Orbitofrontal Connectivity with Resting-State Networks Is Associated with Midbrain Dopamine D3 Receptor Availability

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Animal research and human postmortem evidence highlight the importance of brain dopamine D3 receptor (D3R) function in multiple neuropsychiatric disorders, including addiction. Separate anatomical and functional neuroimaging findings implicate disrupted frontal cortical connectivity with distributed brain networks in processes relevant for these diseases. This potential conjunction between molecular and functional markers has not, however, been tested directly. Here, we used a novel combination of [11C]-((+)-PHNO) positron emission tomography and resting-state functional magnetic resonance imaging in the same healthy individuals to investigate whether differences in midbrain D3R availability are associated with functional interactions between large-scale networks and regions involved in reward processing and cognition. High midbrain D3R availability was associated with reduced functional connectivity between orbitofrontal cortex (OFC) and networks implicated in cognitive control and salience processing. The opposite pattern was observed in subcortical reward circuitry and the “default mode” network, which showed greater connectivity with OFC in individuals with high D3R availability. These findings demonstrate that differential interactions between OFC and networks implicated in cognitive control and reward are associated with midbrain D3R availability, consistent with the hypothesis that dopamine D3R signaling is an important molecular pathway underlying goal-directed behavior.

Keywords: dopamine, d3 receptor, networks, resting state, reward

Introduction

Dopamine has been widely implicated in motivated behavior, reward processing, and related neuropsychiatric disorders, particularly substance dependence (see e.g., Schultz 2002; Wise 2004). Preclinical studies suggest that the D2-like family of dopamine receptors (D2, D3, and D4) mediates reward-related behavior (Dalley et al. 2007; Heidbreder and Newman 2010). D3 receptor (D3R) function specifically has been related to sensitivity to positive reinforcement, contributing to reward-seeking and motivated behaviors (Caine and Koob 1993; Guillon et al. 2001; Sokoloff et al. 2006). D3Rs in the substantia nigra/ventral tegmental area (SN/VTA) of the midbrain are thought to act as inhibitory modulators of postsynaptic dopamine release in regions implicated in reward processing, including orbitofrontal cortex (OFC) and striatum (Levant 1997; Buckholtz et al. 2010; Heidbreder and Newman 2010; Koob and Volkow 2010; Sesack and Grace 2010). However, while the D2 receptor (D2R) system has been explored extensively, little has been discovered about the role of the D3 system in human brain function due to the lack, until recently, of D3R-selective imaging probes (Rabiner and Laruelle 2010).

Studies in primates and patients with frontal lobe lesions have shown that the OFC is crucial for decision-making processes subserving inhibitory control and goal-directed behavior (Damasio 1990; Bechara et al. 2000; Izquierdo et al. 2004, 2005). Functional magnetic resonance imaging (fMRI) studies have expanded on these findings, identifying task-specific blood oxygen level-dependent (BOLD) activity in functionally heterogeneous regions of human OFC that are associated with distinct aspects of reward processing and goal-directed behavior (Damasio et al. 2003, 2001). Thus, OFC functions strongly parallel those often attributed to the D3R system. Furthermore, primate OFC has been shown to experience reduced blood flow following D3-prefering agonist administration (Black et al. 2002). The OFC also has extensive reciprocal anatomical connections with cortical, subcortical, and midbrain regions, consistent with the notion that it regulates the functioning of multiple neuronal networks underlying behavioral decision making (Kringelbach and Rolls 2004; Frank and Claus 2006; Price 2006). Despite these functional similarities, and although large-scale brain networks are known to underlie many of the complex behaviors contributing to reward and addiction (e.g., Cole, Beckmann, et al. 2010; Koob and Volkow 2010), little is currently known about the potential role of midbrain D3R in modulating interactions between orbitofrontal regions and networks relevant to cognitive control, motivation, and reward processing.

We used positron emission tomography (PET) with the D3-prefering radioligand [11C]-((+)-PHNO and resting-state FMRI in the same individuals to examine midbrain D3R availability in conjunction with measures of OFC functional connectivity with “resting-state” networks (RSNs). RSN connectivity measures map the brain’s functional network architecture, highlighting the integration of spontaneous synchronized BOLD signal fluctuations across distinct distributed neural systems.
We hypothesized that RSNs spanning frontoparietal cortical regions, previously shown to support cognitive control and salience processing (Gusnard et al. 2001; Greicius et al. 2004; Seeley et al. 2007, 2009), would show variation in OFC functional connectivity between subjects, associated with individual differences in midbrain D3R availability. In line with reported dissociations between cognitive control and reward-related brain activity in addiction and other neuropsychiatric disorders (Baler and Volkow 2006; Cools 2006), we further hypothesized that an opposing relationship would exist between D3R availability and OFC connectivity with RSNs comprising reward-processing structures in the limbic system and basal ganglia.

