Longitudinal functional MRI for animal studies of alterations in neurovascular coupling

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Introduction: Preclinical fMRI is a valuable tool in the understanding of disease mechanisms in animal models, and in preclinical drug development. The non-invasive nature of MRI can facilitate translation between studies on animal models and human subjects. However, preclinical fMRI does have its drawbacks. Animals require anaesthesia for scanning and commonly used anaesthetics such as isoflurane inhibit vascular reactivity. Anaesthetics without this effect such as urethane and alpha-chloralose are carcinogenic and so are restricted for terminal scans.[1] This has led to a lack of longitudinal preclinical fMRI studies, with those that do exist reporting inconsistent findings, due to the limitations of the anaesthetic protocol.[2]. Also, no preclinical studies exist of changes in the haemodynamic response in normal ageing, which limits the application of fMRI in studying the progression of age-related disease. Here we test the suitability of using a novel, minimally invasive anaesthesia protocol in combination with a functional MRI protocol to assess alterations in neuronal activity due to physiological aging. Data is presented from the first two time points of an ongoing 18 month aging study in rats.

Methods: This study was conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986 and following institutional ethical approval. 11 female Wistar Han rats aged 3 months on arrival were housed in standard cages and given daily access to a playpen. Animals were scanned at 7 months and 9 months of age and anaesthetised with 3% isoflurane. Tail vein cannulation was performed before transferring the animals to the MRI bed. A bolus of 9mg/kg propofol[3] was administered over 1 minute and isoflurane was gradually reduced to 0%. Respiration was monitored using a respiration pillow, temperature monitored using a rectal probe, and heart rate and blood oxygen saturation monitored using a pulse oximeter. Copper electrodes were inserted subcutaneously into the dorsal surface of the right forepaw between digits 1-2 and 2-3. 3 minutes after the bolus ended, a continuous infusion of propofol was given at 54mg/kg/hr for the duration of the scan. Coronal scout images were used to locate bregma, and three 1.5mm slices were selected with bregma in the middle slice. A shimming voxel with dimensions 10x9x4.5mm was positioned to cover the centre of all three slices, excluding non-brain tissue or tissue outside the slices of interest. FASTMAP was used to shim these slices to a 50% linewidth between 20-35Hz. Rats were switched from breathing oxygen to room air and fMRI was performed for 9 minutes using a rapid EPI sequence (TR=250ms, TE=22ms, kzero=8, shots=2, data matrix =128x128). The forepaw was stimulated at 10mV, 10Hz, pulse width 1us, with a block design of 60s off, 30s on. A standard fMRI analysis pipeline was performed using FSL (www.fmrib.ox.ac.uk/fsl). Motion correction (MCFLIRT), brain extraction (rBET), bias field correction (FAST) and independent component analysis for artefact removal (MELODIC) were performed prior to time-series analysis in FEAT to visualise the BOLD response.

Results: Animals' oxygen saturation stayed between 80-98% while breathing room air, and breathing rate was maintained between 40 and 70bpm, with brief increases in response to the stimulus. S1FL activation was quantified in three ways: number of active voxels, mean BOLD signal change across the ROI, and maximum BOLD signal change within the ROI. No significant difference in any of these measures was found between the two time points. Mean number of voxels was 399.5 at 7 months and 532.75 at 9 months. Max signal change was 5.045% at 7 months and 6.027% at 9 months, and mean signal change was 1.583 at 7 months and 1.808 at 9 months.

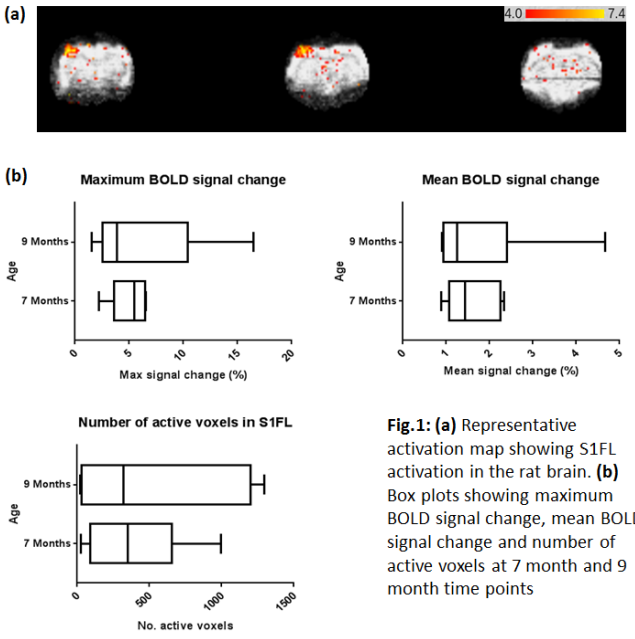

Fig.1: (a) Representative activation map showing S1FL activation in the rat brain. **(b)** Box plots showing maximum BOLD signal change, mean BOLD signal change and number of active voxels at 7 month and 9 month time points

Discussion:

This study presents the first two time points of an 18 month ageing study in rats. The anaesthetic protocol showed no adverse effects from repeat imaging, a robust and reproducible BOLD response, with rapid recovery of the animals. Though some outliers were present, the minimal change in signal between the two time points supports the reproducibility of the protocol and potential to detect age-related changes at future time points. The median values for maximum signal change and mean signal change across the ROI are both lower at 9 months than 7 months, while the number of active voxels shows a much greater interquartile range at this time. If this trend continues as the rats age, this would support data from human studies[4] reporting that the BOLD response has a larger area distribution and is less intense in older participants. This protocol is also being used to study rats for 6 weeks following surgically-induced transient stroke, and will also be applied to an in vivo model of hypertension.

References

[1] Tremoleda et al (2012) *EJNMMI Res* 2(1):44. **[2]** Dijkhuizen et al (2012) *Trans Stroke Res* 3(1):36. **[3]** Griffin et al (2010) *NeuroImage* 51(4):1395. **[4]** Tombari et al (2004) *NeuroImage* 23(3): 827