

Midbrain dopamine function in schizophrenia and depression: a post-mortem and positron emission tomographic imaging study

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Elevated *in vivo* markers of presynaptic striatal dopamine activity have been a consistent finding in schizophrenia, and include a large effect size elevation in dopamine synthesis capacity. However, it is not known if the dopaminergic dysfunction is limited to the striatal terminals of dopamine neurons, or is also evident in the dopamine neuron cell bodies, which mostly originate in the substantia nigra. The aim of our studies was therefore to determine whether dopamine synthesis capacity is altered in the substantia nigra of people with schizophrenia, and how this relates to symptoms. In a post-mortem study, a semi-quantitative analysis of tyrosine hydroxylase staining was conducted in nigral dopaminergic cells from post-mortem tissue from patients with schizophrenia ($n = 12$), major depressive disorder ($n = 13$) and matched control subjects ($n = 13$). In an *in vivo* imaging study, nigral and striatal dopaminergic function was measured in patients with schizophrenia ($n = 29$) and matched healthy control subjects ($n = 29$) using ^{18}F -dihydroxyphenyl-L-alanine (^{18}F -DOPA) positron emission tomography. In the post-mortem study we found that tyrosine hydroxylase staining was significantly increased in nigral dopaminergic neurons in schizophrenia compared with both control subjects ($P < 0.001$) and major depressive disorder ($P < 0.001$). There was no significant difference in tyrosine hydroxylase staining between control subjects and patients with major depressive disorder, indicating that the elevation in schizophrenia is not a non-specific indicator of psychiatric illness. In the *in vivo* imaging study we found that ^{18}F -dihydroxyphenyl-L-alanine uptake was elevated in both the substantia nigra and in the striatum of patients with schizophrenia (effect sizes = 0.85, $P = 0.003$ and 1.14, $P < 0.0001$, respectively) and, in the voxel-based analysis, was elevated in the right nigra ($P < 0.05$ corrected for family wise-error). Furthermore, nigral ^{18}F -dihydroxyphenyl-L-alanine uptake was positively related with the severity of symptoms ($r = 0.39$, $P = 0.035$) in patients. However, whereas nigral and striatal ^{18}F -dihydroxyphenyl-L-alanine uptake were positively related in control subjects ($r = 0.63$, $P < 0.001$), this was not the case in patients ($r = 0.30$, $P = 0.11$). These findings indicate that elevated dopamine synthesis capacity is seen in the nigral origin of dopamine neurons as well as their striatal terminals in schizophrenia, and is linked to symptom severity in patients.

Keywords: dopamine; psychosis; depression; substantia nigra; schizophrenia; F-DOPA; dihydroxyphenyl-L-alanine; brain imaging; striatum; tyrosine; post-mortem

Abbreviations: DOPA = dihydroxyphenyl-L-alanine

Introduction

The dopamine hypothesis of schizophrenia proposes that subcortical dopamine dysfunction underlies the illness (Davis *et al.*, 1991; Howes and Kapur, 2009). Recent revisions of the hypothesis have specified a critical role for presynaptic dopamine dysfunction, in contrast to the earlier focus on postsynaptic dopamine D2 receptors (Howes and Kapur, 2009; Lyon *et al.*, 2011). Supporting this there is now a large body of evidence for presynaptic dopaminergic dysfunction in the striatum in schizophrenia (Howes *et al.*, 2007; Meisenzahl *et al.*, 2007; Lyon *et al.*, 2011). Specifically schizophrenia is associated with elevations in striatal dopamine synthesis capacity, as measured using the PET radiotracers L-[β - ^{11}C]-dihydroxyphenyl-L-alanine or 6-[^{18}F]fluoro-dihydroxyphenyl-L-alanine (^{18}F -DOPA; Reith *et al.*, 1994; Hietala *et al.*, 1995, 1999; Dao-Castellana *et al.*, 1997; Lindstrom *et al.*, 1999; Elkashef *et al.*, 2000; Meyer-Lindenberg *et al.*, 2002; McGowan *et al.*, 2004; Kumakura *et al.*, 2007; Bose *et al.*, 2008; Howes *et al.*, 2009b; Nozaki *et al.*, 2009; Shotbolt *et al.*, 2011; Demjaha *et al.*, 2012), and elevated baseline and stimulated striatal dopamine levels (Laruelle *et al.*, 1996; Breier *et al.*, 1997; Abi-Dargham *et al.*, 1998, 2000, 2009; Mizrahi *et al.*, 2012). Increased striatal dopamine synthesis capacity is also evident in individuals with prodromal symptoms of schizophrenia, suggesting that the dopaminergic abnormality predates the onset of frank psychosis (Howes *et al.*, 2009b, 2011a,b; Egerton *et al.*, 2013). However, it is not elevated in non-psychotic depression (Martinot *et al.*, 2001) or in people with persistent subclinical psychotic symptoms who have not developed a psychotic disorder (Howes *et al.*, 2011b, 2013), suggesting specificity to psychotic illness (Howes *et al.*, 2007, 2009a).

