Nitrous Oxide for Treatment-Resistant Major Depression: a Proof-of-Concept Trial

Peter Nagele M.D., M.Sc., Andreas Duma M.D., M.Sc., Michael Kopec M.D., Marie Anne Gebara M.D., Alireza Parsoei M.D., Marie Walker M.D., Ph.D., Alvin Janski Ph.D., Vassilis N. Panagopoulos M.D., Pilar Cristancho M.D., J. Philip Miller A.B., Charles F. Zorumski M.D., Charles Conway M.D.

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Nitrous Oxide for Treatment-Resistant Major Depression: a Proof-of-Concept Trial

Short Title: Nitrous Oxide and Treatment-Resistant Depression

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Abstract

Background: NMDA receptor antagonists, such as ketamine, have rapid antidepressant effects in patients with treatment-resistant depression (TRD). We hypothesized that nitrous oxide, an inhalational general anesthetic and NMDA receptor antagonist, may also be a rapidly acting treatment for TRD.

Methods: In this blinded, placebo-controlled crossover trial 20 TRD patients were randomized to a 1-hour inhalation of 50% nitrous oxide/50% oxygen or 50% nitrogen/50% oxygen (placebo control). Primary endpoint was the change on HDRS-21 24 hours after treatment.

Results: Mean duration of nitrous oxide treatment was 55.6 ± 2.5 (SD) minutes at a median inspiratory concentration of 44% (37 – 45%, IQR). In two patients nitrous oxide treatment was briefly interrupted and in three discontinued. Depressive symptoms improved significantly at 2 hours and 24 hours after receiving nitrous oxide compared to placebo (mean HDRS-21 difference at 2 hours: -4.8 points, 95% CI -1.8 to – 7.8 points, p= 0.002; at 24 hours: -5.5 points, 95% CI -2.5 to -8.5 points, p<0.001; comparison between nitrous oxide and placebo: p<0.001). Four patients (20%) had treatment response (reduction ≥50% on HDRS); three patients (15%) a full remission (HDRS ≤ 7 points) after nitrous oxide, compared to one patient (5%) and none after placebo (odds ratio [OR] for response 4.0, 95% CI 0.45 – 35.79; OR for remission 3.0, 95% CI 0.31 – 28.8). No serious adverse events occurred; all adverse events were brief and of mild to moderate severity.

Conclusions: This proof-of-concept trial demonstrated that nitrous oxide has rapid and marked antidepressant effects in patients with treatment-resistant depression.

The trial was registered at clinicaltrials.gov (NCT02139540)
Treatment-resistant major depression (TRD) is a severe form of major depressive disorder (1). Affecting one in three patients with major depression (estimated prevalence in the United States is 10 million adults), patients with TRD often fail multiple treatments with standard antidepressants and have an unfavorable long-term prognosis (2). Therapeutic options for TRD are very limited.

There is a strong biological rationale supporting the potential therapeutic use of nitrous oxide in TRD. Although nitrous oxide is known to modulate several CNS targets (3-14), like ketamine, the primary target of nitrous oxide appears to be the NMDA receptor, where nitrous oxide acts as a non-competitive inhibitor (15-17). NMDA receptor signaling has been implicated in the neurobiology of depression and is a key component of CNS information processing (18-20). Consistent with the relevance of NMDA receptor signaling in the pathophysiology of major depression, NMDA receptor antagonists, such as ketamine - a general, dissociative anesthetic - have been shown to provide rapid and sustained antidepressant effects at subanesthetic doses in TRD (21-27).

Given the similar mechanisms of action, we hypothesized that nitrous oxide may also have rapid antidepressant effects in TRD. This proof-of-concept trial assessed the immediate (2 hours) and sustained (24 hours) antidepressant effects of nitrous oxide in a population of well-characterized patients with treatment-resistant major depression.
Methods

Study Design and Oversight

This study was designed as a randomized, placebo-controlled crossover pilot clinical trial testing the antidepressant effects of nitrous oxide in 20 patients with treatment-resistant depression. In this study, patients had two treatment sessions that were 1 week apart (nitrous oxide or placebo). The sequential order of the sessions was assigned by a random number generator. Other than the gas mixture administered, both sessions were indistinguishable in setting, setup, and monitoring.

