



Nitrous oxide as a putative novel dual-mechanism treatment for bipolar depression: Proof-of-concept study design and methodology

Mikaela K. Dimick^{a,b}, Danielle Omrin^b, Bradley J. MacIntosh^{b,e}, Rachel H.B. Mitchell^{b,d}, Daniel Riegert^{b,c}, Anthony Levitt^{b,d}, Ayal Schaffer^{b,d}, Susan Belo^{b,c}, John Iazzetta^{b,f}, Garfield Detzler^b, Mabel Choi^{b,c}, Stephen Choi^{b,c}, Beverley A. Orser^{b,c}, Benjamin I. Goldstein^{a,b,d,*}

^a Pharmacology and Toxicology Department, University of Toronto, Toronto, Ontario, Canada

^b Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada

^c Department of Anesthesia, University of Toronto, Toronto, Ontario, Canada

^d Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada

^e Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

^f Pharmacy Department, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada

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ABSTRACT

Introduction: Depressive symptoms predominate in the course of bipolar disorder (BD) and there is an urgent need to evaluate novel application of repurposed compounds that act on pre-specified treatment targets. Several lines of reasoning suggest that nitrous oxide (N₂O) is an ideal medication to study as a potential treatment and as a strategy to identify the underlying pathophysiology of bipolar depression. N₂O is a potent cerebral vasodilator and there is compelling evidence of reduced frontal cerebral blood flow (CBF; i.e. hypoperfusion) in depression. Therefore, N₂O may increase CBF and thereby improve symptoms of depression. The goal of this randomized, double-blind trial is to study the effect of a single administration of N₂O versus the active comparator midazolam on mood and CBF in adults with treatment-resistant bipolar depression.

Methods: Participants with BD-I/-II currently experiencing a major depressive episode will be randomized to one of two conditions (n = 20/group): 1) inhaled N₂O plus intravenous saline, or 2) inhaled room air plus intravenous midazolam. Montgomery-Asberg Depression Rating Scale scores will serve as the primary endpoint. CBF will be measured via arterial spin labelling magnetic resonance imaging.

Conclusions: N₂O is a potential novel treatment for bipolar depression, as it causes cerebral vasodilation. This proof-of-concept study will provide valuable information regarding the acute impact of N₂O on mood and on CBF. If N₂O proves to be efficacious in future larger-scale trials, its ubiquity, safety, low cost, and ease of use suggest that it has great potential to become a game-changing acute treatment for bipolar depression.

1. Introduction

Reducing the burden of bipolar depression has been described as one of the greatest challenges in modern psychiatry [1]. Bipolar depression is associated with high rates of suicide, prolonged episodes, frequent recurrences, and pronounced functional impairment [1–4]. Adults with bipolar disorder (BD)-I and BD-II spend 30–50% of their lives with depression, compared to 1–10% of their lives with mania/hypomania [5]. Although guidelines suggest many different potential

approaches [6], few efficacious treatments exist for bipolar depression [2,3,7–10], resulting in an urgent need to evaluate repurposed existing compounds with rapid-acting novel mechanisms of action that engage pre-specified treatment targets [11,12].

Several lines of reasoning suggest that it is important to study nitrous oxide (N₂O), both to understand the pathophysiology of bipolar depression and as a potential treatment. An inhaled anesthetic drug, N₂O has a rapid onset of action and short half-life. Thus, it can be studied as a biologic probe within a single neuroimaging session. The

* Corresponding author Centre for Youth Bipolar Disorder, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Toronto, ON, M4N 3M5 T, Canada.
E-mail address: benjamin.goldstein@sunnybrook.ca (B.I. Goldstein).

effects of N₂O on consciousness and cognition has been previously studied using neuroimaging [13,14]; however, this approach has not been applied to study bipolar depression. Further, N₂O is relatively safe and well-tolerated and remains widely used for conscious sedation, analgesia, and as an adjunctive anesthetic [15].

