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# Glycine Transporter Inhibitor Attenuates the Psychotomimetic Effects of Ketamine in Healthy Males: Preliminary Evidence

**Deepak Cyril D'Souza*[1,2,3], Nagendra Singh[1,2], Jacqueline Elander[1,2], Michelle Carbuto[1,2,3], Brian Pittman[1,3], Joanna Udo de Haes[4], Magnus Sjogren[4], Pierre Peeters[4], Mohini Ranganathan[1,2,3,5] and Jacques Schipper[4,5]**

*[1]Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA; [2]VA Connecticut Healthcare System, West Haven, CT, USA; [3]Clinical Neuroscience Research Unit, New Haven, CT, USA; [4]Merck, Sharpe and Dohme (formerly Organon NV), Oss, The Netherlands*

Enhancing glutamate function by stimulating the glycine site of the NMDA receptor with glycine, D-serine, or with drugs that inhibit glycine reuptake may have therapeutic potential in schizophrenia. The effects of a single oral dose of *cis-N*-methyl-*N*-(6-methoxy-1-phenyl-1,2,3,4-tetrahydronaphthalen-2-ylmethyl) amino-methylcarboxylic acid hydrochloride (Org 25935), a glycine transporter-1 (GlyT1) inhibitor, and placebo pretreatment on ketamine-induced schizophrenia-like psychotic symptoms, perceptual alterations, and subjective effects were evaluated in 12 healthy male subjects in a randomized, counter-balanced, within-subjects, crossover design. At 2.5 h after administration of the Org 25935 or placebo, subjects received a ketamine bolus and constant infusion lasting 100 min. Psychotic symptoms, perceptual, and a number of subjective effects were assessed repeatedly before, several times during, and after completion of ketamine administration. A cognitive battery was administered once per test day. Ketamine produced behavioral, subjective, and cognitive effects consistent with its known effects. Org 25935 reduced the ketamine-induced increases in measures of psychosis (Positive and Negative Syndrome Scale (PANSS)) and perceptual alterations (Clinician Administered Dissociative Symptoms Scale (CADSS)). The magnitude of the effect of Org 25935 on ketamine-induced increases in Total PANSS and CADSS Clinician-rated scores was 0.71 and 0.98 (SD units), respectively. None of the behavioral effects of ketamine were increased by Org 25935 pretreatment. Org 25935 worsened some aspects of learning and delayed recall, and trended to improve choice reaction time. This study demonstrates for the first time in humans that a GlyT1 inhibitor reduces the effects induced by NMDA receptor antagonism. These findings provide preliminary support for further study of the antipsychotic potential of GlyT1 inhibitors.
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**Keywords:** ORG 25935; glycine transporter inhibitor; ketamine; healthy subjects; psychosis; cognition

## INTRODUCTION

*N*-methyl D-aspartate (NMDA) receptor antagonists have been shown to induce an array of transient schizophrenia-like positive and negative symptoms, neuropsychological deficits, sensory deficits, eye movement dysfunction, and impairments in electrophysiological indices of information processing in healthy human volunteers (reviewed in Cho *et al*, 2008; Kantrowitz and Javitt, 2010). NMDA receptor antagonists have also been shown to exacerbate symptoms in schizophrenic patients (Lahti *et al*, 1995; Malhotra *et al*, 1997). Accordingly, deficits in NMDA receptor function are thought to contribute to the neurobiology of schizophrenia.

The therapeutic implications of the NMDA deficit hypothesis are that facilitation of NMDA receptor function may have the potential as a treatment for schizophrenia and that the reversal of NMDA receptor antagonist effects may be predictive of antipsychotic-like activity of drugs. Direct activation of NMDA receptors is associated with excitoxicity (Lynch and Guttmann, 2002) and is therefore not safe. Stimulation of other modulatory sites on the NMDA receptor offer safer alternatives. Endogenous glycine and D-serine both act as co-agonists at the strychnine-insensitive glycine-B (Gly-B) site on the NMDA receptor, and along with glutamate, co-activate the receptor (Dingledine *et al*, 1990; Thomson, 1990). Furthermore, forebrain synaptic glycine and D-serine levels are regulated by the glycine transporter-1 (GlyT1) (Aragon and Lopez-Corcuera, 2005; Betz *et al*, 2006; Borowsky and Hoffman, 1998; Borowsky *et al*, 1993; Harsing *et al*, 2003) and the alanine–serine–cysteine

*Correspondence: Dr DC D'Souza, Psychiatry Service 116A, Yale University School of Medicine, VA Connecticut Healthcare System, 950 Campbell Avenue, West Haven, CT 06516, USA, Tel: +1 203 932 5711ext2594, Fax: +1 203 937 4860, E-mail: deepak.dsouza@yale.edu
[5]These authors share senior authorship
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transporter-1 (Asc-1) (Hayashi *et al*, 1997; Ribeiro *et al*, 2002), respectively. Thus, drugs with agonist effects at the Gly-B site, such as glycine and D-serine, or drugs that inhibit the GlyT1 or Asc-1 may offer a safe approach to facilitate NMDA receptor function. There is considerable interest in these approaches for the treatment of schizophrenia.

