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Dose-controlled aerosol-lung delivery and standardized airway hyperresponsiveness (AH) measurements in mice

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Introduction

Airway hyperresponsiveness (AH) often represents a primary outcome in respiratory studies. The measurement protocol involves aerosolized delivery of a broncho-constrictor (methacholine, MCH). It has been shown that the delivery protocol (respiratory and nebulizer para-meters) affects the measured AH making it difficult to compare different studies (Robichaud, 2015).

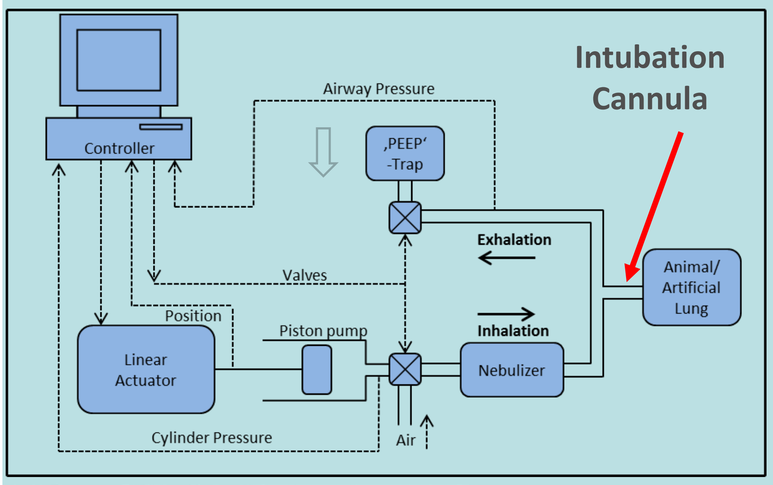
Hypothesis

Standardized AH measurements are possible, if the inhaled instead of the nominal MCH dose is used.

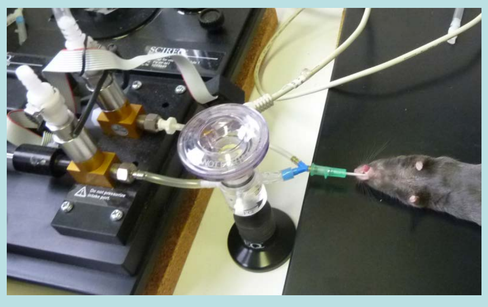
Devices and Methods

- Aerosolized delivery of saline or MCH solutions with a flexivent system (Legacy, EMKA/Scireq) for lung function measurements in mice
- Inhaled aerosol dose was determined gravimetrically for various delivery protocols (different nebulizers, nebulizer settings, respiratory parameters; n=3-5 each)
- AH measurements in healthy wild-type C57BL/6 mice

Flexivent system for aerosol (MCH) delivery to intubated mice lungs



Aeroneb nebulizer
(Aerogen Inc., Ireland)

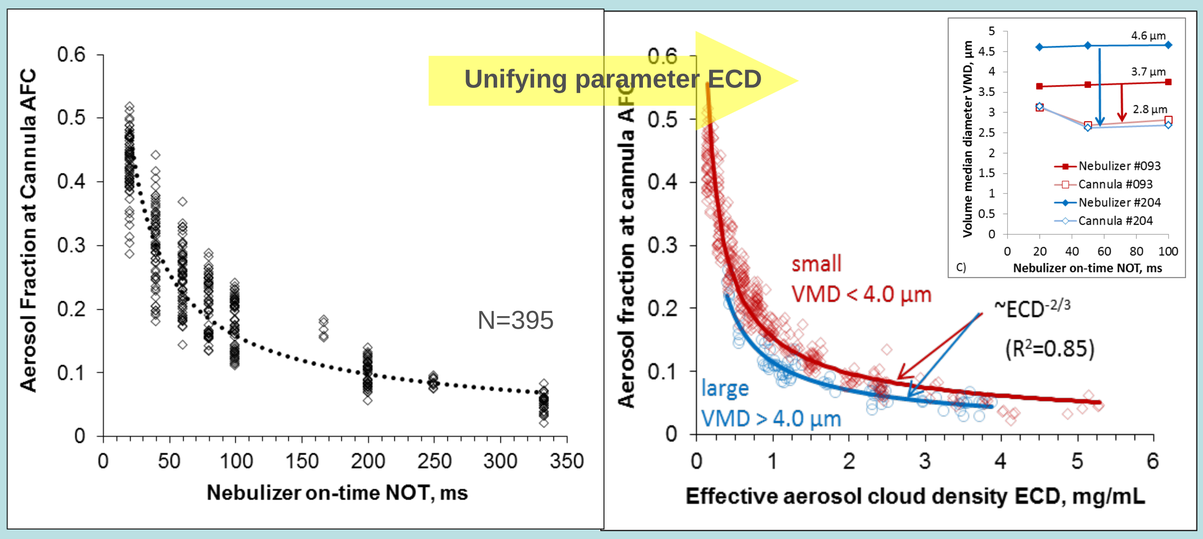


Aeroneb nebulizers and their droplet size
VMD = Volume Median Diameter
Large droplets, Pro, VMD = 4.0 µm-6.0 µm (#204)
Small droplets, Lab, VMD = 3.5 µm-4.0 µm (#093)

