

# Selective Augmentation of Striatal Functional Connectivity Following NMDA Receptor Antagonism: Implications for Psychosis

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The psychotomimetic effect of the *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine is thought to arise from a functional modulation of the brain's fronto-striato-thalamic (FST) circuits. Animal models suggest a pronounced effect on ventral 'limbic' FST systems, although recent work in patients with psychosis and high-risk individuals suggests specific alterations of dorsal 'associative' FST circuits. Here, we used functional magnetic resonance imaging to investigate the effects of a subanesthetic dose of ketamine on measures of functional connectivity as indexed by the temporal coherence of spontaneous neural activity in both dorsal and ventral FST circuits, as well as their symptom correlates. We adopted a placebo-controlled, double-blind, randomized, repeated-measures design in which 19 healthy participants received either an intravenous saline infusion or a racemic mixture of ketamine (100 ng/ml) separated by at least 1 week. Compared with placebo, ketamine increased functional connectivity between the dorsal caudate and both the thalamus and midbrain bilaterally. Ketamine additionally increased functional connectivity of the ventral striatum/nucleus accumbens and ventromedial prefrontal cortex. Both connectivity increases significantly correlated with the psychosis-like and dissociative symptoms under ketamine. Importantly, dorsal caudate connectivity with the ventrolateral thalamus and subthalamic nucleus showed inverse correlation with ketamine-induced symptomatology, pointing to a possible resilience role to disturbances in FST circuits. Although consistent with the role of FST in mediating psychosis, these findings contrast with previous research in clinical samples by suggesting that acute NMDAR antagonism may lead to psychosis-like experiences via a mechanism that is distinct from that implicated in frank psychotic illness. *Neuropsychopharmacology* (2015) **40**, 622–631; doi:10.1038/npp.2014.210; published online 17 September 2014

## INTRODUCTION

The *N*-methyl-D-aspartate receptor (NMDAR) hypofunction is thought to play a primary role in the pathophysiology of psychosis (Javitt and Zukin, 1991; Olney and Farber, 1995). Its effects are commonly modeled in healthy volunteers by administration of ketamine, a potent antagonist of NMDARs on cortical GABAergic cells that leads to disinhibited glutamatergic stimulation of non-NMDA (ie,

kainate and AMPA) receptors (Moghaddam *et al*, 1997; Olney and Farber, 1995). These effects have a downstream impact on mesocortical and mesolimbic dopamine pathways (Adams and Moghaddam, 1998; Moghaddam *et al*, 1997). In addition, ketamine infusion increases blood flow and metabolic activity in the prefrontal cortex, striatum, and thalamus (Holcomb *et al*, 2001; Vollenweider *et al*, 1997), the effects that strongly correlate with the emergence of dissociative and psychosis-like symptoms (Driesen *et al*, 2013; Holcomb *et al*, 2001; Vollenweider *et al*, 1997). Disruption of fronto-striato-thalamic (FST) circuitry is thought to play a major role in the pathogenesis of schizophrenia and dissociative phenomena (Pantelis *et al*, 1992).

FST circuits comprise a set of parallel yet integrated loops serving distinct and complementary functions (Alexander *et al*, 1986). This functional specificity raises the question of

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whether ketamine exerts its psychiatric effects through selective modulation of activity in one circuit over another. Animal models suggest that NMDAR inhibition leads to schizophrenia-like behavior and increased dopamine in the ventral, the so-called 'limbic' FST circuit, linking the medial prefrontal cortex and the ventral/limbic striatum (ie, nucleus accumbens) (Moghaddam *et al*, 1997). In contrast, recent human molecular imaging studies have found that both patients with psychotic disorders and those with an 'at-risk mental state' (ARMS) for psychosis show increased dopamine transmission in the dorsal, the so-called 'associative' striatum (Howes *et al*, 2009; Kegeles *et al*, 2010), and that these changes correlate with symptom severity and abnormal dorsal prefrontal cortical activation (Fusar-Poli *et al*, 2011). Such findings suggest that the psychotomimetic effects of ketamine may arise, in part, from its influence on dopamine transmission in dorsal FST circuits. Alternatively, ketamine might induce psychosis-like experiences through an independent mechanism, given evidence that dopamine D2 receptor blockade does not attenuate ketamine-induced psychotic symptoms (Krystal *et al*, 1999; Lahti *et al*, 1995).

In light of these possibilities, we sought to characterize the effects of a subanesthetic dose of ketamine on the temporal coherence—ie, functional connectivity—of spontaneous neural activity of dorsal and ventral FST circuits as measured using resting-state functional magnetic resonance imaging (fMRI) in healthy young adults. Resting-state fMRI offers a powerful means to map the functional integrity of large-scale brain networks and their modulation with pharmacological agents (Cole *et al*, 2012; Driesen *et al*, 2013). Our own recent work has identified a prominent reduction of functional connectivity in dorsal FST circuits in ARMS subjects, first-episode schizophrenia patients, and their unaffected first-degree relatives (Dandash *et al*, 2013b; Fornito *et al*, 2013). As such, if the psychotomimetic effects of ketamine arise through a mechanism similar to that seen in patients with psychosis and ARMS individuals, ketamine should act primarily by reducing functional connectivity in dorsal FST circuitry. A distinct mode of action would be suggested by alternative FST functional connectivity changes.