Drugs that stimulate the Gly-B site either directly or by inhibiting the reuptake of glycine have been shown to reduce the effects NMDA receptor antagonists at many levels. The GlyT1 inhibitor, such as (*R*)-(*N*-[3-(40-fluorophenyl)-3-(40-phenylphenoxy)propyl]sarcosine (NFPS), has been reported to potentiate NMDAR currents in the presence of glycine (Chen *et al*, 2003), and NFPS combined with risperidone, but not clozapine, potentiates these currents (Konradsson *et al*, 2006). Preclinical studies have also demonstrated that a number of Gly-B agonists and GlyT1 inhibitors attenuate a wide range of effects produced by NMDA receptor antagonists PCP, MK-801, and ketamine, including locomotor stimulatory effects, stereotypies, prepulse inhibition deficits, c-fos expression, cortical stimulation, and recognition memory deficits (Contreras, 1990; Gozzi *et al*, 2008; Hashimoto *et al*, 2008; Javitt and Frusciante, 1997; Javitt *et al*, 1997; Kanahara *et al*, 2008; Karasawa *et al*, 2008; Kinney *et al*, 2003; Nilsson *et al*, 1997; Tanii *et al*, 1991, 1994; Toth and Lajtha, 1986). However, this has not yet been demonstrated in humans. Most recently, the GlyT1 inhibitor PF-3463275 was shown to reduce ketamine-induced deficits in spatial working memory, but not ketamine-induced hallucinatory-like behaviors in non-human primates (Roberts *et al*, 2010). Taken collectively, the evidence indicates that glycine sites are not saturated *in vivo* and furthermore that stimulating the Gly-B site can facilitate NMDA receptor neurotransmission and attenuate the effects of NMDA receptor antagonists.

Organon NV developed *cis-N*-methyl-*N*-(6-methoxy-1-phenyl-1,2,3,4-tetrahydronaphthalen-2-ylmethyl) amino-methylcarboxylic acid hydrochloride (Org 25935), a highly selective inhibitor of GlyT1 ($IC_{50}$ $\sim$100 nM) (Walker *et al*, 2007). In functional assays of [$^3$H]glycine assays, it behaves as a mixed non-competitive inhibitor. Org 25935 has no significant interactions with GlyT2- or Na/Cl-dependent transporters (including NA, DA, 5HT, and GABA) and it does not have any measurable affinity across a broad range ($>$50) of receptors, ion channels, and enzymes, including either the strychnine-sensitive or -insensitive glycine receptors. Org 25935 is a potent sarcosine-related analog that easily passes the blood–brain barrier and produces dose-related increases in brain glycine levels (Walker *et al*, 2007). In preclinical studies, Org 25935 reversed PCP-induced behavioral deficits observed in the novel object recognition (NOR) model (Snigdha *et al*, 2007) and Org 25935 attenuated the hyperdopaminergic state induced by chronic PCP administration (Javitt *et al*, 2004). Furthermore, the Org 25935-induced locomotor activity in mice can be reversed by MK-801. Accordingly, we hypothesized that Org 25935 would attenuate the psychotomimetic effects of ketamine in healthy human volunteers.

A preliminary open-label, non-placebo-controlled phase, dose-escalation study was conducted to establish the highest dose of Org 25935 that could be safely administered in the ketamine paradigm. In this preliminary study, two of the six subjects studied received either a single dose of 4 8, or 16 mg Org 25935, followed by ketamine infusion in an open, sequential, escalating dose design. The preliminary study showed that 16 mg Org 25935 pretreatment followed by ketamine infusion was safe and well tolerated.

## MATERIALS AND METHODS

### General Study Design

The effects of highest dose of Org 25935 (16 mg) and placebo pretreatment on the ketamine response was evaluated in 12 healthy male subjects in a randomized, counter-balanced, crossover design spread over two test days separated by 1 week.

### Setting

The study was conducted at VA Connecticut Healthcare System, West Haven, CT, USA.

### Approvals

The study was conducted with the approval of the Institutional Review Boards of VA Connecticut Healthcare System and Yale University School of Medicine, and the US Food and Drug Administration. The study was conducted in full accordance with the Good Clinical Practice Consolidated Guideline approved by the International Conference on Harmonization. Written informed consent was obtained from each subject before they underwent any study procedures or assessments.

### Sample

Male subjects deemed medically and psychiatrically healthy on the basis of an extensive screening process were included.

### Study Drugs

*Org 25935.* The 16 mg dose of Org 25935 was selected on its demonstrated safety in both other phase I studies and in a preliminary open-label, dose-escalation study, described earlier. This dose of Org 25935 has also been shown to result in a two- to threefold increase in CSF glycine levels in humans (data on file). Org 25935 manufactured by Organon NV was administered per oral in tablet form as were visually matching placebo tablets.

*Ketamine.* The dose of ketamine (0.23 mg/kg bolus over 1 min, followed by 0.58 mg/kg per h $\times$ 30 min, followed by 0.29 mg/kg per h $\times$ 69 min) is well within the range of doses that were used in previous studies and has been shown to be safe. This dosing paradigm was selected with the goal of being able to conduct a battery of assessments while ketamine blood levels were at steady-state levels.

### Schedule of Procedures

On each test day, subjects received Org 25935 or placebo, followed 2.5 h later by a ketamine bolus (0.23 mg/kg bolus over 1 min), followed by a constant infusion (0.58 mg/kg per h $\times$ 30 min, followed by 0.29 mg/kg per h $\times$ 69 min). There

was a wash-out of at least 7 days between test days. The timing of the ketamine infusion was selected to coincide with the peak effects of Org 25935. The subjects were fasting until the end of the ketamine infusion. Behavioral, cognitive, and ketamine and Org 25935 blood level data were obtained on each test day. The schedule of procedures is described in Supplementary Table 1 and the outcome measures are described in further detail in the Supplementary section.

