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N-Methyl-D-Aspartate Receptor Antagonist Effects on Prefrontal Cortical Connectivity Better Model Early Than Chronic Schizophrenia

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ABSTRACT

BACKGROUND: Prefrontal cortex (PFC) function contributes to schizophrenia onset and progression. However, little is known about neural mechanisms behind PFC functional alterations along illness stages. Recent pharmacologic studies indicate that glutamate dysfunction may produce increased functional connectivity. However, pharmacologic models of schizophrenia overlook effects of illness progression on PFC function. This study compared *N*-methyl-D-aspartate glutamate receptor (NMDAR) antagonist effects in healthy volunteers with stages of schizophrenia with respect to PFC functional connectivity.

METHODS: First, we tested ketamine effects on PFC functional connectivity in healthy volunteers in a data-driven way ($n = 19$). Next, we compared healthy subjects ($n = 96$) with three clinical groups: individuals at high risk for schizophrenia ($n = 21$), people early in their course of schizophrenia (EC-SCZ) ($n = 28$), and patients with chronic illness ($n = 20$). Across independent analyses, we used data-driven global brain connectivity techniques restricted to PFC to identify functional dysconnectivity.

RESULTS: Results revealed robust PFC hyperconnectivity in healthy volunteers administered ketamine (Cohen's $d = 1.46$), resembling individuals at high risk for schizophrenia and EC-SCZ. Hyperconnectivity was not found in patients with chronic illness relative to EC-SCZ patients. Results provide the first evidence that ketamine effects on PFC functional connectivity resemble early course but not chronic schizophrenia.

CONCLUSIONS: Results suggest an illness phase-specific relevance of NMDAR antagonist administration for prefrontal dysconnectivity associated with schizophrenia. This finding has implications for the neurobiology of illness progression and for the widespread use of NMDAR antagonists in the development of therapeutics for schizophrenia.

Keywords: Chronic schizophrenia, Disinhibition, First episode, Glutamate, High-risk, Ketamine, NMDA receptor, Prefrontal connectivity

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Schizophrenia is a prevalent neuropsychiatric syndrome and one of the most disabling medical conditions worldwide (1), yet its neural mechanisms remain poorly understood. It is well established that schizophrenia is associated with cognitive deficits associated with prefrontal cortex (PFC) function (2,3). Many studies attempting to understand schizophrenia pathophysiology, however, focused on striatal dopaminergic hyperactivity (4–8). This approach did not explain cortical and PFC alterations associated with the progression of this illness (9). This work is now complemented by studies characterizing glutamate neurotransmission in schizophrenia (10–13), pointing to alterations in the balance of excitation and inhibition in the cortical microcircuitry resulting from the hypofunction of the *N*-methyl-D-aspartate glutamate receptor (NMDAR) (14,15). This hypothesis is based on a key observation: subanesthetic doses of noncompetitive NMDAR antagonists such as ketamine produce cardinal symptoms resembling those of schizophrenia in healthy humans, including positive, negative, and cognitive aspects of the illness (16,17). Such pharmacologic studies provide insights into how disturbances in glutamate signaling might contribute to schizophrenia (14), but the effects of NMDAR antagonists on PFC functional connectivity remain unknown (18).

However, there are important differences between acute pharmacologic models and schizophrenia. For instance, NMDAR antagonists increase pyramidal cell activity, extracellular glutamate levels (19), cortical metabolism (20–24), and functional connectivity (25). Conversely, chronic schizophrenia has been associated with reduced cortical connectivity (26) and activation (27), especially in the PFC (28). This important discrepancy between ketamine effects and clinical observations needs to be reconciled to elucidate the neurobiology of schizophrenia and to inform applications of the ketamine model commonly used in drug development. It is important

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to examine whether the ketamine model best applies to an identifiable subgroup of patients or illness phase. For example, ketamine tends to produce symptoms associated with incipient illness stages, rather than fully developed auditory hallucinations typical of chronic schizophrenia (16,20,29–31). Similarly, increased glutamate levels appear to be a feature of early illness course rather than chronic schizophrenia (32). It is unknown, however, whether ketamine effects on PFC functional connectivity are more similar to the early or later phases of schizophrenia.

To address these questions, the purpose of the current study was threefold. First, based on recent evidence suggesting that NMDAR antagonists induce hyperconnectivity (25), we tested the hypothesis that acute ketamine administration would be associated with PFC functional hyperconnectivity. We quantified PFC connectivity via a data-driven tool called restricted global brain connectivity (rGBC) (33,34). Such data-driven approaches have proven successful in identifying connectivity alterations in chronic schizophrenia (27) and psychotic bipolar illness (35). Similar techniques have been applied to assay whole-brain connectivity under ketamine (24). Second, we directly compared PFC functional connectivity in early course of schizophrenia and chronic patients to test the hypothesis that PFC functional connectivity differs across illness stages. Third, we evaluated whether ketamine effects qualitatively resemble early course but not chronic schizophrenia findings. We tested these hypotheses focusing on PFC in particular, given evidence implicating PFC functional network alterations in schizophrenia (36,37), to maximize statistical power, given the smaller search space, and to programmatically build upon recent studies using similar approaches in chronic schizophrenia (28) and psychotic bipolar patients (33). Collectively, this study informs NMDAR antagonist effects on PFC functional connectivity and establishes relevance of these effects to specific schizophrenia illness stages.

METHODS AND MATERIALS

Participants

Healthy Volunteer Recruitment for Pharmacologic Neuroimaging. All healthy volunteers recruited for the pharmacologic ketamine arm of the study provided informed consent approved by Yale University's Institutional Review Board. The final sample included 19 neurologically and psychiatrically intact right-handed volunteers (10 male volunteers) with a mean ± SD age of 27.5 ± 6.3 years. All volunteers were initially screened using a detailed telephone interview. If deemed eligible, participants underwent a subsequent diagnostic interview using the Structured Clinical Interview for the DSM-IV (SCID) (38), a physical exam by a physician, and a urine toxicology screen. All recruited participants tolerated the infusions well and were able to complete the protocol successfully. Pharmacologic neuroimaging protocol details are presented in Supplement 1. All recruitment details have been described previously in our published work (10), where ketamine effects were analyzed in relation to a cognitive activation task. Resting-state data were never examined in any prior publications.

