The impact of common dopamine D2 receptor gene polymorphisms on D2/3 receptor availability: C957T as a key determinant in putamen and ventral striatum

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Supplementary Material

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Supplementary Extrastriatal ROI Analysis

To test for extrastriatal BP_{ND} effects of C957T, we extracted BP_{ND} from 11 anatomical ROIs obtained from the Automated Anatomical Labeling (AAL) atlas [Tzourio-Mazoyer *et al.*, 2002] or Brodmann Areas from the WFU Pickatlas [Maldjian *et al.*, 2003] to approximate those found to display a CC>CT>TT effect by Hirvonen et al. [Hirvonen *et al.*, 2009] The ROIs were: 1) AAL Anterior Cingulate Cortex (ACC); 2) AAL Angular Gyrus; 3) AAL Middle Temporal Lobe; 4) AAL Posterior Cingulate Cortex (PCC); 5) AAL Orbital Frontal Cortex (OFC), which consisted of the combination of the Frontal_Sup_Orb, Frontal_Mid_Orb, Frontal_Inf_Orb, and Frontal_Med_Orb ROIs from the AAL; 6) AAL Supramarginal Gyrus; 7) AAL Superior Temporal Lobe; 8) AAL Amygdala; 9) AAL Hippocampus; 10) AAL Thalamus; and 11) Dorsolateral Prefrontal Cortex (dlPFC), created by combining Brodmann areas 9 and 46. We also calculated η^2 effect sizes (controlling for age and sex) for BP_{ND} obtained from these ROIs.

Supplementary Multilocus Analyses

To test whether Ins/Ins vs Del carrier status significantly increased the predictive power of C957T T allele status on BP_{ND} in the striatum, we ran a stepwise multiple regression analysis with BP_{ND} from the combined C957T + Ins/Ins score clusters as our outcome variable. At the first level, we controlled for sex and age while at the second level we entered C957T T allele number as a predictor. As expected, C957T significantly predicted BP_{ND} in the clusters (β=0.383 in left striatum, 0.362 in right striatum, both $p<0.005$, explaining 20.3% and 25.6%, of the variance

respectively). In neither cluster did the addition of Ins/Ins status lead to significant improvement in predicting BP_{ND} (F-change=2.762, p=0.10 & F-change=0.329, p=0.57 for right and left striatum, respectively).

We also tested for additive effects of the three SNPs in anatomically defined striatal ROIs (see Methods) to ensure that the results of the multilocus analyses were not biased by the method of identifying significant clusters in our voxelwise analysis. In the right putamen, C957T accounted for 6.6% of the variance in BP_{ND} after controlling for age and sex while the addition of Ins/Del or Taq1A status decreased explanatory power in this region to ~5.0%. Results were similar for the left putamen (C957T accounting for 5.8% of variance in BP_{ND}). In the ventral striatum, we also found significant effects for C957T on both right (F-change=4.41, p=0.039; accounting for 13.0% of variance) and left (F-change=11.35, p=0.001; accounting for 17.2% of variance) BP_{ND}, whereas no effects were seen for the other two SNPs. Figure 3 displays the C957T effect on BP_{ND} in anatomically-defined putamen and ventral striatum. In contrast to these two striatal subdivisions, when looking at the right or left caudate, there was no significant effect of C957T on BP_{ND} after controlling for age and sex (a finding which is consistent with the greater involvement of putamen than caudate voxels in the voxelwise analyses). Furthermore, the addition of the other DRD2 SNPs did not predict caudate BP_{ND}.

A limitation of the multilocus score approach is that it assumes an additive effect of each SNP. However, there could be nonlinear effects of these polymorphisms. We therefore tested whether there were any statistical interactions between C957T and Taq1a or -141C Ins/Ins in the striatal ROIs. However, we found no evidence for an interaction between C957T and the other two SNPs (C957T*Taq1A: max $F_{(2,75)}$=0.46, min p=0.64; C957T*InsDel: max $F_{(1,77)}$=0.22, min p=0.64).