We undertook several measures to ensure treatment blinding. First, we completely separated personnel and location of the team providing nitrous oxide treatment from the team performing psychiatric evaluations. The two locations were physically separated from each other and no team member was allowed to enter the other space while a study patient was present. Second, records for the nitrous oxide/placebo treatment administration were kept separate from the psychiatric assessment case report forms until completion of the study. Third, all equipment used to provide treatments was identical between nitrous oxide and placebo sessions. Lastly, all patients were informed that they would receive either nitrous oxide or an air mixture with a high nitrogen component (placebo); hence, patients were blinded as to the nature of the inhaled gas at each inhalation session.

A data and safety monitoring board monitored the trial. The study was approved by the Washington University in St. Louis Institutional Review Board, and all patients provided written, informed consent. The trial was registered at clinicaltrials.gov (NCT02139540).
Patients

Patients were recruited from an existing database of TRD patients administered by the Washington University Department of Psychiatry as well as from the “Volunteers for Health” patient pool (individuals with various medical/psychiatric conditions who volunteer to participate in clinical research) within Washington University School of Medicine. Inclusion criteria were: (1) 18-65 years of age; (2) meeting the DSM-IV-TR criteria for major depressive disorder without psychosis, as determined using a structured clinical interview (the Mini International Neuropsychiatric Interview [MINI](28)); (3) a pre-treatment score >18 on the Hamilton Depression Rating Scale-21 (HDRS-21); (4) meeting criteria for treatment-resistant depression, defined as having had at least two adequate dose-duration, antidepressant medication failures in the current depressive episode and a lifetime failure of at least three antidepressant medication trials. Exclusion criteria were: (1) a history of bipolar disorder, schizophrenia, schizoaffective disorder, obsessive-compulsive disorder, panic disorder, or documented Axis II diagnoses; (2) active or recent substance abuse or dependence (“recent” defined as within the past 12 months; exception was made for nicotine use disorder); (3) the presence of acute medical illness that could interfere with study participation, including, but not limited to, significant pulmonary disease; (4) active suicidal intention; (5) active psychosis; (6) previous administration of NMDA-receptor antagonists (such as ketamine); (7) ongoing electroconvulsive therapy (ECT) treatment; (8) pregnant or breastfeeding women; (9) contraindications against the use of nitrous oxide: pneumothorax, middle ear occlusion, elevated intracranial pressure, chronic cobalamin and/or folate deficiency treated with folic acid or vitamin B\textsubscript{12}. Patients were instructed to continue their current standard of care treatment for major depression and were required to maintain a stable medication or psychotherapy regimen without changes for 4 weeks prior to initiation of the study and continue on the same dose throughout the study.
Treatment

Patients received either an admixture of up to a maximum of 50% nitrous oxide and 50% oxygen (“active treatment”) or 50% nitrogen/50% oxygen for 1 hour (“placebo”). The inspiratory nitrous oxide concentration was titrated during the first 10 minutes until 50% was achieved. 50% nitrous oxide concentration was selected in this pilot trial based on clinical experience for sedation in dentistry and obstetric analgesia where 50% nitrous oxide has been used for decades with an excellent safety and effectiveness record. Furthermore, we decided to maintain an equal oxygen concentration (50%) in the placebo treatment to limit the variability between treatment and placebo. The gas mix was administered via a standard anesthesia facemask through tubing connected to an anesthesia machine. A small sample connector line was inserted into the facemask allowing the measurement of inhaled and exhaled gas concentrations. Total gas flow was between 4-8 L/min. Patients were monitored during and after the treatment according to American Society of Anesthesiologists standard which includes continuous 3-lead ECG, pulse oximetry, non-invasive blood pressure, and end-tidal CO2 under the supervision of an attending-level anesthesiologists. After the one-hour treatment session, patients were transferred to a recovery area and monitored for 2 hours. A study team physician determined if the patients met criteria for discharge before patients were allowed to leave the treatment facility.

