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ORIGINAL ARTICLE

Transiently increased glutamate cycling in rat PFC is associated with rapid onset of antidepressant-like effects

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Several drugs have recently been reported to induce rapid antidepressant effects in clinical trials and rodent models. Although the cellular mechanisms involved remain unclear, reports suggest that increased glutamate transmission contributes to these effects. Here, we demonstrate that the antidepressant-like efficacy of three unique drugs, with reported rapid onset antidepressant properties, is coupled with a rapid transient rise in glutamate cycling in the medial prefronal cortex (mPFC) of awake rats as measured by *ex vivo* ^{1}H-[^{13}C]-nuclear magnetic resonance spectroscopy. Rats were acutely pretreated by intraperitoneal injection with a single dose of ketamine (1, 3, 10, 30 and 80 mg kg^{-1}), Ro 25-6981 (1, 3 and 10 mg kg^{-1}), scopolamine (5, 25 and 100 μg kg^{-1}) or vehicle (controls). At fixed times after drug injection, animals received an intravenous infusion of [1,6-$^{13}C_2$]glucose for 8 min to enrich the amino-acid pools of the brain with ^{13}C, followed by rapid euthanasia. The mPFC was dissected, extracted with ethanol and metabolite ^{13}C enrichments were measured. We found a clear dose-dependent effect of ketamine and Ro 25-6981 on behavior and the percentage of ^{13}C enrichment of glutamate, glutamine and GABA (γ-aminobutyric acid). Further, we also found an effect of scopolamine on both cycling and behavior. These studies demonstrate that three pharmacologically distinct classes of drugs, clinically related through their reported rapid antidepressant actions, share the common ability to rapidly stimulate glutamate cycling at doses pertinent for their antidepressant-like efficacy. We conclude that increased cycling precedes the antidepressant action at behaviorally effective doses and suggest that the rapid change in cycling could be used to predict efficacy of novel agents or identify doses with antidepressant activity.

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INTRODUCTION

A growing number of studies are now reporting that a novel class of antidepressant drugs is capable of inducing a rapid antidepressant response in patients with previously treatment-resistant mood disorders.[1] The strongest evidence of this rapid-acting antidepressant (RAAD) effect has been established for ketamine, a non-selective *N*-methyl-D-aspartate receptor (NMDAR) antagonist.[2] However, RAAD effects were also reported for other NMDAR targeting drugs, such as lanicemine, the low trapping non-subunit-selective NMDA receptor channel blocker,[3] and CP-101 606,[4] an NR2B-selective receptor antagonist. In addition, there is now evidence suggesting that the anticholinergic drug scopolamine (Scop) also possesses RAAD-like effects.[5]

Other findings suggest that a transient activation of AMPA receptors is a necessary event in the generation of the RAAD effects induced by NMDAR antagonists and Scop in rodent models.[6–9] In light of earlier work showing ketamine's ability to rapidly increase glutamate efflux in the frontal cortex of the rat,[10] possibly due to effects on inhibitory GABAergic interneurons,[11] it has been hypothesized that this brief ketamine-induced surge in glutamate release is a critical event for the RAAD activity. However, the question remains whether this surge in glutamate release is a mechanism common to other drugs with RAAD-like properties, and whether the increase in glutamate release is related to the more durable antidepressant-like behavioral effects of these RAAD drugs.

Having previously found that subanesthetic doses of ketamine led to rapid increases in glutamate, GABA (γ-aminobutyric acid) and glutamine cycling,[12] and considering previous reports of inverted-U-type dose–response relationships between ketamine-induced effects on glutamate efflux,[10] as well as antidepressant-like behavioral responses and cellular changes in rats,[6] we first sought to confirm and extend the dose–response relationship between RAAD properties of ketamine and glutamate cycling. We next sought to determine the time dependence of ketamine's effect on glutamate/GABA-glutamine cycling in the medial prefrontal cortex (mPFC) of rats. Last, to determine whether the increase in glutamate cycling is generalizable to drugs of other classes with RAAD properties, we investigated the effects of Ro 25-6981, an NR2B-selective NMDAR antagonist, and Scop, a muscarinic receptor M1 antagonist[13] that has been shown to require AMPA activation to produce the antidepressant-like effects in rodents,[9] on both cycling and behavior.

