JAMA Psychiatry | Original Investigation

Utility of Imaging-Based Biomarkers for Glutamate-Targeted Drug Development in Psychotic Disorders
A Randomized Clinical Trial

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IMPORTANCE Despite strong theoretical rationale and preclinical evidence, several glutamate-targeted treatments for schizophrenia have failed in recent pivotal trials, prompting questions as to target validity, compound inadequacy, or lack of target engagement. A key limitation for glutamate-based treatment development is the lack of functional target-engagement biomarkers for translation between preclinical and early-stage clinical studies. We evaluated the utility of 3 potential biomarkers—ketamine-evoked changes in the functional magnetic imaging (fMRI) blood oxygen level–dependent response (pharmacoBOLD), glutamate proton magnetic resonance spectroscopy (^{1}H MRS), and task-based fMRI—for detecting ketamine-related alterations in brain glutamate.

OBJECTIVE To identify measures with sufficient effect size and cross-site reliability to serve as glutamatergic target engagement biomarkers within early-phase clinical studies.

DESIGN, SETTING, AND PARTICIPANTS This randomized clinical trial was conducted at an academic research institution between May 2014 and October 2015 as part of the National Institute of Mental Health–funded Fast-Fail Trial for Psychotic Spectrum Disorders project. All raters were blinded to study group. Healthy volunteers aged 18 to 55 years of either sex and free of significant medical or psychiatric history were recruited from 3 sites. Data were analyzed between November 2015 and December 2016.

INTERVENTIONS Volunteers received either sequential ketamine (0.23 mg/kg infusion over 1 minute followed by 0.58 mg/kg/h infusion over 30 minutes and then 0.29 mg/kg/h infusion over 29 minutes) or placebo infusions.

MAIN OUTCOMES AND MEASURES Ketamine-induced changes in pharmacoBOLD, ^{1}H MRS, and task-based fMRI measures, along with symptom ratings. Measures were prespecified prior to data collection.

RESULTS Of the 65 volunteers, 41 (63%) were male, and the mean (SD) age was 31.1 (9.6) years; 59 (91%) had at least 1 valid scan. A total of 53 volunteers (82%) completed both ketamine infusions. In pharmacoBOLD, a highly robust increase (Cohen d = 5.4; P < .001) in fMRI response was observed, with a consistent response across sites. A smaller but significant signal (Cohen d = 0.64; P = .04) was also observed in ^{1}H MRS–determined levels of glutamate+glutamine immediately following ketamine infusion. By contrast, no significant differences in task-activated fMRI responses were found between groups.

CONCLUSIONS AND RELEVANCE These findings demonstrate robust effects of ketamine on pharmacoBOLD across sites, supporting its utility for definitive assessment of functional target engagement. Other measures, while sensitive to ketamine effects, were not sufficiently robust for use as cross-site target engagement measures.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT02134951

JAMA Psychiatry. 2018;75(1):11-19. doi:10.1001/jamapsychiatry.2017.3572
Published online November 22, 2017.

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All currently approved treatments for schizophrenia, including both typical and atypical antipsychotics, function primarily by blocking dopamine D_2 receptors.[1,2] Nevertheless, these compounds are fully effective for only a minority of individuals with schizophrenia, indicating the need for alternative treatment approaches.[3,4] Recent theories implicate dysfunction of brain glutamatergic systems in general and of neurotransmission mediated by *N*-methyl-D-aspartate-type glutamate receptors (NMDAR) in particular. This latter theory is based on the ability of phencyclidine, ketamine, MK-801 (dizocilpine), or other NMDAR antagonists to induce clinical symptoms and neurocognitive dysfunction closely resembling those of schizophrenia.[5-7] In rodent studies, effects of NMDAR antagonists such as ketamine[8,9] are mediated, at least in part, by stimulation of presynaptic glutamate release in frontal brain regions and are reversed by compounds acting either to enhance postsynaptic NMDAR function or to diminish presynaptic glutamate release, which either reverse the primary deficit in NMDAR dysfunction or block the NMDAR antagonist–induced increase in presynaptic glutamate release.[9-11]

Nevertheless, a major limitation to glutamate-targeted treatment development is the lack of appropriate biomarkers to evaluate this effect in early-stage clinical trials to confirm target engagement.[12,13] The present study evaluates 3 potential biomarkers of functional target engagement by ketamine, reversal or blockade of which would serve as functional evidence of a brain effect of novel glutamatergic agents in schizophrenia. The biomarkers include ketamine-evoked changes in (1) the functional magnetic imaging (fMRI) blood oxygen level–dependent (BOLD) response (pharmacoBOLD), (2) glutamate proton magnetic resonance (^{1}H MRS) spectroscopy, and (3) task-based fMRI. Our primary goal was to identify biomarkers with a magnitude of effect at least equivalent to that observed in rodent studies (eg, $z > 2.3$[11]), which successfully detected effects of metabotropic glutamate receptors (mGluR) 2 and 3 agonists in animal models.