Outcomes

Outcomes were assessed at six time points for each patient (three per session; two sessions): at baseline (pre-treatment), 2 hours, and 24 hours after treatment for each session. A one week outcome assessment was not formally planned, but was available as part of the baseline assessment for the second treatment session. Primary study endpoint was the change in the HDRS-21 at 24 hours after treatment. Secondary endpoints included change on the Quick Inventory of Depressive Symptoms Self Report (QIDS –SR) scale. The primary mood
assessment was selected to be administered at 24 hours to ensure that any acute euphoric effects of nitrous had dissipated by this time (nitrous oxide euphoric effects typically cease shortly after discontinuation of nitrous oxide administration). Psychiatric safety endpoints were assessed via careful clinical observations and questioning for dangerousness to self (suicidality), as well as for emergence of psychosis (hallucinations/delusions/disorganized thinking). Other safety endpoints included cardiovascular, respiratory, and central nervous system adverse events determined by hemodynamic and respiratory monitoring. The extent of nitrous oxide-induced inactivation of vitamin B₁₂ was determined by measurement of plasma total homocysteine before and after treatment.

Statistical Analysis

The primary outcome (HDRS-21) was analyzed with a repeated measures mixed effects linear model using restricted maximum likelihood estimation. To adjust for the observed carryover effect, the model included a randomization group term and a three-way interaction (treatment x time x randomization group). Furthermore, we performed a similar repeated-measures mixed model for only the first treatment session (with a two-way interaction). These analyses were repeated for the QIDS scale.

To compare the rates of treatment responses and remissions between the two treatments (using the paired data structure), an exact binomial test was used (and corresponding odds ratios calculated) as the number of discordant pairs was <20. Data are presented as mean ± SD or 95% confidence intervals, or as median and interquartile range.

Because this was the first in-human patient pilot study, no prior knowledge existed for adequate sample size determination. We based our sample size (20 treatment-resistant patients) on available results from ketamine trials in similar populations, where a significant antidepressant effect was observed in less than 20 patients. JMP Pro 11.1 and SAS 9.3 (SAS Institute, Cary, NC), as well as Prism 6.04 (GraphPad Software, Inc., La Jolla, CA) were used for the statistical
analysis and graphing. All reported p-values are two-sided and a p-value of <0.05 was considered statistically significant.

Results

Patients

Between November 2012 and February 2014, we enrolled 24 patients with TRD into the trial. After excluding three patients for screen failure, 21 patients were randomly assigned to a study group (CONSORT diagram, Figure S1). One patient withdrew after the first session and before any outcomes could be assessed, leaving an evaluable patient population of 20 patients who received both treatments and completed the follow-up assessment. All results are reported from these 20 evaluable patients (modified intention-to-treat).

Patients had an average 19 lifetime years of major depressive disorder, failed a median of 8 (adequate-dose/duration) antidepressant drug treatments, and were taking a median of two antidepressants at time of study participation (Table 1). The median HDRS-21 score at enrollment was 23.5 (IQR 22.3 – 25.0) and the median QIDS was 19 [IQR 15.3 – 20.8], indicative of severe depression.

Treatment

Fifteen patients completed the full 60-minute treatment with nitrous oxide; in two patients the treatment was interrupted for five minutes and in three patients discontinued (at 55, 28 and 18 minutes, respectively, for emotional discomfort; regurgitation; claustrophobia; nausea and vomiting, see Table 2 for adverse events). The mean duration of nitrous oxide treatment was 55.6 ± 2.5 (SD) minutes at an average inspiratory nitrous oxide concentration of 44% (37 – 45%, median, IQR). All patients completed the full 60-minute placebo treatment.

Study Outcomes
Patients experienced a significant improvement in depressive symptoms at 2 hours and 24 hours after receiving nitrous oxide compared to placebo (mean difference in HDRS-21 score at 2 hours: -4.8 points, 95% CI -1.8 to – 7.8 points, p= 0.002; at 24 hours: -5.5 points, 95% CI -2.5 to -8.5 points, p<0.001), compared to placebo at 2 hours: -2.3 points, 95% CI 0.8 to -5.3 points, p=0.14; at 24 hours: -2.8 points, 95% CI 0.2 to -5.8 points, p=0.07; comparison between nitrous oxide and placebo: p<0.001; Figure 1). Figure 2 shows the response within individual symptoms from the HDRS-21 that showed the biggest change: depressed mood, guilt, suicidal ideation, and psychic anxiety. On the self-reported QIDS scale, patients experienced a significant reduction at 24 hours after nitrous oxide treatment (mean -3.2 points, 95% CI -1.3 to -5.0 points, p=0.001 between baseline and 24 hours) compared to placebo (mean -1.0, 95% CI 0.9 to -2.8 points; p=0.32) (Comparison nitrous oxide vs. placebo: p=0.003; Supplemental Figure S2).

