MRI reveals brain asymmetry following 6-OHDA lesions in the mouse brain

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Introduction

Injection of the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA) into the striatum or medial forebrain bundle (MFB) is classically used to model Parkinsonism, but is increasingly used in a range of disease models [1]. Standard histological techniques are generally used to study the development and extent of the lesion, but it is not possible to use these techniques *in-vivo*. MRI offers an opportunity to perform non-invasive longitudinal studies of lesion development. Here we present some early work investigating brain morphology *ex vivo* by looking at brain symmetry following a unilateral lesion.

Methods

Six R6/2 [2] and six wild-type (C57/Bl6 x CBA) mice were anaesthetised with isoflurane at twelve weeks and their heads placed in a stereotaxic frame. A craniotomy was made and 6-OHDA injected at the level of the medial forebrain bundle (MFB). Two weeks post-surgery the mice were killed and the brains were perfusion-fixed with 4% PFA. To avoid damage to the cranial surface, olfactory bulbs and paraflocculars lobes of the cerebellum, the brains were not removed from the skull prior to imaging.

Brains were scanned in a proton-free fluid FluorInert FC-70 (3M) using a 4.7T Bruker Pharmascan system with a RARE sequence (TR/TE 2000/32 ms, ETL 8, 2 NEX, FOV 1.79×1.34×0.90cm^3 resolution 70µm isotropic in 3.5 hours). A previously-described mouse brain atlas [2] was made symmetric by flipping along the left-right (LR) axis and averaging with itself and applying a 70µm Gaussian smoothing kernel. Each brain was linearly registered to this image and a copy was flipped LR. Both images were then registered separately again to the symmetric target using a non-linear registration algorithm [4] based on *b*-splines (control point spacing: 10 voxels with a normalised mutual information cost function). Paired images were created of the flipped and unflipped Jacobian determinants in the transformation to the symmetric atlas.

SPM5 [5] was then used to perform a paired *t*-test on the brains to find statistically-significant differences in the local volume changes between left and right sides with genotypic effects excluded as a covariate. False discovery rate (FDR) was used to correct for multiple comparisons.

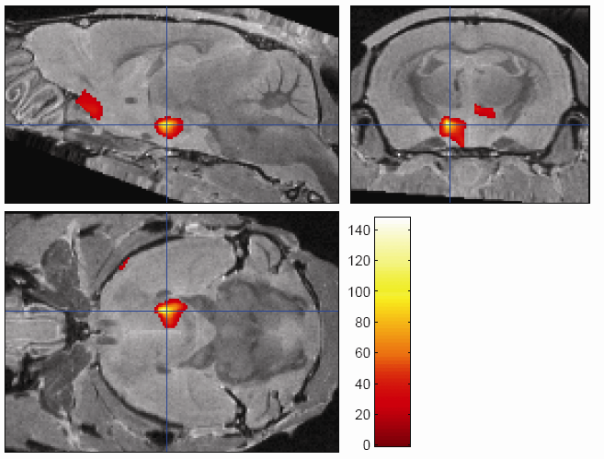
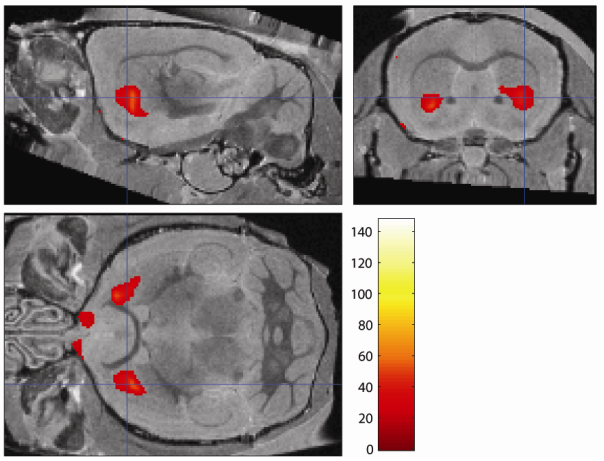


Figure 1 Significant findings shown on a single specimen. Crosshair centred on the MFB lesion site. (scale $F_{1,10}, p_{FDR} < 0.1$)

Figure 2 Differences seen downstream of MFB lesion in the striatum, scale as in Figure 1. Crosshair centred on left caudate putamen.



Results

The method shows a significant asymmetric volume change at the lesion site (MFB) in addition to the anterior striatal regions. As 6-OHDA is known to have a downstream effect on dopaminergic neurons, these results are in line with expectations, as well as histological findings in the literature.

Conclusions

The technique reveals asymmetry in the lesioned brains. Importantly, as it stands this does not discriminate between asymmetry caused by consequences of lesioning and natural asymmetry in the brain. It is intended, however, that this method be used in a longitudinal *in-vivo* study with non-lesioned littermate controls that would avoid these problems to distinguish normal brain asymmetry from that following the lesions and we plan to do this in future studies. The general linear model framework of SPM would readily accommodate these refinements, but this pilot study is promising as it shows MRI can be used to automatically assess the morphological effects of lesioning in the brain.

References

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