### Behavioral and Subjective Measures

Psychotomimetic symptoms were measured using a version of the Positive and Negative Syndrome Scale (PANSS) (Kay *et al*, 1989) adapted for repeated measurements within a short time period (D'Souza *et al*, 2004). The items of the PANSS for passive social avoidance (N4) and active social avoidance (G16) were excluded because for both these items, the bases of rating are reports from primary care workers and family. Perceptual alterations were measured using the self and clinician administered subscales of the Clinician Administered Dissociative Symptoms Scale (CADSS) (Bremner *et al*, 1998), a scale consisting of 19 self-report items and 8 clinician-rated items rated from 0 (not at all) to 4 (extremely). The scale evaluates aspects of altered environmental perception, time perception, body perception, feelings of unreality, and memory impairment. The CADSS has been shown to be sensitive to the effects of a number of psychoactive drugs, including ketamine (Krystal *et al*, 1994). A number of feeling states, including 'high', 'anxious', 'drowsy', 'irritable', 'tired', 'hungry', and 'energetic', were measured using a Visual Analog Scales of Mood State (VAS). Ratings were conducted by experienced and trained research staff who participated in regular inter-rater reliability sessions led by the principal investigator. Intraclass correlation coefficients for the PANSS have been consistently $>0.85$.

### Cognitive Measures

Verbal learning was assessed using the Rey Auditory Verbal Learning Test (RAVLT), a 15 word list that is not semantically organized (Rey, 1958). Attention, executive function, spatial working memory, visual recognition memory, choice reaction time, and motor function were assessed using the Cambridge Neuroscience Test Automated Battery (CANTAB) (Sahakian and Owen, 1992). Intelligence quotient was measured as part of the CANTAB using the National Adult Reading Test. To reduce practice effects, subjects were trained on the CANTAB at screening.

### Neurochemical Measures

Ketamine, its major active metabolite norketamine, and Org 25935 were sampled at various time-points (Supplementary Table 1) and assayed to determine whether there are any pharmacokinetic interactive effects.

### Safety Assessments

Orientation to time, place, and person was assessed at the end of each study day using items from the Mini-Mental State Exam (Folstein *et al*, 1975) to document that subjects were at baseline at the time that they were discharged. Clinical safety included continuous monitoring of adverse events and routine clinical laboratory parameters, ECG, and vital signs.

A telephone check-up was performed one day after each test day and 1 week, 1 month, and 3 months after the last study drug intake. Furthermore, approximately 2 weeks after the last study, drug subjects were evaluated with a physical examination, adverse event questionnaire, vital signs, and 12-lead ECG recording, as well as a hematology, biochemistry, and urinalysis assessment.

### Statistical Analyses

Initially, data were examined descriptively using means, standard deviations, and graphs. Each outcome was assessed for normality visually by normal probability plots and histograms and Kolmogorov–Smirnov test statistics. All analyses were performed on an intent-to-treat basis—therefore, data from non-completers were also included in the analysis.

All PANSS variables were highly skewed and exhibited floor effects. These outcomes were analyzed using the nonparametric approach for repeated measures data by Brunner (2002), where the data were first ranked, and then fitted using a mixed-effects model with an unstructured variance–covariance matrix and *p*-values adjusted for ANOVA-type statistics (ATS). All other outcomes were sufficiently normal or successfully normalized by log transformation (eg, some VAS outcomes) as indicated in the results.

Linear mixed models were used to analyze normal outcomes. Each PANSS and CADSS model included treatment (Placebo *vs* Org) and time ($-175$, $-30$, $+5$, and $+180$ min) as within-subjects explanatory factors. Models for each VAS outcome included these same factors and a random subject effect. These models allowed for the testing of the treatment $\times$ time interaction. Similar models were used for blood ketamine levels measured at $-10$, $+30$, $+70$, and $+180$ min. As visible in the figures, although some outcomes (eg, PANSS, CADSS) were assessed over time, there was insufficient variance at any time-point, except at the 5-min post-ketamine infusion, the analysis was restricted to the peak change from baseline and included only treatment as a within-subjects variable. Learning measured by the RAVLT was analyzed in a model, including treatment and trial (1–5) as within-subjects factors and a random subject effect. The remaining RAVLT outcomes, and raw CANTAB outcomes, were assessed only once per test day and analyzed with treatment as a within-subjects variable. In each model described above, the best-fitting correlation structure was selected according to Akaike Information Criterion and Schwartz Bayesian criterion. Order effects were tested for, but as they were not present, were excluded from the model. Although power to detect carryover and order effects was limited, none was observed in any model. Ketamine area under the curve was used as a covariate in all analyses. Data were analyzed using SAS, version 9.1 (SAS Institute, Cary, NC). All results were considered statistically significant using the two-sided $\alpha=0.05$ threshold. In this report, *p*-values between 0.05 and 0.1 were considered trend

level significance. Given the preliminary nature of this study together with the limited sample size, we chose to report *p*-values unadjusted for multiple testing.

## RESULTS

In all, 20 subjects consented for the study, 18 were screened and 15 enrolled in the study. Of these, 12 subjects completed both test days. Three subjects prematurely terminated the study because they were unable to tolerate the psychotomimetic effects of ketamine following either Org 25935 administration (1 subject) or placebo administration (2 subjects). All data were used in the analysis. The demographics of the sample are described in Table 1.

### Ketamine and Norketamine Levels

Over time, ketamine ($F_{(2,64)} = 117.9$, $p < 0.001$) and norketamine ($F_{(2,64)} = 51.67$, $p < 0.001$) levels increased from baseline, reaching a peak around +70 min and then decreasing (Supplementary Table 2). There were no statistically significant differences in ketamine levels ($F_{(1,64)} = 2.98$, $p = 0.088$) or norketamine levels ($F_{(1,64)} = 0.17$, $p = 0.68$) between the active and placebo Org 25935 conditions. There were no treatment by time interactions on ketamine ($F_{(2,64)} = 0.68$, $p = 0.51$) or norketamine ($F_{(2,64)} = 0.23$, $p = 0.79$) levels.