## MATERIALS AND METHODS

### Participants

A total of 21 participants (10 males; mean age 28.7 years, SD = 3.2 years) were recruited via advertisements placed throughout central Cambridge, UK. All participants underwent a screening interview in which they were asked whether they had previously been diagnosed or treated for any mental health problems and whether they had ever taken any psychotropic medications. Participants reporting a personal history of any mental health problems or a history of any treatment were excluded from the study. All participants were right-handed, were free of current of previous psychiatric or neurological disorder or substance abuse problems, and had no history of cardiovascular illness or family history of psychiatric disorder/substance abuse. All participants provided written informed consent in accordance with ethics committee guidelines.

### Study Design

Participants were assessed on two occasions, separated by at least 1 week. On one occasion, they received a continuous computer-controlled intravenous infusion of a racemic ketamine solution (2 mg/ml) until a targeted plasma concentration of 100 ng/ml was reached. This concentration was sustained throughout the protocol. A saline infusion was administered on the other occasion. Infusion order was randomly counterbalanced across participants. The infusion was performed and monitored by a trained anesthetist (RA) who was unblinded for safety reasons, but who otherwise had minimal contact with participants. At all other times, participants were supervised by investigators blinded to the infusion protocol (AF and RG). The participants remained blinded until both assessments were completed. All MRI and assessment procedures were identical across assessment occasions.

### Infusion Protocol

Bilateral intravenous catheters were inserted into volunteers' forearms, one for infusion, and the other for serial blood sampling. We used a validated and previously implemented (Corlett *et al*, 2006) three-compartment pharmacokinetic model to achieve a constant plasma concentration of 100 ng/ml (Domino *et al*, 1982) using a computerized pump (Graseby 3500, Graseby Medical, UK). The infusion continued for 15 min to allow stabilization of plasma levels. Blood samples were drawn before and after the resting fMRI scan and then placed on ice. Plasma was obtained by centrifugation and stored at  $-70^{\circ}\text{C}$ . Plasma ketamine concentrations were measured by gas chromatography-mass spectrometry.

### Clinical Measures

Once the infusion had stabilized and before MRI, participants underwent a clinical rating of positive psychotic symptoms as assessed by the Rating Scale for Psychotic Symptoms (RSPS) (Chouinard and Miller, 1999) and the Brief Psychiatric Rating Scale (BPRS) expanded version 4 (Ventura *et al*, 1993). Seven key items on the BPRS representing positive symptoms of the psychosis prodrome (somatic concerns, anxiety, depression, elevated mood, grandiosity, hallucination, and unusual thought content) were selected. Dissociative symptoms were assessed by the Clinician Administered Dissociative States Scale (CADSS) (Bremner *et al*, 1998). In addition, participants completed assessments of psychosis proneness (Chapman magical ideation (Eckblad and Chapman, 1983) and perceptual aberration scales (Chapman *et al*, 1978) and the Peters delusion inventory (Peters *et al*, 1999)) before the first scan (see Supplementary Materials and Methods, Supplementary Table S1, and Supplementary Figure S2 for further information on scoring properties as well as distribution and descriptive statistics of the psychosis-proneness scales).

### MRI Acquisition

Scanning was performed using a 3.0 T MRI scanner (Siemens Magnetom, Trio Tim, Erlangen, Germany)

equipped with a 12-channel array coil located at the Wolfson Brain Imaging Centre, Addenbrooke's Hospital, Cambridge, UK. T2\*-weighted echo-planar images were acquired under eyes-closed resting-state conditions. Participants were instructed to close their eyes and let their minds wander without going to sleep. Subsequent participant debriefing ensured that no participants fell asleep during the scan. Imaging parameters were:  $3 \times 3 \times 3.75$  mm voxel size, with a time-to-repetition (TR) of 2000 ms, time-to-echo (TE) of 30 ms, flip angle of  $78^\circ$  in  $64 \times 64$  matrix size, and 240 mm field of view (FOV). A total of 300 volumes comprising 32 slices each were obtained. In addition, high-resolution anatomical T1 images were acquired using a three-dimensional magnetic-prepared rapid gradient echo (MPPRAGE) sequence. In all, 176 contiguous sagittal slices of 1.0 mm thickness using a TR of 2300 ms, TE of 2.98 ms, flip angle of  $9^\circ$ , and a FOV of 256 mm in  $240 \times 256$  matrix were acquired with a voxel size of  $1.0 \text{ mm}^3$ .