The goal of this study is to examine the effect of a single treatment with N₂O or midazolam on depressive symptoms and cerebral blood flow (CBF), along with other potential biomarkers of treatment response, in adults with treatment-resistant bipolar depression. Specifically, the acute effect of N₂O versus the active comparator midazolam on depression symptoms (via the Montgomery-Asberg Depression Rating Scale [MADRS]) [16] will be measured 24-h post-treatment. In addition, CBF (via arterial spin labelling [ASL] MRI) will be measured during the administration of N₂O. We hypothesize that N₂O will significantly reduce depression symptoms versus midazolam, and that N₂O will significantly increase frontal CBF versus midazolam. The choice of midazolam as a comparator was driven by a number of factors: 1) similar to N₂O, it is fast-acting, has a short elimination half life [17], and yields noticeable psychoactive effects and thus, optimizing the integrity of the double-blind design [18]; 2) it does not inhibit N-methyl-D-aspartate (NMDA) receptors and has no vasodilatory properties and thus is mechanistically distinct from N₂O [19]; and 3) precedent exists as a ketamine comparator [20,21].

Our second objective is to identify potential biomarkers that predict effects of N₂O on depression symptoms. We expect greater increases in frontal CBF following N₂O treatment compared to following midazolam treatment. In addition, we hypothesize poorer peripheral endothelial function, a possible biomarker of response as determined via reactive hyperemia peripheral arterial tonometry (RH-PAT) index (RHI), is associated with greater improvement in depression symptoms following N₂O. Lastly, we will examine for an association between genotype and neurophysiology and treatment response. This study will allow us to generate data to inform future studies targeting personalized medicine.

2. Methods

2.1. Participant eligibility

Informed written consent will be obtained from all participants. The study protocol is approved by the local research ethics committee at Sunnybrook Health Sciences Centre and is registered with Health Canada and on [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT02351869).

Adults aged 20–60 years old with bipolar depression will be recruited from Sunnybrook Health Sciences Centre and the community via advertisements. Eligible participants must have a diagnosis of BD-I or BD-II and currently experiencing a major depressive episode of minimum four weeks duration, based on the Structured Clinical Interview for Axis I DSM-IV Disorders-Patient version (SCID) [22], with a MADRS 10 score of ≥ 22 . Participants must be treated with at least one anti-manic mood stabilizing medication/s (i.e. anti-manic anti-convulsant, antipsychotic, and/or lithium). Exclusion criteria include any new treatments (including medications, electroconvulsive therapy, or other biological/physical interventions) or dosing changes in the two weeks prior to the study; current significant manic symptoms (Young Mania Rating Score (YMRS) [23] score ≥ 12); current significant suicidality (MADRS item 10 score of ≥ 4); current psychosis; substance abuse within the preceding three months; active major medical conditions including hepatic, renal, respiratory, or cardio/cerebrovascular disease, diabetes, esophageal reflux, sleep apnea or B₁₂ deficiency/disorders (due to the possibility of demyelination [24]); any MRI contraindication; history of adverse anesthetic reactions; anesthesia American Society of Anesthesiologists (ASA) class ≥ 3 ; and scuba diving in the preceding week [25]. Female participants must not be

pregnant or breastfeeding and must be using a reliable contraception method.

2.2. Study design

Participants will be randomly assigned to a single administration of either N₂O or a comparator midazolam (n = 20 per group). Participants attend three in-person study visits; four follow up interviews (via phone) and self-reports are scheduled for completion 7 days following the last study visit.

Visit 1 includes screening to ensure eligibility criteria are met. Clinical interviews will be completed to confirm the diagnosis of BD and depression criteria. Study psychiatrists assess participants to confirm all inclusion and exclusion criteria.

