Data supplement for Price et al., A Novel, Brief, Fully Automated Intervention Extends the Antidepressant Effect of a Single Ketamine Infusion: A Randomized Clinical Trial. Am J Psychiatry (doi: 10.1176/appi.ajp.20220216)

Supplemental Methods & Materials

Ethical Oversight

The study was performed at the University of Pittsburgh and approved by the Internal Review Board of the University of Pittsburgh STUDY19040414, “Testing a Synergistic, Neuroplasticity-Based Intervention for Depressive Neurocognition”. All participants provided informed consent prior to any study procedure. The study was further overseen by an external Data Safety & Monitoring Board (DSMB) and was monitored by the Clinical Research Education, Support, and Training (CREST) Program through the funding sponsor (NIMH).

Participants and Assessments

Participants were recruited and enrolled from 12/01/2017 to 09/30/2021 (clinicaltrials.gov: NCT03237286). Inclusion criteria specified that participants: 1) be between the ages of 18 and 60 years; 2) have not responded to one or more adequate trials of FDA-approved antidepressants within the current depressive episode, determined by the Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (ATRQ); 3) score ≥ 25 on the Montgomery Asberg Depression Rating Scale (MADRS)(1); 4) score >1SD above the normative mean on the Cognitive Triad Inventory(2) "self" subscale *OR* <1SD below the normative mean on the Rosenberg Self-Esteem Scale(3), which was used to ensure a mechanistic fit between the cognitive target of the ASAT intervention (enhancing self-worth) and the depressed patient subpopulation endorsing low self-esteem as a prominent symptom of depression [NOTE: in our screening procedures, <4% of patients meeting all other inclusions/exclusions failed to meet this criterion and were ruled out due to insufficiently low self-esteem (n=7 out of >200 patients screened)—suggesting broad generalizability and applicability of our intervention within the pool of treatment-seeking patients with moderate-to-severe, treatment-resistant depression]; 5) possess a level of understanding sufficient to agree to all tests and examinations required by the

protocol and must sign an informed consent document; and 6) agree to sign a release of information (ROI), identifying another individual [friend, family member, etc.] as a contact person while the patient is enrolled in the study. These criteria produced an enrolled sample in which all patients met diagnostic criteria for Major Depressive Disorder (MDD), and all but one had a primary diagnosis of MDD (one patient had a primary diagnosis of Posttraumatic Stress Disorder and a secondary diagnosis of MDD). The most prevalent current comorbid diagnosis in the sample was Generalized Anxiety Disorder (n=52; 33.8%) followed by Social Anxiety Disorder (n=48; 31.2%). DSM-5 diagnoses were established by experienced master's-level (or higher) clinicians using the MINI International Neuropsychiatric Interview(4).

Clinical trial exclusion criteria included the following:

1. Presence of lifetime bipolar, psychotic, or autism spectrum; current problematic substance use (e.g., substance use disorder); or lifetime recreational ketamine or PCP use
2. Use of a Monoamine Oxidase Inhibitor (MAOI) within the previous 2 weeks
3. Failure to meet standard MRI inclusion criteria: those who have cardiac pacemakers, neural pacemakers, cochlear implants, metal braces, or other non-MRI-compatible metal objects in their body, especially in the eye. Dental fillings do not present a problem. Plastic or removable dental appliances do not require exclusion. History of significant injury or surgery to the brain or spinal cord that would impair interpretation of results.
4. Current pregnancy or breastfeeding, or failure to engage in an effective birth control strategy throughout the duration of the study
5. Acute suicidality or other psychiatric crises requiring treatment escalation.
6. Changes made to treatment regimen within 4 weeks of baseline assessment
7. Reading level <6th grade
8. For study entry, patients must be reasonable medical candidates for ketamine infusion, as determined by a board-certified physician co-investigator during study screening. Serious, unstable medical illnesses including respiratory [obstructive sleep apnea, or history of

difficulty with airway management during previous anesthetics], cardiovascular [including ischemic heart disease and uncontrolled hypertension], and neurologic [including history of severe head injury] will be exclusions.