Recent findings have shown that $[^{11}C]$-(-)PHNO can be used to quantify the in vivo availability of both D3R and D2R subtypes (Rabiner et al. 2009; Searle et al. 2010). $[^{11}C]$-(-)PHNO binding in the midbrain SN/VTA is attributable specifically to D3R availability ($>90\%$ of the total signal), while the majority of binding ($>90\%$) in the dorsal putamen (DPU) is attributable to D2R (Tziortzi et al. 2011). Based on this and other emerging evidence of anatomical and functional dissociations between D2R and D3R (van Gaalen et al. 2009; Heidbreder and Newman 2010), a secondary objective of the study was to investigate possible differential effects on RSN connectivity patterns associated with D2R, relative to D3R availability.

### Materials and Methods

#### Participants

Participants were 12 right-handed healthy male volunteers (mean age = 37.3 years, range = 31.2-52.4 years). No subjects had a history of medical, neurological, or psychiatric illness nor were any taking concomitant medications or nonprescription drugs. Suitability for inclusion in the study was assessed by complete medical history as confirmed by general practitioner and a qualified study physician, review of systems, physical examination, psychiatric interview, routine blood tests, urine toxicology, and electrocardiogram. Subjects were excluded from the study if any history of neuropsychiatric illness was established, including substance use disorder. Screening prior to scanning included urine testing to exclude subjects positive for recent alcohol or nicotine use and other drugs of abuse. Subjects were excluded if presenting with a history of regular alcohol consumption within 6 months of the study, defined as an average weekly intake of $\geq 37.0$ units for men and $\geq 21.0$ units for women. Subjects with urinary cotinine levels indicative of smoking or history or regular use of tobacco- or nicotine-containing products within 6 months prior to study enrollment were also excluded. Subjects abstained from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) for 24 h prior to their scans.

#### Study Design

Each subject was PET scanned with $[^{11}C]$-(-)PHNO. This was followed on the same day by resting-state BOLD FMRI acquired twice in a single session. PET scanning was carried out for 90 min, and participants were transferred for FMRI scanning within 60 min of PET scan completion. During FMRI, each subject underwent two 5-min resting-state scans separated by 20 min, both of which were subsequently analyzed. Participants completed a cognitive task between the 2 resting scans. All participants provided written informed consent. Imaging data were collected during baseline sessions of a larger drug study. The study protocol was approved by the UK Health Protection Agency Administration of Radioactive Substances Advisory Committee and the Welwyn Clinical Pharmacology Ethics Committee, University of Hertfordshire.

$[^{11}C]$-(-)PHNO Production

$[^{11}C]$-(-)PHNO was prepared from the N-despropyl PHNO, (+)-3,4,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9-ol, hydrochloride, by $[^{11}C]$-propionyl chloride acylation followed by reduction of the $[^{11}C]$-amide intermediate (Wilson et al. 2005). This reaction was fully automated to reliably produce high-purity $[^{11}C]$-(-)PHNO in a radiochemical decay-corrected yield of $16 \pm 5\%$ after a synthesis time of 35 min. Injected activity of $[^{11}C]$-(-)PHNO ranged from 114.5 to 404.7 MBq across subjects ($mean = 222.8 \pm 96.5$ standard deviation [SD]) and injected mass ranged from 1.04 to 4.24 $\mu$g ($mean = 2.01 \pm 0.91$ SD).

#### PET Image Acquisition

Dynamic PET scans were acquired in 3D mode using a Siemens Biograph 61 Hi-REZ PET-CT scanner. A CT scan was acquired for attenuation correction of PET data prior to administration of $[^{11}C]$-(-)PHNO. The radioligand was administered as an intravenous bolus over 30 s. Dynamic PET data were acquired for 90 min, binned into 26 frames (durations: $8 \times 15$ s, $5 \times 60$ s, $5 \times 2$ min, $5 \times 5$ min, and $5 \times 10$ min), then reconstructed using Fourier rebinning and 2D filtered back projection with a ramp filter at Nyquist cutoff frequency. Image data were smoothed with a Gaussian kernel of 5 mm full-width at half-maximum (FWHM).