### Preprocessing

An established procedure was used to characterize cortico-striatal functional connectivity in relation to six seed regions located in ventral and dorsal areas of the caudate nucleus and putamen per hemisphere (Dandash *et al*, 2013b; Di Martino *et al*, 2008; Fornito *et al*, 2013). Seeds were defined in both hemispheres as 3.5 mm radial spheres at the following stereotaxic coordinates: dorsal caudate ( $x = \pm 13$ ,  $y = 15$ ,  $z = 9$ ); ventral striatum/nucleus accumbens ( $x = \pm 9$ ,  $y = 9$ ,  $z = -8$ ); dorsal-caudal putamen ( $x = \pm 28$ ,  $y = 1$ ,  $z = 3$ ), and ventral-rostral putamen ( $x = \pm 20$ ,  $y = 12$ ,  $z = -3$ ). Image preprocessing was performed using statistical parametric mapping software (SPM8) and included motion correction and coregistration of functional images with subjects' anatomical scans that were concurrently normalized to SPM-T1 template. The resulting transformation matrix was applied to the functional data to achieve accurate spatial normalization across individuals. The anatomical scans were also segmented using a unified normalization and segmentation approach. Segmented white matter and CSF images were thresholded at 50% tissue probability and binarized to create nuisance variable masks, together with a binary mask of the global brain volume (summed from the gray matter, white matter, and CSF segments). Although it has been suggested that the removal of the global signal can possibly induce negative or anti-correlated relationships between brain regions (Murphy *et al*, 2009), a large body of competing evidence suggests that the removal of the global signal significantly improves the specificity of resting-state fMRI results (see Van Dijk *et al*, 2010 for a detailed analysis and an extensive list of supporting evidence). In addition, it is highly unlikely that the between-condition comparisons are confounded by this preprocessing step, given that the removal of the global signal was identically performed across both conditions, ketamine and placebo.

Functional images were smoothed using a Gaussian filter (full width at half maximum, 8 mm). All image sequences were routinely inspected for potential normalization artifacts. Mean time series were then extracted from each seed region and each nuisance mask for each participant. Two participants were excluded for excessive head motion

( $>2$  mm translation and  $>2^\circ$  rotation), resulting in a final sample of 19 subjects.

### First-Level, Within-Subject Analysis

For each participant, functional connectivity maps were estimated using whole-brain general linear models as implemented in SPM8. Before model estimation, each of the three nuisance covariates (white matter, cerebrospinal fluid, and global mean signals) and six head motion parameters (three translation and three rotation) were orthogonalized with respect to each seed's time series, resulting in a model comprising six 'noise-cleaned' regions and nine nuisance variables. Time courses extracted from each striatal subregion were entered into a single general linear model that included all striatal seeds together with the nine nuisance variables and used as a variable of interest in a whole-brain regression analysis. Data were high-pass filtered ( $\sim 0.008$  Hz cutoff) and contrast images were generated for each participant by estimating the regression coefficient between all brain voxels and each region's time series separately for left and right hemisphere seeds. The results were largely replicated when employing a more comprehensive first-level motion correction procedure as recently recommended (Satterthwaite *et al*, 2013; see Supplementary Figure S1).

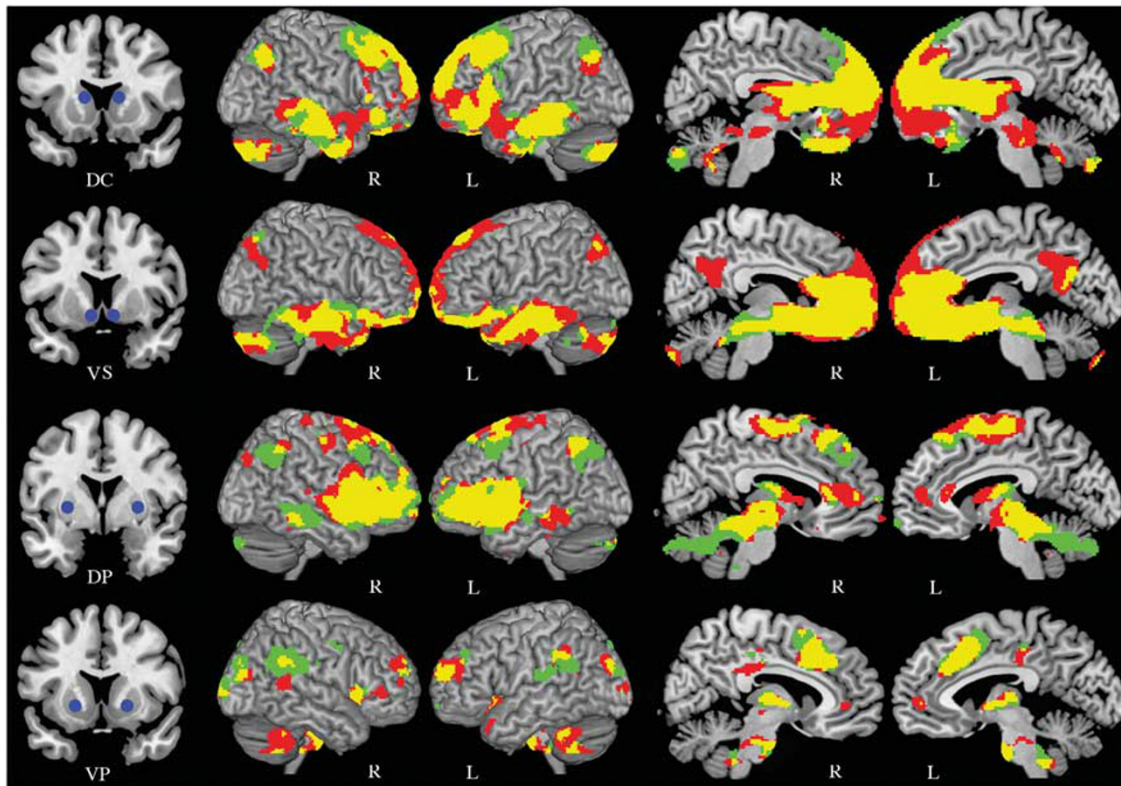
### Second-Level, Between-Condition Analysis