The first approach, pharmacoBOLD, leverages effects of glutamate on brain energy metabolism. During normal brain homeostasis, recycling of glutamate released from presynaptic glutamatergic terminals accounts for approximately 50% of energy expenditure.[14] In animals, NMDAR antagonist–induced increases in glutamate release are accompanied by increased local metabolism and cerebral blood volume that reflect increased energy expenditure.[11,15] These effects are prevented along with NMDAR antagonist–induced behaviors by compounds that block presynaptic glutamate release, such as lamotrigine or mGluR2/3 agonists.[15,16] Compared with ketamine, dopaminergic agents, such as methylphenidate, have limited effect on cerebral blood volume in adults.[17] In fMRI, increases in regional metabolism are associated with increased local blood flow and BOLD signal.[18] Here, we evaluated these effects compared with placebo in a multicenter trial setting.

Second, we evaluated effects of ketamine on the local concentration of glutamate and metabolites determined using ^{1}H MRS. In ^{1}H MRS, the concentration of glutamate, glutamine, or glutamate+glutamine (Glx) can be measured directly.[19-21] Prior studies have shown dysregulated glutamate release in the medial prefrontal or anterior cingulate cortex in patients diagnosed as having acutely psychotic, unmedicated schizophrenia,[22-26] as well as those at high clinical risk.[27] Elevated cingulate glutamate levels may also persist throughout later stages of illness in individuals who are antipsychotic resistant but not in those who are antipsychotic responsive[28,29] and correlate with common allelic variants in glutamate-related risk genes.[30] Several small studies have also shown ketamine-induced alterations in brain glutamate metabolism in the medial prefrontal or anterior cingulate cortex in healthy volunteers,[31-33] but to our knowledge, the consistency of these findings in cross-site studies has not been evaluated.

Finally, we examined the effect of ketamine on functional activations observed during the Relational and Item-Specific Encoding (RISE) task,[34,35] which is known to the engage dorsolateral prefrontal cortex (DLPFC) and hippocampal regions involved in long-term memory. Patients diagnosed as having schizophrenia show reduced DLPFC BOLD and hippocampus activation during relational encoding,[36] suggesting task-based fMRI readouts involving DLPFC or hippocampus involvement during the RISE task might serve as biomarkers as well.

The primary goal of the study was to compare both the magnitude of response and feasibility of implementation of imaging-based biomarkers for glutamate-targeted drug development in psychotic disorders across measures within the context of a multicenter clinical trial as a method for validating a biomarker for future early-stage glutamatergic drug development in schizophrenia. In general, we hypothesized that ketamine effects would be in the direction observed in both animal studies with NMDAR antagonists and in schizophrenia—ie, toward increased BOLD response, increased ^{1}H-MRS Glx levels, and reduced activation during the RISE task.

Key Points

Question Can imaging-based biomarkers be used for glutamatergic drug development in psychiatry?

Findings In this randomized clinical trial including 53 volunteers, a direct comparison of 3 proposed neuroimaging-based target engagement biomarkers for glutamatergic treatment development in the context of a multicenter ketamine challenge study was performed. Significant ketamine effects were observed for the ketamine-evoked changes in the functional magnetic imaging blood oxygen level–dependent (BOLD) response, with lesser degrees of change for magnetic resonance spectroscopy and task-based BOLD responses.

Meaning This trial validates the ketamine-evoked increases in functional magnetic imaging BOLD response as a cross-site biomarker for glutamatergic drug development in schizophrenia.

Methods

Participants

The study was approved by the Yale University Institutional Review Board, the University of California, Davis Institutional Review Board, and the New York State Psychiatric Institute Institutional Review Board. Written informed consent was obtained from all participants prior to participation. The

trial protocol can be found in Supplement 1. Volunteers were medically healthy men and women aged 18 to 55 years without current or past Axis I or II psychiatric or substance history,[37] as detailed in the eMethods in Supplement 2.

Design

Participants were randomized to ketamine or placebo in a 2:1 ratio. Volunteers participated in 2 magnetic resonance imaging sessions at least 2 weeks apart, with the first magnetic resonance imaging session always being ^{1}H MRS only and the second session incorporating both pharmacoBOLD and task-based fMRI. In the ^{1}H MRS session, an initial structural scan was conducted for voxel placement. Anterior cingulate cortex Glx levels were then obtained in 15-minute blocks starting 30 minutes prior to infusion (2 blocks) and continuing until the end of the 1-hour infusion (4 blocks). Participants maintained their blinded group assignment across scanning sessions.

Ketamine/Placebo Infusion

Racemic ketamine hydrochloride (0.23 mg/kg infusion over 1 minute followed by 0.58 mg/kg/h infusion over 30 minutes and then 0.29 mg/kg/h infusion over 29 minutes[38,39]) or placebo (normal saline) was administered.

Clinical and Safety Measures

Participants were rated with the Brief Psychiatric Rating Scale (BPRS), Profile of Mood States (POMS), and Clinician Administered Dissociative States Scale (CADSS)[6] tests as well as the Psychotomimetic States Inventory[40] after the scanning period ended.