^{1}H-[^{13}C]-nuclear magnetic resonance (NMR) spectroscopy was used to examine the effects of the drugs on glutamate release in

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the mPFC of rats and recycling into glia (glutamate cycling), and neuronal and glial energy metabolism. ^{13}C-labeled *glucose* is metabolized mainly in the neuronal TCA cycle and labels neuronal glutamate and GABA, which are released and taken up by astrocytes, followed by conversion to (and labeling of) glutamine, suggesting that ^{13}C-labeled glucose studies provide information on glutamate and GABA neurotransmitter cycling as well as neuronal (mainly) and glial (partly) cell metabolism, reflecting the neurotransmitter activity[12,14,15] (see Supplementary Figure S1). As several studies have specifically demonstrated increased rates of mPFC metabolism in rodents following treatment with subanesthetic doses of ketamine,[16–19] and similar regions are believed to mediate several of ketamine's behavioral effects,[20–22] all studies were performed in rat mPFC.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Charles River, Raleigh, NC, USA) weighing 180–220 g were group-housed and maintained under standard conditions of constant temperature (25 °C) and humidity with a 12-h light/dark cycle, with *ad libitum* access to food and water. All experiments were conducted in accordance with the National Institutes of Health guidelines and the protocols approved by the Yale University Animal Care and Use Committee.

Plasma ketamine measures

Separate groups of male rats (170–200 g at delivery; $n = 4$ per group) were dosed intraperitoneally with ketamine (1, 3, 10, 30 and 80 mg kg^{-1}) and plasma samples were collected at time points corresponding to the midpoint of glucose utilization determinations in the study. Plasma aliquots (50 µl) were treated with acetonitrile (200 µl) followed by vortex mixing for 2 min. The supernatant was then separated from the precipitated proteins by centrifugation (10 min at 1479 *g*), and 150 µl was transferred to a 96-well plate. An aliquot of supernatant (5 µl) was injected onto the ultra-high-performance liquid chromatography column for liquid chromatography/mass spectrometry/mass spectrometry-based analysis.

NMR experiments

Dose–response studies of ketamine, Ro 25-6981 and Scop. Rats (6–8 per group) were treated with different doses of ketamine reported to have antidepressant behavioral efficacy as well as lower and higher doses (1, 3, 10, 30 and 80 mg kg^{-1}),[6,8] diluted in either saline (0.9%) or vehicle (vehicle) 10 min before the beginning of [1,6-$^{13}C_2$]glucose infusion. Ro 25-6981 (supplied by BMS) dissolved in vehicle was tested at doses of 1, 3 and 10 mg kg^{-1} intraperitoneally, based on previous reports of behavioral efficacy,[6,23,24] alongside the ketamine studies at 1, 30 and 80 mg kg^{-1}. Effects of all treatments were compared with unique control groups receiving intraperitoneal infusions of saline as described previously[12] (for the ketamine 3 and 10 mg) and vehicle (for ketamine 1, 30 and 80 mg kg^{-1} and Ro 25-6981 1, 3 and 10 mg kg^{-1}) as Ro 25-6981 was not soluble in saline. The vehicle consisted of 5% dimethyl sulfoxide (DMSO, Sigma, St Louis, MO, USA)/10% propylene glycol/0.0375% methylcellulose/70% water. Scop (Sigma) 25 µg kg^{-1} was studied based on behavioral findings and previous reports of behavioral efficacy.[9] All drugs were administered intraperitoneally in volumes of 2 ml kg^{-1}, with the exception of the highest ketamine dose (80 mg kg^{-1}), which was 3 ml kg^{-1}.

Time dependence of single-dose ketamine effects. Rats (six rats per time point) received a single injection of ketamine (30 mg kg^{-1}, intraperitoneally) at 0, 10, 30 or 60 min before [1,6-$^{13}C_2$]glucose infusion. The 30 mg kg^{-1} dose of ketamine was chosen because it had the largest metabolic effect in the dose–response study. The effects of a single injection of ketamine (10 mg kg^{-1}, intraperitoneally) at 24 h was also measured to match the dosing previously shown to have significant effects on dendritic spine density at 24 h.[6,25]

[1,6-$^{13}C_2$]glucose infusions. Tail vein catheters were placed under brief isoflurane anesthesia and animals were allowed to recover for at least 30 min before drug/vehicle injections. Ten minutes after injection of ketamine, 7 min after injection of Scop and 30 min after injection of Ro 25-6981 or vehicle (times determined by the Cmax and onset of observable behavioral changes with each compound), a solution of [1,6-$^{13}C_2$]glucose (99 atom%; Cambridge Isotopes, Andover, MA, USA) dissolved in water (0.75 mol l^{-1} per 200 g body weight) was infused for 8 min as described previously.[12] Immediately following the 8 min infusion of [1,6-$^{13}C_2$]glucose, the rats were quickly killed using focused-beam microwave irradiation as described in the Supplementary Material.