Schizophrenia, High-Risk, and Healthy Comparison Subject Recruitment. All participants recruited for the clinical arm of the study signed a written informed consent approved by both the Institutional Review Boards of China Medical University and Yale University. Here, we independently studied healthy comparison subjects (HCS, $n = 96$) matched to three groups: individuals at high risk for schizophrenia (HR, $n = 21$), patients early in their illness course (EC-SCZ, $n = 28$), and chronic schizophrenia patients (C-SCZ, $n = 20$) (Table 1). We

Table 1. Characteristics of Clinical Groups and Comparison Subjects

	HCS ($n = 96$)		C-SCZ ($n = 20$)		EC-SCZ ($n = 28$)		HR ($n = 21$)		Significance	
Characteristic	M	SD	M	SD	M	SD	M	SD	*F*/*t* Value Chi-Square	*p* Value (Two-Tailed)
Age (in Years)	28.84	10.51	31.43	8.20	25.00	9.70	19.95	5.24	6.73	<.001[a]
Gender (% Male)	45	–	45	–	43	–	57	–	1.23	.75
Father's Occupational Status	37.63	22.69	28.79	18.25	34.54	19.39	30.22	14.43	1.28	.28
Mother's Occupational Status	36.67	21.89	37.06	22.48	34.00	19.72	31.26	15.14	.38	.77
Participant's Education (in Years)	14.79	3.11	11.48	3.52	11.54	3.02	12.70	2.83	13.06	<.001[a]
Handedness (% Right)	88.54	–	90.00	–	78.57	–	71.43	–	8.97	.44
Medication – CPZ Equivalents	–	–	240.00	132.22	96.40	71.33	–	–	2.67	<.01[a]
Percent Treated	–	–	95.00		43.00		–	–	13.86	<.001[a]
BPRS Total Symptoms	–	–	25.56	10.58	36.67	15.68	18.11	.46	14.42	<.001[a]
Duration of Illness (Months)	–	–	64.45	38.26	4.27	3.20	–	–	8.32	<.001[a]
Signal-to-Noise	177.47	49.82	195.58	52.87	173.44	57.37	160.93	41.30	2.10	.10
% Frames Scrubbed	8.63	12.69	8.26	6.62	8.07	6.46	12.52	13.01	0.67	.61

Age and education levels are expressed in years; duration of illness is expressed in months. No participants had current alcohol/drug use or past history of drug dependence. The occupation status (socioeconomic status) of the participants' parents was used as a proxy for educational attainment and was scored according to The International Socio-Economic Index of Occupational Status (65). CPZ equivalents were calculated using standard approaches (40). Note: Across groups, education level and age differed; however, adjusting for these differences did not alter the reported findings. Also, reported findings did not change when we covaried for medication presence/absence or medication dose (Figures 4 and 5).

ANOVA, analysis of variance; BPRS, Brief Psychiatric Rating Scale; CPZ, chlorpromazine; C-SCZ, chronic schizophrenia patients; EC-SCZ, early course schizophrenia patients; HCS, healthy comparison subjects; HR, high risk subjects; M, mean; SD, standard deviation.

[a]Denotes significant *F* statistic for the one-way between-group ANOVA.

examined the HR group to provide a comparison to interpret clinical effects. If HR individuals exhibit some functional alterations resembling those found during early course, then HR effects should also be distinct from those found in chronic patients.

Participants from both clinical groups (C-SCZ, EC-SCZ), as well as HR participants, were recruited from outpatient clinics of the Department of Psychiatry, First Affiliated Hospital of China Medical University. Patients were diagnosed with schizophrenia, schizophreniform disorder, or brief psychotic disorder according to DSM-IV criteria and had no other Axis I disorders. All adult participants were diagnosed by two psychiatrists and diagnoses were confirmed via consensus using the SCID. Where appropriate, all diagnoses were confirmed via long-term follow-up. Legal guardians provided consent for any participants <18 years old and different diagnostic tools were used for individuals <18 years old. As noted, SCID (34) was used to diagnose participants >18 years old, whereas the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (38) was used for participants <18 years old. Participants were not excluded based on nicotine or alcohol use history (to provide a more representative clinical sample), but current nicotine, alcohol, or drug abuse/dependence was not allowed. No participants reported past history of alcohol and drug dependence. This is typical based on the community from which participants were recruited (F. Wang, Ph.D., oral communication). Illness duration was calculated by deducting the age at first symptom onset from the patient's age at the time of the scan, reported by participants and confirmed by secondary sources (medical records and reports from relatives).

Of the 48 patients who were diagnosed with schizophrenia and participated in the study, 28 were within 1 year of initial symptom presentation and met the criteria for early course illness (Table 1). EC-SCZ patients were followed longitudinally to confirm diagnosis according to DSM-IV criteria by a trained psychiatrist. HR participants were offspring of individuals diagnosed with schizophrenia (at least one parent), and all were under the age of peak illness risk (<30 years) to ensure subjects were within the elevated risk period for developing the illness. All HR participants, defined based on parental risk, were assessed using the same clinical measures (Table 1). Chronic patients were defined as having met diagnostic criteria for at least 12 consecutive months (mean = 64.45 months of illness duration). Ninety-six HCS subjects were recruited from the community, mean-matched to each of the other three groups by age, sex, ethnicity, handedness, and parental socioeconomic status (Table 1). HCS had no current or lifetime Axis I psychiatric disorder (determined by a psychiatrist), no history of any serious medical or neurological conditions, and no history of psychotic, mood, or other Axis I disorders in first-degree relatives (as reported by detailed family history).

Exclusionary criteria were the same across all clinical and comparison groups: history of neurological conditions (e.g., epilepsy, migraine, head trauma with loss of consciousness), magnetic resonance imaging contraindications, or any concomitant major medical disorder. Brief Psychiatric Rating Scale (39) was used to assess symptoms. All antipsychotic doses were converted to chlorpromazine equivalents using standard procedures (40) (Table 1). Of note, resting-state data used for the clinical component of the study were analyzed in a previous paper, focusing on an independent question (41).

Functional Neuroimaging

Neuroimaging acquisition, processing, and analysis details are presented in Supplement 1. All methods followed previously validated and published procedures (28,33,42). Given that healthy volunteers undergoing pharmacologic neuroimaging were recruited at a different location from the clinical samples (Yale versus China Medical University), we took great care to ensure that all primary pharmacologic and clinical analyses were conducted independently of each other and were orthogonal to possible confounds arising from scanner differences (see Supplement 1 for detailed considerations).

RESULTS

PFC Connectivity Is Increased Following Ketamine Administration

We first tested whether ketamine administration in healthy volunteers was associated with PFC hyperconnectivity at rest.

