Measuring *CFTR* function in CF rats: Optimisation of the nasal potential difference technique

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Introduction

- Pathophysiology of CF results from loss of function of the CFTR chloride channel and sodium epithelial channel (ENaC).
- CFTR and ENaC ion transport in nasal epithelium induces a nasal transepithelial voltage (NPD).
- CFTR function can be investigated by perfusing the nasal membrane with solutions that either activate or inhibit these channels.
- Preliminary investigations of NPD in our new CFTR knock out (KO) rat model were performed as non-recovery as rats displayed heighten sensitivity to fluid in the airways and respiratory difficulties.

Aims:

1. To optimise NPD measurement technique in rats.
2. Test the recovery and repeatability of the optimised technique on cohorts of normal and CF rats.

Methods

- Alterations to existing mouse NPD setup (Fig. 1A, C) were made including changes to fluid management, equipment design and procedural monitoring.
- Rats were anaesthetised with medetomidine:ketamine and placed in the prone position on custom platform (Fig. 1B, D).
- Cannula was inserted in nasal cavity (4 - 7 mm) and a reference electrode inserted subcutaneously into abdomen.
- The nasal cavity was perfused at rate of 10 µl/min with one of four solutions. Once a plateau of 1 - 2 minutes was obtained, the solution was changed.
- NPD tracings (Fig. 2) were interpreted by an experienced assessor blinded to the animal genotype.