**Table 1** Demographics

| | |
|---|---|
| *Race* | |
| Caucasian | 9 |
| Asian | 5 |
| Black | 1 |
| Native American | 0 |
| *Ethnicity* | |
| Hispanic or Latino | 1 |
| Not Hispanic or Latino | 14 |
| *Smoking status* | |
| Smokers | 3 |
| Nonsmokers | 12 |
| *Handedness* | |
| Right handed | 15 |
| Left handed | 0 |
| | **Mean (SD)** |
| Age (years) | 33.2 (9.05) |
| Education (years) | 17.13 (2.56) |
| Weight (kg) | 81.53 (8.59) |
| Height (m) | 1.79 (0.06) |
| BMI | 25.36 (2.23) |
| National Adult Reading Test (NART), IQ | 114.93 (4.5) |

### Org 25935 Levels

The concentration-*vs*-time curves were in good agreement with previously encountered exposure levels for Org 25935 (Supplementary Figure 1).

### PANSS

Org 25935 reduced the peak increase in ketamine-induced Total PANSS scores significantly ($F_{(1,11)} = 6.55$, $p = 0.02$) with an effect size (Cohen's *d*) of 0.71 (Figure 1; Supplementary Table 3). To rule out the possibility that Org 25935 reduction in Total PANSS scores was not merely related to a nonspecific sedating effect of Org 25935, *post-hoc* analysis of Total PANSS was conducted adjusting for the peak change from baseline of VAS 'drowsy'. The effect of Org 25935 persisted despite adjusting for sedation ($F_{(1,11)} = 5.47$, $p = 0.039$).

Org 25935 trended ($F_{(1,11)} = 4.12$, $p = 0.067$) to reduce the peak increase in PANSS Positive Symptoms Subscale scores, and significantly decreased ($F_{(1,11)} = 7.41$, $p = 0.019$) scores on the PANSS General Symptoms Subscale, but not the PANSS Negative Symptoms Subscale ($F_{(1,11)} = 0.05$, $p = 0.83$).

### Perceptual Alterations

Org 25935 reduced the peak increase in CADSS clinician-rated scores ($F_{(1,11)} = 13.23$, $p = 0.0039$) with an effect size (Cohen's *d*) of 0.98 (Figure 1; Supplementary Table 3). To rule out the possibility that the decrease in ketamine-induced perceptual alterations by Org 25935 was not merely related to a nonspecific sedating effect of Org 25935, *post-hoc* analysis of the CADSS clinician-rated scores was conducted adjusting for the peak change from baseline of VAS 'drowsy'. The effect of Org 25935 persisted despite adjusting for sedation ($F_{(1,11)} = 11.21$, $p = 0.0065$). Org 25935 did not have any effect on ketamine-induced subject-rated CADSS scores ($F_{(1,14)} = 0.25$, $p = 0.62$).

### Feeling States

The results of the analysis of feeling states are presented in Table 2 (raw data in Supplementary Table 4). VAS scores of 'drowsy' ($F_{(1,89)} = 7.69$, $p = 0.0068$) and 'irritable' ($F_{(1,89)} = 4.2$, $p = 0.043$) were significantly higher on the active *vs* placebo Org 25935. There were no significant differences in any of the other VAS feeling states between the active *vs* placebo Org 25935. VAS scores of 'talkative,' 'happy,' 'energetic,' 'calm,' 'anxious,' 'fearful,' and 'hungry' decreased over time (ketamine effect), whereas VAS scores of 'high' and 'drowsy' increased over time. There were interactive effects of Org 25935 and time on VAS 'talkative' scores ($F_{(1,89)} = 2.65$, $p = 0.053$) that trended towards significance with *post-hoc* analysis revealing a significant effect at the +5 min time-point ($F_{(1,89)} = 4.71$, $p = 0.032$). There were significant interactive effects of Org 25935 and time on VAS 'happy' scores ($F_{(1,89)} = 3.08$, $p = 0.031$), with *post-hoc* analysis revealing a significant effect at the −30 min time-point ($F_{(1,89)} = 5.7$, $p = 0.019$). There were significant interactive effects of Org 25935 and time on VAS 'high' scores ($F_{(1,89)} = 2.99$, $p = 0.035$), with *post-hoc* analysis revealing a significant effect at the baseline (−175 min)

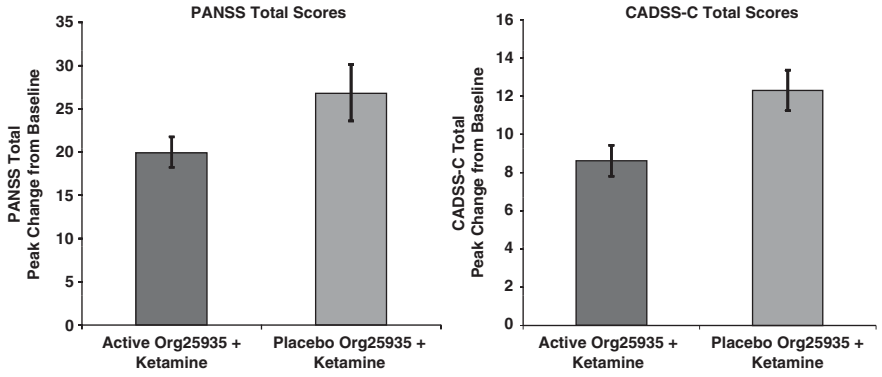


**Figure 1** Org 25935 effects on ketamine-induced psychotomimetic effects.

time-point ($F_{(1,89)} = 5.47$, $p = 0.021$). There were no significant interactive effects of Org 25935 and time on any other feeling states' (Figure 2).

