

# Dopamine-Dependent Architecture of Cortico-Subcortical Network Connectivity

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**Maladaptive dopaminergic mediation of reward processing in humans is thought to underlie multiple neuropsychiatric disorders, including addiction, Parkinson's disease, and schizophrenia. Mechanisms responsible for the development of such disorders may depend on individual differences in neural signaling within large-scale cortico-subcortical circuitry. Using a combination of functional neuroimaging and pharmacological challenges in healthy volunteers, we identified opposing dopamine agonistic and antagonistic neuro-modulatory effects on distributed functional interactions between specific subcortical regions and corresponding neocortical "resting-state" networks, known to be involved in distinct aspects of cognition and reward processing. We found that, relative to a placebo, levodopa and haloperidol challenges, respectively, increased or decreased the functional connectivity between (1) the midbrain and a "default mode" network, (2) the right caudate and a right-lateralized frontoparietal network, and (3) the ventral striatum and a fronto-insular network. Further, we found drug-specific associations between brain circuitry reactivity to dopamine modulation and individual differences in trait impulsivity, revealing dissociable drug–personality interaction effects across distinct dopamine-dependent cortico-subcortical networks. Our findings identify possible systems underlying pathogenesis and treatment efficacy in disorders of dopamine deficiency.**

**Keywords:** dopamine, functional connectivity, impulsivity, pharmacological FMRI, resting-state networks

## Introduction

Dopamine neurotransmission is intimately and consistently linked with reward-seeking and impulsive behaviors (Pessiglione et al. 2006; Buckholz et al. 2010). Specific neuroreceptor proteins regulating dopaminergic signaling are thought to mediate individual differences in sensitivity to pharmacological manipulation and, accordingly, the probability of developing and sustaining symptoms of pathological reward, inhibitory, or salience processing in disorders such as addiction, Parkinson's disease, and schizophrenia (Schafer et al. 2001; Dalley et al. 2007; Dagher and Robbins 2009; Buckholz et al. 2010). It has been hypothesized further that the complex cognitive processes and personality factors relevant for reward-related behavior and impulsivity are mediated by large-scale neuronal systems, communicating via cortico-subcortical pathways (Koob and Volkow 2010). Evidence suggests that dopaminergic influences in the brain can be

investigated through functional magnetic resonance imaging (fMRI) of analogous networks (Honey et al. 2003). However, broad-spectrum dopaminergic manipulations of cortico-subcortical connections within multiple large-scale networks and, moreover, interactions with individual difference measures relevant for psychopharmacological modulation of pathological processing have not yet been investigated.

With fMRI, communication between remote neuronal populations at the systems level can be probed via measures of synchronization over time, or "functional connectivity," between spatially distinct blood-oxygenation level-dependent (BOLD) signals (Biswal et al. 1995; Smith et al. 2009; Lee et al. 2010). Specifically, pharmacological fMRI research demonstrates that measures of connectivity between distributed brain regions are sensitive to the effects of dopaminergic challenge, using agonist or antagonist drugs that either increase or decrease neurotransmission (Honey et al. 2003; Achard and Bullmore 2007; Kelly et al. 2009; Tost et al. 2010).

Our study used BOLD fMRI to investigate in detail the functional connectivity relationships between subcortical regions known to comprise core dopaminergic transmission pathways (Pessiglione et al. 2006; Buckholz et al. 2010; Koob and Volkow 2010) and multiple, distributed neocortical networks thought to underlie specific aspects of cognition (e.g. Greicius et al. 2004; Beckmann et al. 2005; Seeley et al. 2007). Measured during undirected wakefulness, these systems are known as "resting-state" networks (RSNs) and comprise the fundamental functional architecture of the human brain (Beckmann et al. 2005; Smith et al. 2009; Biswal et al. 2010). We focussed specifically on RSNs relevant for cognitive control, impulsivity, and reward processing (Seeley et al. 2007; Smith et al. 2009; Cole et al. 2010; Koob and Volkow 2010; Shannon et al. 2011), which have also been implicated as dysfunctional in neuropsychiatric disorders regularly treated with dopamine-targeting medications (e.g. Castellanos et al. 2008; Kelly et al. 2009; Wolf et al. 2011). Through a novel combination of multivariate and univariate fMRI data analysis techniques, along with "clinical" fixed-dose dopamine agonistic (100 mg levodopa; L-dopa) and antagonistic (3 mg haloperidol) pharmacological challenges, we were able to map in healthy humans the dopamine-dependent architecture of subcortical functional connectivity with these RSNs and, further, to relate variability in drug effects on these systems-level connectivity patterns to individual differences in impulsivity.

