CHRONIC EXPOSURE OF NEUROTOXIC DOSES OF D-AMPHETAMINE POTENTIATES THE CENTRAL EFFECT OF AN ACUTE CHALLENGE WITH METHYLPHENIDATE.

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Introduction:

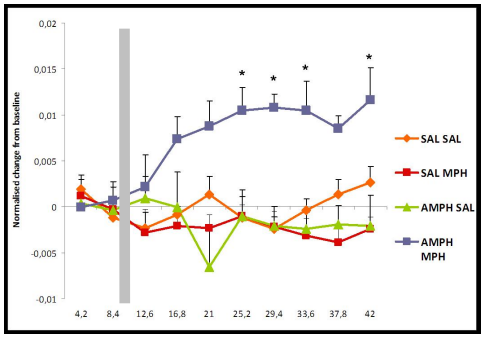
It has been shown that pharmacological magnetic resonance imaging (phMRI) can assess loss of dopamine (DA) neurons by mapping hemodynamic changes following a dopaminergic drug challenge. Chen et al. (1997) demonstrated that unilateral dopaminergic 6-OHDA lesions in rats resulted in a blunted BOLD response following a d-amphetamine (dAMPH) challenge compared to controls. In addition, Jenkins et al. (2004) observed a diminished response to AMPH in MPTP-induced hemiparkinsonian primates. However, it is unclear whether phMRI is able to visualise more subtle changes in the DA system. In order to assess the sensitivity of phMRI as a valid tool to study DA dysfunction, we administered dAMPH to rodents to induce subtle changes in the DA system. We expected that dAMPH treatment would result in a blunted response in brain activity triggered by acute challenge with methylphenidate (MPH) when compared to saline treated animals.

Methods:

Male Sprague-Dawley rats (N=28, Charles River, UK) were treated with dAMPH (5mg/kg s.c. 4 times 2 hrs apart) or saline followed by a 5 day washout before the phMRI experiment. Animals were anaesthetised with 1.5% isoflurane in a 70:30 mixture of medical air and oxygen and then tracheostomised and mechanically ventilated for better control of the pCO2. The right femoral artery was cannulated for blood gas (RapidLab 348, Siemens diagnostic) and blood pressure (Biopac Systems Corp., USA) monitoring. An i.p. cannula was placed for the acute injection of MPH (4mg/kg) or saline during the phMRI experiments. MRI data were acquired on a 4.7T direct drive Varian (now Agilent) MR system using 72 mm inner diameter volume RF coil and 4 phase array coils (m2m Imaging Corp., Cleveland OH, USA)working in volume transmit surface receive mode. For each animal, a T2 weighted anatomical image volume was acquired using a fast spin echo multi-slice sequence (fsems) with an echo train of 8, matrix= 256x256, FOV=35mm, 24 contiguous interleaved 1 mm coronal slices, 4 averages, 2 dummy scans, TR= 5112 ms, and TEeff=60ms. The time series were acquired using a gradient echo multi-slice (gems) sequence with 16 contiguous interleaved 1 mm slices centred to the same position as the anatomical image with TR=260ms, TE=14ms, flip angle 40 deg, 2 averages, 2 dummy scans, FOV=35mm and matrix size of 128x96 (zero-fill to 128x128). Fifty time points (acquisition time per time series volume is 50.5s; total scan time of approximately 42 minutes) were acquired with an injection of the pharmacological challenge after acquisition of volume 12. The data were spatially normalised to a stereotaxic rat brain MRI atlas template (Schwarz et al., 2006). FSL-FEAT 5.90 (Woolrich et al. 2001) was then used for the group analysis using a GLM model of the expected BOLD response. Following the time series acquisition animals were perfused transcardially with saline followed by 4% paraformaldehyde in 0.1m phosphate buffer for subsequent immunohistochemical assessments.

Results:

Group analysis revealed both cortical and subcortical increases in BOLD signal intensity only in the AMPH pre-treated group that received a MPH (AMPH-MPH) challenge compared to the saline pre-treated groups (SAL-SAL and SAL-MPH) and the AMPH pre-treated rats with a saline challenge (AMPH-SAL) (Figure 1 and 2).



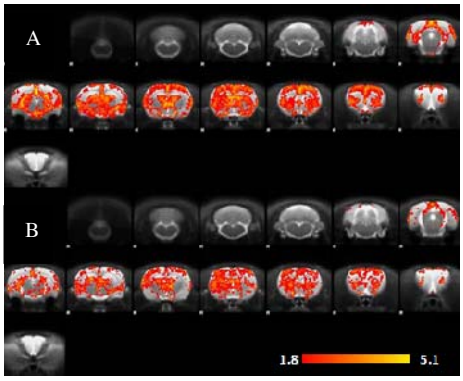


Figure 2. Statistical parametric map of the positive BOLD activation in the AMPH-MPH group (A) and F-test of the interaction between treatment and challenge (B)
(FEAT Version 5.90, part of FSL). Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>1.8 and a (corrected) cluster significance threshold of P=0.05.

Figure 1. Average timecourse of normalised signal intensity change in the striatum. MPH (4 mg/kg i.p.) or saline challenge was administered at volume 12.
* indicate timepoints where groups differ significantly as tested with the Kruskal-Wallis test (p<0.05). The grey bar indicates time of MPH injection.

Discussion:

In contrast to our hypothesis, we observed an increase, rather than a decrease, in brain activity induced by the MPH challenge in the dAMPH pre-treated group. This potentiation of activation induced by MPH reflects the repeated exposure to dAMPH. Further immunohistochemical analysis of the perfusion-fixed brains is ongoing to pinpoint the contribution of different receptor subtypes and transporters to the phMRI signal. Regardless of the underlying mechanism, our results suggest that phMRI is sensitive enough to be able to investigate abnormalities of brain function induced by chronic exposure to drugs.

References:

Chen et al., Mag Res Med 1997; 38(3): 389-398; Jenkins et al., J Neurosci 2004; 24(43): 9553-9560; Schwarz et al., Neuroimage 2006; 32(2):538-550; Woolrich et al., NeuroImage 2001; 14(6):1370-1386