# *In Utero Exposure to Nicotine: The Effects of Postnatal Withdrawal on the Developing Breathing Pattern in Neonates*

By
*Cassie Andreas*
*March 30, 2015*




**Allegheny College**
***Biology Program***

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements
for a Bachelor of Science Degree from Allegheny College

***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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*Dr. Coates*

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*Dr. Mumme*

**Abstract:**

Sudden Infant Death Syndrome (SIDS) is the death of an infant under one year of age that is unexplained after all possible causes have been investigated. One of the leading risk factors for SIDS is prenatal nicotine exposure. Prenatal nicotine exposure can cause defects in cardiorespiratory control of infants that demonstrates the ventilatory response to stress, hypoxia, and hypercapnia. Previous studies have examined the effects of prenatal nicotine exposure on a neonate, yet no studies have addressed the issue of nicotine withdrawal following the gestational period. In this study, four breeding pairs of mice were exposed to 3 mg/kg/day of nicotine drinking water prior to and during gestation. Of these four breeding pairs, two litters were continuously exposed to nicotine following birth and two litters were withdrawn from nicotine following birth. Upon birth, pressure plethysmography was used to measure the ventilatory response of the mice to hypercapnia on postnatal days 1, 3, 5, 7, 9, and 11. Frequency, tidal volume and minute ventilation were analyzed and represented as functions of percent $CO_2$ to determine the effects of hypercapnia on postnatal nicotine withdrawal. I found that postnatal nicotine withdrawal delays the neonate's insensitivity to $CO_2$ during the postnatal period of maturation. The delayed period on insensitivity to $CO_2$ may allow for this period to occur outside of the critical window for SIDS, reducing the infants vulnerability to SIDS.

**Introduction:**

Sudden infant death syndrome, more commonly known as SIDS, is defined as the death of an infant under the age of one year by unexplainable causes following a case investigation, autopsy, crime scene investigation, and thorough review of clinical history (Willinger *et al.*, 1991). In 1992, the incidence of SIDS was 120 for every 100,000 births (American Academy of Pediatrics, 2011). It was observed that in many of the SIDS incidences, the babies were sleeping in the prone (on their stomach) position spawning the "Back to Sleep" campaign in 1994. This campaign advised mothers and caregivers to place infants on their backs to sleep, rather than in the prone position. Infants sleeping in the prone position have an increased risk of SIDS by up to two-fold because it increases the likelihood that expired gases will be inhaled again. Over a fifteen-year period this campaign increased the positioning of infants in the supine (on their back) position by 72% (American Academy of Pediatrics, 2011). During this same period, the "Back to Sleep" campaign decreased SIDS by over 50% (Trachtenburg *et al*., 2012). However, there are still a number of risk factors that place infants at risk of SIDS in cases that could have been preventable.

In 1994, Filiano and Kinney proposed the Triple-Risk Model, which stated that there are three factors that when present increases the infant's risk of SIDS. These factors include: a vulnerable infant, a critical developmental period, and exogenous stressor(s) (Figure 1). A vulnerable infant is considered by the criteria that they have

central nervous system and autonomic function abnormalities, postneonatal abnormalities in cardiac and ventilatory patterns, and maternal risk factors for SIDS which include young maternal age, maternal cigarette smoking or cocaine use during pregnancy. The second factor is the critical developmental period in which most SIDS related deaths occur in the first six months of life, particularly in the two to four month range. This critical period is identified by the physiological changes that occur during this time including: autonomic control, ventilation, temperature regulation and circadian rhythms. The brain undergoes significant changes during this critical period in which it transitions to homeostatic control. The third factor states that infants who may appear clinically normal are exposed to exogenous stressors within the first six months. Examples of exogenous stressors include the prone sleeping position, elevated $CO_2$ increased skin temperature, increased baseline heart rate, and recent infection of the upper airway. In order for an infant to be at risk for SIDS, the exogenous stressor must match the vulnerability of that infant (Filiano and Kinney, 1994).

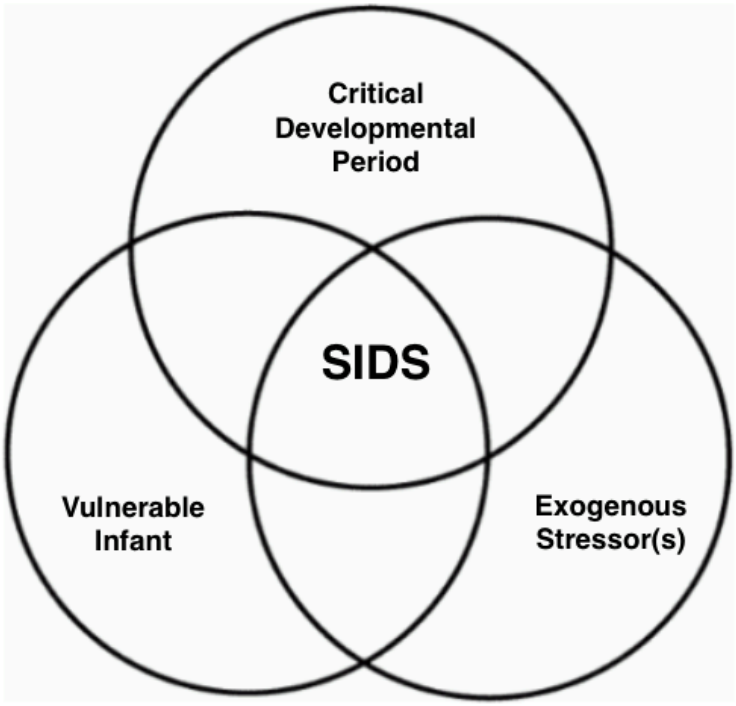



**Figure 1. Triple-Risk Model for SIDS (Filiano and Kinney, 1994).** Each circle represents one of the factors that are a key link to SIDS. The diagram represents that all three factors are needed in order for one to be at risk of SIDS.

Low birthweight, preterm birth, smoking during pregnancy, lack of breastfeeding, lack of prenatal care, sleeping in the prone position, and use of any drugs or alcohol are all risk factors of SIDS (Hoffman *et al.*, 1988). Cigarette smoking is the only legal consumer product that kills a large proportion of its users with over 5 million deaths resulting from tobacco use each year, making it the leading preventable cause of death in women and newborns (Coleman, 2012; DiFranza and Lew, 1995). Prenatal or postnatal exposure to cigarette smoke increases the chance of addictive behaviors in adolescence and adulthood (Rogers, 2009). Maternal smoking is not the only form of tobacco use that is harmful to the fetus. In studies that examined smokeless tobacco exposed prenatally, there were similar effects on the developing fetus as of cigarettes

(Wickstrom, 2007). Nicotine is the key factor that links smoking during pregnancy to cardiorespiratory problems (Eugenin *et al.,* 2008). It is well known that smoking during pregnancy can be harmful, sometimes even fatal to the fetus, yet 25% of women in the United States continue to use tobacco products while pregnant (Ernst *et al*., 2001). Approximately 34-40% of women in the United States either quit or decreased their smoking habits throughout the duration of their pregnancy (Godding *et al*., 2004). Smoking while pregnant has several implications for the newborn including low birth weight, perinatal deaths, asthma, and effects on the normal development of the central nervous system (DiFranza and Lew, 1995). While cigarettes contain many toxins, nicotine is defined as a neuroteratogen by itself affecting critical stages of brain development during pregnancy (Rogers, 2009; Slotkin, 1995).

Nicotine exposure to the fetus is the leading risk factor for SIDS (Robinson *et al*., 2002). The risk of SIDS is increased by up to fourfold in utero exposure of cigarettes (Bamford *et al.,* 1996). It is estimated that one-third of SIDS related deaths could be prevented by eliminating maternal smoking during pregnancy (American Academy of Pediatrics, 2011). With a quit rate of approximately 37% during pregnancy, the prevalence of mothers returning to smoking after pregnancy is equal to what it was prior the pregnancy (DiFranza and Lew, 1995). Those that are most impacted are the newborns of younger, less educated women who are less likely to quit smoking while pregnant, than women of higher education (Rogers, 2009). It is believed that chronic hypoxia (decrease in oxygen) plays a role in death by SIDS which corresponds to defects in cardiorespiratory control during fetal development as a result of nicotine exposure

(Bamford *et al*., 1996). The implications of *in utero* nicotine exposure linking to SIDS are diminished heart rate responses to stress, alterations in brainstem regions related with respiratory control, and decreased growth and development of the fetal lungs (Rogers, 2009).

Cigarette use is not the only method of nicotine exposure to neonatal infants, as it extends to all forms of smokeless tobacco. Smokeless tobacco refers to all types of tobacco-containing products such as chewing tobacco, oral tobacco, and spitting tobacco. The various forms of smokeless tobacco contain many of the same chemicals present in cigarettes, mainly nicotine. Chewing tobacco for a minimum of thirty minutes is the equivalent of smoking three cigarettes within that small period of time (NIH Medline Plus, 2011). Regardless of the form of tobacco for which one chooses to use, the risk and exposure to nicotine still poses prominent effects on the infant.

In mice, *in utero* exposure to nicotine via osmotic minipumps has been used to model maternal smoking of mothers and the effects on their newborn. When a mouse is exposed to nicotine prior to and during gestation, the mouse pups are observed to hypoventilate and have reduced responses to air enriched with 10% $CO_2$ (which is almost three times the normal exposure) suggesting that fetal nicotine exposure interferes with central respiratory chemoreception (Eugenin *et al*., 2008). By exposing the mothers to nicotine prior to gestation and throughout the term, it allows for the nicotine to play a role in affecting the development of all systems of the infant, particularly the respiratory system. The reduced ventilatory responses and

hypoventilation of these mice pups suggests that when nicotine interferes in central respiratory chemoreception, it changes the hypoxic and hypercapnic (elevated carbon dioxide) chemoreflexes (Eugenin *et al*., 2008). In agreement with other studies, Eugenin *et al.* (2008) identified nicotine as having a critical period following the exposure of nicotine around postnatal days three and four. Those results are consistent with a study in which the ventilatory responses of mice were being examined in response to hypercapnia. It was found that around postnatal day 8, the rats had a lower-sensitivity to $CO_2$ in comparison to all other days tested, representing a critical period of insensitivity (Stunden *et al.,* 2001). This period is critical because it highlights a specific time when a mouse pup can be highly vulnerable and might fall susceptible to hypercapnia (Eugenin *et al*., 2008).

When a mouse is only exposed to nicotine on the tenth day of gestation and for the rest of the pregnancy, delayed respiratory effects on the pups exposed to nicotine in comparison to the unexposed pups were reported. The delayed respiratory effects were a result of hypoxic levels of 7.4% $O_2$ compared to the normal oxygen level in the air (21%) is quite low. Similar respiratory responses were seen for the mouse pups on day one of testing but began to differ at approximately day three. The data from this experiment suggest that there are delayed developmental changes in a newborn's breathing pattern (Robinson *et al*., 2002). This is not a typical model of human behavior, as it is unlikely a mother would start smoking halfway through her pregnancy, but it does suggest that nicotine's half-life is of key-importance when studying the amount of nicotine which the fetus is exposed to, so the results can be interpreted in a

situational manner. Both of these studies suggest that impaired respiratory responses to hypercapnia during a mouse pup's initial postnatal days increases the vulnerability to SIDS. They also suggest that infants of smoking mothers have a delayed development of their breathing patterns resulting in impaired respiratory control (Robinson *et al*., 2002).

In the mouse studies described above, osmotic minipumps were placed dorsally, under the skin of the mother before or during gestation, however, there are various critiques to this method of drug delivery. The surgical implantation of a minipump and the following recovery may cause stress on the mother and/or her litter. Aside from the stress, the minipump does not accurately model the smoking habit seen in people addicted to smoking because it is not a phasic exposure to nicotine. The minipump continuously supplies the mouse with a constant dose of nicotine absorbed into the bloodstream, but that is not true of real smokers. Administering nicotine in the drinking water is a better model for cigarette smoking in that the nicotine is constantly available in the cage but the mice choose when they want it, modeling the addictive behavior. The mice drink the nicotine water when they are thirsty and then develop the addictive behavior that will model a phasic pattern of nicotine delivery.

The studies of Eugenin et al. (2008) and Robinson et al. (2002) examined the effects of nicotine respiratory development in newborns that were exposed during and after gestation, but there are currently no studies that examine the withdrawal of nicotine following birth after constant exposure throughout the gestational period. In 2012, it was determined that prenatal nicotine exposure caused both lower and higher

sensitivity to hypercapnia in neonatal mice. This study exposed mice to 3 mg/kg/day of nicotine through a drinking solution and exhibited both lower and higher sensitivities in response to increased levels of $CO_2$ (Brzoza, 2012). Following that study, similar results were observed in a study that exposed mice to either low or high dosages of nicotine prenatally and postnatally. The results indicated that there was an impairment of the neonatal ventilatory responses regardless of whether the neonate is exposed to low or high levels of nicotine (Culig, 2014).

My study models the situation in which an infant is exposed to nicotine prenatally but then removed from the environment which exposes them to nicotine following birth. This represents newborns that are born from mothers who smoked while pregnant and then were given up for adoption or raised by another family member (in a smoke-free environment), implying nicotine withdrawal symptoms in the newborn. This is in comparison to newborns that are raised in a household where the mother smokes (and possibly other family members) and the newborn is continuously exposed to nicotine through the breast milk of the mother or the expired smoke from cigarettes throughout gestation and maturation. I recorded ventilatory responses for the two experimental groups in response to elevated levels of $CO_2$ to examine possible increases or decreases in respiration that can represent nicotine's effects on the newborn's respiratory development. Using the protocol of Culig (2014) and Brzoza (2012) I exposed mice to nicotine drinking water and tested the neonate's respiratory response to hypercapnia using plethysmography every other day for eleven days. I compared both of my groups (continuous nicotine exposure and nicotine withdrawal) to

the controls in their experiments to provide a baseline for comparison of normal values of frequency, tidal volume and minute ventilation, because it was expected that both of my groups would experience changes in ventilatory responses. My objective for this study was to determine if the postnatal withdrawal of nicotine from a neonates' environment would have any effect on their breathing response to hypercapnia. The results of this study showed that postnatal withdrawal of nicotine delayed the neonates' insensitivity to elevated levels of $CO_2$. This suggests that the postnatal withdrawal of nicotine may delay the respiratory response in human infants outside of the critical period in which infants are most at risk of SIDS.

**Methods:**

*Animals.* All experiments were carried out using 4 litters of C57 BL/6 mice (totaling 23 pups) that were bred and housed in the Allegheny College Biology department's animal colony in a room on a 12 hour light/dark cycle. Food and water were provided *ad libitum*. In order to track individual mouse pups through the 11 days of testing, the animals were tattooed on the first day of testing (P1) in sequential order by using combinations of dots on their paws.

*Prenatal Nicotine Exposure*. Nicotine was provided in sucrose-flavored drinking water, with 3 mg/kg/day of nicotine dissolved in 2% sucrose, one week prior to mating and throughout gestation, which is approximately 21 days. Three mg/kg/day was the chosen dosage because 1.5 mg/kg/day did not produce any major effects on the ventilatory responses in mouse pups (Culig, 2014). Nicotine intake was measured by weighing the drinking water daily. Previous studies have shown that 2% sucrose must be added to the water to mask the bitter taste of the nicotine and reinforce water consumption (Culig, 2014). The daily dosage of nicotine in the sucrose solution was calculated by multiplying the dosage of nicotine (3 mg/kg/day) by the weight of the mouse and then dividing that by the average daily consumption assumed of mice. Nicotine water was measured every other day to keep track of the amount being taken in by the mice. After birth, the adult males were removed and the dams and mouse pups were either continuously exposed to the nicotine drinking water or withdrawn from the nicotine by having the drinking water replaced by regular water (Figure 2).

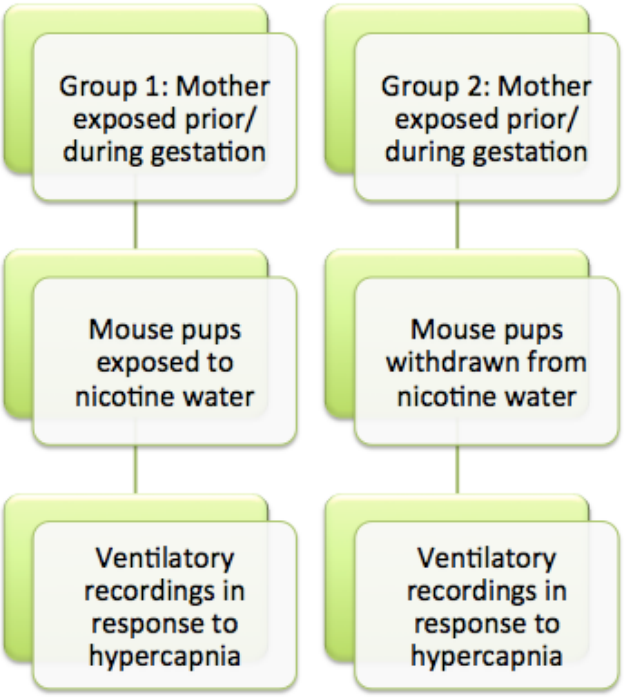
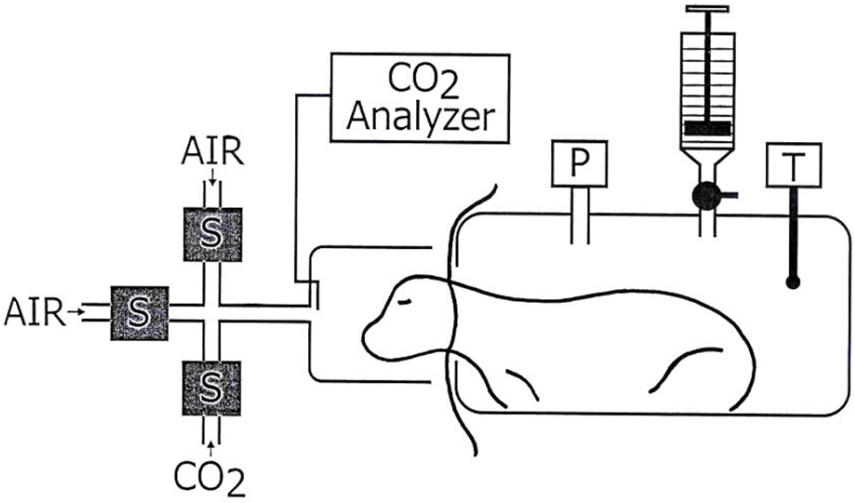



**Figure 2. Schematic of the experimental groups.**

*Experimental Protocol*. Neonates were weighed every other day after birth. Head-out, whole body plethysmography was used to record ventilation from pups on days P1, P3, P5, P7, P9 and P11, with P0 representing the day of birth (Figure 3).




From Coates and Silvis, 1999

**Figure 3. Head-out plethysmography chamber which mouse pumps were placed into to receive gas stimulation.** S, solenoid valve; P, pressure transducer; T, temperature probe. (Coates and Silvis, 1999).

Mouse pups were placed in a plethysmography chamber and acclimated for approximately four minutes. To provide the pups with a constant temperature, a heating pad, set at 33°C, was placed under the plethysmography chamber (Coates and Silvis, 1999). Mouse pup's baseline breathing was recorded for 3-5 minutes. As seen in Figure 4, increases in $CO_2$ concentration were administered every 3 minutes in 2% increments. After 3 minutes of 6% $CO_2$ inhalation, the $CO_2$ was shut off and the mouse pup was removed from the plethysmography chamber and returned to the mother.

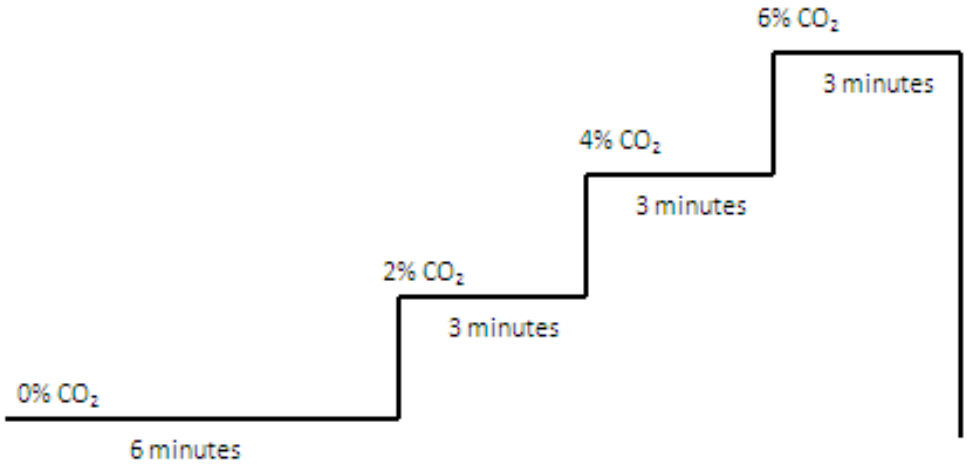



**Figure 4. Schematic of how % $CO_2$ was administered.**

*Data Analysis.* For each mouse pup, on each testing day (P1, P3, P5, P7, P9 and P11), 10 seconds of the breathing record was analyzed at the end of the three minute period of each $CO_2$ concentration. The number of breaths in 10 seconds was measured and multiplied by 6 to determine the frequency of breaths per minute (*f* ) (Figure 5).

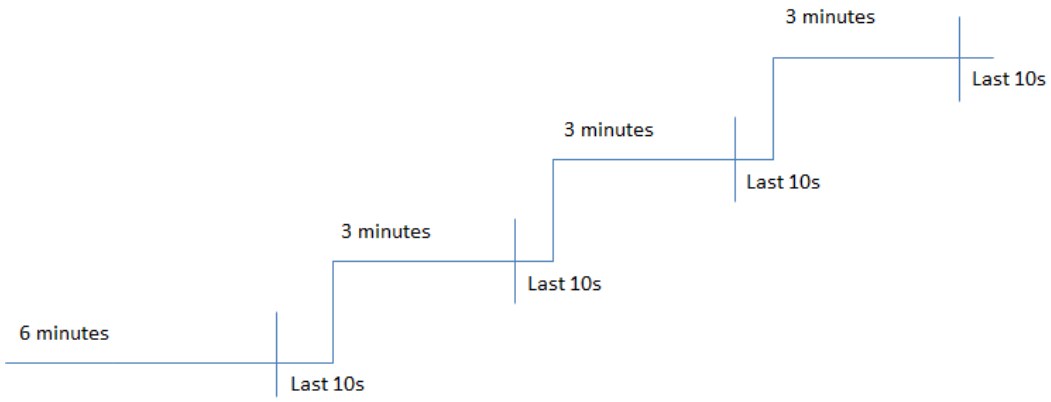



**Figure 5. Schematic representation of data collection and analysis.** *f*, $V_T$, and $V_E$ were recorded during the last 10 seconds of $CO_2$ inhalation at levels of 0, 2, 4 and 6%.

The area under the breathing record was also measured during the same 10 seconds and this represented the total volume of air breathed. After dividing this number by the

number of breaths and the weight of the animal in grams, a raw value for the volume of air per breath, or tidal volume ($V_T$) was determined (Figure 6).

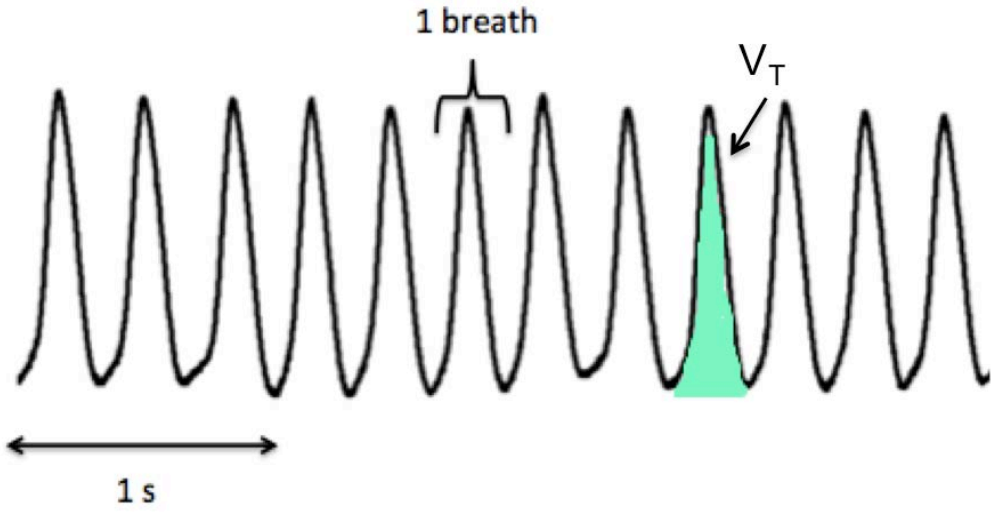



**Figure 6. Sample four second breathing recording, showing area under curve and frequency.** *F* is the frequency of breaths per minute, while $V_T$ is the average area under the curve per breath.

A calibration syringe, attached to the chamber, was used to deliver a known volume of air to calibrate the tidal volume. Minute ventilation ($V_E$) defined as milliliters per minute, is the product of *f* (breaths/min) and $V_T$ (mls/breath/gram). Graphs of *f* as % of $CO_2$ as a response to hypercapnia for each animal on each testing day were made. Using a linear best-fit line, the slopes of the ventilatory response to hypercapnia were analyzed for each of the ventilatory parameters (Figure 7).

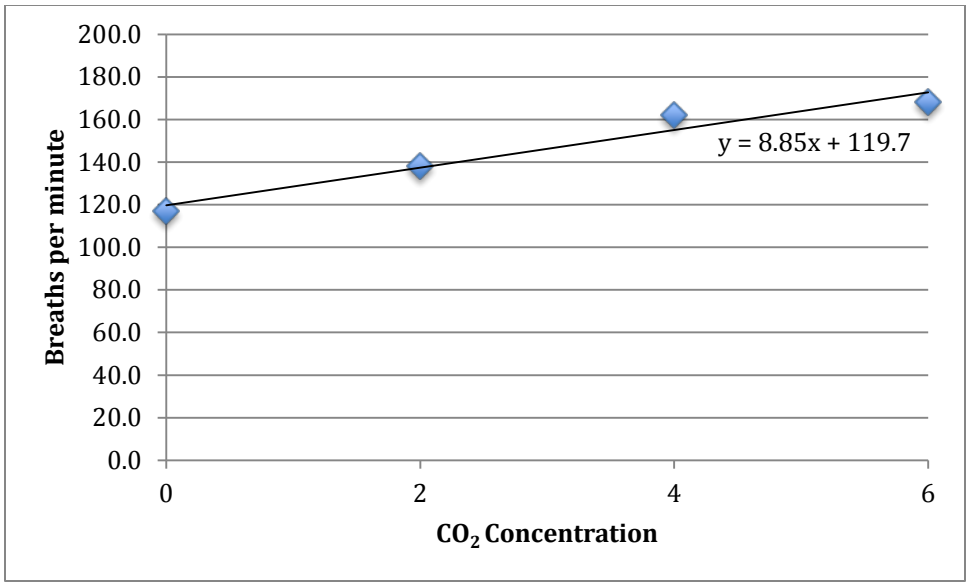



**Figure 7. Graph of individual mouse pup frequency of breaths as $CO_2$ concentrations increase.** Linear trend line gives the slope of an individual mouse pups breathing frequency. Equation represents the slope of the linear trendline.

