HIGH-RESOLUTION SYNCHROTRON X-RAY IMAGING OF LIVE MOUSE AIRWAYS: OVERCOMING CHALLENGES IN PHYSIOLOGICAL ASSESSMENT

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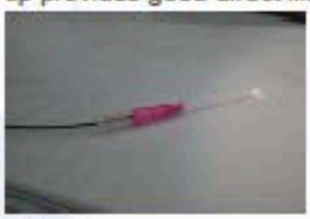
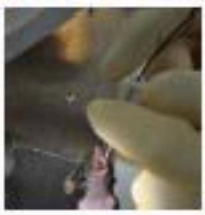
BACKGROUND: Small animal models are useful for studying respiratory diseases such as CF. However, the complexity of physiological studies is increased when imaging live animal airways using high-resolution synchrotron phase contrast X-ray imaging (PCXI). Over the last 5 years we have developed techniques at the Japanese SPring-8 synchrotron to facilitate accurate and reliable visualisation of mouse airways using high-resolution 2D imaging. Here we present our effective approaches and continuing challenges.

HUTCH SETUP: PCXI is performed on the SPring-8 BL20XU undulator beamline using 25keV monochromatic X-rays. Imaging is confined to a specialised hutch, a lead-lined room attached to the end of a synchrotron beamline. When imaging live animals it is necessary to perform remote animal monitoring, maintain stable anaesthesia and remotely deliver any test substances or pharmaceuticals.

STRAIN: Fur can produce strong phase effects and cause PCXI image artifacts. Using nude strains (Crl:CD1-*Foxn1*^{nu} / HOS:HR-1) allows us to acquire images without fur artifacts, but compared to normal mice these strains may exhibit other physiological differences that may affect our respiratory studies. Using only hairless strains also precludes imaging other useful strains such as transgenic CF mice. In preference we remove fur from the imaging area (ie trachea) of normal C57BL/6 mice using depilatory cream, producing images free from fur artifacts.

AIRWAY ACCESS: Airway access via tracheotomy or intubation facilitates mechanical ventilation, pulmonary function testing and pharmaceutical delivery. Tracheotomy is a relatively slow and invasive procedure and can alter airway biology including allowing blood to enter the trachea. In considering our future need for repeat-imaging studies, tracheal intubations are now performed via the mouth since they can be rapid, minimally invasive and readily repeatable. We use a 0.5mm plastic fiber optic guide as an introducer, and a 20Ga i.v. catheter as the endotracheal (ET) tube. The end of the fiber is attached to a bright light source so that the tip provides good direct illumination to visualize the epiglottis.

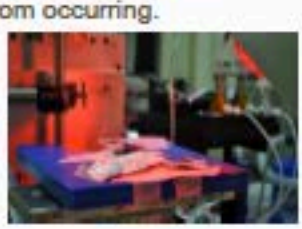


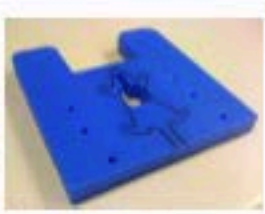


ANAESTHESIA: Due to Japanese government regulations only pentobarbital (~72 mg/kg, i.p.) and isoflurane (2% in oxygen, passively humidified) are available at SPring-8. Pentobarbital is limited by the induction of unpredictable leg "kick" movements despite deep anesthesia and the potential for overdose. Isoflurane anaesthesia is preferred as it can be easily adjusted from outside the hutch, does not produce leg movements and has a wide therapeutic index.

VENTILATION: Mice are ventilated using a flexiVent mouse ventilator, which allows respiratory system mechanics to be measured, coordinated delivery of aerosols, pharmaceuticals or test substances, and respiratory-gated image acquisition to minimize respiratory movements. In some studies a length of heat-thinned PE10 tubing is fed through the wall of the inspiratory tube to the tip of the ET tube to allow test substances to be delivered to the trachea or lung airways.

ANIMAL POSITIONING: The fixed X-ray beam location and orientation limits experimental flexibility in terms of animal restraint and positioning. Mice are tethered to an imaging board using surgical tape to minimise movement. For anterior-posterior (AP) imaging it is necessary to mount the animals head-high, but for lateral imaging they can be head-high or supine. Despite being well anesthetized, after approximately 25-30 minutes of imaging some mice mounted head-high appeared unsettled and displayed uncontrollable and unpredictable respiratory excursions that degraded image quality, limiting usable imaging time to less than 30 minutes. Using a supine imaging position prevents these movements from occurring.





DISCUSSION AND CONCLUSION: Synchrotron PCXI is a valuable technique for studying live mouse airways and despite its limitations there are currently no other imaging modalities with these capabilities. Attention to animal-handling and imaging techniques will permit continued development of novel, high-resolution, live animal airway physiology imaging for use in respiratory research.

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