Recent evidence indicates that these alterations in dopamine function are particularly apparent in the associative subdivision of the striatum (Howes *et al.*, 2009b; Kegeles *et al.*, 2010), which predominantly receives projections from the substantia nigra (Haber *et al.*, 2000, 2006; Joel and Weiner, 2000).

This suggests that they may reflect changes to dopaminergic neurons in the substantia nigra. Dopamine is synthesized from tyrosine in a two-step process: tyrosine hydroxylase converts tyrosine into dihydroxyphenyl-L-alanine (DOPA), which is then converted by aromatic acid decarboxylase into dopamine (Fernstrom and Fernstrom, 2007). Both enzymes are regulated, but the rate limiting step in dopamine synthesis is the conversion of tyrosine to dihydroxyphenyl-L-alanine (Zhu *et al.*, 1993; Cumming *et al.*, 1995, 1997; Fernstrom and Fernstrom, 2007).

Post-mortem studies have found altered tyrosine hydroxylase messenger RNA levels, increased tyrosine hydroxylase activity and increased variability in tyrosine hydroxylase levels in the substantia nigra of patients with schizophrenia compared to control subjects in some (Toru *et al.*, 1988; Mueller *et al.*, 2004; Perez-Costas *et al.*, 2012) but not all studies (Ichinose *et al.*, 1994). In addition one PET study has reported increased turnover of dopamine in the midbrain of patients with schizophrenia compared with matched control subjects (Kumakura *et al.*, 2007). However, as the midbrain region of interest was an order of magnitude larger than the substantia nigra (Ahsan *et al.*, 2007; Duzel

et al., 2009), it is unclear from this study whether the abnormalities reported represent dysregulated nigral dopaminergic function or dysfunction in other midbrain neurons. We have recently shown that it is possible to reliably index dopaminergic function in the substantia nigra *in vivo* using ^{18}F -DOPA-PET in humans (Egerton *et al.*, 2009), enabling it to be specifically studied in schizophrenia.

We hypothesized that dopamine synthesis capacity in schizophrenia will be increased in the substantia nigra as well as in the striatum. We tested this hypothesis in two complementary studies of different aspects of dopamine synthesis in the substantia nigra. The first study used post-mortem tissue to investigate tyrosine hydroxylase levels *ex vivo*. Non-disorder specific factors, such as suicide as cause of death, are potential confounds in post-mortem studies of psychiatric disorders (Biegon and Fieldust, 1992). For this reason we also included a psychiatric comparator group comprising samples from patients diagnosed with major depressive disorder. We hypothesized that tyrosine hydroxylase levels would be elevated in schizophrenia in comparison with both healthy control and major depression groups. The second study used ^{18}F -DOPA imaging *in vivo*, which measures the uptake and conversion of ^{18}F -DOPA into ^{18}F -fluorodopamine by aromatic acid decarboxylase in dopamine neurons (Kumakura and Cumming, 2009). We hypothesized that this would also be elevated in the substantia nigra in schizophrenia in comparison with healthy control subjects.

Materials and methods

Post-mortem study

Sample

This study was approved by the London South West Local Ethics Committee. Post-mortem tissue from 12 patients with schizophrenia was compared with that from 13 patients with major depressive disorder and 13 healthy control subjects matched for age and post-mortem interval (see Supplementary Tables 1 and 2 for case details). To be included, patients had to have met International Classification of Disease-10 criteria for schizophrenia or recurrent major depressive disorder. Controls were required to have no history of psychiatric or neurological disorder. Exclusion criteria for all subjects were: any evidence of neurodegenerative, neurovascular or infectious pathology, including Parkinson's disease, at post-mortem conducted by a consultant pathologist, or any recorded history of alcohol or drug abuse. Samples were selected at random from the Corcellis Tissue Bank (Supplementary material).

Tissue preparation and immunohistochemical staining

Transverse sections of the substantia nigra at the level of the superior colliculus were obtained from formalin-fixed, paraffin-embedded tissue (Supplementary Fig. 2). Each block was sectioned in coronal plane at 10 μm thickness using a microtome (Thermo Shandon Finesse) and mounted onto electrostatic glass slides. The first mounted 10 μm section of each specimen was stained with haematoxylin and eosin for anatomical identification of the substantia nigra as described in the Supplementary material. The adjacent section in each hemisphere, giving two sections per case, was then prepared for

immunohistochemical staining (Supplementary material), which was conducted by incubation with horseradish peroxidase-conjugated secondary antibody (Dako K4002) for 30 min at room temperature before further washing (Supplementary material), counterstaining and mounting. Slides were randomized and blinded by an independent investigator before image capture.