For each striatal region, participant-specific contrast images were included in a random-effects  $2 \times 2$  factorial design (Condition (placebo, ketamine) by Hemisphere (left, right)). Within-condition statistical maps were thresholded at a false discovery rate (FDR) of  $P < 0.05$  for the whole brain volume (Figure 1). Between-condition effects (main effects of condition and condition  $\times$  hemisphere interactions) were mapped by implicitly masking the  $t$ -contrasts (one tailed) with a global conjunction of the within-subject striatal seed main effect across ketamine and placebo for the combined left and right hemispheres. Nuisance covariates included four additional summary measures of head motion unaccounted for by the first-level correction procedure (Van Dijk *et al*, 2012). These covariates included: (1) the number of significant micromovements (instances of  $>0.10$  mm relative displacement between adjacent volumes), (2) mean head displacement, (3) maximum head displacement, and (4) mean head rotation. Consistent with our past work (Dandash *et al*, 2013a; Fornito *et al*, 2013), between-condition statistical maps were thresholded using a  $P < 0.05$  family-wise error (FWE) cluster corrected threshold determined by a permutation test based on the AlphaSim algorithm (1000 permutations with a cluster-forming threshold of  $P < 0.01$  uncorrected), as implemented in the REST toolbox (Song *et al*, 2011). A more stringent cluster-forming threshold of  $P < 0.001$  uncorrected was additionally used to test the robustness of the findings (see Results).

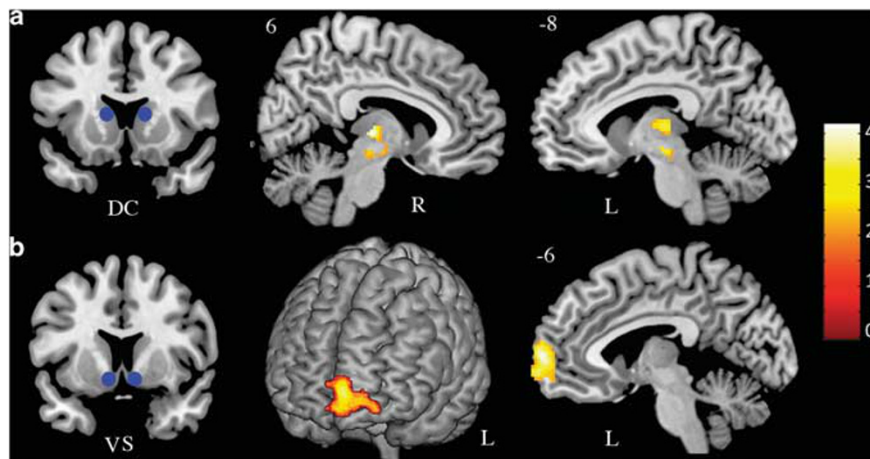
### Brain-Behavior Analysis

The difference between symptom scores obtained under placebo and ketamine (ketamine-placebo) was computed to





**Figure 1** Significant within-group functional connectivity maps of the dorsal caudate (DC), ventral striatum/nucleus accumbens (VS), dorsal putamen (DP), and ventral putamen (VP) seeds (in blue). Green indicates connectivity under placebo, red indicates connectivity under ketamine infusion, and yellow indicates areas of overlap. R, right hemisphere; L, left hemisphere. Sagittal slices are displayed at  $x = \pm 6$ . Because of the large magnitude of seed main effects, these maps were corrected using voxel-wise threshold of  $P < 0.05$  (FDR corrected).



**Figure 2** Z-score statistical maps of significant between-condition differences in functional connectivity of the (a) dorsal caudate (DC) and (b) ventral striatum (VS). R, right hemisphere; L, left hemisphere. Coordinates correspond to MNI space. Results are displayed at  $P < 0.05$  (FWE cluster corrected).

obtain change scores for each scale, denoted by  $\Delta$ BPRS,  $\Delta$ RSRS, and  $\Delta$ CADSS. These change scores were correlated, on a voxel-wise basis, with subject-specific parameter estimates for the effect of ketamine (vs placebo) on striatal functional connectivity, masked by regions showing a significant ketamine effect on functional connectivity (Figure 2 and Table 2). The four aforementioned summary

measures of head motion were included as nuisance variables in these analyses. All results were displayed at a FWE cluster corrected level of  $P < 0.05$ , with appropriate correction for the search volume employed. Secondary analyses tested for associations between functional connectivity changes and trait-like measures of psychosis proneness (ie, the Chapman and Peters scales).

