Pharmacological MRI and Tensor-Based Morphometry in the 6-OHDA Rat Model of Parkinson’s Disease

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Introduction The neurotoxin 6-hydroxydopamine (6-OHDA), when administered into the medial forebrain bundle of rats, damages the dopaminergic nigrostriatal pathway. This models parkinsonian features such as reduced dopamine and dopaminergic neural terminals in the striatum. The 6-OHDA rat has been widely used as a test-bed for many symptomatic and neuroprotective agents. The aim of this study was to further evaluate the 6-OHDA rat in terms of structural and functional brain abnormalities in order to identify new biomarkers and provide new targets for therapeutic interventions.

Methods Male adult Sprague-Dawley rats were lesioned by a stereotaxic unilateral injection of 11.76 μg 6-OHDA (n=18) or saline (sham group, n=18) into the left medial forebrain bundle (AP -4.4, ML +1, DV -7.8). Serial structural MR images (sMRI, 7T Varian scanner, FSE T2W images, TEeff 60ms, TR 4000ms, voxel dimensions 0.25x0.125x0.5mm) were acquired before surgery (baseline) and at 48 hours, 3 days, 7 days, 2 weeks, 4 weeks, and 5 weeks post-surgery. Images were analyzed using a tensor-based morphometry (TBM) approach. After rigid-body realignment, scans were non-rigidly registered (fluid) to the group-mean sham baseline images and unequal variance t-tests of the log-Jacobian determinants at each voxel were performed. Significance was assessed using permutation statistics. Resulting statistical parametric maps (SPM’s) are of significant (p<0.01, uncorrected) difference between the lesion and the sham group. At the final (5th week) time-point, pharmacological MRI (phMRI) was performed using a BOLD contrast-sensitive gradient echo technique: 270 whole brain volumes over time, 20 seconds per volume, TE 5,10,15ms, TR 500ms and voxel dimension 0.5x0.5x1mm. The rats were thus continually imaged for 30 min before and 60 min after the administration of dopamine agonist apomorphine (0.1 mg/kg sc). PhMRI data were pre-processed (realigned, normalized to rat brain template and Gaussian smoothed 2x in plane resolution) and statistically analysed (regressor based on behavioural data (not shown) GLM, 2nd order) by SPM8 software (Wellcome Trust Centre for Neuroimaging). Raw time course signal from selected regions of interest (ROI) was extracted and average BOLD signal changes over 10 minutes after injection, relative to baseline, were tested for significant difference by a Student’s t-test.

Results PhMRI revealed a stronger BOLD signal in the lesioned (ipsilateral) than in contralateral side of the 6-OHDA rat brains in several regions including thalamus, frontal cortex and the brain stem, in contrast to the sham where only the bilateral thalamic BOLD signal increase was observed, with very small changes in the other areas (Fig 1). ROI analysis of BOLD signal confirmed a significant asymmetry between the ipsilateral and contralateral thalamus and frontal cortex, and a slight (non-significant) change in the pedunculopontine nuclei (PPN) in the 6-OHDA rats (Fig 2). No such asymmetry was observed in the shams (not shown). The TBM analysis showed an apparent tissue volume contraction in the ipsilateral brain stem at 2 days and 5 weeks post-surgery, and in the ipsilateral frontal cortex at 5 weeks (Fig 3).

Discussion This phMRI data visualizes, for the first time, bilateral thalamic activation after the dopamine agonist apomorphine challenge that is, however, impaired in the unilaterally 6OHDA-lesioned rats. Given that the thalamus is a major relay of efferent transmission from the basal ganglia (BG) to the cortex, and that the unilateral 6-OHDA lesion leads to an increased sensitivity of dopamine receptors (D2) in the ipsilateral striatum, the increased BOLD signal in ipsilateral thalamus in lesioned rats might be related to increased transmission within the BG-thalamo-cortical loop. The increased BOLD signal in the ipsilateral brain stem is less clear but might be linked to an altered neural activity within the brain stem PPN nuclei that are considered as an important contributor to the BG locomotor control. Structural changes within the brain stem (PPN) and frontal cortex raise the question about their relationship to the observed functional impairment. The frontal cortex tissue ‘shrinkage’ is only evident at five weeks indicating long-term effect of the lesioning. However, structural changes in the brain stem already appear two days after 6-OHDA lesioning and are therefore likely to be initiated by a direct effect of 6-OHDA induced neuronal cell loss of the substantia nigra, presumably caused by retrograde dying-back of neurons. This is underpinned by the fact that ~40% of PPN’s neurons send projections to the dopamine-containing neurons of the substantia nigra pars compacta. The structural changes in the brain stem that coincide with functional abnormality in the same area give rise to a structure-function relation which has been shown to be true for PPN in PD patients. Moreover, it could be postulated that in addition to striatal dopamine depletion, PPN degeneration enforces thalamic impairment seen by phMRI.

Conclusion The visualization of structural and functional alterations in the integrity of thalamus, frontal cortex and brain stem in this animal model lends further credence to the validity of 6-OHDA rat for modelling multiple aspects of human PD and may broaden its application for therapeutic intervention targeting the PPN-thalamus-cortex pathway.