### Cambridge Neuroscience Test Automated Battery

The results of the analysis of the cognitive battery are presented in Table 3. Except for choice reaction time that trended to be faster ($F_{(1,10)} = 3.38$, $p = 0.09$) in the active *vs* the placebo Org 25935 condition, there were no significant differences on performance in active *vs* placebo Org 25935 on the rapid visual information processing task, the spatial working memory task, the delayed match to sample task, and the Stocking of Cambridge task.

### Verbal Learning

Subjects recalled more words with each successive trial ($F_{(4,102)} = 58$, $p < 0.001$), and while Org 25935 treatment did not have any significant effects, there was a significant interaction between Org 25935 and trial 5 ($F_{(4,102)} = 2.47$, $p < 0.049$) (Figure 3). *Post-hoc* analyses revealed that relative to the placebo condition, subjects recalled fewer words on the 5th trial on the Org 25935 condition ($F_{(1,102)} = 3.83$, $p < 0.053$), which trended towards significance. There were no significant effects of Org 25935 on total free recall. However, Org 25935 significantly impaired both delayed free recall ($F_{(1,10)} = 8.37$, $p < 0.016$) and delayed recognition recall ($F_{(1,10)} = 7.7$, $p < 0.019$), with subjects recalling fewer words in the Org 25935 condition than the placebo condition.

*Post-hoc* analyses were conducted using the peak change from baseline of VAS 'drowsy' scores as a covariate. The effect of Org 25935 on immediate recall ($F_{(4,102)} = 2.47$, $p < 0.049$), delayed free recall ($F_{(1,10)} = 13.62$, $p < 0.004$), and delayed recognition recall ($F_{(1,10)} = 7.08$, $p < 0.023$) persisted despite adjusting for sedation. This suggests that there is no (linear) relationship between drowsiness peak scores and cognitive task results.

### Safety Results

There were no differences in the end of test day Mini-Mental State Exam scores across the two test days ($F_{(1,12)} = 0.12$, $p = 0.73$). There were no serious adverse events associated with this study. Three subjects prematurely terminated the study due to mild-to-moderate adverse events (discomfort, drug intolerance, disturbance in attention, and/or feeling abnormal) during ketamine infusion following either Org 25935 administration (1 subject) or placebo administration (2 subjects). All of these events were considered to be probably related to the administration of ketamine. Moderate adverse events were only reported in the placebo condition (3 subjects). About 85% of subjects experienced some adverse events after administration of Org 25935, and 53% experienced adverse events after placebo treatment. Ketamine-related adverse events were reported by 39% after Org 25935–ketamine and 53% after placebo–ketamine treatment. Ketamine-related adverse events included disturbance in attention (1 subject), nausea (1 subject), discomfort (1 subject), drug intolerance (2 subjects), feeling abnormal (1 subject), dizziness (1 subject), and increased blood pressure (3 subjects). Further events, for which a relationship to ketamine could not be completely ruled out, included blurred vision, dizziness, headache, somnolence, fatigue, asthenia, and sluggishness.

Org 25935-related adverse events mainly included visual symptoms and CNS effects. The percentage of subjects with such adverse events was higher after Org 25935 treatment compared with placebo treatment (77 *vs* 7% and 54 *vs* 20%, respectively). Visual symptoms included single mild cases of metamorphopsia and visual impairment as well as blurred vision. CNS effects included mild dizziness, headache, and somnolence. No clinically significant abnormal routine laboratory result was observed during this study. There were no clinically relevant changes in vital signs or ECG parameters during this study. Finally, at the face-to-face safety assessment 2 weeks post-study and the telephone evaluations 1 week, 1 month, and 3 months post-study, there was no evidence of any negative outcomes.

## DISCUSSION

To our knowledge this is the first study in humans, demonstrating that pretreatment with a GlyT1 inhibitor significantly attenuates the effects of the NMDA receptor antagonism.

### Implications for Psychosis and Cognition

Consistent with other studies, ketamine produced psychotomimetic effects captured by the PANSS and Clinician