**Table 1** Symptom Scores of Healthy Participants Before and After the Administration of Ketamine

Psychotic and dissociative symptoms measures (n = 19)	Placebo mean (SD)	Ketamine mean (SD)	Wilcoxon signed-ranks Z, P
Brief Psychotic Rating Scale (BPRS)	7.7 (2.3)	9.7 (3.7)	− 2.53, <i>P</i> = 0.011
Rating Scale for Psychotic Symptoms (RSPS)	2.3 (4.3)	20.2 (16.8)	− 3.55, <i>P</i> < 0.001
Clinician Administered Dissociative States Scale (CADSS)	0.5 (1.2)	18.2 (16.7)	− 3.73, <i>P</i> < 0.001
<b>Psychosis-proneness measures (n = 16)<sup>a</sup></b>	<b>Baseline mean (SD)</b>	—	—
Chapman Magical Ideation scale	3.5 (3.6)	—	—
Chapman Perceptual Aberration scale	4.1 (6.7)	—	—
Peters Delusion Inventory	36.8 (31.1)	—	—

<sup>a</sup>Values for three subjects were not available.

**Table 2** Brain Regions Demonstrating Significant Between-Condition (Ketamine Greater Than Placebo) Differences in Functional Connectivity and Association with Symptom Change Scores (Ketamine–Placebo)

Main effect (Figure 2)	Anatomical region	Hemisphere	MNI peak coordinates x, y, z	Z (df = 1, 68)	Voxels
(a) DC	Thalamus	Right	6, − 18, 2	3.64	552
	Thalamus	Left	− 14, − 18, 6	3.38	
	Midbrain	Left	− 18, − 16, − 12	3.38	
	Midbrain	Right	12, − 16, − 10	2.88	
(b) VS	Superior ventromedial prefrontal cortex	Left	− 8, 64, 2	3.62	582
	Frontopolar cortex	Left	− 6, 68, − 6	3.22	
	Inferior ventromedial prefrontal cortex	Left	− 16, 62, − 2	3.06	
Behavioral association (Figure 3)	Anatomical region	Hemisphere	MNI peak coordinates x, y, z	Z (df = 1, 31)	Voxels
(a) DC (BPRS)	Midbrain	Right	10, − 14, − 10	5.08	84
(b) DC (RSPS)	Midbrain	Right	8, − 12, − 10	2.65	12
(c) DC (CADSS)	Ventrolateral thalamus and subthalamic nucleus	Left	− 18, − 12, 0	2.86	18
(c) DC (RSPS)	Ventrolateral thalamus and subthalamic nucleus	Left	− 12, − 14, 2	3.11	31
(d) VS (BPRS)	Superior ventromedial prefrontal cortex	Left	0, 58, 8	3.58	31
(e) VS (CADSS)	Inferior ventromedial prefrontal cortex	Left	− 6, 60, − 10	4.04	47

Abbreviation: DC, dorsal caudate; VS, ventral striatum.

Results are thresholded at *P* < 0.05 (FWE) cluster corrected.

