# Alterations of resting-state functional activity and connectivity in the rat brain induced by acute ketamine treatment – Implications for Schizophrenia

Dany D'Souza[1], Andreas Bruns[1], Basil Kuennecke[1], Daniela Alberati[1], Edilio Borroni[1], Markus von Kienlin[1], Annemie Van der Linden[2], and Thomas Mueggler[1]
[1]*pRED, Pharma Research & Early Development, DTA Neuroscience, F. Hoffmann-La Roche AG, Basel, Switzerland,* [2]*Bio-Imaging Lab, University of Antwerp, Antwerp, Belgium*

**Background:** Translation of resting-state fMRI (rs-fMRI) applications to small animals has experienced growing interest and functional connectivity (FC) networks are now increasingly being explored [1]. Rs-fMRI bears a great potential in pre-clinical imaging as it allows probing of FC in rodent models of various disorders of the nervous system non-invasively, thus aiding in the development of new therapies. In schizophrenia, dysregulation of the glutamate neurotransmitter system has been implicated in the pathophysiological mechanism underlying the disease [2]. NMDA receptor antagonists are widely used as pharmacological models of schizophrenia due to their ability to induce positive and negative symptoms as well as cognitive impairments in healthy subjects and to exacerbate symptoms in schizophrenic patients. In rodents an equivalent dose of ketamine induces abnormal behavior including deficits in gating of auditory evoked potentials with concomitant increase in the power of spontaneously occurring gamma oscillations in the cortex, effects reminiscent of what is clinically observed during psychosis [3]. However, less is known about the brain regions and circuitry involved in the neuropsychiatric effects of ketamine. In this study, we performed rs-fMRI in rats subcutaneously (s.c.) injected with ketamine, and sought to unravel the large-scale FC circuitry potentially underlying behavioral abnormalities observed in this model.

**Methods:** Seven Sprague-Dawley rats were subjected to two imaging sessions, one week apart, in a randomized cross-over design. Animals received an injection (s.c.) of either ketamine (10mg/kg) or vehicle (NaCl) in each session, 45 minutes prior to rs-fMRI scans. Sedation protocol consisted of an induction by isoflurane (2%, 8min.), whereas during the rs-fMRI experiments sedation was maintained using a continuous infusion of medetomidine (0.1 mg/kg/h, s.c.). Body temperature was kept at 37.0±0.5 °C. Breathing-, heart-rate and blood oxygen saturation were monitored throughout the experiment. Acquisition was performed on a 9.4 T/20 cm BioSpec scanner (Bruker, Germany) using a quadrature transmit volume coil and a receive-only surface coil tailored for rat head. $T_2$-weighted images were acquired using a Turbo-RARE sequence ($TR/TE_{eff}$=2500/33 ms; 256x256 matrix; 32x32 $mm^2$ FOV; 22 coronal slices; 1 mm thickness). Rs-fMRI data were acquired using a $T_2$*-weighted single shot gradient echo EPI sequence ($TR/TE_{eff}$=2000/17.5 ms; 128x128 matrix; 32x32 $mm^2$ FOV; 20 coronal slices; 1 mm thickness). Two scans of 165 EPI volumes were acquired per subject. EPI data were preprocessed in FSL v5.0 (steps: brain extraction, motion correction, high-pass filter >0.007 Hz, regression of motion parameters), and normalized to an in-house rat brain MRI template prior to spatially smoothing (0.5x0.5 $mm^2$). Rs-fMRI time courses were extracted from 36 brain regions, and subjected to two types of analytical approaches. First, we quantitatively characterized the complexity of resting-state signals from individual brain regions by measuring the Shannon entropy using the Matlab Wavelet Toolbox ('wentropy.m') [4]. Secondly, we estimated FC by computing Pearson correlation coefficients of the rs-fMRI time courses, between all pairs of brain regions, resulting in a 36x36 covariance matrix, which was subsequently Fisher z-transformed for normality. Using two-sample paired t-tests, we investigated if there were any differences of the entropy and FC values between vehicle and ketamine treatments.

**Results:** The average wavelet entropy of regional rs-fMRI time series from all brain regions significantly decreased with ketamine treatment (Fig. 1a). Cortical areas (auditory, AU; visual, V; association temporal and parietal, TeA & PtA; prelimbic cortex, PrL) showed a significant decrease in entropy after ketamine administration (Fig. 1b), implicating an increase in ordering of the spontaneous BOLD fluctuations. As shown in Figure 2a & b modulation in FC after ketamine administration was predominantly observed in cortical areas whereas no changes were seen in subcortical brain regions. Significant modulations of FC with ketamine were observed intra-cortically in areas associated with primary sensory and motor (AU, V, M1), associative (TeA, PtA), and executive functions (PrL, Cg). Altered FCs are plotted on a sagittal view of the rat brain shown in Fig. 3.