## Materials and Methods

### Participants and Study Design

We recruited 55 healthy male volunteers, naive to the experimental drugs, who were assigned randomly to 3 groups (l-dopa, haloperidol, or placebo). Data are reported from 49 participants who completed the study in full (mean age = 22.4 years  $\pm$  4.1 SD; Table 1). Eligibility criteria were: no current (or history of) psychiatric problems as determined by the Mini-International Neuropsychiatric Interview (Sheehan et al. 1998); no medical history indicating a risk in using l-dopa or haloperidol (e.g. cardiac illness, depressive disorders, thyroid disorders, and glaucoma); and no current or recent use (<12 weeks before participation) of psychopharmacological medication and other medications or psychotropic drugs that might interfere with the central nervous system action of l-dopa or haloperidol (e.g. cannabis or cocaine). Each participant gave signed, informed consent in which confidentiality, anonymity, and the opportunity to withdraw without penalty were assured.

Participants received a fixed dose of 3 mg haloperidol (Haldol;  $N = 18$ ) 4 h prior to scanning ( $T_{\max} = 3\text{--}6$  h, half-time = 14–36 h) or 100 mg levodopa combined with 25 mg carbidopa (Sinemet;  $N = 16$ ) 1 h prior ( $T_{\max} = 45$  min, half-time = 1–2 h) or placebo ( $N = 15$ ). Drug administration was double-blind and followed a previously published, “placebo-counterbalanced” protocol (Pessiglione et al. 2006), ensuring that resting-state fMRI data were acquired at projected peak plasma concentrations for both drugs. All tablets were over-encapsulated to ensure that participants and experimenters were blind to the dosages and could not compare or identify the drugs. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center and carried out in accordance with the standards of the Declaration of Helsinki.

### Questionnaires

Participants completed questionnaires immediately after ingestion of the first pill. To assess individual differences in impulsivity, the Barratt Impulsiveness Scale (BIS-11; Patton et al. 1995) was administered to all subjects (Table 1; data absent for a single subject in the l-dopa group).

### Image Acquisition

Imaging was carried out on a 3 T Achieva MRI scanner (Philips, Best, The Netherlands) using an 8-channel SENSE head coil. A T1-weighted structural volume was acquired for registration purposes. For the resting-state fMRI scan, 220 whole-brain volumes of T2\*-weighted gradient echo planar images (EPIs) sensitive to BOLD contrast were obtained in the axial direction (repetition time = 2.2 s, echo time = 30 ms, flip angle = 80°, isotropic voxels of 2.75 mm, slice gap = 0.25 mm, 38 slices). Participants were instructed to remain awake with their eyes closed throughout.

### Image Preprocessing

Resting-state fMRI data were preprocessed and initially analyzed in individual subject/session-level EPI space. Image preprocessing was performed with tools from the FMRIB Software Library (FSL; [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl); Smith et al. 2004). The first 4 volumes were removed from each fMRI data set to allow for magnetic equilibration, resulting in a 216-data point BOLD time series at each voxel per

session. Preprocessing techniques applied to these data included motion correction, brain extraction, spatial smoothing with a Gaussian kernel of 5 mm full width at half maximum, and high-pass temporal filtering at 100 s.

Seed-based “partial” correlation analysis (SBCA; O’Reilly et al. 2010) was carried out separately for each subject within an anatomically derived subcortical seed mask incorporating regions with established dopamine-dependent functionality or connectivity (Honey et al. 2003; Kelly et al. 2009; Buckholz et al. 2010; Koob and Volkow 2010). Every voxel within each “individualized” subcortical seed mask was tested quantitatively in terms of its connectivity with each of a number of RSN “target” maps, which collectively covered the majority of neocortex.