#### PET Analysis

Dynamic PET images were registered to the subject’s $T_1$-weighted anatomical volume (see FMRI acquisition, below) and corrected for motion using a frame-to-frame registration process with a mutual information cost function (as implemented in SPM5; Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm). Bilateral SN/VTA regions of interest (ROIs) were defined manually on each subject’s PET integral image, using a previously published method (Searle et al. 2010). DPU ROIs were bilaterally and manually defined on each subject’s $T_1$ image, using a technique shown to reliably distinguish the region from others in the striatum (Tziortzi et al. 2011). Examples of subject-specific ROIs are provided in Supplementary Figure S1. ROIs were applied to the dynamic PET data to derive regional time-activity curves. Nondisplaceable binding potential ($BP_{ND}$) values were calculated for both bilateral ROIs via the basis function implementation of the simplified reference tissue model (Lammertsma and Hume 1996; Gunn et al. 1997). The whole cerebellar gray matter, defined using an in-house atlas (see Tziortzi et al. 2011), was used as the reference region in this analysis.

$$BP_{ND} = \frac{f_{ND}B_{max}}{K_0}$$

(1)

The $[^{11}C]$-(-)PHNO binding potential relative to the nondisplaceable compartment ($BP_{ND}$) is equal to the product of receptor density ($B_{max}$), affinity of ligand for the target ($1/K_0$), and the free fraction of the ligand in the brain ($f_{ND}$).

This produced values of $[^{11}C]$-(-)PHNO $BP_{ND}$ in SN/VTA and DPU regions for each subject. $BP_{ND}$ values were demeaned prior to inclusion in the FMRI analyses.

#### FMRI Acquisition

BOLD contrast-sensitive $T_2$-weighted echo-planar images were acquired continuously on a 3-T Siemens Trio scanner with a 32-channel head coil. Each scan consisted of 154 volumes of 38-slice acquisition (time repetition [TR] = 2100 ms, time echo [TE] = 31 ms, flip angle = 78°, $3.5 \times 3.5 \times 3.0$ mm voxels). It is well established that OFC regions can exhibit signal dropout with echo-planar imaging (see, e.g., Kringelbach and Rolls 2004). Therefore, we used the following acquisition techniques to recover signal in these regions: 1) slice acquisition was angled 30° coronally upward from the anterior-posterior commissural plane; 2) slice thickness was minimized to 3 mm. These measures minimized OFC signal dropout but led to loss of coverage over a small posterior portion of the superior parietal lobe in some subjects. The first 4 volumes were removed from each FMRI data set to allow for magnetic equilibration, resulting in a 150-datapoint BOLD time series at each voxel per acquisition.
Additional high-resolution T1-weighted anatomical scans (magnetization prepared rapid gradient echo; TR = 3000 ms, TE = 5.66 ms, flip angle = 9°, voxel size = 1 mm³, 208 slices) were acquired with whole-brain coverage for each subject to aid with both FMRI and PET image coregistration and PET ROI definition (see Supplementary Fig. S1). For the resting-state scans, subjects were instructed to lie still with their eyes closed but to stay awake.

FMRI Analysis
Image preprocessing and analyses were performed with FSL (FMRIB Software Library; www.fmrib.ox.ac.uk/fsl; Smith et al. 2004). Preprocessing techniques applied to the resting-state FMRI data included motion correction, brain extraction, spatial smoothing with a Gaussian kernel of 5 mm FWHM, and high-pass temporal filtering at 100 s. Volumes from each FMRI acquisition were registered to a common space, first by linear registration to the individual’s own high-resolution anatomical volume, then via nonlinear spatial normalization to the Montreal Neurological Institute (MNI) 152 2 mm³ stereotactic template. Both resting-state acquisitions from each subject were included in the analysis. To identify independent components, including RSNs, expressing consistent spatiotemporal coherence across scans and subjects, all data were entered into probabilistic multisession independent component analysis (ICA) with temporal concatenation (as implemented in FSL, MELODIC; Beckmann and Smith 2004; Beckmann et al. 2005). This group ICA approach decomposes the concatenated 4D data set (150 volumes per scan × 2 resting-state scans per subject × 12 subjects = 3600 image volumes) into spatial maps of structured component signals in the data (and associated time courses). We here identified 33 components displaying maximal spatial independence effects. The number of components for the group 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).