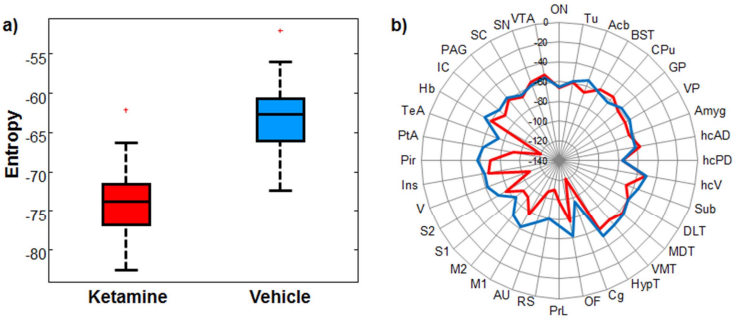


**Figure 1: Wavelet Entropy**. a) Comparison of average entropy from all brain regions between treatment conditions (ketamine<vehicle; p<0.05 FDR corrected). b) Region-wise entropy values (ketamine (red)<vehicle (blue): PrL p<0.05, V p<0.05, AU p<0.05; PtA p<0.05; TeA p<0.05; FDR corrected).

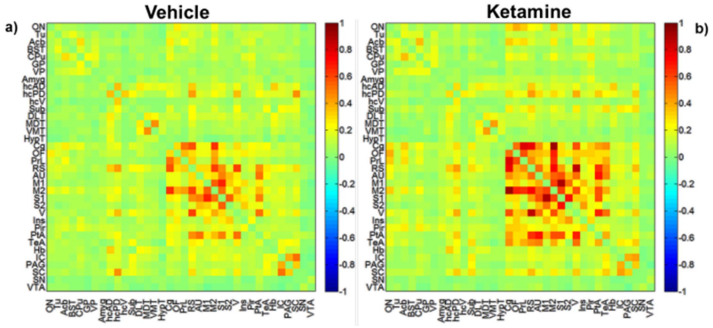


**Figure 2: Functional connectivity between treatments**. a) Mean FC matrix after vehicle treatment. b) Mean FC matrix after ketamine treatment. Color-coding indicates the Fisher z-transformed correlation coefficients.

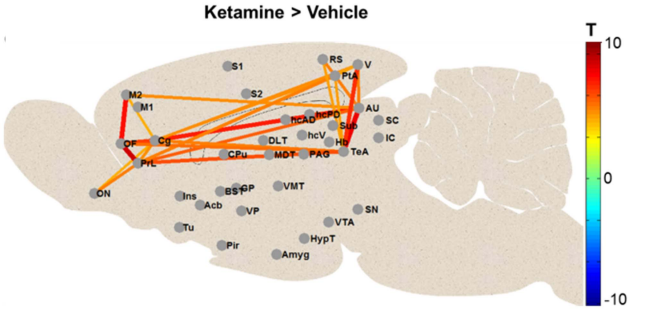


**Figure 3: Significant altered FCs plotted on sagittal view of the rat brain**. (Color-coding indicates T-value of treatment comparisons (ketamine>vehicle; p<0.05; FDR corrected).

**Discussion:** In our study acute treatment with a single dose of ketamine resulted in altered FC nearly exclusively between cortical brain areas (prelimbic PrL, auditory AU, visual cortex V). These region-specific alterations are in line with NMDA receptor models of schizophrenia predicting sensory (auditory and visual processing) cortical and higher cognitive prefrontal dysfunction [2]. As only systemic administrations of ketamine but not local injection in frontal cortex in rats affect the spontaneous discharge of prefrontal neurons, an indirect effect through increased function of glutamatergic neurons projecting from midline thalamic neurons onto local cortical inhibitory GABAergic interneurons has been proposed [5]. Under the assumption that changes in low-frequency BOLD fluctuations are correlated with gamma-band activity (and hence synchronization), the increased connectivity and altered regional entropy we measured after acute ketamine administration may reflect gamma oscillation abnormalities observed in the disease and believed to underlie deficits in the correct integration of sensory inputs.

**Conclusion:** We have shown that rs-fMRI FC values in the rat under medetomidine sedation can be perturbed by acute pharmacological intervention with an NMDA receptor antagonist. Our finding of connectivity alterations specifically in prefrontal, auditory and visual cortical areas may be linked to the psychotic-like behavior observed in this model reminiscent of positive symptoms (e.g. hallucination) observed in schizophrenic patients.

## References

1. Jonckers et al., PLoS One, 2011. 6(12): e18876
2. Javitt et al., Schizophr Bull., 2012. 38(5): 958-996
3. McNally et al., Neuroscience, 2011.199: 51-63
4. Bassett et al., Neuroimage, 2012. 59(3): 2196-207
5. Kargieman et al., Neurotox Research, 2008. 14(2,3): 129-14