9. Clinically significant abnormal findings of laboratory parameters [including urine toxicology screen for drugs of abuse], physical examination, or ECG.
10. Uncontrolled or poorly controlled hypertension, as determined by a board-certified physician co-investigator's review of vitals collected during screening and any other relevant medical history/records.
11. Patients with one or more seizures without a clear and resolved etiology.
12. Patients starting hormonal treatment (e.g., estrogen) in the 3 months prior to Screening. Birth control is not an exclusion.
13. Past intolerance or hypersensitivity to ketamine.
14. Patients taking medications with known activity at the NMDA or AMPA glutamate receptor [e.g., riluzole, amantadine, lamotrigine, memantine, topiramate, dextromethorphan, D-cycloserine], or the muopioid receptor.
15. Patients taking any of the following medications: St John's Wort, theophylline, tramadol, metrizamide
16. Patients who have received ECT in the past 6 months prior to Screening.
17. Patients currently receiving treatment with vagus nerve stimulation (VNS) or repetitive transcranial stimulation (rTMS).

Cognitive Outcome Measure: Pictorial Implicit Association Test

The Implicit Association Test (IAT (5)) assesses the strength of association between a concept (e.g., frown) and an attribute (e.g., me/myself). The task measures implicit associations based on the relative speed with which an individual classifies words or images when the same manual key response is required for a given concept-attribute pairing (e.g., “frown” and “me”).

Stimuli appear one at a time in the center of the screen and must be dichotomously classified according to a concept (e.g., smile/frown) or attribute (e.g., me/not me) listed at the upper right of the screen (right key press) and upper left of the screen (left key press). The critical comparison in behavioral performance is made when the concept-attribute pairing (e.g., “me” and “frown”) is swapped (such that “me” and “smile” now require the same key press). If such a swap in pairings results in decreased efficiency on the task in a given participant, the implication is that the second concept-attribute pairing is less strongly associated than the first.

The present analyses focus on a pictorial IAT which was selected from a larger assessment battery as representing the closest analogue for the target stimuli and trained self-associations that were presented during Automated Self-Association Training (ASAT; as described below). Thus, this measure was used to index “target engagement,” or the success with which the automated training altered the targeted aspects of implicit cognition. The IAT was administered and scored in accordance with recommended procedures (6) and as described in numerous previous reports. Reaction times (RTs) and accuracy were recorded for each picture. If an incorrect key response was made, a red X appeared on the screen above the word, and the current trial continued until the participant made a correct response. D-scores were calculated for each participant, for each IAT, where D=[(mean RT during “Frown”=Me block) – (mean RT during “Smile”=Me block) ÷ (SD of RT across all trials)]. Thus, faster responses when “frown” pictures and “me” pictures both required a left finger response, relative to when “smile” pictures and “me” pictures both required a left finger response, indicated an implicit association between frowning strangers and me, and resulted in a positively valenced D-score.

The IAT was completed on a Dell laptop computer running E-Prime 2.0 software. Participants were instructed to classify stimuli appearing at the center of the screen by pressing the “e” key for pictures belonging to categories on the left of the screen and the “i” key for pictures

belonging to categories on the right. A series of images related to the negative concept (frown) or a corresponding positive concept (smile) were presented in random order, intermixed with a series of self-relevant digital photos or other-relevant (standardized) photos of strangers. Category labels ('Frown,' 'Smile,' 'Me,' 'Not Me') remained in the upper left and right corners of the screen throughout the task.

"Me" pictures consisted of digital photographs taken of the participant at the screening visit, who were coached to use a neutral facial expression. Head-shot photographs were taken at a standardized distance, filling the full vertical frame, at three standardized angles (head-on, 45° left, 45° right). "Not-me" pictures consisted of digital photographs of a gender-matched stranger in the same three orientations, taken from the neutral expression images in the standardized Karolinska Directed Emotional Faces (KDEF) image set (Lundqvist, D., Flykt, A., & Öhman, A.; 1998). "Smile" and "Frown" concept images consisted of the angry and happy facial expressions of two male and two female actors in the standardized NimStim image set (7).

The IAT followed a standardized "5-block" IAT design, with 60 trials in each of the two critical blocks (i.e., blocks where concepts and attributes were categorized simultaneously). The order of which type of critical block occurred first (frown=me vs. smile=me) was counterbalanced across participants, but fixed within participants across study visits, to reduce within-subject variability. As recommended to reduce order effects across participants, 40 practice trials were included during the fourth "switch" block, in which participants practiced the newly reversed key-mappings for the negative/positive concepts.