At 24 hours, four patients (20%) had treatment response (defined as reduction in depressive symptoms ≥50% on the HDRS) after receiving nitrous oxide compared to one patient (5%) after placebo treatment (odds ratio [OR] 4.0, 95% CI 0.45 – 35.79; Figure 3A). Three patients (15%) had a full remission after nitrous oxide treatment (defined as complete resolution of depressive symptoms, HDRS ≤ 7 points), and none after placebo (OR 3.0, 95% CI 0.31 – 28.8; Figure 3B). Subdividing the HDRS scale into five levels of depression severity on the HDRS (normal/ mild/ moderate/ severe/ very severe), 7 of twenty patients (35%) had at least a 2-level improvement 24 hours after receiving nitrous oxide, i.e. from severe to mild, compared to two patients receiving placebo (10%; p=0.06; Table 3). Supplemental Table S1 shows the response on the QIDS scale.

First treatment session-only analysis

In this crossover trial, we expected depressive symptoms to revert to baseline after one week when patients returned for their second treatment session. However, several patients showed
markedly lower HDRS-21 scores after the one-week interval, indicating a significant carryover effect (p=0.02). The heat map in Figure 4 shows a significant difference between HDRS scores of the 10 patients who received nitrous oxide first and the 10 patients who received placebo (p=0.02 for difference between randomization groups). To address this carryover effect, we additionally analyzed the first treatment session only, i.e., compared the 10 patients who received nitrous oxide to 10 who received placebo, akin to a parallel group design. This analysis allowed us to include 1-week outcomes as it represents the baseline assessment for the second treatment session.

Patients who received nitrous oxide first (n=10) had a significant improvement of their depressive symptoms at 2 hours, 24 hours and 1 week (mean reduction of HDRS-21 at 2 hours: -7.1 points, 95% CI -2.4 to -11.8 points; at 24 hours: -8.6 points, 95% CI -4.4 to -12.8 points; at 1 week: -5.5 points, 95% CI -0.8 to -10.2 points) compared to placebo (n=10) at 2 hours: -2.9 points, 95% CI 1.7 to -7.6 points; at 24 hours: -4.7 points, 95% CI -0.0 to -9.4 points; at 1 week: -4.4 points, 95% CI 0.3 to -9.1 points; Figure 5A+B).

Safety

No serious adverse event occurred. All adverse events (Table 2) were temporary. No increase in plasma total homocysteine was observed after nitrous oxide or placebo treatment, indicating minimal inactivation of vitamin B₁₂-dependent metabolism by nitrous oxide. (Supplemental Figure S3).
Discussion

This proof-of-concept trial demonstrated that nitrous oxide has rapid antidepressant effects in patients with treatment-resistant major depression. These antidepressants effects were sustained for at least 24 hours and in some patients for 1 week. Nitrous oxide resulted in a treatment response in 20% of the TRD patients and remission in 15%. Although a subset of patients experienced adverse events requiring either a short interruption or discontinuation of treatment, the mild to moderate nature and immediate reversibility of these events (nausea, anxiety, vomiting) suggest an acceptable risk/benefit ratio for nitrous oxide use in the setting of TRD.

The internal validity of our crossover trial was affected by the observed carryover effect, i.e., patients having a different baseline at different treatment sessions. In our study, several patients who returned for their second treatment session had markedly lower depression scores. Typically, carryover effects bias results towards the null hypothesis, i.e., reduce the observable effect size (29, 30). This was the case in our study: the ten patients who received nitrous oxide treatment first had a mean reduction in depressive symptoms of 8.6 points on HDRS compared to 5.5 points for the full cohort. This observation supports the notion that nitrous oxide has true antidepressant efficacy. A second effect that influenced the internal validity of our trial was the presence of a placebo effect. Placebo effects are common in trials of antidepressants (22, 31, 32) and may introduce bias by masking or exaggerating treatment effects.