The value of the slope represents the sensitivity of the mouse to increasing amounts of $CO_2$. For example a positive slope indicates a greater response to increasing levels of $CO_2$ and a negative slope or slope of zero represents a negative response or an absence of a response to hypercapnia.

*Statistical Analysis*

A Mixed Model ANOVA was used to analyze the *f* and $V_E$ for both experimental groups over the eleven-day testing period. Random effect was used to control the longitudinal tracking of mice for each day and for each $CO_2$ concentration because the measurements on the same mice are non-independent.

**Results:**

*Survivability, Growth and Behavioral Observations*

Eight breeding pairs of mice were put together and provided with nicotine in their drinking water, and five of the dams gave birth, resulting in a 62.5% breeding rate. Of those five pairs, four gave birth to live pups, giving four testable litters composed of a total of 29 mouse pups. Of those 29 pups, 24 survived past postnatal day 5 (Table 1).

Pups were weighed every other day and the weights were recorded and graphed for litters continuously exposed to nicotine versus litters withdrawn from nicotine. The graph shows that nicotine did not affect weight gain whether it was continuously consumed or withdrawn the day of birth. Using Culig (2014) control mice as a baseline for growth, the experimental groups did not differ (Figure 8).

Behaviors of the two groups of pups were recorded to demonstrate the behavioral changes impacted by nicotine exposure. Litters withdrawn from nicotine took longer periods of time to settle during the acclimation period which consisted of 0% $CO_2$. Litters that were continuously exposed to nicotine showed frequent apneas on postnatal day 1 but not much on the following days of testing. The litters withdrawn from nicotine experienced frequent apneas on day 1 and day 3. Agitation and irritability to the breathing chamber was noted for all litters on various testing days. The litters continuously exposed to nicotine showed agitation and restlessness as early as day 5,

where the litters withdrawn from nicotine did not demonstrate restlessness until day 7/day 9.

*Water Consumption*

Nicotine water was measured every other day to record the average intake of each breeding pair. The average amount of nicotine water consumed by all four tested litters was 7.86 grams per day (Appendix A).

*Continuous Nicotine Exposure: Breathing Frequency*

Figure 9A shows each individual mouse pup's slope (breathing frequency as a function of % $CO_2$) over the eleven-day span of testing. The average slope of this litter started at 6.94 and proceeded to decrease down to day 5, which then proceeded to increase (significant) each day thereafter (Figure 9B). The slope on day 5 demonstrates a small breathing response of only 1.09, and it should be noted that a slope of 0 indicates no ventilatory response to $CO_2$. By postnatal day 11, the slope had resumed to that of day 1.

Figure 10A shows each individual mouse pup's slope (breathing frequency as a function of % $CO_2$) for the second litter continuously exposed to nicotine. The average slope follows a similar pattern to the first litter, day 1 the slope was 5.46 which then increased on day 3 and then decreased (significant) by day 5, increasing each day thereafter (Figure 10B). On day 5 the slope decreased to 2.52 but by day 11 the slope had increased to 7.8, demonstrating an increasing breathing response to hypercapnia.

Figure 11 shows the average slope of ventilatory response to $CO_2$ for the two litters continuously exposed to nicotine, demonstrating what was shown in both Figure 9B and Figure 10B. The average slope began at 6.2 on day 1 and then increased (significant) on day 3, but then decreased on day 5 to 2.59 (Figure 11). It increased (significant) each day after and on day 11 the slope was 10.05.

*Nicotine Withdrawal: Breathing Frequency*

Figure 12A shows the individual mouse pups from the first litter of pups withdrawn from nicotine water, over the eleven-day span of testing. The average slope of the individual mouse pups within that litter starts at 2.52 on day 1 and then proceeds to increase on day 3, but following day 3 it begins a slow progression of decreasing (no significant change) slopes with the lowest point being a negative slope of -2.186 on day 9 and a slight increase following on day 11 (Figure 12B).

Figure 13A shows the individual mouse pups from the second litter of pups withdrawn from nicotine water over the eleven-day span. The average slope of this litter started off at 4.8 on day 1 and proceeded to increase on day 3, this was followed by a slight decrease (no significant change) until day 7 where the slope reached the low point of 2.6, the slopes then increased until day 11 (Figure 13B). The average slope on day 4 was 4.4, very similar to the slope of 4.4 on day 1.

Figure 14 is the average of the two litters that were continuously exposed to nicotine over the eleven-day postnatal period. The average slope begins at 3.66 and then increase on day 3, following that the average slope decreases (no significant

changes) until it reaches its lowest point on day 9 where the slope was 0.91 and then increases back up to 5.43 on day 11 (Figure 14).

*Minute Ventilation: Continuous Nicotine Exposure and Nicotine Withdrawal*

Breathing frequency and minute ventilation are representative of the similar data but $V_E$ includes the variable of tidal volume. $V_E$ represents the total output from the mouse pup while breathing frequency is just a measurement of the rate of breathing. The litters that were continuously exposed to nicotine had negative slopes on P5 and P7, with the lowest overall slope on P5, indicating no respiratory response to elevated levels of $CO_2$ (Appendix E). The slopes of $V_E$ were calculated and demonstrated that there was little to no respiratory response in the presence of elevated levels of $CO_2$ on P7 for mice withdrawn from nicotine (Appendix F). There was a significant difference on P11 for which the $V_E$ for litters withdrawn from nicotine was greater than that of the litters continuously exposed to nicotine. Tables were made of the minute ventilation values and they were similar to that of the slope of breathing frequencies for both continuous and nicotine withdrawal. $V_T$ needed to be recorded in order to calculate $V_E$, but it is not displayed.

| | Day 0 | Day 1 | Day 3 | Day 5 | Day 7 | Day 9 | Day 11 | Survival |
|---|---|---|---|---|---|---|---|---|
| Litter 1-Continuous | 9 | 6 | 5 | 5 | 5 | 5 | 5 | 55% |
| Litter 4-Continuous | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 100% |
| Litter 2-Withdrawal | 6 | 5 | 5 | 5 | 5 | 5 | 5 | 83% |
| Litter 3-Withdrawal | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 85% |

**Table 1**. Sizes of the four tested litters on the day of birth until last day of testing accompanied with the survival rates of each litter.

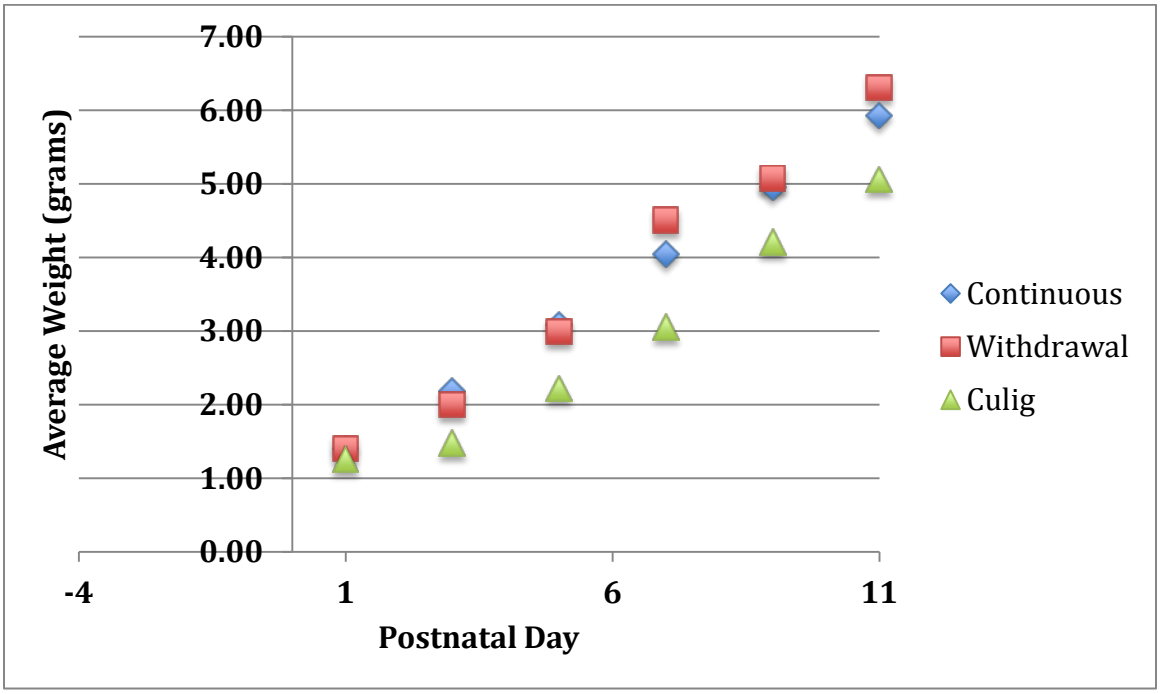



**Figure 8. Average growth rates of litters continuously exposed to nicotine and litters withdrawn from nicotine** (Culig, 2014).

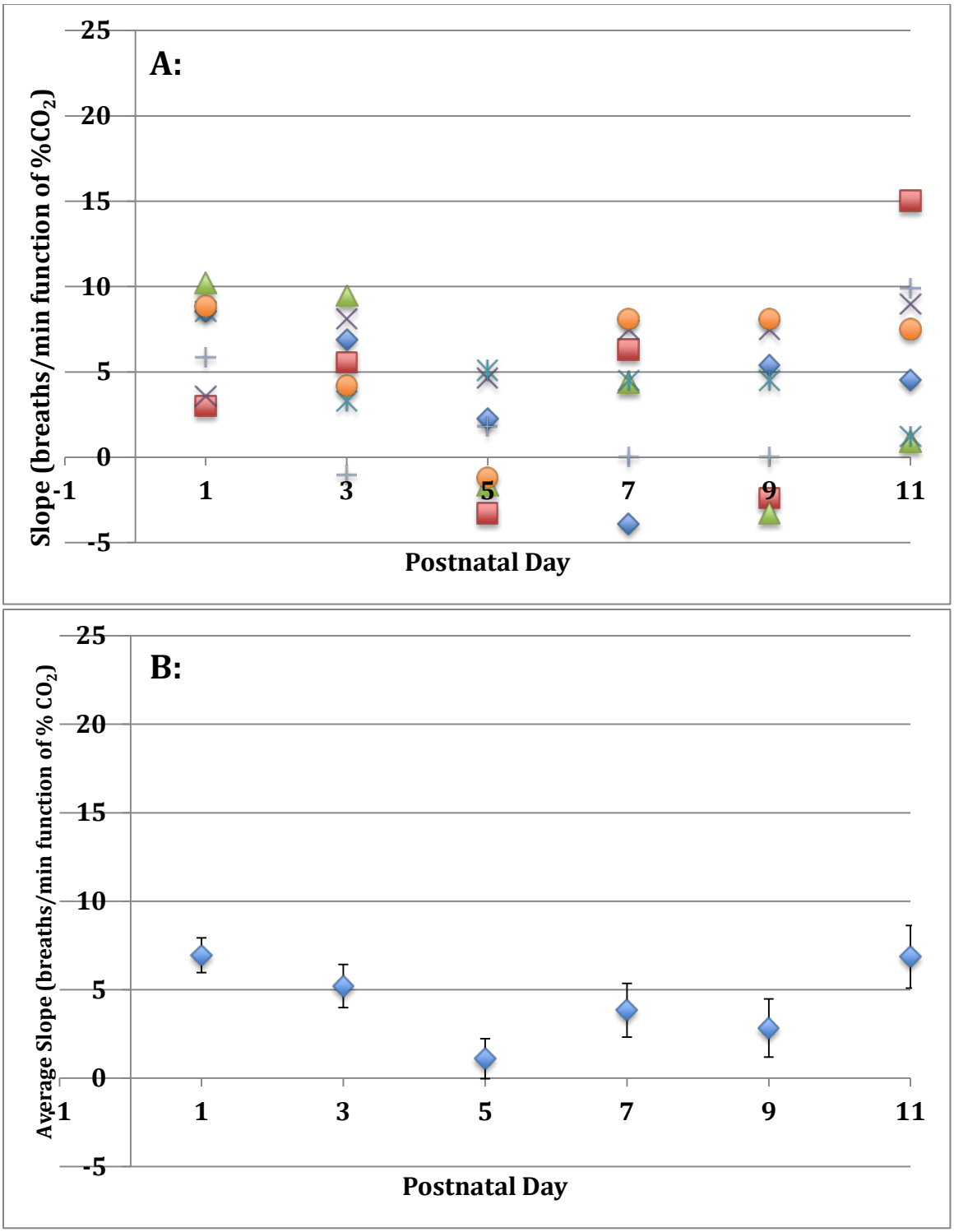



**Figure 9. Slope of breathing response (breaths/minute as a function of % $CO_2$) in the first litter of mouse pups continuously exposed to nicotine water for 11 days following birth. A:** Slopes of 7 individual mouse pups, with each symbol representing a different pup. **B:** Average slope (± SEM) of all mouse pups in the first litter. Note that the absolute values on the y-axes for figures 9, 10, 12 and 13 are different but the overall scale is equivalent (30 units of slope).

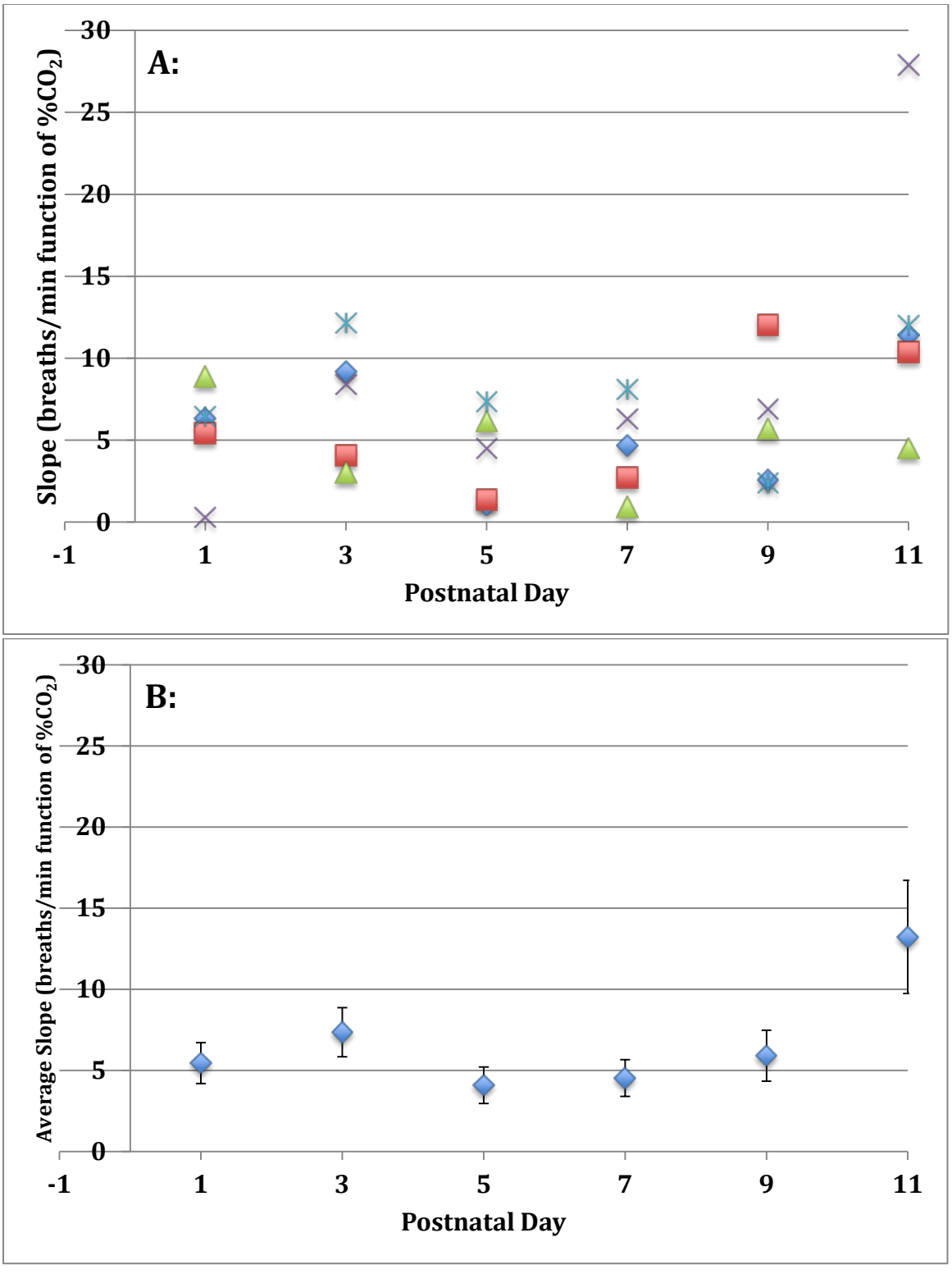



**Figure 10. Slope of breathing response (breaths/minute as a function of % $CO_2$) in the second litter of mouse pups continuously exposed to nicotine water for 11 days following birth. A:** Slopes of 6 individual mouse pups, with each symbol representing a different pup. **B:** Average slope (± SEM) of all mouse pups in the second litter.

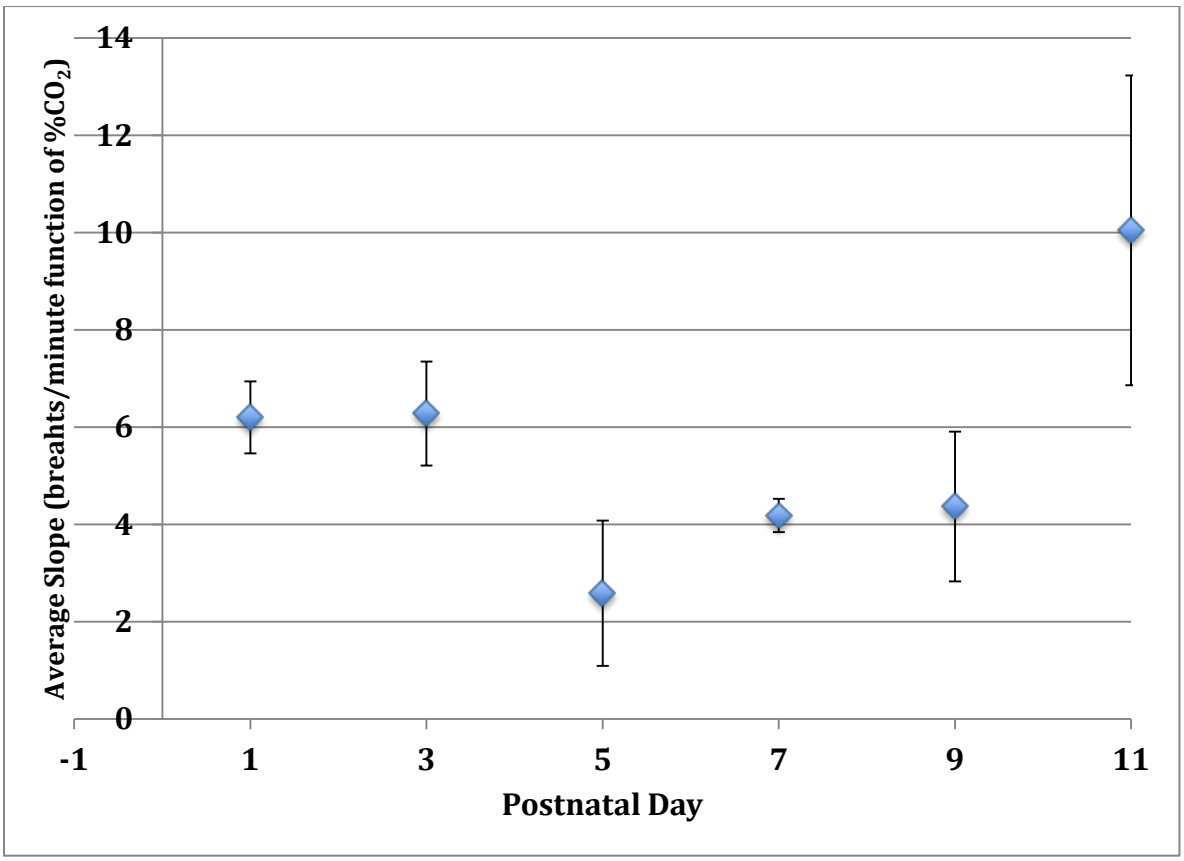



**Figure 11. Average slope of breathing responses for two litters continuously exposed to nicotine.** n= 13 mouse pups

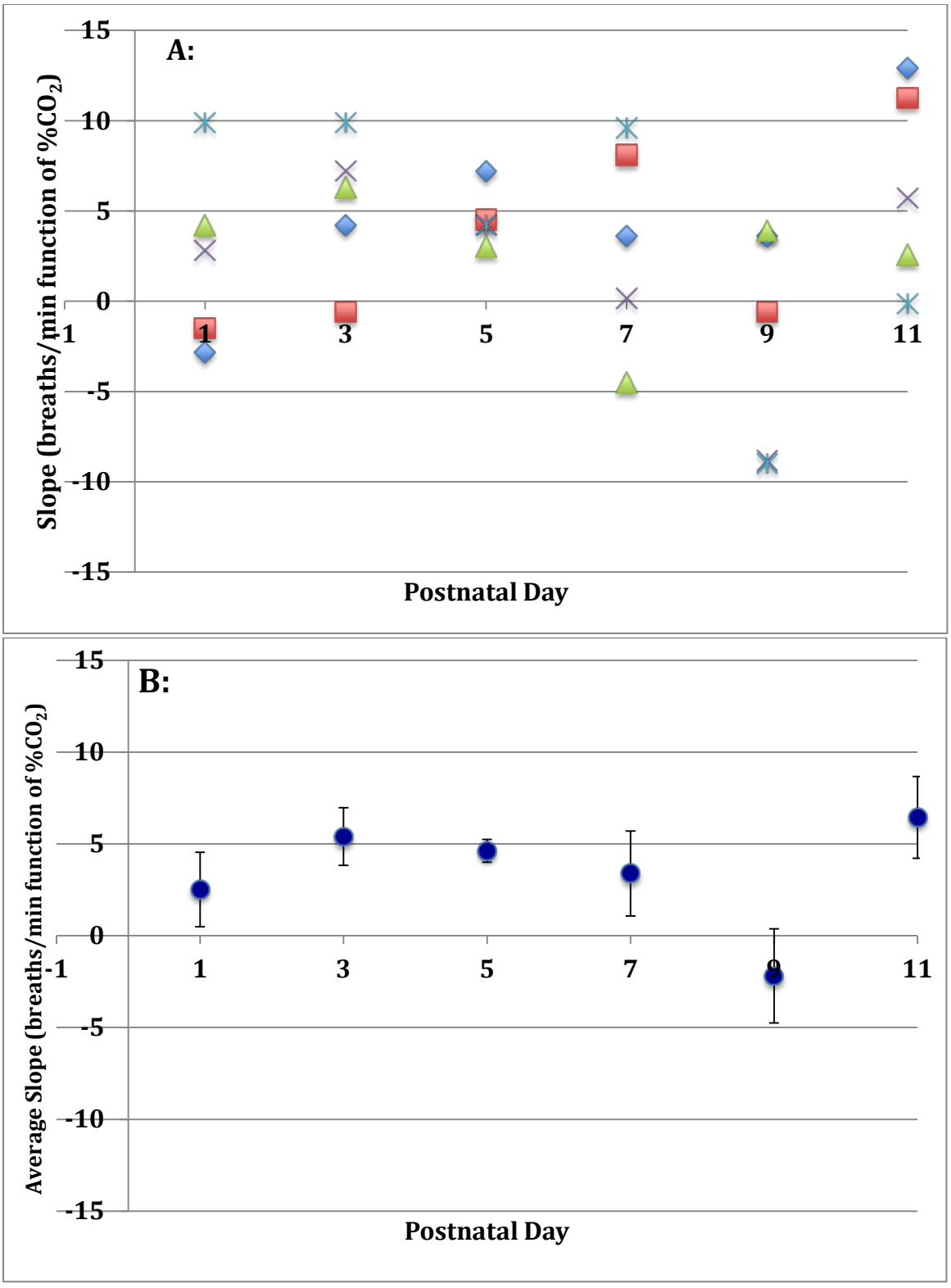



**Figure 12. Slope of breathing response (breaths/minute as a function of % $CO_2$) in the first litter of mouse pups withdrawn from nicotine water for 11 days following birth.** **A:** Slopes of 5 individual mouse pups. **B:** Average slope (± SEM) of all mouse pups in the first litter.

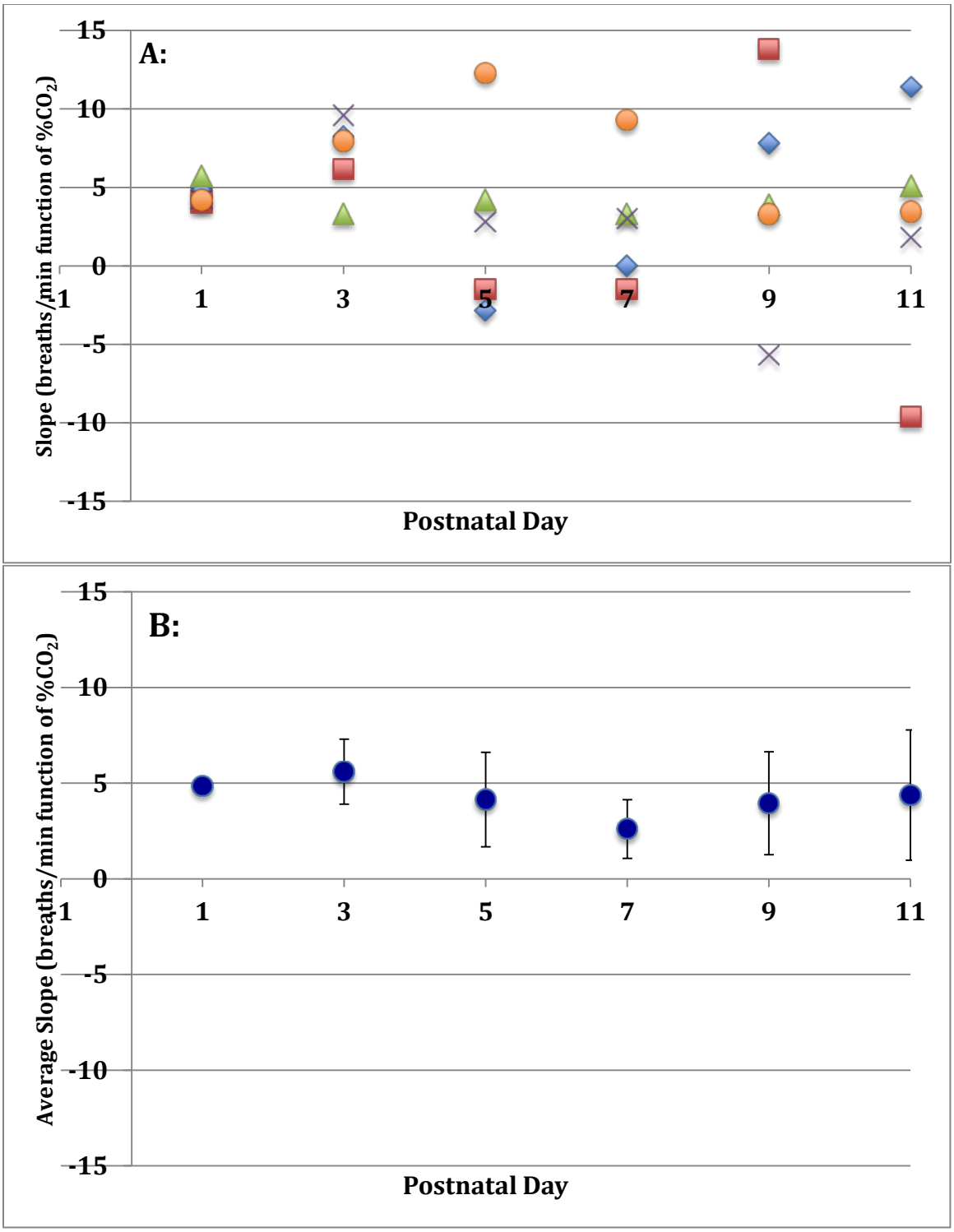



**Figure 13. Slope of breathing response (breaths/minute as a function of % $CO_2$) in the second litter of mouse pups withdrawn from nicotine water for 11 days following birth. A:** Slopes of 6 individual mouse pups. **B:** Average slope (± SEM) of all mouse pups in the second litter.

