An investigation into the effects of anaesthesia upon regional functional modulation in the rat brain: a phMRI study

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Background: The use of anaesthesia in small animal MRI is widely accepted, facilitating replication of results across laboratories, reducing movement artefacts and the stress of the animal[1] as well as aiding in interpretation of results[2]. Numerous anaesthetic agents are currently utilised for fMRI and phMRI studies including isoflurane[3], alpha chloralose[4], halothane[5] and urethane[6]: each conferring their own advantages and disadvantages for preclinical BOLD imaging studies. However, there is no standardization for anaesthetic protocols. BOLD signal contrast data interpretation is dependant upon neurovascular coupling[7] and therefore it is pertinent to choose an agent with minimal cardiovascular/ respiratory effects. To assess the effects of anaesthetics upon BOLD signal contrast, we compared the effects of the anaesthetic agents isoflurane and urethane on cerebral activation in the rat following an acute fluoxetine challenge using phMRI.

Methods: Adult male Sprague Dawley rats (n=36) were anaesthetised with either urethane (1.4g/kg) or isoflurane (4% induction, 1.5 – 2% maintenance ventilated in 0.9 l/min medical oxygen) and were scanned in a 4.7T magnet for 180mins, using a continuous three echo, gradient echo sequence (TE = 5,10,15 ms; TR = 940ms; acquisition matrix = 64X64X40). One whole brain volume was acquired every minute for the entire scan. After 30mins the animals received vehicle or fluoxetine (10mg/kg i.p.) (all groups n=9). SPM99 was used to identify brain regions where changes in BOLD contrast correlated with the known pharmacokinetic profile of fluoxetine.

Results: Following acute fluoxetine administration, statistical parametric maps ($p < 0.05$, corrected for multiple comparisons) (figure 1) demonstrated differing patterns of BOLD activation/deactivation depending upon the anaesthetic used. Isoflurane anaesthetised animals exhibited significant negative BOLD in the cerebellum, retrosplinal cortex, sensory cortex 1, motor cortices 1 and 2 and the frontal association cortex. Significant increases in BOLD were present unilaterally in the caudate putamen, substantia nigra, dentate gyrus and cerebral peduncle. Urethene anaesthetised animals exhibited an increase in BOLD contrast within the hypothalamus and a decrease in BOLD signal within the mesencephalic nuclei.

Discussion: This experimental work demonstrates that cerebral activations visualised through phMRI following an acute fluoxetine challenge, are dependant upon the type of anaesthetic administered. Data derived from urethane anaesthetised animals confirms the neuroanatomical response seen in previous studies. Furthermore, the data appears to highlight the initial blockade of the SERT protein and subsequent sensitization of the somatodendritic $5HT1_A$ autoreceptor[8]. Following isoflurane administration, animals produced BOLD spatial activation patterns in contrast with previous preclinical research on acute fluoxetine. This highlights the influence of anaesthesia on the BOLD signal and the importance of choosing an appropriate anaesthetic regimen in preclinical fMRI/pHMRI studies. Urethane encompasses a simple induction process, producing long lasting, stable anaesthesia during which normal respiratory and cardiovascular function is maintained[9&10]. Additionally, urethane promotes linear coupling between neuronal activity and the BOLD response[11]. Despite maintaining neurovascular coupling at levels of 2%[12], isoflurane is a potent vasodilator and increases regional cerebral blood flow[13], a confound when using BOLD imaging. However, despite urethane's obvious advantages, it is carcinogenic[14], so precludes recoverable experiments. If isoflurane's problems can be overcome, it will prove a very popular choice for functional MRI studies, promoting non invasive, recoverable studies with translational potential. It is especially advantageous for therapeutic assessment of novel psychiatric compounds as chronic dosing is almost ubiquitous to achieve clinical efficacy within this scientific discipline.

Conclusions: This study compliments previous work in highlighting the importance of using different anaesthetic protocols in order to identify the most appropriate anaesthetic agent for a particular study. It is essential to promote continuity throughout preclinical fMRI/pHMRI to aid in meaningful interpretation, comparison and contribution of data to the many fields of neuroscience.

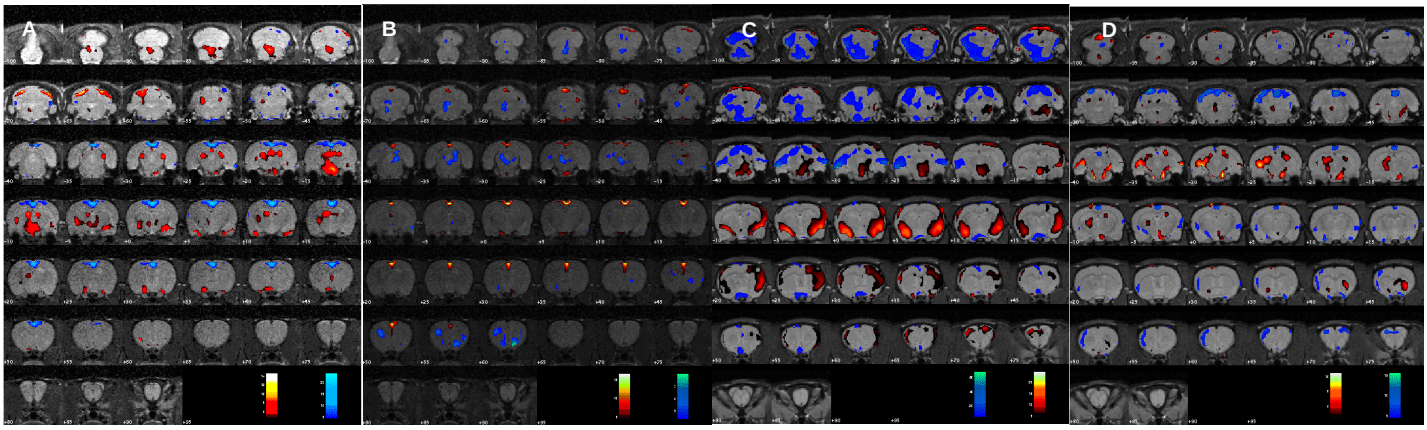

Figure 1 : SPM {t} distribution maps of BOLD signal change overlaid onto co-registered spin echo anatomical templates of a) an acute vehicle administration under urethane and c) an acute vehicle administration under isoflurane, b) the main effects of an acute fluoxetine challenge under urethane and d) the main effects of an acute fluoxetine challenge under isoflurane correlated with the known PD profile of fluoxetine. Coloured pixels represent significant correlation (thresholded at $p < 0.05$ corrected for multiple comparisons, $T > 4.28$) of signal time course with the pharmacodynamic profile of fluoxetine. Red = positive correlation, blue = negative correlation, (all groups, n=9).

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