Visit 2 includes obtaining a blood sample, ECG, and evaluation of height, weight and blood pressure. RHI will be measured via peripheral arterial tonometry (using EndoPAT, Itamar Medical, Israel). Participants will be fitted with a face mask and undergo a MRI practice session during which they are positioned supine in the MRI scanner with the face mask in place. This allows participants to be acclimated to the MRI facility to ensure they are comfortable in the scanner and familiar with the environment.

Visit 3 will occur after participant's ECG and blood work have been reviewed by a study anesthesiologist and a study psychiatrist. Study visit 3 begins with psychiatric interviews and self-reported measures of psychiatric symptoms and side-effects. Participants must refrain from using alcohol, benzodiazepines or illicit drugs for at least 24 h prior to study visit and must be fasting for at least 8 h prior to drug administration (due to concerns about the potential for vomiting/aspiration with anesthetic agents). In addition, blood pressure and heart rate will be measured before, during and after treatment. Follow up interviews and self-reports will be completed at 1, 2, 3, and 7 days post treatment.

2.3. Compensation

Participants will be compensated for their participation in each of the study visits and for completing the post-treatment interviews and self-reports.

2.4. Measures

Diagnoses will be determined with the SCID-Axis I for DSM-IV [22]. Mood symptom severity will be determined with the MADRS 10¹⁶, Hamilton Depression Rating Scale (HRSD; 17-items) [26], Beck Depression Inventory (BDI) [27], YMRS [23], Brief Psychiatric Rating Scale (BPRS) [28], Clinician Administered Dissociative States Scale (CADSS) [29], Hamilton Anxiety Rating Scale (HAM-A) [30], Visual Analog Scale (VAS) [31], and modified Patient Rated Inventory of Side Effects (PRISE) [32]. Weight will be measured on a TANITA scale, and height will be measured using a separate stadiometer, with participants in bare or stocking feet and light clothing. Body mass index (BMI) percentiles for age and sex will be determined using the Center for Disease Control norms. Obesity is defined as BMI ≥ 95 th percentile.

2.5. Study intervention

Participants will be randomized to one of two treatment conditions: 1) inhaled N₂O plus intravenous saline injection, or 2) inhaled medical air plus intravenous midazolam injection. An anesthesiologist will oversee pre-anesthetic assessment, drug administration, monitoring (vitals, pulse oximetry) and post-treatment recovery. All participants wear a face mask connected to a non-rebreathing circuit including a 2-L reservoir. All participants undergo venous cannulation

which will remain in situ for the duration of the neuroimaging procedures. A team of seven is required on the day of treatment administration, including: study psychiatrist, study anesthesiologist, two anesthesia assistants, two clinical research staff, and an MR imaging technologist.

2.5.1. Dosing

The concentration of 25% N₂O in oxygen and the duration of 20 min of administration of N₂O were selected based on the existing literature, which suggests that inhalation of 25% concentration of N₂O provides an optimized balance between the desired changes in mood and physiological effects versus adverse effects observed at higher doses [33]. Nausea and vomiting is a common adverse event associated with N₂O administration and was reported in 15% of MDD patients administered at 50% concentration of N₂O [25,33]. One study with healthy males assessing functional EEG changes with different doses of N₂O and found 60% concentration of N₂O condition resulted in nausea and emesis compared to 20% and 40% N₂O [34]. Midazolam 2 mg total dose is expected to yield subjective psychoactive effects that will optimize blinding. In three trials comparing ketamine to midazolam as an active placebo a midazolam dose of 0.045 mg/kg was used [21,35,36]. One study assessing ketamine versus midazolam in BD used a lower dose of 0.02 mg/kg midazolam [37]. Compared to ketamine trials using midazolam as an active comparator [21,37–39], we chose a lower dose of midazolam in order to minimize risks in an MR suite.