**Table 2** Feeling States (Adjusted for Plasma Ketamine Levels)

| | Effect | Num df | Den df | F value | *p*-Value | *Post-hoc* test |
|---|---|---|---|---|---|---|
| 'Talkativeness' | Org 25935 | 1 | 89 | 2.04 | 0.16 | Org 25935 effect at time +5 min |
| | Time | 3 | 89 | 4 | 0.01 | ($F_{(1,89)} = 4.71$, $p = 0.03$) |
| | Org 25935 × Time | 3 | 89 | 2.65 | 0.05 | |
| 'Happy'[a] | Org 25935 | 1 | 89 | 0.92 | 0.34 | Org 25935 effect at time −30 min |
| | Time | 3 | 89 | 4.83 | 0.004 | ($F_{(1,89)} = 5.7$, $p = 0.02$) |
| | Org 25935 × Time | 3 | 89 | 3.08 | 0.031 | |
| 'High'[a] | Org 25935 | 1 | 89 | 0.03 | 0.86 | Org 25935 effect at time −175 min |
| | Time | 3 | 89 | 36.62 | <0.0001 | ($F_{(1,89)} = 5.47$, $p = 0.0215$) |
| | Org 25935 × Time | 3 | 89 | 2.99 | 0.04 | |
| 'Energetic' | Org 25935 | 1 | 89 | 0.91 | 0.34 | |
| | Time | 3 | 89 | 6.27 | 0.0007 | |
| | Org 25935 × Time | 3 | 89 | 1.73 | 0.17 | |
| 'Tired'[a] | Org 25935 | 1 | 89 | 2.38 | 0.13 | Org 25935 effect at time +5 min |
| | Time | 3 | 89 | 2.27 | 0.086 | ($F_{(1,89)} = 3.64$, $p = 0.059$) |
| | Org 25935 × Time | 3 | 89 | 1.11 | 0.35 | |
| 'Drowsy' | Org 25935 | 1 | 89 | 7.69 | 0.007 | Org 25935 effect at time −30 min |
| | Time | 3 | 89 | 2.86 | 0.041 | ($F_{(1,89)} = 13.8$, $p = 0.0004$) |
| | Org 25935 × Time | 3 | 89 | 1.82 | 0.15 | Org 25935 effect at time +5 min ($F_{(1,89)} = 3.32$, $p = 0.07$) |
| 'Tense'[a] | | | No significant effects | | | |
| 'Sad'[a] | | | No significant effects | | | |
| 'Depressed'[a] | | | No significant effects | | | |
| 'Calm' | Org 25935 | 1 | 89 | 1.35 | 0.25 | |
| | Time | 3 | 89 | 3.14 | 0.03 | |
| | Org 25935 × Time | 3 | 89 | 0.6 | 0.62 | |
| 'Anxious'[a] | Org 25935 | 1 | 89 | 0 | 0.97 | |
| | Time | 3 | 89 | 9.6 | <0.0001 | |
| | Org 25935 × Time | 3 | 89 | 0.69 | 0.56 | |
| 'Fearful'[a] | Org 25935 | 1 | 89 | 0.34 | 0.56 | |
| | Time | 3 | 89 | 3.85 | 0.012 | |
| | Org 25935 × Time | 3 | 89 | 0.13 | 0.94 | |
| 'Mellow' | | | No significant effects | | | |
| 'Angry'[a] | | | No significant effects | | | |
| 'Mania'[a] | | | No significant effects | | | |
| 'Irritable'[a] | Org 25935 | 1 | 89 | 4.2 | 0.04 | |
| | Time | 3 | 89 | 1.74 | 0.16 | |
| | Org 25935 × Time | 3 | 89 | 0.26 | 0.85 | |
| 'Hungry' | Org 25935 | 1 | 89 | 0.04 | 0.85 | |
| | Time | 3 | 89 | 13.97 | <0.0001 | |
| | Org 25935 × Time | 3 | 89 | 0.64 | 0.59 | |

[a]Analysis conducted on log-transformed data.

Administered Dissociative Symptoms Scale (Anand *et al*, 2000; Krystal *et al*, 1998a, 1999, 2004, 2005a). Ketamine also increased VAS 'high' scores similar to other studies (Krystal *et al*, 1998b). Org 25935 reduced ketamine-induced increases in Total PANSS, Positive Symptoms subscale score, and General Symptoms subscale score, but not Negative Symptoms subscale scores. Org 25935 also reduced CADSS Clinician-Rated subscale scores. The effects of Org 25935 in reducing ketamine-induced psychotomimetic effects cannot be explained by differences in ketamine levels or