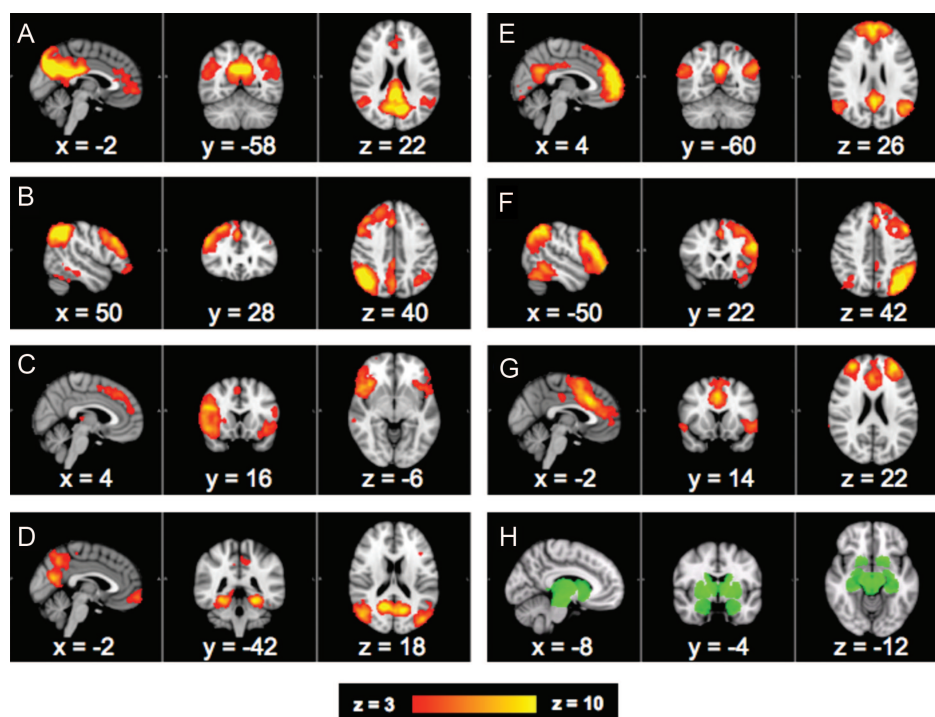
To construct subject-specific subcortical seed masks, T1 structural images were segmented using FSL FIRST. Bilateral regions included in these masks were the entire striatum (comprising regions of caudate, putamen, and ventral striatum), globus pallidus, amygdala, hippocampus, and thalamus (and midbrain, discussed subsequently). The unthresholded versions of these segmented structures (i.e. without boundary correction; Patenaude et al. 2011) were combined into a single, “liberal” mask image for each subject. To include mid-brain voxels within our masks, we carried out nonlinear warp transformation (as implemented in FSL FNIRT) of 6 binary, bilateral volumes from the Talairach Daemon atlas (Lancaster et al. 2000; labels = midbrain, substantia nigra, subthalamic nucleus, red nucleus, mammillary body, and medial geniculate body) to the high-resolution space of each subject. This midbrain information was then added to the mask containing subjects’ other subcortical regions. These subject-specific combined masks were then affine-registered to EPI space using FSL FLIRT and used in subsequent subject-wise SBCA, to quantify subcortical functional connectivity with neocortical RSNs.

To construct RSN masks for use as target neural functional connectivity networks at the subject level in SBCA, we obtained 20 binary RSN spatial maps (7 of interest and 13 “nuisance,” see Higher-Level Analysis) from a probabilistic group independent component analysis (ICA) of the subjects given placebo. Placebo data only were entered into probabilistic multisession ICA with temporal concatenation (as implemented in FSL MELODIC; Beckmann and Smith 2004; Beckmann et al. 2005), to avoid biasing this spatial target selection toward the larger haloperidol group. This group ICA approach decomposed the concatenated 4-D data set (216 volumes per scan  $\times$  15 subjects = 3240 image volumes) into spatial maps of structured component signals in the data (and associated time courses), identifying component maps, including RSNs, displaying consistent spatiotemporal coherence within scans and maximal spatial independence across subjects. The number of components for the data set was estimated automatically using the Laplace approximation to the Bayesian evidence for the model order in a probabilistic principal component model (for details, see Beckmann and Smith 2004). We identified 43 independent components in total in the placebo group fMRI data. Twenty of these were selected for further analyses based on their neuroanatomical configurations and neurophysiological feasibility as RSNs, in comparison with previous literature (Beckmann et al. 2005; Kiviniemi et al. 2009; Smith et al. 2009; Cole et al. 2010). The remaining 23 components were deemed artifacts of motion, non-neuronal physiology, or magnetic susceptibility (Kiviniemi et al. 2009) and thus not included in further analyses. Networks identified by the group ICA and entered into further analyses have been described previously by multiple groups and comprise core systems and subsystems implicated in multiple sensory, motor, and cognitive functions (Biswal et al. 1995; Vincent et al. 2006, 2008; Seeley et al. 2007; Smith et al. 2009; Andrews-Hanna et al. 2010; Cole et al. 2010). All 20 template RSN maps were thresholded (at  $z > 3$ ), then binarized, and transformed from MNI152 “standard” space (Montreal Neurological Institute, McGill University, Quebec, Canada) to the data space of each subject’s EPI session via FLIRT affine registration: first via the space of the associated high-resolution T1 structural scan and then to functional EPI space. In high-resolution space (prior to registration to EPI space), voxels with less than 20% probability of containing gray matter in the equivalent T1 structural (as calculated using FSL FAST) were removed from all “subject-specific” RSN spatial maps.

**Table 1**

Descriptive statistics of subject variables for each drug group and associated 1-way analysis of variance results

	Haloperidol ( $N = 18$ )	Placebo ( $N = 15$ )	l-dopa ( $N = 16$ ; 15 for BIS-11)	$F$ ( $P$ )
Age (mean $\pm$ SD)	22.25 $\pm$ 3.53	21.47 $\pm$ 3.05	23.38 $\pm$ 5.30	0.86 (0.43)
BIS-11 total (mean $\pm$ SD)	66.06 $\pm$ 6.46	63.53 $\pm$ 9.01	66.67 $\pm$ 11.58	0.51 (0.61)



**Figure 1.** RSNs of interest and group subcortical seed mask. (A–G) Seven RSNs subjected to higher-level post-SBCA analysis of dopamine-dependent subcortical functional connectivity. (A) Postero-centric DMN, (B) right-lateralized FPN, (C) inferior fronto-insular SEN, (D) hippocampal-parietal/ventral DMN, (E) antero-centric DMN, (F) left-lateralized FPN, and (G) dorsal medial-lateral frontal SEN. (H) Subcortical mask used in higher-level analyses. Axial and coronal slices are presented in radiological orientation (left = right).