2.5.2. Administration

For participants in the N₂O treatment arm, the anesthesiologists will deliver an initial dose of N₂O 10% in oxygen for 5 min, followed by a 5 min wash-out period, followed by N₂O 25% in oxygen for 20 min, followed by medical air for 3 min to flush any residual N₂O. N₂O participants will also receive intravenous injection of 0.5 ml saline concomitantly with 10% N₂O, and intravenous injection of 1.5 ml saline with 25% N₂O. Participants in the midazolam treatment arm will receive an intravenous injection of 0.5 mg midazolam (concurrent with the first gas administration epoch), followed by an intravenous injection of 1.5 mg midazolam (concurrent with the second gas administration epoch), followed by medical air. Treatments will be given in two epochs, with a smaller test dose administered first as a safety precaution within the MR environment.

2.5.3. Safety

The study will be monitored by a Data Safety and Monitoring Board that holds semi-annual meetings to review all adverse events and offer study oversight. Although N₂O is safe for the vast majority of people, prolonged or frequent use may lead to immunosuppression and/or macrocytosis or neuropathy (via B₁₂ deactivation) [40,41]. Neurological toxicity has occurred after single exposures in patients with undiagnosed B₁₂ deficiency [24]. Side effects at the proposed dose include mild elation, dizziness, impaired concentration, and tingling [42]. Additional details can be found in the medical gas datasheet [25]. Common side effects of midazolam in adults with unipolar major depressive episodes include nausea, dizziness, blurred vision, and impaired concentration, although rarely to the point of distress [38]. N₂O and midazolam, particularly when used in conjunction with psychiatric medications, may potentially result in unpredictable psychiatric (e.g. mania, psychosis) and/or physical side-effects [43,44]. For this reason, study participants will be monitored on site for a minimum of 2 h following treatment, and medical clearance from both anesthesiology and psychiatry will be required prior to participants leaving the facility.

2.6. Blinding

The anesthesiologist, anesthesia assistants, pharmacist, and the study monitor will be aware of treatment condition; all other study staff will remain blinded to treatment condition. Study visits and all procedures are identical for both treatment arms. Upon bringing the study participant to the MRI suite, the clinical raters and study psychiatrist will be stationed outside of the MRI suite for the duration of the scan. Sealed randomization envelopes containing the treatment assignment will be given to the anesthesiologists on the day of the exam and source documents containing gas levels are kept in a sealed envelope. To measure the integrity of the blind, clinical raters and participants will complete questionnaires guessing which treatment the participant received following the 60-min post-treatment interview.

2.6.1. Potential biomarkers and role of genetics

B₁₂ and nitric oxide (NO) levels will be examined as predictors of response owing to known associations with the mechanism of action of N₂O [45–49]. B₁₂ will be examined in screening clinical blood work. Serum NO will be analyzed off-site using the Griess reaction (a two-step analytical chemical test to determine the amount of nitrite ion in a solution) [50,51]. We will examine genetic markers that are relevant to BD, vascular function, and/or NMDA. Examples include, but are not limited to, the alpha 1C subunit of the L-type voltage-gated calcium channel (*CACNA1C*), Vascular Endothelial Growth Factor (*VEGF*), brain-derived neurotrophic factor (*BDNF*), Neurogenic locus notch homolog protein 4 (*NOTCH4*), and nitric oxide synthase, variable number of tandem repeats (*NOS-I VNTR*). Exploratory analyses focusing on these biomarkers will yield preliminary data to inform the selection of moderators in future studies.

2.6.2. Magnetic resonance imaging

MRI scans will take place on a 3 T MRI system. MRI protocol consists of localizer scans to plan subsequent images, an anatomical structural image of the brain, a time-of-flight angiography image of brain arteries, resting state networks and perfusion images. Standard T1-weighted acquisition will quantify brain gray and white matter and co-register participants to a standard space reference brain. Pseudo-continuous ASL (PC-ASL) imaging are used to obtain CBF levels in absolute units of mL/100 g of tissue/min on a voxel-by-voxel basis, with spatial resolution of approximately 3 × 3 × 5 mm. Approximately 90 min of scanner time are needed to allow for participant preparation and the following in-scanner schedule: 1) time of flight angiography; 2) venous oxygenation (T0: zero denotes baseline); 3) carotid and vertebral arteries phase contrast angiography flow velocity; 4) baseline (T0) ASL; 5) resting state functional MRI (T0); 6) inhalation of 10% N₂O or medical air to reach 95% concentration in brain [52]; 7) post-inhalation (T1) ASL; 8) 25% N₂O or medical air; 9) resting-state functional MRI (T1); 10) post-inhalation (T2) ASL; 11) structural scan; 12) venous oxygenation (T1); 13) end-of-study (T3) ASL.