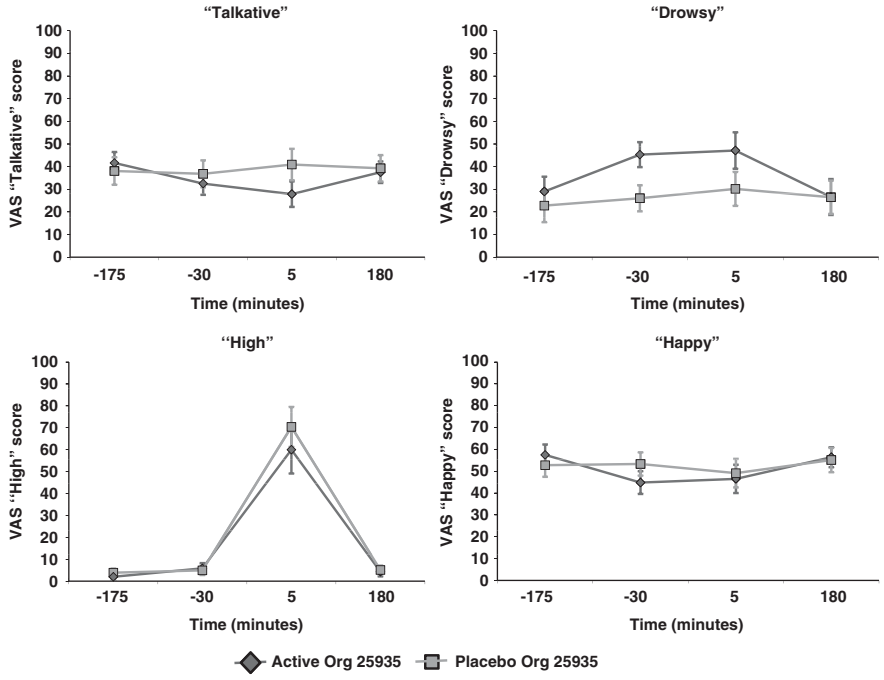


**Figure 2** Org 25935 effects on ketamine-induced subjective effects.

**Table 3** Effects on Cognition (CANTAB) (Adjusted for Plasma Ketamine Levels)

| Task | Outcome variable | Org 25935 (mean+(SD)) | | Num df | Den df | F value | *p*-Value |
|---|---|---|---|---|---|---|---|
| | | Placebo | Active | | | | |
| Choice reaction time | | 362.49 (51.89) | 336.74 (47.1) | 1 | 10 | 3.38 | 0.095 |
| Rapid visual processing | A′ | 0.97 (0.021) | 0.98 (0.015) | 1 | 10 | 1.12 | 0.31 |
| | B′ | 0.6 (0.72) | 0.70 (0.58) | 1 | 10 | 0.61 | 0.45 |
| Spatial working memory | Between errors | 15 (14.63) | 12.92 (15) | 1 | 10 | 1.1 | 0.32 |
| Stocking of Cambridge (5 moves) | Problems solved in minimum moves | 5.85 (1.46) | 5.83 (1.11) | 1 | 10 | 0.01 | 0.94 |
| | Mean initial thinking time | 6444 (3529) | 7263 (2745) | 1 | 10 | 0.91 | 0.36 |
| | Mean no. of moves | 6.77 (1.69) | 6.83 (1.2) | 1 | 10 | 0.01 | 0.92 |
| Delayed match to sample | Total correct | 14.54 (1.45) | 14.75 (0.97) | 1 | 10 | 0.01 | 0.92 |

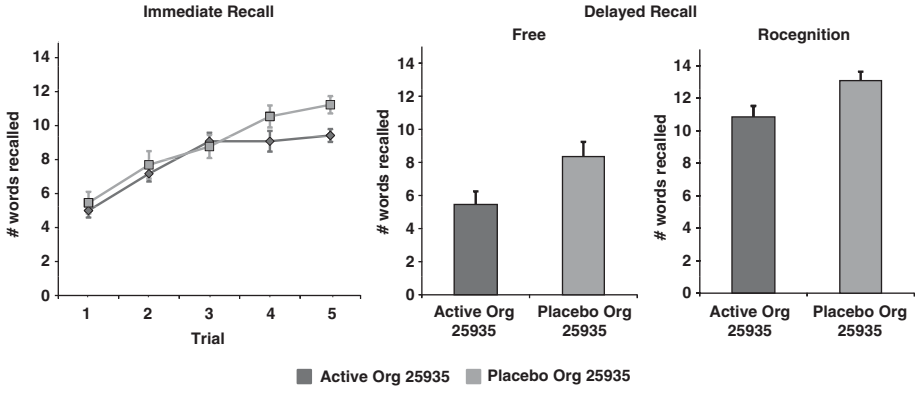


**Figure 3** Org 25935 effects on verbal memory (AVLT).

nonspecific sedating effects of Org 25935, as these effects survived adjustment for ketamine levels and Org 25935-induced sedation, respectively. Furthermore, the magnitude of the effects of Org 25935 on ketamine-induced psychotomimetic effects was moderate to large. These findings provide preliminary support for testing GlyT1 inhibitors in

the treatment of the positive symptoms and perceptual alterations associated with psychotic disorders.

PANSS negative symptom subscale scores were lower on the active *vs* the placebo Org 25935 condition, but the differences were not statistically significant. The lack of statistically significant effects of Org 25935 on ketamine-induced negative symptoms in healthy subjects contrasts with a recent report that the addition of RG1678, a GlyT1 inhibitor, to atypical antipsychotic treatment resulted in an improvement of negative symptoms in schizophrenia patients (Umbricht, 2010). However, it should be noted that there are important differences in the design (single dose *vs* multiple chronic dosing), samples (healthy volunteers *vs* schizophrenia patients), and target (ketamine-induced symptoms *vs* schizophrenia-related negative symptoms) between the studies.

Contrary to the study hypothesis, Org 25935 impaired verbal learning and delayed recall. This is important if Org 25935 is to be developed as a treatment for schizophrenia, as verbal memory is already impaired in schizophrenia. This effect seemed specific to verbal learning, because Org 25935 did not affect performance on other measures of memory, for example, visual recognition memory and spatial working memory, assessed by the CANTAB.

There are several possible explanations for why Org25925 worsened verbal memory. First, there appears to be an inverted 'U' dose-shaped regulation of NMDAR function in the hippocampus (Zhang *et al*, 2008b), such that elevation of glycine levels beyond a certain point might actually decrease NMDAR activity. Consistent with an inverted 'U' response, other studies have shown that high doses of glycine impair prepulse inhibition (O'Neill *et al*, 2010) and also reduce mismatch negativity amplitude (Leung *et al*, 2008) in humans. Second, in the hippocampus, glycine can modulate hippocampal neurotransmission by effects on excitatory strychnine-insensitive NMDARs (MacDonald *et al*, 2006; Mohler *et al*, 2008) and inhibitory strychnine-sensitive glycine receptors (GlyRs) (Becker *et al*, 1993; Chattipakorn and McMahon, 2003; Malosio *et al*, 1991; Racca *et al*, 1998; Thio *et al*, 2003). In fact, Zhang *et al* (2008a) showed that GlyT1 inhibition induced depression of hippocampal excitatory post-synaptic potential spike potentiation, a phenomenon relevant to learning and memory. Extrapolating these preclinical data to the current study, it is possible that a consequence of increasing glycine in the hippocampus might include a disruption in the balance of inhibitory and excitatory influences mediated by GlyR and NMDAR, respectively, leading to a disruption of input–output functions that in turn could impair learning and memory. A third possibility may be related to differences in the response of different subunit compositions of NMDAR to glycine. Extracellular glycine has been shown to differentially activate NR2A- or NR2B-containing NMDARs, which may lead to variable physiological consequences (Kohr, 2006; Liu *et al*, 2004; Loftis and Janowsky, 2003). Thus, it is conceivable that Org 25935-induced increases in glycine levels might have different effects in different brain regions based on the subunit composition of NMDARs.