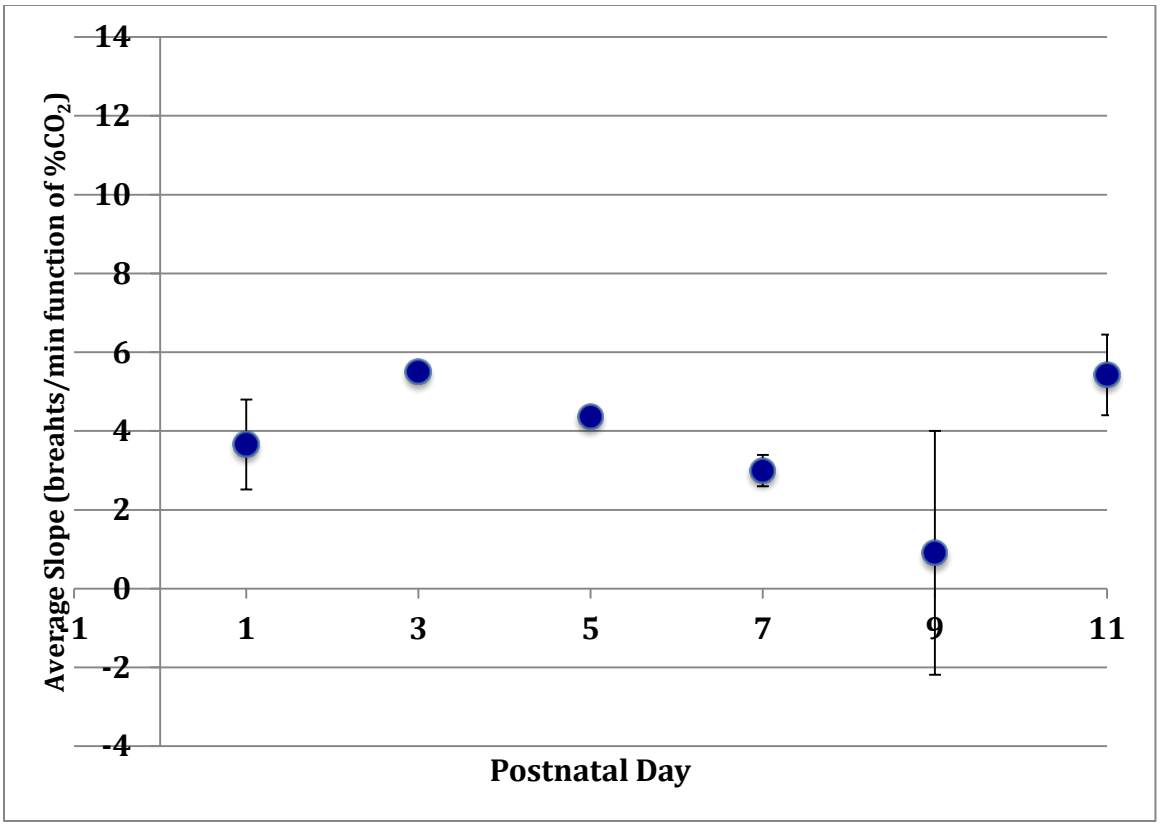



**Figure 14. Average slope of breathing responses for two litters withdrawn from nicotine.** n=11 mouse pups

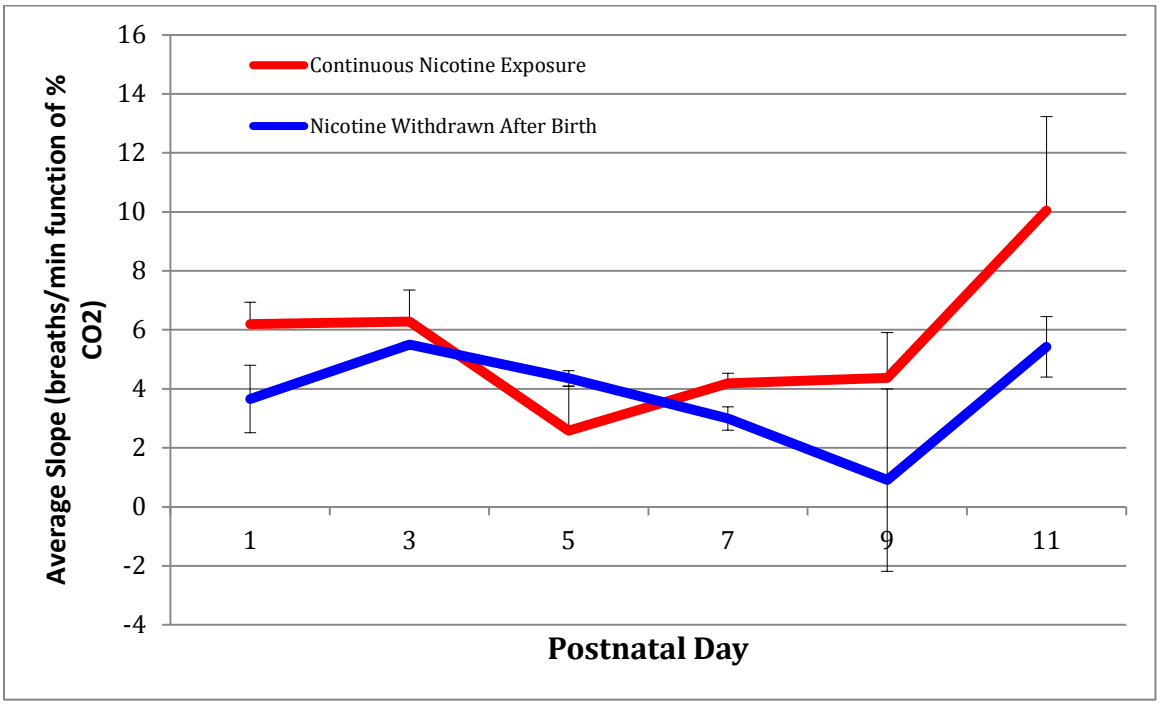



**Figure 15. Average slopes of litters continuously exposed and withdrawn from nicotine following a biphasic pattern.**

**Discussion:**

The objective of my study was to determine if the withdrawal of nicotine following birth in neonatal mice would change their breathing response to $CO_2$. In general my results suggest that the withdrawal of nicotine from a neonate following birth delays the reduced breathing response to hypercapnia to around days 7-9 where it occurs at days 5-7 in mice that are continuously exposed to nicotine (Figure 11 & Figure 14). These results suggest that the withdrawal of nicotine may inhibit the development of the respiratory system or potentially allow the respiratory system to return to its normal capacity over time. Therefore, that the critical period in which infants are susceptible to experiencing insensitivities to elevated levels of $CO_2$ could be delayed thus inhibiting the vulnerability to SIDS.

Water consumption was comparable to the intake reported in another study that showed a range around 7-8 militers of water consumed per day (Brzoza, 2012). The average water consumption was calculated by dividing the amount consumed per day by the number of days in between measurements and then also by the number of mice within the breeding pair that was in the cage. Error could have occurred here because the water consumption of the male mice was not known but assumed to be half of the total consumed. While water bottles were tested and examined daily, there were still bottles that experienced heavy leakage and thus water consumption was not calculated for those days. The nicotine drinking solution did not cause the mice to drink more than was expected, but they also didn't consume less than expected, indicating that the 2% sucrose did in fact mask the bitter taste of the nicotine.

The weight gain of pups for both continuous nicotine consumption and withdrawal groups was on average similar throughout the eleven-day experiment. The weight gain of control mouse pups (unexposed to nicotine) was on average equal to the weight gain of mouse pups continuously exposed or withdrawn from nicotine (Culig, 2014). This demonstrates that nicotine did not have a physical effect on the growth or weight gain of the pups in this study. Even though some studies have reported that nicotine caused smaller birth weights and stunted the growth in mouse pups, my experimental groups grew at a normal rate comparable to the controls from Culig's (2014) study. This is also consistent with Eugenin et al. (2008) who observed that even high doses of nicotine (60 mg/kg/day) did not have an effect on the birth weight or weight gain of the pups.

While it was expected that the mouse pups continuously exposed to nicotine would experience apneas within the first few days of life after being prenatally exposed to nicotine (Huang *et al.,* 2004), frequent apneas were observed in both experimental groups. It was previously seen that prenatal nicotine exposure is commonly associated with longer and more frequent apneas during the first two postnatal days (Huang *et al.,* 2004). In my study the litters withdrawn from nicotine were observed to experience frequent and longer-lasting apneas throughout the first five days following birth. This is similar to what Huang et al., (2004) observed where apneas were not observed past P6, even though their dosage of nicotine (6 mg/kg/day) was double the dosage that was used in my study.

Eugenin et al. (2008) tested the effects of prenatal nicotine exposure on ventilatory responses and central chemoreception in the brainstem of mouse pups. It is difficult to compare my study to Eugenin et al. (2008) due to differences in methodology. Osmotic minipumps were used as a method of delivery of nicotine, in comparison to my study which used a drinking solution. Also, their study was not longitudinal, but rather, tested various mice of various ages. Despite these differences, there are comparable findings that resulted from the both of these studies. The breathing frequencies of neonates continuously exposed to nicotine (postnatally) increased overall during the eleven day period, but the insensitivities to $CO_2$ were seen around P5 and P7 (Figure 9B & Figure 10B). The nicotine interaction by day was statistically significant for the continuously exposed group, which is what was expected. The linear progression of breathing frequency in response to increases in $CO_2$ concentration were found in the Eugenin et al. (2008) study in which they found that increased $CO_2$ frequently caused the mouse pups to hypoventilate as a respiratory response. In that study, the hypoventilation occurred during the early postnatal days (P0-P3) and often saw increases in breathing response as the pups matured over the eleven-day testing period (Eugenin *et al.,* 2008). However, in my study the period of little to no respiratory response to hypercapnia was observed during the later postnatal days (P5-P9) because nicotine may potentially delay the maturation of the respiratory system in neonates (Figure 11 & Figure 14). In both studies frequent apneas occurred that lasted for longer durations, especially during the first couple days of life (Eugenin *et*

*al.,* 2008). These ventilatory abnormalities that were observed in this study, hint at the possible causal link between nicotine and SIDS.

In the litters that were withdrawn from nicotine following birth, the smallest slope of breathing frequencies occurred around P7 and P9, which indicates that there was very little respiratory response to elevated levels of $CO_2$ during that time (Figure 12B & Figure 13B). However despite these observations, statistical analysis determined that the withdrawal of nicotine was not a significant factor affecting the breathing response to elevated levels of $CO_2$. Stunden et al. (2001) observed that hypercapnia of any concentration is a major stimulus for an animal's increase in ventilation. This was demonstrated in my study for all litters (continuously exposed and withdrawn from nicotine) in which high concentrations of $CO_2$ greatly increased the breathing frequency. Postnatal day 7 demonstrated insensitivities to elevated levels of $CO_2$ for both experimental groups. This period of time during a neonate's maturation was described as a critical window in which a rat transitions between the neonatal phase into the adult phase (Stunden *et al.,* 2001). Litters continuously exposed to nicotine saw a depression of the respiratory response to $CO_2$ around P5 however litters withdrawn from nicotine saw a similar depression around P7-P9. This could indicate that the transition to the adult mediated breathing pattern around this time is delayed due to the physical effects of nicotine withdrawal (Stunden *et al.,* 2001). In humans this critical window for SIDS is between one and four months of age (Hoffman *et al.,* 1988). This period of time could be adapted to months instead of days (in mice) and thus indicate that the critical window could be shifted back in the infant's age reducing the risk of

SIDS. It is difficult to compare a mouse model to a rat model in that the metabolic rates of the two animals differ, as well as the metabolic rate of humans differ, but similar results were found nonetheless.

Minute ventilation demonstrated a linear increase in respiratory response to increasing levels of $CO_2$. However, similar to the breathing frequency, the smallest recorded breathing responses occurred around P7, with a slight variation to the days before and after, reaching statistical significance. Similar effects were seen, when the $CO_2$ was increased from 0% to 10% causing a linear increase (Stunden *et al.,* 2001). Minute ventilation was observed to be significantly lower in the withdrawal animals on average. Statistical significance was observed on P11 between the continuous nicotine exposure and nicotine withdrawal experimental groups. The litters withdrawn from nicotine had greater $V_E$ which may indicate that nicotine withdrawal eventually leads to a normalized breathing pattern in the neonate's maturing life. Although, I measured $V_E$ and it demonstrated for both experimental groups (continuously exposed and withdrawn) that the lowest respiratory response was apparent around P7, the breathing frequency data is trusted better. Minute ventilation calculation is dependent on the tidal volume parameter which is variable due to how difficult it is to accurately measure.

A mouse model for this study worked as an applicable representation for the effects of nicotine on a neonate. It allowed for one variable to be adjusted, while other factors remained consistent. With previous knowledge/expectations of weight gain, water consumption, and gestation/birth adjusting variables was easily

managed. However, when using a mouse model for human representation there are many limitations that are experienced. One of the limitations of my study was the inability to control litter size of the mice; whereas humans typically give birth to 1-2 infants, mice can give birth to anywhere from 4-12 mice at a time. Also, with larger litter sizes the distribution of consumed nicotine is dispersed and may not be equivalent amongst the various pups allowing for various amounts of nicotine to be consumed. Also, the metabolic rate in mice is greater than that of humans, so the rate at which nicotine is metabolized and transmitted into the blood stream, can vary greatly from that of humans. The assumed method of consumption of nicotine water for the continuous litters was through the dam's breast milk. Mouse pups rely on their mother's breast milk as a nutritional source for the first few weeks of life, encompassing the length of my study. Yet the competition for breast milk is that much greater when there are large litter sizes, making it difficult to determine how much nicotine the pups are constantly being exposed to. Contrary to that, it is difficult to know how long the nicotine stays in the bloodstream and breast milk of the dams whose litters are withdrawn from the nicotine drinking solution. This factor then makes it hard to pinpoint when the withdrawal actually occurs in the mouse pups.

Future studies should investigate the long-term effects of withdrawal to determine if there are further implications on the neonate's respiratory development. Since the time that nicotine withdrawal occurs in neonatal mice is unknown, the effects on the respiratory system may not be observed until further along in the mouse pup's maturation, past the eleven days of testing that took place in my

study. Mice are not weaned from their mothers until three weeks after they are born, indicating that development occurs for longer than my study lasted. These effects may indicate that while there was initially a delay in the insensitivity to $CO_2$, the mouse's respiratory system may not develop to its full capacity resulting in increased insensitivities during the mouse pup's life. This could potentially be expected due to the withdrawal effects exhibited in mice that include: grooming, chewing, scratching, shaking, leg tremors and hyperalgesia (Salas *et al.,* 2004). However, the withdrawal effects may exceed the physical attributes and inhibit the mechanical pathways that assists in respiratory and neurological development. Due to the agitation and irritability associated with plethysmography chamber, data collected past the mouse pup's second week of life may be hard to gather or be skewed.

Epidemiological studies should also be an area of future study to examine the statistics between SIDS and adoption. By conducting an epidemiological study relating SIDS cases to statistics regarding adoption, many areas can be further explored to examine nicotine as the corresponding variable. This could be examined further to explore how many infants who are put up for adoption come from smoking mothers or the homes of smoke-filled environments. A study of this kind would allow for nicotine to be explored as an individual risk factor and how it has impacted SIDS cases in the past, and its potential to impact cases in the future. This area of future study would allow for a more applicable model and set of results regarding the link between prenatal nicotine exposure and SIDS. The implications of an epidemiological study could lead to

what protocols need to be taken for infants following birth when they were exposed to nicotine prenatally.

Although the breathing frequencies of the nicotine withdrawal litters lack statistical significance, it can be inferred based on the data collected that the mouse pups insensitivity to elevated levels of $CO_2$ was delayed to a later period of time. Also, the minute ventilation demonstrated that the litters withdrawn from nicotine had increased their respiratory responses to $CO_2$ by P11, suggesting that the breathing pattern may eventually normalize over time in comparison to the litters that were continuously exposed to nicotine. This could indicate that the critical window in which infants are at risk for SIDS could be delayed by removing the infant from the nicotine exposure following birth.

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**Appendix:**

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

| Continuous | | Withdrawn | |
|---|---|---|---|
| **Female 1** | **Female 2** | **Female 1** | **Female 2** |
| 7.61 | 9.10 | 6.24 | 9.42 |
| 11.30 | 9.48 | 7.72 | 9.42 |
| 7.08 | 5.76 | 6.14 | 9.79 |
| 6.78 | 10.11 | 7.75 | 6.00 |
| 5.50 | 6.87 | 7.49 | 8.57 |
| 8.46 | 5.56 | 9.13 | 5.71 |
| 7.88 | 8.91 | 9.33 | 5.72 |
| 5.53 | 8.19 | 8.05 | 8.55 |
| 6.44 | 6.03 | 6.70 | 5.37 |
| 8.30 | 10.05 | 9.80 | 7.00 |
| 11.11 | 7.58 | 7.80 | 10.72 |
| 7.89 | | 7.84 | |
| **Average= 7.86** | | | |

Appendix B. Average weight of pups by day.

| Litter 1 | P1 | P3 | P5 | P7 | P9 | P11 |
|---|---|---|---|---|---|---|
| 1 | 1.508 | 2.301 | 3.166 | 4.239 | 5.205 | 5.981 |
| 2 | 1.415 | 2.05 | 3.049 | 4.088 | 5.017 | 6.051 |
| 3 | 1.569 | 2.43 | 3.43 | 4.536 | 5.384 | 6.645 |
| 4 | 1.125 | 1.78 | 2.525 | 3.702 | 4.48 | 5.901 |
| 5 | 1.504 | 2.257 | 3.301 | 4.499 | 5.382 | 6.52 |
| AVERAGE | 1.4242 | 2.1636 | 3.0942 | 4.2128 | 5.0936 | 6.2196 |
| | | | | | | |
| Litter 2 | | | | | | |
| 1 | 1.624 | 2.407 | 3.285 | 3.998 | 4.12 | 5.65 |
| 2 | 1.34 | 1.967 | 2.816 | 4.123 | 5.02 | 6.194 |
| 3 | 1.391 | 2.008 | 2.808 | 3.615 | 4.759 | 6.54 |
| 4 | 1.638 | 2.377 | 3.385 | 4.65 | 5.76 | 6.56 |
| 5 | 1.64 | 2.376 | 3.284 | 4.192 | 5.193 | 6.25 |
| AVERAGE | 1.5266 | 2.227 | 3.1156 | 4.1156 | 4.9704 | 6.2388 |
| | | | | | | |
| Litter 3 | | | | | | |
| 1 | 1.18 | 1.68 | 2.732 | 4.176 | 5.106 | 6.164 |
| 2 | 1.313 | 1.746 | 2.824 | 8.846 | 5.07 | 6.139 |
| 3 | 1.242 | 1.772 | 2.914 | 4.05 | 5.342 | 6.595 |
| 5 | 1.348 | 1.872 | 3.027 | 4.206 | 5.287 | 6.608 |
| 6 | 1.243 | 1.723 | 2.783 | 3.85 | 4.876 | 6.046 |
| 7 | 1.329 | 1.804 | 2.963 | 4.253 | 5.407 | 6.735 |
| AVERAGE | 1.2758 | 1.7662 | 2.8738 | 4.8968 | 5.1813 | 6.3812 |
| | | | | | | |
| Litter 4 | | | | | | |
| 1 | 1.309 | 2.112 | 2.98 | 3.809 | 4.674 | 5.459 |
| 2 | 1.329 | 2.157 | 3.125 | 3.963 | 4.829 | 5.55 |
| 3 | 1.414 | 2.164 | 3.183 | 3.957 | 4.763 | 5.566 |
| 4 | 1.446 | 2.354 | 3.057 | 3.766 | 4.664 | 5.445 |
| 5 | 1.585 | 2.435 | 3.354 | 4.205 | 5.185 | 6.228 |
| 6 | 1.447 | 2.241 | 3.18 | 3.981 | 4.99 | 5.748 |
| 7 | 1.195 | 1.975 | 2.72 | 3.536 | 4.657 | 5.487 |
| AVERAGE | 1.3893 | 2.2054 | 3.0856 | 3.8881 | 4.8231 | 5.6404 |

Appendix C. Data from litters continuously exposed to nicotine by day.

**Day 1**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L1M1 | 17.0 | 23.0 | 28.0 | 28.0 | 30.0 | |
| | L1M2 | 19.0 | 22.0 | 23.0 | 26.0 | 27.0 | |
| | L1M3 | 16.0 | 19.5 | 23.0 | 27.0 | 28.0 | |
| | L1M4 | 14.0 | 20.0 | 20.0 | 24.0 | 19.0 | |
| | L1M5 | 25.5 | 22.0 | 25.0 | 28.5 | 28.0 | |
| **Freq (60s)** | L1M1 | 102.0 | 138.0 | 168.0 | 168.0 | 180.0 | 6.3 |
| **(breaths/min)** | L1M2 | 114.0 | 132.0 | 138.0 | 156.0 | 162.0 | 5.4 |
| | L1M3 | 96.0 | 117.0 | 138.0 | 162.0 | 168.0 | 8.85 |
| | L1M4 | 84.0 | 120.0 | 120.0 | 144.0 | 114.0 | 0.3 |
| | L1M5 | 153.0 | 132.0 | 150.0 | 171.0 | 168.0 | 6.45 |
| | **Avg** | **109.8** | **127.8** | **142.8** | **160.2** | **158.4** | |
| | **Std** | **26.5** | **8.9** | **17.7** | **10.7** | **25.7** | |
| | **SEM** | **10.0** | **3.4** | **6.7** | **4.1** | **9.7** | |
| **Vt (Total)** | L1M1 | 0.10176 | 0.10299 | 0.12042 | 0.13666 | 0.17703 | |
| | L1M2 | 0.06788 | 0.10008 | 0.07730 | 0.12394 | 0.12039 | |
| | L1M3 | 0.08475 | 0.15262 | 0.10005 | 0.14965 | 0.18437 | |
| | L1M4 | 0.08894 | 0.01632 | 0.04724 | 0.01992 | 0.03698 | |
| | L1M5 | 0.10400 | 0.11899 | 0.12278 | 0.14541 | 0.18197 | |
| **Vt (avg vol/breath)** | L1M1 | 0.0059859 | 0.0044778 | 0.0043007 | 0.0048807 | 0.0059010 | |
| **(raw)** | L1M2 | 0.0035726 | 0.0045491 | 0.0033609 | 0.0047669 | 0.0044589 | |
| | L1M3 | 0.0052969 | 0.0078267 | 0.0043500 | 0.0055426 | 0.0065846 | |
| | L1M4 | 0.0063529 | 0.0008160 | 0.0023620 | 0.0008300 | 0.0019463 | |
| | L1M5 | 0.0040784 | 0.0054086 | 0.0049112 | 0.0051021 | 0.0064989 | |
| **Vt (avg vol/breath)** | L1M1 | 0.03059 | 0.02288 | 0.02198 | 0.02494 | 0.03015 | |
| **(actual)** | L1M2 | 0.01826 | 0.02325 | 0.01717 | 0.02436 | 0.02278 | |
| | L1M3 | 0.02707 | 0.03999 | 0.02223 | 0.02832 | 0.03365 | |
| | L1M4 | 0.03246 | 0.00417 | 0.01207 | 0.00424 | 0.00995 | |
| | L1M5 | 0.02084 | 0.02764 | 0.02510 | 0.02607 | 0.03321 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L1M1 | 0.020284 | 0.015174 | 0.014573 | 0.016539 | 0.019996 | 1.508 |
| **(actual/gram)** | L1M2 | 0.012902 | 0.016428 | 0.012137 | 0.017215 | 0.016102 | 1.415 |
| | L1M3 | 0.017251 | 0.025490 | 0.014167 | 0.018051 | 0.021445 | 1.569 |
| | L1M4 | 0.028856 | 0.003706 | 0.010729 | 0.003770 | 0.008841 | 1.125 |
| | L1M5 | 0.013857 | 0.018376 | 0.016686 | 0.017335 | 0.022081 | 1.504 |
| | | | | | | | 1.35 |
| | **Avg** | **0.018630** | **0.015835** | **0.013659** | **0.014582** | **0.017693** | |
| | **Std** | **0.006419** | **0.007867** | **0.002300** | **0.006068** | **0.005467** | |
| | **SEM** | **0.002426** | **0.002974** | **0.000869** | **0.002293** | **0.002066** | |
| **Minute Ventilation** | L1M1 | 2.0689 | 2.0939 | 2.4483 | 2.7785 | 3.5993 | 0.242312 |
| **(mL/min/g)** | L1M2 | 1.4708 | 2.1685 | 1.6749 | 2.6855 | 2.6086 | 0.116541 |
| | L1M3 | 1.6561 | 2.9824 | 1.9551 | 2.9243 | 3.6028 | 0.141526 |
| | L1M4 | 2.4239 | 0.4448 | 1.2874 | 0.5429 | 1.0078 | 0.04723 |
| | L1M5 | 2.1201 | 2.4257 | 2.5029 | 2.9643 | 3.7096 | 0.215649 |
| | **Avg** | **1.947976** | **2.023058** | **1.973747** | **2.379103** | **2.905617** | |
| | **Std** | **0.381984** | **0.948623** | **0.516220** | **1.032570** | **1.151458** | |
| | **SEM** | **0.144376** | **0.358546** | **0.195113** | **0.390275** | **0.435210** | |