Imaging Methods

Detailed imaging methods are provided in the eMethods in Supplement 2. Briefly, for pharmacoBOLD, T2-weighted echo-planar imaging scans were collected for 10 minutes prior to and during each ketamine infusion, with a repetition time of 2 seconds and with 32 slices at a resolution of 3.4 × 3.4 × 4.0 mm. A prespecified region of interest analysis was performed using the midcingulate cortex.[18] In addition, an exploratory whole-brain analysis was performed using multiple linear regression. Voxel-wise thresholds were set at $P < .05$ and were corrected for multiple comparisons using gaussian random field theory ($P < .05$).

For ^{1}H MRS, the region of interest was placed in the medial prefrontal cortex anterior to the genu of the corpus callosum, oriented along the anterior commissure–posterior commissure line, and centered on the interhemispheric fissure, with dimensions 2.5 × 3.0 × 2.5 cm (volume, 18.8 cm^3). The MRS acquisition used a standard point-resolved spectroscopy pulse sequence (repetition time/echo time = 2000/80 milliseconds) with 336 excitations. Four acquisitions (15 minutes each) were conducted over the 1-hour infusion period and compared with the preinfusion baseline. For data analysis, 8-channel phased-array coil data were combined into a single regular time-domain free-induction decay signal, using the unsuppressed voxel tissue water signal to derive the relative phased-array coil sensitivities. The primary outcome measure consisted of the pre/post ketamine change score for the Glx to creatinine ratio.

The RISE task was implemented in accordance with Ragland et al.[36] Briefly, participants viewed a series of visual depictions of objects during either an item-specific or relational encoding phase (eMethods in Supplement 2). The predesignated primary outcome measure was DLPFC BOLD activation during the task.

Group-level contrasts were made for encoding (relational minus item-specific), item recognition (hits minus misses for item-specific and hits minus misses for relational encoding), and associative recognition (hits minus misses). Structural masks from the WFU_PickAtlas[41] were used to restrict analyses to subgroups of activated voxels within left and right DLPFC (Brodmann areas 9, 46, and 9/46). Secondary analysis assessed hippocampal effects.[36]

Statistical Analysis

The ketamine effect on pharmacoBOLD and the RISE task were tested using a 2-sample t test (ketamine vs placebo). Ketamine effects on the ^{1}H MRS response measures were tested with repeated-measures analysis of variance of the Glx responses across time during the infusion with 4 time intervals (0-15, 15-30, 30-45, and 45-60 minutes), treatment group, and time × treatment group with post hoc contrasts. Across-site differences in each biomarker outcome were tested using an analysis of variance, with treatment, site, and treatment × site as fixed effects.

Ketamine effects on clinical measurements (ie, BPRS, POMS, and CADSS measures) were assessed using change score measures (postinfusion minus preinfusion scores), except for the Psychotomimetic States Inventory measure, which was only collected after ketamine infusion. Associations among biomarker responses, clinical measures, and blood levels of ketamine and metabolites were assessed using Spearman correlations. Ratings on clinical measures were averaged across the 2 days for associations with biomarkers, and blood levels were taken from the specific day. Finally, logistic regression was used to model the odds of assignment to the ketamine group on the main response measures. The optimal cut points for sensitivity and specificity were selected using Youden J statistic (sensitivity + specificity − 1).

Between-group analyses were conducted using t tests. Effect sizes were assessed using the Cohen d statistic. Correlations between variables were assessed using nonparametric (Spearman) correlations. Values in the text are means and standard deviations unless indicated otherwise. All P values were 2-tailed, and significance was set at $P < .05$.

Results

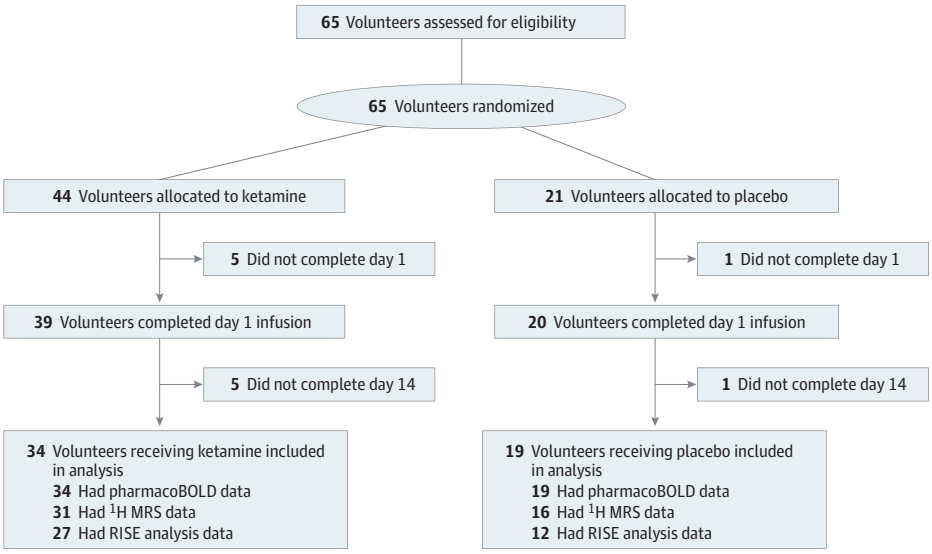
Sample

Sixty-five participants were randomized (**Figure 1**). The mean (SD) age was 31.1 (9.6) years and was similar across groups. No serious or unexpected adverse events were reported in the study.

