Selective modulation of functional connectivity in the brain's reward pathway by D3 receptor antagonism

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Introduction:

The dopamine D_3 receptor presents a focal distribution in the brain with high expression in the so-called "reward pathways" associated with pleasure seeking, and is over-expressed in the brain of cocaine overdose fatalities. Consistent with this, selective dopamine D_3 receptor antagonists are effective in blocking drug-seeking behaviour in animal models of drug dependence [1]. However, the system-level mechanisms underlying this efficacy remain unclear. Here, we investigated the effects of pre-treatment with a selective D_3 receptor antagonist (SB277011A) in modifying the functional connectivity network activated in response to acute *d*-amphetamine challenge, a pharmacological probe of the dopaminergic system. This extends the application of correlation-based analyses of the pharmacologically activated brain [2] to antagonist-agonist protocols and shows that treatment-induced *modulations* in such functional connectivity patterns can be detected using this phMRI-based approach.

Methods:

Animal preparation: Male Sprague-Dawley rats (N=36, weight mean±SEM=289±6g) were surgically prepared and monitored as detailed previously [3,4] and imaged under 0.8% halothane maintenance anaesthesia, neuromuscular blockade and artificial ventilation.

MR acquisition: Data were acquired using a Bruker Biospec 4.7T system with a quadrature "rat brain" surface receive coil. Spatially coincident T_2-weighted anatomical and functional time series image volumes were acquired using the RARE sequence (phMRI parameters: RARE factor 32, 128×128, FOV 40mm, 16x1mm slices, TR_{eff} = 2700ms, TE_{eff} = 100ms, TA=20s/NEX=4 => dt=80s, N_t=64). 2.67ml/kg of blood pool contrast agent Endorem (Guerbet, France) was injected after 5 time points so that subsequent signal changes would reflect alterations in relative cerebral blood volume (rCBV).

Protocol: 20mg/kg SB277011A or vehicle i.p. 30 min. after contrast agent followed by 1mg/kg *d*-amphetamine (Sigma, Milan, Italy) or vehicle (saline) i.v. a further 30 min. later. Subsequent signal changes were tracked for twenty minutes, capturing the robust initial rCBV changes following i.v. d-amphetamine injection [3]. The study comprised three arms: veh/amp (*N*=17), SB277011A/amp (*N*=12) and veh/veh (*N*=7). (SB277011A did not elicit significant rCBV changes *per se* [3].)

Time series processing: (1) Conversion to rCBV; (2) Spatial normalisation to template [5]; (3) Brain parenchyma mask; (4) 0.8mm (~2.5 pixels) FWHM spatial smoothing; (5) Individual post-injection response amplitude maps calculated using general linear model (GLM) implemented within the FSL package (FEAT).

VOIs: (1) Anatomical VOI delineation using atlas structures co-registered with template [5]; (2) Average time courses for selected VOIs extracted and amplitude quantified using the same design matrix as above.

Correlation maps: Higher-level GLM in each cohort using (a) group mean response and (b) zero-meaned response amplitude across subjects from a selected VOI as explanatory variables. Correlation maps were thresholded using spatially extended clusters of voxels determined by z>2.3 and a corrected cluster significance of p=0.05

Results:

The correlation patterns underlying the rCBV response to *d*-amphetamine present a rich structure, including a focal network of brain regions corresponding to mesolimbic dopamine projections from the ventral tegmental area (VTA) (Fig. 1(b)). In the cohort pre-treated with the selective D_3 receptor antagonist SB277011A, the correlation pattern for *d*-amphetamine was altered with the strongest effect being a region-specific reduction in correlation with the VTA (Fig. 1(c,d)). In particular, a reversal from positive to negative correlation between the VTA and the mediodorsal thalamus (thalMD) was observed (Figs. 1,2).

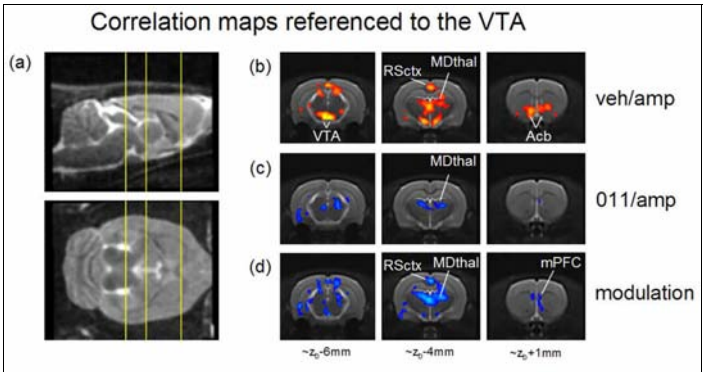

Figure 1: Correlation maps referenced to the VTA. (a) Position reference for slices in (b-d). (b) Control group, showing a focal pattern of correlated responses corresponding to the mesolimbic dopamine projections. (c) Group pre-treated with SB277011A. (d) Map of differences in correlation between the two groups (011/amp – veh/amp).

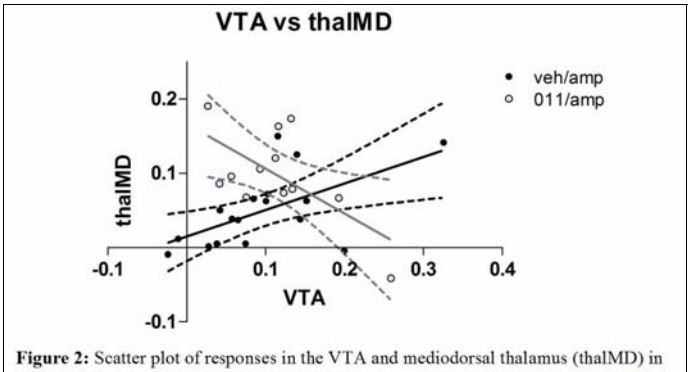

Figure 2: Scatter plot of responses in the VTA and mediodorsal thalamus (thalMD) in control (filled circles) and treated (open circles) groups. Linear regression fits with 95% confidence bands are shown for each group in black and grey respectively. Regression equations are: control group, MDthal = 0.015 + 0.36×VTA, r=0.60, p=0.011; treated group, MDthal = 0.17 – 0.60×VTA, r=-0.64, p=0.026; change, Δr=-1.24, p=0.00075.

Discussion and conclusions:

This study investigated how correlations in the phMRI response to acute *d*-amphetamine between brain regions were modified by the presence of a selective D_3 receptor antagonist. Interpreting correlated responses as an indication of functional connectivity [6], these results suggest that selective blockade of D_3 receptors modifies the functional connectivity in brain regions strongly associated with the VTA and the mesolimbic reward circuit, implicated in drug-seeking behaviour and the process of addiction. The thalamus and hippocampus are also important components of a limbic brain circuit thought to be involved in drug addiction [7], providing modulatory inputs to the prefrontal cortex, a key brain region involved in goal-directed behaviour. The present findings suggest that a modified functional connectivity within the mesolimbic reward circuit may also play a key role in the efficacy of selective dopamine D_3 receptor antagonists in attenuating drug-seeking behaviour [1].

Methodologically, this study demonstrates the applicability of inter-subject functional connectivity analyses of the phMRI response [2] to experiments of an antagonist-agonist design, detecting treatment-induced modulations in the correlation structure underlying the response to a probe signal.

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