Pilot studies, such as this early phase II clinical trial, are designed to detect an efficacy signal in a small group of patients and cannot provide robust and definitive measures of effectiveness. Thus, pilot trials should be interpreted with caution as results must be replicated in larger cohorts. Although the antidepressant efficacy results in this trial are promising, several potential limitations should be taken into consideration. First, although our study team went to great
lengths to maintain blinding, the euphoric effects of nitrous oxide inhalation are difficult to mask. Nitrous oxide induces sedation and has a slightly sweet smell and taste. Hence, it is possible that some patients were able to determine whether they were receiving nitrous oxide or placebo inhalation. Regrettably, we did not test patients to determine if they were aware of their group assignment, and this limits our conclusions. We intentionally selected the 24-hour post-inhalation mark as the primary measure to minimize acute euphoric effects. However, there remains the possibility that nitrous oxide inhalation may have produced a "masking" of depressive symptoms, i.e., the depressive symptoms were not really altered, but rather, "covered up" by other effects. Symptom "masking" has been observed with rapidly acting psychostimulants (methylphenidate and cocaine), which promote a transient alteration in mood, but not a true antidepressant effect. (33, 34)

Second, although we clinically assessed the presence of euphoria and psychosis at each time point, we did not do standardized testing of either. In general, at 2 hours and 24 hours, the patients did not report euphoric feelings. Third, the use of the HDRS-21 and QIDS scales to measure rapid antidepressant action was a limitation as both scales assess symptom changes occurring over the course of days and weeks rather than hours, including questions related to sleep and weight, and thus are not ideal for assessing changes in antidepressant action that occur rapidly. Different scales, such as the Positive and Negative Affect Schedule (e.g., I-PANAS-SF) or a visual-analog scale (VAS) might have been superior. Fourth, we had no prior knowledge about dosing in this patient population and opted to use a 50% inspiratory concentration of nitrous oxide, a dose commonly used in dentistry and obstetric analgesia. Subsequent studies may determine that different dosing regimens improve efficacy and tolerance.

Compared to ketamine, the most commonly investigated NMDA receptor antagonist drug in major depressive disorder, nitrous oxide had a similarly rapid onset of antidepressant action
(within two hours), but appeared to be devoid of psychotomimetic side effects seen with ketamine (delusions, illusions, hallucinations), which may result from nitrous oxide’s more favorable pharmacokinetics as its offset occurs on the order of minutes.(22, 27, 35-37). The fact that both ketamine and nitrous oxide have antidepressant effects in patients with treatment-resistant depression supports the notion that NMDA receptor signaling plays a crucial role in the neurobiology of major depressive disorder.(18-21, 38, 39) However, recent data indicate that other neurotransmitter receptor systems, including nicotinic acetylcholine receptors, may be important contributors to rapid antidepressant actions.(40, 41)

We can only speculate why certain NMDA receptor antagonists (ketamine, nitrous oxide) appear to have rapid antidepressant properties while others such as memantine do not. Differences in NMDA receptor channel blocking seem unlikely to contribute because differences between ketamine and memantine are often observable only under extreme depolarization or pathological receptor activation (simulated ischemia).(42) The presence of extracellular magnesium may distinguish the effects of ketamine and memantine on NMDA receptors, with memantine being relatively ineffective against NMDA receptor-mediated synaptic currents in magnesium.(43) This latter effect also appears to contribute to differences in the ability of the two drugs to promote BDNF production. Differences in mode of administration and pharmacokinetics may also contribute to observed clinical differences between ketamine and memantine. While nitrous oxide, like ketamine, is a non-competitive NMDA receptor antagonist, it differs from ketamine in lacking use-dependence and is not a trapping open channel blocker (15). Thus, nitrous oxide represents an alternative way to modulate NMDA receptor function clinically.