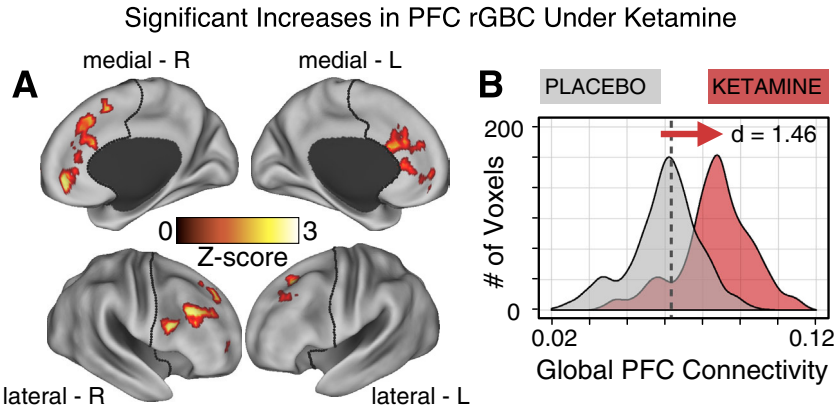

Figure 1. Acute ketamine administration is associated with increased prefrontal cortex (PFC) connectivity. **(A)** Paired *t* test results testing effects of acute ketamine infusion on PFC restricted global brain connectivity (rGBC) in healthy volunteers. All presented clusters survived appropriate type I error correction, as done for the clinical analyses in Figure 2. **(B)** Signal was extracted from all clusters showing a significant effect in panel **(A)**. Effect sizes highlight a marked increase in PFC connectivity following ketamine infusion (Cohen's $d = 1.46$, $p < .00001$), computed across subjects. Of note, this effect was identified specifically for the healthy volunteer sample undergoing ketamine administration and is thus fully independent of Figure 2 clinical effects. Gray vertical dashed line marks the mean for the saline control condition. See Table 2 for all region coordinates and pair-wise comparisons. L, left; R, right.

We specifically examined whether ketamine induced PFC hyperconnectivity via fully data-driven methods (36). Results revealed differences across three medial PFC clusters (Figure 1), reflecting an increase in PFC rGBC following ketamine administration. Critically, no regions showed a connectivity decrease following ketamine administration following described analyses, consistent with prior whole-brain effects (25). To illustrate the robustness of this finding, we computed formal effect size estimates of ketamine-based modulation across all identified areas (Cohen's $d = 1.46$, $p < .00001$) (Table 2).

PFC Connectivity Differs Between Chronic and Early Course Schizophrenia

Prior meta-analytic evidence (32) points to increased PFC glutamate levels early in the illness course but a decrease at later stages (32). We tested whether EC-SCZ and HR groups could be associated with elevated PFC rGBC relative to HCS, possibly reflecting cortical disinhibition (43) similar to ketamine effects. Conversely, we tested if the C-SCZ group could be associated with PFC rGBC decreases, possibly reflecting loss of PFC structural integrity (9).

Primary clinical results revealed two areas exhibiting significant between-group differences (tested via a one-way between-group analysis of variance [ANOVA]; Table 2): a right superior lateral prefrontal cortex (LPFC) region and a superior medial prefrontal cortex (MPFC) region (Figure 2A,B; Table 2). The LPFC effect was driven by a significant connectivity reduction for the C-SCZ group relative to the HCS group (Figure 2C), verified via formal effect sizes (Cohen's $d = 1.08$, $p < 4.7^{-5}$) (44). This replicated prior reports showing reduced lateral PFC rGBC in chronic patients (28), increasing validity of reported findings. Conversely, the MPFC cluster exhibited an effect whereby both EC-SCZ and HR groups showed increased PFC rGBC relative to HCS (EC-SCZ vs. HCS: Cohen's $d = .9$, $p < .00025$; HR vs. HCS: Cohen's $d = .8$, $p < .001$). However, there was no evidence for such an increase in the C-SCZ group (Figure 2D). Instead, as hypothesized, C-SCZ showed significant PFC connectivity reductions relative to EC-SCZ for the LPFC cluster (EC-SCZ vs. C-SCZ, Cohen's $d = .84$, $p < .007$) and the MPFC cluster (EC-SCZ vs. C-SCZ, Cohen's $d = .95$, $p < .002$) (Figure 2C,D).

As noted, the HR group provides important convergent evidence. HR individuals exhibited effects qualitatively similar to EC-SCZ but differed significantly from C-SCZ for both areas (LPFC: Cohen's $d = .7$, $p < .035$, MPFC: Cohen's $d = .85$, $p < .015$). None of the clinical findings were driven by medication or age. These clinical results highlight that C-SCZ was associated with a reduction in lateral PFC functional

Table 2. Region Coordinates, *p* Values, *t* Values, and Effect Size Estimates for the Primary Pharmacologic and Clinical Analyses

Regions Identified via Primary Pharmacologic Ketamine-Placebo Analyses									
X	Y	Z	Hemisphere	Anatomical Landmark	Cluster Size (mm^3)	Comparison	Cohen's *d*	*t* Value	*p* Value
−9	47	33	Left	Superior frontal gyrus (BA 9)	11340	Ketamine vs. Placebo	.96	4.30	.0004[a]
26	9	58	Right	Middle frontal gyrus (BA 6)	2592	Ketamine vs. Placebo	1.46	4.85	.0001[a]
−9	47	33	Left	Superior frontal gyrus (BA 9)	11340	Ketamine vs. Placebo	.92	3.29	.004[b]
Regions Identified via Primary Clinical Between-Group Analyses									
X	Y	Z	Hemisphere	Anatomical Landmark	Cluster Size (mm^3)	Comparison	Cohen's *d*	*t* Value	*p* Value
						HCS vs. C-SCZ	.03	.13	.90
						HCS vs. EC-SCZ	.88	3.78	.0002[a]
						HCS vs. HR	.79	3.40	.0001[a]
−9	47	33	Left	Superior frontal gyrus (BA 9)	11340	HR vs. C-SCZ	.85	2.69	.01[b]
						HR vs. EC-SCZ	.07	.23	.82
						C-SCZ vs. EC-SCZ	.95	3.29	.002[b]
						HCS vs. C-SCZ	1.08	4.23	.00005[a]
						HCS vs. EC-SCZ	.22	1.02	.31
						HCS vs. HR	.26	1.16	.25
26	9	58	Right	Middle frontal gyrus (BA 6)	2592	HR vs. C-SCZ	.70	2.19	.034[c]
						HR vs. EC-SCZ	.06	.23	.82
						C-SCZ vs. EC-SCZ	.84	2.84	.007[b]

Top: PFC rGBC results for the primary pharmacologic analyses (ketamine vs. placebo) for the three discovered foci (corresponding to Figure 1 effects). Bottom: Primary clinical between-group ANOVA analyses with region coordinates and relevant pair-wise statistics reported (corresponding to Figure 2 effects). Here, for completeness, we present all pair-wise comparisons across groups. Effect sizes show standard Cohen's *d* estimates. Cohen's *d* was obtained by extracting the average Fisher's *r*-to-Z connectivity value for each subject across the entire identified cluster. This was done to characterize the magnitude of between-group effects across voxels surviving the type I error correction and to provide a guide regarding sample sizes needed for future replications (63). Cross-validation statistics are shown in Table 3.