Following group ICA, we calculated subject-specific measures of functional connectivity for all independent components (including RSNs) via a dual regression approach described previously by multiple groups (Filippini et al. 2009; Cole, Beckmann, et al. 2010; Roosendaal et al. 2010). The dual regression procedure is applied separately to each individual FMRI data set and operates within a multiple regression framework. For each independent component, voxelwise maps of functional connectivity strength (regression coefficients) at the subject/scan level, representing “individualized” versions of the group-level components, were calculated as follows. Step 1: The full set of thresholded spatial component maps (including RSNs) identified by group ICA of the FMRI data was entered into a consecutive linear model fits (spatial regression) against preprocessed FMRI data sets from each resting-state acquisition. These regressions produced separate 33-column matrices describing the mean temporal dynamics, at the individual subject/scan level, of each equivalent group-level component (one per column). Step 2: These matrices were then used in consecutive linear model fits (temporal regression) against the same associated functional data sets. This produced, for each FMRI acquisition, a set of 33 individualized spatial maps, each one the subject/scan-specific instantiation of an equivalent group-level component, including RSNs. These 3D maps contain voxelwise regression coefficient measures of network functional connectivity, which we define here as a measure of the synchronization between the BOLD temporal dynamics at a given voxel and the mean, or “characteristic,” scan-specific BOLD time series of the individualized (RSN) component. The individualized maps corresponding to 6 separate RSNs of interest were then concatenated across subjects, creating a 4D file per RSN, one subject/scan per volume. Final analyses comparing individual differences in RSN connectivity with PET measures were carried out on these data.

We selected RSNs from the group ICA results for further analyses based on their neuroanatomical configurations, in comparison with previous literature (e.g., Beckmann et al. 2005; Seeley et al. 2007; Kiviniemi et al. 2009; Smith et al. 2009) and through spatial cross-correlation with RSN templates from 2 previously published group ICA data sets (Beckmann et al. 2005; Cole, Beckmann, et al. 2010). We were primarily interested in networks thought to be relevant for cognitive control and goal-directed behavior (Supplementary Table S1). These were 1) 3 bilateral frontoparietal RSNs supporting high-level cognitive and motivational processes, with nodes located within 3 or more of the dorsal anterior cingulate, dorsolateral prefrontal, anterior insular, and inferior parietal cortices (typically referred to as “salience” or “executive control” networks) we will refer to here as SEN1, SEN2, and SEN3; 2) the “default mode” network (DMN; Fig. 1D), and 3) a network comprising basal ganglia/limbic regions (the BGLN; Fig. 1E), with potential relevance to dopamine function (Kelly et al. 2009; Sesack and Grace 2010). A sixth somatomotor cortical network served as a control RSN in our analyses (see Results).

PET-FMRI Comparison
Four-dimensional files comprising subject-wise whole-brain maps of voxelwise functional connectivity with each RSN of interest (from dual regression) were hypothesis tested to find regions where individual differences in RSN connectivity were significantly positively or negatively associated with PET BPND measures. For this, we used voxelwise random-effects nonparametric permutation testing (as implemented in FSL randomise; Nichols and Holmes 2002), with 5000 permutations and 5 mm variance smoothing. Our primary interest was in relating D3R availability to OFC-RSN connectivity patterns, so [11C]-[(+)-PHNO BPND values from SN/VTA were entered as a covariate of interest in voxelwise analyses of separate RSN maps. Of secondary interest were effects associated with D2R availability, so DPU [11C]-(+)-PHNO BPND scores were also included as a covariate in these analyses. To control for the effects of a task carried out between the 2 resting FMRI scans in each session, we included an additional order covariate, differentially modeling and contrasting for potential pre- and post-task resting session effects across subjects. Normalized voxels displaying zero signal in at least one individual acquisition or falling below a 20% threshold of containing gray matter based on a study population–specific probabilistic atlas calculated using FAST (part of FSL; Zhang et al. 2001), were exempt from all random-effects regression analyses. Significant regional effects were defined using cluster-mass thresholding (ts = 2.3, P < 0.05) correcting for family-wise error (FWE).

