Supplementary Online Content


eAppendix. Supplementary Material

This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix. Supplementary Material

Statistical Power

Given lack of prior studies in psychotic bipolar disorder, we used data from our prior studies in schizophrenia 1–3, which show effect sizes of 1.1-1.25 for the elevation in dopamine synthesis capacity, to inform the power calculation. To be conservative we used the lower estimate in a power calculation using G*Power, which determined that a sample size of at least 15 would have greater than 80% power to detect a difference between groups and at least 21 to detect a moderate or greater correlation with symptoms both with an alpha value of <0.05 (two tailed).

\(^{18}\text{F-DOPA PET imaging}\)

\(^{18}\text{F-DOPA synthesis and PET data acquisition}\)

A 17MeV GE PET-trace cyclotron was used for radionuclide production. The gas target was filled with \(^{18}\text{O}_2\) and bombarded at 40 mA for 30 mins followed by a passivation bombardment of 0.1% \(\text{F}_2\) in argon at 20 mA for 20 min. This produced \(^{18}\text{F}-\text{F}\) by the \(^{18}\text{O}\ (p, n)\) \(^{18}\text{F}\) reaction. An electrophilic fluorination procedure was then used to synthesize 6-\(^{18}\text{F}\) fluoro-L-DOPA. In brief, \([^{18}\text{F}]{\text{F}}_2\) was bubbled through a solution of 6-trimethylstannyl-L-DOPA (60 mg) stirring in Deutero-chloroform (5 ml) over 20 mins at 5 1C. 6 M HCl (2 ml) was added and the chloroform evaporated at 70°C. The resulting aqueous mixture was heated at reflux for 10 mins before allowing to cool. The cooled crude mixture was purified by semi-prep high-pressure liquid chromatography polymer column eluting with ammonium acetate buffer. The peak corresponding to \(^{18}\text{F}-\text{L-DOPA}\) eluted at 15 mins was stabilized with 1 mg
ascorbic acid and sodium phosphate dibasic. For quality assurance purposes, a sample was taken from each synthesis and analyzed by reverse phase high-pressure liquid chromatography to confirm identity and purity. To proceed with the injection, a radiochemical purity of 95.0% or higher was required.

**PET data analysis**

Correction for head movement during the scan was performed by denoising the non-attenuation-corrected dynamic images using a level 2, order 64 Battle-Lemarie wavelet filter. Frames were realigned to a single reference frame, acquired 20 mins post-injection, employing a mutual information algorithm. The transformation parameters were then applied to the corresponding attenuated-corrected dynamic images, creating a movement-corrected dynamic image, which was used in the analysis. Realigned frames were then summated to create an individual motion-corrected reference map for the brain tissue segmentation.

**Striatal sub-divisions**

The striatum shows a topographical distribution of inputs, with predominant inputs to antero-ventral regions (the limbic striatum) from orbitofrontal, hippocampal and other limbic brain regions, those to anterior caudate and putamen (associative striatum) from associative cortical regions such as the dorso-lateral prefrontal cortex, and those to posterior putamen (sensorimotor striatum) from sensorimotor cortex.

**PET parametric mapping**

We implemented a previously established method in which the $K_i^{\text{ser}}$ parametric images of the brain were constructed from motion-corrected images using a wavelet-based approach. The parametric image for each participant was then normalized into Montreal
Neurological Institute standard space (matrix dimension: 91x109x91; voxel size: 2mm isotropic) using the participant’s PET summation image and the $^{18}$F-DOPA template. Statistical parametric mapping was conducted using SPM8 using a striatal mask to compare striatal dopamine synthesis capacity between groups, using an independent t-test. Results are presented corrected for multiple comparisons as applied in SPM8 (family wise error rate (FWE) corrected).

**Supplementary Results**

**Striatal subdivisions**

Exploratory analysis of striatal functional subdivisions showed a significant effect of group on $K_i^{cer}$ for limbic ($F (2, 57)=7.54$, $p=0.001$), associative ($F (2, 57)=6.67$, $p=0.002$) and sensorimotor ($F (2, 57)=3.72$, $p=0.03$) subdivisions (see Table 2). Pairwise comparisons showed a significant elevation in the bipolar relative to control group for the limbic ($p=0.001$, Cohen’s $d=1.13$), associative ($p=0.002$, Cohen’s $d=1.01$), and sensorimotor ($p=0.04$, Cohen’s $d=0.67$) subdivisions, and trend for significant elevations in the schizophrenia group relative to control groups for the associative striatum ($p=0.05$, Cohen’s $d=0.91$), though not limbic or sensorimotor subdivisions ($p=0.13$, $p=0.09$). There were no significant differences between the schizophrenia and bipolar groups for any subdivision (associative, $p=0.7$, limbic, $p=0.26$, sensorimotor, $p=0.99$).

**Substantia Nigra**

There was no significant effect of group on $K_i^{cer}$ for the three conditions; bipolar (Mean=$7.2 \times 10^{-3} \text{min}^{-1}$, SD=$0.93 \times 10^{-3} \text{min}^{-1}$), schizophrenia (Mean=$7.18 \times 10^{-3} \text{min}^{-1}$, SD=$0.68 \times 10^{-3} \text{min}^{-1}$) and controls (Mean=$7.08 \times 10^{-3} \text{min}^{-1}$, SD=$0.8 \times 10^{-3} \text{min}^{-1}$), $F (2,57)=1.04$ ($p=0.36$)
SUV reference region analysis

There was no significant effect of group on standardized uptake values in the reference region when tested with ANOVA (F(2,57)=1.99, p=0.168), indicating tracer delivery to cerebellum was not significantly different between groups.

In case differences in uptake in the reference region influenced the findings and given that the direction of effect of the SUV analysis indicated a possible, although non-significant, effect of group on the SUV, we repeated the analysis of the effect of group on striatal $K_{i^c}^{cer}$ using an ANCOVA, with SUV in the reference region as a covariate. This did not change the results, which continued to show a significant effect of group on $K_{i^c}^{cer}$ (F (2,57)=5.74, p=0.01).

Voxel-wise analysis of striatal dopamine synthesis capacity

The voxel-based analysis identified a significantly greater $K_{i}^{cer}$ in the bipolar group relative to the controls, with the peak in the right striatum (Figure 2; Montreal Neurological Imaging (MNI) coordinates for the peak: x=8, y=10, z=-8; p<0.05 corrected for multiple comparisons using the family-wise error rate). The control group > bipolar group contrast revealed no voxels where dopamine synthesis capacity was significantly greater in controls relative to the bipolar group even using a liberal statistical threshold (p<0.05 uncorrected).

The voxel-based analysis identified significantly greater $K_{i}^{cer}$ in the schizophrenia group relative to the controls, with the peak in the left putamen (Figure 3.) The control group > schizophrenia group contrast revealed no significant difference even at a liberal statistical threshold (p<0.05 uncorrected).

The voxel-based analysis did not show any difference between bipolar and schizophrenia groups, irrespective of the contrast applied and, even at a liberal statistical threshold (p<0.05 uncorrected).

References