**Day 1**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L4M1 | 14.0 | 16.0 | 15.0 | 18.0 | 24.5 | |
| | L4M2 | 23.0 | 24.0 | 25.0 | 23.0 | 28.0 | |
| | L4M3 | 18.0 | 17.0 | 22.0 | 23.0 | 28.0 | |
| | L4M4 | 22.0 | 23.0 | 24.0 | 27.0 | 26.0 | |
| | L4M5 | 21.0 | 20.0 | 24.0 | 25.5 | 29.0 | |
| | L4M6 | 25.0 | 22.0 | 26.0 | 27.0 | 31.5 | |
| | L4M7 | 22.0 | 21.5 | 23.0 | 23.0 | 28.0 | |
| **Freq (60s)** | L4M1 | 84.0 | 96.0 | 90.0 | 108.0 | 147.0 | 8.55 |
| (breaths/min) | L4M2 | 138.0 | 144.0 | 150.0 | 138.0 | 168.0 | 3 |
| | L4M3 | 108.0 | 102.0 | 132.0 | 138.0 | 168.0 | 10.2 |
| | L4M4 | 132.0 | 138.0 | 144.0 | 162.0 | 156.0 | 3.6 |
| | L4M5 | 126.0 | 120.0 | 144.0 | 153.0 | 174.0 | 8.55 |
| | L4M6 | 150.0 | 132.0 | 156.0 | 162.0 | 189.0 | 8.85 |
| | L4M7 | 132.0 | 129.0 | 138.0 | 138.0 | 168.0 | 5.85 |
| | | | | | | | |
| | | | | | | | |
| | **Avg** | **124.3** | **123.0** | **136.3** | **142.7** | **167.1** | |
| | **Std** | **21.8** | **18.1** | **21.8** | **18.8** | **13.3** | |
| | **SEM** | **8.3** | **6.8** | **8.3** | **7.1** | **5.0** | |
| **Vt (Total)** | L4M1 | 0.03932 | 0.06718 | 0.06843 | 0.09326 | 0.15170 | |
| | L4M2 | 0.10696 | 0.05788 | 0.06869 | 0.09120 | 0.15594 | |
| | L4M3 | 0.16360 | 0.08792 | 0.07245 | 0.08488 | 0.17797 | |
| | L4M4 | 0.15308 | 0.22294 | 0.14616 | 0.23777 | 0.08092 | |
| | L4M5 | 0.06529 | 0.04624 | 0.06129 | 0.08980 | 0.08549 | |
| | L4M6 | 0.09750 | 0.08002 | 0.08498 | 0.08252 | 0.19568 | |
| | L4M7 | 0.09139 | 0.07409 | 0.05568 | 0.05370 | 0.09712 | |
| **Vt (avg vol/breath)** | L4M1 | 0.0028086 | 0.0041988 | 0.0045620 | 0.0051811 | 0.0061918 | |
| (raw) | L4M2 | 0.0046504 | 0.0024117 | 0.0027476 | 0.0039652 | 0.0055693 | |
| | L4M3 | 0.0090889 | 0.0051718 | 0.0032932 | 0.0036904 | 0.0063561 | |
| | L4M4 | 0.0069582 | 0.0096930 | 0.0060900 | 0.0088063 | 0.0031125 | |
| | L4M5 | 0.0031090 | 0.0023120 | 0.0025538 | 0.0035216 | 0.0029479 | |
| | L4M6 | 0.0039000 | 0.0036373 | 0.0032685 | 0.0030563 | 0.0062121 | |
| | L4M7 | 0.0041541 | 0.0034460 | 0.0024209 | 0.0023348 | 0.0034686 | |
| **Vt (avg vol/breath)** | L4M1 | 0.01435 | 0.02146 | 0.02331 | 0.02648 | 0.03164 | |
| (actual) | L4M2 | 0.02376 | 0.01232 | 0.01404 | 0.02026 | 0.02846 | |
| | L4M3 | 0.04644 | 0.02643 | 0.01683 | 0.01886 | 0.03248 | |
| | L4M4 | 0.03556 | 0.04953 | 0.03112 | 0.04500 | 0.01590 | |
| | L4M5 | 0.01589 | 0.01181 | 0.01305 | 0.01800 | 0.01506 | |
| | L4M6 | 0.01993 | 0.01859 | 0.01670 | 0.01562 | 0.03174 | |
| | L4M7 | 0.02123 | 0.01761 | 0.01237 | 0.01193 | 0.01772 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L4M1 | 0.010964 | 0.016391 | 0.017809 | 0.020226 | 0.024171 | 1.309 |
| (actual/gram) | L4M2 | 0.017881 | 0.009273 | 0.010565 | 0.015246 | 0.021414 | 1.329 |
| | L4M3 | 0.032846 | 0.018690 | 0.011901 | 0.013337 | 0.022970 | 1.414 |
| | L4M4 | 0.024589 | 0.034254 | 0.021521 | 0.031120 | 0.029030 | 1.446 |
| | L4M5 | 0.010023 | 0.007454 | 0.008233 | 0.011353 | 0.009504 | 1.585 |
| | L4M6 | 0.013773 | 0.012845 | 0.011542 | 0.010793 | 0.021938 | 1.447 |
| | L4M7 | 0.017764 | 0.014736 | 0.010352 | 0.009984 | 0.014832 | 1.195 |
| | **Avg** | **0.018263** | **0.016235** | **0.013132** | **0.016009** | **0.020551** | |
| | **Std** | **0.008108** | **0.008851** | **0.004737** | **0.007517** | **0.006432** | |
| | **SEM** | **0.003064** | **0.003345** | **0.001790** | **0.002841** | **0.002431** | |
| **Minute Ventilation** | L4M1 | 0.9210 | 1.5735 | 1.6028 | 2.1844 | 3.5532 | 0.336949 |
| (mL/min/g) | L4M2 | 2.4676 | 1.3353 | 1.5847 | 2.1040 | 3.5975 | 0.374953 |
| | L4M3 | 3.5474 | 1.9064 | 1.5709 | 1.8405 | 3.8590 | 0.427824 |
| | L4M4 | 3.2458 | 4.7271 | 3.0991 | 5.0415 | 4.5287 | -0.08743 |
| | L4M5 | 1.2630 | 0.8945 | 1.1856 | 1.7371 | 1.6537 | 0.042322 |
| | L4M6 | 2.0659 | 1.6955 | 1.8006 | 1.7485 | 4.1462 | 0.517579 |
| | L4M7 | 2.3448 | 1.9009 | 1.4286 | 1.3778 | 2.4918 | 0.201352 |
| | **Avg** | **2.265049** | **2.004737** | **1.753182** | **2.290526** | **3.404294** | |
| | **Std** | **0.957773** | **1.251264** | **0.622554** | **1.241623** | **0.998173** | |
| | **SEM** | **0.362004** | **0.472934** | **0.235303** | **0.469290** | **0.377274** | |

**Day 3**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L1M1 | 29.0 | 27.0 | 26.5 | 30.0 | 36.0 | |
| | L1M2 | 34.0 | 29.5 | 33.0 | 33.0 | 34.0 | |
| | L1M3 | 29.0 | 29.0 | 25.5 | 29.5 | 31.0 | |
| | L1M4 | 25.0 | 27.5 | 30.0 | 35.5 | 35.0 | |
| | L1M5 | 24.0 | 20.0 | 26.0 | 29.0 | 32.5 | |
| **Freq (60s)** | L1M1 | 174.0 | 162.0 | 159.0 | 180.0 | 216.0 | 9.15 |
| **(breaths/min)** | L1M2 | 204.0 | 177.0 | 198.0 | 198.0 | 204.0 | 4.05 |
| | L1M3 | 174.0 | 174.0 | 153.0 | 177.0 | 186.0 | 3 |
| | L1M4 | 150.0 | 165.0 | 180.0 | 213.0 | 210.0 | 8.4 |
| | L1M5 | 144.0 | 120.0 | 156.0 | 174.0 | 195.0 | 12.15 |
| | **Avg** | **169.2** | **159.6** | **169.2** | **188.4** | **202.2** | |
| | **Std** | **23.8** | **23.0** | **19.3** | **16.6** | **11.9** | |
| | **SEM** | **9.0** | **8.7** | **7.3** | **6.3** | **4.5** | |
| **Vt (Total)** | L1M1 | 0.09211 | 0.09787 | 0.04983 | 0.09358 | 0.13752 | |
| | L1M2 | 0.07565 | 0.10163 | 0.07228 | 0.07603 | 0.10682 | |
| | L1M3 | 0.13989 | 0.14889 | 0.13876 | 0.18329 | 0.15091 | |
| | L1M4 | 0.10442 | 0.09015 | 0.10491 | 0.14517 | 0.13255 | |
| | L1M5 | 0.10512 | 0.08173 | 0.10795 | 0.14228 | 0.24970 | |
| **Vt (avg vol/breath)** | L1M1 | 0.0031762 | 0.0036248 | 0.0018804 | 0.0031193 | 0.0038200 | |
| **(raw)** | L1M2 | 0.0022250 | 0.0034451 | 0.0021903 | 0.0023039 | 0.0031418 | |
| | L1M3 | 0.0048238 | 0.0051341 | 0.0054416 | 0.0062132 | 0.0048681 | |
| | L1M4 | 0.0041768 | 0.0032782 | 0.0034970 | 0.0040893 | 0.0037871 | |
| | L1M5 | 0.0043800 | 0.0040865 | 0.0041519 | 0.0049062 | 0.0076831 | |
| **Vt (avg vol/breath)** | L1M1 | 0.01623 | 0.01852 | 0.00961 | 0.01594 | 0.01952 | |
| **(actual)** | L1M2 | 0.01137 | 0.01760 | 0.01119 | 0.01177 | 0.01605 | |
| | L1M3 | 0.02465 | 0.02624 | 0.02781 | 0.03175 | 0.02488 | |
| | L1M4 | 0.02134 | 0.01675 | 0.01787 | 0.02090 | 0.01935 | |
| | L1M5 | 0.02238 | 0.02088 | 0.02122 | 0.02507 | 0.03926 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L1M1 | 0.007054 | 0.008050 | 0.004176 | 0.006927 | 0.008483 | 2.301 |
| **(actual/gram)** | L1M2 | 0.005546 | 0.008588 | 0.005460 | 0.005743 | 0.007831 | 2.05 |
| | L1M3 | 0.010144 | 0.010796 | 0.011443 | 0.013066 | 0.010237 | 2.43 |
| | L1M4 | 0.011991 | 0.009411 | 0.010039 | 0.011739 | 0.010872 | 1.78 |
| | L1M5 | 0.009917 | 0.009252 | 0.009400 | 0.011108 | 0.017395 | 2.257 |
| | **Avg** | **0.008930** | **0.009219** | **0.008104** | **0.009717** | **0.010964** | |
| | **Std** | **0.002588** | **0.001036** | **0.003122** | **0.003194** | **0.003803** | |
| | **SEM** | **0.000978** | **0.000392** | **0.001180** | **0.001207** | **0.001438** | |
| **Minute Ventilation** | L1M1 | 1.2273 | 1.3041 | 0.6640 | 1.2469 | 1.8324 | 0.108396 |
| **(mL/min/g)** | L1M2 | 1.1314 | 1.5200 | 1.0810 | 1.1371 | 1.5976 | 0.0144476 |
| | L1M3 | 1.7650 | 1.8786 | 1.7508 | 2.3126 | 1.9041 | 0.0319154 |
| | L1M4 | 1.7986 | 1.5528 | 1.8070 | 2.5005 | 2.2831 | 0.1442226 |
| | L1M5 | 1.4280 | 1.1103 | 1.4664 | 1.9328 | 3.3920 | 0.3655835 |
| | **Avg** | **1.470078** | **1.473144** | **1.353850** | **1.825991** | **2.201850** | |
| | **Std** | **0.304265** | **0.288616** | **0.481140** | **0.615035** | **0.709470** | |
| | **SEM** | **0.115001** | **0.109087** | **0.181854** | **0.232461** | **0.268155** | |

**Day 3**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L4M1 | 24.0 | 22.0 | 25.0 | 27.0 | 29.0 | |
| | L4M2 | 29.0 | 34.0 | 30.0 | 35.0 | 38.5 | |
| | L4M3 | 26.0 | 31.0 | 30.0 | 36.0 | 39.5 | |
| | L4M4 | 27.5 | 29.0 | 28.0 | 34.0 | 36.0 | |
| | L4M5 | 27.0 | 30.0 | 27.0 | 32.0 | 32.0 | |
| | L4M6 | 28.0 | 32.0 | 32.0 | 34.0 | 36.0 | |
| | L4M7 | 27.0 | 32.0 | 30.0 | 29.5 | 31.0 | |
| **Freq (60s)** | L4M1 | 144.0 | 132.0 | 150.0 | 162.0 | 174.0 | 6.9 |
| **(breaths/min)** | L4M2 | 174.0 | 204.0 | 180.0 | 210.0 | 231.0 | 5.55 |
| | L4M3 | 156.0 | 186.0 | 180.0 | 216.0 | 237.0 | 9.45 |
| | L4M4 | 165.0 | 174.0 | 168.0 | 204.0 | 216.0 | 8.1 |
| | L4M5 | 162.0 | 180.0 | 162.0 | 192.0 | 192.0 | 3.3 |
| | L4M6 | 168.0 | 192.0 | 192.0 | 204.0 | 216.0 | 4.2 |
| | L4M7 | 162.0 | 192.0 | 180.0 | 177.0 | 186.0 | -1.05 |
| | | | | | | | |
| | | | | | | | |
| | **Avg** | **161.6** | **180.0** | **173.1** | **195.0** | **207.4** | |
| | **Std** | **9.6** | **23.2** | **14.0** | **19.4** | **23.8** | |
| | **SEM** | **3.6** | **8.8** | **5.3** | **7.3** | **9.0** | |
| **Vt (Total)** | L4M1 | 0.08491 | 0.06825 | 0.11552 | 0.08055 | 0.10082 | |
| | L4M2 | 0.10894 | 0.12045 | 0.10184 | 0.13381 | 0.15840 | |
| | L4M3 | 0.11956 | 0.11345 | 0.10932 | 0.12354 | 0.13294 | |
| | L4M4 | 0.11979 | 0.12897 | 0.10667 | 0.09576 | 0.14238 | |
| | L4M5 | 0.12492 | 0.11539 | 0.07225 | 0.12845 | 0.14600 | |
| | L4M6 | 0.13144 | 0.12729 | 0.08907 | 0.10775 | 0.12479 | |
| | L4M7 | 0.11011 | 0.13439 | 0.11918 | 0.11701 | 0.17143 | |
| **Vt (avg vol/breath)** | L4M1 | 0.0035379 | 0.0031023 | 0.0046208 | 0.0029833 | 0.0034766 | |
| **(raw)** | L4M2 | 0.0037566 | 0.0035426 | 0.0033947 | 0.0038231 | 0.0041143 | |
| | L4M3 | 0.0045985 | 0.0036597 | 0.0036440 | 0.0034317 | 0.0033656 | |
| | L4M4 | 0.0043560 | 0.0044472 | 0.0038096 | 0.0028165 | 0.0039550 | |
| | L4M5 | 0.0046267 | 0.0038463 | 0.0026759 | 0.0040141 | 0.0045625 | |
| | L4M6 | 0.0046943 | 0.0039778 | 0.0027834 | 0.0031691 | 0.0034664 | |
| | L4M7 | 0.0040781 | 0.0041997 | 0.0039727 | 0.0039664 | 0.0055300 | |
| **Vt (avg vol/breath)** | L4M1 | 0.01808 | 0.01585 | 0.02361 | 0.01524 | 0.01777 | |
| **(actual)** | L4M2 | 0.01920 | 0.01810 | 0.01735 | 0.01954 | 0.02102 | |
| | L4M3 | 0.02350 | 0.01870 | 0.01862 | 0.01754 | 0.01720 | |
| | L4M4 | 0.02226 | 0.02273 | 0.01947 | 0.01439 | 0.02021 | |
| | L4M5 | 0.02364 | 0.01965 | 0.01367 | 0.02051 | 0.02331 | |
| | L4M6 | 0.02399 | 0.02033 | 0.01422 | 0.01619 | 0.01771 | |
| | L4M7 | 0.02084 | 0.02146 | 0.02030 | 0.02027 | 0.02826 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L4M1 | 0.008560 | 0.007506 | 0.011180 | 0.007218 | 0.008412 | 2.112 |
| **(actual/gram)** | L4M2 | 0.008899 | 0.008393 | 0.008042 | 0.009057 | 0.009747 | 2.157 |
| | L4M3 | 0.010859 | 0.008642 | 0.008605 | 0.008103 | 0.007947 | 2.164 |
| | L4M4 | 0.009456 | 0.009654 | 0.008270 | 0.006114 | 0.008585 | 2.354 |
| | L4M5 | 0.009709 | 0.008072 | 0.005616 | 0.008424 | 0.009575 | 2.435 |
| | L4M6 | 0.010704 | 0.009070 | 0.006347 | 0.007226 | 0.007904 | 2.241 |
| | L4M7 | 0.010552 | 0.010866 | 0.010279 | 0.010263 | 0.014308 | 1.975 |
| | **Avg** | **0.009820** | **0.008886** | **0.008334** | **0.008058** | **0.009497** | |
| | **Std** | **0.000911** | **0.001112** | **0.001974** | **0.001364** | **0.002242** | |
| | **SEM** | **0.000344** | **0.000420** | **0.000746** | **0.000516** | **0.000847** | |
| **Minute Ventilation** | L4M1 | 1.2326 | 0.9908 | 1.6770 | 1.1693 | 1.4636 | -0.025628 |
| **(mL/min/g)** | L4M2 | 1.5485 | 1.7121 | 1.4476 | 1.9020 | 2.2515 | 0.0954312 |
| | L4M3 | 1.6940 | 1.6074 | 1.5489 | 1.7503 | 1.8835 | 0.0404612 |
| | L4M4 | 1.5602 | 1.6798 | 1.3893 | 1.2472 | 1.8544 | 0.0469056 |
| | L4M5 | 1.5729 | 1.4529 | 0.9097 | 1.6174 | 1.8383 | 0.1109126 |
| | L4M6 | 1.7983 | 1.7415 | 1.2186 | 1.4742 | 1.7073 | 0.0539564 |
| | L4M7 | 1.7094 | 2.0863 | 1.8502 | 1.8165 | 2.6613 | 0.0882131 |
| | **Avg** | **1.587980** | **1.610108** | **1.434468** | **1.568133** | **1.951434** | |
| | **Std** | **0.182122** | **0.333550** | **0.307915** | **0.282723** | **0.391223** | |
| | **SEM** | **0.068836** | **0.126070** | **0.116381** | **0.106859** | **0.147868** | |

**Day 5**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L1M1 | 32.0 | 32.5 | 34.0 | 36.0 | 33.0 | |
| | L1M2 | 36.0 | 40.5 | 39.0 | 45.0 | 40.0 | |
| | L1M3 | 34.5 | 28.0 | 43.0 | 36.5 | 37.0 | |
| | L1M4 | 36.5 | 36.0 | 33.0 | 36.0 | 40.0 | |
| | L1M5 | 28.5 | 29.5 | 33.0 | 35.0 | 37.0 | |
| **Freq (60s)** | L1M1 | 192.0 | 195.0 | 204.0 | 216.0 | 198.0 | 1.05 |
| **(breaths/min)** | L1M2 | 216.0 | 243.0 | 234.0 | 270.0 | 240.0 | 1.35 |
| | L1M3 | 207.0 | 168.0 | 258.0 | 219.0 | 222.0 | 6.15 |
| | L1M4 | 219.0 | 216.0 | 198.0 | 216.0 | 240.0 | 4.5 |
| | L1M5 | 171.0 | 177.0 | 198.0 | 210.0 | 222.0 | 7.35 |
| | **Avg** | **201.0** | **199.8** | **218.4** | **226.2** | **224.4** | |
| | **Std** | **19.8** | **30.3** | **26.7** | **24.7** | **17.3** | |
| | **SEM** | **7.5** | **11.5** | **10.1** | **9.3** | **6.5** | |
| **Vt (Total)** | L1M1 | 0.07709 | 0.07521 | 0.12250 | 0.14784 | 0.16882 | |
| | L1M2 | 0.09763 | 0.07381 | 0.03843 | 0.15491 | 0.15491 | |
| | L1M3 | 0.12046 | 0.07364 | 0.18459 | 0.11600 | 0.14681 | |
| | L1M4 | 0.07681 | 0.11454 | 0.06910 | 0.07675 | 0.08796 | |
| | L1M5 | 0.09110 | 0.11353 | 0.10054 | 0.19039 | 0.25668 | |
| **Vt (avg vol/breath)** | L1M1 | 0.0024091 | 0.0023142 | 0.0036029 | 0.0041067 | 0.0051158 | |
| **(raw)** | L1M2 | 0.0027119 | 0.0018225 | 0.0009854 | 0.0034424 | 0.0038728 | |
| | L1M3 | 0.0034916 | 0.0026300 | 0.0042928 | 0.0031781 | 0.0039678 | |
| | L1M4 | 0.0021044 | 0.0031817 | 0.0020939 | 0.0021319 | 0.0021990 | |
| | L1M5 | 0.0031965 | 0.0038485 | 0.0030467 | 0.0054397 | 0.0069373 | |
| **Vt (avg vol/breath)** | L1M1 | 0.01231 | 0.01183 | 0.01841 | 0.02099 | 0.02614 | |
| **(actual)** | L1M2 | 0.01386 | 0.00931 | 0.00504 | 0.01759 | 0.01979 | |
| | L1M3 | 0.01784 | 0.01344 | 0.02194 | 0.01624 | 0.02028 | |
| | L1M4 | 0.01075 | 0.01626 | 0.01070 | 0.01089 | 0.01124 | |
| | L1M5 | 0.01633 | 0.01967 | 0.01557 | 0.02780 | 0.03545 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L1M1 | 0.003888 | 0.003735 | 0.005815 | 0.006628 | 0.008257 | 3.166 |
| **(actual/gram)** | L1M2 | 0.004545 | 0.003054 | 0.001651 | 0.005769 | 0.006491 | 3.049 |
| | L1M3 | 0.005202 | 0.003918 | 0.006395 | 0.004735 | 0.005911 | 3.43 |
| | L1M4 | 0.004259 | 0.006439 | 0.004238 | 0.004315 | 0.004450 | 2.525 |
| | L1M5 | 0.004948 | 0.005957 | 0.004716 | 0.008421 | 0.010739 | 3.301 |
| | **Avg** | **0.004568** | **0.004621** | **0.004563** | **0.005974** | **0.007170** | |
| | **Std** | **0.000525** | **0.001485** | **0.001839** | **0.001639** | **0.002417** | |
| | **SEM** | **0.000199** | **0.000561** | **0.000695** | **0.000620** | **0.000913** | |
| **Minute Ventilation** | L1M1 | 0.7466 | 0.7283 | 1.1863 | 1.4317 | 1.6349 | 0.14824972 |
| **(ml./min/g)** | L1M2 | 0.9817 | 0.7422 | 0.3864 | 1.5577 | 1.5577 | 0.18089299 |
| | L1M3 | 1.0768 | 0.6583 | 1.6500 | 1.0369 | 1.3123 | 0.067452 |
| | L1M4 | 0.9327 | 1.3908 | 0.8391 | 0.9319 | 1.0681 | -0.0437679 |
| | L1M5 | 0.8461 | 1.0545 | 0.9338 | 1.7684 | 2.3841 | 0.24116537 |
| | **Avg** | **0.916775** | **0.914820** | **0.999127** | **1.345328** | **1.591409** | |
| | **Std** | **0.126526** | **0.306783** | **0.464746** | **0.352687** | **0.495764** | |
| | **SEM** | **0.047822** | **0.115953** | **0.175657** | **0.133303** | **0.187381** | |

