Ketamine-evoked Functional Connectivity Changes in Isoflurane Anaesthetised Rats

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Purpose: In recent years synchronised low-frequency spontaneous fluctuations detected by fMRI BOLD signal has been widely applied to study functional connectivity networks in human subjects [1]. Although, the nature of functional connectivity detected by spontaneous BOLD fMRI and its underlying neural correlates have not been well understood yet [2]. Animal studies provide wider possibility of invasive and non-invasive methods. Hence, the applications of BOLD fMRI functional connectivity in animal studies are expected to facilitate in-depth understanding of low-frequency BOLD fluctuations. In particular, using psychoactive compound to study changes in functional connectivity would allow us to study changes in a given animal on same background neural activity. This study examined the spatiotemporal dynamics under isoflurane anaesthesia and whether existing spatiotemporal dynamics alter following ketamine intervention.

Method: All experiments were performed on male Lister hooded rats n = 6 (Charles River, UK) were maintained on a 12:12 h light/dark schedule and all procedures were carried out in accordance with the local regulations and U.K Animals (Scientific Procedures) Act, 1986. Anaesthesia was induced with 4% isoflurane and the level was maintained at 2.0% throughout surgery to ensure areflexia; during imaging isoflurane was maintained at 1.75% and adjunct gases of 50:50% nitrous oxide and oxygen was used. Mean arterial blood pressure, respiration, heart rate, peripheral oxygen saturation and body temperature was monitored throughout the experiments. Data was acquired using a 7T animal MR scanner (Bruker, Karlsruhe) using a receive only head coil. Imaging parameters were GE-EPI sequence with TR/TE = 2000/23 ms, 128x128 matrix, 15 axial slices and FOV 30mm^2. In each experiment 20 minutes baseline was followed by intraperitoneal saline injection. Ketamine (25mg/kg) was injected on completion of 20 minutes post saline period and further 1 hour post-ketamine scans were acquired. Time courses were motion corrected and spurious noise related to the global, muscle and motion signals were regressed out. Analysis was performed on 10 minutes time bins using FSL v4.1.5 (FMRIB Centre, University of Oxford) to assess low frequency power, independent components andseed-based correlations.

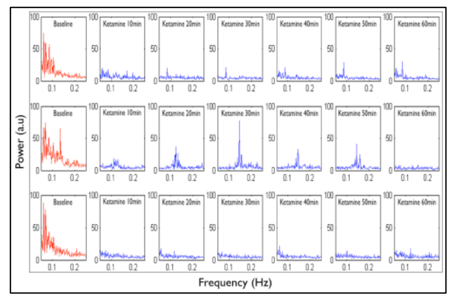

Fig 1. Average power spectra (n =6 rats) of BOLD time courses from ROIs during baseline and following 10-60 minutes after ketamine (25mg/kg i.p.) injection. Upper panel: rMC, right primary motor cortex; middle panel: rHC, right hippocampus ; lower panel: rACC, right anterior cingulate cortex .

Results: 1. Power spectral density and the extent of seed-based correlations significantly decreased following ketamine administration in regions of right primary motor cortex, hippocampus and anterior cingulate as shown in Fig1. Model-free functional network ICA analysis revealed clearly defined bilaterally connected cortical networks at baseline (isoflurane anaesthesia) with two main resting state networks in the rat brain: the interhemispheric cortical network (Fig2) and the cingulate cortex network (Fig3). The bilateral cortex network include primary and secondary motor area, primary and secondary somatosensory area, primary visual and auditory areas were detected as one component. The cingulate cortex network comprises the anterior and posterior cingulate. Following ketamine administration reduced connectivity was also observed in the extent of ICA detectd RSN (Fig4). There were no changes following a control saline administration.

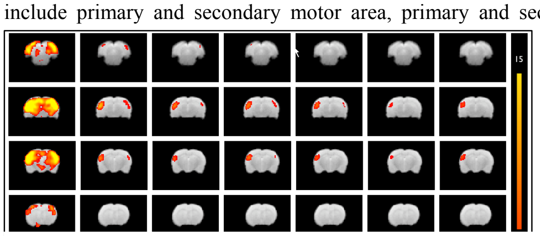

Fig 2. Group ICA detected cortex network during baseline and following ketamine treatment. There is a clear bilateral connectivity decrease after ketamine (25mg/kg) injection. Each column shows different slices of the same condition. All analysis were performed on 10 minutes time bins.

Discussion: Here, an exploration of the functional connectivity of nitrous oxide-isoflurane anaesthetised rats and the changes following ketamine administration was performed using three different methods including power spectra analysis, seed-based analysis and ICA. This study found consistent and strong reduction of the functional connectivity following ketamine injection in all investigated regions and by all three methods. Our findings of baseline bilateral connectivity in rat brain are furthermore consistent with recent reports based on seed-based and power spectral analysis [2]. To the best of our knowledge only one previous study used ICA to examine resting state networks in Long-Evans rats using infomax algorithm based ICA approach as implemented in GIFT software [3]. They were able to detect 12 independent networks in the cortical and subcortical regions. But, in the present study only 2 networks were detected. This could be due to the difference in the anaesthetic level and numbers of independent components. In conclusion, this study demonstrated bilateral cortical networks in 1.75% isoflurane anaesthetised rats. 25mg/kg ketamine significantly disrupted both the interhemispheric and cingulated resting state network.

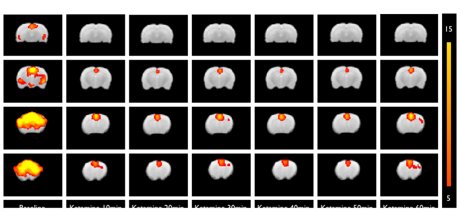

Fig 3. Group ICA detected cingulate network during baseline and after ketamine (25mg/kg, i.p.) injection. There is a clear connectivity decrease after ketamine injection. Each column shows different slices of the same condition. All analysis were performed on 10 minutes time bins.

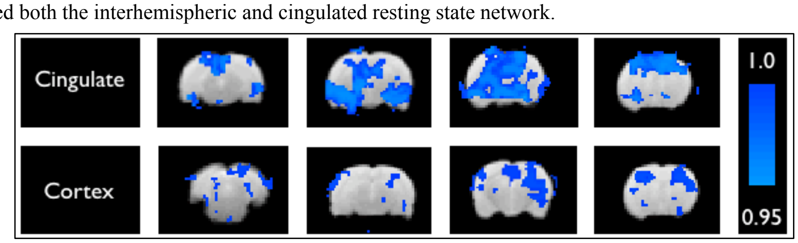

Fig 4. Permutation test comparison between baseline (10-20 minutes) and post ketamine (20-30 minutes) conditions of ICA detected networks. First row, ICA detected cingulate network decrease following ketamine administration. Second row, ICA detected cortex network decrease after ketamine injection. In each column shows an image slice, in which the significant change was detected. Images were thresholded at $p < 0.05$.

References:

1. Auer, D.P., *Spontaneous low-frequency blood oxygenation level-dependent fluctuations and functional connectivity analysis of the 'resting' brain.* Magn Reson Imaging, 2008. **26**(7): p. 1055-64.
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3. Hutchison, R.M., et al., *Functional networks in the anesthetized rat brain revealed by independent component analysis of resting-state FMRI.* J Neurophysiol. **103**(6): p. 3398-406.