Tissue extraction and NMR sample preparation. Metabolites in the mPFC were extracted from frozen tissue (65–85 mg) as described by Chowdhury *et al.*[12] (see Supplementary Material). Brain and plasma samples were loaded into 5 mm tubes for NMR analysis.

NMR spectroscopy analysis of ^{13}C incorporation. Total concentrations and ^{13}C enrichments of mPFC amino acids and metabolites and plasma glucose were determined from fully relaxed ^{1}H-[^{13}C]-NMR spectra acquired at 11.7 T (^{1}H frequency of 500.13 MHz; Bruker AVANCE; Bruker Instruments, Billerica, MA, USA) as described in the Supplementary Material.

Behavioral tests

Drug administration for behavioral tests. Animals received a single intraperitoneal injection of vehicle (saline and/or DMSO); ketamine at 3, 30 and 80 mg kg^{-1}; Ro 25-6981 at 3 and 10 mg kg^{-1}; or Scop at 5, 25 and 100 µg kg^{-1}, 24 h after the first day of swimming. Ketamine and Scop were dissolved with saline (0.9%). Ro 25-6981 was dissolved in a solution mixed with saline: DMSO (v:v = 5.6:1). Animals for each drug treatment were tested for behavior using assays at different time points.

Forced swim test. Antidepressant effects of ketamine, Ro 25-6981 and Scop were assessed using the forced swim test (FST). Each rat was placed in the plexiglass cylinder (65 cm in height and 30 cm in diameter) filled with 24–25 °C water to a depth of 45 cm for 15 min on day 1 and 10 min on day 3. On day 3 and 24 h after the drug injections, animals were recorded from the side with a video camcorder. Time immobile was scored, separating two blocks of 5 min for analysis. Immobility was defined as minimum movement to stay afloat.

Stereotypy. Effects of ketamine, Ro 25-6981 and Scop on stereotypic behaviors were assessed immediately after the injection on day 2. Animals were placed in a new cage and videotaped from the side. Orofacial and aspecific stereotypy was scored by bins of 1 min every 5 min (i.e. minutes 5–6 and 10–11). Occurrence of each stereotypic behavior and not their frequency for each minute bin was scored. The stereotypy counts for each animal represent the sum of stereotypic occurrence for the five bins. Orofacial stereotypy included mouth movement, bite, self-gnaw, jaw tremor and aspecific head stereotypy including head bob, head sway and taffy pull.

Statistical analysis

The statistical significance of differences in concentrations and ^{13}C enrichments between control and ketamine, Ro 25-6981 or Scop in treated rats were assessed using analysis of variance (ANOVA) followed by Dunnett's multiple comparisons procedure where each treatment is compared with a single control group. Overall, ANOVA effects were considered significant at $P < 0.05$ and *post hoc* tests significant at $P_{adj} \leqslant 0.05$ using Dunnett's test. FST immobility times were separated into two bins (first 5 min and last 5 min), and were analyzed using linear models with treatment assignment included as a between-subjects factor and time as a within-subjects factor. The group by time interaction was modeled. Stereotypic behavioral outcomes were analyzed using one-way ANOVA followed by Dunnett's test.

RESULTS

Plasma levels for all doses of ketamine at each time point examined are shown in Supplementary Table 1. Glucose concentrations were similar in plasma samples from all groups (ranging from mean ± s.e.m.; 15.1 ± 1.0 to 17.8 ± 0.5 mmol l^{-1}, n = 6–8 per group). Similar results were found for the percent ^{13}C enrichments: ranging from mean ± s.e.m.; 45.7 ± 1.5 to 49.4 ± 2.4%). In addition, total levels of amino acids and metabolites were determined in the different groups by averaging