Results

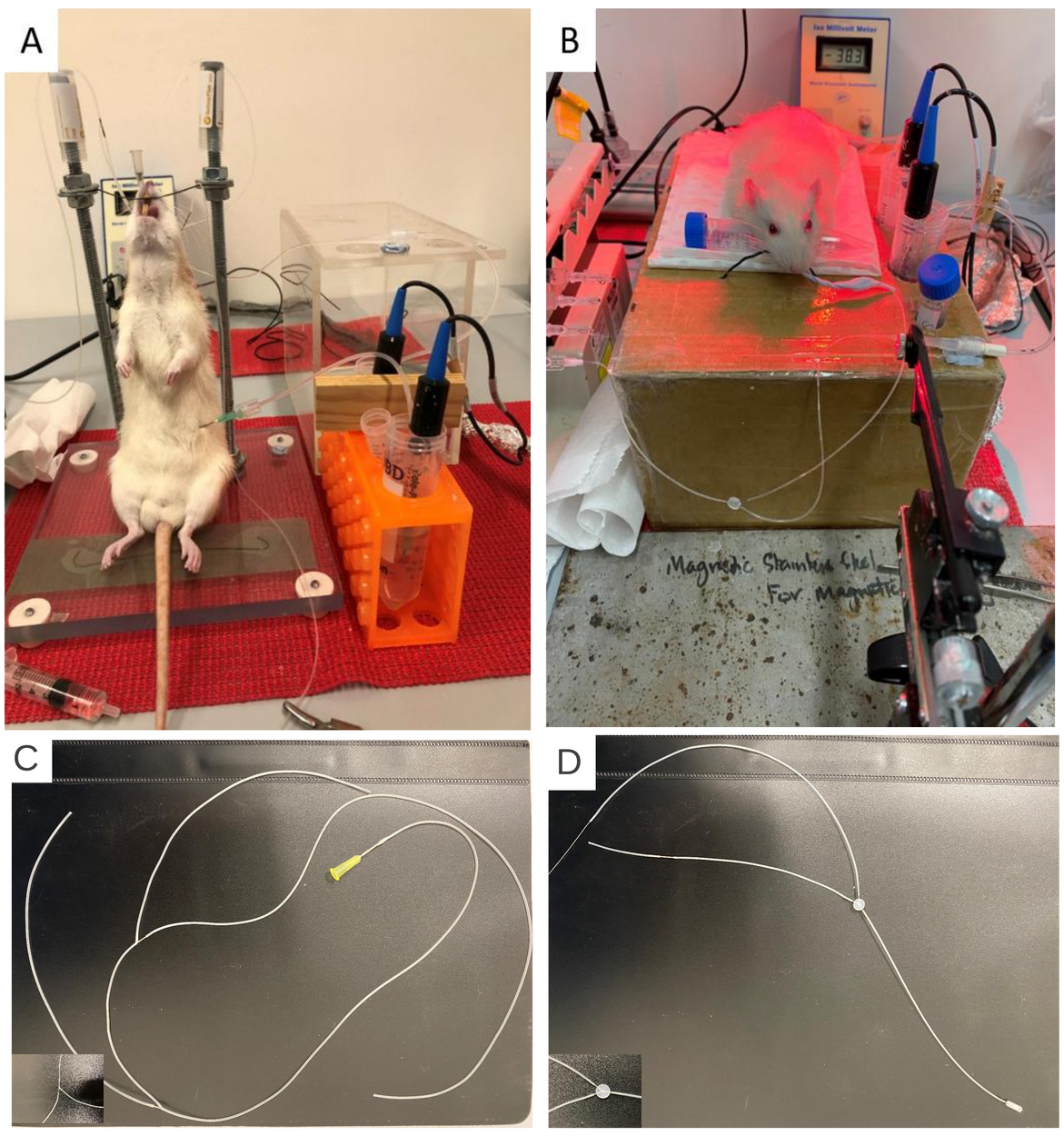
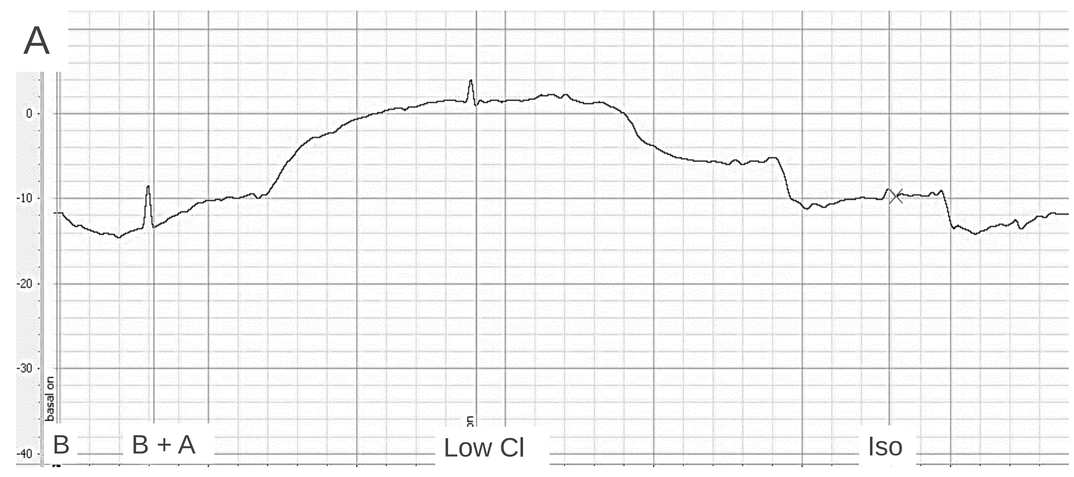


Figure 1: Optimisation of NPD setup
A) Original NPD set up with perfusion tubing (C) and (B) new set up with modified perfusion tubing and connectors (D). In the optimised setup the position of rat was changed to prone and a micro-positioner was included to assist with nasal cannula placement/stability. Fluid delivery was redesigned to aid in bubble reduction including new perfusion tubing with pin-port and pin-port connectors (insert D), compared to the original (insert C).