PharmacoBOLD

The mean ketamine-evoked response in the dorsal midcingulate cortex showed a very large effect size ($t_{49} = 7.18$; Cohen

Figure 1. CONSORT Diagram



^{1}H MRS indicates glutamate proton magnetic resonance spectroscopy; pharmacoBOLD, functional magnetic imaging blood oxygen level–dependent response.

Table 1. Biomarker Results

Measure	Mean (SE)		Cohen *d*[a]	*P* Value
	Ketamine	Placebo		
Resting BOLD response				
No. of volunteers	34	19	NA	NA
Mean amplitude	0.91 (0.10)	−0.27 (0.14)	5.4	<.001
Glx[b]				
No. of volunteers	31	16	NA	NA
0-15 min	0.015 (0.002)	0.007 (0.003)	0.64	.04
15-30 min	0.009 (0.002)	0.012 (0.003)	−0.22	.47
30-45 min	0.011 (0.002)	0.010 (0.003)	0.04	.89
45-60 min	0.014 (0.002)	0.017 (0.003)	−0.17	.57
RISE task				
No. of volunteers	27	12	NA	NA
Discriminability index (d′)	−0.15 (0.10)	−0.10 (0.16)	−0.11	.77
Left DLPFC activation, overall	−1.12 (1.37)	2.24 (2.06)	−0.42	.18
Left DLPFC activation, relational item	−1.69 (1.94)	5.01 (2.91)	−0.79	.06
Right hippocampus hit miss	−0.53 (0.58)	−0.71 (0.90)	0.08	.87

Abbreviations: BOLD, blood oxygen level–dependent; DLPFC, dorsolateral prefrontal cortex; Glx, glutamate+glutamine; NA, not applicable; RISE, Relational and Item-Specific Encoding.

[a] Cohen *d* calculated as difference in means divided by standard deviation of the placebo group.

[b] Change in Glx level to creatine ratio relative to baseline acquisition.

d = 5.4; *P* < .001) (**Table 1**) (**Figure 2**A), with no significant difference across treatment sites ($F_{2,50}$ = 0.31; within-site effect size range, 4.6-5.6; *P* = .73). A voxelwise analysis (*P* < .05) demonstrated that most of the cortex was activated by ketamine administration (Figure 2B); only the orbitofrontal cortex did not show a positive BOLD response. The largest activations (*z* > 6) were found primarily in the dorsal anterior cingulate cortex (Montreal Neurological Institute brain atlas region 4, 22, and 32) and anterior insula (Montreal Neurological Institute brain atlas region 38, 28, 0; −32, −28, −2).

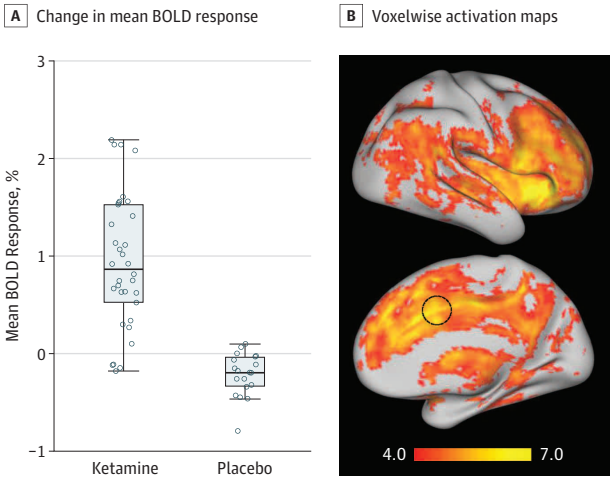
^{1}H MRS

A moderate effect size in between-group difference in acute Glx level increase was noted within the first 15-minute interval following ketamine administration (t_{126} = 2.09; Cohen *d* = 0.64; *P* = .04) (**Figure 3**) (Table 1). However, no significant between-group differences were seen after the first acquisition, leading to statistically similar increases in Glx levels across the remaining 45 minutes of the infusion period and a nonsignificant group difference across the entire infusion period ($F_{3,126}$ = 1.54; *P* = .21). There was not a significant difference in ketamine effects by site ($F_{3,114}$ = 2.51; *P* = .06).