We additionally explored correlations between SN/VTA (D3) BPND, DPU (D2) BPND, and subject age to test whether these factors were significantly associated (e.g., Volkow et al. 1998). For completeness, we also correlated age (post hoc) with individual scores from any BPND-related OFC-RSN connectivity effects identified by our PET-FMRI comparison (see Results).

We assessed connectivity correlations across the single-network voxelwise analyses (Fig. 1; see also Supplementary Tables S2 and S3), we carried out an additional random-effects multiple regression with identical parameters and covariates to those already described, this time entering the 4D concatenated subject-specific 3D spatial maps of 2 RSNs (the DMN and SEN1) displaying inverse D3-dependent relationships, heavily overlapping the same OFC region (Fig. 2). Whole-brain differential contrasts were carried out between the 2 networks, in order to fully examine the D3-related dependencies of this, and other, patterns of regional connectivity with one RSN relative to the other.

Results
PET Measures of D3 and D2 Receptor Availability
We estimated midbrain D3R availability for 12 healthy male subjects by calculating the specific binding potential (BPND) of [11C]-(+)-PHNO in the SN/VTA (mean = 1.59 ± 0.30 SD, range = 1.12–2.06). [11C]-(+)-PHNO BPND values from the putamen region of the dorsal striatum (DPU) were calculated to provide measures of D2R availability (mean = 2.47 ± 0.21 SD, range = 2.09–2.78). We found no correlation between D3R and D2R availabilities (r = −0.03, P = 0.93) in our sample. There was also no correlation between BPND measures and age (D3: r = 0.01, P = 0.98; D2: r = 0.29, P = 0.36) in our sample. Similarly, there were no significant correlations between subject age and any of
the BPND-related OFC-RSN connectivity measures identified below (D3: all \( P > 0.37 \); D2: all \( P > 0.22 \)).

**Resting-State Functional Connectivity Analysis Identifies Networks Implicated in Cognitive Control and Goal-Directed Behavior**

Using group-level ICA we identified 33 components in the resting-state fMRI data (24 scans in total, 2 per subject), including RSNs commonly found in previous studies (e.g., Beckmann et al. 2005; Kiviniemi et al. 2009). We selected 6 RSNs of interest for further analyses. This included 3 frontoparietal SENs (Fig. 1A–C) with spatial patterns previously characterized as relevant to cognitive and motivational processing (e.g., Seeley et al. 2007). We examined the DMN (Fig. 1D) because of its putative role in spontaneous cognition (Gusnard et al. 2001; Raichle et al. 2001; Buckner et al. 2008), and the functional relevance of its “anticorrelated” relationship with SENs identified previously (Fox et al. 2005; Cole, Beckmann, et al. 2010). We also examined a basal ganglia/limbic network (BGLN) including the striatum, amygdala, thalamus, and ventral anterior cingulate (Fig. 1E), as many of these regions have high D3R concentrations (Rabiner et al. 2009; Searle et al. 2010; Tziortzi et al. 2011), and this network has been strongly implicated in reward processes and dopamine function (Kelly et al. 2009; Sesack and Grace 2010). A somatomotor RSN spanning bilateral superior parietal cortices, thought to relate to primary sensory and motor processing (Biswal et al. 1995), served as a control RSN (i.e., not expected to be influenced by D3R availability) in our analyses. The spatial patterns of functional connectivity for these RSNs
association between midbrain D3R availability and midbrain D3R availability (overlap = 438 mm$^3$, Fig. 2).

Variation in somatomotor RSN connectivity with the OFC showed no relationship to individual differences in midbrain D3 BP$_{ND}$ (Supplementary Table S2). We also found no evidence of significant order effects on the functional connectivity of any of the RSNs studied by testing for differences between the 2 resting FMRI scans acquired in each session.

**Effects of Putamen D2R Availability on RSN Connectivity Differ from Those of Midbrain D3R Availability**

Brain regions in which subject-specific RSN functional connectivity differences were associated with DPU $[^{11}$C]-(+)-PHNO BP$_{ND}$ variation (reflecting D2R availability) were much less abundant than those associated with variation in D3R availability and also showed different anatomical patterns (Supplementary Table S3). We did not find D2-related connectivity associations between SENs and clusters in distinct or overlapping OFC subregions. However, 2 RSNs (the BGLN and the DMN) showed effects of D2R availability on connectivity with clusters located in (or extending to) OFC (Fig. 3; BGLN: peak $t = 5.28, x = 4, y = 56, z = -12$; DMN: $t = 5.14, x = 50, y = 40, z = -10$). These relationships were opposite in direction (negative) to the (positive) associations found between D3R availability and OFC functional connectivity with the same 2 networks (see Fig. 1D,E).

