Neurophysiological underpinnings of ketamine-induced negative BOLD response.

N. Dashdorj[1], M. I. Schubert[1], M. Prior[2], R. Mason[3], and D. Auer[1]

[1]Academic Radiology, University of Nottingham, Nottingham, United Kingdom, [2]Brain and Body Centre, University of Nottingham, Nottingham, United Kingdom, [3]School of Biomedical Sciences, University of Nottingham, Nottingham, United Kingdom

Purpose: Pharmacological fMRI is an increasingly used tool to assess and map drug-induced changes of neural activity. Brain function is typically probed by comparing blood oxygen level dependant (BOLD) signals before and after drug administration. In analogy to fMRI pharmacologically induced BOLD signal changes are thought to reflect respective changes in neuronal activity, despite additional drug-induced physiological confounds (1,2). Negative BOLD responses are more common in pharmacological fMRI than stimulus-induced fMRI and particularly challenging to interpret. The neural correlates of BOLD signal changes were directly studied in few animal studies (3,4) . It remains however unclear whether a similar relation between LFP and BOLD signal change exist for pharmacological fMRI. In this study we compared ketamine-induced BOLD changes with electrophysiological recordings in rodent brain.

Method: All experiments were performed on male Lister hooded rats n=12 (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 recording/imaging isoflurane was maintained at 1.75%. Mean arterial blood pressure, respiration, heart rate, peripheral oxygen saturation and body temperature was monitored throughout the experiments. For the electrophysiological experiments (n=6 rats) 8-channel electrode arrays were placed stereotaxically in the right hippocampus and multiple-single unit (MUA) and local field potential (LFP) neural activity was recorded using a Plexon system (5). Ephys recording site was verified by post mortem histology and region-of-interest was chosen accordingly for the imaging analysis (Fig1).Physiological and anaesthetic conditions for extracellular neuronal recording and phMRI experiment were kept as similar as feasible. Baseline scans lasted always 30 minutes with one hour of neuronal recording/ phMRI scanning following i.p injection of ketamine (25mg/kg). phMRI experiments were performed using a 7T animal scanner (Bruker, Karlsruhe) using an receive only coil. Imaging parameters were GE-EPI sequence with TR/TE = 2000/23 ms, 96x96 matrix, 15 axial slices and FOV 30mm^2. Off-line neuronal MUA and LFP analysis were undertaken with Plexon Offline-Sorter, Neuroexplorer and in house Matlab scripts. phMRI data was analysed by SPM5and FSL41.

Results: ***1.*** *Ketamine-induced neuronal activity MUA and LFP:* Ketamine i.p administration evoked three types of responses (44cells/6rats): A decrease in firing rate (61.4%, n=27); an increase (25%, n=11), or firing rate was not affected (13.6%, n=6). Following ketamine injection there was a significant LFP power reduction ($p<0.001$ Student t-test from 0.0213+/- 0.002mV^2 to 0.0109+/- 0.0008 mV^2). ***2.*** *Ketamine-induced BOLD signal changes:* Ketamine induced negative BOLD responses in the hippocampal area (Fig1b). Signal ratio of WM and hippocampus showed significance decrease in n=5 (ANOVA $p<0.0001$). ***3.*** *Comparison of Ephys and BOLD time course*: As the experiments were set up in parallel we compared dynamic signal changes of BOLD, MUA and LFP during baseline and post ketamine period (Fig2) time-locked to the ketamine application. Non-parametric Spearman correlation analysis resulted in r=0.911 for MUA & LFP (Fig3), r=0.611 for LFP&BOLD and r=0.589 for MUA&BOLD.

Discussion: These parallel experiments suggest that ketamine-induced BOLD signal decrease is associated and temporally correlated with decreased neuronal activity with similar relation to MUA and LFP. Our results of ketamine-induced negative BOLD change in conflict with previous reports (6). In contrast, our Ephys results are well in line with single unit recording study (7). These discrepancies are might be due to different anaesthetics and its depth. Previous studies reported different effects with various anaesthetics and anaesthetic depths (8, 9). From a behavioural aspect ketamine is known to induce psychotic symptoms and it has been shown to reduce hippocampal blood flow in schizophrenic subjects(10).

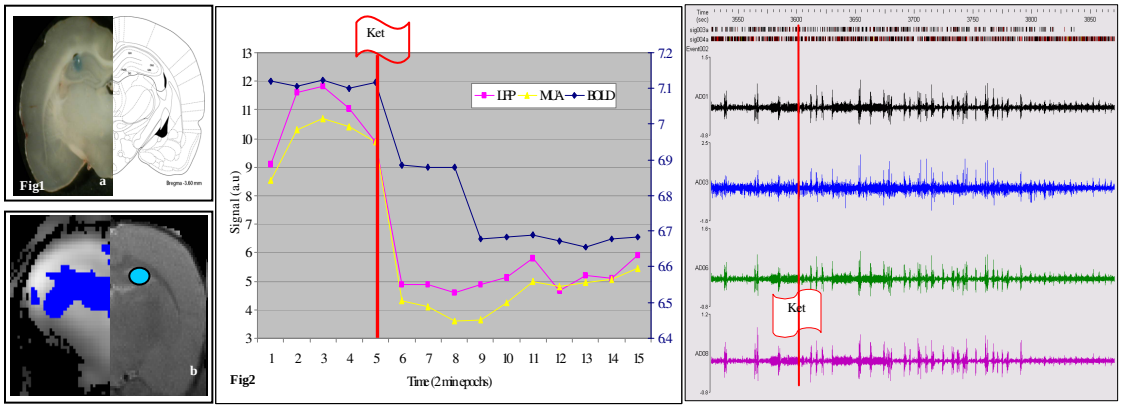

Fig1. Region-of-interest **(a)** right hippocampus recording site identified with post-mortem histology in comparison with Paxinos& Watson atlas. **(b)** Representative animal right hippocampus showing negative BOLD signal ($p<0.01$ corrected for multiple comparisons) after ketamine injection in comparison with ROI used for time series extraction. **Fig2.** Right hippocampus phMRI BOLD signal and neural activity MUA, LFP changes time series. BOLD signal in here is represented by WM and ROI time series ratio actual ratio is shown in the secondary Y-axis in blue. For viewing purpose actual numbers of LFP power were increased 500 times. **Fig3.** Representative animal Ephys recording MUA and LFP correlation. MUA shown as raster, 4 LFP channels are shown in coloured continues recording.

Conclusion: To the best of our knowledge this is the first study to investigate drug-induced negative BOLD changes and its underlying neuronal activity changes. Ketamine-induced negative BOLD changes in the right hippocampus were correlated with decreased neuronal activity namely LFP and MUA. This study is limited by small sample size and measurements made on parallel experiments.

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