RISE Task

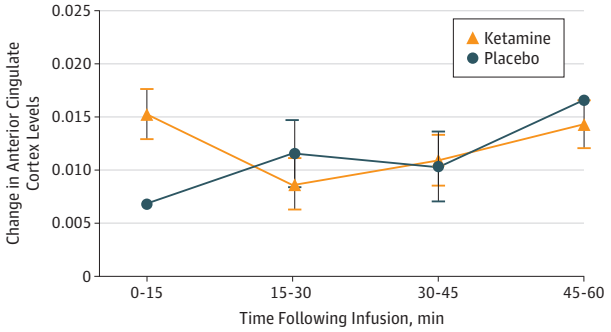
No significant behavioral difference was seen between groups in the a priori analysis of change in the discriminability index (d′) of item recognition following relational encoding (t_{37} = 0.30; Cohen *d* = −0.11; *P* = .77). Also, no significant ef-

Figure 2. Ketamine-Evoked Changes in the Functional Magnetic Imaging Blood Oxygen Level–Dependent (BOLD) Response



A, Change in mean BOLD response following ketamine administration in the predefined region of interest (dorsal midcingulate cortex) (Cohen d = 5.4; P < .001). Each point represents an individual participant. The box indicates the 25th and 75th percentiles; the line, the median value; error bars, the data range excluding outliers. B, Voxelwise activation maps. Peak activations were located in the dorsal anterior cingulate cortex, insula, and thalamus. z Statistic maps were thresholded at z > 4.0. The circle indicates the region of interest used for primary analysis.

Figure 3. Magnetic Resonance Spectroscopy Response



Change in glutamate+glutamine (Glx) to creatine ratio within the anterior cingulate cortex following ketamine vs the preinfusion baseline by glutamate proton magnetic resonance spectroscopy. The difference was significant (Cohen d = 0.64; P = .04) for the first 15-minute interval but was not significant for other intervals.

fect in activation was detected in the DLPFC during the task (t_{37} = 1.36; Cohen d = −0.42; P = .18). However, a strong group × site interaction was observed (P = .005), reflecting both significant and nonsignificant (P range, .03-.12) but opposite direction tendencies across the sites. Similarly, there was no significant activation effect in the hippocampus (t_{35} = 0.16; Cohen d = 0.08; P = .87).

Clinical Ratings

As expected, large effect size and between-group differences were seen for all behavioral scales (**Table 2**). On BPRS, only the positive subscale showed significant changes. Similar changes in clinical ratings were seen in the ketamine group on days 1 and 14 for all clinical measures except the CADSS, which was significantly higher on day 1.

Ketamine Level

Levels of ketamine, norketamine, and dehydronorketamine were obtained after the volunteers were removed from the scanner. Similar changes were seen on days 1 and 14 for ketamine (mean [SD] ng/mL, 108.5 [33.0] vs 111.9 [31.0]; t_{30} = 1.59; P = .12) and dehydronorketamine (mean [SD] ng/mL, 53.7 [16.2] vs 67.0 [19.1]; t_{30} = 1.57; P = .13). For norketamine, significantly lower levels were observed on day 1 vs day 14 (mean [SD] ng/mL, 28.2 [15.8] vs 33.4 [22.5]; t_{30} = 3.35; P = .002).

There were no significant associations with same-day ketamine levels for any of the primary outcome variables. There were also no significant associations between any of the primary outcome variables and levels of either norketamine or dehydronorketamine.

Relationship With Symptom Measures

Correlational analyses were conducted both across groups and within the ketamine group individually. Across groups, significant associations were seen between pharmacoBOLD increases and changes in the Psychotomimetic States Inventory score (r_s = 0.37; P = .007), BPRS (r_s = 0.43; P = .001), CADSS (r_s = 0.56; P < .001), and POMS (r_s = 0.30; P = .03) (eTable in Supplement 2). However, associations were not significant within the ketamine treatment group. If across-group correlations were corrected for multiple comparisons, the association with POMS would no longer be significant.

In contrast to pharmacoBOLD, no significant changes were observed for ^{1}H MRS Glx levels relative to symptoms. By contrast, left DLPFC activation to relational encoding items was significantly associated with both BPRS negative symptoms (r_s = 0.43; P = .03) and in ketamine-treated individuals alone (r_s = 0.46; P = .02). No significant changes were observed for hippocampal activation relative to symptoms.

Group Assignment

A final analysis evaluated the degree to which the proposed biomarkers could be used to predict group membership (ketamine vs placebo group). As expected, pharmacoBOLD response (χ^2 = 12.9; P < .001) significantly differentiated the groups, with sensitivity of 85.3% and specificity of 89.5%. Left DLPFC activation on the RISE task, despite absence of significant between-group difference, significantly predicted group membership (χ^2 = 6.18; P = .01), with relatively high specificity (83.3%) relative to sensitivity (48.1%). Despite the significant between-group differences, Glx levels did not significantly predict group membership. A combined measure of pharmacoBOLD+RISE activation was highly predictive, with 90% sensitivity and 88.9% specificity, leading to correct classification of 90% of participants. For pharmacoBOLD, a cutoff value of 0.5% increase in BOLD signal eliminated all placebo responders while retaining 80% of ketamine responders.