**Midbrain D3R Availability Modulates Inverse Connectivity between OFC and RSNs**

Anatomically overlapping, but inverse, associations between anterolateral OFC functional connectivity with the DMN relative to SEN1, which were explained by midbrain $[^{11}$C]-(+)-PHNO BP$_{ND}$ (Fig. 2), were identified by separate analyses of functionally distinct RSNs. To confirm that this was indicative of D3-dependent differences in connectivity between the OFC region and one RSN relative to another, we conducted a further whole-brain multiple regression analysis (see Materials and Methods). We found that inverse functional connectivity with the DMN relative to SEN1 in regions including right anterolateral OFC was associated with variation in midbrain D3R availability across subjects (Fig. 4). High D3R availability was associated with greater (less negative) right anterolateral OFC connectivity with the DMN and lower positive functional connectivity with SEN1 (peak $t = 6.81$; MNI coordinates: $x = 24, y = 60, z = -8$). This contrast also revealed inverse RSN connectivity effects dependent on midbrain D3R availability (Fig. 4) in the left dorsomedial prefrontal cortex ($t = 6.67$; --22, 62, 12), right inferior parietal cortex ($t = 6.03$; 42, --58, 52), and the precuneus ($t = 5.33; 2, -46, 40$), as well as the cerebellum ($t = 6.99$; --14, --54, --52). Therefore, higher midbrain D3R availability across individuals was associated with a significant reduction in negative functional connectivity between the DMN and SEN1, as measured within a number of their key cortical regions. Based on the combined and single-network analyses, the OFC in particular appears to influence interactions of large-scale cognitive RSNs to a greater degree in individuals with low midbrain D3R availability.

**Discussion**

The results presented here relate molecular signaling through a specific dopamine neuroreceptor system to activity...
Figure 3. OFC-RSN functional connectivity patterns associated with putamen D2R availability are sparse relative to D3-related effects and opposite in direction. In contrast to relationships between midbrain D3R availability and OFC connectivity with all frontoparietal and limbic RSNs, individual differences in DPU D2R availability (N = 12) only significantly explain OFC functional connectivity with the DMN (green) and the BGLN (blue), but with no SEN systems (cluster t = 2.3, P < 0.05, FWE-corrected). These negative relationships are in the opposite direction to those associated with D3R availability found between OFC regions and these 2 RSNs (see Fig. 1; plots as described therein; see also Supplementary Table S3).

Figure 4. High midbrain D3 receptor availability explains reduced inverse functional connectivity with the DMN relative to SEN1, in right anterolateral OFC and key regions of cognitive RSN overlap. Higher midbrain D3 receptor availability is associated, across individuals (N = 12), with a significant reduction of the inverse functional connectivity patterns with the DMN relative to the SEN1 system displayed by (reading left to right) 1) the right anterolateral OFC, 2) left dorsomedial prefrontal cortex, 3) bilateral precuneus, and 4) right inferior parietal cortex (cluster t = 2.3, P < 0.05, FWE-corrected).
modulations in brain RSNs. Together with recent studies of pharmacological or genetic effects (see, e.g., Achard and Bullmore 2007; Liu et al. 2010; Gordon et al. 2011; Kunisato et al. 2011; Wiggins et al. 2011), these findings provide important insights into the molecular bases of large-scale functional connectivity patterns. Parallels between resting-state BOLD FMRI activity fluctuations and those evoked by specific cognitive tasks are becoming increasingly apparent (Fox and Raichle 2007; Buckner et al. 2008, Smith et al. 2009; Cole, Smith, et al. 2010), reinforcing the notion that these phenomena reflect core aspects of neural processing relevant for cognitive and behavioral functioning. Furthermore, the same large-scale networks show altered activity in certain neuropsychiatric disease states (e.g., Greicius et al. 2004; Seeley et al. 2009; Cole, Beckmann, et al. 2010; Koob and Volkow 2010).

