

Rapid *in vivo* nasal transepithelial potential difference measurement in mice

Patricia Cmielewski¹⁻³ and David Parsons¹⁻³

1. Respiratory and Sleep Medicine, Women's and Children's Hospital, Adelaide, SA, Australia
2. Robinson Research Institute, and 3. Adelaide Medical School, University of Adelaide, South Australia,



Women's & Children's
Hospital

Introduction

To determine the effectiveness of a putative cystic fibrosis (CF) cell or gene therapy to improve the CFTR ion channel defect in live intact CF mice, we have used a well-established transepithelial potential difference (PD) technique in nasal airways, where mice are oriented vertically to enable placement of the electrode tip into the nares and onto ciliated epithelium (Fig. 1). This method used a slow infusion rate (1 $\mu\text{l}/\text{min}$) of sequential Krebs buffers, with the flow-rate limited largely by the ability of the animal to cope with the fluid volume delivered. With the use of additional krebs buffers perfusions, we have now assessed a more rapid PD protocol using higher fluid rates, in mice that are non-surgically intubated to eliminate fluid ingress into the lung.

Methods

- Normal C57Bl/6 and CF (unc-FABp) mice were anaesthetised and non-surgically intubated with a 20G cannula (BD Insite™ IV catheter) to permit normal breathing during the perfusion solution deliveries.
- We first examined the feasibility of infusion rates of 10, 20 and 50 $\mu\text{l}/\text{min}$ and compared these to our standard 1 $\mu\text{l}/\text{min}$ in the same animal, using normal basal (B) and low chloride (LC) krebs buffers to determine an optimal flow rate.
- The standard slow infusion rate was then compared to the optimal infusion rate in both normal and CF mice, using the addition of the sodium channel blocker amiloride (A) and the β -agonist Isoproterenol (Iso) in the sequence.
- Nasal perfusions of B, B+A, LC+A, and LC+A+Iso buffers were performed 1 week apart to allow for complete recovery of the airway epithelium from any physical disturbances that may have occurred due to previous placement of the recording/perfusion cannula.

