Sub-anesthetic ketamine modulates intrinsic BOLD connectivity between the hippocampus and the prefrontal cortex in the rat

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INTRODUCTION

Low-frequency (<0.1 Hz) fluctuations in Blood Oxygen Level Dependent (BOLD) signal are temporally synchronized at rest over spatially distributed brain regions and reflect functionally connected networks. Resting-state fMRI (rsfMRI) is well-established in humans and has been shown to be sensitive to pharmacological modulation. More recently, consistent intrinsic connectivity networks have been demonstrated in the rat, but drug effects on rsfMRI are only beginning to be characterized in the rodent. The utility of rsfMRI as a translational biomarker depends on (1) its sensitivity to pharmacological modulation in preclinical species, and (2) the degree of convergence with effects using the same compound in humans. Ketamine, a potent N-methyl-D-aspartate (NMDA) receptor antagonist, is of substantial current interest both as a pharmacological model of glutamatergic dysfunction in psychiatric disease [1], and as a rapidly acting antidepressant, effective in treatment-resistant depressive patients [2]. The aim of this work was to systematically characterize the effects of ketamine on rsfMRI in the rat. As a first step of the analysis, we chose the hippocampal-prefrontal system, since it is involved in the pathophysiology of many psychiatric and neurodegenerative disorders [3,4,5], and we have recently characterized in detail the connectivity patterns within this circuit in the rat brain [6]. The strongest correlation is observed between the posterior (subiculum) region of the hippocampus and the prefrontal cortex. In the current work we used a similar hippocampal parcellation, and we administered three different subanesthetic doses of S(+)-ketamine to observe the effects at 15 and 30 min post-injection, when ketamine levels reach their peak.

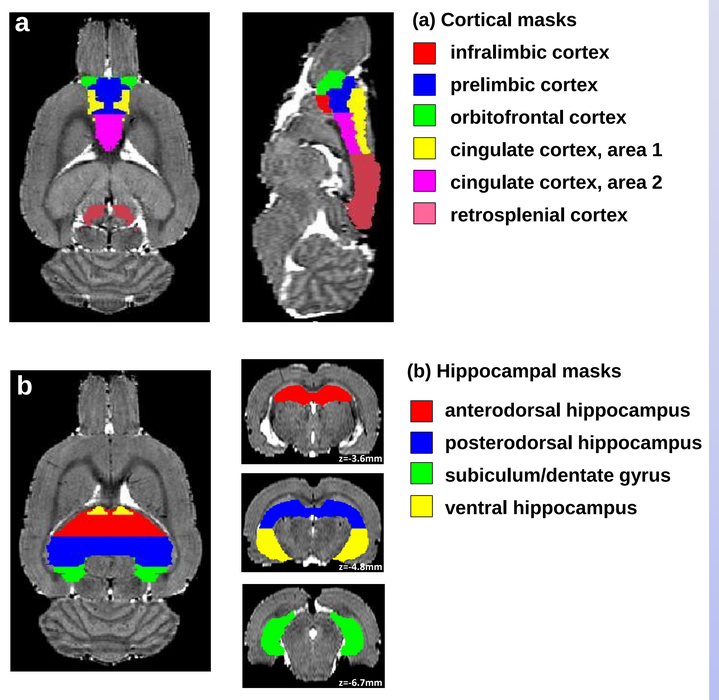
METHODS

- **40 Sprague-Dawley male rats** (368-447 g)
- **4 parallel groups**: s.c. **5, 10, 25 mg/kg S-Ketamine** and saline; 10 rats in each group; randomized parallel design
- initially anaesthetized with 4% isoflurane in a mixture of N2 (70%) and oxygen (30%)
- **medetomidine** anesthesia: 0.07 mg/kg bolus, then discontinuation of isoflurane within 10 min, 0.14 mg/kg/h
- **EPI (echo-planar imaging) scanning sessions:**
  (1) before injection;
  (2) 15 min post-injection
  (3) 30 min post-injection
- **Scanner:** 94/20 Bruker Biospec MRI scanner (**9.4 T;** Bruker BioSpec, Ettlingen, Germany)
- **EPI sequence:** TR/TE 1700/17.5 ms, flip angle 60°, 1 segment, 1 average, 29 coronal slices, 96x96 imaging matrix, field of view 35x35 mm^2, slice thickness 0.5 mm with 0.2 mm gap, 300 acquisitions, acquisition time 8.5 min. In-plane linear voxel dimension 0.365 mm; the slice stack covered the brain from the cerebellum (z ~ zbregma – 13 mm) to the posterior olfactory bulb (z ~ zbregma + 6 mm)