ANOVA, analysis of variance; BA, Brodmann area; C-SCZ, chronic schizophrenia patients; EC-SCZ, early course schizophrenia patients; HCS, healthy comparison subjects; HR, high risk subjects; PFC, prefrontal cortex; rGBC, restricted global brain connectivity.

[a]Significant between-group difference $p < .001$.

[b]Significant between-group difference $p < .01$.

[c]Significant between-group difference $p < .05$.

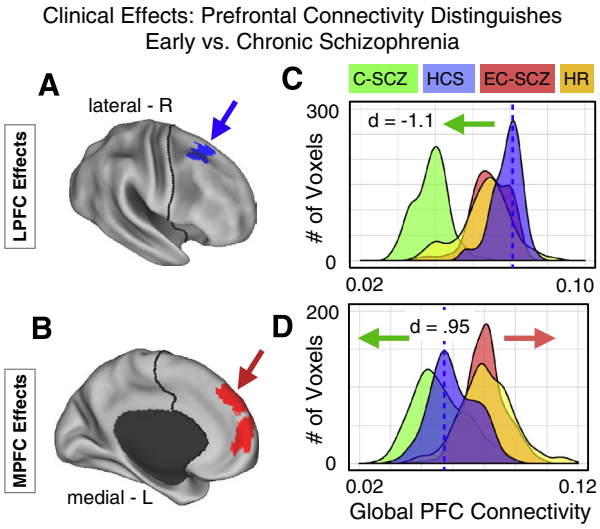

Figure 2. Prefrontal cortex (PFC) connectivity distinguishes between early course and chronic schizophrenia. **(A, B)** Clusters mark regions surviving the one-way between-group analysis of variance *F* test. The blue cluster marks a lateral PFC (LPFC) region where the chronic schizophrenia patient (C-SCZ) group showed decreased global PFC connectivity relative to individuals at high risk for schizophrenia (HR), patients early in their illness course (EC-SCZ), and healthy comparison subjects (HCS), replicating and extending prior effects (28). The red cluster marks a medial PFC (MPFC) region where C-SCZ group showed decreased global PFC connectivity, but the HR and EC-SCZ showed increased PFC connectivity relative to HCS. **(C, D)** All formal effect size calculations were computed via standard approaches across subjects via Cohen's *d* (44) by extracting the Fisher's Z value for all subjects across all voxels within a cluster showing main effects for each analysis. This was done to characterize the magnitude of between-group effects across voxels surviving the type I correction, as done previously (63), and to provide a guide for future studies (64). Effect sizes (Cohen's *d*) indicate a robust difference between EC-SCZ and C-SCZ across both clusters (the voxel-wise distribution plots are illustrative). Blue vertical dashed line marks the mean for the HCS group. See Table 2 for all region coordinates and all pair-wise comparisons. L, left; R, right.

connectivity, replicating prior reports (28), unlike reported ketamine effects (Figure 1). However, results significantly differed for early-course patients, who showed increased medial PFC functional connectivity. These data-driven cross-sectional analyses support the possibility that PFC functional connectivity dissociates between schizophrenia illness stages.

Secondary Analyses: Cross-Validating Pharmacologic and Clinical Findings

In our primary analyses, we identified hyperconnectivity following NMDAR antagonism. We also identified differences across illness stages with respect to PFC connectivity, some of which resembled ketamine effects (i.e., early course patients). These two primary analyses were statistically orthogonal, as they involved independent samples. Our secondary aim was to utilize the areas from each of the two independent analyses and re-compute a focused cross-validation for the other analysis.

First, we examined ketamine effects within the two identified regions that showed significant clinical effects (Figure 3), given statistical independence from ketamine analyses by design. Across both areas identified via the clinical analyses, there was a consistent increase in PFC rGBC for ketamine versus control placebo infusion (MPFC: Cohen's $d = .38$, $p = .09$, trend; LPFC: Cohen's $d = .35$, $p = .14$, trend) (Figure 3, Table 3). While these secondary effects were trend level, they were qualitatively distinct from findings observed in C-SCZ (Figure 2E,F).

To provide further convergent evidence, we examined effects for the clinical groups within regions identified via pharmacologic analyses (i.e., areas modulated by ketamine), which were again statistically independent from clinical effects (Figure 1). We extracted the signal out of identified clusters for all subjects from the HR, EC-SCZ, C-SCZ, and HCS groups for further analyses (Figure 3E–F, bottom panel). Results again supported qualitative difference between C-SCZ and EC-SCZ groups: medial and left lateral PFC clusters modulated by ketamine were also found to differentiate between EC-SCZ and C-SCZ patients (Cohen's $d = .52$, $p < .045$ and Cohen's $d = .6$, $p < .03$, respectively). Again, HR effects differed from C-SCZ findings for the medial PFC cluster (Cohen's $d = .84$, $p = .001$). These effects were also not explained by medication/age (Figures 4 and 5). Collectively, these secondary analyses, while statistically more modest, were congruent with primary data-driven effects (Figures 1 and 2).

DISCUSSION

Consistent with predictions, NMDAR antagonism increased PFC functional connectivity demonstrated via data-driven analyses. Present findings are consistent with the hypothesis that ketamine could induce a state of excessive glutamatergic signaling, which may alter the functional coupling of low-frequency blood oxygen level-dependent fluctuations at rest (18,25). Second, we cross-sectionally studied alterations in PFC functional connectivity across schizophrenia stages. We found that chronic patients exhibited a reduction in lateral PFC connectivity, in contrast to HR individuals and EC-SCZ individuals who showed increased medial PFC connectivity. These results suggest that distinct aspects of PFC connectivity differ across schizophrenia stages. These data point to possible qualitative differences between NMDAR antagonist and C-SCZ effects, suggesting that ketamine's effect on PFC connectivity may be more relevant to particular illness stages, which has implications for the utility of the NMDAR antagonist model in understanding schizophrenia broadly.