To facilitate concurrent fMRI data collection with fixed block lengths, the length of each IAT block was standardized based on the expected typical duration needed to complete the number

of trials in each block (depending on block type). Thus, a small subset of subjects completed blocks that “timed out” before the total (maximum) number of trials was completed. This was not made apparent to the participant, and never resulted in a reduction of more than 2 trials in any given block. Whenever participants completed the target number of trials within the allocated block duration, a blank screen with a fixation cross remained until the end of the block.

Automated Self-Association Training (ASAT) and Sham Training Technical Details

All ASAT (active or sham) procedures were delivered in a private office on a laptop computer running E-Prime 2 software (Psychology Software Tools; Pittsburgh PA) and a Windows operating system. Positive and neutral word lists and photographs were comprised from previously validated stimulus sets used in prior research.

Each ASAT session was comprised of three consecutive blocks: (1) a subliminal lexical evaluative conditioning block; (2) a supraliminal lexical evaluative condition block; and (3) a pictorial evaluative conditioning block.

In the subliminal lexical evaluative conditioning block, 60 trials were presented consecutively in randomized order. Each trial consisted of a fixation string in the center of the screen (‘XXXX’; 500ms), followed by a single letter probe (active ASAT: ‘I’, implicitly denoting ‘self’; sham ASAT: ‘A’) presented for 17ms; followed by a positive word (active ASAT) or a neutral word (sham ASAT) presented for 17ms; followed by a final probe string comprised of random letters, presented until a response was made. The participant’s task was to press a key to indicate whether the final random letter string began with a vowel (left key; 50% of trials) or a consonant (right key; 50% of trials).

In the supraliminal lexical evaluative conditioning block, 60 trials were presented consecutively in randomized order. Each trial consisted of a fixation string (‘XXXX’; 500ms), followed by a single letter probe (active ASAT: either ‘X’ or ‘I’; sham ASAT: either ‘X’ or ‘A’)

presented for 500ms; followed by either a real word or a random letter string, presented until a response was made. In the active ASAT group, the letter ‘X’ always preceded random letter strings and the letter ‘I’ (implicitly denoting ‘self’) always preceded positive words. In the sham ASAT group, the letter ‘X’ always preceded random letter strings and the letter ‘I’ always preceded neutral words. The participant’s task was to press a key to indicate whether the final letter string was a word (left key; 50% of trials) or a non-word (right key; 50% of trials).

In the pictorial evaluative conditioning block, 480 trials were presented consecutively in randomized order (with one self-paced break at the midpoint). Each trial consisted of a screen divided into 4 quadrants, and a single photographic probe presented until the participant made a mouse click in the correct quadrant; after mouse click, the first photograph was immediately replaced by a second photograph in the same location, which was presented for 400ms. In the active ASAT condition, the first (probe) pictures on 33% of the trials consisted of one of three digital photographs taken of the participant at the screening visit, who were coached to use a neutral facial expression. Head-shot photographs were taken at a standardized distance, filling the full vertical frame, at three standardized angles (head-on, 45° left, 45° right). Whenever the first picture was a participant’s self-photo, the second, replacement photo condition consisted of a happy facial expression, randomly selected from the happy expressions of seven male and seven female actors in the standardized NimStim image set (7). On the remaining 66% of trials in the active ASAT condition, the first (probe) picture consisted of digital photographs of strangers in the same three orientations, taken from the neutral expression images in the standardized Karolinska Directed Emotional Faces (KDEF) image set (Lundqvist, D., Flykt, A., & Öhman, A.; 1998); and the second (replacement) photo consisted of either a second neutral facial expression (33% of trials) or an angry facial expression (33% of trials), randomly selected from the standardized angry and neutral expressions of seven male and seven female actors in the standardized NimStim image set (7). In the sham ASAT condition, the first (probe) pictures were all comprised of neutral expression images in the standardized Karolinska Directed

Emotional Faces (KDEF) image set (Lundqvist, D., Flykt, A., & Öhman, A.; 1998). The second, replacement photos consisted of either a second neutral facial expression (66% of trials) or an angry facial expression (33% of trials), randomly selected from the standardized angry and neutral expressions of seven male and seven female actors in the standardized NimStim image set (7).