2.6.3. Analytic plan

This study began recruitment in 2016 and is expected to be completed in fall 2020. Modified intention-to-treat analyses will include all randomized participants with at least one post-baseline measurement. The primary outcome is a reduction in MADRS scores 24 h post-treatment. Although other time-points will be examined, 24 h was selected as the primary outcome to minimize the impact of acute sedation and psychoactive effects and to align with similar ketamine studies for refractory depression [37,38]. Response rates (≥50% reduction in MADRS) will be examined as a secondary outcome. For the primary analyses, repeated measures analysis of covariance (ANCOVA) models will examine reductions in MADRS at 24 h covarying for baseline MADRS, and logistic regression analyses will examine response, as a function of treatment group, controlling for baseline MADRS.

For our secondary hypothesis examining cortical perfusion, the primary outcome is mean change (i.e. difference in CBF as in: T3-T0) in ASL across anterior cingulate cortex (ACC), striatum, and ventral prefrontal cortex (VPFC). Secondary analyses will examine these regions of interest individually. ANCOVA will examine ASL change scores (T3-T0), as a function of treatment group controlling for T0 ASL. Pearson correlation coefficients will be computed to examine the association of frontal CBF and RHI with change in MADRS scores 24 h post-treatment. If correlations ≥ 0.3 are observed, we will compute linear regression models with change in MADRS as the dependent variable. The threshold for significance will be $p < 0.05$ for all tests. Exploratory analyses will examine B₁₂ and NO blood levels, and selected genetic markers, as predictors of changes in MADRS scores. Exploratory analyses will also examine secondary measures in place of MADRS. This preliminary study will not control for multiple comparisons on secondary or exploratory analyses.

2.6.4. Power

Assuming a medium effect size ($d \geq 0.4$), 20 participants per cell will offer sufficient power ($\beta = 0.8$) to detect a difference in the primary outcome across the two groups at $\alpha = 0.05$ (one-tailed).

2.7. Limitations

This is a highly novel and unprecedented study. As such, the primary limitation is uncertainty about the effective concentration and duration of N₂O treatment. We could have opted for higher concentration and/or longer duration of treatment. We have taken a conservative approach that minimizes safety concerns. We will integrate ASL measures as a proof-of-concept, but also as a proximal intermediate phenotype in the spirit of prevailing calls for action regarding target engagement with novel antidepressants [53]. We opted for ASL over SPECT because of concerns regarding radiation exposure. Also, we have not applied restrictive medication exclusions, opting to enroll the “real-life” clinical sample in which we foresee N₂O potentially being employed. Finally, we could have opted for repeated treatments rather than a single session. In our view, preliminary promising findings from the proposed study, in terms of efficacy and safety/tolerability, would be needed to justify a longer course of treatment with N₂O.

2.8. Design-specific challenges

Obtaining MR approval for this population, which have frequent medical comorbidities, requires extensive screening including X-rays and reviewing medical records. Each participant requires the coordination of seven staff members, and coordinating availability of a study psychiatrist, study anesthesiologist, and MR technician is a unique challenge which requires study visit 3 (drug administration) to occur during the weekends. In addition, as drug administration occurs within an MR environment custom fitting of face masks is required to avoid MR contraindicated mask components.