Effects of Ketamine on Prefrontal Network Connectivity in Relation to Illness Stages

Present results illustrate that ketamine administration is associated with increased PFC rGBC, a data-driven measure designed to assay connectivity in a way that differs from traditional seed-based analyses. A difference in PFC rGBC may reflect areas/networks in which coordination of information processing is affected across large-scale neural systems (in this case the entire PFC). Increased PFC rGBC may suggest an aberrant temporal synchronization of functional networks, observed here following ketamine administration. Thus, present pharmacologic effects suggest that NMDAR

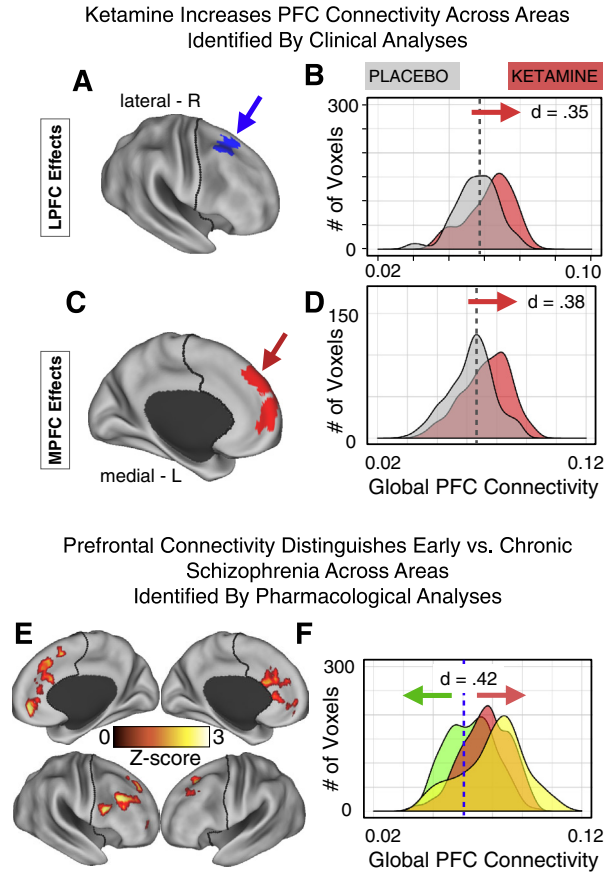

Figure 3. Cross-validating prefrontal cortex (PFC) connectivity between clinical and pharmacologic analyses. **(A–D)** We extracted the signal out of the identified lateral PFC (LPFC) **(A)** and the medial PFC (MPFC) **(C)** regions for the independent sample of healthy volunteers that underwent a saline (gray distributions) followed by a ketamine infusion (red distributions) to evaluate the direction of pharmacologic effects in the same regions relative to clinical findings (identified in Figure 2). For the two regions that were identified via the clinical analyses **(A, C)**, there was a consistent increase in PFC connectivity following ketamine infusion. **(E, F)** Given statistical independence, we also extracted the signal from all clusters showing significant pharmacologic effects, which were identified in Figure 1 analyses, to further explore the pattern of connectivity alterations across clinical groups for clusters explicitly modulated by ketamine. Blue vertical dashed line marks the mean for the healthy comparison subject group (we did not present the healthy comparison subject distribution to avoid obscuring the clinical groups). These analyses again revealed a qualitative difference in PFC connectivity between the chronic schizophrenia patients and the patients early in their illness course groups, particularly for the medial PFC and left lateral PFC clusters (Cohen's $d = .52$, $p < .045$ and Cohen's $d = .6$, $p < .03$, respectively, see Table 3 for all pair-wise comparisons). L, left; R, right.

antagonism may increase the functional coupling of PFC networks, particular along the medial PFC surface. These effects are consistent with reports of whole-brain hyperconnectivity under ketamine and prior focused seed-based studies (18,25). Moreover, these analyses suggest that ketamine does not reduce PFC functional coupling at rest.

Critically, across analyses, such hyperconnectivity was not observed in C-SCZ, either when tested relative to other clinical groups or when tested within independently defined regions showing alterations under ketamine. Therefore, pharmacologic effects, in addition to prior clinical studies (28), suggest that acute ketamine administration may not always match chronic schizophrenia findings, potentially reflecting unique pathophysiological mechanisms across different phases of this complex neurodevelopmental disease (9,45). Conversely, qualitative similarities between ketamine-induced hyperconnectivity and EC-SCZ suggest that increased PFC connectivity may be a marker of functional changes that occur during early stages of schizophrenia (or acute illness phases), perhaps reflecting glutamatergic alterations (32). Such markers may help to iteratively test and refine novel pharmacotherapies. These results underscore the utility of data-driven methods, such as the rGBC approach, for detecting functional network alterations in clinical conditions (28,33) and following pharmacologic manipulations (25). It is important to note that regions that showed the most robust ketamine effect on connectivity in the pharmacologic experiment at times showed only modest group differences in the clinical analysis (at times not significant, see Tables 2 and 3). This discrepancy potentially challenges the conclusion that the effects observed in the clinical samples are completely related to glutamate dysregulation observed under ketamine. This is somewhat mitigated by the anatomical proximity between increased medial PFC rGBC in EC-SCZ and HR samples and under ketamine. That is, findings collectively point to a medial PFC abnormality characterized by hyperconnectivity during early illness stages, which qualitatively resembled ketamine effects. There may occur a possible normalization of this medial PFC connectivity in chronic patients but a progressive reduction of lateral PFC connectivity. While this lateral/medial dissociation was somewhat surprising, it may be possible that such differences reflect dissociable relationships between these areas and thalamostriatal circuits or perhaps their differential sensitivity to glutamatergic disruption.