### Analysis of Neocortical RSN Connectivity with Subcortical Regions

In SBCA, the subcortical seed masks from each subject were examined individually, in EPI space, for their voxel-wise spatial distributions of functional connectivity strength with the characteristic activity of each of the 20 RSNs (7 subjected to higher-level analysis and 13 nuisance). All 20 non-artifactual components from group ICA were included in this first-level analysis to ensure that potential extraneous interactions, or temporally overlapping relationships, between any of the 7 RSNs of interest (see Higher-Level Analysis; Fig. 1A–G) and any of the 13 nuisance RSNs (e.g. visual, auditory, or somatosensory networks) could be factored out of the analysis, in effect treating the latter as confound regressors. Voxel-wise connectivity strengths were quantified by calculating partial correlation coefficients between the BOLD signal time series at each mask voxel and that of the weighted principal eigenvariate associated with each RSN (the latter calculated via subject-wise principal component analyses; O'Reilly et al. 2010). Voxel-wise coefficients are termed partial because the analysis associated with a given target RSN controlled, in turn, for the seed voxel's activity relationship with each of the other 19 RSNs examined as targets in separate correlation analyses. In these analyses, we also controlled for the confounding influences of structured noise from white matter (WM) and cerebrospinal fluid (CSF) tissue types and residual motion artifacts. To this end, binary T1-segmented maps of WM and CSF (calculated using FSL FAST) were registered to EPI space using FLIRT and, for each session, used as masks against the associated, preprocessed functional data sets, in order to extract confound time series that were calculated as the mean BOLD signal within these tissue masks. In addition to the WM and CSF confounds, 6 time series resulting from the motion correction procedure describing individual subject head motion parameters were also regressed out of the SBCA.

### Higher-Level Analysis

Further analyses examining drug effects on RSN-subcortical functional connectivity focussed on a subset of 7 RSNs (Fig. 1A–G) of interest due to their reported involvement in higher cognitive control and

motivational processes potentially relevant for impulse control, reward processing, or dopamine function (Greicius et al. 2004; Vincent et al. 2006, 2008; Seeley et al. 2007; Kelly et al. 2009; Smith et al. 2009; Andrews-Hanna et al. 2010; Cole et al. 2010, 2011; Koob and Volkow 2010; Shannon et al. 2011; Wolf et al. 2011). In line with this literature, these RSNs are here referred to as the (1) antero-centric and (2) postero-centric subsystems of the “default mode” network (DMN), (3) left- and (4) right-lateralized frontoparietal networks (FPNs), (5) fronto-insular and (6) dorsal medial-lateral frontal salience/executive networks (SENs), and (7) the hippocampal-parietal/ventral DMN.

**Table 2**

Clusters displaying significant linear effects of dopamine modulation on RSN functional connectivity: spatial information and associations with impulsivity

RSN (Fig. 1)	Subcortical cluster anatomical location	Cluster MNI <i>x, y, z</i> coordinates (peak <i>t</i> -statistic) and volume	Association between drug and RSN-subcortical connectivity	Association with BIS-11 scores
(A) Postero-centric DMN	Midbrain (bilateral posterior)	−6, −28, −6 (5.08), 1680 mm <sup>3</sup>	Linear: L-dopa (>placebo) > haloperidol	Significant negative correlation in haloperidol group; significantly different from L-dopa group
(B) Right FPN	Right dorsal caudate	12, 8, 6 (4.08), 1656 mm <sup>3</sup>	L-dopa (>placebo) > haloperidol	Positive trend in haloperidol group; close-to-significantly different from placebo group
(C) Fronto-insular SEN	Left ventral striatum	−16, 12, −10 (4.21), 1160 mm <sup>3</sup>	L-dopa (>placebo) > haloperidol	n.s.
(F) Left FPN	Bilateral ventro-medial thalamus	6, −14, 2 (4.00), 1392 mm <sup>3</sup>	L-dopa (>placebo) > haloperidol	n.s.

Note: n.s., none significant.