We have shown in healthy subjects that individual differences in midbrain dopamine D3 receptor availability relate to distinct variations in functional connectivity between the OFC and multiple large-scale brain networks (RSNs). High midbrain D3R availability was associated with reduced connectivity between the OFC and frontoparietal RSNs involving brain regions implicated in executive control and salience processing (Seeley et al. 2007). Conversely, OFC connectivity with the basal ganglia and with the DMN was greater in individuals with high D3R availability. Our analyses further emphasized that subregions of the anterolateral OFC could be distinguished on the basis of their relative D3-related (and not their D2-related) connectivity with one or more RSNs. Previous work has indicated that dopaminergic mechanisms may cause frontal cortical and basal ganglia activity patterns to dissociate during aberrant reward processing (Baler and Volkow 2006; Cools 2006), while the DMN is often “downregulated” during cognitive processes associated with increased salience or executive network activity (Greicius et al. 2003; Fox et al. 2005). Our results therefore provide novel evidence of connectivity dissociations in humans, in line with these findings, relating the functional modulation of neocortical pathways relevant to cognitive control, motivation, and reward processing to D3R-mediated dopaminergic signaling.

Our results demonstrate that functional interactions between OFC and networks implicated in cognitive control and reward are associated with midbrain D3R signaling. Preclinical, clinical, and postmortem evidence implicates dysfunction of both the D3R system and the salience/executive RSNs in a number of neuropsychiatric disorders (Staley and Mash 1996; Gurevich et al. 1997; Allman et al. 2005; Sokoloff et al. 2006; Seeley et al. 2009; Kim et al. 2011) and in aspects of emotional and social behaviors in healthy subjects (Seeley et al. 2007; Allman et al. 2010). Primary “nodes” of SEN systems in frontoinsular and anterior cingulate cortices contain large quantities of long-range bipolar projection neurons found only in humans and a minority of other mammals with highly evolved neocortices (Nimchinsky et al. 1999; Allman et al. 2010). Moreover, regions rich in these cells exhibit strong anatomical connections with anterior orbitofrontal cortices (Allman et al. 2010), and immunocytochemical evidence shows these neurons to be labeled abundantly with D3R antibodies (Allman et al. 2005). Together with our findings, the collective evidence therefore is suggestive of an association between D3R function, specialized frontal lobe cytoarchitecture, and large-scale network connectivity patterns underlying complex behavior in humans.

An earlier study reported a positive association between D2/D3 receptor availability in the striatum and metabolism in OFC, measured using $[^{11}C]$raclopride PET and $[^{18}F]$fluorodeoxyglucose PET, respectively (Volkow et al. 2001). However, radioligands such as $[^{11}C]$raclopride do not distinguish between D2 and D3 receptor subtypes (Graff-Guerrero et al. 2008). We were able to make use of the relative selectivity of $[^{11}C]$. (+)-PHNO binding in midbrain SN/VTA and DPU to delineate specific measures of D3R and D2R availability, respectively (Rabiner et al. 2009; Searle et al. 2010). By combining this information with FMRI data in the same subjects, we were further able to characterize associations between D3R availability and the relative functional connectivity of large-scale networks (RSNs) with anatomically distinct OFC subregions. Future work needs to apply these novel multimodal neuroimaging techniques to characterize clinical and behavioral changes associated with this systems-level connectivity modulation and, further, determine a causal link between D3R function and OFC large-scale connectivity patterns.

OFC regions are thought to be crucial for regulating impulsive and adaptive behaviors related to reward sensitivity (Bechara et al. 2000; Izquierdo et al. 2004; Kringelbach and Rolls 2004) and goal-directed decision making (Frank and Claus 2006; Walton et al. 2010). A previous meta-analytic review of functional imaging studies relates activity in anterior and lateral OFC regions analogous to those described in the current study to “impending changes in behavior” in response, for example, to “punisher” stimuli signaling negative emotional, social-, or reward-related connotations for maintaining a behavioral strategy (Kringelbach and Rolls 2004). This kind of adaptive behavior across multiple facets of complex cognition and emotional processing is precisely that represented in the functional correlates of activity in SENs (Seeley et al. 2007). The current results expand upon these interpretations by showing that anterolateral OFC integration in reward and cognitive control systems is explained to some extent by midbrain D3R signaling.