Table 2. Clinical Symptoms

Variable (Range)	Difference (Preinfusion vs Postinfusion), Mean (SD)		Difference, Mean (SE)	Cohen *d*[a]	*P* Value
	Ketamine	Placebo			
Day 1					
No. of volunteers	44	21	NA	NA	NA
POMS total (0-260)	13.89 (17.76)	−0.93 (7.29)	14.81 (5.60)	2.03	.01
BPRS					
Total (19-133)	4.59 (5.07)	0.96 (1.24)	3.63 (1.09)	2.93	.001
Positive (4-28)	2.16 (2.07)	0.21 (0.46)	1.95 (0.44)	4.24	<.001
Negative (3-21)	0.46 (1.02)	0.14 (0.31)	0.32 (0.21)	1.03	.14
Activation (3-21)	0.49 (1.21)	0.11 (0.38)	0.38 (0.27)	1.01	.16
Hostility (4-28)	0.33 (0.75)	0.07 (0.36)	0.26 (0.16)	0.72	.12
CADSS total (0-92)	14.21 (13.14)	2.25 (2.43)	11.95 (2.84)	4.92	<.001
PSI[b] (0-144)	20.18 (12.10)	8.17 (4.28)	12.00 (4.59)	2.80	.01
Day 14					
No. of volunteers	34	19	NA	NA	NA
POMS total (0-260)	12.40 (14.22)	4.45 (5.88)	7.95 (3.44)	1.35	.02
BPRS					
Total (19-133)	3.12 (5.50)	0.28 (1.11)	2.84 (1.25)	2.56	.03
Positive (4-28)	1.41 (2.07)	0.13 (0.40)	1.29 (0.48)	3.23	.01
Negative (3-21)	0.47 (1.14)	0.13 (0.32)	0.34 (0.27)	1.06	.20
Activation (3-21)	0.43 (1.31)	0.02 (0.47)	0.41 (0.31)	0.87	.20
Hostility (4-28)	0.06 (0.75)	0.06 (0.47)	0.004 (0.18)	0.01	.98
CADSS total (0-92)	7.75 (8.12)	1.72 (2.07)	6.03 (1.90)	2.91	.003
PSI[b] (0-144)	15.58 (8.80)	7.67 (4.69)	7.91 (3.48)	1.69	.03

Abbreviations: BPRS, Brief Psychiatric Rating Scale; CADSS, Clinician Administered Dissociative States Scale; NA, not applicable; POMS, Profile of Mood States; PSI, Psychotomimetic States Inventory.

[a] Cohen *d* calculated as difference in means divided by standard deviation of the placebo group.

[b] PSI has only postinfusion measure; hence, means are the mean of postinfusion, not change scores.

Discussion

Most psychiatric drugs in clinical use derive from serendipitous discoveries that were then reverse engineered to develop comparable or incrementally improved medications. Although there have been dramatic increases in the pathophysiological understanding of disorders, such as schizophrenia, these have not yet translated into innovations in pharmacotherapeutics or enhanced therapeutic outcome. One of the major obstacles to developing novel neurotherapeutic agents at present is the limited availability of validated biomarkers to establish a dose that reaches the desired target in the brain and produces some functional effect that may lead to a therapeutic response.[12,42] There is a critical absence of validated biomarkers in the case of glutamate-targeted treatments, which are presumed to work at least in part by modulation of presynaptic glutamate release. Ideal biomarkers not only have large associated magnitudes of effect but also have high translatability across sites, permitting use in multicenter clinical trials.[13]

The present study evaluated 3 potential biomarkers of glutamate-based functional target engagement—pharmacoBOLD, ^{1}H MRS Glx, and task-based fMRI (RISE task)—based on their sensitivity to ketamine administration in healthy volunteers. Critical issues include magnitude of effect and feasibility for cross-site implementation. Although each measure has been studied in isolation, to our knowledge, this is the first study to investigate them in parallel in the same sample in a rigorously designed, randomized, placebo-controlled, blinded, multisite investigation.

The primary finding is that the pharmacoBOLD results were very robust, with both a large magnitude of effect (Cohen *d* = 5.4) and strong cross-site consistency, suggesting its potential as a biomarker durable enough to be applied in multicenter studies, provided that adequate quality control, methodology, and minimum-capability 3-T magnetic resonance imaging systems from one of the major vendors (ie, Siemens, GE, or Philips) are used. Other measures provided complementary findings and may possess additional biomarker utility, depending on the therapeutic target and experimental compound.

An important feature of the pharmacoBOLD measure is that it recapitulates the effects of NMDAR antagonists in rodents, where acute administration of NMDAR antagonists increases blood flow, as measured by cerebral blood volume.[11,15] Effects are reversed by glutamatergic agents, such as mGluR2/3 receptor agonists,[11,15] and NMDAR glycine-site modulators, such as D-serine or glycine transport inhibitors,[43] as well as atypical antipsychotics, such as clozapine.[11] Therefore, the pharmacoBOLD biomarker may be particularly relevant to developing mGluR2/3 and NMDAR modulator-type compounds, establishing appropriate doses, and implementing experimental medicine type drug development principles.

