Neurophysiological underpinnings of ketamine-induced negative BOLD response and interactions with anaesthesia

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Purpose: Pharmacological magnetic resonance imaging (phMRI) is an increasingly popular tool to study drug effects on brain function using the blood oxygen level dependent (BOLD) signal [1]. However, the nature of the drug induced BOLD signal change is still not well understood; in particular its relationship with the underlying neural activity. Additionally, the majority of animal phMRI studies require anaesthesia, which may interact with the investigated drug [2]. The neurophysiological underpinnings of BOLD signal changes were directly studied in few hallmark studies [3, 4]. It remains however unclear whether a similar relation between LFP and BOLD signal change exist for pharmacological fMRI under different anaesthetic protocol. In this study we compared ketamine-induced BOLD changes with electrophysiological recordings in rodent brain under two different anaesthetic protocols.

Method: All experiments were performed on male Lister hooded rats n=26 (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% with two different adjunct gases. In ***group A***, rats were anaesthetised with 1.75% isoflurane with 50:50% N_2O and O_2, while in ***group B***, rats were kept under 1.75% isoflurane and medical air anaesthesia. MABP, respiration, HR, SpO_2 and body temperature were monitored throughout the experiments. For the electrophysiological experiments multiple-single unit (MUA) and local field potential (LFP) neural activity was recorded using a Plexon system from 8-channel electrode arrays, that were stereotaxically placed in the right hippocampus (rHC) and medial prefrontal cortex (mPFC) [5]. In ***group A***, recordings were made only from rHC and in ***group B*** recordings were made both from rHC and mPFC. Ephys recording site was verified by post mortem histology and ROI was chosen accordingly for the imaging analysis (Fig1). Physiological and anaesthetic conditions for neuronal recording and phMRI experiment were kept as similar as feasible. In all experiments, a twenty minutes baseline was followed by another twenty minutes post-control saline injection and further one hour data acquisition following an i.p ketamine (25mg/kg) administration. phMRI experiments were performed using a 7T animal scanner (Bruker, Karlsruhe) with a receive only head coil and standard GE-EPI sequence (TR/TE = 2000/23 ms, 128x128 matrix, 15 axial slices and FOV 30mm^2). Off-line neuronal MUA and LFP analysis was undertaken with Plexon Offline-Sorter, Neuroexplorer and in house Matlab scripts. phMRI data was analysed using SPM5and FSL41.

Results: ***1.*** *Ketamine-induced neuronal activity MUA and LFP in* ***group A****:* Ketamine evoked a mean firing rate decrease in the rHC ($F_{6, 133} = 2.8$; $P < 0.01$) and there was a significant LFP power reduction ($p < 0.05$ repeated measures ANOVA test). ***2.*** *Ketamine-induced BOLD signal changes in* ***group A****:* Ketamine induced negative BOLD responses in the hippocampal area (Fig2A). ***3.*** *Ketamine-induced neuronal activity MUA and LFP in* ***group B****:* Ketamine (25mg/kg) did not change hippocampal mean firing rate. While, ketamine exacerbated MUA decrease in the mPFC (18 cells/5 rats); from baseline firing rate 4.26 ± 0.43 spikes/sec to post ketamine 1.7 ± 0.11 spikes/sec ($F_{1, 34} = 9.86$; $P < 0.01$). There was a significant ketamine-induced decrease in total LFP signal in both mPFC and rHC ($p < 0.05$ repeated measures ANOVA test). ***4.*** *Ketamine-induced BOLD signal changes in* ***group B****:* There were little or no significant changes following ketamine administration in ***group B***, with the exception of a decrease in the anterior cingulate cortex at uncorrected level ($p = 0.05$; Fig2B). ***5.*** *Comparison of Ephys and BOLD time course*: Ketamine administration (25mg/kg, i.p.) in ***group A*** rats evoked negative BOLD signal changes were compared with neural activity changes detected by MUA and LFP during baseline and post ketamine period time-locked to the ketamine application. The Pearson correlation analysis demonstrated a significant correlation of BOLD and MUA ($r = 0.38$, $p < 0.01$), BOLD and LFP correlation of $r = 0.4$ and $p < 0.01$ and higher correlation between LFP and MUA activity with $r = 0.55$ and $p < 0.01$ (Fig2). Ketamine led to decreases in BOLD, MUA and LFP with respective group average reductions of 11.2% ± 3.2% in BOLD, 37.1% ± 7% in LFP and 39% ± 11% in MUA. The correlation analysis of ketamine effects on ***group B*** rats in the rHC area demonstrated a significant correlation between BOLD signal and LFP activity ($r = 0.3$, $p < 0.01$; Fig3). Whereas, there were no significant correlation between BOLD signal and MUA ($r = 0.13$; $p = 0.24$). Finally, the correlation between LFP and MUA activity was statistically significant ($p < 0.05$) Pearson correlation of 0.23 (Fig2C). Group level percentage change of the measured signals were 2.14% ± 5.8% and 43.6% ± 9.6% decrease in BOLD and LFP activity, respectively. In contrast, there was no significant MUA change. The Pearson correlation of mPFC neural activity signatures (LFP, MUA) were compared with BOLD time series for the ROI of mPFC. While mPFC BOLD signal time course did not show any significant correlation with MUA entity (Fig6), there was a significant correlation between BOLD time course and LFP activity ($r = 0.25$, $p < 0.05$). Neural activity signatures of medial prefrontal cortex LFP and MUA were highly correlated ($r = 0.72$, $p < 0.01$). The percentage change in the mPFC BOLD signal was 5.6% ± 5.7% decrease. Neural signatures of the region were a substantial reduction of 47.9% ± 13.4% and 44.5% ± 19.4% in LFP and MUA, respectively.