**Day 5**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L4M1 | 32.0 | 36.0 | 39.0 | 34.5 | 40.0 | |
| | L4M2 | 39.0 | 40.0 | 38.0 | 36.0 | 37.0 | |
| | L4M3 | 32.0 | 37.0 | 37.5 | 32.0 | 37.0 | |
| | L4M4 | 32.0 | 26.0 | 28.0 | 27.0 | 31.5 | |
| | L4M5 | 34.0 | 38.0 | 36.0 | 32.0 | 45.0 | |
| | L4M6 | 39.0 | 38.0 | 37.0 | 42.0 | 35.0 | |
| | L4M7 | 29.0 | 28.0 | 25.0 | 28.0 | 29.0 | |
| **Freq (60s)** | L4M1 | 192.0 | 216.0 | 234.0 | 207.0 | 240.0 | 2.25 |
| **(breaths/min)** | L4M2 | 234.0 | 240.0 | 228.0 | 216.0 | 222.0 | -3.3 |
| | L4M3 | 192.0 | 222.0 | 225.0 | 192.0 | 222.0 | -1.65 |
| | L4M4 | 192.0 | 156.0 | 168.0 | 162.0 | 189.0 | 4.65 |
| | L4M5 | 204.0 | 228.0 | 216.0 | 192.0 | 270.0 | 5.1 |
| | L4M6 | 234.0 | 228.0 | 222.0 | 252.0 | 210.0 | -1.2 |
| | L4M7 | 174.0 | 168.0 | 150.0 | 168.0 | 174.0 | 1.8 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | **Avg** | **203.1** | **208.3** | **206.1** | **198.4** | **218.1** | |
| | **Std** | **22.8** | **32.6** | **33.1** | **30.5** | **31.8** | |
| | **SEM** | **8.6** | **12.3** | **12.5** | **11.5** | **12.0** | |
| **Vt (Total)** | L4M1 | 0.03932 | 0.06718 | 0.06843 | 0.09326 | 0.15170 | |
| | L4M2 | 0.10696 | 0.05788 | 0.06869 | 0.09120 | 0.15594 | |
| | L4M3 | 0.16360 | 0.08792 | 0.07245 | 0.08488 | 0.17797 | |
| | L4M4 | 0.15308 | 0.22294 | 0.14616 | 0.23777 | 0.18092 | |
| | L4M5 | 0.06529 | 0.04624 | 0.06129 | 0.08980 | 0.08549 | |
| | L4M6 | 0.09750 | 0.08002 | 0.08498 | 0.08252 | 0.19568 | |
| | L4M7 | 0.09139 | 0.07409 | 0.05568 | 0.05370 | 0.09712 | |
| **Vt (avg vol/breath)** | L4M1 | 0.0012288 | 0.0018661 | 0.0017546 | 0.0027032 | 0.0037925 | |
| **(raw)** | L4M2 | 0.0027426 | 0.0014470 | 0.0018076 | 0.0025333 | 0.0042146 | |
| | L4M3 | 0.0051125 | 0.0023762 | 0.0019320 | 0.0026525 | 0.0048100 | |
| | L4M4 | 0.0047838 | 0.0085746 | 0.0052200 | 0.0088063 | 0.0057436 | |
| | L4M5 | 0.0019203 | 0.0012168 | 0.0017025 | 0.0028063 | 0.0018998 | |
| | L4M6 | 0.0025000 | 0.0021058 | 0.0022968 | 0.0019648 | 0.0055909 | |
| | L4M7 | 0.0031514 | 0.0026461 | 0.0022272 | 0.0019179 | 0.0033490 | |
| **Vt (avg vol/breath)** | L4M1 | 0.00628 | 0.00954 | 0.00897 | 0.01381 | 0.01938 | |
| **(actual)** | L4M2 | 0.01401 | 0.00739 | 0.00924 | 0.01295 | 0.02154 | |
| | L4M3 | 0.02612 | 0.01214 | 0.00987 | 0.01355 | 0.02458 | |
| | L4M4 | 0.02444 | 0.04382 | 0.02667 | 0.04500 | 0.02935 | |
| | L4M5 | 0.00981 | 0.00622 | 0.00870 | 0.01434 | 0.00971 | |
| | L4M6 | 0.01278 | 0.01076 | 0.01174 | 0.01004 | 0.02857 | |
| | L4M7 | 0.01610 | 0.01352 | 0.01138 | 0.00980 | 0.01711 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L4M1 | 0.002107 | 0.003200 | 0.003009 | 0.004635 | 0.006503 | 2.98 |
| **(actual/gram)** | L4M2 | 0.004485 | 0.002366 | 0.002956 | 0.004143 | 0.006892 | 3.125 |
| | L4M3 | 0.008208 | 0.003815 | 0.003102 | 0.004258 | 0.007722 | 3.183 |
| | L4M4 | 0.007996 | 0.014333 | 0.008726 | 0.014720 | 0.009601 | 3.057 |
| | L4M5 | 0.002926 | 0.001854 | 0.002594 | 0.004275 | 0.002894 | 3.354 |
| | L4M6 | 0.004017 | 0.003384 | 0.003691 | 0.003157 | 0.008984 | 3.18 |
| | L4M7 | 0.005920 | 0.004971 | 0.004184 | 0.003603 | 0.006292 | 2.72 |
| | **Avg** | **0.005094** | **0.004846** | **0.004037** | **0.005542** | **0.006984** | |
| | **Std** | **0.002379** | **0.004302** | **0.002133** | **0.004077** | **0.002192** | |
| | **SEM** | **0.000899** | **0.001626** | **0.000806** | **0.001541** | **0.000829** | |
| **Minute Ventilation** | L4M1 | 0.4045 | 0.6912 | 0.7040 | 0.9595 | 1.5608 | -0.1278343 |
| **(mL/min/g)** | L4M2 | 1.0494 | 0.5679 | 0.6739 | 0.8948 | 1.5300 | -0.1448161 |
| | L4M3 | 1.5759 | 0.8469 | 0.6979 | 0.8176 | 1.7143 | -0.0727496 |
| | L4M4 | 1.5353 | 2.2360 | 1.4659 | 2.3847 | 1.8146 | -0.0156293 |
| | L4M5 | 0.5968 | 0.4227 | 0.5603 | 0.8209 | 0.7815 | -0.1064625 |
| | L4M6 | 0.9400 | 0.7715 | 0.8193 | 0.7956 | 1.8867 | -0.0990376 |
| | L4M7 | 1.0302 | 0.8351 | 0.6276 | 0.6053 | 1.0947 | 0.01778731 |
| | **Avg** | **1.018880** | **0.910180** | **0.792712** | **1.039773** | **1.483210** | |
| | **Std** | **0.435838** | **0.604061** | **0.307176** | **0.603039** | **0.403624** | |
| | **SEM** | **0.164731** | **0.228314** | **0.116101** | **0.227927** | **0.152556** | |

**Day 7**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L1M1 | 39.0 | 41.0 | 39.5 | 43.0 | 45.0 | |
| | L1M2 | 48.0 | 38.0 | 36.0 | 42.0 | 39.0 | |
| | L1M3 | 38.0 | 42.0 | 40.0 | 43.0 | 42.0 | |
| | L1M4 | 27.0 | 34.0 | 40.0 | 34.0 | 43.0 | |
| | L1M5 | 40.0 | 40.0 | 41.0 | 44.0 | 48.0 | |
| **Freq (60s)** | L1M1 | 234.0 | 246.0 | 237.0 | 258.0 | 270.0 | 4.65 |
| (breaths/min) | L1M2 | 288.0 | 228.0 | 216.0 | 252.0 | 234.0 | 2.7 |
| | L1M3 | 228.0 | 252.0 | 240.0 | 258.0 | 252.0 | 0.9 |
| | L1M4 | 162.0 | 204.0 | 240.0 | 204.0 | 258.0 | 6.3 |
| | L1M5 | 240.0 | 240.0 | 246.0 | 264.0 | 288.0 | 8.1 |
| | **Avg** | **230.4** | **234.0** | **235.8** | **247.2** | **260.4** | |
| | **Std** | **45.0** | **19.0** | **11.5** | **24.5** | **20.2** | |
| | **SEM** | **17.0** | **7.2** | **4.4** | **9.3** | **7.6** | |
| **Vt (Total)** | L1M1 | 0.11385 | 0.11490 | 0.11381 | 0.17787 | 0.20727 | |
| | L1M2 | 0.25405 | 0.21915 | 0.27480 | 0.10882 | 0.08798 | |
| | L1M3 | 0.13772 | 0.17813 | 0.19190 | 0.28341 | 0.29658 | |
| | L1M4 | 0.10290 | 0.10121 | 0.13388 | 0.13866 | 0.24028 | |
| | L1M5 | 0.15666 | 0.09694 | 0.20969 | 0.20913 | 0.30851 | |
| **Vt (avg vol/breath)** | L1M1 | 0.0029192 | 0.0028024 | 0.0028813 | 0.0041365 | 0.0046060 | |
| (raw) | L1M2 | 0.0052927 | 0.0057671 | 0.0076333 | 0.0025910 | 0.0022559 | |
| | L1M3 | 0.0036242 | 0.0042412 | 0.0047975 | 0.0065909 | 0.0070614 | |
| | L1M4 | 0.0038111 | 0.0029768 | 0.0033470 | 0.0040782 | 0.0055879 | |
| | L1M5 | 0.0039165 | 0.0024235 | 0.0051144 | 0.0047530 | 0.0064273 | |
| **Vt (avg vol/breath)** | L1M1 | 0.01492 | 0.01432 | 0.01472 | 0.02114 | 0.02354 | |
| (actual) | L1M2 | 0.02705 | 0.02947 | 0.03901 | 0.01324 | 0.01153 | |
| | L1M3 | 0.01852 | 0.02167 | 0.02452 | 0.03368 | 0.03608 | |
| | L1M4 | 0.01947 | 0.01521 | 0.01710 | 0.02084 | 0.02855 | |
| | L1M5 | 0.02001 | 0.01238 | 0.02613 | 0.02429 | 0.03284 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L1M1 | 0.003519 | 0.003378 | 0.003473 | 0.004986 | 0.005552 | 4.239 |
| (actual/gram) | L1M2 | 0.006616 | 0.007209 | 0.009542 | 0.003239 | 0.002820 | 4.088 |
| | L1M3 | 0.004083 | 0.004778 | 0.005405 | 0.007425 | 0.007955 | 4.536 |
| | L1M4 | 0.005261 | 0.004109 | 0.004620 | 0.005629 | 0.007713 | 3.702 |
| | L1M5 | 0.004448 | 0.002753 | 0.005809 | 0.005398 | 0.007300 | 4.499 |
| | **Avg** | **0.004785** | **0.004445** | **0.005770** | **0.005336** | **0.006268** | |
| | **Std** | **0.001203** | **0.001722** | **0.002289** | **0.001498** | **0.002145** | |
| | **SEM** | **0.000455** | **0.000651** | **0.000865** | **0.000566** | **0.000811** | |
| **Minute Ventilation** | L1M1 | 0.8235 | 0.8311 | 0.8232 | 1.2865 | 1.4992 | 0.123381 |
| (mL/min/g) | L1M2 | 1.9054 | 1.6436 | 2.0610 | 0.8162 | 0.6599 | -0.20981 |
| | L1M3 | 0.9309 | 1.2040 | 1.2971 | 1.9156 | 2.0047 | 0.151022 |
| | L1M4 | 0.8522 | 0.8382 | 1.1088 | 1.1484 | 1.9900 | 0.174746 |
| | L1M5 | 1.0676 | 0.6606 | 1.4290 | 1.4252 | 2.1024 | 0.216082 |
| | **Avg** | **1.115910** | **1.035512** | **1.343814** | **1.318374** | **1.651222** | |
| | **Std** | **0.451354** | **0.393529** | **0.461004** | **0.403200** | **0.601922** | |
| | **SEM** | **0.170596** | **0.148740** | **0.174243** | **0.152395** | **0.227505** | |

**Day 7**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L4M1 | 31.0 | 31.0 | 30.0 | 32.0 | 26.0 | |
| | L4M2 | 38.0 | 37.0 | 38.0 | 41.0 | 43.0 | |
| | L4M3 | 34.0 | 33.0 | 30.0 | 37.0 | 35.5 | |
| | L4M4 | 35.0 | 34.0 | 37.0 | 41.0 | 41.0 | |
| | L4M5 | 36.0 | 35.0 | 39.0 | 39.0 | 40.0 | |
| | L4M6 | 34.0 | 35.0 | 39.0 | 39.0 | 44.0 | |
| | L4M7 | 35.0 | 36.0 | 37.0 | 39.0 | 40.0 | |
| **Freq (60s)** | L4M1 | 186.0 | 186.0 | 180.0 | 192.0 | 156.0 | -3.9 |
| **(breaths/min)** | L4M2 | 228.0 | 222.0 | 228.0 | 246.0 | 258.0 | 6.3 |
| | L4M3 | 204.0 | 198.0 | 180.0 | 222.0 | 213.0 | 4.35 |
| | L4M4 | 210.0 | 204.0 | 222.0 | 246.0 | 246.0 | 7.5 |
| | L4M5 | 216.0 | 210.0 | 234.0 | 234.0 | 240.0 | 4.5 |
| | L4M6 | 204.0 | 210.0 | 234.0 | 234.0 | 264.0 | 8.1 |
| | L4M7 | 210.0 | 216.0 | 222.0 | 234.0 | 240.0 | 4.2 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | **Avg** | **208.3** | **206.6** | **214.3** | **229.7** | **231.0** | |
| | **Std** | **12.8** | **11.9** | **23.9** | **18.6** | **36.9** | |
| | **SEM** | **4.8** | **4.5** | **9.0** | **7.0** | **13.9** | |
| **Vt (Total)** | L4M1 | 0.04586 | 0.06954 | 0.05986 | 0.06718 | 0.13049 | |
| | L4M2 | 0.10696 | 0.08945 | 0.06998 | 0.05788 | 0.14598 | |
| | L4M3 | 0.12596 | 0.09053 | 0.10349 | 0.08792 | 0.16697 | |
| | L4M4 | 0.12431 | 0.05699 | 0.14616 | 0.22294 | 0.56033 | |
| | L4M5 | 0.09236 | 0.09448 | 0.07984 | 0.04624 | 0.04895 | |
| | L4M6 | 0.09750 | 0.08595 | 0.06948 | 0.08002 | 0.19568 | |
| | L4M7 | 0.08697 | 0.08394 | 0.05098 | 0.07409 | 0.12395 | |
| **Vt (avg vol/breath)** | L4M1 | 0.0014794 | 0.0022432 | 0.0019953 | 0.0020994 | 0.0050190 | |
| **(raw)** | L4M2 | 0.0028147 | 0.0024176 | 0.0018416 | 0.0014117 | 0.0033950 | |
| | L4M3 | 0.0037047 | 0.0027434 | 0.0034498 | 0.0023762 | 0.0047034 | |
| | L4M4 | 0.0035517 | 0.0016762 | 0.0039503 | 0.0054376 | 0.0136666 | |
| | L4M5 | 0.0025655 | 0.0026994 | 0.0020472 | 0.0011856 | 0.0012239 | |
| | L4M6 | 0.0028676 | 0.0024557 | 0.0017816 | 0.0020518 | 0.0044473 | |
| | L4M7 | 0.0024849 | 0.0023318 | 0.0013779 | 0.0018997 | 0.0030988 | |
| **Vt (avg vol/breath)** | L4M1 | 0.00756 | 0.01146 | 0.01020 | 0.01073 | 0.02565 | |
| **(actual)** | L4M2 | 0.01438 | 0.01235 | 0.00941 | 0.00721 | 0.01735 | |
| | L4M3 | 0.01893 | 0.01402 | 0.01763 | 0.01214 | 0.02403 | |
| | L4M4 | 0.01815 | 0.00857 | 0.02019 | 0.02779 | 0.06984 | |
| | L4M5 | 0.01311 | 0.01379 | 0.01046 | 0.00606 | 0.00625 | |
| | L4M6 | 0.01465 | 0.01255 | 0.00910 | 0.01048 | 0.02273 | |
| | L4M7 | 0.01270 | 0.01192 | 0.00704 | 0.00971 | 0.01583 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L4M1 | 0.001985 | 0.003009 | 0.002677 | 0.002816 | 0.006733 | 3.809 |
| **(actual/gram)** | L4M2 | 0.003629 | 0.003117 | 0.002375 | 0.001820 | 0.004378 | 3.963 |
| | L4M3 | 0.004784 | 0.003543 | 0.004455 | 0.003069 | 0.006074 | 3.957 |
| | L4M4 | 0.004819 | 0.002274 | 0.005360 | 0.007378 | 0.018544 | 3.766 |
| | L4M5 | 0.003118 | 0.003280 | 0.002488 | 0.001441 | 0.001487 | 4.205 |
| | L4M6 | 0.003681 | 0.003152 | 0.002287 | 0.002634 | 0.005709 | 3.981 |
| | L4M7 | 0.003591 | 0.003370 | 0.001991 | 0.002745 | 0.004478 | 3.536 |
| | **Avg** | **0.003658** | **0.003107** | **0.003090** | **0.003129** | **0.006772** | |
| | **Std** | **0.000976** | **0.000407** | **0.001285** | **0.001963** | **0.005464** | |
| | **SEM** | **0.000369** | **0.000154** | **0.000486** | **0.000742** | **0.002065** | |
| **Minute Ventilation** | L4M1 | 0.3691 | 0.5598 | 0.4818 | 0.5408 | 1.0504 | 0.07504 |
| **(mL/min/g)** | L4M2 | 0.8275 | 0.6921 | 0.5414 | 0.4478 | 1.1294 | 0.060646 |
| | L4M3 | 0.9760 | 0.7015 | 0.8019 | 0.6812 | 1.2937 | 0.075772 |
| | L4M4 | 1.0120 | 0.4640 | 1.1899 | 1.8150 | 4.5618 | 0.603793 |
| | L4M5 | 0.6734 | 0.6889 | 0.5821 | 0.3372 | 0.3569 | -0.05854 |
| | L4M6 | 0.7509 | 0.6619 | 0.5351 | 0.6163 | 1.5070 | 0.127975 |
| | L4M7 | 0.7541 | 0.7279 | 0.4421 | 0.6424 | 1.0748 | 0.066846 |
| | **Avg** | **0.766153** | **0.642277** | **0.653485** | **0.725806** | **1.567722** | |
| | **Std** | **0.214025** | **0.095278** | **0.263191** | **0.494957** | **1.366992** | |
| | **SEM** | **0.080894** | **0.036012** | **0.099477** | **0.187076** | **0.516674** | |

**Day 9**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L1M1 | 50.5 | 48.5 | 54.0 | 49.0 | 53.0 | |
| | L1M2 | 41.0 | 33.0 | 49.0 | 44.0 | 48.0 | |
| | L1M3 | 38.0 | 39.0 | 34.0 | 38.0 | 44.0 | |
| | L1M4 | 39.5 | 42.0 | 41.0 | 52.0 | 46.0 | |
| | L1M5 | 51.0 | 46.0 | 42.0 | 44.0 | 48.0 | |
| **Freq (60s)** | L1M1 | 303.0 | 291.0 | 324.0 | 294.0 | 318.0 | 2.55 |
| **(breaths/min)** | L1M2 | 246.0 | 198.0 | 294.0 | 264.0 | 288.0 | 12 |
| | L1M3 | 228.0 | 234.0 | 204.0 | 228.0 | 264.0 | 5.7 |
| | L1M4 | 237.0 | 252.0 | 246.0 | 312.0 | 276.0 | 6.9 |
| | L1M5 | 306.0 | 276.0 | 252.0 | 264.0 | 288.0 | 2.4 |
| | **Avg** | **264.0** | **250.2** | **264.0** | **272.4** | **286.8** | |
| | **Std** | **37.5** | **36.5** | **46.3** | **32.2** | **20.1** | |
| | **SEM** | **14.2** | **13.8** | **17.5** | **12.2** | **7.6** | |
| **Vt (Total)** | L1M1 | 0.17809 | 0.15352 | 0.22811 | 0.17749 | 0.42056 | |
| | L1M2 | 0.11678 | 0.08613 | 0.23300 | 0.28595 | 0.27221 | |
| | L1M3 | 0.13206 | 0.14459 | 0.14370 | 0.17909 | 0.36359 | |
| | L1M4 | 0.12619 | 0.11472 | 0.20424 | 0.32822 | 0.30249 | |
| | L1M5 | 0.16341 | 0.12519 | 0.12279 | 0.27376 | 0.38868 | |
| **Vt (avg vol/breath)** | L1M1 | 0.0035265 | 0.0031654 | 0.0042243 | 0.0036222 | 0.0079351 | |
| **(raw)** | L1M2 | 0.0028483 | 0.0026100 | 0.0047551 | 0.0064989 | 0.0056710 | |
| | L1M3 | 0.0034753 | 0.0037074 | 0.0042265 | 0.0047129 | 0.0082634 | |
| | L1M4 | 0.0031947 | 0.0027314 | 0.0049815 | 0.0063119 | 0.0065759 | |
| | L1M5 | 0.0032041 | 0.0027215 | 0.0029236 | 0.0062218 | 0.0080975 | |
| **Vt (avg vol/breath)** | L1M1 | 0.01802 | 0.01617 | 0.02159 | 0.01851 | 0.04055 | |
| **(actual)** | L1M2 | 0.01455 | 0.01334 | 0.02430 | 0.03321 | 0.02898 | |
| | L1M3 | 0.01776 | 0.01894 | 0.02160 | 0.02408 | 0.04223 | |
| | L1M4 | 0.01632 | 0.01396 | 0.02546 | 0.03225 | 0.03360 | |
| | L1M5 | 0.01637 | 0.01391 | 0.01494 | 0.03179 | 0.04138 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L1M1 | 0.003462 | 0.003108 | 0.004147 | 0.003556 | 0.007790 | 5.205 |
| **(actual/gram)** | L1M2 | 0.002901 | 0.002658 | 0.004843 | 0.006619 | 0.005776 | 5.017 |
| | L1M3 | 0.003298 | 0.003519 | 0.004011 | 0.004473 | 0.007843 | 5.384 |
| | L1M4 | 0.003644 | 0.003116 | 0.005682 | 0.007200 | 0.007501 | 4.48 |
| | L1M5 | 0.003042 | 0.002584 | 0.002776 | 0.005907 | 0.007688 | 5.382 |
| | **Avg** | **0.003270** | **0.002997** | **0.004292** | **0.005551** | **0.007320** | |
| | **Std** | **0.000302** | **0.000382** | **0.001076** | **0.001511** | **0.000873** | |
| | **SEM** | **0.000114** | **0.000144** | **0.000407** | **0.000571** | **0.000330** | |
| **Minute Ventilation** | L1M1 | 1.0490 | 0.9043 | 1.3437 | 1.0455 | 2.4773 | 0.221041 |
| **(mL/min/g)** | L1M2 | 0.7137 | 0.5264 | 1.4239 | 1.7475 | 1.6635 | 0.186756 |
| | L1M3 | 0.7520 | 0.8234 | 0.8183 | 1.0199 | 2.0705 | 0.197146 |
| | L1M4 | 0.8636 | 0.7851 | 1.3978 | 2.2463 | 2.0702 | 0.235182 |
| | L1M5 | 0.9309 | 0.7132 | 0.6995 | 1.5595 | 2.2142 | 0.268158 |
| | **Avg** | **0.861853** | **0.750470** | **1.136638** | **1.523733** | **2.099149** | |
| | **Std** | **0.135857** | **0.143003** | **0.348566** | **0.513807** | **0.294814** | |
| | **SEM** | **0.051349** | **0.054050** | **0.131746** | **0.194201** | **0.111429** | |

**Day 9**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L4M1 | 46.0 | 42.0 | 49.0 | 43.0 | 50.0 | |
| | L4M2 | 38.0 | 39.0 | 36.0 | 37.0 | 36.0 | |
| | L4M3 | 43.0 | 42.0 | 40.0 | 41.0 | 38.0 | |
| | L4M4 | 32.0 | 32.0 | 38.0 | 39.0 | 40.0 | |
| | L4M5 | 37.0 | 41.0 | 40.0 | 42.0 | 48.0 | |
| | L4M6 | 39.0 | 39.0 | 43.0 | 44.0 | 45.0 | |
| | L4M7 | 31.0 | 33.0 | 35.0 | 36.0 | 39.0 | |
| **Freq (60s)** | L4M1 | 276.0 | 252.0 | 294.0 | 258.0 | 300.0 | 5.4 |
| (breaths/min) | L4M2 | 228.0 | 234.0 | 216.0 | 222.0 | 216.0 | -2.4 |
| | L4M3 | 258.0 | 252.0 | 240.0 | 246.0 | 228.0 | -3.3 |
| | L4M4 | 192.0 | 192.0 | 228.0 | 234.0 | 240.0 | 7.5 |
| | L4M5 | 222.0 | 246.0 | 240.0 | 252.0 | 288.0 | 6.9 |
| | L4M6 | 234.0 | 234.0 | 258.0 | 264.0 | 270.0 | 5.7 |
| | L4M7 | 186.0 | 198.0 | 210.0 | 216.0 | 234.0 | 5.7 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | **Avg** | **228.0** | **229.7** | **240.9** | **241.7** | **253.7** | |
| | **Std** | **32.5** | **24.9** | **28.4** | **18.2** | **32.3** | |
| | **SEM** | **12.3** | **9.4** | **10.8** | **6.9** | **12.2** | |
| **Vt (Total)** | L4M1 | 0.04905 | 0.05794 | 0.06799 | 0.05839 | 0.23094 | |
| | L4M2 | 0.10400 | 0.04689 | 0.02388 | 0.03905 | 0.18779 | |
| | L4M3 | 0.15039 | 0.08693 | 0.06499 | 0.06934 | 0.10842 | |
| | L4M4 | 0.14039 | 0.03422 | 0.12340 | 0.19320 | 0.14503 | |
| | L4M5 | 0.08393 | 0.04855 | 0.08794 | 0.05993 | 0.09384 | |
| | L4M6 | 0.07399 | 0.09993 | 0.09843 | 0.09374 | 0.19568 | |
| | L4M7 | 0.09384 | 0.03948 | 0.06232 | 0.07959 | 0.18403 | |
| **Vt (avg vol/breath)** | L4M1 | 0.0010664 | 0.0013795 | 0.0013876 | 0.0013580 | 0.0046188 | |
| (raw) | L4M2 | 0.0027369 | 0.0012024 | 0.0006634 | 0.0010554 | 0.0052165 | |
| | L4M3 | 0.0034975 | 0.0020699 | 0.0016248 | 0.0016913 | 0.0028532 | |
| | L4M4 | 0.0043873 | 0.0010695 | 0.0032474 | 0.0049538 | 0.0036258 | |
| | L4M5 | 0.0022684 | 0.0011841 | 0.0021985 | 0.0014270 | 0.0019551 | |
| | L4M6 | 0.0018973 | 0.0025624 | 0.0022891 | 0.0021305 | 0.0043484 | |
| | L4M7 | 0.0030270 | 0.0011965 | 0.0017807 | 0.0022110 | 0.0047188 | |
| **Vt (avg vol/breath)** | L4M1 | 0.00545 | 0.00705 | 0.00709 | 0.00694 | 0.02360 | |
| (actual) | L4M2 | 0.01399 | 0.00614 | 0.00339 | 0.00539 | 0.02666 | |
| | L4M3 | 0.01787 | 0.01058 | 0.00830 | 0.00864 | 0.01458 | |
| | L4M4 | 0.02242 | 0.00546 | 0.01659 | 0.02531 | 0.01853 | |
| | L4M5 | 0.01159 | 0.00605 | 0.01123 | 0.00729 | 0.00999 | |
| | L4M6 | 0.00969 | 0.01309 | 0.01170 | 0.01089 | 0.02222 | |
| | L4M7 | 0.01547 | 0.00611 | 0.00910 | 0.01130 | 0.02411 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/g)** | L4M1 | 0.001166 | 0.001508 | 0.001517 | 0.001485 | 0.005050 | 4.674 |
| (actual/gram) | L4M2 | 0.002896 | 0.001272 | 0.000702 | 0.001117 | 0.005520 | 4.829 |
| | L4M3 | 0.003752 | 0.002221 | 0.001743 | 0.001815 | 0.003061 | 4.763 |
| | L4M4 | 0.004807 | 0.001172 | 0.003558 | 0.005428 | 0.003972 | 4.664 |
| | L4M5 | 0.002236 | 0.001167 | 0.002167 | 0.001406 | 0.001927 | 5.185 |
| | L4M6 | 0.001943 | 0.002624 | 0.002344 | 0.002182 | 0.004453 | 4.99 |
| | L4M7 | 0.003321 | 0.001313 | 0.001954 | 0.002426 | 0.005178 | 4.657 |
| | **Avg** | **0.002874** | **0.001611** | **0.001998** | **0.002265** | **0.004166** | |
| | **Std** | **0.001218** | **0.000578** | **0.000872** | **0.001466** | **0.001291** | |
| | **SEM** | **0.000460** | **0.000218** | **0.000329** | **0.000554** | **0.000488** | |
| **Minute Ventilation** | L4M1 | 0.3218 | 0.3801 | 0.4460 | 0.3830 | 1.5149 | -0.20013 |
| (mL/min/g) | L4M2 | 0.6603 | 0.2977 | 0.1516 | 0.2479 | 1.1923 | -0.12475 |
| | L4M3 | 0.9681 | 0.5596 | 0.4184 | 0.4464 | 0.6979 | 0.000488 |
| | L4M4 | 0.9229 | 0.2250 | 0.8112 | 1.2700 | 0.9534 | -0.21911 |
| | L4M5 | 0.4963 | 0.2871 | 0.5200 | 0.3544 | 0.5549 | -0.08346 |
| | L4M6 | 0.4546 | 0.6140 | 0.6048 | 0.5760 | 1.2023 | -0.09651 |
| | L4M7 | 0.6178 | 0.2599 | 0.4103 | 0.5240 | 1.2116 | -0.18367 |
| | **Avg** | **0.634548** | **0.374776** | **0.480337** | **0.543113** | **1.046765** | |
| | **Std** | **0.239674** | **0.153124** | **0.201870** | **0.338528** | **0.332675** | |
| | **SEM** | **0.090588** | **0.057875** | **0.076300** | **0.127952** | **0.125739** | |