Although a single administration of 50% nitrous oxide/oxygen has been found generally safe (4% non-serious adverse event rate among 25,828 patients receiving sedation(44)), two
potential safety concerns exist: First, nitrous oxide administration had to interrupted or discontinued in a subset of our patients (typically near the end of the 1-hour treatment session), and the adverse event profile indicates that some patients may experience emotional discomfort, paradoxically increased anxiety levels, and nausea during nitrous oxide administration. Although nearly all side effects were limited to the immediate treatment period and disappeared shortly after discontinuation, their nature suggests that perhaps a shorter treatment duration or lower nitrous concentration may be advantageous.

A second potential safety concern relates to nitrous oxide’s inactivation of vitamin B₁₂.(45, 46) While a single exposure is unlikely to result in clinically relevant hematological or neurological complications(47, 48), the risk for such complications is substantially higher when nitrous oxide administrations are repeated within short periods of time (5). Hematological and neurological complications, such as megaloblastic anemia and myelopathy, have been reported among chronic nitrous oxide abusers (49) or patients with chronic disturbances of folate metabolism(50, 51). It is likely that for sustained antidepressant effect, nitrous oxide must be administered several times which would increase the risk for such complications. Moreover, nitrous oxide is a drug of abuse and its abuse potential represents a potential limitation for its clinical utility in major depressive disorder. Our pilot study was not designed to address this safety concern.

Conclusions
In summary, this preliminary, proof-of-concept clinical trial provides the first evidence that nitrous oxide may have rapid and marked antidepressant effects in patients with treatment-resistant depression. Subsequent studies will be required to determine optimal antidepressant dosing strategies, as well as the risk/benefit ratio of nitrous oxide in a larger and more diverse population of patients with treatment-resistant major depression.
References:


Acknowledgments

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Conflicts of Interest:

Dr. Nagele has filed for intellectual property protection related to the use of nitrous oxide in major depression, and has no other conflicts of interest related to this work. He has received research support from Roche Diagnostics, Abbot, and Express Scripts unrelated to this work.

Dr. Zorumski serves on the Scientific Advisory Board of Sage Therapeutics. Sage Therapeutics was not involved in this study.

Dr. Conway was previously on the speaker's bureau for Bristol-Myers Squibb and Otsuka Pharmaceuticals. He has received research funding from Bristol-Myers Squibb, Cyberonics, the Stanley Baer Foundation and the Brain and Behavior Research Foundation.

All other authors report no biomedical financial interests or potential conflicts of interest.

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Role of the funding sources:

The sponsoring departments had no role in the collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript.

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – yrs</td>
<td>48 [30 – 55]</td>
</tr>
<tr>
<td>Female Sex</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Race - White</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Depression history - yrs</td>
<td>19 [11 – 27]</td>
</tr>
<tr>
<td>Number of failed treatments</td>
<td>8 [4 – 12]</td>
</tr>
<tr>
<td>Baseline HDRS-21 score</td>
<td>23.5 [22.3 – 25.0]</td>
</tr>
<tr>
<td>Baseline QIDS score</td>
<td>19 [15.3 – 20.8]</td>
</tr>
<tr>
<td>Vagus nerve stimulator</td>
<td>3 (15)</td>
</tr>
<tr>
<td>History of ECT</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Number of current antidepressant medications</td>
<td>2 [0 – 2]</td>
</tr>
<tr>
<td>History of migraine</td>
<td>10 (50)</td>
</tr>
<tr>
<td>History of hypothyroidism</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Current medication</td>
<td></td>
</tr>
<tr>
<td><strong>Antidepressants</strong></td>
<td></td>
</tr>
<tr>
<td>SSRI</td>
<td>6 (30)</td>
</tr>
<tr>
<td>SNRI</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Bupropion</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>1 (5)</td>
</tr>
<tr>
<td><strong>Atypical Antipsychotics</strong></td>
<td></td>
</tr>
<tr>
<td>Quetiapine</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>1 (10)</td>
</tr>
<tr>
<td><strong>Anticonvulsants</strong></td>
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<tr>
<td>Lamotrigine</td>
<td>3 (15)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Atomoxetine</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>
Dextroamphetamine | 1 (5)
Methylphenidate | 1 (5)