Table S1: BP_{ND} and Genotype Effects for C957T SNP Across Striatal ROIs							
Area	**SRTM BP_{ND} mean±SEM (95% CI), by genotype**			**Omnibus Effect**	**η^2 for Each Pairwise BP_{ND} Comparison (if omnibus sign)**		
	CC (n=30)	**CT (n=40)**	**TT (n=14)**	***F*, *p*, η^2**	**CT>CC**	**TT>CT**	**TT>CC**
Full Striatum	17.02±0.32 (16.38, 17.66)	18.29±0.28 (17.74, 18.85)	18.11±0.48 (17.16, 19.06)	4.63, 0.012, 0.104	0.114	0.003	0.104
Right Caudate	15.41±0.37 (14.67, 16.16)	16.46±0.32 (15.82, 17.10)	15.43±0.52 (14.33, 16.53)	2.75, 0.072, 0.064			
Left Caudate	15.17±0.37 (14.43, 15.92)	16.32±0.32 (15.68, 16.96)	15.83±0.55 (14.73, 16.93)	2.67, 0.076, 0.062			
Right Putamen	23.49±0.47 (22.57, 24.42)	25.23±0.40 (24.43, 26.03)	25.60±0.69 (24.24, 26.97)	5.02, 0.009, 0.111	0.104	0.002	0.178
Left Putamen*	22.96±0.46 (22.05, 23.87)	24.94±0.39 (24.15, 25.72)	24.51±0.68 (23.16, 25.86)	5.46, 0.006, 0.117	0.138	0.008	0.077
Right VS	14.56±0.45 (13.66, 15.47)	16.12±0.39 (15.34, 16.90)	15.89±0.67 (14.56, 17.23)	3.51, 0.035, 0.071	0.081	0.002	0.063
Left VS*	16.45±0.56 (15.32, 17.57)	18.16±0.48 (17.20, 19.12)	19.68±0.83 (18.03, 21.34)	5.62, 0.005, 0.109	0.062	0.048	0.172

Table S1. BP_{ND} Data and Differences Across C957T Genotype Groups, controlling for age and sex, in Mawlawi Anatomical Striatal ROIs. *, subregion effect is significant using Bonferroni correction of $p<0.008$; VS, ventral striatum

Table S2: BP_{ND} and Genotype Effects for C957T SNP Across Extrastriatal ROIs				
Area	**SRTM BP_{ND} mean±SEM (95% CI), by genotype**			**Omnibus Effect**
	CC (n=30)	**CT (n=40)**	**TT (n=14)**	***F*, *p*, η^2**
ACC	0.59±0.024 (0.54, 0.64)	0.59±0.02 (0.55, 0.63)	0.64±0.04 (0.57, 0.71)	0.71, 0.49, 0.016
Angular Gyrus	0.60±0.04 (0.53, 0.67)	0.54±0.03 (0.48, 0.60)	0.51±0.05 (0.41, 0.62)	1.04, 0.36, 0.024
Middle Temporal	0.87±0.04 (0.79, 0.95)	0.83±0.04 (0.76, 0.90)	0.81±0.06 (0.69, 0.93)	0.37, 0.69, 0.009
OFC	0.64±0.02 (0.59, 0.69)	0.64±0.02 (0.60, 0.68)	0.68±0.04 (0.61, 0.75)	0.49, 0.62, 0.009
PCC	0.33±0.02 (0.30, 0.37)	0.33±0.02 (0.30, 0.36)	0.33±0.03 (0.28, 0.38)	0.07, 0.93, 0.0009
Supramar Gyrus	0.62±0.04 (0.56, 0.69)	0.58±0.03 (0.52, 0.64)	0.55±0.05 (0.44, 0.65)	0.86, 0.43, 0.019
Superior Temporal	0.80±0.04 (0.73, 0.87)	0.77±0.03 (0.71, 0.84)	0.73±0.06 (0.63, 0.84)	0.50, 0.61, 0.011
Amygdala	3.60±0.21 (3.18, 4.01)	3.75±0.18 (3.39, 4.11)	4.26±0.31 (3.65, 4.88)	1.57, 0.21, 0.033
Hippo	1.88±0.08 (1.72, 2.04)	1.91±0.07 (1.77, 2.04)	1.93±0.12 (1.70, 2.17)	0.09, 0.92, 0.002
Thalamus	2.57±0.07 (2.44, 2.71)	2.65±0.06 (2.53, 2.77)	2.53±0.10 (2.33, 2.74)	0.63, 0.53, 0.014
dlPFC	0.38±0.02 (0.34, 0.42)	0.37±0.02 (0.34, 0.40)	0.39±0.03 (0.33, 0.45)	0.20, 0.82, 0.004

Table S2. BP_{ND} data and Differences Across C957T Genotype Groups, controlling for age and sex, in extrastriatal ROIs. We extracted BP_{ND} data from extrastriatal regions of interest (using the AAL atlas and Broadmann Areas) that were constructed to be similar to that used by Hirvonen et al., 2009 in an FLB-457 PET study. BP_{ND} did not differ across any of the ROIs when controlling for age and sex.

ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; PCC, posterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex (defined here as Brodmann area 9+46); Supramar, supramarginal; Hippo, hippocampus

Table S3: BP_{ND} Data and Genotype Effects Across Striatal ROIs by Taq1A A1 Carrier Status			
Area	**SRTM BP_{ND} mean±SEM (95% CI)**		**Genotype Effect**
	A1 Carriers (n=36)	**A2A2 (n=48)**	***F*, *p*, η^2**
Full Striatum	17.69±0.31 (17.08, 18.30)	17.90±0.27 (17.37, 18.42)	0.25, 0.62, 0.003
Right Caudate	16.05±0.37 (15.32, 16.78)	15.81±0.29 (15.25, 16.34)	0.26, 0.61, 0.003
Left Caudate	15.70±0.38 (14.99, 16.48)	15.92±0.27 (15.38, 16.49)	0.22, 0.64, 0.003
Right Putamen	24.28±0.46 (23.38, 25.18)	24.96±0.38 (24.20, 25.67)	1.38, 0.24, 0.017
Left Putamen	23.94±0.48 (22.98, 24.93)	24.32±0.35 (23.60, 25.01)	0.42, 0.52, 0.005
Right VS	15.74±0.41 (14.87, 16.56)	15.37±0.39 (14.49, 16.12)	0.38, 0.54, 0.005
Left VS	17.37±0.54 (16.26, 18.41)	18.12±0.49 (17.15, 19.06)	1.12, 0.29, 0.014

Table S3. BP_{ND} Data and Differences Across Taq1A Genotype Groups, controlling for age and sex, in Mawlawi Anatomical Striatal ROIs

Table S4: BP_{ND} Data and Genotype Effects Across Striatal ROIs by Del Carrier Status			
Area	**SRTM BP_{ND} mean±SEM (95% CI)**		**Genotype Effect**
	InsIns (n=59)	**Del Carrier (n=25)**	***F*, *p*, η^2**
Full Striatum	17.91±0.24 (17.43, 18.38)	17.58±0.37 (16.85, 18.31)	0.57, 0.45, 0.007
Right Caudate	15.85±0.28 (15.30, 16.41)	16.06±0.34 (15.39, 16.75)	0.18, 0.68, 0.002
Left Caudate	15.79±0.27 (15.24, 16.28)	15.92±0.41 (15.11, 16.75)	0.07, 0.79, 0.001
Right Putamen	24.88±0.37 (24.17, 25.55)	24.18±0.38 (23.49, 24.94)	1.22, 0.27, 0.015
Left Putamen	24.32±0.37 (23.58, 25.04)	23.77±0.35 (23.10, 24.49)	0.79, 0.38, 0.010
Right VS	15.82±0.34 (15.18, 16.52)	14.84±0.50 (13.70, 15.83)	2.67, 0.11, 0.032
Left VS	18.10±0.43 (17.28, 18.95)	17.11±0.64 (15.88, 18.35)	1.66, 0.20, 0.020

Table S4. BP_{ND} Data and Differences Across Ins/Del Genotype Groups, controlling for age and sex, in Mawlawi Anatomical Striatal ROIs

Table S5: C957T T Allele Dose + Taq1A A2 Allele Dose Score Positively Correlates with BP_{ND}			
Area	**MNI coordinates**	**# voxels**	**T value, pFDR**
Right Striatum	26, 10, -4	405	4.30, 0.085
Left Striatum	-24, 2, 0	342	3.55, 0.085

Table S5. Results from an SPM regression analysis of C957T T allele + Taq1A A2 allele dose on Fallypride BP_{ND}. The C957T + Taq1A multilocus score was associated with high BP_{ND} in similar areas observed in the C957T regression analysis. However, the addition of Taq1A decreased the significance in the striatum, resulting in clusters that did not meet significance after correcting for multiple comparisons. Only clusters with pFDR<0.1 are reported.

Table S6: C957T T Allele Dose + Ins/Ins Score Positively Correlates with BP_{ND}			
Area	**MNI coordinates**	**# voxels**	**T value, pFDR**
Right Striatum	26, 8, -4	1019	4.20, 0.002
Left Striatum	-20, 8, -8	488	3.41, 0.043
Midbrain/Pons	2, -26, -28	353	4.30, 0.087

Table S6. Results from an SPM regression analysis of C957T T allele + presence of the InsIns genotype on Fallypride BP_{ND}. The addition of Ins/Ins allele status resulted in similar striatal effects as observed in the C957T regression analysis. Multilocus score was related to BP_{ND} in midbrain, though neither cluster survived corrections for multiple comparisons. Conventions as per Table S1.

Table S7: C957T T Allele Dose + Ins/Ins + Taq1A A2 Allele Dose Score Positively Correlates with BP_{ND}			
Area	**MNI coordinates**	**# voxels**	**T value, pFDR**
Right Striatum	26, 10, -4	622	4.49, 0.013
Left Striatum	-24, 0, 0	435	3.58, 0.027

Table S7. Results from an SPM regression analysis of C957T T allele + InsIns + Taq1A A2 multilocus score on Fallypride BP_{ND}. The addition of all three SNPs result in significant effects on striatal BP_{ND} that are qualitatively similar to that observed with C957T alone. Conventions as per Table S1.