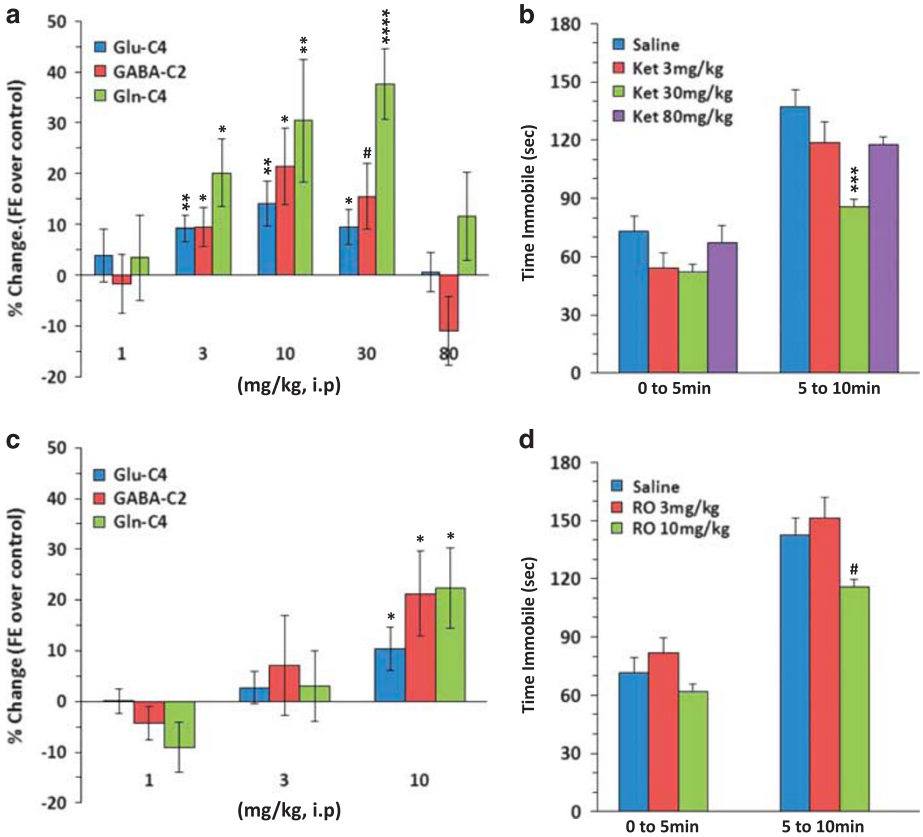

Figure 1. Effects of ketamine and Ro 25-6981 on medial prefronal cortex (mPFC) glutamate/γ-aminobutyric acid (GABA)-glutamine metabolism and forced swim test (FST) behavior. (**a**) Percent change in PFC glutamate-C4, GABA-C2 and glutamine-C4 fractional enrichments over an interval from 10 to 18 min after intraperitoneal doses of 1, 3, 10, 30 and 80 mg kg^{-1} compared with vehicle-injected animals. (**b**) Ketamine 30 mg kg^{-1} decreased average time immobile in the FST but not 3 and 10 mg kg^{-1}. The effect was more pronounced in the last 5 min of the test. (**c**) Percent change in PFC glutamate-C4, GABA-C2 and glutamine-C4 enrichments over an interval from 30 to 38 min after intraperitoneal doses of 1, 3 and 10 mg kg^{-1} Ro 25-6981 relative to vehicle-treated controls. (**d**) Averaged over the two blocks of 5 min, the Ro 25-6981 10 mg kg^{-1} animal group shows a trend for decreased time immobile with a more pronounced effect for the last 5 min ($^{\#}P_{unadj} = 0.05$; $P_{Bonferroni} = 0.1$). *Interrogation time* is the 8-min interval after ketamine injection during which the ^{13}C-glucose was infused (means+/-s.e.m.) ($^{\#}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$).

the respective [1,6-$^{13}C_2$]glucose infusion data; total mPFC concentrations of the metabolites examined were similar in all animal groups (see Supplementary Tables S1 and S2). These results indicate that changes in mPFC levels of the ^{13}C-labeled amino acids found in this study are not related to general metabolic alterations or changes in total amino-acid concentrations.

Dose–response effects of ketamine and Ro 25-6981 on rat mPFC glutamate/GABA-glutamine cycling