Image capture and quantification

Images were captured using an Olympus Vanox AHBT 3 microscope with Q Imaging Micropublisher RTV 3.3 camera and analysed using Image-Pro Plus 5.1 software (Media Cybernetics). The nigra was identified to ensure measures incorporated the entire nucleus (Supplementary material). Sample cells showing no staining, moderate staining and heavy staining were captured at $\times 400$ magnification and used as standards (Supplementary Fig. 1). The medial region of each tyrosine hydroxylase-stained nigral section was imaged at $\times 40$ magnification. All dopaminergic cells (identified as large, oval stained neurons containing neuromelanin and a counterstained nucleus) were tagged. A random number generator (www.graphpad.com) was used to select eight unique neurons which were imaged at $\times 400$ magnification and semi-quantitatively assessed for tyrosine hydroxylase expression using the following scale: 0 = no staining visible in the cytoplasm; 1 = moderate staining visible in the cytoplasm distinct from neuromelanin; 2 = heavy staining forming a clear halo around the nucleus and intense staining observed elsewhere in the cytoplasm (Supplementary Fig. 1).

In vivo imaging study

Subjects

The study was approved by the Hammersmith Hospital research ethics committee. Following complete description of the study, all subjects gave written informed consent to participate. Twenty-nine subjects meeting International Classification of Disease-10 criteria for schizophrenia were recruited from outpatient clinics in London, and included 13 antipsychotic-free patients (no antipsychotic treatment for at least 3 months before scanning, drug free $n = 8$; drug naive $n = 5$) and 16 antipsychotic-treated patients (drugs listed in Supplementary material). They were compared with 29 healthy control subjects recruited contemporaneously from the same geographical area of London. See Supplementary material for further details on the subjects.

Clinical assessment

Subjects were assessed at the time of scanning using the Comprehensive Assessment of Symptoms and History to assess current symptoms and clinical characteristics (Andreasen, 1987). Symptom severity scores were calculated for positive, negative and affective symptoms by summing the scores for symptoms in each domain, and for all symptoms combined (total symptom severity score). Additionally all subjects received assessment of recreational exposure to psychoactive substances by semi-structured questionnaire [modified from Barkus *et al.* (2006)].

Positron emission tomography scanning

The PET data were acquired using the 966 ECAT/EXACT3D PET scanner (Siemens/CTI). All subjects were positioned in the tomograph so that the orbitomeatal line was parallel to the transaxial plane of the tomography, and head position was monitored via laser crosshairs and a camera. A 5-min transmission scan was carried out using a 150 MBq

cesium-137 rotating point source for attenuation and scatter correction. Data were acquired in list mode for 95 min.

Image analysis

All PET scan image preprocessing and analysis was performed using fully automated methods and blind to group status using a region of interest approach to test the primary hypothesis. Standardized regions in Montreal Neurologic Institute (MNI) space were defined in the cerebellum (the reference region) and regions of interest: substantia nigra (left and right sides combined; Supplementary Fig. 2A); striatum (left and right sides combined) using the HamNet atlas, a probabilistic atlas of standard brain regions (Ahsan *et al.*, 2007). This approach has shown good reliability in delineating these structures, including after spatial normalization (Egerton *et al.*, 2009). Although it was not the focus of this study, as recent findings suggest that dopaminergic alterations are most marked in the associative subdivision of the striatum (Howes *et al.*, 2009b; Kegeles *et al.*, 2010), we also conducted exploratory analyses of the striatal functional subdivisions defined as previously described (Howes *et al.*, 2009b). The region of interest map was normalized together with an ^{18}F -DOPA template (which aids normalization) to each individual PET summation image using statistical parametric mapping (Statistical Parametric Mapping 5; Wellcome Department of Cognitive Neurology, London, England) and then used to sample the dynamic PET images. The positioning of the nigral region of interest following normalization to individual dynamic images is illustrated in Supplementary Fig. 2B. A Patlak graphical analysis was used to calculate the influx constant (K_1^{cer} value) for the specific uptake of ^{18}F -DOPA in the regions of interest relative to uptake in the reference region (Patlak *et al.*, 1983). To investigate subregional and whole-brain differences, a voxel-wise analysis was conducted using statistical parametric mapping (Supplementary material).

Statistical analysis

All statistical analyses were performed using Windows SPSS 18.0.

Post-mortem study

Analysis of variance was used to determine if there was an effect of group on age, sex or post-mortem interval. The primary analysis used a Kruskal-Wallis test to determine if there was an effect of diagnostic group (control, schizophrenia, major depressive disorder) on staining score. Where there was a significant effect of group, planned *post hoc* Mann-Whitney U-tests adjusted for multiple comparisons using Bonferroni correction, were used to determine if there was a significant difference between the schizophrenia, major depressive disorder and control groups. To determine if age, sex, or post-mortem interval moderated the effect, a secondary analysis of variance was used to determine the effect of diagnostic group on staining score co-varying for these variables.