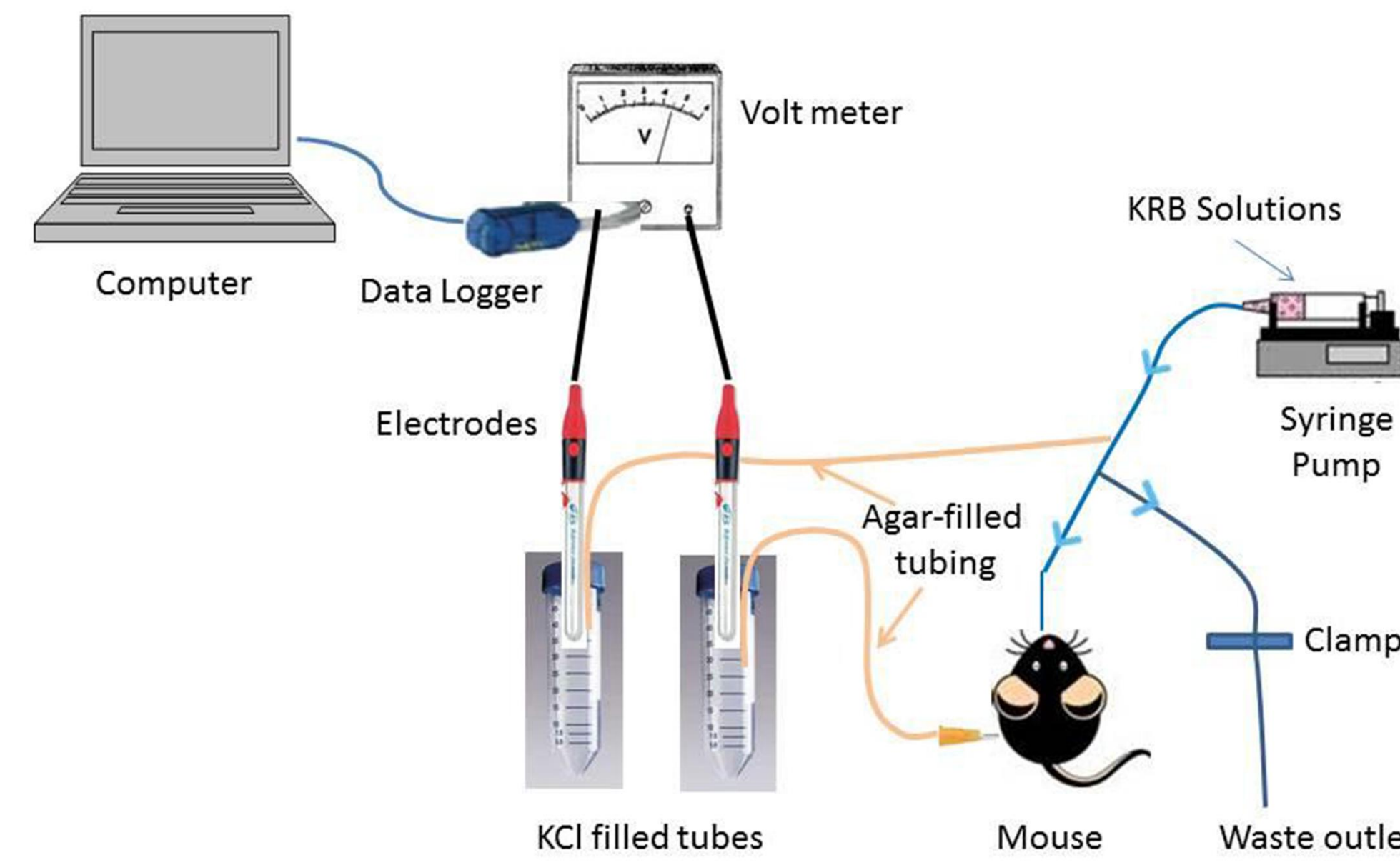


Figure 1. Diagrammatic representation of nasal PD setup

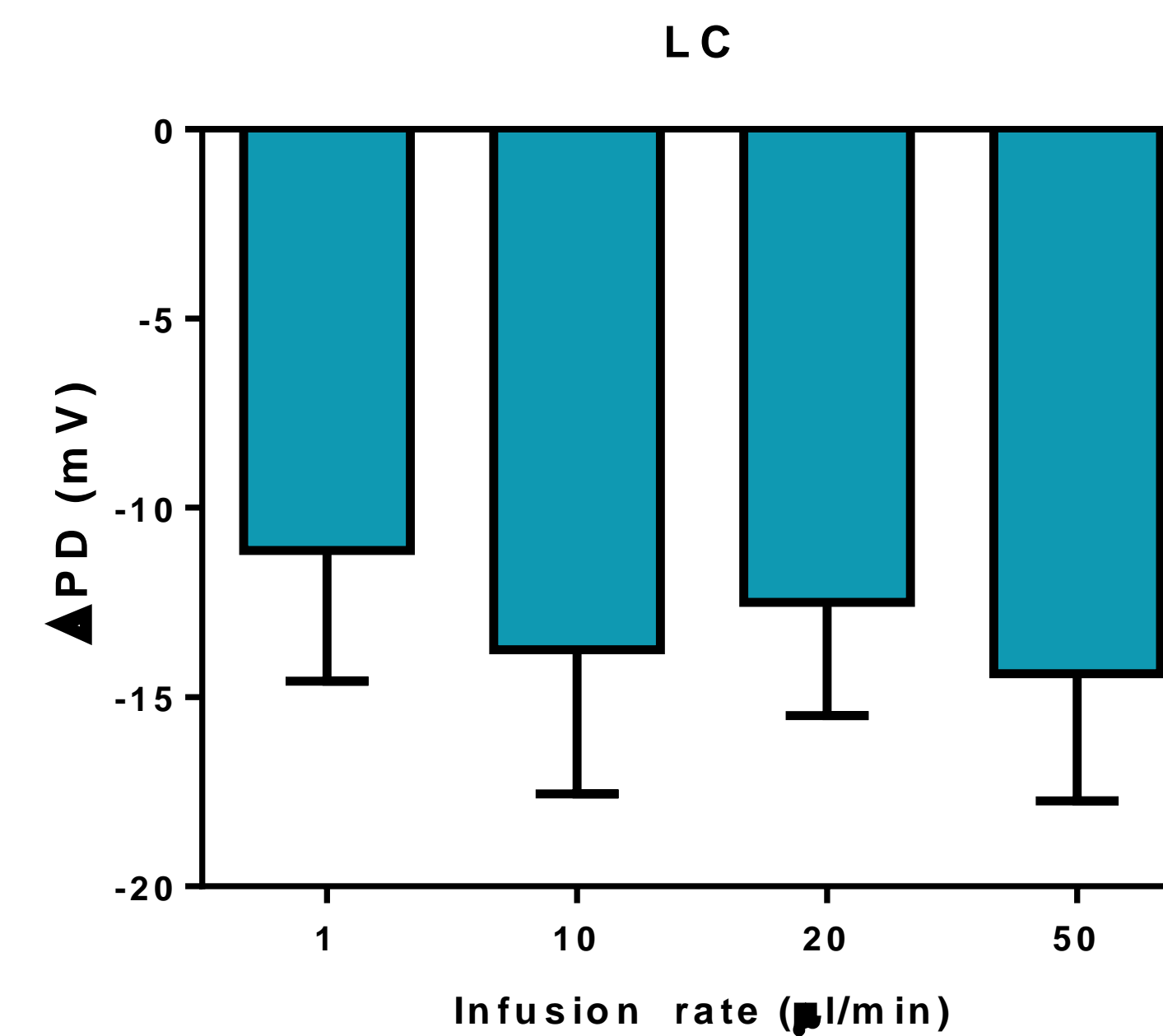


Figure 2. The change in nasal PD (ΔPD) in normal mice after different infusion rates (n.s., ANOVA)