Figures 1a and c illustrate the dose–response relationships between the percent ^{13}C enrichment of treatment relative to controls for ketamine and Ro 25-6981, respectively. Lower doses of ketamine and Ro 25-6981 (1 mg kg^{-1} each) had no effect on ^{13}C-labeled incorporation into glutamate-C4 ($F(2,21) = 0.53$, $P = 0.60$), GABA-C2 ($F(2,21) = 0.35$, $P = 0.71$) or glutamine-C4 ($F(2,21) = 1.78$, $P = 0.19$) (Supplementary Figure S2A). At the mid-dose, there was a significant main effect of treatment on both glutamate-C4 ($F(2,20) = 3.5$, $P < 0.05$) and glutamine-C4 ^{13}C enrichment ($F(2,20) = 15.0$, $P < 0.0001$) (Supplementary Figure S2B). Specifically, ketamine at 30 mg kg^{-1} increased glutamate-C4 enrichment by 9% above vehicle ($P_{adj} < 0.05$), and increased glutamine-C4 enrichment by 38% above vehicle ($P_{adj} < 0.0001$). There was a trend suggesting that the mid-doses altered GABA-C2 enrichment ($F(2,20) = 2.68$, $P = 0.09$), with the 30 mg kg^{-1} dose of ketamine increasing enrichment by 22% ($P_{adj} = 0.06$). R0 6981 at 3 mg kg^{-1} did not show any effect on ^{13}C enrichment of any of the amino acids ($P_{adj} > 0.6$, for all). There was a significant main effect of treatment on the ^{13}C enrichment of all three amino acids, glutamate-C4 ($F(2,23) = 3.92$, $P < 0.05$), GABA-C2 ($F(2,23) = 8.16$, $P < 0.01$) and glutamine-C4 enrichment ($F(2,23) = 3.56$, $P < 0.05$) at the highest dose of the drugs tested (Supplementary Figure S2C). Here it was Ro 25-6981 10 mg kg^{-1} that significantly increased ^{13}C enrichment ($P_{adj} < 0.05$) of all three amino acids, whereas the higher anesthetic dose of ketamine (80 mg kg^{-1}) showed no effect on the enrichment ($P_{adj} \geqslant 0.3$, for all).

Two additional studies examining intermediate ketamine doses of 3 (Supplementary Figure S2D) and 10 mg kg^{-1} (Supplementary Figure S2E) demonstrated effects of treatment on the ^{13}C enrichment of glutamate-C4 ($P < 0.01$ for both), GABA-C2 ($P < 0.05$ for both) and glutamine-C4 ($P < 0.05$ and $P < 0.01$, respectively) compared with their respective saline control.

Dose–response effects of ketamine and Ro 25-6981 on behavior

We were further able to align the changes in cycling with the RAAD effects of ketamine by demonstrating that the measured antidepressant-like performances in the FST 24 h after the ketamine administration mirrors the dose-dependent effects on cycling (Figure 1b). A significant overall ketamine effect was observed ($F(3,25) = 5.08$, $P < 0.01$) owing to decreased average

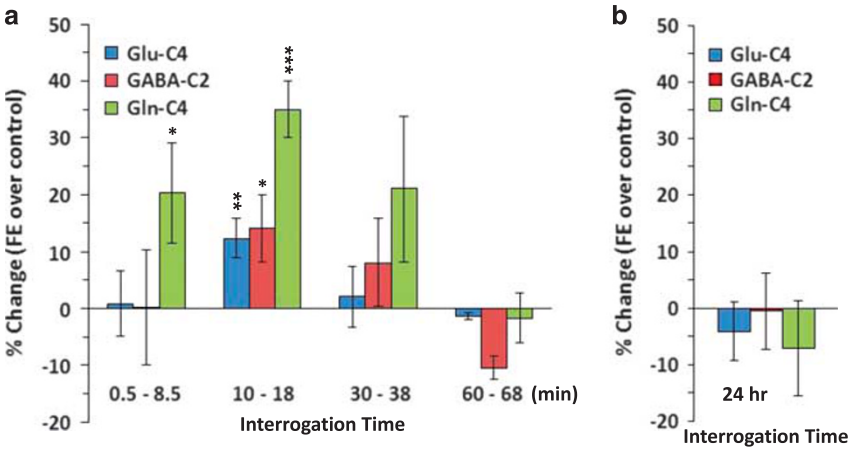

Figure 2. Effects of time after ketamine injection on glutamate, γ-aminobutyric acid (GABA) and glutamine ^{13}C labeling compared with control animals. (**a**) The figure depicts the percent change in fractional enrichment compared with vehicle-injected animals over time. Amino-acid labeling reflects the interval from 0.5 to 8.5 min, 10 to 18 min, 30 to 38 min and 60 to 68 min after intraperitoneal injection of ketamine 30 mg kg^{-1}. (**b**) There was no significant effect of a single 10 mg kg^{-1} intraperitoneal dose of ketamine on glutamate, GABA and glutamine ^{13}C labeling from ^{13}C-glucose when interrogated for 8 min at 24 h ($^{\#}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

time immobile in the FST at 30 mg kg^{-1} ($P_{adj} = 0.002$) but not 3 and 80 mg kg^{-1} (all $P_{adj} > 0.14$). There was a significant interaction between group and time ((F3,25) = 5.37, $P < 0.01$) where the observed ketamine 30 mg kg^{-1} effects were more pronounced during the last 5 min ($P_{Bonferroni} < 0.001$).

