In vivo mapping of functional connectivity in active neurotransmitter systems using pharmacological MRI

A. J. Schwarz[1], A. Gozzi[1], T. Reese[1], and A. Bifone[1]

[1]Neuroimaging, GlaxoSmithKline / Psychiatry CEDD, Verona, Veneto, Italy

Introduction:

Pharmacological MRI (phMRI) methods map the haemodynamic response to drug challenge as a surrogate for changes in neuronal activity. Interestingly, there is often variability across subjects not only in overall magnitude but in the *spatial profile* of the response. While this can be unhelpful when calculating univariate group 'activation' maps, it can be harnessed to derive convincing inter-subject correlation maps referenced to selected brain structures. Here we apply an inter-subject analysis of correlations in the amplitude of the phMRI response to map functionally connected brain regions recruited in response to acute pharmacological challenge. Application to *d*-amphetamine and fluoxetine reveals neurotransmitter pathways and functional connectivity patterns underlying the group mean signal changes detected via standard methods. To the best of our knowledge this is the first time these *functional* connections underlying the acute response to a pharmacological stimulus have been visualised *in vivo*.

Methods:

Acquisition: PhMRI time series data were acquired from male Sprague-Dawley rats under 0.8% halothane anaesthesia with the T_2w RARE sequence on a 4.7T Bruker Biospec, using the blood pool contrast agent Endorem (Guerbet, France) to detect signal changes proportional to relative cerebral blood volume (rCBV) [1-4]. The pharmacological challenges for the studies reported here were (1) 1mg/kg *d*-amphetamine (*N*=17) or saline (*N*=7) i.v.; (2) 10mg/kg fluoxetine (*N*=7) or water (*N*=4) i.p.
Analysis: (1) All subjects spatially co-registered to a stereotaxic rat brain template with associated anatomical atlas [5]. (2) Maps of response amplitude (magnitude of post-injection signal change elicited by the acute drug challenge) calculated for each subject. (3) Time courses extracted from atlas-specified VOIs and associated response amplitudes calculated. (4) Correlation maps referenced to selected reference ('seed') VOIs calculated using the cross-subject vector of response amplitudes from step 3. (Correlations with peripheral blood pressure changes were also mapped – neither group evidenced any significant correlation.)

Results:

D-amphetamine: Amphetamine *per se* induced widespread rCBV increases as captured by the univariate group map, with strong but variable responses in most cortical regions and most significant responses in the insular cortex (Fig.1a). However, the correlation map referenced to the ventral tegmental area (VTA), the source of mesolimbic dopamine projections to the ventral forebrain, showed a striking bilateral delineation of these pathways including projections to the lateral habenular region in the mediodorsal thalamus (Fig.1b). The substantia nigra showed a similar, but yet more restricted pattern. In contrast, correlation maps referenced to forebrain cortical regions were more widespread and reminiscent of the group mean response.

Fluoxetine: The univariate group comparison with vehicle showed a predominantly cortical effect (Fig. 2a). Although serotonin projections include extensive innervations of the cerebral cortex, many sub-cortical regions also involved in these pathways and known to be involved in the central effect of fluoxetine were not identified in the group mean map. In contrast, correlation maps referenced to the raphe nuclei and structures in the amygdala, hippocampus, striatum and thalamus indicated a sub-cortical network of correlated responses, including a delineation of the serotonergic projections from the raphe to the forebrain (Fig. 2b).

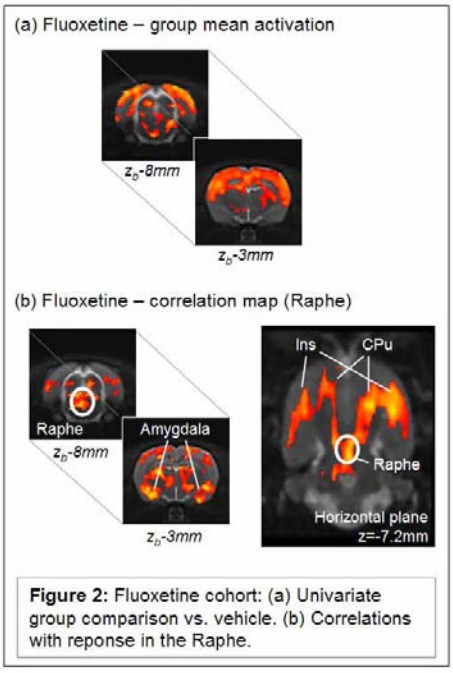

Figure 2: Fluoxetine cohort: (a) Univariate group comparison vs. vehicle. (b) Correlations with reponse in the Raphe.