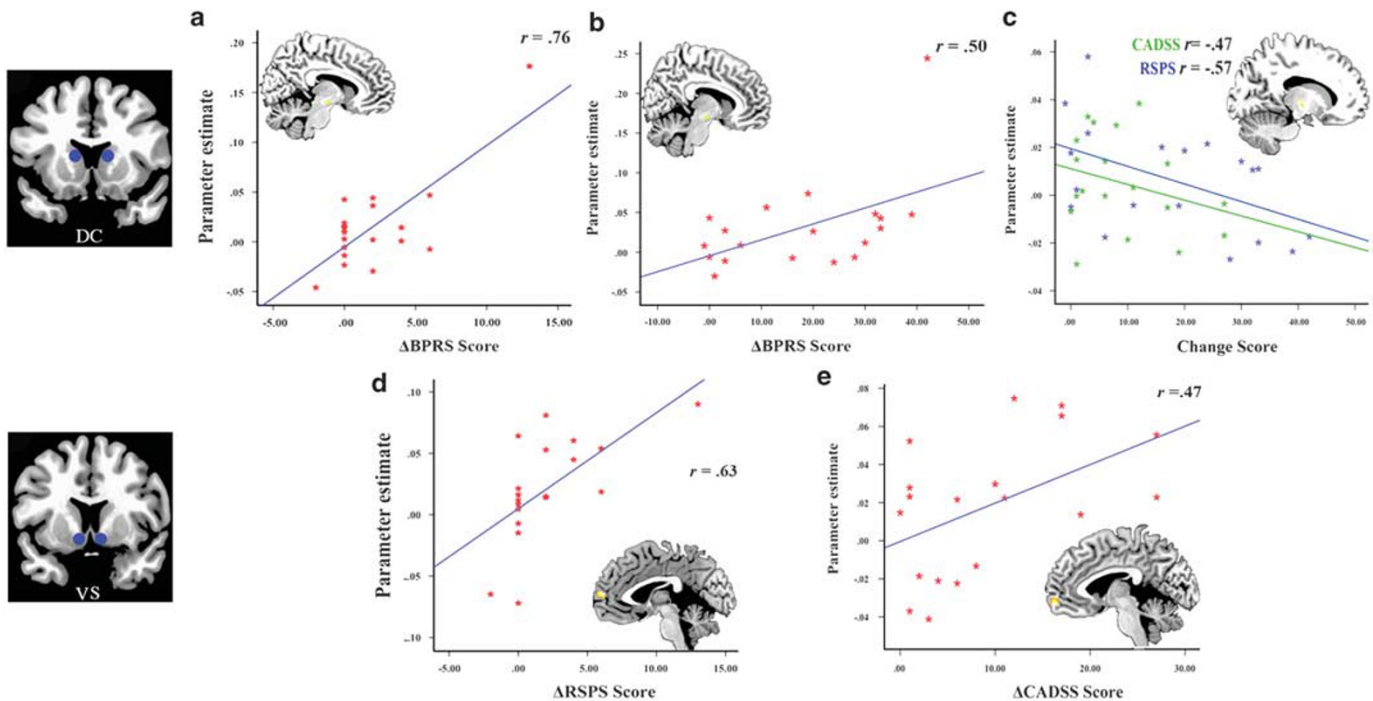
## RESULTS

### Plasma Concentrations and Symptom Ratings

The mean plasma concentration of ketamine before and after scanning was  $68.6 \pm 43.6$  ng/ml. Ketamine caused a significant increase in psychosis-like and dissociative symptoms as indexed by the RSPS, BPRS, and the CADSS, respectively (Table 1). Endorsed RSPS items pertained mainly to somatosensory perturbations and loss of attention focus; BPRS items included grandiosity, elevated mood, anxiety, delusional thoughts, and hallucinations; and CADSS items pertained to external and reality perception. These symptoms concur with previous reports (Krystal *et al*, 1994). Ketamine-induced symptomatology was not accompanied by changes in head motion as found by a formal between-condition comparison.

### Functional Connectivity Analyses

Collapsed across placebo and ketamine conditions, the four seed-specific striatal functional connectivity maps closely recapitulated previous studies (Figure 1; Di Martino *et al*, 2008; Harrison *et al*, 2009). Compared with placebo, ketamine augmented functional connectivity of the dorsal caudate and ventral striatum/nucleus accumbens seeds only (Figure 2 and Table 2). Specifically, functional connectivity was increased between the dorsal caudate and regions in the thalamus and midbrain bilaterally (Figure 2a). Connectivity was also increased between the ventral striatum and left anterior and ventromedial prefrontal cortex (Figure 2b). All effects except for the ketamine-induced increase of dorsal caudate–midbrain connectivity were replicated using a cluster-forming threshold of *P* = 0.001 (*P* < 0.05, FWE cluster corrected). There were no significant effects of



**Figure 3** Z-scores statistical maps of significant brain-behavior association between estimates of functional connectivity of striatal seeds and positive psychotic and dissociative symptoms caused by ketamine infusion, displayed at  $P < 0.05$  (FWE cluster corrected). For visualizing purposes with the midbrain (a and b), ventral thalamus/subthalamic nucleus (c) and medial prefrontal cortex (d and e), parameter estimates were extracted from peak voxels in the shown brain maps and correlated with symptom change scores. Results remained significant after the exclusion of outliers.

ketamine on putamen functional connectivity, no significant condition-by-hemisphere interactions, and no significant functional connectivity decreases observed under ketamine relative to placebo.

### Brain-Behavior Associations

A stronger ketamine-related increase of functional connectivity between the dorsal caudate and right midbrain was correlated with higher  $\Delta$ BPRS scores (Figure 3a). Conversely, a stronger effect of ketamine on functional connectivity between dorsal caudate and left ventrolateral thalamus, as well as between dorsal caudate and subthalamic nucleus, was associated with lower  $\Delta$ RSPS and lower  $\Delta$ CADSS scores (Figure 3b), suggesting a possible protective role in combating the psychotogenic effect of ketamine. In the ventral system, higher ketamine-induced functional connectivity between ventral striatum and left ventromedial prefrontal cortex was associated with higher  $\Delta$ BPRS scores (Figure 3c), whereas higher  $\Delta$ CADSS scores were associated with a stronger ketamine effect on functional connectivity between the ventral striatum and an adjacent ventromedial prefrontal region (Figure 3d). All results were FWE cluster corrected ( $P < 0.05$ ) for multiple comparisons. Theoretical and empirical models suggest a competitive or antagonistic relationship between dorsal and ventral frontostriatal function (O'Doherty *et al*, 2004). In support of this view, an exploratory analysis of the association between participant-specific parameter estimates of ketamine-induced functional connectivity changes in dorsal (dorsal caudate-subthalamic nucleus connectivity) and ventral (ventral striatal-frontopolar cortex connectivity) systems

found a significant negative correlation ( $r = -0.5$ ,  $P < 0.029$ ). In other words, ketamine-related enhancement of connectivity in one circuit reduced connectivity in the other.