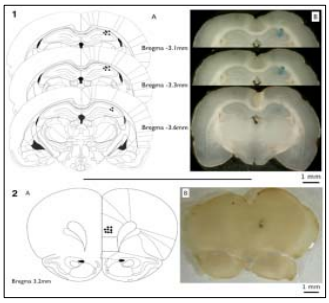

Fig1.Representative histology confirmations of the right hippocampus (1) and right medial prefrontal cortex (2). (A) Schematic representation of multi-electrode array placement. The distance posterior to bregma is indicated in mm beside each coronal section. (B) Histology of array placement within right hippocampus (1) and right mPFC (2).

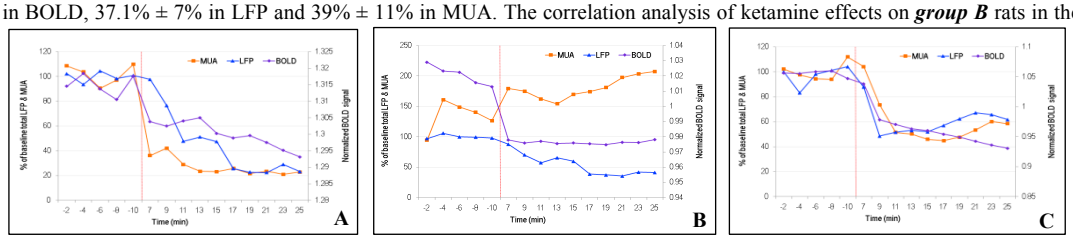

Fig2. A comparison of ketamine-evoked BOLD, with MUA & LFP activity changes. (A) rHC changes in ***group A*** (n = 6 rats). (B) rHC changes in ***group B*** *(n =5 rats)*. (C) mPFC changes in ***group B*** *(n =5 rats)*. The graph is showing rHC phMRI BOLD signal and neural (MUA and LFP) activity time series changes. Dotted line indicates the point of ketamine administration time. Group mean data is shown.

Discussion: These parallel experiments suggest that ketamine-induced BOLD signal decrease is associated with temporally correlated 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 might be due to different depth of anaesthesia and effect from N_2O, ***group B*** results show that presence of N_2O alters the BOLD and Ephys response, but does not sufficiently explain differences from previous studies. Nevertheless, previous studies reported already heterogenous drug effects dependent on the type and depth of anaesthesia [2, 8]. From a behavioural aspect ketamine is both a sedative and known to induce psychotic symptoms so both inhibitory and stimulatory effects can be expected. In fact ketamine has been shown to reduce hippocampal blood flow in schizophrenic subjects [9].

Conclusion: To the best of our knowledge this is the first study to investigate drug-induced BOLD changes under different anaesthetic protocols in parallel with their underlying neuronal activity changes. The study found consistently significant correlations between ketamine-induced BOLD signal and the LFP activity in both anaesthetic protocols and both brain regions. Correlations between MUA and BOLD activity were highly variable and dependant on the anaesthetic protocol. This study is limited by small sample size and measurements made on parallel experiments.

Acknowledgements: This research was partially funded by University of Nottingham Institute of Neuroscience Postgraduate Research funding.

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