Measurement of Covariates

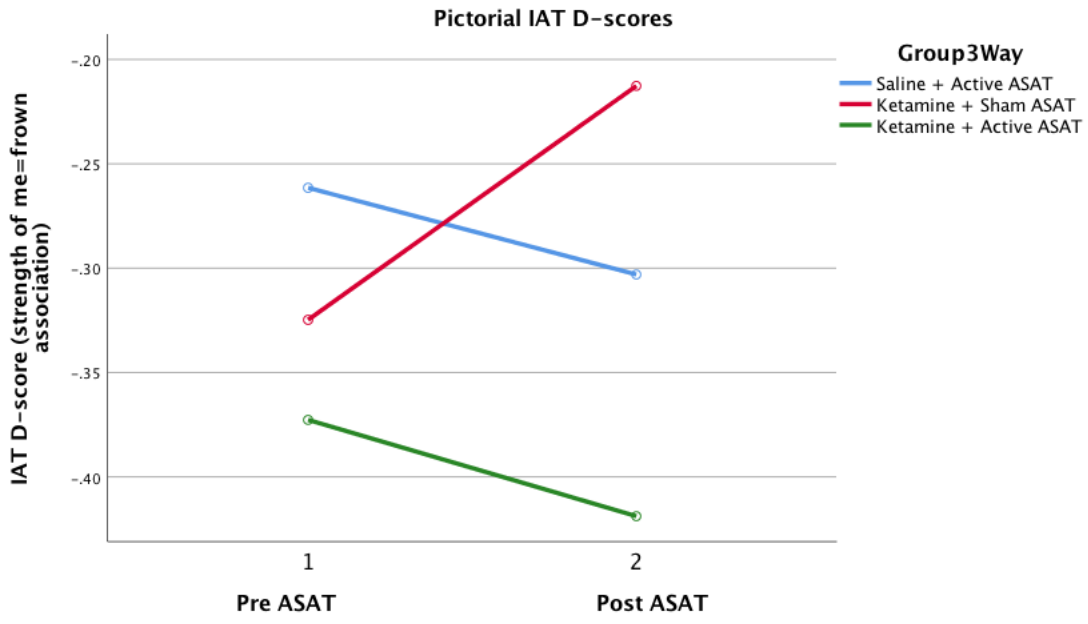
At the screening assessment, participants completed general self-report demographic and medical history forms and supplied a full list of their current medications (including dose and indication). This information was used to quantify the following indices for use as covariates in sensitivity analyses: age, biological sex assigned at birth (as listed on one's birth certificate), and concurrent use of any concomitant psychotropic medication. Patients were queried on the date of their last psychotropic medication change to ensure >4 weeks between the last change and the screening assessments (which equated to roughly 6 weeks stable dose prior to infusion date), and were asked to maintain stable doses of medications and other psychiatric therapies (e.g., psychotherapy) until the infusion day and then throughout the 30-Day post-infusion acute phase study period. Severity of past treatment resistance to FDA-approved antidepressant medications, assessed by the clinical interviewer using the ATRQ and dichotomized as moderate (<3 failed trials in the current episode) or severe (≥3 failed trials in the current episode), was used to stratify randomization (in addition to sex assignment at birth) and as an additional covariate in sensitivity analyses.

Supplemental Analyses

Impact of ASAT on Pictorial Implicit Association Test

IAT D-scores indexing the strength of association between self (patient's self-photos) and frowning faces (negative self-association) were analyzed from pre- to post-ASAT as a function of timepoint (24-hour visit, just prior to the start of ASAT session 1; Day 5 visit, 1 day after the completion of the final ASAT session) and ASAT allocation (active vs. sham). These analyses revealed a significant time*group interaction ($F(1,136)=5.16$; $p=.025$), such that negative self-associations decreased following active ASAT (irrespective of drug condition) and increased following sham ASAT (see **Figure S1** below). Thus, the active form of ASAT appeared effective in bolstering positive self-associations at the implicit level of cognition, as intended.

FIGURE S1



Note that, given that sham ASAT contained no self-referential content whatsoever, and all sham ASAT patients had previously received ketamine prior to the first assessment point, the observed increase in negative self-associations within this group could reflect the start of a gradual return of depressive symptoms following ketamine infusion, and/or a non-specific effect of study procedures and task demands, rather than an impact of sham ASAT per se. Since all mean D-scores remained below 0 at both pre- and post-ASAT timepoints, even patients in the Sham ASAT group retained relatively positive self-associations (i.e., stronger me=smile than me=frown associations), according to conventional interpretations of IAT D-scores.