No significant associations were observed between measures of psychosis proneness (ie, the Chapman and Peters scales) and ketamine-induced functional connectivity changes. However, exploratory analyses revealed a significant association between the Chapman magical ideation and RSPS difference scores ( $r = 0.51$ ,  $P < 0.045$ ). A trend was also observed between Chapman perceptual abnormality and RSPS scores ( $r = 0.48$ ,  $P < 0.058$ ), suggesting that individuals who had higher psychotic traits experienced more severe psychotic-like symptoms under ketamine.

### DISCUSSION

Ketamine increased functional connectivity between specific components of both dorsal and ventral FST circuits and these increases correlated with the emergence of psychosis-like and dissociative phenomena assessed with the BPRS, RSPS, and the CADSS, respectively. These drug-induced functional connectivity changes contrast with the alterations found in patients with first-episode psychosis, their unaffected relatives, and ARMS individuals (Dandash *et al*, 2013b; Fornito *et al*, 2013) who show reduced functional connectivity in dorsal FST systems and less consistent evidence for ventral circuit alterations (Dandash *et al*, 2013b; Fornito *et al*, 2013). Specifically, two primary differences were noted. First, ketamine led to a general increase, rather than decrease, in both dorsal and ventral



circuit functional connectivity. Second, the dorsal circuit increases were primarily subcortical, affecting functional coupling of the dorsal caudate with midbrain and thalamus (Figure 2a), rather than the frontostriatal changes seen in clinical populations. These findings imply that the psychosis-like experiences elicited by an acute dose of ketamine arise from FST alterations that are distinct from those associated with the primary pathophysiological disturbance in psychosis.

The general increase in FST functional connectivity is consistent with past reports that ketamine augments regional resting cerebral blood flow, metabolic and electrophysiological activity, and resting-state functional connectivity in these and other regions (Driesen *et al*, 2013; Holcomb *et al*, 2001; Vollenweider *et al*, 1997). This effect is thought to arise from excitation-inhibition imbalance (Anticevic *et al*, 2012) caused by ketamine's blockade of NMDARs on inhibitory GABAergic interneurons that leads to the disinhibition of glutamate release, resulting in excess glutamatergic binding at AMPA and kainate receptors (Moghaddam *et al*, 1997). The striatum receives afferent glutamatergic input from the prefrontal cortex and sends efferents to the thalamus via the pallidum and midbrain. The thalamus projects back to the originating prefrontal area completing a FST loop (Alexander *et al*, 1986). Influential models of psychosis posit that increased flow of sensory information from the striatum to the thalamus results in a failure of the thalamus to 'filter-out' irrelevant stimuli before they reach the cortex, thus predisposing individuals to psychotic symptoms (Carlsson, 1988). The associations we found between the emergence of psychosis-like symptoms and ketamine-induced increases of functional connectivity (Figure 3) support this view, under the assumption that increased functional connectivity reflects increased AMPA/kainate-mediated feed-forward frontostriatal and striatothalamic signaling. However, this result is at odds with the lack of subcortical functional connectivity increases observed in first-episode patients and ARMS individuals (Dandash *et al*, 2013b; Fornito *et al*, 2013).

Dysregulation of dopamine likely plays a central role in the risk-related FST changes observed in clinical samples, given robust evidence for elevated dopaminergic function, particularly in dorsal striatal regions, in both patients (Kegeles *et al*, 2010) and high-risk individuals (Howes *et al*, 2009). Either too much or too little dopamine in FST circuits is expected to reduce neural signal-to-noise ratio (Cools and D'Esposito, 2011) and thus lower functional connectivity (Fornito *et al*, 2013). NMDAR hypofunction has been implicated as a candidate upstream cause of dopaminergic abnormalities associated with psychosis onset (Carlsson and Carlsson, 1990) and some human studies suggest that ketamine augments dopamine release in a manner that parallels observations in clinical sample (Kegeles *et al*, 2000), while also affecting activation of frontostriatal systems (Corlett *et al*, 2006). However, other studies have failed to demonstrate that ketamine increases striatal dopamine transmission, even with very high doses (Aalto *et al*, 2002), that ketamine has a limited effect on D2 receptor availability (Kegeles *et al*, 2002), and that treatment with Haloperidol has no impact on ketamine-induced psychotic symptoms (Krystal *et al*, 1999; Lahti *et al*, 1995). These findings suggest a mode of action for ketamine

that diverges from the pathophysiological alterations seen in psychosis patients.

