SYNCHROTRON X-RAY IMAGING EXPLAINS GENE EXPRESSION PATTERNS IN MOUSE NASAL AIRWAYS

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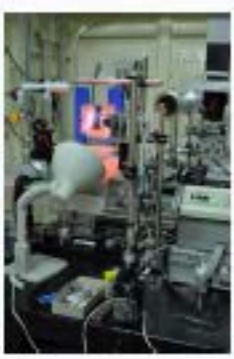
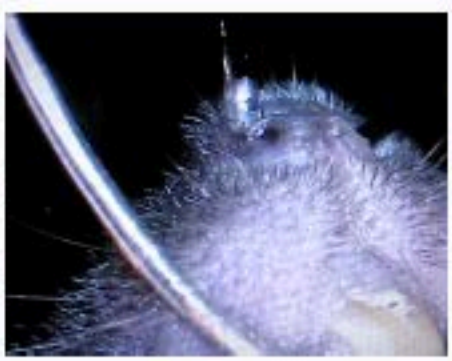
BACKGROUND: Most groups use the mouse nose as an *in vivo* model site for developing gene treatments for CF airway disease. Reliable dosing of an airway pre-treatment followed by a lentiviral vector is essential for the success of our gene transfer protocols. Despite using standardised delivery techniques we see variability in reporter-gene histological assessments and electrophysiological measurements. The aim of this experiment was to use synchrotron X-ray imaging at SPring-8 **(F1)** to accurately determine the fate of fluids delivered into live mouse nasal airways.

(F1)

METHODS: Six nembutal anaesthetised C57Bl/6 mice were imaged on the BL20B2 beamline **(F2)** at the SPring-8 synchrotron in Japan. Images were captured at 1Hz. After 1 minute of baseline a 4 µl sample of iodine-based contrast fluid (airway pre-treatment surrogate) was delivered **(F3)** over 10 sec. After 10 min of data collection an additional 20 µl bolus (gene-vector surrogate) was delivered over 30 sec. Imaging continued for a further 10 min. Fluid motion was revealed using a background subtraction method.

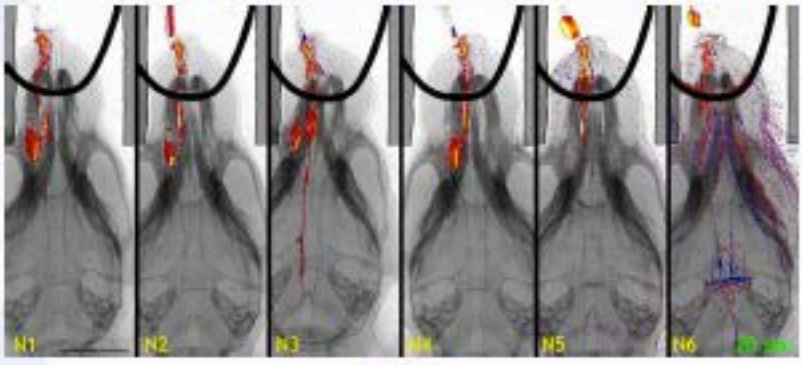


(F2)

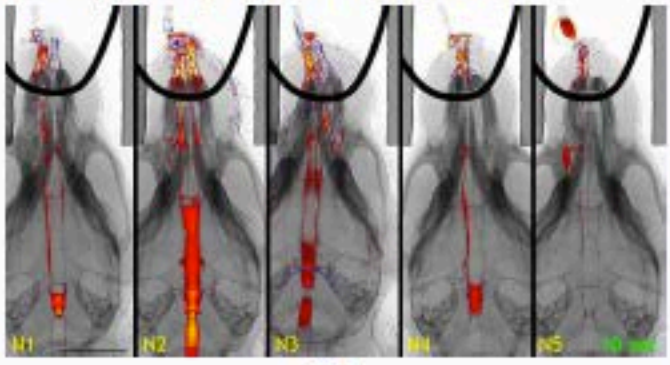


(F3)

RESULTS: The **4 µl dose (F4)** was retained on the treated side, supporting the strength of gene expression seen in nasal ciliated epithelium and validating the use of a 4 µl dose to retain an untreated nasal airway as a within-animal control. These images also confirm why gene expression is limited to the treated side when it extends into the tubular nasopharynx. Although the dose also distributes into the lateral transitional/olfactory region, expression from the gene vector was restricted to respiratory epithelium in our gene transfer studies. The **20 µl dose (F5)** overwhelms the 'holding capacity' of the dosed nostril, with some fluid continuing into the trachea and likely into deeper lung airways. This supports our finding of long term lung expression using a LV-Luc vector delivered to nose and suggests smaller vector doses may produce the same levels of expression in nasal airways. Finally, these results support the proposed LPC mechanism of action since we only observe gene expression in areas reached by the 4 µl dose, despite demonstrating the wide reach of the 20 µl surrogate gene vector dose.



(F4)



(F5)

CONCLUSION: Synchrotron imaging can help explain the mechanisms underlying published outcomes from our gene-transduction protocols in mouse nasal airways.

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