In vivo imaging study

All variables showed a normal distribution and equal variances with the exception of cigarette smoking and symptom scores. Group differences in radiochemistry, demographic and clinical measures were tested using independent *t*-tests for parametric variables and Mann-Whitney U-tests for non-normally distributed data. Regional K_1^{cer} values in schizophrenic patients versus control subjects were compared using independent *t*-tests. The relationship between nigral K_1^{cer} values and clinical variables was explored using Pearson's correlation coefficients. Where there was a group difference in nigral or striatal K_1^{cer} values, planned *post hoc* independent *t*-tests with Bonferroni

correction for multiple comparisons were used to determine if this was also the case when the analysis was restricted to the subgroup of patients who were drug-free compared with control subjects. All tests were two-tailed and $P < 0.05$ was considered significant. Glass' delta effect sizes were calculated for significant differences between groups. Exploratory analyses compared K_i^{cer} values in striatal subdivisions in schizophrenic patients versus control subjects using independent t -tests.

Results

Post-mortem study

There was no significant effect of group on age [$F(2,37) = 2.9$; $P = 0.07$], sex [$F(2,37) = 3.1$; $P = 0.06$], or post-mortem interval [$F(2,37) = 2.4$; $P = 0.11$]; see Supplementary Tables 1 and 2 for details.

There was a significant main effect of group on tyrosine hydroxylase staining score ($X^2 = 21.9$, $df = 2$, $P < 0.001$, Fig. 1). *Post hoc* tests showed that tyrosine hydroxylase staining score was significantly greater in the schizophrenia group compared with both the control group [median (interquartile range, IQR) score for control group = 0.38 (0.31); schizophrenia group = 1.56 (0.51); Bonferroni corrected $P < 0.001$] and the major depressive disorder group [median (IQR) score major depressive disorder group = 0.37 (0.31); Bonferroni corrected $P < 0.001$]. There was no significant difference in staining score between the control and major depressive disorder groups ($P > 0.9$). The effect of group on tyrosine hydroxylase staining score was also highly significant after co-varying for age, sex and post-mortem interval [$F(2,37) = 30.3$; $P < 0.001$].

In vivo imaging study

Radiochemistry, demographic and clinical characteristics

There were no significant differences between groups in the dose of ^{18}F -DOPA injected [mean (SD) for control subjects = 136.8 (17.8) and schizophrenia = 129.6 (19.3) MBq; $t(54) = 1.5$, $P = 0.15$], or its specific activity [mean (SD) for control subjects = 27.8 (17.8) and schizophrenia = 20.2 (14.3) MBq/ μmol ; $t(53) = 1.7$, $P = 0.09$].

There was no significant difference in age or sex between groups [mean age (SD): control subjects = 29.3 (7.5), schizophrenia = 33.7 (10.6) years, $t(56) = 1.8$, $P = 0.07$; sex: control subjects = 22 males, 75.8%; schizophrenia = 26 males, 89.7%; $z = 1.4$, $P = 0.17$].

There was no significant difference between groups in cigarette consumption or alcohol use [median (IQR) cigarette use for control subjects = 0.1 (7) and schizophrenia < 0.1 (10) cigarettes/day, $Z = 0.6$, $P = 0.54$; median (IQR) alcohol use for control subjects = 6 (10) and schizophrenia = 2 (8) units/week, $Z = 1.2$, $P = 0.24$].

In the schizophrenia group the mean (SD) symptom severity scores were: total symptoms = 77.6 (47.6); positive symptoms = 38.3 (30); affective symptoms = 2.0 (3.9); negative symptoms = 31.9 (22.9).

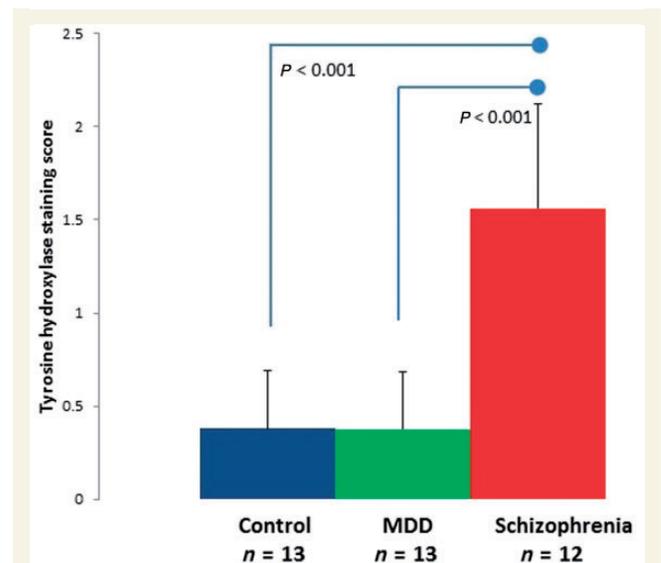


Figure 1 Median (IQR) staining scores for tyrosine hydroxylase levels in substantia nigra for control subjects ($n = 13$); major depressive disorder (MDD, $n = 13$); and schizophrenia ($n = 12$).