Breathing and cardiac rates were monitored using a respiration pad placed beneath the chest (Small Animal Instruments Inc., NY, USA) and a pulse oximeter attached to the hindpaw. Signals were recorded (10 ms resolution) using a signal breakout module (Small Animal Instruments Inc., NY, USA) and a 4-channel recorder (Velleman ® N.V., Gavere, Belgium)

→ Realign and unwarp distorted EPI (*SPM8*)
→ Movement correction (regression of motion parameters with *FSL*)
→ Filtering out physiological noise (respiratory and cardiac signal with *Aztec*)
→ Slice-timing correction (*SPM8*)
→ Band-pass filter 0.01-0.1 Hz (*AFNI*)
→ Spatial normalization to Paxinos space (*SPM8*): Schwarz atlas template →

- Differences relative to pre-injection baseline considered.
- **ROI-ROI:**
  - **Cross-correlation analysis** between 10 bilateral brain regions of interest (ROIs) located in hippocampal and prefrontal regions: calculation of all-pairs correlation coefficients.
  - GLM analysis with dose, time-point (15 or 30 min) as factors.
  - PK/PD modelling.
- **Seed-region functional connectivity maps**:
  – subiculum/dentate gyrus.
  – infralimbic cortex.
  – Correlations with dose and plasma concentration mapped.



RESULTS

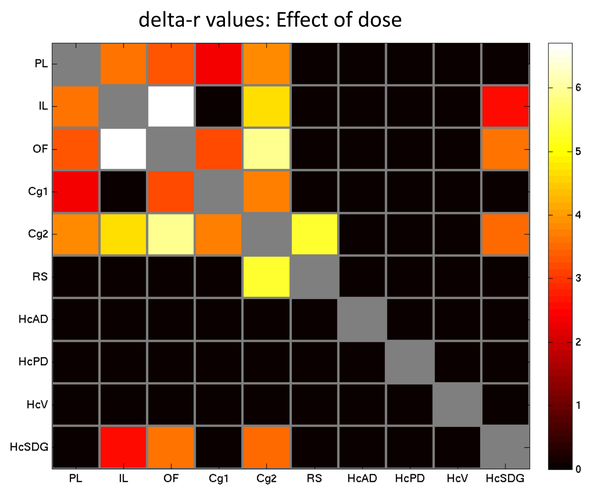


Fig.1. Pairs of hippocampal-prefrontal brain regions whose functional connectivity was significantly altered by ketamine dose (FDR; q<0.05). Black squares: region-of-interest pairs not achieving statistical significance.

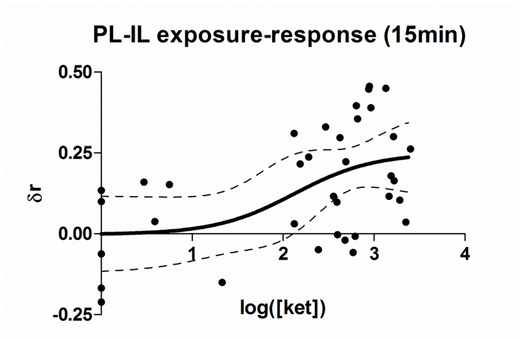


Fig. 2. Relationship between change in PL-IL connectiviy and ketamine plasma levels (15 min).