In the present study, effect sizes for the pharmacoBOLD response were larger than those noted in prior studies using this approach, which have generally been in the range of 2.0 to 3.0 SD units.[18,44] The larger effect size is likely because of the greater infusion of ketamine given (0.23 mg/kg) vs prior

studies (0.1 mg/kg). As in prior studies, the BOLD response peaked within the first 5 minutes and was likely driven by the initial infusion, suggesting that sustained ketamine infusion is likely not required. In the present study, a cutoff value of 0.5% mean signal change fully separated individuals receiving ketamine from placebo and could be used in target engagement-type studies to maximize power to detect a significant medication effect.

The pharmacoBOLD response was also significantly associated with symptoms across but not within groups. This finding may reflect nonlinear relationships between pharmacoBOLD measures and psychosis within the ketamine group, such as saturation of the pharmacoBOLD response at high levels, as well as threshold levels needed to induce symptoms. Additionally, symptom ratings were not obtained until the end of the scan session, potentially weakening the associations. The pharmacoBOLD response was highly significant despite being obtained in the second scan, raising the possibility that even greater results and potentially stronger correlations with behavior might have been obtained if no prior scan was performed. In prior studies, high test-retest reliability of the pharmacoBOLD response has been observed across administrations.[18]

In contrast to the pharmacoBOLD response, Glx levels measured by ^{1}H MRS showed much smaller, albeit significant, effect-size differences between treatments (Cohen d = 0.64; P = .04). The lower sensitivity of ^{1}H MRS to ketamine effects may reflect difficulties in distinguishing synaptic from extrasynaptic glutamate.

Finally, effects of task-based fMRI in this study were less robust than those of pharmacoBOLD. Nevertheless, a moderate to large effect size difference in left DLPFC activation in response to relational items was observed (Cohen d = −0.42; P = .06). These changes also correlated with severity of ketamine-induced negative symptoms, both across treatment groups (r = 0.36; P = .03) and in the ketamine group itself (r = 0.43; P = .03).

Changes in left DLPFC activation also contributed to the differentiation of ketamine and placebo treatment groups over and above contributions of pharmacoBOLD. In particular, changes were highly specific to the ketamine group, albeit with relatively low sensitivity. Ketamine is also known to affect other NMDAR-dependent functions, such as working memory,[45] smooth pursuit eye movement,[46] and auditory information processing.[47] Therefore, the combination of task-based measures with pharmacoBOLD may help refine the biomarker algorithm.

Limitations

Our study has limitations. We selected a single sequence—point-resolved spectroscopy echo time 80 acquisition with linear combination model difference method analysis—that could be reliably implemented at each of the sites. It is possible that alternative ^{1}H MRS approaches might have yielded stronger results. A nonsignificant (P = .06) site-to-site variability was observed even with the consensus sequence and might be exacerbated by site-specific measurement protocols.

Conclusions

One of the major hurdles to neuroscience-based treatment development in psychiatry is the lack of appropriate functional target engagement measures. The present cross-site, randomized trial demonstrates that ketamine-induced pharmacoBOLD provides a robust and reliable measure of excess cortical glutamate release and may therefore serve as an informative measure for the development of novel glutamate-targeted therapeutic agents. The study also supports the potential utility of MRS and task-based fMRI, pending appropriate method refinements, and enables future target-engagement based research for development of glutamatergic medications in schizophrenia.

ARTICLE INFORMATION

Accepted for Publication: October 3, 2017.

Published Online: November 22, 2017. doi:10.1001/jamapsychiatry.2017.3572

Author Contributions: Dr Javitt had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Obtained funding: Javitt, Krystal, Kantrowitz, Girgis, Kegeles, Robinson, Lieberman.
Administrative, technical, or material support: Javitt, Carter, Krystal, Kantrowitz, Girgis, Maddock, Lesh, Tanase, Corlett, Rothman, Mason, Qiu, Robinson, Potter, Carlson, Lieberman.
Study supervision: Javitt, Carter, Kantrowitz, Girgis, Kegeles, Maddock, Lesh, Corlett, Robinson, Lieberman.

Conflict of Interest Disclosures: Dr Javitt has received consulting payments or honoraria from Forum, Takeda, Pfizer, Autifony, Glytech, and Lundbeck; has served on the scientific advisory board for Promentis and Phytonics; holds equity in Glytech, AASI, and NeuroRx; and holds intellectual property for use of by *N*-methyl-D-aspartate receptor agonists in schizophrenia and movement disorders as well as by *N*-methyl-D-aspartate receptor antagonists in depression, obsessive-compulsive disorder, and posttraumatic stress disorder. Dr Krystal has served as a consultant for AstraZeneca, Biogen, Biomedisyn, Janssen, LEK Otsuka, Pragma Therapeutics, SK Life Science, Spring Care, Sunovion, Takeda, Taisho, and Teva; has received research support from AstraZeneca and Pfizer; has served on scientific advisory boards for biOasis Technologies, Biohaven Pharmaceuticals, Blackthorn Therapeutics, Broad Institute, Lohocla Research Corporation, Luc Therapeutics, Pfizer, and TRImaran Pharma; holds equity in Biohaven Pharmaceuticals, Blackthorn Therapeutics, Luc Therapeutics, and Spring Care; and holds intellectual property for glutamate modulating agents in treatment of mental disorders and intranasal ketamine for treatment of depression. No other disclosures were reported.