Nigral and striatal dopaminergic function

Region of interest analysis

The mean K_i^{cer} value in the substantia nigra was significantly elevated in the schizophrenic group compared with the control group with an effect size of 0.85 [$t = 3.2$ (56), $P = 0.003$; Fig. 2 and Table 1]. *Post hoc* tests showed that this remained the case when the analysis was restricted to the drug-free schizophrenic subjects ($n = 13$; Bonferroni corrected $P = 0.016$; effect size = 1.02, Table 1). However, there was no significant difference between control subjects and drug-treated patients ($n = 16$; Bonferroni corrected $P = 0.086$, Table 1).

The mean K_i^{cer} value in the striatum was also significantly elevated in the schizophrenic group compared to the control group with an effect size of 1.14 [$t = 4.4$ (56), $P < 0.0001$; Table 1]. This remained the case when the analysis was restricted to the drug-free schizophrenic subjects ($n = 13$; Bonferroni corrected $P < 0.0001$; effect size = 1.57; Table 1), and when restricted to drug-treated patients ($n = 16$; Bonferroni corrected $P = 0.012$; effect size = 0.87; Table 1). There was no significant difference between K_i^{cer} values in drug-free and drug treated patients in either the nigra ($P = 0.28$) or striatum ($P = 0.20$). In the patients who were receiving antipsychotic treatment, there was no relationship between antipsychotic treatment duration and nigral ($r = 0.35$, $P = 0.21$) or striatal ($r = 0.05$, $P = 0.85$) K_i^{cer} values.

Exploratory analyses of the striatal subdivisions indicated that there was a significant elevation in schizophrenia in the associative [mean (SD) K_i^{cer} value/min for control subjects = 0.0131 (0.0015), schizophrenia = 0.0146 (0.0017); $P = 0.001$], sensorimotor [mean (SD) K_i^{cer} value/min for control subjects = 0.0149 (0.0019), schizophrenia = 0.0167 (0.0020); $P = 0.001$] and limbic [mean (SD) K_i^{cer} value/min for control subjects = 0.0138 (0.0016), schizophrenia = 0.0148 (0.0015); $P = 0.017$] subdivisions.

Voxel-based analysis

The voxel-based analysis within the nigra identified a significant elevation in schizophrenia in the right substantia nigra (Fig. 3, peak: family-wise error corrected $P = 0.031$; MNI coordinates 12, -18 , -10), and no voxels where the control subjects were greater than the schizophrenic group (even at an uncorrected $P < 0.05$). The whole brain voxel-based analysis supported the region of interest analyses, showing significantly elevated dopaminergic function in schizophrenia in two clusters, one including the striatum and the other with a peak in the nigra (Supplementary Fig. 5A; peak right nigra: MNI coordinates 12, -18 , -10), whereas the contrast control subjects $>$ patients did not identify any regions where there were significant elevations in control subjects (Supplementary Fig. 5B).

The relationship between nigral and striatal ^{18}F -DOPA uptake

There was a significant positive relationship between nigral K_i^{cer} values and striatal K_i^{cer} values in the control subjects ($r = 0.631$,

$P < 0.001$, Supplementary Fig. 3) that was not evident in the schizophrenic group as a whole ($r = 0.303$, $P = 0.11$), or in either the drug-free ($r = 0.28$, $P = 0.36$) or drug-treated subgroups ($r = 0.26$, $P = 0.33$). However, there was no significant difference between these correlation coefficients ($Z > 1.1$, $P > 0.11$ in all cases).

The relationship between nigral dopaminergic function and symptoms

There was a positive relationship between total symptom scores and nigral K_i^{cer} values ($r = 0.39$, $P = 0.035$, Supplementary Fig. 4A) but not with striatal K_i^{cer} value ($r = 0.27$, $P = 0.16$). Sub-analyses found there was a significant direct relationship between nigral K_i^{cer} values and positive symptom subscale score ($r = 0.37$, $P = 0.048$), but this did not survive correction for multiple comparisons and was not significant when the sample was restricted to the drug-free or drug-treated patients. There was also a significant direct relationship between striatal K_i^{cer} values and positive symptom subscale score in the drug-free patients ($r = 0.60$, $P = 0.029$), although this did not survive correction for multiple comparisons and was not significant in the whole sample or drug-treated patients alone. There were no significant relationships between nigral