An ANOVA analysis of the effects of Ro 25-6981 in the FST test revealed a significant group effect (F(2,42) = 5.02, $P < 0.05$). Averaged over the two blocks of 5 min, Ro 25-6981 at 10 mg kg^{-1} animal group showed a trend difference from control ($P_{unadj} = 0.04$; $P_{adj} = 0.08$), with a more pronounced effect for the last 5 min ($P_{unadj} = 0.05$; $P_{Bonferroni} = 0.1$) (Figure 1d).

Time dependence of ketamine effect on glutamate/GABA-glutamine cycling in rat mPFC and behavior

To examine the duration of ketamine's effects on cycling, we chose to examine the effects of a single 30 mg kg^{-1} intraperitoneal injection of ketamine, the dose showing the largest magnitude of effect in the dose–response study above. Ketamine injection led to an elevation in glutamine-C4 ^{13}C enrichment over the 8-min interval from 0.5 to 8.5 min ($P < 0.05$), 10 to 18 min ($P < 0.001$) and 30 to 38 min (NS, $P = 0.17$) compared with vehicle-injected rats, but not the 60 to 68 min ($P = 0.81$) time point (Figure 2a). ^{13}C labeling of glutamate-C4 ($P < 0.01$) and GABA-C2 ($P < 0.05$) was significantly increased only at the 10 to 18 min time point (see Supplementary Figure 3 for individual ^{13}C fractional enrichments). Thus, this postinjection period of increased ^{13}C labeling of mPFC amino acids was transient, with a peak rise occurring in < 30 min and disappearing within 1 h.

There was no significant effect of a single 10 mg kg^{-1} intraperitoneal dose of ketamine (dose most frequently shown to have delayed behavioral effects in tests of antidepressant action) at 24 h on glutamate-C4, GABA-C2 and glutamine-C4 ^{13}C labeling from ^{13}C-glucose (Figure 2b and Supplementary figure 4 for individual fractional enrichments).

Scop effects on rat mPFC glutamate/GABA-glutamine behavior and cycling

We first examined the antidepressant-like effects of Scop in the FST using three doses. Scop decreased average time immobile in the FST at 25 μg kg^{-1} but not 5 and 100 μg kg^{-1}. There was a significant interaction between drug and time (F(3,48) = 4.03, $P < 0.05$), with a more pronounced effect with Scop 25 μg kg^{-1} during the last 5 min ($P_{unadj} = 0.007$; $P_{Bonferroni} = 0.04$) (Figure 3a).

A single 25 μg kg^{-1} intraperitoneal dose of Scop increased ^{13}C enrichment of glutamate-C4, GABA-C2 and glutamine-C4 over control from ^{13}C-glucose for all three amino acids ($P < 0.05$ for all) (Figure 3b and see Supplementary Figure 5 for individual fractional enrichments).

Effect of ketamine, Ro 25-6981 and Scop on orofacial and aspecific head stereotypy

The effect of rapid-acting antidepressant drugs on orofacial and head stereotypy is described in Supplementary Figure S6. Ketamine increased stereotypy counts within 30 min after injection; the effect was significant at the dose of 30 mg kg^{-1} ($P_{adj} < 0.05$) when compared with the vehicle-injected group. It is important to note that animals receiving ketamine at 80 mg kg^{-1} were practically asleep 20 min after the injection; however, they showed occurrence of more diverse stereotypic behaviors during the first few bins. In contrast, Ro 25-6981 injections had no effect on stereotypy, whereas Scop's slight numeric increase in stereotypic behaviors at the dose of 25 μg kg^{-1} was not significant.

DISCUSSION

To the best of our knowledge, this study provides the first experimental evidence directly suggesting that a transient surge in amino-acid neurotransmitter cycling is associated with the induction of RAAD-like effects. The study found evidence of inverted U-shaped dose–response relationship between ketamine and amino-acid neurotransmitter cycling. We also found similar evidence of an inverted U-shaped dose–response relationship between ketamine and immobility on the FST. These findings are generally similar to previously established dose–response relationships for ketamine-induced effects on glutamate efflux,[10] ketamine-associated antidepressant-like behavioral responses and a variety of accompanying cellular changes in rats.[6] The plasma levels obtained at the 14 min time point for 10 mg kg^{-1} ketamine dose are consistent with the levels reported in Li *et al.*[6] for the same intraperitoneal dose adjusted for time, and are in the similar range of the peak concentrations seen in the human studies dosing at 0.5 mg kg^{-1} (typically around 100–200 ng ml^{-1} at the 40 min time point, see Shaffer *et al*[26]). However, the 30 mg kg^{-1} dose of ketamine, which induces clear increases in labeling and antidepressant-like effects in the rats, produces plasma levels that are well above that typically seen with doses