Adequacy of blinding

At the final (+30 days) visit, adequacy of both patient and clinical rater blinding, for both infusion and ASAT allocation, was assessed using a 0-100 sliding scale (0=definitely placebo/sham; 50=I don't know; 100=definitely ketamine/active). As shown in **Table S1** below, both patients and the rater were functionally unblinded to infusion condition (ketamine vs. saline). While 35% of saline-treated patients incorrectly guessed that they received ketamine, 99% of ketamine-treated patients (all but one) correctly guessed that they received ketamine. In both groups (ketamine and saline-treated participants), clinician ratings of allocation were significantly correlated with degree of symptom reduction (% change from baseline MADRS) at the timepoint when blinding was assessed (infusion +30 days; r's≥.46;*p*'s≤.001). By contrast, patient ratings of allocation were significantly correlated with degree of symptom reduction in the saline arm (r=.49;*p*=.001), but not the ketamine arm (r=.01;*p*=.95). These patterns suggest that, whereas clinician's correct guesses as to allocation may have been more uniformly driven by degree of clinical improvement, patient's first-hand knowledge of other infusion day experiences (e.g., dissociative side effects) likely contributed to their correct guesses (irrespective of clinical improvement).

In contrast, both patients and the rater were adequately blinded to ASAT condition (**Table S1**). Patients' mean response with respect to both conditions (active=54%; sham=49%; t_{143}=1.28;p=.20) reflected the "I don't know" mid-point portion of the 0-100% response choice scale, as did the clinicians' responses (active=55%; sham=53%; t_{147}=0.60;p=.55).

TABLE S1. Patient and clinician guesses regarding treatment allocation, as a function of true allocations

Measure	Actual patient allocation	Guessed probability of active treatment[a]	T-test statistics[b]	% of sample guessing active treatment was received (dichotomous)
Patient ratings of infusion allocation	Received ketamine	91.5% (SD=15.1)	t_{143}=13.2;p<.001	99%
	Received saline	40.3% (SD=31.8)		35%
Clinician ratings of infusion allocation	Received ketamine	64.3% (SD=25.5)	t_{148}=2.8;p=.006	75%
	Received saline	51.5% (SD=27.8)		49%
Patient ratings of ASAT allocation	Received active ASAT	54.4% (SD=22.6)	t_{143}=1.28;p=.20	57%
	Received sham ASAT	49.0% (SD=26.0)		43%
Clinician ratings of ASAT allocation	Received active ASAT	55.3% (SD=19.7)	t_{147}=0.60;p=.55	48%
	Received sham ASAT	53.4% (SD=13.8)		41%

[a]Responses recorded on 0-100% slider scale; 0="Definitely" placebo/sham; 50%="I don't know"; 100%="Definitely" active treatment.
[b]Within each respondent/measure, continuous probability ratings were compared across active and control groups via unpaired t-tests.

Though very few prior ketamine studies have reported explicit measurements of blinding success, the prospect of inadequate blinding has been noted consistently as a challenge when conducting RCTs of ketamine given its unique, acute impact on cognition—even when utilizing a psychoactive comparison arm (e.g., midazolam)(8,9). Here, an inert placebo was selected because a large body of prior work already substantiated ketamine's clinical efficacy in depression, relative to both inert and psychoactive placebo conditions)(8,10-12), and the

present design facilitated clean comparisons of ketamine's potential synergy with ASAT, without introducing the potential ambiguity or confounding (either positive or detrimental) neurocognitive effects of a second psychoactive substance. The fact that a significant response to saline (25% responders at 24-hours; reductions from baseline maintained throughout the 30-day assessment period—**Figure 2**, main text) was observed in our sample provides some reassurance that the saline arm successfully controlled for many non-specific factors (including, but not limited to, expectancy effects) that influence outcomes in depression treatment trials(13). Future work would benefit from stronger blinding efforts, e.g., through the use of an independent, off-site rater pool where raters can remain fully blinded to session/visit number, the patient's prior ratings, adverse events, and other contextual factors(14,15).