Present results also revealed functional distinctions between C-SCZ patients versus HR and EC-SCZ groups. Importantly, only some HR individuals will proceed to develop schizophrenia as their primary diagnosis. Other individuals in this HR group could develop different psychiatric diagnoses or never develop a neuropsychiatric disorder. The inability to classify HR individuals by their true schizophrenia risk likely introduced noise from these participants. Therefore, longitudinal studies will need to determine whether severity of reported HR effects map onto frank schizophrenia diagnosis. Relatedly, there is growing recognition that the revised psychiatric diagnostic scheme (DSM-V) does not cleanly map onto the underlying neurobiology or genetics of psychiatric disorders (45,46). One possibility is that the increases in PFC functional connectivity is a marker of the canonical schizophrenia diagnosis, as outlined by the updated Diagnostic and Statistical Manual of the American Psychiatric Association (http://www.dsm5.org). Alternatively, it needs to be determined if observed PFC connectivity alterations are a better marker for executive deficits that contribute to functional disability cutting across psychiatric diagnoses, consistent with the approach taken by the National Institute of Mental Health Research Domain Criteria initiative (46). Similarly, future studies should carefully consider how identified areas of hypoconnectivity

Table 3. Region Coordinates, *p* Values, *t* Values, and Effect Size Estimates for the Secondary Cross-Validation Analyses

Regions Identified via Primary Clinical Analyses Ketamine-Placebo Cross-Validation									
X	Y	Z	Hemisphere	Anatomical Landmark	Cluster Size (mm^3)	Comparison	Cohen's *d*	*t* Value	*p* Value
−9	47	33	Left	Superior frontal gyrus (BA 9)	11340	Ketamine vs. Placebo	.38	1.79	.09 (trend)
26	9	58	Right	Middle frontal gyrus (BA 6)	2592	Ketamine vs. Placebo	.35	1.52	.146
Regions Identified via Primary Pharmacologic Analyses - Clinical Cross-Validation									
X	Y	Z	Hemisphere	Anatomical Landmark	Cluster Size (mm^3)	Comparison	Cohen's *d*	*t* Value	*p* Value
						HCS vs. C-SCZ	.12	1.03	.307
						HCS vs. EC-SCZ	.42	2.18	.03[a]
						HCS vs. HR	.17	1.14	.256
−9	47	33	Left	Superior frontal gyrus (BA 9)	11340	HR vs. C-SCZ	.30	1.37	.177
						HR vs. EC-SCZ	.29	1.42	.162
						C-SCZ vs. EC-SCZ	.52	2.11	.04[a]
						HCS vs. C-SCZ	.30	1.57	.120
						HCS vs. EC-SCZ	.08	.93	.354
						HCS vs. HR	.17	1.25	.213
26	9	58	Right	Middle frontal gyrus (BA 6)	2592	HR vs. C-SCZ	.43	1.72	.09 (trend)
						HR vs. EC-SCZ	.25	1.31	.197
						C-SCZ vs. EC-SCZ	.23	.77	.448
						HCS vs. C-SCZ	.51	2.26	.026[a]
						HCS vs. EC-SCZ	.02	.73	.468
						HCS vs. HR	.35	1.83	.07 (trend)
−9	47	33	Left	Superior frontal gyrus (BA 9)	11340	HR vs. C-SCZ	.85	2.96	.005[b]
						HR vs. EC-SCZ	.36	1.64	.107
						C-SCZ vs. EC-SCZ	.58	2.31	.025[a]

Top: PFC rGBC results for the pharmacologic comparison (ketamine vs. placebo) cross-validated specifically within the two discovered foci obtained from the primary clinical analyses (corresponding to Figure 3A–D effects). These cross-validation pharmacologic effects were statistically modest and trend-level but moved in the same qualitative direction as the primary effects in Figure 1. Bottom: Clinical between-group comparisons cross-validated specifically within the three discovered foci obtained from the primary pharmacologic analyses (corresponding to Figure 3E,F effects). As in Table 2, for completeness, we present all pair-wise comparisons across groups. Effect sizes show standard Cohen's *d* estimates as in Table 2.

BA, Brodmann area; C-SCZ, chronic schizophrenia patients; EC-SCZ, early course schizophrenia patients; HCS, healthy comparison subjects; HR, high risk subjects; PFC, prefrontal cortex; rGBC, restricted global brain connectivity.

[a]Significant between-group difference $p < .05$.

[b]Significant between-group difference $p < .01$.

in PFC relate to task-based deficits in cognition typically reported in SCZ (47).

Implications for the Neurobiology of Schizophrenia Progression

The current study uses cross-sectional data, with longitudinal implications (see Limitations). Emergence of schizophrenia may be associated with abnormally increased functional connectivity, but its advancement may be associated with the transition to connectivity reductions, consistent with progressive decline in white-matter integrity and gray-matter volume described in structural magnetic resonance imaging studies and analyses of postmortem tissue (48–52). This model is also consistent with the shift from elevated cortical glutamate levels early in the illness course to glutamate deficits in chronic schizophrenia (32). Thus, early course functional hyperconnectivity may contribute to the later connectivity decline, in line with preclinical data showing that repeated ketamine administration activates corticolimbic circuits leading to local volume loss in activated areas (53). Present data are not completely congruent with this model, as chronic patients exhibited hypoconnectivity in distinct lateral PFC areas in the current sample (although this could be a limitation of the cross-section design since the same individuals were not studied longitudinally), or possibly related to important analysis nuances of resting-state signal (54). An alternative interpretation is that PFC functional connectivity differs between patients that exhibit distinctive alterations in their underlying neurobiology, irrespective of chronicity. Therefore, longitudinal studies combining structural and functional connectivity are needed to arbitrate between these possibilities and further inform the viability of pharmacologic manipulations such as ketamine for modeling specific illness aspects.

Implication for Medication Development

Limiting the relevance of ketamine effects on functional connectivity to EC-SCZ may paradoxically increase the impact of this model psychosis in the long run by establishing ketamine's specificity for future investigations testing