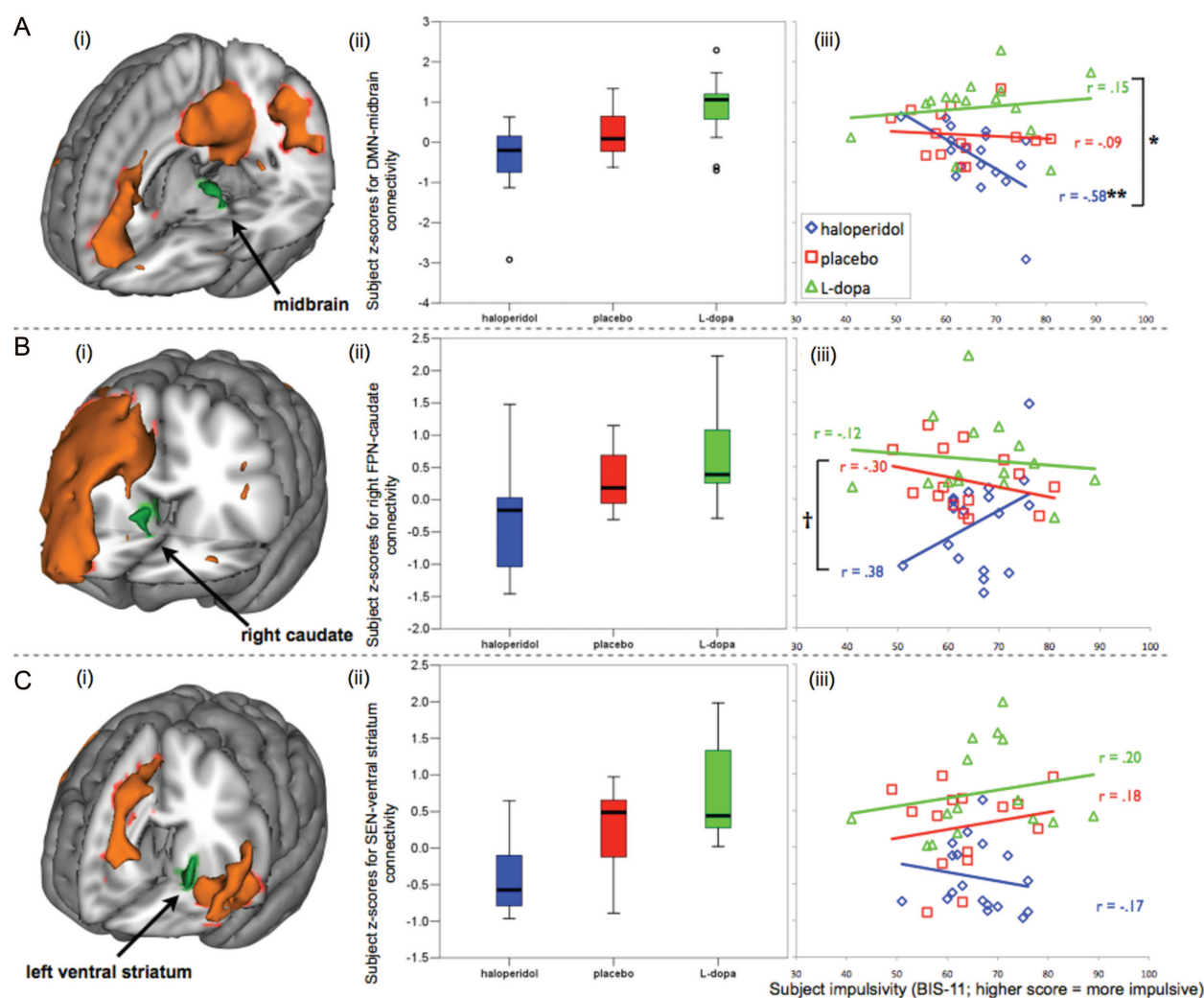


To test the correlation maps resulting from SBCA for between-group differences (7 RSNs  $\times$  49 subjects = 343 maps), they were transformed to a common stereotactic space, first by affine registration to high-resolution structural space and then by FNIRT non-linear warp transform to the MNI152 template space. Correlation maps were then arranged in a single 4-D file per RSN, containing, for each subject, a subcortical map of connectivity with said RSN (thus 49 per RSN). These RSN-specific subcortical connectivity maps were then analyzed within the framework of the general linear model, using nonparametric permutation testing (5000 permutations; as implemented in FSL randomise) to identify subcortical regions in which functional connectivity with a given RSN of interest differed between dopamine drug treatment groups, in terms of being more strongly or weakly positive or negative. Explicitly, we hypothesized for linear drug effects on RSN-subcortical connectivity (L-dopa > placebo > haloperidol and the inverse contrast). Significant effects were defined by cluster-mass thresholding ( $t = 2.3$ ,  $P < 0.05$ ) with family-wise error (FWE) correction for multiple comparisons across the group subcortical mask and are presented in Table 2. For the group subcortical mask used in these analyses, to be inclusive and to

allow for small intersubject structural variations, we nonlinearly warped the subcortical masks from each subject to MNI152 space (using FSL FNIRT), summed them, and then binarized the resulting image (Fig. 1H).

### Measuring Associations Between Connectivity and Impulsivity

To examine drug-specific network functional connectivity associations with impulsivity, FMRI results were correlated, within-group, with subject BIS-11 total scores. Significant clusters identified from the post-SBCA higher-level analysis were thus used as masks to extract mean connectivity scores from normalized (Fisher's  $z$ -transformed) versions of the RSN-specific correlation maps initially used as inputs to higher-level analyses. The resulting values were then grouped by drug condition and correlated (Pearson's  $r$ ) with BIS-11 total scores to find significant within-group associations ( $P < 0.05$ , 2-tailed). We then compared these correlations across groups to investigate drug-personality interactions, by testing for significant differences between the resulting (Fisher's  $z$ -transformed) correlation coefficients.