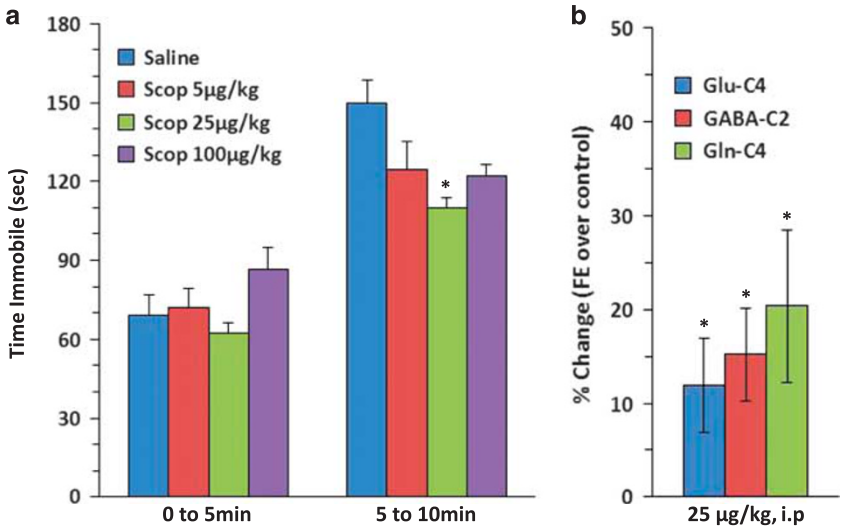

Figure 3. Effects of Scopolamine (Scop) on forced swim test (FST) immobility and glutamate, γ-aminobutyric acid (GABA) and glutamine ^{13}C labeling compared with control animals. (**a**) Scop decreased average time immobile in the FST at 25 µg kg^{-1} but not 5 and 100 µg kg^{-1}. Averaged over the two blocks of 5 min, there was a significant interaction between drug and time ($F3,48 = 4.03$, $P < 0.05$), with a more pronounced effect with Scop 25 µg kg^{-1} for the last 5 min (*$P_{Bonferroni} < 0.05$) when compared with the vehicle-injected animal group. (**b**) Effects of a single 25 µg kg^{-1} intraperitoneal dose of Scop on magnitude change of glutamate, GABA and glutamine ^{13}C labeling from glucose over control (*$P < 0.05$ when compared with vehicle-injected animal group).

used in the treatment of mood disorders, and more in line with anesthetic doses as reported by Domino *et al*.[27] In spite of this, the 30 mg kg^{-1} dose did not produce hypnotic effects in the rats, indicating that there are species-specific dose effects related to the hypnotic and anesthetic properties of ketamine. Interpretation of the plasma level results at the lower dose range are limited by the variability of the individual measures, making it difficult to show meaningful differences in the plasma levels between the 1 and 3 mg kg^{-1} doses. Taken together, these studies provide evidence that the subanesthetic doses of ketamine are associated with rapid increases in amino-acid neurotransmitter cycling and antidepressant-like effects on the FST, whereas higher anesthetic doses fail to have the same effects on either measure.

We further examined the relationship between glutamate cycling and antidepressant-like behavior by also analyzing the effects of an NR2B-selective NMDA receptor antagonist, Ro 25-6981. Although the dose range with Ro 25-6981 was truncated by the limited solubility of the drug, there was a dose-dependent increase in cycling and, to a lesser extent, behavior in the FST. Only the highest dose of Ro 25-6981 (10 mg kg^{-1}) induced significant increases in glutamate-C4, GABA-C2 and glutamine-C4 ^{13}C enrichment and immobility on the FST over vehicle-treated animals. The dose effect of Ro 25-6981 in the FST was similar to that previously reported in mice,[23] with the largest behavioral response being seen at the highest dose, 10 mg kg^{-1}. These studies demonstrate that both ketamine and Ro 25-6981 can dose-dependently induce changes in amino-acid neurotransmitter cycling, and suggest that NMDAR antagonist-induced increases in mPFC glutamate release and cycling are critical events in generating the antidepressant-like response of the drugs.