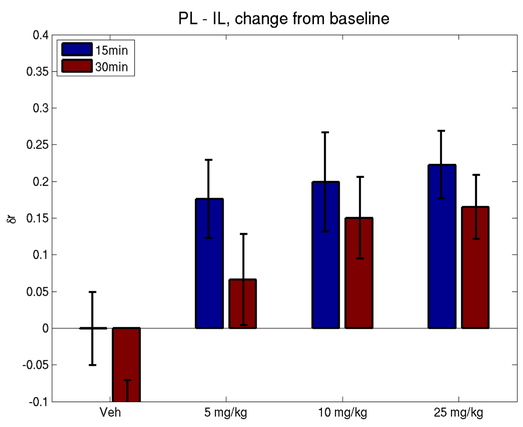


Fig.3A. Dose-response for coupling between the prelimbic and infralimbic cortices.

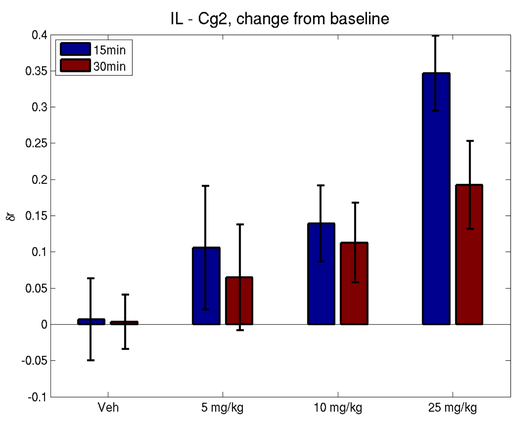


Fig.3B. Dose-response for coupling between the infralimbic cortex and area 2 of cingulate cortex.

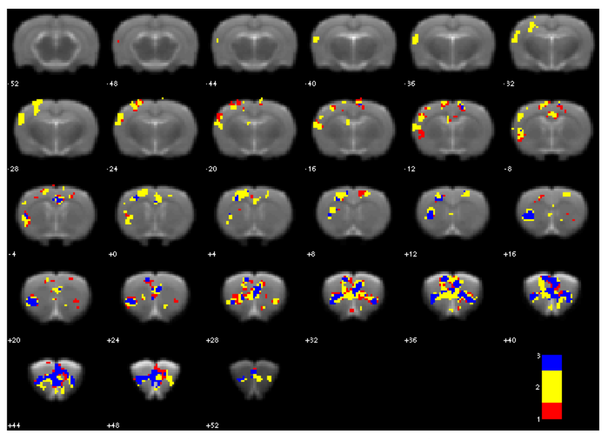


Fig.4A. Map of functional connectivity for HcSDG seed; ANOVA dose-response analysis, 15min and 30min post-injection (maps here and further thresholded at p=0.01).

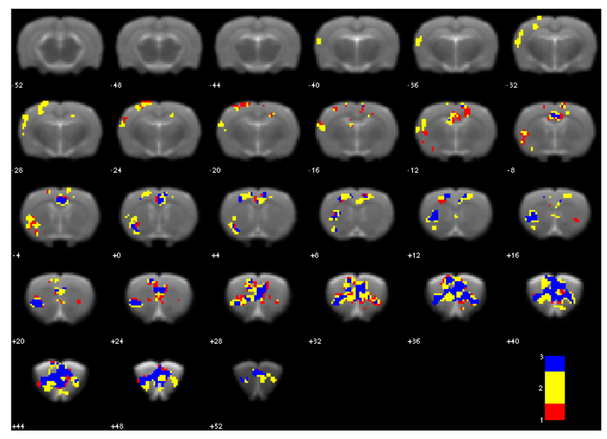


Fig.4B. Map of functional connectivity for HcSDG seed; correlation with plasma levels, 15min and 30min post-injection.