## Day 11

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L1M1 | | 45.0 | 44.0 | 40.0 | 59.0 | |
| | L1M2 | | 40.5 | 47.0 | 44.0 | 53.0 | |
| | L1M3 | | 44.0 | 36.0 | 42.0 | 47.0 | |
| | L1M4 | | 31.0 | 37.0 | 58.0 | 55.0 | |
| | L1M5 | | 40.0 | 47.0 | 51.0 | 52.0 | |
| **Freq (60s)** | L1M1 | | 270.0 | 264.0 | 240.0 | 354.0 | 11.4 |
| (breaths/min) | L1M2 | | 243.0 | 282.0 | 264.0 | 318.0 | 10.35 |
| | L1M3 | | 264.0 | 216.0 | 252.0 | 282.0 | 4.5 |
| | L1M4 | | 186.0 | 222.0 | 348.0 | 330.0 | 27.9 |
| | L1M5 | | 240.0 | 282.0 | 306.0 | 312.0 | 12 |
| | **Avg** | **#DIV/0!** | **240.6** | **253.2** | **282.0** | **319.2** | |
| | **Std** | **#DIV/0!** | **33.2** | **32.1** | **44.5** | **26.3** | |
| | **SEM** | **#DIV/0!** | **12.5** | **12.1** | **16.8** | **9.9** | |
| **Vt (Total)** | L1M1 | | 0.17073 | 0.26533 | 0.22619 | 0.27068 | |
| | L1M2 | | 0.13608 | 0.24722 | 0.20861 | 0.37215 | |
| | L1M3 | | 0.16683 | 0.18181 | 0.22906 | 0.26709 | |
| | L1M4 | | 0.06975 | 0.29635 | 0.30240 | 0.41619 | |
| | L1M5 | | 0.16182 | 0.17182 | 0.26406 | 0.50209 | |
| **Vt (avg vol/breath)** | L1M1 | 0.0037940 | 0.0037940 | 0.0060302 | 0.0056548 | 0.0045878 | |
| (raw) | L1M2 | 0.0033600 | 0.0033600 | 0.0052600 | 0.0047411 | 0.0070217 | |
| | L1M3 | 0.0037916 | 0.0037916 | 0.0050503 | 0.0054538 | 0.0056828 | |
| | L1M4 | 0.0022500 | 0.0022500 | 0.0080095 | 0.0052138 | 0.0075671 | |
| | L1M5 | 0.0040455 | 0.0040455 | 0.0036557 | 0.0051776 | 0.0096556 | |
| **Vt (avg vol/breath)** | L1M1 | 0.01939 | 0.01939 | 0.03081 | 0.02890 | 0.02344 | |
| (actual) | L1M2 | 0.01717 | 0.01717 | 0.02688 | 0.02423 | 0.03588 | |
| | L1M3 | 0.01938 | 0.01938 | 0.02581 | 0.02787 | 0.02904 | |
| | L1M4 | 0.01150 | 0.01150 | 0.04093 | 0.02664 | 0.03867 | |
| | L1M5 | 0.02067 | 0.02067 | 0.01868 | 0.02646 | 0.04934 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/** | L1M1 | 0.003241 | 0.003241 | 0.005152 | 0.004831 | 0.003920 | 5.981 |
| (actual/gram) | L1M2 | 0.002837 | 0.002837 | 0.004442 | 0.004004 | 0.005930 | 6.051 |
| | L1M3 | 0.002916 | 0.002916 | 0.003884 | 0.004194 | 0.004370 | 6.645 |
| | L1M4 | 0.001948 | 0.001948 | 0.006936 | 0.004515 | 0.006553 | 5.901 |
| | L1M5 | 0.003171 | 0.003171 | 0.002865 | 0.004058 | 0.007567 | 6.52 |
| | **Avg** | **0.002823** | **0.002823** | **0.004656** | **0.004320** | **0.005668** | |
| | **Std** | **0.000517** | **0.000517** | **0.001524** | **0.000348** | **0.001517** | |
| | **SEM** | **0.000195** | **0.000195** | **0.000576** | **0.000131** | **0.000573** | |
| **Minute Ventilation** | L1M1 | 0.8752 | 0.8558 | 1.3601 | 1.1595 | 1.3876 | 0.06974 |
| (mL/min/g) | L1M2 | 0.6895 | 0.8002 | 1.2526 | 1.0570 | 1.8857 | 0.153042 |
| | L1M3 | 0.7698 | 0.6298 | 0.8389 | 1.0569 | 1.2324 | 0.101284 |
| | L1M4 | 0.3624 | 0.4325 | 1.5398 | 1.5712 | 2.1624 | 0.261052 |
| | L1M5 | 0.7610 | 0.8941 | 0.8080 | 1.2417 | 2.3611 | 0.241728 |
| | **Avg** | **0.691563** | **0.722476** | **1.159878** | **1.217263** | **1.805809** | |
| | **Std** | **0.195578** | **0.190990** | **0.323998** | **0.212486** | **0.486214** | |
| | **SEM** | **0.073921** | **0.072188** | **0.122460** | **0.080312** | **0.183772** | |

**Day 11**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| **Freq (10s)** | L4M1 | 31.0 | 32.0 | 32.0 | 35.0 | 36.0 | |
| | L4M2 | 35.0 | 31.0 | 43.0 | 45.0 | 47.0 | |
| | L4M3 | 45.0 | 43.0 | 43.0 | 40.0 | 45.0 | |
| | L4M4 | 37.0 | 38.0 | 40.0 | 40.0 | 48.0 | |
| | L4M5 | 49.0 | 44.0 | 45.0 | 37.0 | 48.0 | |
| | L4M6 | 35.0 | 35.0 | 36.0 | 40.0 | 42.0 | |
| | L4M7 | 39.0 | 40.0 | 34.0 | 40.0 | 49.0 | |
| **Freq (60s)** | L4M1 | 186.0 | 192.0 | 192.0 | 210.0 | 216.0 | 4.5 |
| **(breaths/min)** | L4M2 | 210.0 | 186.0 | 258.0 | 270.0 | 282.0 | 15 |
| | L4M3 | 270.0 | 258.0 | 258.0 | 240.0 | 270.0 | 0.9 |
| | L4M4 | 222.0 | 228.0 | 240.0 | 240.0 | 288.0 | 9 |
| | L4M5 | 294.0 | 264.0 | 270.0 | 222.0 | 288.0 | 1.2 |
| | L4M6 | 210.0 | 210.0 | 216.0 | 240.0 | 252.0 | 7.5 |
| | L4M7 | 234.0 | 240.0 | 204.0 | 240.0 | 294.0 | 9.9 |
| | | | | | | | |
| | | | | | | | |
| | **Avg** | **232.3** | **225.4** | **234.0** | **237.4** | **270.0** | |
| | **Std** | **37.6** | **30.8** | **30.2** | **18.6** | **27.7** | |
| | **SEM** | **14.2** | **11.6** | **11.4** | **7.0** | **10.5** | |
| **Vt (Total)** | L4M1 | 0.05849 | 0.08943 | 0.09684 | 0.07832 | 0.12043 | |
| | L4M2 | 0.10947 | 0.06794 | 0.09747 | 0.09472 | 0.15303 | |
| | L4M3 | 0.15030 | 0.08923 | 0.07352 | 0.08343 | 0.14764 | |
| | L4M4 | 0.12083 | 0.10932 | 0.15030 | 0.23777 | 0.15033 | |
| | L4M5 | 0.10948 | 0.06949 | 0.12043 | 0.08980 | 0.13053 | |
| | L4M6 | 0.08934 | 0.08234 | 0.07293 | 0.03893 | 0.17520 | |
| | L4M7 | 0.08430 | 0.07953 | 0.06939 | 0.06793 | 0.18302 | |
| **Vt (avg vol/breath)** | L4M1 | 0.0018869 | 0.0027948 | 0.0030263 | 0.0022378 | 0.0033453 | |
| **(raw)** | L4M2 | 0.0031278 | 0.0021917 | 0.0022668 | 0.0021049 | 0.0032560 | |
| | L4M3 | 0.0033400 | 0.0020751 | 0.0017098 | 0.0020858 | 0.0032809 | |
| | L4M4 | 0.0032657 | 0.0028769 | 0.0037576 | 0.0059443 | 0.0031319 | |
| | L4M5 | 0.0022343 | 0.0015794 | 0.0026762 | 0.0024270 | 0.0027194 | |
| | L4M6 | 0.0025526 | 0.0023526 | 0.0020259 | 0.0009733 | 0.0041715 | |
| | L4M7 | 0.0021615 | 0.0019884 | 0.0020409 | 0.0016983 | 0.0037351 | |
| **Vt (avg vol/breath)** | L4M1 | 0.00964 | 0.01428 | 0.01546 | 0.01144 | 0.01709 | |
| **(actual)** | L4M2 | 0.01598 | 0.01120 | 0.01158 | 0.01076 | 0.01664 | |
| | L4M3 | 0.01707 | 0.01060 | 0.00874 | 0.01066 | 0.01677 | |
| | L4M4 | 0.01669 | 0.01470 | 0.01920 | 0.03038 | 0.01600 | |
| | L4M5 | 0.01142 | 0.00807 | 0.01368 | 0.01240 | 0.01390 | |
| | L4M6 | 0.01304 | 0.01202 | 0.01035 | 0.00497 | 0.02132 | |
| | L4M7 | 0.01105 | 0.01016 | 0.01043 | 0.00868 | 0.01909 | |
| | | | | | | | Weight |
| **Vt (avg vol/breath/** | L4M1 | 0.001766 | 0.002616 | 0.002833 | 0.002095 | 0.003131 | 5.459 |
| **(actual/gram)** | L4M2 | 0.002880 | 0.002018 | 0.002087 | 0.001938 | 0.002998 | 5.55 |
| | L4M3 | 0.003066 | 0.001905 | 0.001570 | 0.001915 | 0.003012 | 5.566 |
| | L4M4 | 0.003065 | 0.002700 | 0.003526 | 0.005579 | 0.002939 | 5.445 |
| | L4M5 | 0.001833 | 0.001296 | 0.002196 | 0.001991 | 0.002231 | 6.228 |
| | L4M6 | 0.002269 | 0.002091 | 0.001801 | 0.000865 | 0.003708 | 5.748 |
| | L4M7 | 0.002013 | 0.001852 | 0.001901 | 0.001582 | 0.003478 | 5.487 |
| | **Avg** | **0.002413** | **0.002068** | **0.002273** | **0.002281** | **0.003071** | |
| | **Std** | **0.000578** | **0.000478** | **0.000681** | **0.001513** | **0.000467** | |
| | **SEM** | **0.000218** | **0.000181** | **0.000257** | **0.000572** | **0.000176** | |
| **Minute Ventilation** | L4M1 | 0.3285 | 0.5023 | 0.5439 | 0.4399 | 0.6764 | 0.005171 |
| **(mL/min/g)** | L4M2 | 0.6048 | 0.3753 | 0.5385 | 0.5233 | 0.8454 | 0.021598 |
| | L4M3 | 0.8279 | 0.4915 | 0.4050 | 0.4596 | 0.8133 | 0.009858 |
| | L4M4 | 0.6804 | 0.6156 | 0.8463 | 1.3388 | 0.8465 | 0.026758 |
| | L4M5 | 0.5390 | 0.3421 | 0.5929 | 0.4421 | 0.6426 | 0.015346 |
| | L4M6 | 0.4766 | 0.4392 | 0.3890 | 0.2077 | 0.9345 | 0.011862 |
| | L4M7 | 0.4710 | 0.4444 | 0.3877 | 0.3796 | 1.0227 | 0.018506 |
| | **Avg** | **0.561167** | **0.458638** | **0.529047** | **0.541559** | **0.825904** | |
| | **Std** | **0.161851** | **0.090106** | **0.163309** | **0.365303** | **0.133817** | |
| | **SEM** | **0.061174** | **0.034057** | **0.061725** | **0.138072** | **0.050578** | |

Appendix D. Data from litters withdrawn from nicotine by day.

**Day 1**

**Measurement of last 10 sec**

| | Mouse | 0 | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 24.0 | 25.0 | 24.0 | 25.0 | 21.5 | |
| | L2M2 | 19.0 | 20.0 | 18.0 | 19.0 | 18.0 | |
| | L2M3 | 23.0 | 22.0 | 23.0 | 25.0 | 26.0 | |
| | L2M4 | 20.0 | 24.0 | 26.0 | 26.5 | 27.0 | |
| | L2M5 | 22.0 | 18.0 | 22.0 | 25.0 | 28.0 | |
| Freq (60s) | L2M1 | 144.0 | 150.0 | 144.0 | 150.0 | 129.0 | -2.85 |
| (breaths/min) | L2M2 | 114.0 | 120.0 | 108.0 | 114.0 | 108.0 | -1.5 |
| | L2M3 | 138.0 | 132.0 | 138.0 | 150.0 | 156.0 | 4.2 |
| | L2M4 | 120.0 | 144.0 | 156.0 | 159.0 | 162.0 | 2.85 |
| | L2M5 | 132.0 | 108.0 | 132.0 | 150.0 | 168.0 | 9.9 |
| | Avg | 129.6 | 130.8 | 135.6 | 144.6 | 144.6 | |
| | Std | 12.4 | 17.2 | 17.8 | 17.5 | 25.3 | |
| | SEM | 4.7 | 6.5 | 6.7 | 6.6 | 9.6 | |
| Vt (Total) | L2M1 | 0.22142 | 0.14165 | 0.04981 | 0.06436 | 0.06155 | |
| | L2M2 | 0.15194 | 0.19039 | 0.16297 | 0.09669 | 0.08887 | |
| | L2M3 | 0.06456 | 0.05093 | 0.07603 | 0.02611 | 0.15007 | |
| | L2M4 | 0.11000 | 0.06961 | 0.07896 | 0.06645 | 0.09367 | |
| | L2M5 | 0.06053 | 0.05302 | 0.08863 | 0.10977 | 0.08878 | |
| Vt (avg vol/breath) | L2M1 | 0.0092258 | 0.0056660 | 0.0020753 | 0.0025744 | 0.0028627 | |
| (raw) | L2M2 | 0.0079968 | 0.0095195 | 0.0090539 | 0.0050889 | 0.0049374 | |
| | L2M3 | 0.0028070 | 0.0023150 | 0.0033057 | 0.0010444 | 0.0057719 | |
| | L2M4 | 0.0055000 | 0.0029004 | 0.0030369 | 0.0025075 | 0.0034693 | |
| | L2M5 | 0.0027514 | 0.0029456 | 0.0040286 | 0.0043908 | 0.0031707 | |
| Vt (avg vol/breath) | L2M1 | 0.04714 | 0.02895 | 0.01060 | 0.01316 | 0.01463 | |
| (actual) | L2M2 | 0.04086 | 0.04864 | 0.04627 | 0.02600 | 0.02523 | |
| | L2M3 | 0.01434 | 0.01183 | 0.01689 | 0.00534 | 0.02949 | |
| | L2M4 | 0.02811 | 0.01482 | 0.01552 | 0.01281 | 0.01773 | |
| | L2M5 | 0.01406 | 0.01505 | 0.02059 | 0.02244 | 0.01620 | |
| | | | | | | | Weight |
| Vt (avg vol/breath/g) | L2M1 | 0.029030 | 0.017828 | 0.006530 | 0.008100 | 0.009007 | 1.624 |
| (actual/gram) | L2M2 | 0.030495 | 0.036302 | 0.034526 | 0.019406 | 0.018829 | 1.34 |
| | L2M3 | 0.010312 | 0.008504 | 0.012144 | 0.003837 | 0.021204 | 1.391 |
| | L2M4 | 0.017158 | 0.009048 | 0.009474 | 0.007823 | 0.010823 | 1.638 |
| | L2M5 | 0.008573 | 0.009178 | 0.012553 | 0.013681 | 0.009879 | 1.64 |
| | Avg | 0.019114 | 0.016172 | 0.015045 | 0.010569 | 0.013948 | |
| | Std | 0.010250 | 0.011900 | 0.011155 | 0.006057 | 0.005639 | |
| | SEM | 0.003874 | 0.004498 | 0.004216 | 0.002289 | 0.002131 | |
| Minute Ventilation | L2M1 | 4.1803 | 2.6743 | 0.9403 | 1.2151 | 1.1620 | -0.2131059 |
| (mL/min/g) | L2M2 | 3.4765 | 4.3562 | 3.7289 | 2.2123 | 2.0335 | -0.4242383 |
| | L2M3 | 1.4230 | 1.1226 | 1.6758 | 0.5755 | 3.3078 | 0.272766 |
| | L2M4 | 2.0590 | 1.3030 | 1.4780 | 1.2438 | 1.7533 | 0.055845 |
| | L2M5 | 1.1316 | 0.9912 | 1.6569 | 2.0522 | 1.6598 | 0.12004138 |
| | Avg | 2.454067 | 2.089449 | 1.895984 | 1.459775 | 1.983263 | |
| | Std | 1.322224 | 1.435256 | 1.066892 | 0.671882 | 0.804516 | |
| | SEM | 0.499754 | 0.542476 | 0.403247 | 0.253948 | 0.304078 | |

**Day 1**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 22.0 | 23.0 | 27.0 | 25.0 | 29.0 | |
| | L2M2 | 30.0 | 27.0 | 27.0 | 27.0 | 31.5 | |
| | L2M3 | 24.5 | 22.0 | 24.0 | 26.0 | 29.0 | |
| | L2M5 | 19.0 | 20.0 | 22.0 | 21.0 | 24.0 | |
| | L2M6 | 23.0 | 24.0 | 24.0 | 29.0 | 29.0 | |
| | L2M7 | 22.5 | 22.0 | 26.0 | 25.0 | 27.0 | |
| Freq (60s) | L2M1 | 132.0 | 138.0 | 162.0 | 150.0 | 174.0 | 4.8 |
| (breaths/min) | L2M2 | 180.0 | 162.0 | 162.0 | 162.0 | 189.0 | 4.05 |
| | L2M3 | 147.0 | 132.0 | 144.0 | 156.0 | 174.0 | 5.7 |
| | L2M5 | 114.0 | 120.0 | 132.0 | 126.0 | 144.0 | 4 |
| | L2M6 | 138.0 | 144.0 | 144.0 | 174.0 | 174.0 | 6 |
| | L2M7 | 135.0 | 132.0 | 156.0 | 150.0 | 162.0 | 4.2 |
| | Avg | 141.0 | 138.0 | 150.0 | 153.0 | 169.5 | |
| | Std | 22.0 | 14.2 | 12.0 | 16.0 | 15.1 | |
| | SEM | 8.3 | 5.4 | 4.5 | 6.0 | 5.7 | |
| Vt (Total) | L2M1 | 0.05200 | 0.04752 | 0.04596 | 0.04408 | 0.06807 | |
| | L2M2 | 0.08388 | 0.09175 | 0.08366 | 0.10318 | 0.16918 | |
| | L2M3 | 0.07066 | 0.06490 | 0.06778 | 0.07321 | 0.09833 | |
| | L2M5 | 0.06604 | 0.05054 | 0.05216 | 0.07921 | 0.09886 | |
| | L2M6 | 0.06613 | 0.06165 | 0.05359 | 0.09145 | 0.10099 | |
| | L2M7 | 0.06992 | 0.03830 | 0.07924 | 0.06951 | 0.08708 | |
| Vt (avg vol/breath) | L2M1 | 0.0023636 | 0.0020661 | 0.0017022 | 0.0017632 | 0.0023472 | |
| (raw) | L2M2 | 0.0027960 | 0.0033981 | 0.0030985 | 0.0038215 | 0.0053708 | |
| | L2M3 | 0.0028841 | 0.0029500 | 0.0028242 | 0.0028158 | 0.0033907 | |
| | L2M5 | 0.0034758 | 0.0025270 | 0.0023709 | 0.0037719 | 0.0041192 | |
| | L2M6 | 0.0028752 | 0.0025688 | 0.0022329 | 0.0031534 | 0.0034824 | |
| | L2M7 | 0.0031076 | 0.0017409 | 0.0030477 | 0.0027804 | 0.0032252 | |
| Vt (avg vol/breath) | L2M1 | 0.01208 | 0.01056 | 0.00870 | 0.00901 | 0.01199 | |
| (actual) | L2M2 | 0.01429 | 0.01736 | 0.01583 | 0.01953 | 0.02744 | |
| | L2M3 | 0.01474 | 0.01507 | 0.01443 | 0.01439 | 0.01733 | |
| | L2M5 | 0.01776 | 0.01291 | 0.01212 | 0.01927 | 0.02105 | |
| | L2M6 | 0.01469 | 0.01313 | 0.01141 | 0.01611 | 0.01780 | |
| | L2M7 | 0.01588 | 0.00890 | 0.01557 | 0.01421 | 0.01648 | |
| | | | | | | | Weight |
| Vt (avg vol/breath/g) | L2M1 | 0.010236 | 0.008947 | 0.007371 | 0.007636 | 0.010165 | 1.18 |
| (actual/gram) | L2M2 | 0.010882 | 0.013225 | 0.012059 | 0.014873 | 0.020902 | 1.313 |
| | L2M3 | 0.011866 | 0.012137 | 0.011620 | 0.011585 | 0.013950 | 1.242 |
| | L2M5 | 0.013176 | 0.009579 | 0.008988 | 0.014299 | 0.015615 | 1.348 |
| | L2M6 | 0.011820 | 0.010560 | 0.009180 | 0.012964 | 0.014316 | 1.243 |
| | L2M7 | 0.011771 | 0.006595 | 0.011545 | 0.010532 | 0.012217 | 1.349 |
| | Avg | 0.011625 | 0.010174 | 0.010127 | 0.011981 | 0.014528 | |
| | Std | 0.001001 | 0.002366 | 0.001885 | 0.002677 | 0.003649 | |
| | SEM | 0.000378 | 0.000894 | 0.000712 | 0.001012 | 0.001379 | |
| Minute Ventilation | L2M1 | 1.3511 | 1.2347 | 1.1942 | 1.1453 | 1.7687 | 0.07765035 |
| (mL/min/g) | L2M2 | 1.9587 | 2.1425 | 1.9536 | 2.4094 | 3.9505 | 0.29400208 |
| | L2M3 | 1.7443 | 1.6021 | 1.6732 | 1.8073 | 2.4274 | 0.13049014 |
| | L2M5 | 1.5021 | 1.1495 | 1.1864 | 1.8016 | 2.2486 | 0.19561671 |
| | L2M6 | 1.6312 | 1.5207 | 1.3219 | 2.2557 | 2.4910 | 0.19224782 |
| | L2M7 | 1.5891 | 0.8705 | 1.8010 | 1.5798 | 1.9791 | 0.15524324 |
| | Avg | 1.629416 | 1.419995 | 1.521690 | 1.833186 | 2.477552 | |
| | Std | 0.208286 | 0.441524 | 0.330785 | 0.458153 | 0.771461 | |
| | SEM | 0.078725 | 0.166880 | 0.125025 | 0.173166 | 0.291585 | |

**Day 3**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 27.0 | 31.0 | 33.0 | 35.0 | 35.0 | |
| | L2M2 | 35.0 | 35.0 | 33.0 | 34.0 | 34.0 | |
| | L2M3 | 23.0 | 20.0 | 27.0 | 27.0 | 27.0 | |
| | L2M4 | 26.0 | 25.0 | 27.0 | 31.5 | 31.5 | |
| | L2M5 | 21.0 | 19.0 | 22.0 | 28.0 | 28.0 | |
| Freq (60s) | L2M1 | 162.0 | 186.0 | 198.0 | 210.0 | 210.0 | 4.2 |
| (breaths/min) | L2M2 | 210.0 | 210.0 | 198.0 | 204.0 | 204.0 | -0.6 |
| | L2M3 | 138.0 | 120.0 | 162.0 | 162.0 | 162.0 | 6.3 |
| | L2M4 | 156.0 | 150.0 | 162.0 | 189.0 | 189.0 | 7.2 |
| | L2M5 | 126.0 | 114.0 | 132.0 | 168.0 | 168.0 | 9.9 |
| | Avg | 158.4 | 156.0 | 170.4 | 186.6 | 186.6 | |
| | Std | 32.2 | 41.6 | 28.0 | 21.3 | 21.3 | |
| | SEM | 12.2 | 15.7 | 10.6 | 8.0 | 8.0 | |
| Vt (Total) | L2M1 | 0.20950 | 0.15154 | 0.09332 | 0.11639 | 0.15286 | |
| | L2M2 | 0.06750 | 0.08227 | 0.08996 | 0.13869 | 0.14884 | |
| | L2M3 | 0.06209 | 0.06220 | 0.05304 | 0.02569 | 0.13596 | |
| | L2M4 | 0.12597 | 0.09877 | 0.09767 | 0.10639 | 0.12460 | |
| | L2M5 | 0.06005 | 0.05992 | 0.09004 | 0.12059 | 0.09005 | |
| Vt (avg vol/breath) | L2M1 | 0.0077593 | 0.0048884 | 0.0028279 | 0.0033254 | 0.0043674 | |
| (raw) | L2M2 | 0.0019286 | 0.0023506 | 0.0027261 | 0.0040791 | 0.0043776 | |
| | L2M3 | 0.0026996 | 0.0031100 | 0.0019644 | 0.0009515 | 0.0050356 | |
| | L2M4 | 0.0048450 | 0.0039508 | 0.0036174 | 0.0033775 | 0.0039556 | |
| | L2M5 | 0.0028595 | 0.0031537 | 0.0040927 | 0.0043068 | 0.0032161 | |
| Vt (avg vol/breath) | L2M1 | 0.03965 | 0.02498 | 0.01445 | 0.01699 | 0.02232 | |
| (actual) | L2M2 | 0.00986 | 0.01201 | 0.01393 | 0.02084 | 0.02237 | |
| | L2M3 | 0.01379 | 0.01589 | 0.01004 | 0.00486 | 0.02573 | |
| | L2M4 | 0.02476 | 0.02019 | 0.01848 | 0.01726 | 0.02021 | |
| | L2M5 | 0.01461 | 0.01612 | 0.02091 | 0.02201 | 0.01643 | |
| | | | | | | | Weight |
| Vt (avg vol/breath/g) | L2M1 | 0.016473 | 0.010378 | 0.006004 | 0.007060 | 0.009272 | 2.407 |
| (actual/gram) | L2M2 | 0.005010 | 0.006106 | 0.007082 | 0.010597 | 0.011373 | 1.967 |
| | L2M3 | 0.006870 | 0.007914 | 0.004999 | 0.002421 | 0.012815 | 2.008 |
| | L2M4 | 0.009995 | 0.008150 | 0.007463 | 0.006968 | 0.008160 | 2.477 |
| | L2M5 | 0.005902 | 0.006509 | 0.008447 | 0.008888 | 0.006637 | 2.476 |
| | Avg | 0.008850 | 0.007812 | 0.006799 | 0.007187 | 0.009651 | |
| | Std | 0.004658 | 0.001682 | 0.001333 | 0.003054 | 0.002471 | |
| | SEM | 0.001760 | 0.000636 | 0.000504 | 0.001154 | 0.000934 | |
| Minute Ventilation | L2M1 | 2.6686 | 1.9303 | 1.1887 | 1.4826 | 1.9471 | 0.0172152 |
| (mL/min/g) | L2M2 | 1.0521 | 1.2824 | 1.4022 | 2.1618 | 2.3200 | 0.19362406 |
| | L2M3 | 0.9480 | 0.9497 | 0.8099 | 0.3923 | 2.0760 | 0.14805512 |
| | L2M4 | 1.5592 | 1.2226 | 1.2089 | 1.3169 | 1.5423 | 0.05335484 |
| | L2M5 | 0.7436 | 0.7420 | 1.1150 | 1.4933 | 1.1151 | 0.07487925 |
| | Avg | 1.394319 | 1.225385 | 1.144937 | 1.369347 | 1.800086 | |
| | Std | 0.773083 | 0.449949 | 0.215280 | 0.635135 | 0.475323 | |
| | SEM | 0.292198 | 0.170065 | 0.081368 | 0.240058 | 0.179655 | |

