No reversal of ketamine-induced functional connectivity changes in the rat brain after acute dosing of antipsychotics
Dany D’Souza1, Andreas Brun2, Basil Künnecke2, Daniel Alberati1, Edilio Borroni4, Markus von Kienlin2, and Thomas Mueggler2
1Pharma Research & Early Development Informatics, Disease & Translational Informatics, F. Hoffmann-La Roche Ltd., Basel, Basel Stadt, Switzerland, 2Pharma Research & Early Development, DTA Neuroscience, Behaviour Pharmacology & Preclinical Imaging, F. Hoffmann-La Roche Ltd., Basel, Switzerland, 3Pharma Research & Early Development, DTA Neuroscience, Functional Neuroscience, F. Hoffmann-La Roche Ltd., Basel, Switzerland, 4Pharma Research & Early Development, DTA Neuroscience, Biomarkers and Clinical Imaging, F. Hoffmann-La Roche Ltd., Basel, Switzerland, 5Bio-Imaging Lab, University of Antwerp, Antwerp, Belgium

TARGET AUDIENCE: The presented data are of interest for scientists and clinicians involved in imaging and/or drug development with focus on neuropsychiatric disorders.

PURPOSE: Increased resting-state functional connectivity (FC) across sensory, frontal and association cortices in rats subjected to an acute treatment of psychotomimetic agent ketamine (a potent NMDA receptor antagonist) has recently been reported1,2. Similar alterations in FC in response to acute ketamine challenge have also been observed in a human rs-fMRI study3. In order to further validate this model and to provide an FC-based biomarker in models of glutamatergic dysfunction underlying schizophrenia like symptoms, in the present study, we sought if an acute dose of either a first or second generation antipsychotic (FGA: haloperidol; SGA: clozapine, & risperidone) or an mGlu2/3 agonist (LY354740) can reverse the hyper FC in the rat brain induced by the ketamine challenge.

METHODS: The experimental design comprised five parallel groups of Sprague-Dawley rats (total N=48). All groups received an acute s.c. injection of ketamine (10 mg/kg) 45 minutes prior to rs-fMRI scans. Four out of five groups also received an acute pre-treatment of an antipsychotic (fifth group or control received only vehicle) 30 minutes prior to ketamine injection (Fig 1). Details of group sizes, routes of administration, doses of drugs as follows: 1) Control: n=10, saline p.o.; 2) Clozapine: n=10, p.o., 10 mg/kg; 3) Haloperidol: n=9, p.o., 2 mg/kg; 3) LY354740: n=10, i.p., 10 mg/kg; 5) Risperidone: n=9, p.o., 3 mg/kg. 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, s.c.). Body temperature was kept at 37±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 BiSpec scanner (Bruker, Germany) using a quadrature transmit volume coil and a receive-only surface coil tailored for rat head. T2*-weighted images were acquired using a Turbo-RARE sequence (TR/TE=2500/33 ms; 256x256 matrix; 32x32 mm2 FOV; 1 mm thickness). Rs-fMRI data were acquired using a T2*-weighted single shot gradient echo EPI sequence (TR=2000/17.5 ms; 128x128 matrix; 32x32 mm2 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 (0.5x0.5x0.5 mm3). Rs-fMRI time courses were extracted from 36 brain regions and FC was estimated by computing Pearson’s 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 unpaired t-tests, we investigated if there were any differences of the FC values between vehicle (control) and antipsychotic treatments (FGA and SGA) and blood oxygen saturation were monitored throughout the experiment. Acquisition was performed on a 9.4 T/20 cm BiSpec scanner (Bruker, Germany) using a quadrature transmit volume coil and a receive-only surface coil tailored for rat head. T2*-weighted images were acquired using a Turbo-RARE sequence (TR/TE=2500/33 ms; 256x256 matrix; 32x32 mm2 FOV; 1 mm thickness). Rs-fMRI data were acquired using a T2*-weighted single shot gradient echo EPI sequence (TR=2000/17.5 ms; 128x128 matrix; 32x32 mm2 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 (0.5x0.5x0.5 mm3). Rs-fMRI time courses were extracted from 36 brain regions and FC was estimated by computing Pearson’s 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 unpaired t-tests, we investigated if there were any differences of the FC values between vehicle (control) and antipsychotic treatments (FGA and SGA) or vehicle 30 minutes prior to acute ketamine challenge. Rs-fMRI scans were acquired 45 minutes post ketamine injection.

RESULTS AND DISCUSSION: As previously reported2, acute treatment with a single dose of ketamine resulted in altered FC nearly exclusively between cortical brain areas; preclinical PRL, auditory AU, visual cortex V (Fig 2a). These region-specific alterations are in line with NMDA receptor models of schizophrenia predicting sensory (auditory and visual processing) cortical and higher cognitive prefrontal dysfunction4. We aimed at reversing these altered FCs by pretreating the animals with either a first or second generation antipsychotic or an mGlu2/3 agonist prior to ketamine injection. Overall, acute antipsychotic pretreatment did not reverse ketamine induced modifications of FC indicating that multiple dosing maybe required to effectively block modulation of an acute NMDA antagonist; however, we observed antipsychotic-specific differential effects on the FCs. Haloperidol, targeting predominantly the dopaminergic D2 receptors, showed an increase in FC between olfactory nucleus ON and dorsal lateral thalamus DLT, auditory cortex AU, striatal caudate putamen CPU, nucleus accumbens Acb, globus pallidus GP, olfactory tubercle Tu, motor M1, M2, and association PIA areas, whereas, FC decreased between substantia nigra SN and hippocampal complex hCAd, hCPD, subiculum Sub, sensory (somato S1, visual V), motor M2, retrosplenical RS, and association PtA areas (Fig 2b). Both Clozapine (mode of action on D2 and 5HT2a serotonergic receptors) and LY354740 (mGlu2/3 agonist) decreased FC associated predominately with substantia nigra SN. Clozapine decreased FC between SN, hippocampal complex, insular Ins, and motor cortices, as well as between ventral pallidum VP and temporal association TeA areas (Fig 2c). LY354740 decreased FC between SN and hippocampal complex, motor cortex, prefrontal PRL, and superior colliculus SC, as well as between hippocampus and VP (Fig 2d). Taken together, we identified a set of brain regions, i.e. substantia nigra and motor cortex modulated by haloperidol, clozapine, and LY354740. Whereas for haloperidol and clozapine this effect may be a direct consequence of D2 receptor engagement in the SN, it is likely that risperidone only increased FC (Fig. 2e), in association with olfactory nucleus ON and ventral tegmental area VTA, a key region along the dopaminergic pathway.

CONCLUSIONS: We have demonstrated pharmacological modulation of FC via NMDA antagonism and upon antipsychotic treatment in rats. Whereas acute dosing with FDA and SGA did not reverse the ketamine-induced cortical hyper-connectivity we identified an FC signature pertinent to the mechanism of dopamine antagonism shared by the selected compounds. Further studies are warranted comprising chronic pretreatment to investigate optimal treatment regime to block FC changes after ketamine.


Fig. 2: FC matrices and statistical comparisons. Mean FC matrices are shown for vehicle, ketamine and antipsychotic pre-treatments (vehicle n=10; ketamine n =10; haloperidol n=10; clozapine n = 9; LY354740 n =10; risperidone n=9). Color coding of the matrices indicates Fisher r-transformed correlation coefficients. Significantly altered FCs are shown on a sagittal view of the rat brain. (color coding indicates T-values for different treatment comparisons, p<0.05 uncorrected).