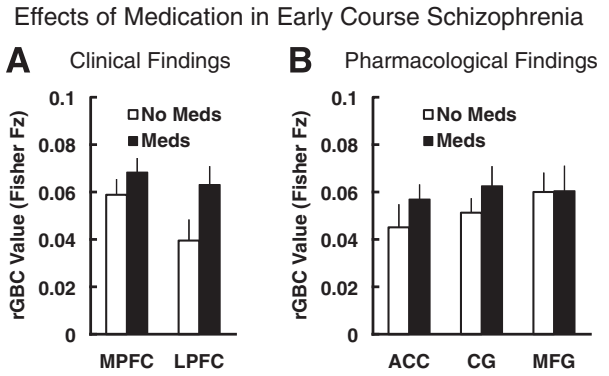

Figure 4. Effects of medication on prefrontal cortex (PFC) restricted global brain connectivity (rGBC) in early course schizophrenia. To rule out possible medication effects, we computed two follow-up analyses within the identified rGBC PFC regions that showed differences between clinical groups (i.e., Figure 2). Because all chronic patients but one were medicated, the analysis that includes both groups would be completely confounded with medication. Therefore, we focused on the early course sample where 16 patients never received medication and 12 patients were medicated. **(A)** We focused on regions identified via clinical analyses. We computed two follow-up between-group *t* tests comparing medicated (black bars) versus unmedicated (white bars) early course patients on rGBC PFC values within the medial PFC (MPFC) and lateral PFC (LPFC) clusters. Across both analyses, there was no significant effect of medication (MPFC: $t_{26} = .96$, $p = .35$, not significant [ns]; LPFC: $t_{26} = 1.57$, $p = .13$, ns). Although there was a slight trend in the LPFC cluster whereby medicated patients showed higher PFC rGBC, this increase was actually in the opposite direction of the chronic patient effects—arguing against medication being the likely cause of reduced LPFC connectivity in the chronic patients. **(B)** To further validate that medication did not relate to any effects within regions identified by pharmacologic analyses, we computed three secondary *t* tests for the rGBC PFC values within the three clusters identified via pharmacologic analyses (i.e., anterior cingulate gyrus [ACC], cingulate gyrus [CG], and the middle frontal gyrus [MFG]). Across all clusters, there was no significant effect of medication (ACC: $t_{26} = .94$, $p = .35$, ns; CG: $t_{26} = 1.1$, $p = .27$, ns; MFG: $t_{26} = .03$, $p = .97$, ns). Collectively, these control analyses strongly argue against medication driving reported effects, at least for the group of patients early in their illness course. Moreover, chlorpromazine equivalent levels for chronic schizophrenia patient with available information ($n = 15$) did not relate to PFC rGBC effects across the two PFC clusters ($r_{MPFC} = -.11$, $p = .7$; $r_{LPFC} = -.47$; $p = .08$, two-tailed), although the LPFC rGBC exhibited a trend effect with chlorpromazine equivalents. Error bars indicate ±1 SEM.

pharmacotherapies. There is widespread implementation of NMDAR antagonist animal models for testing novel pharmacotherapies for schizophrenia. However, such studies identified drugs that attenuated some effects of NMDAR antagonists in animals but were ultimately ineffective in patients (55,56).

Based on current data, there may be two possible reasons for difficulties in translating preclinical effects to human pharmacotherapies. First, animal models may not have employed appropriate dependent measures. Ketamine blocks NMDARs throughout the brain producing both synaptic and large-scale network-based effects (10,57). This study identified a network-level effect whereby ketamine effects resemble EC-SCZ. Second, current data suggest that some ketamine effects may be more relevant for EC-SCZ or perhaps only to subgroups of patients. The potential importance of this observation is supported by studies of metabotropic glutamate receptor-2 (mGluR2) agonists tested for the treatment of schizophrenia. mGluR2 agonists attenuated working memory deficits produced by NMDAR antagonists in animals (58) and humans (59) and were effective in an initial (60) but not subsequent clinical trials. Current data predict that mGluR2 agonists, which suppress glutamate release, may attenuate functional hyperconnectivity associated with EC-SCZ but exacerbate functional connectivity reductions, which were prominent in C-SCZ. Medications that suppress hyperconnectivity during EC-SCZ may play a role in ameliorating the progression of the disorder, perhaps decreasing functional disability. Consistent with this hypothesis, re-analysis of the Lilly mGluR2 agonist clinical trials for schizophrenia provide compelling evidence that this drug may be a more effective medication for EC-SCZ patients but less effective for chronic patients (61). Nevertheless, ketamine may produce other modulations, besides its effects on PFC connectivity, that are relevant for understanding chronic schizophrenia (62). Future studies examining concurrent resting-state and task-based ketamine effects in relation to illness stages will be important to inform this question.

Limitations

The design of the present study does not explicitly allow for longitudinal inferences—follow-up studies are needed to determine whether there is a hyperprogression to hypoprogression within individual subjects as a function of individual illness course. The role of medication also cannot be fully ruled out, as chronic findings could be, in part, medication related (although our follow-up chlorpromazine analyses argue against this). While one strategy would be to temporarily withdraw chronic patients from medication, this would still not fully address long-term polypharmacy. The same issue applies to longitudinal designs, as presumably patients would receive long-term pharmacotherapy. We attempted to address this by comparing EC-SCZ patients evenly divided between medicated and unmedicated individuals. However, medication was not administrated in a randomized design in this case. Future studies with fully unmedicated patients need to replicate present effects. There are some caveats regarding the demographic profiles between healthy volunteers administered ketamine and the clinical populations. Healthy volunteers undergoing pharmacologic neuroimaging were recruited in the United States. Due to logistical reasons, clinical groups were recruited in China (and likely to be exclusively Asian). The critical factor for interpreting clinical findings is that all the participants were formally matched to demographically similar HCS from China ($n = 96$) evenly across clinical groups. Therefore, all clinical effects and pharmacologic experimental findings stand independently of one another, as both study arms yielded statistically orthogonal discoveries (Figure 1 vs. Figure 2). In turn, these effects were partially cross-validated (Figure 3), which provides convergent evidence. Nevertheless, future experiments that explicitly control for demographic and ethnic differences within the same cross-sectional designs are needed to validate present findings. Also, it was difficult to appropriately model age as a covariate (as group and age are likely co-linear, due to little overlap in age across some