MCH delivery protocols

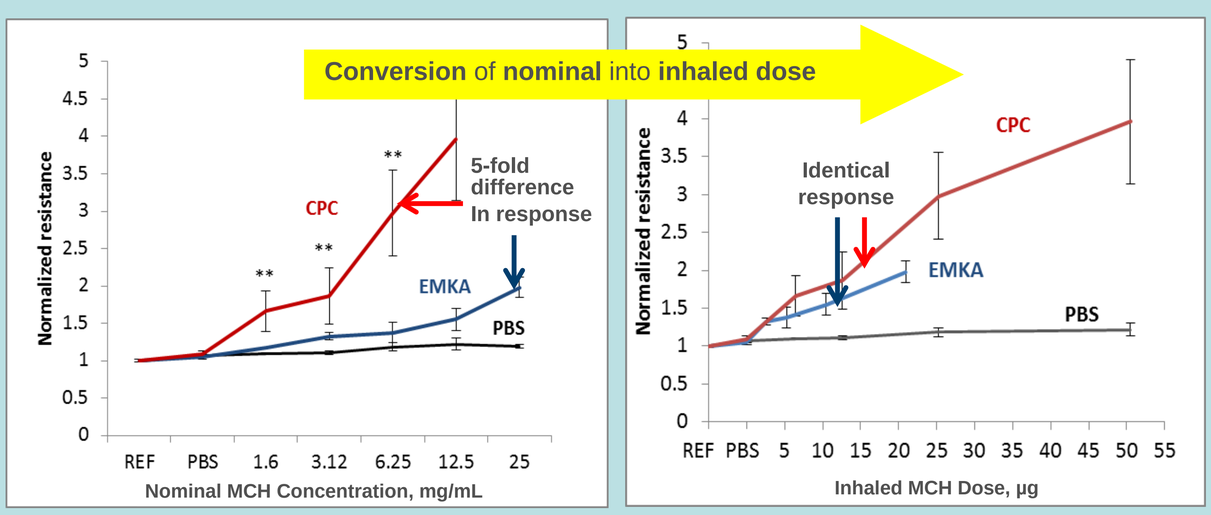
	EMKA (standard)	CPC (optimized)
Respiratory parameters		
Respiratory frequency (bpm)	150	120
Tidal volume (µL)	200	400
Ratio of inhalation/exhalation time	2:3,	2:1
Duty cycle/On-Time of nebulizer per breath	50%/80ms	6%/20ms
Nebulized volume (µL)	10	10
Nebulizer		
Type/ID of Aeroneb vibrating mesh nebulizer	Pro/#204	Lab/#093
Continuous output rate (µL/min)	500	250
Nebulization time per animal (s)	10	70
Mass median droplet diameter (µm)	5.07	3.94
Volume of MCH solution at cannula (µL) (for 10µl of nebulized MCH solution)	**0.84±0.07**	**4.04±0.10**
Dose delivery efficiency (% of invested dose)	**(8.4±0.7)%**	**(40.4±1.0)%**

Results



Delivery Protocol (defined):
- nebulizer parameters (output rate, droplet diameter, duty cycle)
- respiratory parameters (tidal volume V_T, respiratory frequency, inhalation to exhalation ratio).

merged into → **Effective Aerosol Cloud Density (ECD)** → **prediction of** → **AFC** Aerosol Fraction at the Cannula = **inhaled aerosol dose** **AFC ~ ECD $^{-2/3}$**



AH (airway resistance) data of healthy mice for two delivery protocols (EMKA, standard; CPC, optimized).
Only inhaled dose (not nominal dose) provides consistent AH data independent of MCH challenge protocol.

Conclusions / Summary

- The novel characteristic device parameter, **ECD** (= **Effective Cloud Density**) has been introduced for
  - Simple prediction of the **inhaled methacholine (MCH) aerosol dose** from readily available information on nebulizer characteristics and flexiVent (respiratory) parameters.
- With ECD method, apparent differences in AH of a given mouse strain (here: W57BL/6) were resolved by converting nominal (MCH concentration) into inhaled MCH dose.
- The **ECD method has the potential for standardized AH measurement** allowing for
  - direct comparison of AH data taken in different labs,
  - using different nebulizers, flexiVent devices and flexiVent parameters **without the need for inclusion of a "reference mouse strain".**

References: Robichaud, A., Fereydoonzad, L. and Schuessler, T. F. (2015). Delivered dose estimate to standardize airway hyperresponsiveness assessment in mice. Am J Physiol Lung Cell Mol Physiol 308, L837-L846.