**Day 3**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 28.0 | 24.5 | 27.0 | 30.5 | 32.5 | |
| | L2M2 | 27.0 | 30.0 | 35.5 | 38.0 | 36.0 | |
| | L2M3 | 25.0 | 25.0 | 38.0 | 31.0 | 31.0 | |
| | L2M5 | 20.0 | 22.0 | 23.0 | 31.0 | 30.0 | |
| | L2M6 | 24.0 | 31.0 | 29.0 | 28.0 | 29.5 | |
| | L2M7 | 24.0 | 23.0 | 26.0 | 28.5 | 31.0 | |
| Freq (60s) | L2M1 | 168.0 | 147.0 | 162.0 | 183.0 | 195.0 | 8.25 |
| (breaths/min) | L2M2 | 162.0 | 180.0 | 213.0 | 228.0 | 216.0 | 6.15 |
| | L2M3 | 150.0 | 150.0 | 228.0 | 186.0 | 186.0 | 3.3 |
| | L2M5 | 120.0 | 132.0 | 138.0 | 186.0 | 180.0 | 9.6 |
| | L2M6 | 144.0 | 186.0 | 174.0 | 168.0 | 177.0 | -1.65 |
| | L2M7 | 144.0 | 138.0 | 156.0 | 171.0 | 186.0 | 7.95 |
| | Avg | **148.0** | **155.5** | **178.5** | **187.0** | **190.0** | |
| | Std | **16.8** | **22.3** | **34.9** | **21.5** | **14.2** | |
| | SEM | **6.4** | **8.4** | **13.2** | **8.1** | **5.4** | |
| Vt (Total) | L2M1 | 0.14351 | 0.11832 | 0.09877 | 0.12547 | 0.15243 | |
| | L2M2 | 0.14292 | 0.08471 | 0.11446 | 0.08123 | 0.10816 | |
| | L2M3 | 0.07630 | 0.04388 | 0.06488 | 0.05140 | 0.06488 | |
| | L2M5 | 0.13925 | 0.12883 | 0.07061 | 0.15610 | 0.14136 | |
| | L2M6 | 0.12131 | 0.09757 | 0.08658 | 0.07988 | 0.18573 | |
| | L2M7 | 0.07893 | 0.09187 | 0.06739 | 0.09047 | 0.13001 | |
| Vt (avg vol/breath) | L2M1 | 0.0051254 | 0.0048294 | 0.0036581 | 0.0041138 | 0.0046902 | |
| (raw) | L2M2 | 0.0052933 | 0.0028237 | 0.0032242 | 0.0021376 | 0.0030044 | |
| | L2M3 | 0.0030520 | 0.0017552 | 0.0017074 | 0.0016581 | 0.0020929 | |
| | L2M5 | 0.0069625 | 0.0058559 | 0.0030700 | 0.0050355 | 0.0047120 | |
| | L2M6 | 0.0050546 | 0.0031474 | 0.0029855 | 0.0029529 | 0.0062959 | |
| | L2M7 | 0.0032888 | 0.0039943 | 0.0025919 | 0.0031744 | 0.0041939 | |
| Vt (avg vol/breath) | L2M1 | 0.02619 | 0.02468 | 0.01869 | 0.02102 | 0.02397 | |
| (actual) | L2M2 | 0.02705 | 0.01443 | 0.01648 | 0.01092 | 0.01535 | |
| | L2M3 | 0.01560 | 0.00897 | 0.00872 | 0.00847 | 0.01069 | |
| | L2M5 | 0.03558 | 0.02992 | 0.01569 | 0.02573 | 0.02408 | |
| | L2M6 | 0.02583 | 0.01608 | 0.01526 | 0.01458 | 0.03217 | |
| | L2M7 | 0.01681 | 0.02041 | 0.01324 | 0.01622 | 0.02143 | |
| | | | | | | | Weight |
| Vt (avg vol/breath/g) | L2M1 | 0.015590 | 0.014689 | 0.011127 | 0.012513 | 0.014266 | 1.68 |
| (actual/gram) | L2M2 | 0.015492 | 0.008264 | 0.009436 | 0.006256 | 0.008793 | 1.746 |
| | L2M3 | 0.008801 | 0.005062 | 0.004924 | 0.004781 | 0.006035 | 1.772 |
| | L2M5 | 0.019006 | 0.015985 | 0.008380 | 0.013745 | 0.012862 | 1.872 |
| | L2M6 | 0.014568 | 0.009071 | 0.008605 | 0.008222 | 0.018146 | 1.773 |
| | L2M7 | 0.009316 | 0.011314 | 0.007342 | 0.008992 | 0.011880 | 1.804 |
| | Avg | **0.013795** | **0.010731** | **0.008302** | **0.009085** | **0.011997** | |
| | Std | **0.003971** | **0.004113** | **0.002083** | **0.003486** | **0.004231** | |
| | SEM | **0.001501** | **0.001555** | **0.000787** | **0.001317** | **0.001599** | |
| Minute Ventilation | L2M1 | 2.61906 | 2.1593 | 1.8026 | 2.2898 | 2.7818 | 0.11773988 |
| (mL/min/g) | L2M2 | 2.5097 | 1.4875 | 2.0099 | 1.4264 | 1.8993 | 0.03259162 |
| | L2M3 | 1.3202 | 0.7592 | 1.1226 | 0.8893 | 1.1226 | 0.04284095 |
| | L2M5 | 2.2807 | 2.1100 | 1.1565 | 2.5566 | 2.3152 | 0.10079147 |
| | L2M6 | 2.0978 | 1.6873 | 1.4972 | 1.3813 | 3.2118 | 0.22288592 |
| | L2M7 | 1.3415 | 1.5614 | 1.1453 | 1.5376 | 2.2096 | 0.11684451 |
| | Avg | **2.028140** | **1.627455** | **1.455679** | **1.680192** | **2.256722** | |
| | Std | **0.569638** | **0.509331** | **0.381062** | **0.622784** | **0.722156** | |
| | SEM | **0.215303** | **0.192509** | **0.144028** | **0.235390** | **0.272949** | |

**Day 5**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 35.0 | 34.0 | 26.0 | 32.0 | 40.0 | |
| | L2M2 | 34.0 | 38.0 | 27.0 | 39.0 | 39.0 | |
| | L2M3 | 35.0 | 37.0 | 38.0 | 36.0 | 41.0 | |
| | L2M4 | 40.0 | 35.0 | 35.0 | 37.0 | 39.0 | |
| | L2M5 | 32.0 | 34.0 | 37.0 | 36.0 | 39.0 | |
| Freq (60s) | L2M1 | 210.0 | 204.0 | 156.0 | 192.0 | 240.0 | 7.2 |
| (breaths/min) | L2M2 | 204.0 | 228.0 | 162.0 | 234.0 | 234.0 | 4.5 |
| | L2M3 | 210.0 | 222.0 | 228.0 | 216.0 | 246.0 | 3 |
| | L2M4 | 240.0 | 210.0 | 210.0 | 222.0 | 234.0 | 4.2 |
| | L2M5 | 192.0 | 204.0 | 222.0 | 216.0 | 234.0 | 4.2 |
| | Avg | 211.2 | 213.6 | 195.6 | 216.0 | 237.6 | |
| | Std | 17.7 | 10.9 | 34.1 | 15.3 | 5.4 | |
| | SEM | 6.7 | 4.1 | 12.9 | 5.8 | 2.0 | |
| Vt (Total) | L2M1 | 0.10413 | 0.12887 | 0.10056 | 0.10595 | 0.17751 | |
| | L2M2 | 0.09204 | 0.07760 | 0.09051 | 0.11864 | 0.12544 | |
| | L2M3 | 0.10523 | 0.07139 | 0.07773 | 0.10562 | 0.16589 | |
| | L2M4 | 0.08452 | 0.09790 | 0.09389 | 0.16507 | 0.12714 | |
| | L2M5 | 0.07440 | 0.08704 | 0.10381 | 0.10513 | 0.14689 | |
| Vt (avg vol/breath) | L2M1 | 0.0029751 | 0.0037903 | 0.0038677 | 0.0033109 | 0.0044378 | |
| (raw) | L2M2 | 0.0027071 | 0.0020421 | 0.0033522 | 0.0030421 | 0.0032164 | |
| | L2M3 | 0.0030066 | 0.0019295 | 0.0020455 | 0.0029339 | 0.0040461 | |
| | L2M4 | 0.0021130 | 0.0027971 | 0.0026826 | 0.0044614 | 0.0032600 | |
| | L2M5 | 0.0023250 | 0.0025600 | 0.0028057 | 0.0029203 | 0.0037664 | |
| Vt (avg vol/breath) | L2M1 | 0.01520 | 0.01937 | 0.01976 | 0.01692 | 0.02268 | |
| (actual) | L2M2 | 0.01383 | 0.01044 | 0.01713 | 0.01554 | 0.01644 | |
| | L2M3 | 0.01536 | 0.00986 | 0.01045 | 0.01499 | 0.02068 | |
| | L2M4 | 0.01080 | 0.01429 | 0.01371 | 0.02280 | 0.01666 | |
| | L2M5 | 0.01188 | 0.01308 | 0.01434 | 0.01492 | 0.01925 | |
| | | | | | | | Weight |
| Vt (avg vol/breath/g) | L2M1 | 0.004628 | 0.005896 | 0.006016 | 0.005150 | 0.006903 | 3.285 |
| (actual/gram) | L2M2 | 0.004912 | 0.003706 | 0.006083 | 0.005520 | 0.005837 | 2.816 |
| | L2M3 | 0.005471 | 0.003511 | 0.003722 | 0.005339 | 0.007363 | 2.808 |
| | L2M4 | 0.003190 | 0.004223 | 0.004050 | 0.006735 | 0.004921 | 3.385 |
| | L2M5 | 0.003618 | 0.003983 | 0.004366 | 0.004544 | 0.005861 | 3.284 |
| | Avg | 0.004364 | 0.004264 | 0.004847 | 0.005458 | 0.006177 | |
| | Std | 0.000940 | 0.000952 | 0.001121 | 0.000803 | 0.000965 | |
| | SEM | 0.000355 | 0.000360 | 0.000424 | 0.000303 | 0.000365 | |
| Minute Ventilation | L2M1 | 0.9719 | 1.2028 | 0.9386 | 0.9889 | 1.6568 | 0.07061133 |
| (mL/min/g) | L2M2 | 1.0021 | 0.8449 | 0.9855 | 1.2917 | 1.3658 | 0.09344441 |
| | L2M3 | 1.1490 | 0.7795 | 0.8487 | 1.1532 | 1.8113 | 0.17000031 |
| | L2M4 | 0.7655 | 0.8867 | 0.8504 | 1.4951 | 1.1516 | 0.07196269 |
| | L2M5 | 0.6946 | 0.8126 | 0.9692 | 0.9815 | 1.3714 | 0.0844317 |
| | Avg | 0.916628 | 0.905307 | 0.918468 | 1.182098 | 1.471364 | |
| | Std | 0.184707 | 0.170960 | 0.065113 | 0.217036 | 0.261413 | |
| | SEM | 0.069813 | 0.064617 | 0.024611 | 0.082032 | 0.098805 | |

**Day 5**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 35.0 | 32.0 | 36.0 | 32.0 | 37.0 | |
| | L2M2 | 35.0 | 33.0 | 40.0 | 43.0 | 44.0 | |
| | L2M3 | 40.0 | 36.0 | 33.0 | 34.0 | 40.0 | |
| | L2M5 | 24.0 | 24.0 | 29.0 | 30.0 | 30.0 | |
| | L2M6 | 31.0 | 31.0 | 32.0 | 33.0 | 38.0 | |
| | L2M7 | 27.0 | 27.0 | 35.0 | 37.0 | 40.0 | |
| Freq (60s) | L2M1 | 144.0 | 150.0 | 144.0 | 150.0 | 129.0 | -2.85 |
| (breaths/min) | L2M2 | 114.0 | 120.0 | 108.0 | 114.0 | 108.0 | -1.5 |
| | L2M3 | 138.0 | 132.0 | 138.0 | 150.0 | 156.0 | 4.2 |
| | L2M5 | 120.0 | 144.0 | 156.0 | 159.0 | 162.0 | 2.85 |
| | L2M6 | 132.0 | 108.0 | 132.0 | 150.0 | 168.0 | 9.9 |
| | L2M7 | 162.0 | 162.0 | 210.0 | 222.0 | 240.0 | 12.3 |
| | Avg | 135.0 | 136.0 | 148.0 | 157.5 | 160.5 | |
| | Std | 17.3 | 20.0 | 34.3 | 35.3 | 45.0 | |
| | SEM | 6.5 | 7.5 | 13.0 | 13.3 | 17.0 | |
| Vt (Total) | L2M1 | 0.08991 | 0.09078 | 0.09064 | 0.08156 | 0.14554 | |
| | L2M2 | 0.09231 | 0.06262 | 0.10354 | 0.08146 | 0.19098 | |
| | L2M3 | 0.11417 | 0.07386 | 0.08628 | 0.11149 | 0.14384 | |
| | L2M5 | 0.05807 | 0.06844 | 0.16876 | 0.18758 | 0.13220 | |
| | L2M6 | 0.06588 | 0.06803 | 0.07100 | 0.08111 | 0.09344 | |
| | L2M7 | 0.04779 | 0.04855 | 0.09995 | 0.08412 | 0.29928 | |
| Vt (avg vol/breath) | L2M1 | 0.0025689 | 0.0028369 | 0.0025178 | 0.0025488 | 0.0039335 | |
| (raw) | L2M2 | 0.0026374 | 0.0018976 | 0.0025885 | 0.0018944 | 0.0043405 | |
| | L2M3 | 0.0028543 | 0.0020517 | 0.0026145 | 0.0032791 | 0.0036960 | |
| | L2M5 | 0.0024196 | 0.0028517 | 0.0058193 | 0.0062527 | 0.0044067 | |
| | L2M6 | 0.0021252 | 0.0021945 | 0.0022188 | 0.0024579 | 0.0024589 | |
| | L2M7 | 0.0017700 | 0.0017981 | 0.0028557 | 0.0022735 | 0.0074820 | |
| Vt (avg vol/breath) | L2M1 | 0.01313 | 0.01450 | 0.01287 | 0.01302 | 0.02010 | |
| (actual) | L2M2 | 0.01348 | 0.00970 | 0.01323 | 0.00968 | 0.02218 | |
| | L2M3 | 0.01459 | 0.01048 | 0.01336 | 0.01676 | 0.01838 | |
| | L2M5 | 0.01236 | 0.01457 | 0.02974 | 0.03195 | 0.02252 | |
| | L2M6 | 0.01086 | 0.01121 | 0.01134 | 0.01256 | 0.01257 | |
| | L2M7 | 0.00904 | 0.00919 | 0.01459 | 0.01162 | 0.03823 | |
| | | | | | | | Weight |
| Vt (avg vol/breath/g) | L2M1 | 0.004805 | 0.005307 | 0.004710 | 0.004768 | 0.007358 | 2.7318 |
| (actual/gram) | L2M2 | 0.004772 | 0.003434 | 0.004684 | 0.003428 | 0.007854 | 2.824 |
| | L2M3 | 0.005005 | 0.003598 | 0.004585 | 0.005750 | 0.006306 | 2.914 |
| | L2M5 | 0.004085 | 0.004814 | 0.009824 | 0.010555 | 0.007439 | 3.027 |
| | L2M6 | 0.003902 | 0.004029 | 0.004074 | 0.004513 | 0.004515 | 2.783 |
| | L2M7 | 0.003053 | 0.003101 | 0.004925 | 0.003921 | 0.012903 | 2.963 |
| | Avg | #DIV/0! | 0.016172 | 0.026799 | 0.010569 | 0.042868 | |
| | Std | #DIV/0! | 0.011900 | 0.023769 | 0.006057 | 0.039914 | |
| | SEM | #DIV/0! | 0.004498 | 0.008984 | 0.002289 | 0.015086 | |
| Minute Ventilation | L2M1 | 0.6919 | 0.7960 | 0.6782 | 0.7151 | 0.9492 | 0.02482502 |
| (mL/min/g) | L2M2 | 0.5441 | 0.4120 | 0.5059 | 0.3908 | 0.8482 | 0.05967569 |
| | L2M3 | 0.6907 | 0.4749 | 0.6327 | 0.8625 | 0.9837 | 0.08781417 |
| | L2M5 | 0.4902 | 0.6932 | 1.5325 | 1.6783 | 1.2051 | 0.08407626 |
| | L2M6 | 0.5151 | 0.4352 | 0.5378 | 0.6770 | 0.7585 | 0.05546022 |
| | L2M7 | 0.4945 | 0.5024 | 1.0342 | 0.8704 | 3.0968 | 0.34878962 |
| | Avg | 0.571078 | 0.552284 | 0.820214 | 0.869594 | 1.306935 | |
| | Std | 0.095081 | 0.155635 | 0.396764 | 0.434557 | 0.889690 | |
| | SEM | 0.035937 | 0.058824 | 0.149963 | 0.164247 | 0.336271 | |

**Day 7**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 30.0 | 33.0 | 29.0 | 32.0 | 36.0 | |
| | L2M2 | 39.0 | 33.0 | 44.0 | 44.0 | 42.0 | |
| | L2M3 | 38.0 | 39.0 | 31.0 | 31.0 | 34.0 | |
| | L2M4 | 40.0 | 44.0 | 40.0 | 42.0 | 43.5 | |
| | L2M5 | 31.0 | 30.0 | 35.0 | 37.0 | 40.0 | |
| Freq (60s) | L2M1 | 180.0 | 198.0 | 174.0 | 192.0 | 216.0 | 3.6 |
| (breaths/min | L2M2 | 234.0 | 198.0 | 264.0 | 264.0 | 252.0 | 8.1 |
| | L2M3 | 228.0 | 234.0 | 186.0 | 186.0 | 204.0 | -4.5 |
| | L2M4 | 240.0 | 264.0 | 240.0 | 252.0 | 261.0 | 0.15 |
| | L2M5 | 186.0 | 180.0 | 210.0 | 222.0 | 240.0 | 9.6 |
| | Avg | 213.6 | 214.8 | 214.8 | 223.2 | 234.6 | |
| | Std | 28.3 | 33.8 | 37.3 | 34.8 | 24.0 | |
| | SEM | 10.7 | 12.8 | 14.1 | 13.2 | 9.1 | |
| Vt (Total) | L2M1 | 0.02233 | 0.17788 | 0.05508 | 0.09510 | 0.12789 | |
| | L2M2 | 0.09366 | 0.10246 | 0.12038 | 0.21015 | 0.23029 | |
| | L2M3 | 0.14865 | 0.10236 | 0.09615 | 0.10036 | 0.21901 | |
| | L2M4 | 0.19260 | 0.13050 | 0.17991 | 0.17428 | 0.13791 | |
| | L2M5 | 0.12036 | 0.08967 | 0.10461 | 0.20114 | 0.24263 | |
| Vt (avg vol/b | L2M1 | 0.0007443 | 0.0053903 | 0.0018993 | 0.0029719 | 0.0035525 | |
| (raw) | L2M2 | 0.0024015 | 0.0031048 | 0.0027359 | 0.0047761 | 0.0054831 | |
| | L2M3 | 0.0039118 | 0.0026246 | 0.0031016 | 0.0032374 | 0.0064415 | |
| | L2M4 | 0.0048150 | 0.0029659 | 0.0044978 | 0.0041495 | 0.0031703 | |
| | L2M5 | 0.0038826 | 0.0029890 | 0.0029889 | 0.0054362 | 0.0060658 | |
| Vt (avg vol/b | L2M1 | 0.00380 | 0.02754 | 0.00971 | 0.01519 | 0.01815 | |
| (actual) | L2M2 | 0.01227 | 0.01587 | 0.01398 | 0.02441 | 0.02802 | |
| | L2M3 | 0.01999 | 0.01341 | 0.01585 | 0.01654 | 0.03292 | |
| | L2M4 | 0.02460 | 0.01516 | 0.02298 | 0.02120 | 0.01620 | |
| | L2M5 | 0.01984 | 0.01527 | 0.01527 | 0.02778 | 0.03100 | |
| | | | | | | | Weight |
| Vt (avg vol/b | L2M1 | 0.000951 | 0.006890 | 0.002428 | 0.003798 | 0.004541 | 3.998 |
| (actual/gram | L2M2 | 0.002976 | 0.003848 | 0.003391 | 0.005919 | 0.006796 | 4.123 |
| | L2M3 | 0.005530 | 0.003710 | 0.004384 | 0.004576 | 0.009105 | 3.615 |
| | L2M4 | 0.005291 | 0.003259 | 0.004943 | 0.004560 | 0.003484 | 4.65 |
| | L2M5 | 0.004733 | 0.003644 | 0.003643 | 0.006627 | 0.007394 | 4.192 |
| | Avg | 0.003896 | 0.004270 | 0.003758 | 0.005096 | 0.006264 | |
| | Std | 0.001926 | 0.001481 | 0.000964 | 0.001147 | 0.002255 | |
| | SEM | 0.000728 | 0.000560 | 0.000364 | 0.000434 | 0.000852 | |
| Minute Ventil | L2M1 | 0.1712 | 1.3641 | 0.4224 | 0.7293 | 0.9808 | -0.0421594 |
| (mL/min/g) | L2M2 | 0.6965 | 0.7619 | 0.8952 | 1.5627 | 1.7125 | 0.17596594 |
| | L2M3 | 1.2607 | 0.8681 | 0.8155 | 0.8512 | 1.8575 | 0.15018735 |
| | L2M4 | 1.2699 | 0.8605 | 1.1862 | 1.1491 | 0.9093 | 0.00547265 |
| | L2M5 | 0.8803 | 0.6558 | 0.7651 | 1.4711 | 1.7746 | 0.20311153 |
| | Avg | 0.855741 | 0.902101 | 0.816884 | 1.152697 | 1.446934 | |
| | Std | 0.455542 | 0.272329 | 0.274253 | 0.367327 | 0.461733 | |
| | SEM | 0.172179 | 0.102931 | 0.103658 | 0.138837 | 0.174519 | |

**Day 7**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 35.0 | 32.0 | 29.0 | 29.0 | 32.0 | |
| | L2M2 | 39.0 | 39.0 | 45.0 | 37.0 | 40.0 | |
| | L2M3 | 43.0 | 40.0 | 40.0 | 42.0 | 43.0 | |
| | L2M5 | 47.0 | 39.0 | 46.0 | 41.0 | 44.0 | |
| | L2M6 | 38.0 | 40.0 | 41.0 | 40.0 | 42.0 | |
| | L2M7 | 34.0 | 33.0 | 32.0 | 42.0 | 40.0 | |
| Freq (60s) | L2M1 | 210.0 | 192.0 | 174.0 | 174.0 | 192.0 | 0 |
| (breaths/min | L2M2 | 234.0 | 234.0 | 270.0 | 222.0 | 240.0 | -1.5 |
| | L2M3 | 258.0 | 240.0 | 240.0 | 252.0 | 258.0 | 3.3 |
| | L2M5 | 282.0 | 234.0 | 276.0 | 246.0 | 264.0 | 3 |
| | L2M6 | 228.0 | 240.0 | 246.0 | 240.0 | 252.0 | 1.5 |
| | L2M7 | 204.0 | 198.0 | 192.0 | 252.0 | 240.0 | 9.3 |
| | Avg | 236.0 | 223.0 | 233.0 | 231.0 | 241.0 | |
| | Std | 29.6 | 21.9 | 41.5 | 30.1 | 25.9 | |
| | SEM | 11.2 | 8.3 | 15.7 | 11.4 | 9.8 | |
| Vt (Total) | L2M1 | 0.09360 | 0.07160 | 0.09039 | 0.11498 | 0.10020 | |
| | L2M2 | 0.13193 | 0.11404 | 0.16387 | 0.12828 | 0.18239 | |
| | L2M3 | 0.14651 | 0.09985 | 0.09827 | 0.08349 | 0.12203 | |
| | L2M5 | 0.11405 | 0.10860 | 0.11425 | 0.12851 | 0.14154 | |
| | L2M6 | 0.15760 | 0.11090 | 0.09652 | 0.11652 | 0.14258 | |
| | L2M7 | 0.09020 | 0.09081 | 0.09817 | 0.19992 | 0.14996 | |
| Vt (avg vol/b | L2M1 | 0.0026743 | 0.0022375 | 0.0031169 | 0.0039648 | 0.0031313 | |
| (raw) | L2M2 | 0.0033828 | 0.0029241 | 0.0036416 | 0.0034670 | 0.0045598 | |
| | L2M3 | 0.0034072 | 0.0024963 | 0.0024568 | 0.0019879 | 0.0028379 | |
| | L2M5 | 0.0024266 | 0.0027846 | 0.0024837 | 0.0031344 | 0.0032168 | |
| | L2M6 | 0.0041474 | 0.0027725 | 0.0023541 | 0.0029130 | 0.0033948 | |
| | L2M7 | 0.0026529 | 0.0027518 | 0.0030678 | 0.0047600 | 0.0037490 | |
| Vt (avg vol/b | L2M1 | 0.01367 | 0.01143 | 0.01593 | 0.02026 | 0.01600 | |
| (actual) | L2M2 | 0.01729 | 0.01494 | 0.01861 | 0.01772 | 0.02330 | |
| | L2M3 | 0.01741 | 0.01276 | 0.01255 | 0.01016 | 0.01450 | |
| | L2M5 | 0.01240 | 0.01423 | 0.01269 | 0.01602 | 0.01644 | |
| | L2M6 | 0.02119 | 0.01417 | 0.01203 | 0.01489 | 0.01735 | |
| | L2M7 | 0.01356 | 0.01406 | 0.01568 | 0.02432 | 0.01916 | |
| | | | | | | | Weight |
| Vt (avg vol/b | L2M1 | 0.003272 | 0.002738 | 0.003814 | 0.004852 | 0.003832 | 4.176 |
| (actual/gram | L2M2 | 0.004495 | 0.003885 | 0.004838 | 0.004606 | 0.006058 | 3.846 |
| | L2M3 | 0.004299 | 0.003150 | 0.003100 | 0.002508 | 0.003581 | 4.05 |
| | L2M5 | 0.002948 | 0.003383 | 0.003018 | 0.003808 | 0.003908 | 4.206 |
| | L2M6 | 0.005505 | 0.003680 | 0.003125 | 0.003866 | 0.004506 | 3.85 |
| | L2M7 | 0.003188 | 0.003306 | 0.003686 | 0.005719 | 0.004504 | 4.253 |
| | Avg | #DIV/0! | 0.016172 | 0.026799 | 0.010569 | 0.042868 | |
| | Std | #DIV/0! | 0.011900 | 0.023769 | 0.006057 | 0.039914 | |
| | SEM | #DIV/0! | 0.004498 | 0.008984 | 0.002289 | 0.015086 | |
| Minute Venti | L2M1 | 0.6872 | 0.5257 | 0.6636 | 0.8442 | 0.7357 | 0.04052392 |
| (mL/min/g) | L2M2 | 1.0517 | 0.9091 | 1.3064 | 1.0226 | 1.4540 | 0.06754607 |
| | L2M3 | 1.1091 | 0.7559 | 0.7439 | 0.6321 | 0.9238 | 0.01959212 |
| | L2M5 | 0.8314 | 0.7916 | 0.8328 | 0.9368 | 1.0318 | 0.04121532 |
| | L2M6 | 1.2551 | 0.8832 | 0.7687 | 0.9279 | 1.1355 | 0.04580684 |
| | L2M7 | 0.6503 | 0.6547 | 0.7077 | 1.4412 | 1.0811 | 0.10063804 |
| | Avg | 0.930796 | 0.753362 | 0.837189 | 0.967467 | 1.060294 | |
| | Std | 0.244748 | 0.144335 | 0.236819 | 0.267471 | 0.239127 | |
| | SEM | 0.092506 | 0.054553 | 0.089509 | 0.101095 | 0.090382 | |