3. Discussion

Currently, there are few effective treatments for bipolar depression [2,3,7–10]. Using objective measures (i.e. biomarkers of treatment response) in order to personalize treatment selection is important as treatment effectiveness has considerable variation at an individual patient level [54,55]. Furthermore, it is critical to study potential treatments which engage objective treatment targets, such as CBF, in order to better understand the mechanisms of both the disease and the mechanism of action of the treatment [11,56].

N₂O has two mechanisms of action that could be salutary in bipolar depression: antagonism of NMDA receptors [57–59], and cere-

brovascular vasodilation with predominance in the frontal regions of the brain [60]. Glutamate, an excitatory neurotransmitter, is a known target of mood stabilizers and NMDA receptors are one of several glutamate receptors [61]. Several NMDA antagonists have been shown to have anti-depressant effects in animal models both as monotherapy and combined with traditional anti-depressants [61]. N₂O is known to inhibit NMDA-activated currents in a dose-dependent manner within cultured neurons [62]. The effectiveness of NMDA antagonism in the treatment of depression has been suggested in recent landmark studies of ketamine, recently approved by the FDA for treatment-resistant depression [63], although not all NMDA receptor antagonists exhibit antidepressant properties [59,64–66]. N₂O may also be beneficial due to its effects on blood vessels. BD is also strongly associated with premature onset and excessive prevalence of cardiovascular disease (CVD), even compared to major depressive disorder [67,68]. In addition, BD is associated with white matter hyperintensities suggestive of cortical vascular pathology [69–73]. There is evidence that CBF (i.e. perfusion) is reduced in depression, and that increases in CBF are associated with improvements in depression [74–80]. Taken together, this suggests that increasing frontal CBF could improve bipolar depression.

Ketamine shares similar cellular mechanisms of action with N₂O [59]. Ketamine, an NMDA antagonist and anesthetic, has become a prototype for rapid-acting antidepressants [65,81,82]. Randomized controlled add-on trials of intravenous ketamine provide support for efficacy in bipolar depression [66,83]. Superior efficacy versus placebo is evident within 40 min and persists until 3 days post-injection. A recent study with the active comparator midazolam indicates that the antidepressant efficacy of ketamine is not related to transient psychoactive effects [38]. Positive response to ketamine in the treatment of depression has been associated with higher B₁₂ levels, glucose metabolism, ketamine metabolites, functional connectivity during a working memory task, neural activation to fearful faces, spectroscopy, and familial and molecular genetic factors [45,84–91]. Increased B₁₂ levels have been associated with increased antidepressant treatment response in ketamine and other trials [45,92]. Several studies have shown prolonged antidepressant effects of ketamine after multiple treatments over time [93–95].

Numerous studies have examined CBF in relation to depression using various neuroimaging techniques. CBF is consistently reduced among depressed adults, particularly in frontal regions [74,75,78,80,96–101]. Higher pretreatment CBF, particularly in frontal regions, predicts greater antidepressant response [102]. In turn, depression treatment affects CBF, and changes in CBF may correlate with changes in depression symptoms [75–77,98,103–105], although exceptions exist [106,107]. Among adults with bipolar depression, reduced CBF may be most evident in frontal, temporal and parietal regions and in cases with more severe depression symptoms [80,108–110].

N₂O has been in therapeutic use for over 150 years [111]. NMDA antagonism is most widely recognized properties [60,112]. N₂O protects against neurotoxicity of NMDA, produces similar side effects to other NMDA antagonists in animal models, and inhibits NMDA-evoked currents in neurons recorded using whole-cell, patch-clamp techniques [112]. N₂O has also been studied in individuals with a family history of alcohol problems and has been shown as an effective probe for heritable NMDA-receptor dysregulation [113]. A 2015 review highlights the potential value of N₂O as a treatment for refractory depression, underscoring the putative mechanism of NMDA receptor antagonism [59].