There are several explanations for the absence of robust beneficial effects of Org 25935 on the ketamine-induced cognitive deficits in this study. It is unclear whether the dose used was optimal for enhancing cognitive function. While Org 25935 is procognitive in several animal models of cognition such as the NOR, there appears to be an inverse dose–response where robust effects were seen at lower doses, but this response was abolished at higher doses (Snigdha *et al*, 2007). Similarly, in the recent clinical trial with RG1678, a loss of efficacy was observed at higher doses (Umbricht, 2010). Furthermore, preclinical studies suggest that partial (50%) occupancy of brain GlyT1 is necessary to obtain efficacy in behavioral models relevant to schizophrenia (Alberati *et al*, 2011). This study selected the highest dose of Org 25935 that was tested in a preliminary study; therefore, the optimal dose for Org 25935 to have procognitive and other effects cannot be determined. Interestingly, in contrast to the findings of the current study, Roberts *et al* (2010) showed that another GlyT1 (PF-3463275) reduced the spatial working memory deficits induced by ketamine, but not the perceptual alterations induced by ketamine in non-human primates. These contrasting effects raise the possibility that the antipsychotic-like and procognitive effects of GlyT1 inhibitors may be dissociable.

The effects of Org 25935 contrast with the effects of other drugs that have been tested in the ketamine paradigm in healthy human subjects. Pretreatment with glycine (0.1–0.2 g/kg i.v.) failed to attenuate the psychotomimetic effects of ketamine, although initial analyses suggested that it reduced ketamine-induced memory impairments ($n = 38$) (DC D'Souza *et al*, unpublished observations). The fact that large doses of glycine did not block the effects of ketamine and did not produce any visual disturbances, in contrast to Org 25935, suggests that the doses of glycine used did not increase central glycine levels sufficiently. Indeed, one of the limitations of glycine is its poor central bioavailability. Similarly, the effects of Org 25935 on the ketamine response contrast with pretreatment with the benzodiazepine lorazepam (Krystal *et al*, 1998a), the dopamine D2 receptor antagonist haloperidol (Krystal *et al*, 1999), the group II metabotropic glutamate receptor (mGluR) agonist LY354740 (Krystal *et al*, 2004), and nicotine (D'Souza *et al*, 2010). Pretreatment with lamotrigine significantly decreased ketamine-induced psychotomimetic effects, but in contrast to Org 25935, it also reduced ketamine-induced verbal learning and memory impairment ($n = 16$) (Anand *et al*, 2000). Lamotrigine increased the mood-elevating effects of ketamine. The mechanism by which lamotrigine attenuates the effects of ketamine is related to its inhibition of glutamate release. Taken together, only lamotrigine and Org 25935 appear to reduce the psychotomimetic effects of ketamine, even though they have distinct mechanisms of actions.

## Mechanism

This study does not provide insight into the mechanisms underlying the capacity of Org 25935 to attenuate some of the effects of ketamine. The increase in extracellular glycine by GlyT1 inhibition may activate silent receptors and in doing so overcome the effects of those NMDA receptors that are blocked by ketamine. Alternatively, increased glycine levels may increase the 'off rate' for NMDA receptor antagonists (Priestley and Kemp, 1994), or increased levels of glycine may result in internalization of NMDA receptors rendering fewer receptors available for ketamine to bind to.

### Safety

At doses that have been shown to increase brain glycine levels substantially, Org 25935 did not have significant effects on a number of feeling states and was well tolerated. Furthermore, the observation that pretreatment with Org 25935 did not enhance many of ketamine effects suggests that Org 25935 pretreatment is not making more intra-channel sites accessible to ketamine. If that were the case, one would expect an increase in most effects of ketamine.

### Strengths and Limitations

The double-blind, randomized, placebo-controlled within-subjects design, and the use of a well-characterized paradigm (ketamine) are strengths of this study. The use of a bolus and constant infusion paradigm allowed a battery of assessments to be conducted, while ketamine blood levels were at steady-state levels. However, the use of a single dose of Org 25935 did not permit testing to be conducted with Org 25935 at steady-state levels. The use of only one dose level of Org 25935 did not permit detection of a dose–response. The study was limited to men and the results may not generalize to women who show subtle reductions in ketamine response compared to men (Morgan *et al*, 2006). The power to uncover potentially important effects was limited by the small sample size. Furthermore, in contrast to the behavioral and subjective measures, the cognitive measures were captured only once per test day. As there was no placebo ketamine condition, the effects of ketamine alone cannot be determined in the current study design. However, the effects of ketamine on the PANSS, CADSS, VAS, and memory are well known from many previous studies (Anand *et al*, 2000; Krupitsky *et al*, 2001; Krystal *et al*, 1994, 1998a, 1998b, 1999, 2005b; Lahti *et al*, 2001; Malhotra *et al*, 1996). The study design also makes it difficult to ascertain the effects of Org 25935 alone. Many of these limitations can be addressed in a large sample follow-up study with a $2 \times 2$ design. Finally, while laboratory studies in healthy human subjects may be informative, the therapeutic efficacy of a drug should ultimately be tested in the target population.

### Conclusions and Future Directions

In this preliminary study, the novel GlyT1 inhibitor, Org 25935, reduced the psychotomimetic effects of ketamine, indicating that the facilitation of NMDA transmission by raising endogenous glycine levels may be effective in the treatment of psychosis and perceptual alterations associated with hypoglutamatergic states. Furthermore, the reduction of NMDA receptor antagonist effects by Org 25935 may also be relevant to other conditions, including alcohol-related disorders. The results of this study support future studies with larger samples, multiple doses, and more proximal measures of brain function, followed by or in parallel with a clinical trial in the target population.

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