Funding/Support: This study was supported by government contract HHSN271201200007I to the New York State Psychiatric Institute and the Research Foundation for Mental Hygiene from the National Institute of Mental Health.

Role of the Funder/Sponsor: The funder approved the design and conduct of the study; monitored the collection, management, analysis, and interpretation of the data; and mandated submission of the final manuscript. The funder had no role in the preparation, review, or approval of the manuscript.

Additional Information: Data are available in accordance with National Institute of Mental Health data-sharing procedures. Information about access can be found at https://www.nlm.nih.gov/NIHbmic/nih_data_sharing_repositories.html.

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Invited Commentary

Paving the Way for Targeted Drug Development in Schizophrenia

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Serendipity led to the discovery of antipsychotic medications, which are the cornerstone of acute and maintenance treatment for schizophrenia.[1] Unfortunately, these drugs lack efficacy in alleviating negative symptoms and cognitive symptoms. Notwithstanding the substantial advances in our mechanistic understanding of this phenotypically complex syndrome in the decades since their discovery, efforts to translate scientific progress into novel treatments that will address different schizophrenia symptoms dimensions have been largely unsuccessful.[2] Perhaps the greatest disappointments in recent memory have been the traditional multisite clinical trials of several glutamate-based treatments for schizophrenia, which garnered negative results despite a large body of theoretical and experimental evidence indicating that glutamatergic dysfunction is a core feature of the disease.[2]

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It is possible that neuropathological heterogeneity in study populations is a major contributor to apparent drug failures, because the fraction of poor responders might mask beneficial drug effects in more homogenous subgroups of patients. Abandoning the one-size-fits-all clinical trial paradigm in favor of targeted drug development has revolutionized the field of oncology. Discovery of the Philadelphia chromosome mutation in 1 subtype of patients with leukemia resulted in the design of the first genetically engineered cancer drug, imatinib, which has dramatically improved outcomes for these patients and set the stage for a number of tailored cancer treatments.[3] But rational drug design involves a theoretical understanding of fundamental pathophysiological disease mechanisms, the way those mechanisms change under influence of a drug, and which mechanisms lead to the desired therapeutic effects. Because current insights into biological processes underlying schizophrenia are insufficient to directly translate genetic or molecular disease signatures into novel candidate compounds, creative and pragmatic strategies aimed at targeted drug discovery are warranted.

In this issue of *JAMA Psychiatry*, Javitt et al[4] elegantly illustrate a strategy whereby investigators leveraged insights from a pharmacological model of schizophrenia to inform discovery of reliable biomarkers, a hallmark of precision medicine. They gave study participants ketamine, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor blocker that transiently induces positive, negative, and cognitive symptoms[5] and neurobiological changes[6] that resemble those seen in schizophrenia, and then quantified the changes that ketamine induced on selected biomarkers measurable by magnetic resonance imaging. The 3 selected candidate biomarkers tapped into diverse, presumably glutamate-related pathological processes of the illness. Javitt et al tested the effect size and cross-site reliability of these putative in vivo target engagement biomarkers (all of which were noninvasive and nonradioactive), in the hopes of facilitating the identification of novel glutamatergic compounds for the treatment of schizophrenia. First, a direct measure of tissue glutamatergic compounds glutamate-glutamine (Glx) was obtained through magnetic resonance spectroscopy in the medial prefrontal cortex. Second, the blood oxygen level-dependent (BOLD) response, an indirect measure of glutamate-related effects on local energy metabolism and blood flow, was assessed during a resting-state functional magnetic resonance image scan in the anterior cingulate cortex. Third, an indirect measure of glutamate-mediated functional activation in the dorsolateral prefrontal cortex and hippocampus was quantified during a memory task.

The effects of ketamine on these candidate biomarkers were most robust (Cohen *d*, 5.4) and consistent across sites for the resting-state BOLD response. Importantly, a mean signal change cutoff value of 0.5% fully separated the participants receiving ketamine from those receiving placebo. As the authors note,[4] this finding could inform the design of target engagement studies to help maximize power to detect a significant medication effect. It eventually could also be leveraged as a screening tool to select people with schizophrenia who have an altered resting-state BOLD response, which suggests altered glutamate metabolism, as participants in larger-scale clinical trials.

It might appear counterintuitive at first glance that an indirect measure of glutamate outperforms a more direct glutamatergic measure as a marker of glutamate dysfunction. But in the literature on schizophrenia and ketamine, the magnitude of glutamatergic alterations measured with spectroscopy is generally in the range of 10% to 15%,[7] which translates to modest effect sizes, similar to those seen in Javitt et al.[4] Limited effect sizes and technical challenges leading to