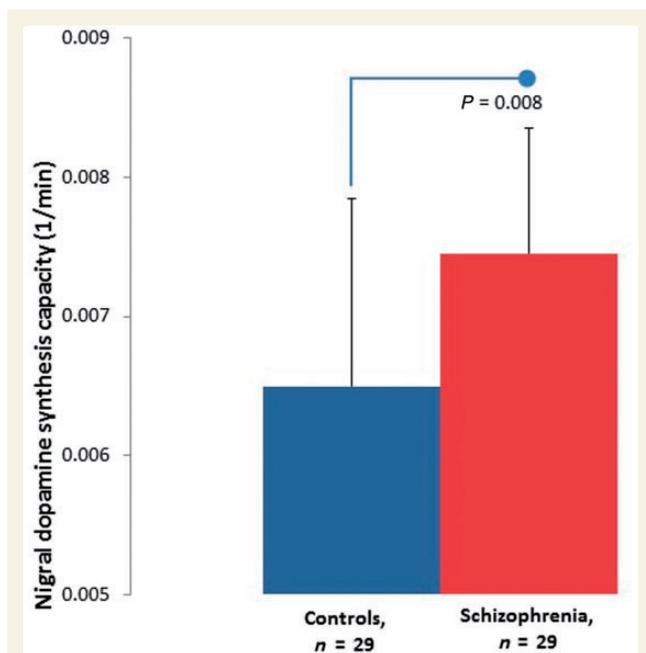


Figure 2 Mean (SD) dopamine synthesis capacity (K_i^{cer} /min) in the substantia nigra in patients with schizophrenia and control subjects. There was a significant elevation in dopamine synthesis capacity in the schizophrenia group compared with control subjects.

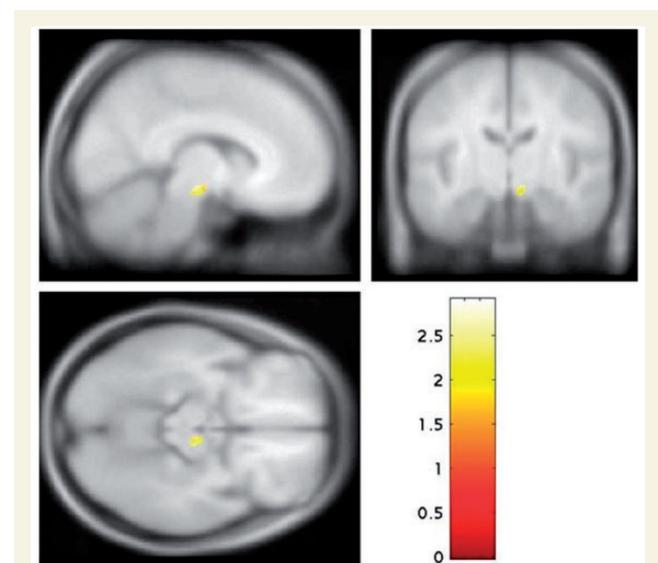


Figure 3 Statistical parametric map of increased K_i^{cer} values in schizophrenia relative to control subjects for the substantia nigra rendered on a T_1 template (peak at MNI coordinates: 12, -18 , -10 ; FWE $P < 0.05$).

Table 1 In vivo dopamine synthesis capacity in control and schizophrenia groups

Region	Control subjects $n = 29$	Schizophrenia total sample, $n = 29$	Schizophrenia drug-treated, $n = 16$	Schizophrenia drug free, $n = 13$
Substantia nigra	0.0066 (0.0017)	0.0075** (0.0009)	0.0073 (0.0009)	0.0077* (0.0009)
Whole striatum	0.0134 (0.0015)	0.0152*** (0.0016)	0.0148* (0.0017)	0.0156*** (0.0013)

Mean [(SD) in min^{-1}] ^{18}F -DOPA K_i^{cer} values in the substantia nigra and striatum. Significant differences between control and schizophrenia groups are indicated: *** $P < 0.0001$, ** $P < 0.005$, * $P < 0.05$.

or striatal K_i^{cer} values and negative symptom subscale scores in the whole sample or either sub-group.

Discussion

Our major findings are that two measures of dopamine synthesis capacity, one *ex vivo* and one *in vivo*, were elevated in the substantia nigra in schizophrenia. In addition, greater nigral ^{18}F -DOPA uptake was linked to greater symptom severity, indicating a link to the clinical expression of the disorder. These findings indicate that elevated dopamine synthesis capacity is evident in the nigral cell bodies of dopamine neurons, and not just in their striatal terminals (Howes *et al.*, 2012a). They extend a previous finding of increased dopamine turnover in the midbrain (Kumakura *et al.*, 2007) to indicate that dopamine synthesis capacity is elevated specifically in the substantia nigra in schizophrenia. Taken with evidence for increased dopamine D2, but not D3 or dopamine transporter, availability in the substantia nigra (Arakawa *et al.*, 2009; Graff-Guerrero *et al.*, 2009; Kessler *et al.*, 2009; Mizrahi *et al.*, 2011), these findings indicate that particular aspects of nigral dopaminergic function are abnormal in schizophrenia.