**Day 9**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 48.0 | 49.0 | 48.0 | 51.0 | 52.0 | |
| | L2M2 | 36.5 | 45.0 | 44.0 | 45.0 | 44.0 | |
| | L2M3 | 43.0 | 38.0 | 38.0 | 39.0 | 42.0 | |
| | L2M4 | 46.0 | 46.0 | 46.5 | 41.0 | 38.0 | |
| | L2M5 | 34.0 | 56.0 | 34.0 | 40.0 | 44.0 | |
| Freq (60s) | L2M1 | 288.0 | 294.0 | 288.0 | 306.0 | 312.0 | 3.6 |
| (breaths/min | L2M2 | 219.0 | 270.0 | 264.0 | 270.0 | 264.0 | -0.6 |
| | L2M3 | 258.0 | 228.0 | 228.0 | 234.0 | 252.0 | 3.9 |
| | L2M4 | 276.0 | 276.0 | 279.0 | 246.0 | 228.0 | 8.85 |
| | L2M5 | 204.0 | 336.0 | 204.0 | 240.0 | 264.0 | -9 |
| | Avg | 249.0 | 280.8 | 252.6 | 259.2 | 264.0 | |
| | Std | 36.2 | 39.2 | 35.5 | 29.5 | 30.6 | |
| | SEM | 13.7 | 14.8 | 13.4 | 11.2 | 11.6 | |
| Vt (Total) | L2M1 | 0.12869 | 0.17783 | 0.13425 | 0.12886 | 0.31359 | |
| | L2M2 | 0.12639 | 0.15231 | 0.16873 | 0.15187 | 0.34077 | |
| | L2M3 | 0.13601 | 0.15343 | 0.15363 | 0.19380 | 0.25444 | |
| | L2M4 | 0.20557 | 0.18391 | 0.16969 | 0.30430 | 0.33943 | |
| | L2M5 | 0.09370 | 0.07891 | 0.13695 | 0.21016 | 0.24989 | |
| Vt (avg vol/b | L2M1 | 0.0026810 | 0.0036292 | 0.0027969 | 0.0025267 | 0.0060306 | |
| (raw) | L2M2 | 0.0034627 | 0.0033847 | 0.0038348 | 0.0033749 | 0.0077448 | |
| | L2M3 | 0.0031630 | 0.0040376 | 0.0040429 | 0.0049692 | 0.0060581 | |
| | L2M4 | 0.0044689 | 0.0039980 | 0.0036492 | 0.0074220 | 0.0089324 | |
| | L2M5 | 0.0027559 | 0.0014091 | 0.0040279 | 0.0052540 | 0.0056793 | |
| Vt (avg vol/b | L2M1 | 0.01370 | 0.01855 | 0.01429 | 0.01291 | 0.03082 | |
| (actual) | L2M2 | 0.01769 | 0.01730 | 0.01960 | 0.01725 | 0.03958 | |
| | L2M3 | 0.01616 | 0.02063 | 0.02066 | 0.02539 | 0.03096 | |
| | L2M4 | 0.02284 | 0.02043 | 0.01865 | 0.03793 | 0.04564 | |
| | L2M5 | 0.01408 | 0.00720 | 0.02058 | 0.02685 | 0.02902 | |
| | | | | | | | Weight |
| Vt (avg vol/b | L2M1 | 0.000333 | 0.000450 | 0.000347 | 0.000313 | 0.000748 | 41.2 |
| (actual/gram | L2M2 | 0.003525 | 0.003445 | 0.003904 | 0.003435 | 0.007884 | 5.02 |
| | L2M3 | 0.003396 | 0.004335 | 0.004341 | 0.005336 | 0.006505 | 4.759 |
| | L2M4 | 0.003965 | 0.003547 | 0.003237 | 0.006584 | 0.007924 | 5.76 |
| | L2M5 | 0.002712 | 0.001387 | 0.003964 | 0.005170 | 0.005589 | 5.193 |
| | Avg | 0.002786 | 0.002633 | 0.003158 | 0.004168 | 0.005730 | |
| | Std | 0.001443 | 0.001636 | 0.001621 | 0.002429 | 0.002954 | |
| | SEM | 0.000546 | 0.000619 | 0.000613 | 0.000918 | 0.001116 | |
| Minute Ventil | L2M1 | 0.0958 | 0.1323 | 0.0999 | 0.0959 | 0.2334 | 0.01495382 |
| (mL/min/g) | L2M2 | 0.7719 | 0.9302 | 1.0305 | 0.9276 | 2.0813 | 0.16750621 |
| | L2M3 | 0.8762 | 0.9885 | 0.9898 | 1.2486 | 1.6392 | 0.11055381 |
| | L2M4 | 1.0942 | 0.9789 | 0.9032 | 1.6198 | 1.8068 | 0.15999889 |
| | L2M5 | 0.5532 | 0.4659 | 0.8086 | 1.2408 | 1.4754 | 0.17303446 |
| | Avg | 0.678280 | 0.699178 | 0.766403 | 1.026517 | 1.447203 | |
| | Std | 0.379454 | 0.384409 | 0.382223 | 0.575112 | 0.714563 | |
| | SEM | 0.143420 | 0.145293 | 0.144467 | 0.217372 | 0.270079 | |

**Day 9**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 36.0 | 38.0 | 37.0 | 45.0 | 44.0 | |
| | L2M2 | 38.0 | 35.0 | 39.0 | 49.0 | 47.0 | |
| | L2M3 | 39.0 | 36.0 | 42.0 | 46.0 | 39.0 | |
| | L2M5 | 45.0 | 44.0 | 38.0 | 40.0 | 37.0 | |
| | L2M6 | 40.0 | 35.0 | 40.0 | 33.0 | 38.0 | |
| | L2M7 | 44.0 | 43.0 | 43.0 | 48.0 | 45.0 | |
| Freq (60s) | L2M1 | 216.0 | 228.0 | 222.0 | 270.0 | 264.0 | 7.8 |
| (breaths/min | L2M2 | 228.0 | 210.0 | 234.0 | 294.0 | 282.0 | 13.8 |
| | L2M3 | 234.0 | 216.0 | 252.0 | 276.0 | 234.0 | 3.9 |
| | L2M5 | 270.0 | 264.0 | 228.0 | 240.0 | 222.0 | -5.7 |
| | L2M6 | 240.0 | 210.0 | 240.0 | 198.0 | 228.0 | 0.6 |
| | L2M7 | 264.0 | 258.0 | 258.0 | 288.0 | 270.0 | 3.3 |
| | Avg | **242.0** | **231.0** | **239.0** | **261.0** | **250.0** | |
| | Std | **21.0** | **24.2** | **13.9** | **36.1** | **25.1** | |
| | SEM | **7.9** | **9.2** | **5.3** | **13.7** | **9.5** | |
| Vt (Total) | L2M1 | 0.18747 | 0.14806 | 0.19834 | 0.24765 | 0.21637 | |
| | L2M2 | 0.21310 | 0.13406 | 0.10105 | 0.25536 | 0.22479 | |
| | L2M3 | 0.19176 | 0.12456 | 0.14329 | 0.20987 | 0.19870 | |
| | L2M5 | 0.11405 | 0.19377 | 0.20199 | 0.20614 | 0.17013 | |
| | L2M6 | 0.19438 | 0.17542 | 0.32951 | 0.19536 | 0.21920 | |
| | L2M7 | 0.18859 | 0.18151 | 0.20228 | 0.27595 | 0.32454 | |
| Vt (avg vol/b | L2M1 | 0.0052075 | 0.0038963 | 0.0053605 | 0.0055033 | 0.0049175 | |
| (raw) | L2M2 | 0.0056079 | 0.0038303 | 0.0025910 | 0.0052114 | 0.0047828 | |
| | L2M3 | 0.0049169 | 0.0034600 | 0.0034117 | 0.0045624 | 0.0050949 | |
| | L2M5 | 0.0025344 | 0.0044039 | 0.0053155 | 0.0051535 | 0.0045981 | |
| | L2M6 | 0.0048595 | 0.0050120 | 0.0082378 | 0.0059200 | 0.0057684 | |
| | L2M7 | 0.0042861 | 0.0042212 | 0.0047043 | 0.0057490 | 0.0072120 | |
| Vt (avg vol/b | L2M1 | 0.02661 | 0.01991 | 0.02739 | 0.02812 | 0.02513 | |
| (actual) | L2M2 | 0.02866 | 0.01957 | 0.01324 | 0.02663 | 0.02444 | |
| | L2M3 | 0.02513 | 0.01768 | 0.01743 | 0.02331 | 0.02603 | |
| | L2M5 | 0.01295 | 0.02250 | 0.02716 | 0.02633 | 0.02350 | |
| | L2M6 | 0.02483 | 0.02561 | 0.04209 | 0.03025 | 0.02948 | |
| | L2M7 | 0.02190 | 0.02157 | 0.02404 | 0.02938 | 0.03685 | |
| | | | | | | | Weight |
| Vt (avg vol/b | L2M1 | 0.005212 | 0.003899 | 0.005365 | 0.005508 | 0.004921 | 5.106 |
| (actual/gram | L2M2 | 0.005652 | 0.003861 | 0.002611 | 0.005253 | 0.004820 | 5.07 |
| | L2M3 | 0.004703 | 0.003310 | 0.003263 | 0.004364 | 0.004874 | 5.342 |
| | L2M5 | 0.002450 | 0.004256 | 0.005138 | 0.004981 | 0.004444 | 5.287 |
| | L2M6 | 0.005093 | 0.005253 | 0.008633 | 0.006204 | 0.006045 | 4.876 |
| | L2M7 | 0.004051 | 0.003989 | 0.004446 | 0.005433 | 0.006816 | 5.407 |
| | Avg | **#DIV/0!** | **0.016172** | **0.026799** | **0.010569** | **0.042868** | |
| | Std | **#DIV/0!** | **0.011900** | **0.023769** | **0.006057** | **0.039914** | |
| | SEM | **#DIV/0!** | **0.004498** | **0.008984** | **0.002289** | **0.015086** | |
| Minute Ventil | L2M1 | 1.1257 | 0.8891 | 1.1910 | 1.4871 | 1.2992 | 0.07633175 |
| (mL/min/g) | L2M2 | 1.2887 | 0.8107 | 0.6111 | 1.5442 | 1.3594 | 0.12895947 |
| | L2M3 | 1.1006 | 0.7149 | 0.8224 | 1.2045 | 1.1404 | 0.08293467 |
| | L2M5 | 0.6614 | 1.1237 | 1.1714 | 1.1954 | 0.9866 | -0.0193604 |
| | L2M6 | 1.2222 | 1.1030 | 2.0719 | 1.2284 | 1.3783 | -0.0008835 |
| | L2M7 | 1.0694 | 1.0292 | 1.1470 | 1.5648 | 1.8403 | 0.14254207 |
| | Avg | **1.078001** | **0.945105** | **1.169133** | **1.370741** | **1.334041** | |
| | Std | **0.219827** | **0.166200** | **0.499534** | **0.178825** | **0.289237** | |
| | SEM | **0.083087** | **0.062818** | **0.188806** | **0.067590** | **0.109321** | |

**Day 11**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 46.0 | 40.0 | 48.0 | 49.0 | 54.0 | |
| | L2M2 | 42.5 | 43.0 | 39.5 | 47.0 | 53.0 | |
| | L2M3 | 43.5 | 42.5 | 42.0 | 43.0 | 45.0 | |
| | L2M4 | 50.0 | 50.5 | 46.0 | 47.0 | 56.5 | |
| | L2M5 | 45.0 | 51.0 | 51.0 | 47.5 | 52.0 | |
| Freq (60s) | L2M1 | 276.0 | 240.0 | 288.0 | 294.0 | 324.0 | 12.9 |
| (breaths/min | L2M2 | 255.0 | 258.0 | 237.0 | 282.0 | 318.0 | 11.25 |
| | L2M3 | 261.0 | 255.0 | 252.0 | 258.0 | 270.0 | 2.55 |
| | L2M4 | 300.0 | 303.0 | 276.0 | 282.0 | 339.0 | 5.7 |
| | L2M5 | 270.0 | 306.0 | 306.0 | 285.0 | 312.0 | -0.15 |
| | Avg | 213.6 | 214.8 | 214.8 | 223.2 | 234.6 | |
| | Std | 28.3 | 33.8 | 37.3 | 34.8 | 24.0 | |
| | SEM | 10.7 | 12.8 | 14.1 | 13.2 | 9.1 | |
| Vt (Total) | L2M1 | 0.18383 | 0.18929 | 0.19291 | 0.24217 | 0.34066 | |
| | L2M2 | 0.14137 | 0.13840 | 0.15818 | 0.28377 | 0.36587 | |
| | L2M3 | 0.16452 | 0.14382 | 0.14080 | 0.22259 | 0.37037 | |
| | L2M4 | 0.18043 | 0.18588 | 0.16962 | 0.15065 | 0.18321 | |
| | L2M5 | 0.16725 | 0.17102 | 0.14940 | 0.21121 | 0.27195 | |
| Vt (avg vol/b | L2M1 | 0.0007443 | 0.0053903 | 0.0018993 | 0.0029719 | 0.0035525 | |
| (raw) | L2M2 | 0.0024015 | 0.0031048 | 0.0027359 | 0.0047761 | 0.0054831 | |
| | L2M3 | 0.0039118 | 0.0026246 | 0.0031016 | 0.0032374 | 0.0064415 | |
| | L2M4 | 0.0048150 | 0.0029659 | 0.0044978 | 0.0041495 | 0.0031703 | |
| | L2M5 | 0.0038826 | 0.0029890 | 0.0029889 | 0.0054362 | 0.0060658 | |
| Vt (avg vol/b | L2M1 | 0.00380 | 0.02754 | 0.00971 | 0.01519 | 0.01815 | |
| (actual) | L2M2 | 0.01227 | 0.01587 | 0.01398 | 0.02441 | 0.02802 | |
| | L2M3 | 0.01999 | 0.01341 | 0.01585 | 0.01654 | 0.03292 | |
| | L2M4 | 0.02460 | 0.01516 | 0.02298 | 0.02120 | 0.01620 | |
| | L2M5 | 0.01984 | 0.01527 | 0.01527 | 0.02778 | 0.03100 | |
| | | | | | | | Weight |
| Vt (avg vol/b | L2M1 | 0.000951 | 0.006890 | 0.002428 | 0.003798 | 0.004541 | 5.65 |
| (actual/gram | L2M2 | 0.002976 | 0.003848 | 0.003391 | 0.005919 | 0.006796 | 6.194 |
| | L2M3 | 0.005530 | 0.003710 | 0.004384 | 0.004576 | 0.009105 | 6.54 |
| | L2M4 | 0.005291 | 0.003259 | 0.004943 | 0.004560 | 0.003484 | 6.56 |
| | L2M5 | 0.004733 | 0.003644 | 0.003643 | 0.006627 | 0.007394 | 6.25 |
| | Avg | 0.003896 | 0.004270 | 0.003758 | 0.005096 | 0.006264 | |
| | Std | 0.001926 | 0.001481 | 0.000964 | 0.001147 | 0.002255 | |
| | SEM | 0.000728 | 0.000560 | 0.000364 | 0.000434 | 0.000852 | |
| Minute Ventil | L2M1 | 0.1712 | 1.3641 | 0.4224 | 0.7293 | 0.9808 | -0.04215 |
| (mL/min/g) | L2M2 | 0.6965 | 0.7619 | 0.8952 | 1.5627 | 1.7125 | 0.175965 |
| | L2M3 | 1.2607 | 0.8681 | 0.8155 | 0.8512 | 1.8575 | 0.150195 |
| | L2M4 | 1.2699 | 0.8605 | 1.1862 | 1.1491 | 0.9093 | 0.005465 |
| | L2M5 | 0.8803 | 0.6558 | 0.7651 | 1.4711 | 1.7746 | 0.20312 |
| | Avg | 0.855741 | 0.902101 | 0.816884 | 1.152697 | 1.446934 | |
| | Std | 0.455542 | 0.272329 | 0.274253 | 0.367327 | 0.461733 | |
| | SEM | 0.172179 | 0.102931 | 0.103658 | 0.138837 | 0.174519 | |

**Day 11**

**Measurement of last 10 sec**

| | Mouse | Air | 0 | 2 | 4 | 6 | |
|---|---|---|---|---|---|---|---|
| Freq (10s) | L2M1 | 46.0 | 40.0 | 46.0 | 48.0 | 52.0 | |
| | L2M2 | 50.0 | 53.0 | 45.0 | 52.0 | 40.0 | |
| | L2M3 | 52.0 | 39.0 | 42.0 | 44.0 | 44.0 | |
| | L2M5 | 48.0 | 47.0 | 49.0 | 40.0 | 52.0 | |
| | L2M6 | 42.0 | 40.0 | 45.0 | 47.0 | 55.0 | |
| | L2M7 | 54.0 | 45.0 | 42.5 | 48.0 | 47.0 | |
| Freq (60s) | L2M1 | 276.0 | 240.0 | 276.0 | 288.0 | 312.0 | 11.4 |
| (breaths/min | L2M2 | 300.0 | 318.0 | 270.0 | 312.0 | 240.0 | -9.6 |
| | L2M3 | 312.0 | 234.0 | 252.0 | 264.0 | 264.0 | 5.1 |
| | L2M5 | 288.0 | 282.0 | 294.0 | 240.0 | 312.0 | 1.8 |
| | L2M6 | 252.0 | 240.0 | 270.0 | 282.0 | 330.0 | 14.1 |
| | L2M7 | 324.0 | 270.0 | 255.0 | 288.0 | 282.0 | 3.45 |
| | Avg | 292.0 | 264.0 | 269.5 | 279.0 | 290.0 | |
| | Std | 25.9 | 32.6 | 15.2 | 24.5 | 34.1 | |
| | SEM | 9.8 | 12.3 | 5.8 | 9.3 | 12.9 | |
| Vt (Total) | L2M1 | 0.20546 | 0.18250 | 0.12128 | 0.13156 | 0.29309 | |
| | L2M2 | 0.19530 | 0.27771 | 0.23562 | 0.44202 | 0.15799 | |
| | L2M3 | 0.24162 | 0.19325 | 0.17620 | 0.19972 | 0.27523 | |
| | L2M5 | 0.17460 | 0.22734 | 0.19929 | 0.22697 | 0.42850 | |
| | L2M6 | 0.20565 | 0.18093 | 0.23082 | 0.27985 | 0.33147 | |
| | L2M7 | 0.20104 | 0.14403 | 0.23940 | 0.29757 | 0.24903 | |
| Vt (avg vol/b | L2M1 | 0.0044665 | 0.0045625 | 0.0026365 | 0.0027408 | 0.0056363 | |
| (raw) | L2M2 | 0.0039060 | 0.0052398 | 0.0052360 | 0.0085004 | 0.0039498 | |
| | L2M3 | 0.0046465 | 0.0049551 | 0.0041952 | 0.0045391 | 0.0062552 | |
| | L2M5 | 0.0036375 | 0.0048370 | 0.0040671 | 0.0056743 | 0.0082404 | |
| | L2M6 | 0.0048964 | 0.0045233 | 0.0051293 | 0.0059543 | 0.0060267 | |
| | L2M7 | 0.0037230 | 0.0032007 | 0.0056329 | 0.0061994 | 0.0052985 | |
| Vt (avg vol/b | L2M1 | 0.02282 | 0.02331 | 0.01347 | 0.01401 | 0.02880 | |
| (actual) | L2M2 | 0.01996 | 0.02678 | 0.02676 | 0.04344 | 0.02018 | |
| | L2M3 | 0.02374 | 0.02532 | 0.02144 | 0.02319 | 0.03196 | |
| | L2M5 | 0.01859 | 0.02472 | 0.02078 | 0.02900 | 0.04211 | |
| | L2M6 | 0.02502 | 0.02311 | 0.02621 | 0.03043 | 0.03080 | |
| | L2M7 | 0.01902 | 0.01636 | 0.02878 | 0.03168 | 0.02708 | |
| | | | | | | | Weight |
| Vt (avg vol/b | L2M1 | 0.004470 | 0.004566 | 0.002639 | 0.002743 | 0.005641 | 5.106 |
| (actual/gram | L2M2 | 0.003937 | 0.005281 | 0.005277 | 0.008567 | 0.003981 | 5.07 |
| | L2M3 | 0.004445 | 0.004740 | 0.004013 | 0.004342 | 0.005984 | 5.342 |
| | L2M5 | 0.003516 | 0.004675 | 0.003931 | 0.005484 | 0.007965 | 5.287 |
| | L2M6 | 0.005131 | 0.004740 | 0.005375 | 0.006240 | 0.006316 | 4.876 |
| | L2M7 | 0.003518 | 0.003025 | 0.005324 | 0.005859 | 0.005007 | 5.407 |
| | Avg | #DIV/0! | 0.016172 | 0.026799 | 0.010569 | 0.042868 | |
| | Std | #DIV/0! | 0.011900 | 0.023769 | 0.006057 | 0.039914 | |
| | SEM | #DIV/0! | 0.004498 | 0.008984 | 0.002289 | 0.015086 | |
| Minute Ventil | L2M1 | 1.2337 | 1.0959 | 0.7283 | 0.7900 | 1.7599 | 0.10269539 |
| (mL/min/g) | L2M2 | 1.1810 | 1.6794 | 1.4249 | 2.6730 | 0.9554 | -0.0461896 |
| | L2M3 | 1.3868 | 1.1091 | 1.0113 | 1.1463 | 1.5797 | 0.07732725 |
| | L2M5 | 1.0125 | 1.3184 | 1.1557 | 1.3162 | 2.4849 | 0.18300894 |
| | L2M6 | 1.2931 | 1.1377 | 1.4514 | 1.7597 | 2.0843 | 0.15740288 |
| | L2M7 | 1.1400 | 0.8167 | 1.3575 | 1.6873 | 1.4121 | 0.05746978 |
| | Avg | 1.207859 | 1.192862 | 1.188167 | 1.562093 | 1.712716 | |
| | Std | 0.129169 | 0.287552 | 0.282082 | 0.650915 | 0.532570 | |
| | SEM | 0.048821 | 0.108685 | 0.106617 | 0.246023 | 0.201293 | |

Appendix E. Minute ventilation of litters continuously exposed to nicotine.

| | Day 1 | Day 3 | Day 5 | Day 7 | Day 9 | Day 11 |
|---|---|---|---|---|---|---|
| L1M1 | 0.2423 | 0.1084 | 0.1482 | 0.1234 | 0.2210 | 0.0697 |
| L1M2 | 0.1165 | 0.0144 | 0.1809 | -0.2098 | 0.1868 | 0.1530 |
| L1M3 | 0.1415 | 0.0319 | 0.0675 | 0.1510 | 0.1971 | 0.1013 |
| L1M4 | 0.0472 | 0.1442 | -0.0438 | 0.1747 | 0.2352 | 0.2611 |
| L1M5 | 0.2156 | 0.3656 | 0.2412 | 0.2161 | 0.2682 | 0.2417 |
| L4M1 | 0.3369 | -0.0256 | -0.1278 | 0.0750 | -0.2001 | 0.0052 |
| L4M2 | 0.3750 | 0.0954 | -0.1448 | 0.0606 | -0.1248 | 0.0216 |
| L4M3 | 0.4278 | 0.0405 | -0.0727 | 0.0758 | 0.0005 | 0.0099 |
| L4M4 | -0.0874 | 0.0469 | -0.0156 | 0.6038 | -0.2191 | 0.0268 |
| L4M5 | 0.0423 | 0.1109 | -0.1065 | -0.0585 | -0.0835 | 0.0153 |
| L4M6 | 0.5176 | 0.0540 | -0.0990 | 0.1280 | -0.0965 | 0.0119 |
| L4M7 | 0.2014 | 0.0882 | 0.0178 | 0.0668 | -0.1837 | 0.0185 |

Appendix F. Minute ventilation of litters withdrawn from nicotine.

| | Day 1 | Day 3 | Day 5 | Day 7 | Day 9 | Day 11 |
|---|---|---|---|---|---|---|
| L2M1 | -0.2131 | 0.0172 | 0.0706 | -0.0422 | 0.0150 | -0.0422 |
| L2M2 | -0.4242 | 0.1936 | 0.0934 | 0.1760 | 0.1675 | 0.1760 |
| L2M3 | 0.2728 | 0.1481 | 0.1700 | 0.1502 | 0.1106 | 0.1502 |
| L2M4 | 0.0558 | 0.0534 | 0.0720 | 0.0055 | 0.1600 | 0.0055 |
| L2M5 | 0.1200 | 0.0749 | 0.0844 | 0.2031 | 0.1730 | 0.2031 |
| L3M1 | 0.0777 | 0.1177 | 0.0248 | 0.0405 | 0.0763 | 0.1027 |
| L3M2 | 0.2940 | 0.0326 | 0.0597 | 0.0675 | 0.1290 | -0.0462 |
| L3M3 | 0.1305 | 0.0428 | 0.0878 | 0.0196 | 0.0829 | 0.0773 |
| L3M5 | 0.1956 | 0.1008 | 0.0841 | 0.0412 | -0.0194 | 0.1830 |
| L3M6 | 0.1922 | 0.2229 | 0.0555 | 0.0458 | -0.0009 | 0.1574 |
| L3M7 | 0.1552 | 0.1168 | 0.3488 | 0.1006 | 0.1425 | 0.0575 |