Our findings additionally highlight in vivo functional distinctions between the influences of dopaminergic signaling through D3 relative to D2 receptor subtypes. There is currently much debate on precisely how postsynaptic dopaminergic inhibition relates to D2/D3 function in midbrain relative to striatal regions (Levant 1997; Volkow et al. 2001; Dalley et al. 2007; Buckholtz et al. 2010; Heidbreder and Newman 2010; Koob and Volkow 2010; Sesack and Grace 2010). High midbrain D3R availability may reflect increased autoreceptor inhibition of dopamine release in frontal and striatal projection areas (Levant 1997; Buckholtz et al. 2010; Heidbreder and Newman 2010; Koob and Volkow 2010; Sesack and Grace 2010), which our data indicate may result in 1) reduced anterolateral OFC modulation of interactions within and between inversely related frontoparietal cognitive RSNs, while 2) increasing the posterior OFC modulation of striatal (BGLN) reward pathways (also associated here with low DPU D2R availability), and 3) perhaps leading to increased compulsive or reward-seeking behaviors (e.g., Volkow et al. 2001). Evidence of such a relationship between these behaviors and the defined brain processes comes, for example, from associations between increased drug cue responsiveness and reduced cognitive control in addictive disorders (see, e.g., Baler and Volkow 2006). Further support for this interpretation stems from recent evidence in nicotine and cocaine addicts showing...

Other PET studies in humans with nonselective D2/D3 ligands support relationships between reward-related personality traits and dopamine receptor availability (Zald et al. 2008; Lee et al. 2009; Buckholtz et al. 2010; Gjedde et al. 2010). Although there are no prior published data in humans specifically linking D3R with behavioral risk factors for addiction, such as impulsivity or reward sensitivity (Zald et al. 2008), nonhuman studies with selective antagonists show that D3-blocking specifically abolishes motivated reward-seeking behaviors (Heidbreder and Newman 2010). Conversely, one rodent study used preferential and selective antagonists for D2R and D3R subtypes, respectively, to demonstrate specific D2R-mediated increases in impulsive behavior following amphetamine challenge (van Gaalen et al. 2009). Our observation that OFC functional connectivity with both the DMN and the BGLN is positively associated with midbrain D3R but negatively associated with DPU D2R availability extends this evidence of functional dissociations between dopamine neuron-receptor subtypes in humans. Moreover, we have identified the OFC as a potentially key region for modulating interactions between SNSs implicated in cognition and motivated behavior, in conjunction with D3R (but not D2R) availability. Such dissociations may relate to existing theories of the “tonic-phasic” hypothesis of dopaminergic influence on cognitive control (e.g., Cools 2006; Sokoloff et al. 2006; Sesack and Grace 2010). Future investigations may seek to link these effects more directly to specific behavioral factors related to addiction or affective disorders, such as salience-dependent motivation, anxiety, or reward sensitivity.

[^11C]-(+)-PHNO binding has been shown to be sensitive to competition from extracellular dopamine in the living human brain (e.g., Willeit et al. 2008; Shotbolt et al. 2011). Thus, extracellular dopamine levels may be a factor in the relationship between midbrain[^11C]-(+)-PHNO binding potentials and OFC-RSN functional connectivity. Additional research is therefore necessary to determine the precise contributions of midbrain D3R density and extracellular dopamine levels to these effects.

This study employed a modest sample size, thus our results should be generalized judiciously. For example, an association between the availability of different dopamine receptor subtypes (D2 and D3) was not found in the current study but could emerge through testing in a different population. Nevertheless, we found highly robust associations between PET measurements of D3R availability and FMRI measurements of functional connectivity across a number of networks involving the OFC, demonstrating that our sample has sufficient power to detect significant effects in accordance with our a priori hypotheses.

In summary, using a novel approach combining[^11C]-(+)-PHNO PET and resting-state FMRI, we have shown directly in healthy humans that variation in midbrain dopamine D3 receptor availability is associated with individual differences in functional connectivity between OFC regions and multiple neural networks underlying cognitive control, goal-directed behavior, and reward. To date, dopaminergic pharmacotherapies have not proven clinically effective in treating most addictions. This work contributes to the validation of D3R as a target for novel treatments for addiction or other motivational disorders. Evidence has already indirectly associated dysfunction of both D3R and frontal cortical networks with a number of neuropsychiatric disorders (Staley and Mash 1996; Gurevich et al. 1997; Allman et al. 2005; Sokoloff et al. 2006; Seeley et al. 2009; Kim et al. 2011). Future studies can extend this to test directly in these disorders for dysfunctional connectivity of the regions of OFC defined here.

### Supplementary Material

Supplementary material can be found at: [http://www.cercor.oxfordjournals.org](http://www.cercor.oxfordjournals.org)

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