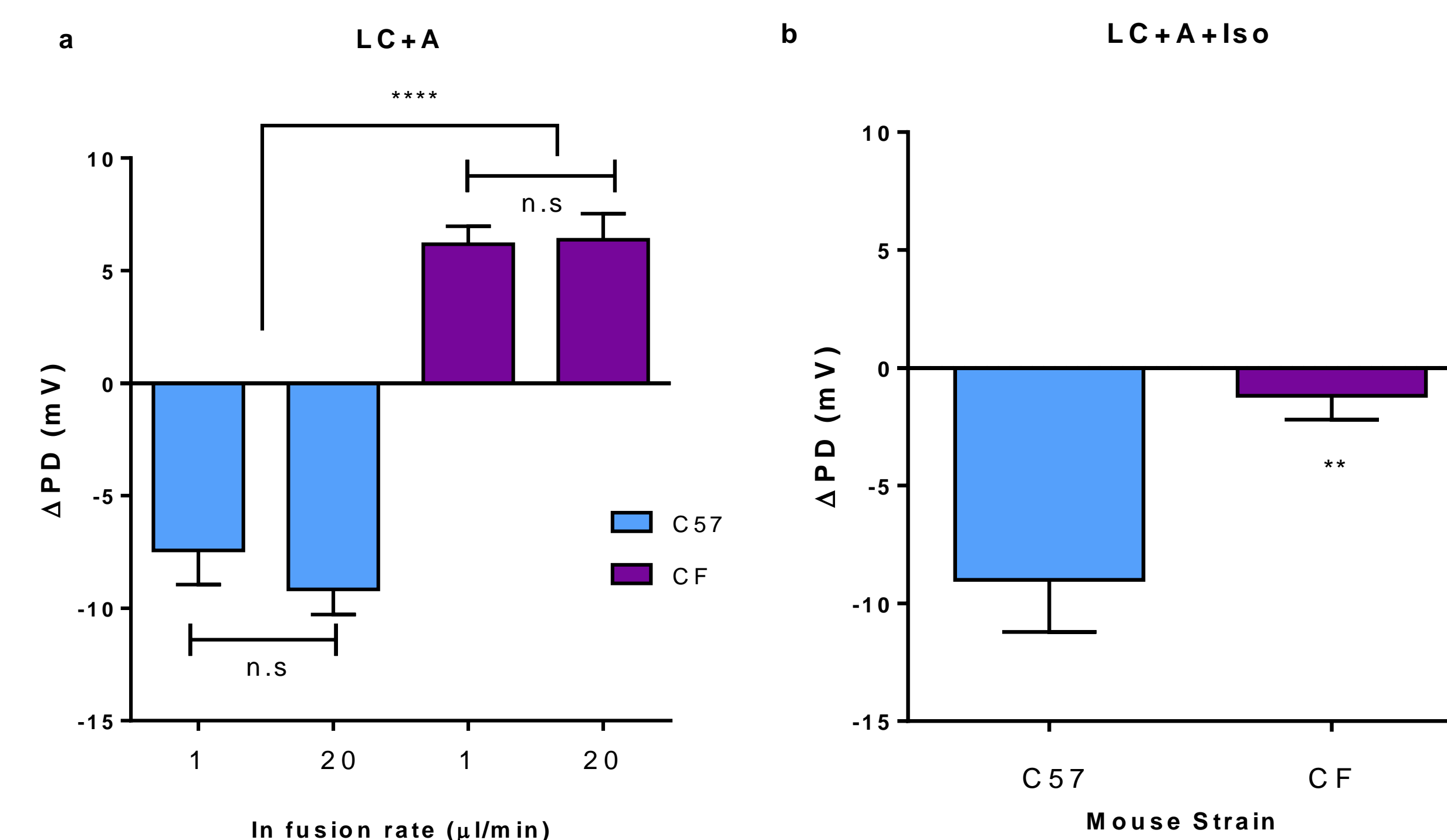


Figure 3. ΔPD following the addition of a) amiloride (LC+A) and b) isoproterenol (LC+A+Iso) in normal (C57) and CF mice (**** $p < 0.0001$, ** $p < 0.01$, t-test).

Results

- There was no significant difference in the change in nasal PD in normal mice under LC conditions at all faster infusion rates compared to the standard rate (Fig. 2, n.s., ANOVA, $n=4$).
- The optimal flow rate for rapid and consistently stabilised PD recordings was the 20 $\mu\text{l}/\text{min}$ infusion rate, and this resulted in a typical reduction in PD assessment time from 40 to 10 mins.
- CF mice displayed a significant difference in their nasal PD response for LC+A perfusion compared to normal mice (Fig. 3a, $p < 0.0001$, t-test, $n=9$), a difference maintained irrespective of infusion rate (n.s., 1 $\mu\text{l}/\text{min}$ vs 20 $\mu\text{l}/\text{min}$, paired t-test, $n=9$).
- The addition of Iso to LC+A perfusion also demonstrated a significant difference between normal mice and CF mice (Fig. 3b, $p < 0.01$, t-test) at the 20 $\mu\text{l}/\text{min}$ infusion rate.

Discussion

The rapid infusion method, in-conjunction with non-surgical intubation, provided equivalent PD measurement capability to our much slower established method, eliminated potential adverse effects due to fluid inhalation, and improved PD assessment throughput 4-fold. The reduced anaesthesia time and fluid load that resulted, allowed for the inclusion of additional perfusion solutions to further discriminate specific changes in the bioelectrical defect responsible for CF. These changes have improved the quality and speed of this nasal PD protocol for *in vivo* assessment of new therapies to correct the basic defect in CF airways.

Acknowledgements

Supported by the USA CF Foundation, the Cure4CF Foundation, and the NHMRC.