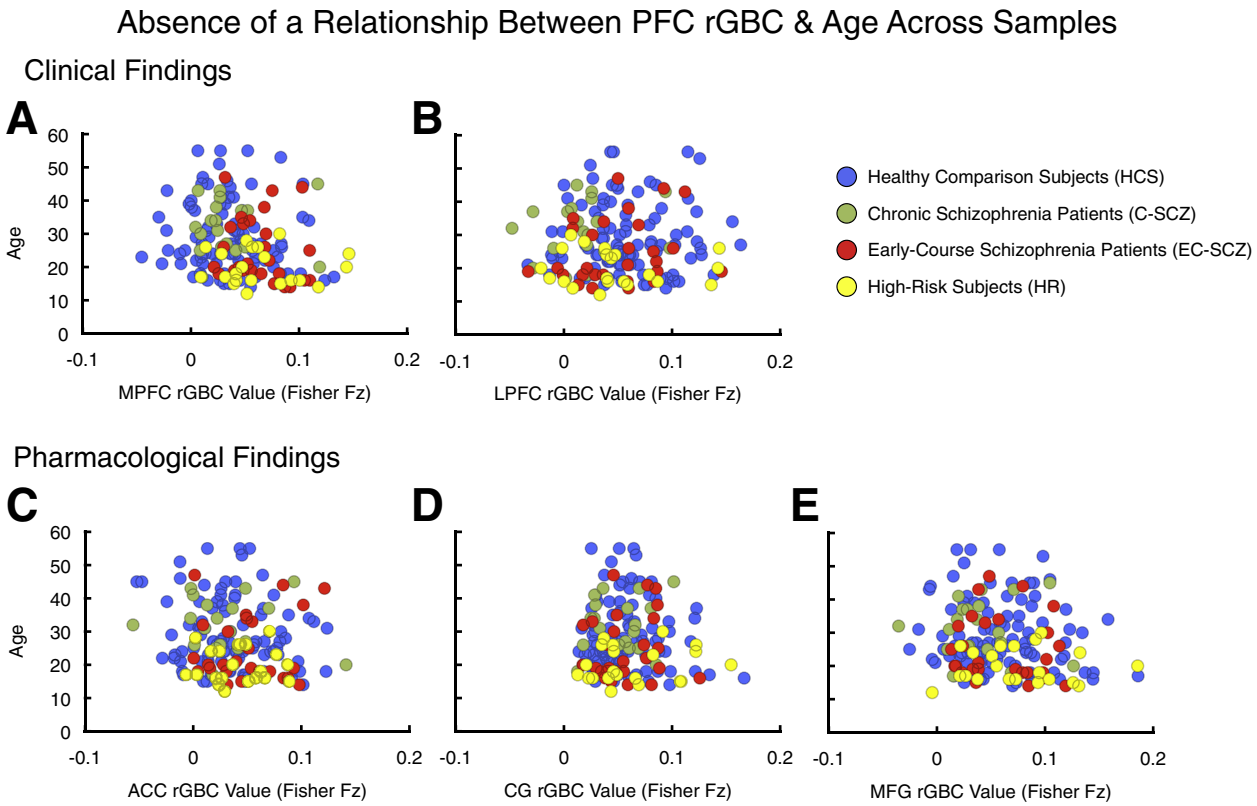

Figure 5. Absence of a relationship between age and prefrontal cortex (PFC) restricted global brain connectivity (rGBC) for clinical group findings. Age is a key variable showing between-group differences across the clinical groups (i.e., HR and EC-SCZ were on average younger than C-SCZ). Age could therefore be related to brain maturity and confound observed across-group clinical differences. To rule this out, we conducted a follow-up validity check analysis to ensure that age was not significantly related to our main between-group effects for the clinical analyses. This issue did not pertain to the ketamine analyses, as healthy volunteers for pharmacologic neuroimaging served as their own control and were thus age-controlled. **(A, B)** First, we examined the relationship between age and PFC rGBC for the effects identified via clinical findings. There was no relationship between age across participants and PFC rGBC connectivity for the two identified clusters. Moreover, there was no significant relationship between age and reported connectivity effects within any of the individual subgroups (all correlation values $<.22$). **(C–E)** To further validate that age did not relate to any effects within regions identified by pharmacologic analyses, we conducted follow-up secondary analyses. There was no relationship between age across participants and PFC rGBC connectivity for the three clusters modulated by ketamine (all correlation values $<.15$). Moreover, there was no significant relationship between age and reported connectivity effects within any of the individual subgroups (all correlation values $<.2$). ACC, anterior cingulate gyrus; CG, cingulate gyrus; LPFC, lateral prefrontal cortex; MFG, middle frontal gyrus; MPFC, medial prefrontal cortex.

groups). Nevertheless, other age-related analyses (Figure 5) mitigate the concern that neuromaturational effects primarily drove reported findings. Finally, relating cognitive functioning to the reported resting-state connectivity profiles represents a vital longer-term research goal. As part of this study, we did not collect detailed neurocognitive functioning and therefore cannot empirically address this question here. This opens an important goal for future studies that combine cognitive task activation paradigms and resting-state acquisition.

Conclusion

The current study combines pharmacologic and clinical neuroimaging to provide three insights regarding the PFC functional network alterations in schizophrenia. First, NMDAR antagonism increases PFC functional connectivity in healthy volunteers. Second, independent clinical results suggest that phase of illness is an important moderator of the PFC functional connectivity in schizophrenia. Third, pharmacologic effects of NMDA antagonists on PFC connectivity are not fully consistent with chronic illness findings but instead may better approximate some early course effects. Collectively, these cross-disciplinary pharmacologic and clinical neuroimaging results could have broad implications for prevention and treatment of this profoundly disabling neuropsychiatric condition.

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John H. Krystal consults for several pharmaceutical and biotechnology companies, with compensation less than $10,000 per year. These companies include AbbVie, Inc.; Amgen; Astellas Pharma Global Development; AstraZeneca Pharmaceuticals; Biomedisyn Corporation; Bristol-Myers Squibb; Easton Associates; Eli Lilly and Co.; F. Hoffman-L Roche Ltd; Forest Laboratories; Gilead Sciences, Inc.; GlaxoSmithKline; Janssen Research & Development; Novartis; Otsuka Pharmaceutical, Development & Commercialization, Inc.; Sage Therapeutics, Inc.; Shire Pharmaceuticals; Sunovion Pharmaceuticals, Inc.; and Takeda Industries. Dr. Krystal is a member of the following scientific advisory boards: CHDI Foundation, Inc.; Lohocla Research Corporation, Mnemosyne Pharmaceuticals, Inc.; Naurex, Inc.; and Pfizer Pharmaceuticals. In addition, Dr. Krystal is a past president of the American College of Neuropsychopharmacology, editor of Biological Psychiatry; and an employee of the Yale University School of Medicine and the Veterans Affairs Connecticut Health System. He is an originator on the following patent: Seibyl JP, Krystal JH, and Charney DS; Dopamine and noradrenergic reuptake inhibitors in treatment of schizophrenia; Patent #:5 447 948; 5 September 1995. In addition, he is an originator of the following relevant pending patents: 1) Vladimir C, Krystal JH, and Sanacora G; Glutamate agents in the treatment of mental disorders; Patent No. 11/399 188; 5 April 2006 (pending); and 2) Intranasal administration of ketamine to treat depression (pending). All other authors report no biomedical financial interests or potential conflicts of interest.

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