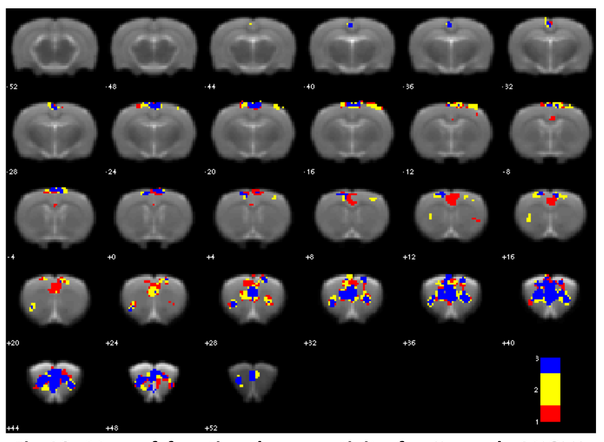


Fig.4C. Map of functional connectivity for IL seed; ANOVA dose-response analysis, 15min and 30min post-injection.

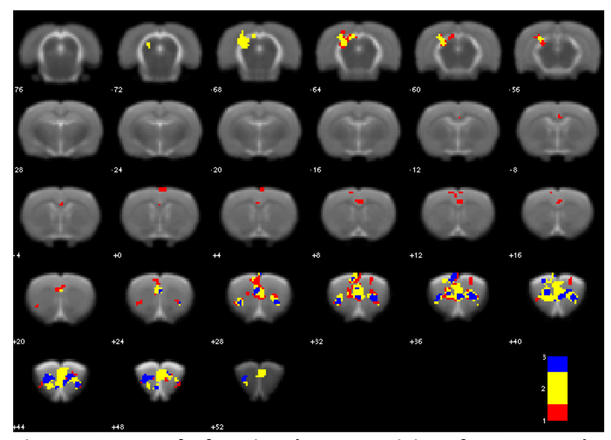


Fig.4D. Map of functional connectivity for IL seed; correlation with plasma levels, 15min and 30min post-injection.

Abbreviations: Cg1 – cingulate cortex, area 1; Cg2 - cingulate cortex, area 2; HcAD – anterodorsal hippocampus; HcPD – posterodorsal hippocampus; HcSDG – subiculum/dentate gyrus region of hippocampus; HcV – ventral hippocampus; IL – infralimbic cortex; OF – orbitofrontal cortex; PL – prelimbic cortex; RS – retropslenial cortex. **Color coding for Fig.4:** red – 15 min post-injection; yellow – 30 min post-injection; blue – overlap between 15 and 30 min post-injection.

DISCUSSION

The main effect of the ketamine injection was a dose-dependent increase of functional connectivity between the hippocampus (subiculum-dentate gyrus) and the prefrontal cortex, and especially within the prefrontal cortex itself. These results well agree with a recent human study which demonstrated an increase in connectivity after ketamine administration [7]. The observed increases in functional connectivity may partially explain established behavioral effects of ketamine, including increased wakefulness and locomotor activity, and are consistent with ketamine-induced increases in cortical EEG gamma band coherence [8]. This pattern of functional connectivity might result from increased levels of glutamate and other excitatory neurotransmitters in the hippocampal-prefrontal regions [9,10]. Glutamate could then bind other types of glutamate receptors (e.g. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors) and in long-term induce synaptogenesis similar to long-term potentiation. Additionally, increased connectivity within the prefrontal cortex could also reflect a psychotomimetic aspect observed after ketamine intake in humans. This study provides further evidence that rsfMRI is a sensitive probe of central pharmacological effects in preclinical species, and characterizes the effects of ketamine, a tool compound of considerable current interest in psychiatry research, on rsfMRI in the rat.

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References: [1] Large, 2007 J Psychopharmacol 21(3):283; [2] Zarate et al, 2006 Arch Gen Psychiatry 63(8):856-64; [3] Meyer-Lindenberg et al, 2005 Arch Gen Psychiatry 62:379–386; [4] Meyer, 2012 Clin Pharmacol Ther 91:201–214; [5] Marlatt & Lucassen 2010, Curr Alzheimer Res 7:113–125; [6] Schwarz et al, 2013 Neuroscience 228:243–258; [7] Driesen et al, 2013 Mol Psych (epub); [8] Phillips et al, 2012 Neuropharmacology 62:1359-1370; [9] Moghaddam et al, 1997 J Neurosci 17(8):2921-7; [10] Lorrain, 2003 Neuropsychopharmacology 28(9):1622-32.