TABLE S2. Adverse events

The AE tables below provides final cumulative tallies for each AE reported as new or worsening during the period proximal to infusion day, separately for the acute infusion day visit (from the infusion start to 4hours post-infusion) and post-acute periods, and as a function of study drug arm (ketamine vs. saline). AEs judged by the study physician to be "unrelated" to any study procedure are not included.

Ketamine-treated patients (n=103)

Adverse Events First Reported <u>on</u> Infusion Day (0-4hrs post-infusion)	**# of patients**	**% of patients**
anxiety	10	9.7
decreased energy	16	15.5
difficulty focusing vision	2	1.9
difficulty sleeping: too little	1	1
difficulty sleeping: too much	5	4.9
difficulty urinating	1	1
dissociative effects	100	97.1
dizziness	70	68
dry mouth	40	38.8
elevated blood pressure[1]	7	6.8
elevated heart rate[1]	3	2.9
emesis	3	2.9
emotional indifference	8	7.8
feeling "discombobulated"	1	1
feeling "high" (per verbal report)	1	1
headache	17	16.5
increased appetite	12	11.7
increased weight	1	1
loss of sexual desire	2	1.9
lowered pulse	1	1
nausea	31	30.1
palpitations	11	10.7
restless	1	1
restlessness	17	16.5
suicidal ideation	1	1
sweating	18	17.5
tinnitus	1	1
tremors	6	5.8
vomiting	1	1

Adverse Events First Reported <u>after</u> Infusion Day (24hrs-30 days post-infusion)	**# of patients**	**% of patients**
anxiety	6	5.8
constipation	7	6.8
decreased energy	6	5.8
dehydration	1	1
diarrhea	6	5.8
difficulty sleeping: too little	6	5.8
difficulty sleeping: too much	4	3.9
dizziness	4	3.9
dry mouth	2	1.9
emotional indifference	5	4.9
fainting during blood draw	1	1
fall	1	1
fatigue	3	2.9
headache	17	16.5
increased appetite	4	3.9
irritability	1	1
lightheadedness	3	2.9
loss of sexual desire	1	1
nausea	7	6.8
palpitations	2	1.9
restlessness	5	4.9
sleeping too much	2	1.9
somnolence	3	2.9
sweating	5	4.9
trouble achieving erection and/or orgasm	4	3.9
vomiting	2	1.9
worsening menstrual cramps	1	1

[1]Blood pressure and heart rate changes were logged as an AE only when they surpassed one or more of the following pre-defined boundaries, based on our hospital system's standing safety protocols for subanesthetic ketamine administration:

- SBP ≥ 180 or ≤ 90 mmHg AND a change of ± 20 mmHg from baseline
- DBP ≥ 105 or ≤ 50 mmHg AND a change of ± 20 mmHg from baseline
- Pulse ≥ 110 or ≤ 50 bpm AND a change of ± 20 bpm from baseline

All such reported changes were transient and resolved shortly after the infusion end, without need for further intervention.

Saline-treated patients (n=51)

Adverse Events First Reported <u>on</u> Infusion Day (0-4hrs post-infusion)	**# of patients**	**% of patients**
anxiety	2	3.9
chest tightness	1	2
decreased blood pressure[1]	1	2
decreased energy	3	5.9
decreased heart rate[1]	1	2
difficulty sleeping: too little	1	2
difficulty sleeping: too much	4	7.8
dissociative side effects	11	21.6
distress	1	2
dizziness	10	19.6
dry mouth	5	9.8
elevated blood pressure[1]	2	3.9
elevated temperature	1	2
emotional indifference	1	2
headache	4	7.8
increased appetite	1	2
nausea	7	13.7
ocular pain	1	2
restlessness	2	3.9
sweating	3	5.9
tremors	1	2
Adverse Events First Reported <u>after</u> Infusion Day (24hrs-30 days post-infusion)	**# of patients**	**% of patients**
anxiety	7	13.7
borborygmi	1	2
constipation	1	2
decreased energy	5	9.8
diarrhea	1	2
difficulty sleeping: too little	8	15.7
difficulty sleeping: too much	2	3.9
disorganized speech; tangentiality	1	2
headache	2	3.9
increased appetite	3	5.9
indigestion	1	2
jittery	1	2

loss of sexual desire	2	3.9
nausea	3	5.9
palpitations	1	2
restlessness	6	11.8
sleeping too much	1	2
sweating	1	2
trouble achieving erection and/or orgasm	2	3.9
visual hallucination	1	2