**Figure 2.** Significant linear effects of antagonistic (haloperidol) and agonistic (L-dopa) dopaminergic neuromodulation on cortico-subcortical RSN functional connectivity and correlations with subject BIS-11 impulsivity scores. (A) (i) DMN-midbrain connectivity shows (ii) a linear effect ( $t > 2.3$ ,  $P < 0.05$ , corrected) of treatment (L-dopa > placebo > haloperidol), which (iii) is negatively correlated with impulsivity within the haloperidol group, differentially to within the L-dopa group. (B) (i) Right FPN-caudate connectivity shows (ii) a similar linear effect and (iii) a trend toward an opposite relationship with impulsivity to the DMN-midbrain result. (C) (i) SEN-ventral striatum connectivity shows (ii) the same linear drug effect but (iii) no significant interaction with impulsivity. Left panels: RSNs presented in orange and subcortical regions in green. Centre panels: box plots represent mean connectivity scores ( $\pm 95\%$  confidence intervals) for each drug group. Right panels: \*\*significant within-group correlation with impulsivity ( $P < 0.05$ ); \*significant difference between 2 correlation coefficients ( $P < 0.05$ ); †near-significant trend toward difference between coefficients ( $P < 0.07$ ).

## Results

### Dopamine-Dependent Connectivity

We found multiple significant linear effects (l-dopa > placebo > haloperidol) of dopaminergic drug group on cortico-subcortical functional connectivity (cluster  $t > 2.3$ ,  $P < 0.05$ , FWE-corrected). These effects of connectivity greater in the l-dopa group and reduced in the haloperidol group were apparent between: (1) the posterocentric DMN and the midbrain (peak  $t = 5.08$ ; MNI coordinates:  $x = -6$ ,  $y = -28$ ,  $z = -6$ ; see Fig. 2A and Table 2 for full results); (2) the right-lateralized (putative cognitive control; Vincent et al. 2008; Smith et al. 2009) FPN and the right dorsal caudate ( $t = 4.08$ ;  $x = 12$ ,  $y = 8$ ,  $z = 6$ ; Fig. 2B); and (3) the inferior fronto-insular salience processing/executive control RSN (Seeley et al. 2007; Smith et al. 2009) and the left ventral striatum ( $t = 4.21$ ;  $x = -16$ ,  $y = 12$ ,  $z = -10$ ; Fig. 2C). (4) A significant drug effect was also found on the connectivity between the left-lateralized FPN and bilateral thalamic regions (Table 2). We found no significant inverse linear effects (haloperidol > placebo > l-dopa) of dopamine modulation on cortico-subcortical connectivity.

### Associations with Impulsivity

We found that the dopamine-dependent connectivity between the posterior DMN and the midbrain was significantly negatively correlated with subject BIS-11 total scores in the haloperidol group ( $r = -0.58$ ,  $P = 0.012$ , 2-tailed). Further, this effect size was significantly different ( $z = 2.1$ ,  $P = 0.038$ , 2-tailed) from an equivalent “positive” (nonsignificant) correlation in the l-dopa group, thereby indicating an interaction effect between impulsivity scores and drug type on network functional connectivity (Fig. 2A). Although both haloperidol and l-dopa groups were found to contain outliers, by removing the scores of these subjects from the respective connectivity–impulsivity correlations, this interaction effect was further amplified ( $z = 2.6$ ,  $P = 0.010$ , 2-tailed). Of potential interest, the drug effects on connectivity between the right FPN and caudate showed an opposing relationship with BIS-11 scores to the midbrain-DMN pattern identified (Fig. 2B). No within-group correlation reached significance for this FPN–caudate relationship, but the haloperidol group correlation was again opposite in direction to the other 2 groups and, in this case, showed a trend toward a significant difference from the BIS-11 correlation with the placebo group ( $z = -1.8$ ,  $P = 0.067$ , 2-tailed). The dopamine-dependent SEN–ventral striatum connectivity pattern showed no within- or between-group associations with BIS-11 scores (Fig. 2C).

## Discussion

Our results provide a novel and important link between dopamine neuromodulation and systems-level signaling within the human brain. We demonstrate opposing effects of agonistic and antagonistic dopaminergic challenges on functional connectivity relationships between specific, dopamine-rich subcortical regions and corresponding neocortical RSNs. Similar large-scale neural circuits have been implicated in aspects of cognition, personality, and reward processing relevant for neuropsychiatric disorders commonly treated with dopamine-targeting medications (e.g. Castellanos et al. 2008; Cole et al. 2010; Koob and Volkow 2010; Tost et al. 2010; Cole et al.