The studies also revealed the effect of the treatment on amino-acid neurotransmitter cycling to be transient. Consistent with the dose–response studies, the effect of ketamine (30 mg kg^{-1}) on cycling was highly significant over the first 18 min after drug injection. Glutamine enrichment is increased within the first 8 min, and the effect is maintained for at least 18 min after injection. However, the effect dissipates over the course of 1 h, and no differences in cycling was found at 24 h, a time point when the antidepressant-like activity of ketamine was seen and repeatedly documented in rodent models.[6,7,25,28] These findings are consistent with other reports demonstrating rapid changes in phencyclidine-induced effects on glutamine levels,[29] and metabolism[30] in rodents that normalize or even reverse by 24 h, and a report suggesting that glutamine levels are rapidly but transiently increased in healthy control subjects receiving a subanesthetic dose of ketamine.[31] The fact that no effects on cycling were observed an hour after the administration of 30 mg kg^{-1} ketamine, despite having blood levels consistent with that found 14 min after 10 mg kg^{-1} dose that did increase cycling, suggest the existence of a hysteresis-like effect. In sum, the findings suggest that although a transient effect on glutamate cycling is associated with initiation of the antidepressant response, maintenance of the effect is not required for the delayed antidepressant-like effects of the treatments.

Another recent study found that Scop increases glutamate efflux, and induces synaptogenesis through AMPA receptor activation in rodent models,[9] suggesting that the surge in glutamate release is also involved in the drugs mechanism of RAAD action. Consistent with that report, we found the 25 µg kg^{-1} dose to produce an antidepressant-like effect and demonstrated that the same dose of Scop increased glutamate cycling rates. These results support the hypothesis that the rapid transient induction of cycling is common to drugs with RAAD properties.

Although the highest stereotypy counts were seen at the doses of ketamine and Scop that were associated with the greatest antidepressant-like effects, this effect was not seen with Ro 25-6981. This suggests that the neuronal mechanisms underlying the onset of stereotypy, behaviors considered related to the psychotomimetic effects of NMDAR antagonists,[32] are not necessarily the same as those underlying the antidepressant-like action.

There are several factors that limit the interpretation of the findings presented in this manuscript. Measures of ^{13}C enrichment are not direct measures of glutamate release. However, the fact that the results align so closely with previous reports of glutamate efflux suggest that the method is reflecting changes in amino-acid release. Moreover, it was not possible to examine the effects of higher doses of Ro 25-6981 because of its limited solubility, precluding the determination of a full dose–response curve for

this compound. We also did not examine the time-dependent effects of Ro 25-6981 and Scop, limiting conclusions on the duration of their effects on glutamate cycling. Last, although the temporal and dose–response relationships between the increase in amino-acid neurotransmitter cycling and the cellular[6] and electrophysiological[33] effects of NMDAR antagonists suggest the rapid increase in cycling is a critical step in the initiation of the antidepressant response, the study does not provide direct mechanistic evidence for this relationship.

CONCLUSION

In sum, these findings are consistent with the hypothesis that a transient glutamate surge is critical in initiating RAAD action. This work defines a timeline of signaling events whereby RAAD drugs initially stimulate a rapid increase in synaptic glutamate release and cycling, but suggests that the effects on amino-acid cycling is not required for the maintenance of the antidepressant-like effects. Rather, the enduring effects of these agents could be related to the sustained effects on synapse number and function that is dependent on glutamate signaling.[34] The novel use of ^{13}C-NMR spectroscopy outlined in the present study can be relatively easily translated to human studies and may be helpful in future attempts to optimize dosing for treatment response in clinical populations.

CONFLICT OF INTEREST

Dr Sanacora has received consulting fees from AstraZeneca, Avanier Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly & Co., Hoffman La-Roche, Merck, Navigen, Naurex, Noven Pharmaceuticals, Servier Pharmaceuticals, Takeda, Teva and Vistagen therapeutics over the past 24 months. He has also received additional research contracts from AstraZeneca, Bristol-Myers Squibb, Eli Lilly & Co., Johnson & Johnson, Hoffman La-Roche, Merck & Co., Naurex and Servier over the past 24 months. Free medication was provided to Dr Sanacora for an NIH-sponsored study by Sanofi-Aventis. In addition, he holds shares in BioHaven Pharmaceuticals Holding Company and is a coinventor on a US patent (no. 8 778 979) held by the Yale University. Dr Duman has received consulting fees from Taisho, Naurex, Sunovion and Johnson & Johnson, and investigator-initiated grants from Forest, Naurex, Sunovion and Eli Lilly & Co. Dr Bristow is an employee of Bristol-Myers Squibb. Dr Schaeffer was an employee of Bristol-Myers Squibb at the time the research was completed and is currently an employee of Janssen Research and Development. Dr Banasr has received research contracts from BioHaven Pharmaceuticals and Servier Pharmaceuticals. Dr Behar holds common stock in Pfizer. The remaining authors declare no conflicts of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)