[1]Blood pressure and heart rate changes were logged as an AE only when they surpassed one or more of the following pre-defined boundaries, based on our hospital system's standing safety protocols for subanesthetic ketamine administration:

- SBP $\geq$ 180 or $\leq$ 90 mmHg AND a change of $\pm$ 20 mmHg from baseline
- DBP $\geq$ 105 or $\leq$ 50 mmHg AND a change of $\pm$ 20 mmHg from baseline
- Pulse $\geq$ 110 or $\leq$ 50 bpm AND a change of $\pm$ 20 bpm from baseline

All such reported changes were transient and resolved shortly after the infusion end, without need for further intervention.

Relationships between response/remission at 24-hours and MADRS symptoms at Day 30

To characterize the relationship between rapid (24-hour) and more enduring (Day-30) changes in depression in the course of our study, we conducted exploratory analyses in which we dichotomized responders (≥50% improvement from baseline MADRS) and remitters (MADRS score≤9) at 24 hours (following either ketamine or saline) and compared MADRS total scores at Day 30 as a function of 24-hour response, drug allocation (ketamine vs. saline), and their interaction. These analyses revealed that patients who were responders at 24 hours also maintained lower average MADRS scores at Day 30 (main effect of 24-hour responder status on Day 30 MADRS: $F(1,136)=14.24$; $p<.001$), and this was seen irrespective of whether patients were randomly allocated to ketamine or saline (no significant treatments*responder interaction: $p=.92$). Similar analyses were not possible for remission, given the very low numbers of remitters in the saline arm at 24hours; however, within the ketamine-treated conditions alone, MADRS scores at Day 30 were likewise lower among those classified as remitters at 24-hours ($t(94)=4.68$; $p<.001$). Overall, these analyses highlight that a significant portion of the variance in depressive symptoms at Day 30 was driven by non-specific, patient-level factors (e.g., placebo responsivity; spontaneous symptom fluctuations) that affected symptom scores in a relatively stable and consistent manner from 24-hours to Day 30, and did so irrespective of treatment allocation. By contrast, the findings presented in the main text highlight a separate (also significant) portion of variance attributable to factors under experimental control within the current design (i.e., randomized treatment allocation).

TABLE S3. Robustness of primary findings in main text when including covariates in models

	Covariate			
Finding	**Age**	**Sex**	**TRD severity**	**Concomitant Medications**
Infusion phase: Group*time interaction for pre-infusion to 24-hours	β=-1.30; t_{150}=-4.29; p<.0001	β=-1.30; t_{150}=-4.29; p<.0001	β=-1.30; t_{150}=-4.29; p<.0001	β=-1.30; t_{150}=-4.29; p<.0001
ASAT phase: Main effect in ketamine+active ASAT group from 24-hours to Day 30	β=-0.62; t_{147}=-3.65; p=.0004	β=-0.62; t_{147}=-3.64; p=.0004	β=-0.60; t_{147}=-3.59; p=.0005	β=-0.61; t_{147}=-3.62; p=.0004
ASAT phase: Group*time interaction effect in ketamine+sham ASAT group from 24-hours to Day 30	β=0.015; t_{568}=2.35; p=.019	β=0.015; t_{568}=2.35; p=.019	β=0.015; t_{568}=2.36; p=.019	β=0.015; t_{568}=2.35; p=.019

Note: Inclusion of these covariates did not change the pattern nor the statistical significance of any finding reported in the main text. Of the covariates examined, only TRD severity (dichotomized as ≥3 adequate failed trials in the current major depressive episode) exhibited a main effect on MADRS scores in lme models (greater severity of treatment resistance associated with higher MADRS scores across all timepoints, in both the infusion phase and the ASAT phase models; *p*'s<.05). Nevertheless, inclusion of this variable as a covariate did not alter any finding reported in the main text (as shown in third covariate column above).

Note: No covariates in the table above exhibited significant moderating (covariate*treatment*day interaction) effects on depression trajectories in either the ketamine phase or the ASAT phase (*p*'s≥.19).

Supplemental References

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