2011; Shannon et al. 2011; Wolf et al. 2011). We observe that l-dopa generally increases cortico-subcortical network connectivity in our study sample, whereas haloperidol tends to decrease it. Furthermore, we show that acute dopamine antagonist modulation of cortico-striatal connectivity, identified previously during task performance (Tost et al. 2010), is also identifiable during rest and, intuitively, acts in opposition to agonistic neuromodulatory effects. This suggests that RSN functional connectivity can, in some cases, provide an indirect measure of dopamine neurotransmission.

It is important to discuss the current results in the context of existing neurobiological and neuropsychiatric findings. For instance, the dopamine-dependent connectivity found here between the ventral striatum and a “salience network,” centered on fronto-insular regions (Seeley et al. 2007), appears in line with the proposed role for frontostriatal dopaminergic mechanisms in mediating reward-related and motivated behaviors relevant for certain psychological functions and dysfunctions (e.g. Pessiglione et al. 2006; Dagher and Robbins 2009; Walter et al. 2009; Koob and Volkow 2010; Sesack and Grace 2010). In addition, the dopaminergic engagement of this neural circuitry is implicated in symptoms of schizophrenia, particularly aberrant salience processing, as well as their augmentation with antipsychotic/neuroleptic drugs such as haloperidol (Goldman-Rakic et al. 1989; Lidow and Goldman-Rakic 1994; Horvitz 2000; Walter et al. 2009; for related opinion, see also Menon 2011; Palaniyappan and Liddle 2012). Similarly, the dopamine-dependent integration of the right caudate within the right-lateralized FPN found here is in line with the proposed role for this circuitry in processes of cognitive control, which has been highlighted using multiple complementary approaches (Alexander et al. 1986; Goldman-Rakic et al. 1989; Liston et al. 2006; Lungu et al. 2007; Cools 2008). Finally, dopamine-dependent connectivity with the posterior DMN was found in posterior regions of the midbrain. This cluster overlaps only minimally with more anterior regions regarded as the “dopaminergic midbrain,” such as the substantia nigra or ventral tegmental area (which contain major dopamine neuronal projections to and from anterior subcortical and cortical circuitry; see e.g. Everitt and Robbins 2005). The Talairach Daemon atlas (Lancaster et al. 2000), which was used here as a basis for defining the midbrain portion of initial seed masks, labels this region predominantly as, simply, “midbrain.” In fact, the cluster primarily covers bilateral portions of the superior colliculi and periaqueductal gray (although extending somewhat into left substantia nigra and red nucleus, as defined by the Talairach Daemon atlas). Interestingly, direct anatomical connections have been reported between the precuneus (a central node of the posterior DMN) and the superior colliculi (Cavanna and Trimble 2006) and also between the latter and the substantia nigra (Comoli et al. 2003). Importantly, the increased sensitivity of functional connectivity methods to polysynaptic connectivity relationships provides complementary information to that achievable in studies of anatomical connectivity (Honey et al. 2009; Lu et al. 2011). Consistent with both this prior anatomical evidence and the current dopamine-dependent functional connectivity results, the superior colliculi have also been implicated in a number of behavioral functions related to dopaminergic activity, particularly the processing of salient stimuli (Comoli et al. 2003; Coizet et al. 2006; Krebs et al. 2012). Furthermore, evidence of both periaqueductal gray and red

nucleus involvement in large-scale networks implicated in similar processes has been provided through prior studies of functional connectivity (Seeley et al. 2007; Nioche et al. 2009). Future work may seek to delineate more directly each of these cortico-subcortical RSN connectivity relationships in terms of specific behavioral correlates sensitive to dopamine modulation.

We note here that the levels of anatomical specificity suggested earlier, particularly with regard to the midbrain, should not be interpreted as definitive, as the whole-brain coverage required for this fMRI study comes at the expense of fine-grained spatial resolution at the level of some subcortical nuclei. Future work using the latest imaging hardware at higher magnetic field strengths (e.g. 7 T), coupled with further development of acquisition sequences and physiological noise correction techniques optimized for obtaining BOLD signal in, for example, the midbrain (Limbrick-Oldfield et al. 2012), will undoubtedly increase the spatial resolution achievable with whole-brain imaging and thus should provide greater insight into subcortical functional connectivity within large-scale networks.

Aside from the gross linear effects of dopaminergic pharmacological challenges on systems-level functioning, we also identify selective associations between individual responses to dopamine modulation of cortico-subcortical network connectivity patterns and impulsive personality traits. The predictive strength and the direction of these patterns differ depending on the RSN involved, perhaps reflecting distinct network functions (Beckmann et al. 2005; Seeley et al. 2007; Smith et al. 2009). For example, the DMN and FPN are thought to subserve opposing aspects of cognition, but are seldom related directly to impulsivity, although meta-analytic work strongly implicates the right-lateralized FPN in inhibitory control (Smith et al. 2009). Here, we expand on previous evidence by highlighting that dopaminergic network differences in impulsivity extend beyond the midbrain and basal ganglia (Dalley et al. 2007; Buckholz et al. 2010) to large-scale, functionally dissociable neocortical systems.