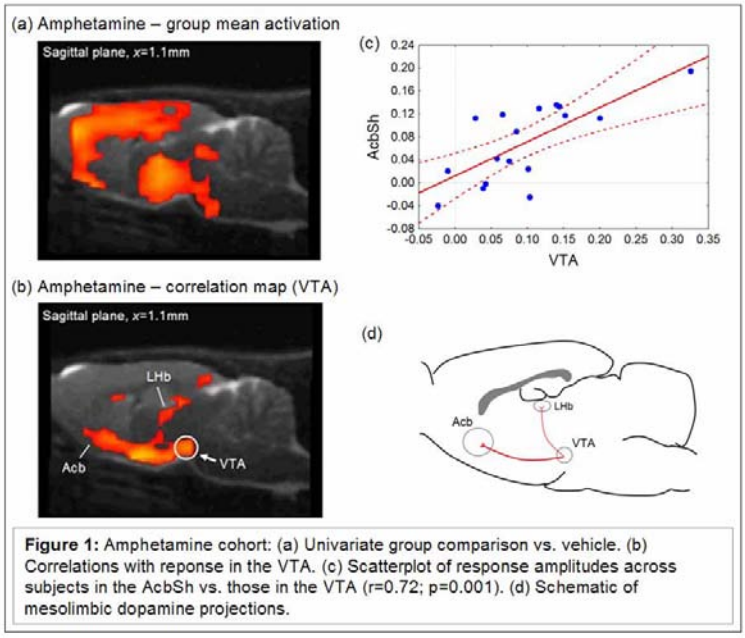

Figure 1: Amphetamine cohort: (a) Univariate group comparison vs. vehicle. (b) Correlations with reponse in the VTA. (c) Scatterplot of response amplitudes across subjects in the AcbSh vs. those in the VTA (r=0.72; p=0.001). (d) Schematic of mesolimbic dopamine projections.

Discussion and conclusions:

Univariate phMRI analyses can identify brain regions involved in the response to the drug, but provide no information regarding the functional relationships between different structures; the resulting maps can be insensitive to weakly responding regions or may integrate the effects of several different neurotransmitter systems or underlying mechanisms; even in the case of selective pharmacology, there can be cross-talk between neurotransmitter systems and recruitment of neural substrates other than those directly targeted by the drug [6]. An analysis of correlated responses provides a more direct indication of functional coupling between different brain structures involved in central drug action. In the present data, inter-subject variability in the spatial response profile of was harnessed to generate correlation maps using the response amplitude across subjects, an approach similar to those used in PET and 2DG.

Correlated response maps were found to delineate key neurotransmitter pathways selectively targeted by these drugs, corroborating the involvement of the targeted neurotransmitter systems in the observed phMRI response. *In vivo* mapping of correlated responses in this way greatly extends the range of information available from phMRI studies and provides a new window into the function of neurotransmitter systems in the active state. This approach may provide important new insights regarding the central systems underlying pharmacological action.

References: [1] Mandeville JB *et al.* (1998) MRM **39** 615. [2] Reese T *et al.* (2000) NMR Biomed **13** 43 [3] Schwarz AJ *et al.* (2004) Synapse **54** 1 [4] Gozzi A *et al.* (2006) Neuropsychopharmacology **31** 1690 [5] Schwarz AJ *et al.* (2006) NeuroImage **32** 538. [6] Reith M *et al.* (1997) Psychopharmacology **134**(3) 309.