We found a direct relationship between striatal and nigral ^{18}F -DOPA uptake in control subjects. This extends to human findings in non-human primates, which have also shown this relationship between striatal and nigral ^{18}F -DOPA uptake (Brown *et al.*, 2013). In contrast, the relationship between striatal and nigral ^{18}F -DOPA uptake was not present in schizophrenia, suggesting a decoupling of the normal relationship between dopamine synthesis capacity in the cell bodies and that in the terminal region. Whereas a non-human primate model of dopamine loss also found a loss of this normal coupling between striatal and nigral ^{18}F -DOPA uptake (Brown *et al.*, 2013), studies in pre-clinical models are required to determine the significance of this decoupling in the context of increased ^{18}F -DOPA uptake, as is the case in schizophrenia. Finally our finding of no alteration in nigral tyrosine hydroxylase staining in patients with major depression extends previous findings in this patient population of altered tyrosine hydroxylase levels in the locus coeruleus (Baumann *et al.*, 1999; Zhu *et al.*, 1999; Gos *et al.*, 2008). It suggests that, although tyrosine hydroxylase alterations in depressive disorder are present in a norepinephrinergic region, they are not present in a dopaminergic region, at least in our sample where the cause of death was predominantly suicide.

Methodological considerations

Some of the schizophrenic patients we studied were taking antipsychotic treatment. Although we cannot exclude an effect of treatment on the post-mortem tyrosine hydroxylase levels (as all the schizophrenia subjects had taken antipsychotics), rodent studies indicate that antipsychotic treatment either does not alter (Perez-Costas *et al.*, 2012) or reduces tyrosine hydroxylase levels (Tejedor-Real *et al.*, 2003). Furthermore the elevation in nigral and striatal K_i^{cer} values in our *in vivo* imaging study was also observed in untreated patients, indicating that the elevation is not secondary to current antipsychotic treatment. Whereas the sub-group

analyses of nigral K_i^{cer} values found that there was a significant elevation in the drug free patients compared to control subjects, there was no significant difference between the drug-treated group and either the drug free or control groups, suggesting the drug-treated group may be intermediate between the other groups. This potential effect of treatment (Grunder *et al.*, 2003) warrants further investigation in a larger sample. Nevertheless tyrosine hydroxylase staining was significantly elevated in patients who had received antipsychotics ante-mortem. This could indicate that there is a greater elevation in tyrosine hydroxylase than aromatic acid decarboxylase, but is probably because of the greater sensitivity of the tyrosine hydroxylase staining and a lack of statistical power in the sub-analysis of treated patients in the imaging study. A study in the midbrain did not find increased K_i^{cer} values in schizophrenia (Kumakura *et al.*, 2007). This apparent discrepancy with our findings could be because, in contrast to our study, this study did not use entacapone to block the formation of the radiometabolite 6-[F-18]fluoro-3-O-methyl-L-dihydroxyphenyl-L-alanine, which can cross the blood-brain barrier and reduce the specific signal. Supporting this interpretation, they did find a significant elevation in ^{18}F -DOPA uptake in the midbrain after they corrected for this plasma metabolite, in line with our findings in the nigra.

A general limitation of post-mortem studies is that clinical information is retrospectively obtained from medical notes. To reduce this risk, in the post-mortem study we only included patients with well documented symptoms who clearly met criteria for either schizophrenia or major depressive disorder, and control subjects where these disorders were positively excluded. Semi-quantitative analysis of staining, as used here, does not provide absolute protein levels and may be less sensitive than fully quantitative approaches, although, as lower sensitivity would militate against finding an elevation in schizophrenia, this is unlikely to change our findings. Our finding of increased nigral tyrosine hydroxylase staining is in agreement with previous findings of increased nigral tyrosine hydroxylase activity (Toru *et al.*, 1988) and tyrosine hydroxylase messenger RNA levels in schizophrenia (Mueller *et al.*, 2004). However, it is important to note that messenger RNA levels have been found to be unaltered in two studies, and, additionally tyrosine hydroxylase protein levels were reduced in one of these studies (Ichinose *et al.*, 1994; Perez-Costas *et al.*, 2012). This discrepancy may reflect the fact that our analysis specifically examined dopamine neurons in the nigra, whereas the other study used homogenates of tissue from the nigra, which, if the tissue samples contained different numbers of dopamine neurons, may have introduced sampling effects.

The cause of death was suicide in the majority (9 of 13) of the major depression cases. This suggests that they had severe illness. Although this serves as a useful psychiatric control for the schizophrenia group, indicating that the findings in the schizophrenia group are unlikely to be due to suicide and/or psychiatric illness *per se*, it may limit the generalizability of the findings to less severe cases of depression.

