Positive allosteric modulation of the metabotropic glutamate receptor subtype 5 modulates dopaminergic brain circuits

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Introduction

Previous preclinical studies have demonstrated the potential of compounds that target the metabotropic glutamate receptor subtype 5 (mGlu5) for the treatment of schizophrenia. As both typical and atypical antipsychotic drugs suppress amphetamine-induced hyperlocomotion in rodents, this model is considered to be able to predict the antipsychotic efficacy of a compound. VU0360172 (N-cyclobutyl-6-((3-fluorophenyl)ethynyl)nicotinamide hydrochloride) is a selective mGlu5 positive allosteric modulator (PAM) that reverses amphetamine-induced hyperlocomotion in rats[1]. The goal of this study was to apply functional connectivity analyses on two phMRI data sets and identify statistically significant differences between circuit correlations observed in (1) the amphetamine response *versus* (2) modulation of the amphetamine response by VU0360172. Through the evaluation of co-varying drug responses, instead of traditional individual regional amplitude calculations alone, functional connectivity analyses can provide additional information on the functional organization of the brain.

Methods

Adult male Sprague-Dawley rats were scanned with a Varian 9.4T scanner using a Doty Litz 38 coil. Structural and functional images were acquired with a T2-weighted fast spin echo (FSE) sequence (11, 1.5mm thick slices). High resolution FSE anatomical images were collected (TR = 2550 ms, TE_{eff} = 40 ms, acquisitions = 2, matrix = 256 × 256) to facilitate registration. After acquiring 7 initial images (TR = 2500 ms, TE_{eff} = 36 ms, acquisitions = 2, matrix = 64× 64, one image every 41.6 s) to determine the pre-contrast baseline, superparamagnetic iron oxide (SPIO) nanoparticles (20 mg/kg, i.v.) were injected. VU0360172 (30 mg/kg i.p.) or Veh was then administered. The post-SPIO functional scan consisted of a 15 min baseline, followed by Amph (1 mg/kg, i.p.) or Veh 30 min after VU0360172 injection, then images were acquired for another 45 min. Following within-subject registration, images from each subject were co-registered with a template rat using AFNI. Fractional CBV changes were calculated as $[\ln (S(t)/S_o)]/[\ln (S_o/S_{pre})]$, where S_o is the average baseline signal and S_{pre} is the average pre-MION signal[2] and time courses were extracted from regions of interest (ROIs). The Pearson linear correlation coefficient between the area under the curve of the CBV response for each ROI pair in the Veh/Amph and VU0152100/Amph groups were computed. Each correlation coefficient, *r*, was converted to a z-score. To identify correlations that were significantly different between the two groups, permutation analysis was performed to generate a Δzperm histogram for each ROI pair; significance was Δz > 95% of Δzperm.

Results

In the VU0360172-Amph drug interaction studies, acute administration of amphetamine (Veh/Amph group) produced robust, sustained CBV increases in cortical, thalamic, and striatal regions in anesthetized rats. Pretreatment with VU0360172 significantly suppressed the amplitude of the CBV response to Amph in the cingulate cortex with trends in retrosplenial cortex, motor cortex, and dorsal striatum. Functional connectivity analysis revealed that the Veh/Amph group had more significant correlations than the VU0360172/Amph group (Figure 1), suggesting modulation of dopaminergic circuits by the mGlu5 PAM. Permutation analysis identified ROI pairs with significant differences between the Veh/Amph and VU0360172/Amph groups, and included hippocampus-entorhinal cortex (Δz = 4.2011); mediodorsal thalamus-entorhinal cortex (Δz = 3.7436); motor cortex-cingulate cortex (Δz = 3.7357); motor cortex-entorhinal cortex (Δz = 3.5392); caudate putamen-retrosplenial cortex (Δz = 3.3252); caudate putamen-nucleus accumbens (Δz = 3.3026); and motor cortex-cingulate cortex (Δz = 3.2042).

Discussion

PhMRI with functional connectivity analysis show the underlying actions of VU0360172 in the rat brain. Correlation analysis of the phMRI responses, a translational approach for characterizing drug-related functional connectivity patterns, provided more information than traditional individual ROI amplitude results, revealing modulation of circuits connected to entorhinal, cingulate, and motor cortices, and striatum. These data support the potential of mGlu5 PAMs for the treatment of the symptoms of schizophrenia.

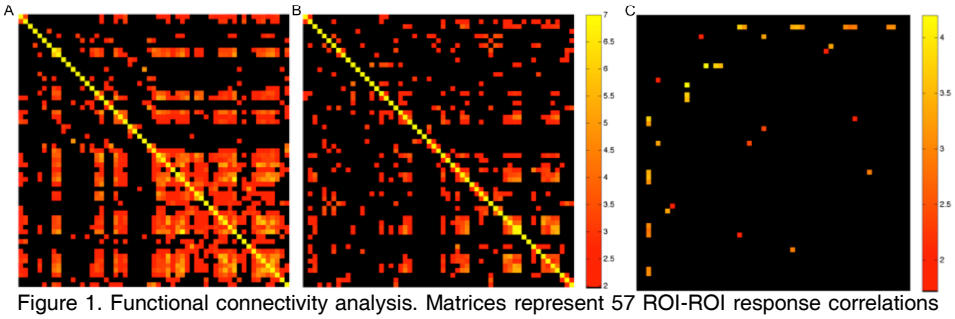

Figure 1. Functional connectivity analysis. Matrices represent 57 ROI-ROI response correlations for the (A) Veh/Amph group and (B) VU0360172/Amph group (n=8-11/group). Pretreatment with the potential antipsychotic VU0360172 modified the amphetamine response; panel B shows decreases in ROI-ROI correlations for the VU0360172/Amph group. Color bar represents Z-scores (thresholded at $p < 0.05$). Permutation analysis identified the significant differences between (A) and (B) shown in matrix C (color bar represents Δz thresholded at $p < 0.05$).

References

1. Rodriguez AL, et al., Mol Pharmacol 2010; 78:1105-23.

2. Mandeville JB et al., Magn Reson Med 1998; 39: 615.