Reviewer's report

Title: Age-dependent differences in lung ventilation impact influenza-induced tachypnea in the cotton rat

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Reviewer: Fadi Xu

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Major Comments

1. Significance
The authors compares the influenza-induced tachypnea in the infant and adults rats, and found an age-dependent hyperventilation characterized by increasing respiratory rate without change in VT in the infant, which is interesting and clinically relevant.

2. Accuracy of title and abstract
The title “Age-dependent differences in lung ventilation impact influenza-induced tachypnea in the cotton rat” should be modified. Logically, it is difficulty to understand how age-related difference in ventilation is causative to developing the influenza-induced tachypnea. It is possible that this difference of influenza-induced tachypnea is somewhat related to the development of the nerves involved in control of breathing. The abstract is not clearly written. Although both preparations were used, the authors only present the data obtained from awake rats using whole body flow plethysmograph, but not those from mechanically-ventilated anesthetized rats. In addition, the time-frame of the viral infection and ventilatory measurements is not clear, so that “transient increase in respiratory rate and enhanced pause (Penh)” appears confusing.

3. Clarity of hypothesis and rationale
The hypothesis is not well addressed.

4. Adequacy of experimental design and methods
P5 –line 6: Ventilation can be substantially affected by body temperature. However, the description how the animal body temperature was measured and maintained at 37.5°C is missing.

P6- Statistical methods: Two-way ANOVA seems to be required for comparison of data obtained from the four groups, i.e., 2 age-groups with and without viral infection, while t-test used in the present study is not sufficient (also see the result section).

P7-parag2: Please clarify the animals groups and rats in each group. The statement that “arithmetic means of each parameter measured in groups of 4 –
10 cotton rats were calculated" is unclear.

5. Quality of data and presentation of results
The results are poorly presented and addition of statistical analysis required (see below).

Table 1: The ventilatory data in control animals should be presented in the table although there were no significant changes after inoculation with an equivalent volume of a 'mock' virus preparation. Compared to the control (day “0”), viral infection did not change VT in infant, but reduced VT in adult, and increased MV in the infant but not in the adult. These data suggest that respiratory frequency is greatly affected by the infection in the infant, but the data of respiratory frequency are missing.

Fig 3: Penh data alone are not sufficient to determine the airway resistance (see Discussion section).

6. Length and appropriateness of discussion
The discussion is generally fine, but there are some concerns described below.

P 9-11: The changes in mechanic capacity of the lung and airway observed in the present study may be related to the ventilatory difference noted in the infant and adult rats before and after viral infection. However, an involvement of development in central respiratory drive may also be responsible for the age-dependent difference. This concern should be discussed.

P11: Although Penh is a tool to measure airway resistance without interference to airway in conscious animals, its reliability has been arguable. Thus, additional supportive data, such as dynamic elastance, resistance, and pleural pressure measured in anesthetized preparation should be included. In fact, these measurements have been performed in this study, but unfortunately, the relevant data were not consistently presented.

P11-12: The authors found that when infant and adult animals were infected with the equivalent virus dose, tachypnea lasted longer, but clearance of virus was faster in the latter than the former. The adequate explanation is lacking.

7. References cited
Recent studies reported that RSV infection altered ventilation and ventilatory response to stimulation of pulmonary C-fibers in both adult and weanling rats and this alteration was much stronger in the latter than the former (Sabogal C, et al., Pediatr Res 57: 819-825, 2005; Pong W, et al., 102: 2201-2206, 2007, JAP). These papers should be cited and discussed.

Minor Comments
Table 1: Some information presented in Table 1 appears confusing. For example, it is hard to understand the superscripts (1 and 2) in the table title and (1-3) in the columns. What is the difference between 3* and *3?
Fig 1: where are the data of MV for time course of post-infection? The time (day or adult) makers are missing in the panels at the bottom. Two way ANOVA seems to be required to analyze these data, which is the same for the following figures.

Fig 2: Do the infant data include age 14, 18, 21 and 28 day? This should be defined in the data analysis section.

Fig 5: Does panel A come from the adult or infant (at which age?)? What is the age of rat used in panel B?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests' below.