Bias in the positioning of region of interests may influence results in neuroimaging studies, but this is unlikely to have been a factor in the *in vivo* imaging, as the image analysis was automated and conducted blind to diagnosis. Our automatic atlas-based

approach could be affected by a systematic group difference in the transformation of the standardized atlas to native space. However, the whole brain analysis identified an elevation in the same regions in schizophrenia and did not identify any regions where there was a significant elevation in ^{18}F -DOPA uptake in control subjects, supporting our region of interest findings. An abnormality in the reference region is also unlikely to explain the results as previous studies have reported elevated striatal dopamine synthesis capacity in schizophrenia using other reference regions (Hietala *et al.*, 1995; McGowan *et al.*, 2004). Partial volume and spill-over effects can affect measurements in small regions of interest but are unlikely to account for the elevation in the nigra as nigral volumes are, if anything, reduced in schizophrenia (Bogerts *et al.*, 1983), and these effects would thus tend to underestimate the K_1^{cer} values. In contrast to recent findings that the striatal dopamine elevation is most marked in the associative subregion (Howes *et al.*, 2009b, 2011b; Kegeles *et al.*, 2010), we found significant elevations in all three striatal subregions. This may reflect the greater power of the larger sample size in our current study to detect small differences. Additionally we cannot exclude spill-over effects, which are more influential for small regions such as the limbic striatum (Kim *et al.*, 2013b). Nevertheless, the smallest alteration was seen in the limbic striatum, in keeping with the previous studies (Howes *et al.*, 2009b, 2011b; Kegeles *et al.*, 2010). Finally, it is possible that less efficient blockade of peripheral ^{18}F -DOPA metabolism in the schizophrenia group could have resulted in elevated brain penetrant radiometabolite levels (Asselin *et al.*, 2007). However, as this would serve to mask an elevation (Asselin *et al.*, 2007), it would not explain our findings. Furthermore, no difference in ^{18}F -DOPA metabolism under carbidopa blockade has been found between patients with schizophrenia and control subjects (Hietala *et al.*, 1999). Nevertheless it would be useful to measure metabolite levels in future studies.

Implications for understanding schizophrenia

The elevated staining for an antibody specific to tyrosine hydroxylase in our post-mortem study indicates increased tyrosine hydroxylase protein levels in schizophrenia that were not seen in matched patients with major depression, suggesting it is not a non-specific indicator of psychiatric illness. Although there is also tyrosine hydroxylase and aromatic-L-amino acid decarboxylase activity in other monoaminergic neurons, most monoaminergic neurons in the nigra are dopaminergic and aromatic-L-amino acid decarboxylase activity in the substantia nigra is abolished by selective dopamine neuron toxin (Yee *et al.*, 2000; Smith and Villalba, 2008). Furthermore there is also a close, direct relationship between nigral ^{18}F -DOPA uptake and both nigral aromatic-L-amino acid decarboxylase activity and nigral dopamine cell numbers (Yee *et al.*, 2000; Forsback *et al.*, 2004; Brown *et al.*, 2013). Our measures are therefore likely to substantially reflect dopaminergic function in the nigra. Taken together our results thus indicate increased activity in the final steps of dopamine synthesis in the nigra in schizophrenia. As post-mortem findings show that the

number of dopamine neurons in the nigra is unaltered in schizophrenia (Bogerts *et al.*, 1983), this elevation is likely to reflect increased capacity to produce dopamine rather than an increase in the number of dopamine neurons.

Given the functional MRI evidence of abnormal nigro-striatal activation in schizophrenia and its prodrome in the context of reward/conditioning tasks (Murray *et al.*, 2008; Waltz *et al.*, 2009; Romaniuk *et al.*, 2010; Gradin *et al.*, 2011; Allen *et al.*, 2012a, b; Nielsen *et al.*, 2012; Roiser *et al.*, 2012; Yoon *et al.*, 2013) and the consistent evidence for increased dopamine release in the striatal terminal regions [see (Laruelle *et al.*, 1996; Breier *et al.*, 1997; Abi-Dargham *et al.*, 2000) and meta-analysis (Howes *et al.*, 2012b)], it seems likely that this reflects an increase in midbrain dopamine neuron population activity, in line with that seen in preclinical developmental models of schizophrenia (Lodge and Grace, 2007, 2011). If this is the case, then it supports the development of treatments that target the control of midbrain dopamine neurons as alternatives to antipsychotics, which target the postsynaptic dopaminergic system (Kim *et al.*, 2012, 2013a). A proof-of-concept for this has been shown using a GABA- α 5 receptor selective agent, which altered the hippocampal drive to the midbrain and was found to reverse aberrant midbrain dopamine neuronal functioning in rodent models of schizophrenia (Gill *et al.*, 2011). Nevertheless future studies are needed to confirm our findings and to examine the breakdown pathways to determine if there is abnormal regulation of dopamine by catabolic processes.

Conclusion

Dopamine synthesis capacity is elevated in the substantia nigra in patients with schizophrenia, as well as in the striatum. This indicates that the pathophysiology of schizophrenia involves the cell bodies of dopamine neurons in addition to their striatal terminals. Nigral tyrosine hydroxylase staining was unaltered in major depression, suggesting that the alterations seen in schizophrenia are not non-specific indicators of psychiatric illness.

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Supplementary material

Supplementary material is available at *Brain* online.

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