Moreover, it seems that subject impulsivity correlates more strongly with the effects of haloperidol, relative to L-dopa, on connectivity. The neurobiological reasons for this are unclear, but may relate to pharmacological differences between the drugs in specificity or potency. Administering exogenous L-dopa indirectly increases neurotransmission by raising existing levels of the dopamine precursor, a process that may have knock-on effects on other neurotransmitter systems (Everett and Borcherting 1970; Dolphin et al. 1976). Conversely, acute haloperidol challenge preferentially blocks neurotransmission at dopamine D2 receptors and is thought to suppress mechanisms governing synaptic plasticity, an effect likely to be reflected in functional connectivity measures (Tost et al. 2010). Our findings raise the possibility that markers of impulsive personality may primarily influence (or be influenced by) these latter, more specific, dopaminergic mechanisms. Indeed, by impacting a broader range of neurochemical systems, L-dopa administration may influence a broader range of behavioral functions with reduced specificity. Nonetheless, we note here that dopamine D2 receptors are implicated in numerous functions other than mediating individual differences in impulsivity, and thus their comparatively specific modulation with haloperidol may also influence functions not addressed in the current study, such as locomotion, reward-based learning, and motivational processing (Volkow

et al. 1999; Vallone et al. 2000; Wise 2004; Johnson and Kenny 2010). Alternatively, in a neuroleptic-naïve population, genetic, metabolic, or neurophysiological differences in susceptible individuals may increase sensitivity to novel dopamine antagonism with clinical doses of haloperidol more dramatically than sensitivity to indirect agonism with the naturally present L-dopa molecule. This may be only indirectly related to high trait impulsivity, although equivalent interactions have previously been identified between impulsivity differences and the functional effects of “direct” dopamine agonists (Cools et al. 2007; Dalley et al. 2007). It should be noted that the objectivity of the BIS-11 self-report scale in assessing multifactorial impulsivity is a subject of some debate (reviewed in Evenden 1999), although as a general construct it displays robust inverse associations with molecular imaging measures of D2/D3 receptor availability pertinent to subcortical dopamine neurotransmission (Buckholz et al. 2010).

Our study incorporated a between-subjects design examining 3 pharmacological conditions in separate groups. As we investigated connectivity in 2 distinct drug conditions and a placebo condition, the envisaged practical benefits of requiring only 1 scanning visit from each participant, rather than requiring the same participant to be scanned on 3 occasions under different conditions, were clearly realized in terms of minimizing subject attrition. However, in many cases, within-subject, double-blind, placebo-controlled studies are regarded as preferable for increasing sensitivity to drug effects. Indeed, it is possible that the additional between-group variability resulting from our design decreases sensitivity to detecting certain types of effect. Nevertheless, our analyses were sensitive enough to reveal the significant systems-level pharmacological effects described. Furthermore, possible differences in the potency of drug effects (at clinical doses) and the subjective psychological experiences thereof might introduce additional order effect biases, even into the results of a randomized design with repeated-measures within-subject. Indeed, some of the apparent divergence between the current findings and those of Kelly et al. (2009), who also tested the effects of L-dopa drug modulation on functional connectivity, may be explained by the fact that their study employed a within-subject, placebo-controlled design. In addition, this previous study used only a single pharmacological (L-dopa) challenge and thus was unable to test hypotheses identical to those examined in our study, specifically of linear dopamine neuromodulatory effects on RSN connectivity (i.e. L-dopa > placebo > haloperidol). Finally, these studies used quite different analytical techniques to define “networks” of functional connectivity. Thus, there are a number of reasons why the 2 approaches are likely to be sensitive to distinct systems-level effects of dopamine modulation. Nonetheless, it will be important for future work to extend the approach described here to elucidate differential drug effects on network connectivity within individuals, particularly with the aim of applying these techniques in clinical or medicines development settings. Ideally, such extensions will also provide the opportunity to obtain, pre- and post-drug administration, behavioral and other measures relevant for individual differences or neuropsychiatric disorders. This will enable experimenters to interpret associations, such as those between impulsivity and dopamine-dependent connectivity, more directly in terms of changes over time or following experimental intervention.



In summary, we find cortico-subcortical network functional connectivity patterns to be affected differentially by dopamine agonist (l-dopa) and antagonist (haloperidol) drugs regularly used to treat neuropsychiatric disorders, relative to a placebo. The systems-level brain response to targeted pharmacological D2 receptor blockade with a selective antagonist may be a more sensitive endophenotype for certain neuropsychiatric indications, such as trait impulsivity, than the response to indirectly increasing dopamine neurotransmission by raising precursor levels. Future studies could extend systems-level investigation of cortico-subcortical connectivity associations with personality, behavioral, or genetic factors to patient populations regularly medicated with selective dopamine receptor agonists or antagonists (Schafer et al. 2001; Dagher and Robbins 2009), revealing the possible impact of brain network functional interactions dependent on these factors on treatment and prognosis.

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## Notes

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