Numbers are listed as median and interquartile range (IQR) or counts and percentages. ADHD – attention deficit hyperactivity disorder; SSRI – selective serotonin reuptake inhibitor; SNRI – serotonin–norepinephrine reuptake inhibitor;

### Table 2. Adverse Outcomes

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Nitrous Oxide – n (%)</th>
<th>Placebo – n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea and vomiting</td>
<td>3 (15%)</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Dizziness/Lightheadedness</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Numbness/Paresthesia</td>
<td>2 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Anxiety</td>
<td>2 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>Panic Attack</td>
<td>1 (5%)</td>
<td>0</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Claustrophobia</td>
<td>1 (5%)</td>
<td>0</td>
</tr>
<tr>
<td>Hyperventilation</td>
<td>1 (5%)</td>
<td>0</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>1 (5%)</td>
<td>0</td>
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Table 3: Change in Level of Depression Severity 24 hours after Treatment

<table>
<thead>
<tr>
<th>Relative Change</th>
<th>Nitrous Oxide</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worst</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Neutral</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Better</td>
<td>7/20 (35%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3/20 (15%)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3/20 (15%)</td>
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</tr>
<tr>
<td></td>
<td>1/20 (5%)</td>
<td>4</td>
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Relative Change after treatment according to the 5 levels of depression severity on the Hamilton Depression Rating Scale (normal; mild; moderate; severe; very severe). Green arrows indicate improvement; red arrows indicate worsening of depressive symptoms compared to baseline. For example, a 2-level improvement would be from severe depressive symptoms to mild.
Figure Legends

Figure 1. Effects of nitrous oxide treatment on depressive symptoms measured on the 21-point Hamilton Depression Rating Scale (HDRS-21). Figure 2A: Absolute change on HDRS-21; Figure 2B: Normalized response (adjusted for baseline) on HDRS-21 Patients were evaluated at three time points: baseline (pre-treatment); 2 hours and 24 hours after treatment completion. Nitrous oxide provided a significantly more pronounced reduction in depressive symptoms compared to placebo (p<0.001). Blue circles = nitrous oxide; squares = control [placebo]. Symbols indicate mean ± 95% CI.

Figure 2: Individual Depressive Symptoms from HDRS-21. The four HDRS-21 depressive symptoms that showed the largest change (depressed mood, guilt, suicidal ideation, and psychic anxiety) are depicted as color-coded bar graphs in order of severity (red – severe, white – absent) between the six different time points of the trial: BL –baseline; 2 h – 2 hours; 24h – 24 hours, N2O – nitrous oxide; placebo

Figure 3. Clinical outcomes after nitrous oxide and placebo treatment. Rates of response (A) (defined as a reduction in HDRS-21 score ≥50%) and remission (B) (defined as complete resolution of depressive symptoms, HDRS ≤7) 24 hours after treatment are shown. Compared to placebo, nitrous oxide had a 4-fold higher response (odds ratio 4.0, 95% CI 0.45 – 35.79) and 3-fold higher remission rate (OR 3.0, 95% CI 0.31 – 28.8).

Figure 4. Cell plot (heat map) of individual responses of first treatment session: nitrous oxide (n=10), left, and placebo (n=10), right, measured on the Hamilton Depression Rating Scale (HDRS) colored to indicate severity of symptoms (red – severe, blue less severe). Each row represents an individual patient. Patients in the left plot are different from the ones on the right.
Figure 5. Effects of nitrous oxide treatment on depressive symptoms for only the first treatment session (10 patients each) measured on the 21-point Hamilton Depression Rating Scale (HDRS-21). Figure 6A shows the absolute, Figure 6B the relative changes on the HDRS-21 compared to baseline, 2 hours, 24 hours and 1 week after treatment. Nitrous oxide provides a significantly stronger reduction in depressive symptoms compared to placebo. HDRS-21 scores at 1 week were derived when patients returned for their second session (= baseline HDRS-21 score for session 2). 1 week HDRS-21 scores after nitrous oxide treatment are significantly lower than at baseline, indicative of a sustained treatment effect.
Fig 2
Fig 3

A

Number of Patients

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<tr>
<td>Placebo</td>
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B

Number of Patients

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<tr>
<td>Placebo</td>
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Fig 5