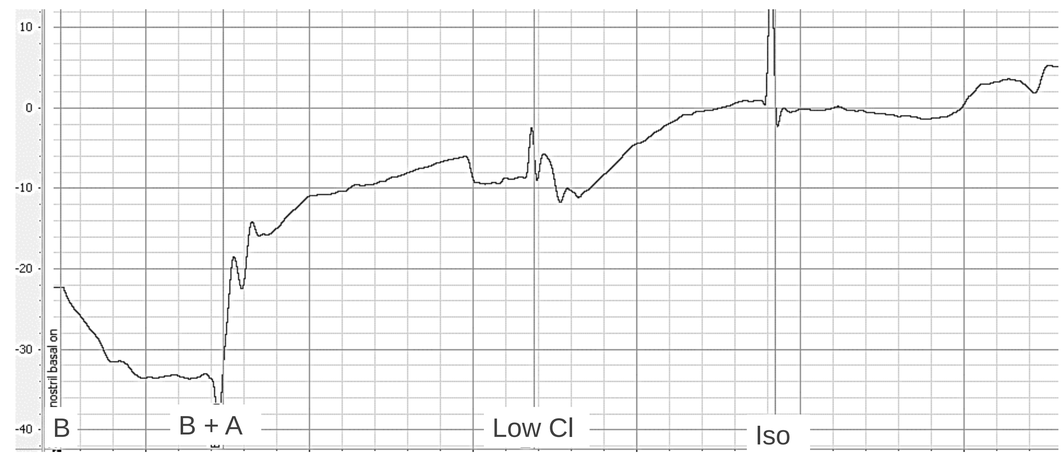


Figure 2: Representative NPD tracings from wild-type rat (A) and (B) KO CF rat. When compared to wild-type, KO CF rat demonstrates classic CF electrophysiological defects in nasal respiratory epithelium. KO CF rats exhibited a more enhanced depolarisation response to amiloride. Low chloride and isoproterenol solutions produced a small hyperpolarisation in CF, whereas a depolarisation was observed in wild-type rats. (B = Basal, B + A = Basal + Amilordie, Low Cl = Low Chloride and Iso = Isoproterenol)

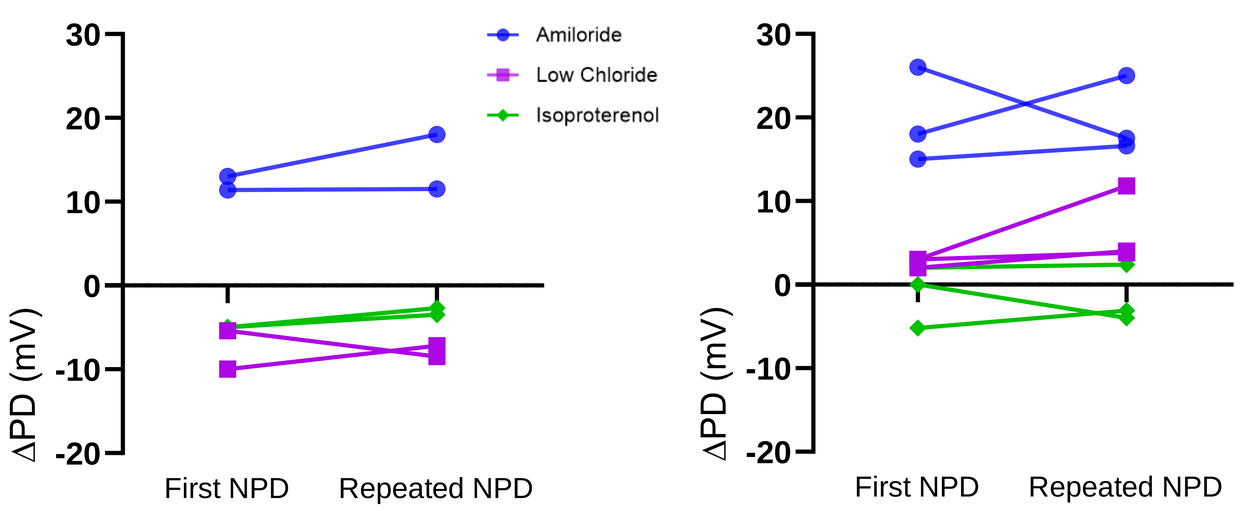


Figure 3: NPD measurements in (A) wild-type rat and (B) KO CF rats from repeated trial. No statistical significances were observed between first PD and repeated PD in both wild-type and KO CF rat in each individual animal for all solutions perfused, showing the repeatability of procedure (paired t-test).

Conclusion

- An optimised technique for conducting electrophysiological measurements of nasal epithelium in rats was developed.
- The optimised technique showed repeatability with no differences between NPD performed in same rat at different time points.
- NPD assessment procedure in rats is reliable and well tolerated that can enable routine assessment of airway CFTR functional correction.
- These improvements will allow us to test various therapeutic strategies in CF rats, including gene-addition therapy.

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