Figure S1

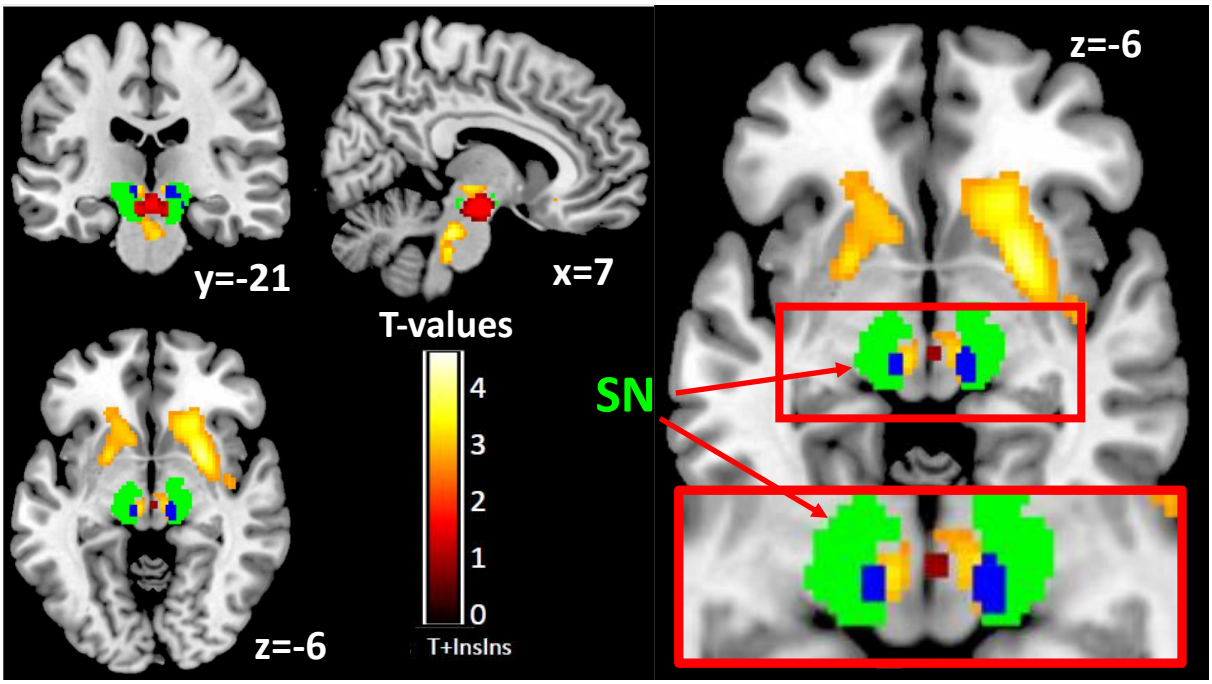

Figure S1: Combined C957T T and Ins/Ins vs Del Carrier multilocus score associated with higher BP_{ND} in midbrain. The combined C957T T + Ins/Ins (0,1) multilocus score effects on BP_{ND} result in similar striatal effects to C957T alone plus the addition of midbrain clusters. Dopaminergic midbrain probabilistic regions of interest (both set at 50% probability) from Murty et al. (2014) are overlaid on the C957T + Ins/Ins BP_{ND} (yellow/orange) with Red representing VTA (visible on coronal and saggital slices) and Green the substantia nigra (visible on coronal and axial slices). The Blue voxels represent overlap of C957T T + Ins/Ins effect in the dopaminergic midbrain. Only a small proportion of significant exstrastriatal voxels from this analysis (k=56; ~11%) overlapped with the substania nigra. SN, substania nigra. Data displayed using a $p<0.005$, uncorrected threshold (T=2.64).

References

Hirvonen, M.M., Lumme, V., Hirvonen, J., Pesonen, U., Nagren, K., Vahlberg, T. *et al.* (2009) C957t Polymorphism of the Human Dopamine D2 Receptor Gene Predicts Extrastriatal Dopamine Receptor Availability in Vivo. *Prog Neuropsychopharmacol Biol Psychiatry* 33: 630-636.

Maldjian, J.A., Laurienti, P.J., Kraft, R.A., and Burdette, J.H. (2003) An Automated Method for Neuroanatomic and Cytoarchitectonic Atlas-Based Interrogation of Fmri Data Sets. *Neuroimage* 19: 1233-1239.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N. *et al.* (2002) Automated Anatomical Labeling of Activations in Spm Using a Macroscopic Anatomical Parcellation of the Mni Mri Single-Subject Brain. *Neuroimage* 15: 273-289.