Another central property of N₂O is that it is also a robust cerebral vasodilator in humans, and associated with changes in CBF [60,112]. In a study of 17 healthy adults, delivery of 30% N₂O for 10 min was associated with a 22.5% increase in gray matter CBF [14]. Region-specific findings include increased CBF in parietal and left frontal gray matter versus sevoflurane, another cerebrovasodilatory anesthetic

used in magnetic resonance imaging (MRI) diagnostics [114], and large increases in frontal regions (+28%) versus modestly increased CBF in the thalamus (+11%), or reduced CBF in cerebellum and occipital lobes (−12% for each). [115] Depression has been associated with an anterior-posterior gradient in CBF, with posterior regions showing increased CBF and anterior regions showing lower CBF [116,117]. N₂O administration has been shown to redistribute the anterior-posterior gradient, such that there are greater CBF increases in anterior compared to posterior blood flow [118]. In addition to CBF and NMDA-related biomarkers, there may be value in examining peripheral indicators of vascular function such as peripheral endothelial functioning, which has been associated with depression [73,119,120].

Studies have examined the psychological effects of N₂O across various applications and populations [42,52,121–126]. Beneficial mood-related effects have included sense of calm [52], reduced irritability [124], and increased pleasant mood [125]. A blinded crossover trial in 20 participants with treatment-resistant unipolar major depression found a significant reduction in depressive symptoms at 2 h (−22.86%) and 24 h (−26.19%) post treatment with N₂O (mixture of 50% nitrous oxide and 50% oxygen) as compared to placebo (mixture of 50% nitrogen, 50% oxygen; −10.95% and −13.33% reduction in depressive symptoms respectively) with 15% of participants (n = 3) achieving full remission versus no participants achieving full remission after placebo [33]. Although the impact of N₂O on manic symptoms in humans has not been systematically examined, N₂O may reduce hyperactivity in children [121] and reduces amphetamine-induced locomotor sensitization in rodents [127], which is one of the leading animal model proxies for mania [128]. A study of 24 healthy adult women found that participants treated with 50% N₂O for 30 min after viewing a “trauma film” had a faster reduction of intrusive analog trauma memories compared to those who received medical air [129]. Finally, preliminary findings suggest that N₂O reduces subjective emotional responses and activation of the amygdala in response to emotional images in healthy volunteers [130].

N₂O is a promising candidate drug as a novel treatment for bipolar depression, owing to its inhibitory effects on NMDA receptors and its cerebral vasodilatory properties. N₂O is inexpensive, widely available, and has a longstanding record of safety and tolerability at sub-anesthetic doses. This study represents the unique collaboration between psychiatrists, anesthesiologists, and neuroimaging specialists. The logistics of team coordination, scheduling and participant recruitment present substantial and unique challenges. While the study of N₂O could have proceeded without the neuroimaging component, the concept of CBF target engagement as a central mechanism to account for the antidepressant properties of N₂O is of central interest. Indeed, the study design will yield insights regarding the neurophysiology of bipolar depression, using target engagement that is relevant to bipolar depression. Moreover, the study integrates putative moderators of N₂O response that are relevant to N₂O, vasodilation, and/or NMDA antagonism (baseline CBF, endothelial function, B₁₂, and NO levels).

This proof-of-concept study will provide valuable information regarding the acute impact of a single acute treatment with N₂O on mood and on CBF. Future studies will be needed to optimize the concentration and duration of exposure to N₂O. Nonetheless, our hope is that this study will spark an entirely new field of research into N₂O as a treatment for bipolar depression (and other brain diseases for which modification of CBF and NMDA receptor dynamics is relevant), and will provide valuable information for this new field regarding the acute impact of N₂O on mood and neurophysiology. If N₂O proves to be efficacious for bipolar depression in future larger-scale trials, its ubiquity, safety, low cost, and ease of use suggest that it has great potential to become a game-changing treatment for bipolar depression.

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Trial registration

The original clinical trial (NCT02351869) is listed in www.clinicaltrials.gov.

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