014642	0.0017921	0.0018900	0.0021624	0.0017720	0.0026965	0.0018233	0.0015420	0.0016168	0.0018657
	7	0.0005788	0.0011530	0.0007734	0.0007366	0.0010415	0.0010289	0.0024217	0.0010694	0.0014786	0.0013434	0.0019466	0.0013526	0.0041577
Vt (avg vol/breath)	1	0.01178	0.01195	0.01315	0.01559	0.01151	0.01227	0.00933	0.00897	0.00886	0.00922	0.01440	0.02336	0.04146
	2	0.02087	0.02100	0.01240	0.01515	0.01757	0.01568	0.01431	0.01565	0.01839	0.01296	0.01430	0.01324	0.01444
	3	0.01960	0.01991	0.01509	0.01245	0.01646	0.01529	0.01440	0.01786	0.01486	0.01644	0.01389	0.01783	0.01366
	4	0.01276	0.00819	0.01219	0.01197	0.01178	0.01698	0.02665	0.05823	0.02766	0.07502	0.15599	0.04882	0.04963
	5	0.01772	0.02562	0.02669	0.02438	0.02145	0.02521	0.02288	0.02387	0.02512	0.03583	0.03078	0.03264	0.03638
	6	0.01283	0.01247	0.01189	0.01233	0.01509	0.01591	0.01821	0.01492	0.02270	0.01535	0.01298	0.01361	0.01571
	7	0.00487	0.00971	0.00651	0.00620	0.00877	0.00866	0.02039	0.00900	0.01245	0.01131	0.01639	0.01139	0.03501
Vt (avg vol/breath/g)	1	0.002990	0.003032	0.003338	0.003957	0.002921	0.003114	0.002367	0.002277	0.002250	0.002341	0.003655	0.005930	0.010523
	2	0.005963	0.006000	0.003544	0.004329	0.005020	0.004479	0.004088	0.004470	0.005253	0.003703	0.004086	0.003783	0.004127
	3	0.005013	0.005093	0.003858	0.003185	0.004210	0.003910	0.003684	0.004569	0.003800	0.004205	0.003552	0.004560	0.003493
	4	0.002954	0.001895	0.002822	0.002771	0.002727	0.003930	0.006170	0.013480	0.006402	0.017366	0.036110	0.011300	0.011489
	5	0.004751	0.006868	0.007155	0.006537	0.005751	0.006758	0.006135	0.006399	0.006735	0.009606	0.008253	0.008752	0.009754
	6	0.003306	0.003214	0.003064	0.003177	0.003889	0.004101	0.004693	0.003845	0.005852	0.003957	0.003346	0.003509	0.004049
	7	0.001361	0.002712	0.001819	0.001732	0.002450	0.002420	0.005696	0.002515	0.003478	0.003160	0.004578	0.003181	0.009779
	Avg	**0.003763**	**0.004116**	**0.003657**	**0.003670**	**0.003853**	**0.004102**	**0.004690**	**0.005365**	**0.004824**	**0.006334**	**0.009083**	**0.005859**	**0.007602**
	Std	**0.001560**	**0.001870**	**0.001674**	**0.001515**	**0.001238**	**0.001358**	**0.001418**	**0.003837**	**0.001677**	**0.005409**	**0.012037**	**0.003073**	**0.003526**
	SEM	**0.000590**	**0.000707**	**0.000633**	**0.000573**	**0.000468**	**0.000513**	**0.000536**	**0.001450**	**0.000634**	**0.002044**	**0.004550**	**0.001162**	**0.001333**
Minute Ventilation	1	0.7356	0.7459	0.8611	0.9971	0.6486	0.5792	0.4829	0.4917	0.4860	0.5898	0.8992	1.3165	2.2098
(mL/min/g)	2	1.1091	1.1879	0.7867	1.0130	1.0241	1.0751	0.9566	0.9924	1.0244	0.7999	0.9805	0.8171	0.9657
	3	0.9324	0.9320	0.8334	0.7070	0.8588	0.8094	0.8621	0.9869	0.7980	0.9083	0.8312	0.9986	0.8174
	4	0.6736	0.4604	0.6856	0.6566	0.6545	0.8724	1.1106	2.7499	1.1908	2.9174	6.2831	1.6950	1.7234
	5	1.0262	1.4423	1.5454	1.3728	1.2423	1.2569	1.3619	1.3822	1.4951	2.0173	1.6835	1.9166	2.1068
	6	0.6348	0.5978	0.5699	0.5910	0.7117	0.7137	0.9292	0.6922	0.9129	0.6410	0.6023	0.7157	0.8502
	7	0.2818	0.4393	0.3165	0.3014	0.3821	0.5082	1.0252	0.5131	0.5842	0.6067	0.7966	0.6680	1.8189
	Avg	**0.770503**	**0.829371**	**0.799812**	**0.805572**	**0.788882**	**0.830677**	**0.961197**	**1.115485**	**0.927349**	**1.211495**	**1.725215**	**1.161095**	**1.498878**
	Std	**0.281106**	**0.379410**	**0.378685**	**0.349663**	**0.281344**	**0.266004**	**0.266652**	**0.785856**	**0.348985**	**0.903556**	**2.038590**	**0.494332**	**0.605159**
	SEM	**0.106248**	**0.143403**	**0.143129**	**0.132160**	**0.106338**	**0.100540**	**0.100785**	**0.297026**	**0.131904**	**0.341512**	**0.770515**	**0.186840**	**0.228729**