Although our findings support a distinct mode of action for ketamine, two considerations merit discussion. The first pertains to dosage. Participants in our study received a lower dose of ketamine than is traditionally used (Aalto *et al*, 2002; Kegeles *et al*, 2002; Krystal *et al*, 1994) to maximize in-scanner tolerance. Although the dose was sufficient to elicit an increase in psychosis-like and dissociative symptoms, their intensity and quality may have been more characteristic of an ARMS than frank psychotic episode. Nonetheless, dorsal FST functional connectivity reductions have still been observed in ARMS individuals (Dandash *et al*, 2013b) and symptom-free genetic high-risk volunteers (Fornito *et al*, 2013), suggesting such changes are present at the earliest signs of illness. Moreover, functional connectivity reductions are by far the most common finding in patients with psychosis across different illness stages and neural systems (Pettersson-Yeo *et al*, 2011), contrasting the generic functional connectivity increases observed in this study and elsewhere, even when using much higher doses (~162 ng/ml) (Driesen *et al*, 2013). These results, combined with other evidence that the dose we used is sufficient to effectively model other psychosis-related brain changes in healthy volunteers (Corlett *et al*, 2006), support the conclusion that not all of the neural effects of ketamine adequately model the brain changes seen in clinical samples, even when they correlate with drug-induced increases in symptomatology (Pomarol-Clotet *et al*, 2006). Thus, ketamine may provide a better model of some risk-related brain changes than others.

A second consideration is regarding the chronicity of exposure. It has been argued that acute NMDAR hypofunction may model neural changes associated with cognitive disturbances that pre-date psychosis onset (eg, akin to an ARMS) (Corlett *et al*, 2007), whereas longer-term deficits may be more characteristic of schizophrenia (Freeman *et al*, 2009). In this regard, early hyperconnectivity of FST circuits may evolve into subsequent hypoconnectivity as psychosis takes hold. This view is supported by reports that striatal glutamate is elevated in early stages of schizophrenia but reduces with antipsychotic treatment (de la Fuente-Sandoval *et al*, 2013), that administration of ketamine is associated with reduced functional connectivity in some brain circuits after 24 h (Scheidegger *et al*, 2012), and that chronic but not acute use of ketamine leads to delusion formation (Freeman *et al*, 2009) that persists after abstinence (Morgan *et al*, 2010). However, the fact that dorsal FST hypoconnectivity is observed in ARMS individuals (Dandash *et al*, 2013b) and symptom-free first-degree relatives of patients populations (Fornito *et al*, 2013) that putatively represent the earliest illness stages suggests that acute ketamine infusion provides an inadequate model of risk-related FST dysconnectivity. Whether chronic exposure better models these changes is an open question.

Interestingly, although ketamine-related increases of functional connectivity were generally associated with more severe psychosis-like and dissociative symptoms, increased connectivity between dorsal caudate and regions of the thalamus and subthalamic nucleus was associated with lower symptom scores (Figure 3b). Others have found that ketamine-induced negative symptoms inversely correlate

with functional connectivity changes of the ventrolateral thalamus with the rest of the brain (Driesen *et al*, 2013). The thalamus and subthalamic nucleus are thought to exert modulatory feedback over striatal output within cortico-striato-thalamic circuits (Parent and Hazrati, 1995). Although the modulatory role of the ventrolateral thalamus on dorsal striatum is less clear, glutamatergic cells in the thalamus and subthalamic nucleus (STN) are proposed to initiate a cascade of events resulting in activation of GABAergic cells in the pallidum, thus dampening outflow from the striatum (Carlsson, 1988; Carlsson and Carlsson, 1990). This feedback is believed to prevent overflow of sensory information, and its failure has been implicated in vulnerability to psychotic experiences (Carlsson and Carlsson, 1990). Accordingly, our findings indicate that individuals who engaged this system to a greater extent experienced fewer psychosis-like and dissociative symptoms under ketamine. Upregulating functional connectivity in this circuit may represent a viable treatment target to combat the deleterious effects of dysfunction elsewhere in the system. This view is consistent with our finding that a stronger ketamine-related increase of dorsal caudate–STN connectivity was associated with a reduced ketamine-induced increase of ventral striatum–frontopolar cortex connectivity, a result that further points to some degree of functional antagonism between these two circuits in mediating the psychotogenic effects of ketamine (O'Doherty *et al*, 2004).

Although we have focused on the known influence of ketamine on ionotropic glutamate receptors and dopaminergic signaling, the drug can affect other modulatory systems (Kapur and Seeman, 2002). However, the average dose used herein provides  $<0.1 \mu\text{M}$  available for binding (assuming plasma binding value of 47% (Dayton *et al*, 1983) and a molecular weight of 274.19 (ketamine hydrochloride)). At this concentration, ketamine demonstrates no binding potential for most other receptors (eg,  $\mu$ -opioid, sigma, dopamine, or 5-HT; Kapur and Seeman, 2002; Smith *et al*, 1987).

In summary, our findings suggest that ketamine affects functional connectivity of both dorsal and ventral FST circuits and that these changes correlate with the severity of psychosis-like and dissociative symptoms induced by the drug. They also point to a potential role for the ventrolateral thalamus and subthalamic nucleus in protecting against disturbances that lead to psychotic experience. Although consistent with the role of FST in mediating psychosis, these effects appear somewhat distinct from the FST changes observed in first-episode patients, unaffected relatives, and ARMS individuals, suggesting that acute ketamine infusion does not completely model the disturbances of FST functional connectivity implicated in risk for psychosis. Investigating the impact of NMDAR agonists as well as glutamate antagonists on the functional connectivity of the ventrolateral thalamus and subthalamic nucleus is important to further delineate their protective role.

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