Nitrous Oxide Exposure Alters Functional Connectivity in Medial Limbic Structures in Treatment-Resistant Major Depression

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Background

Nitrous oxide (N_2O) is an N-methyl-D-aspartate receptor (NMDAR) antagonist that has demonstrated promising therapeutic effects in several psychiatric disorders, including treatment-resistant major depressive disorder (TRMD). Similar to ketamine, N_2O's antidepressant effects are both rapid and sustained and often observed for days to weeks after a single dose, suggesting that N_2O produces lasting CNS changes that persist beyond its acute brain interactions. These lasting effects may be mediated via neural plasticity within brain networks and changes in correlated neural activity, i.e., functional connectivity. However, little is known how N_2O alters brain networks to confer antidepressant effects. This study employed serial resting-state functional magnetic resonance imaging (rs-fMRI) to compare the spatiotemporal effects of inhaled N_2O on brain functional connectivity in TRMD patients and non-depressed healthy controls (CNTL).

Methods

Approval was obtained from the Washington University School of Medicine Human Research Protection Office and registered on ClinicalTrials.gov (NCT02994433). Participant age range was 18-65, and exclusion criteria for TRMD/CNTL participants included severe medical/neurological disorders, psychosis, or severe personality disorders. The CNTL participants did not screen positive for MDD; TRMD participants had a ≥17 score on the Hamilton Depression Rating Scale (17 item) and failure to respond to ≥3 lifetime adequate dose/duration antidepressant treatments, with ≥1 nonresponse in the current episode. Employing serial resting-state functional magnetic resonance imaging (rs-fMRI), we compared spatiotemporal effects of inhaled N_2O on brain functional connectivity in TRD patients (n=14) and non-depressed healthy controls (n=16, CNTL). Participants received sequential, one-hour inhalations of either 50% N_2O/oxygen or air/oxygen (placebo), with sessions separated by at least one month in random cross-over order. BOLD-contrast rs-fMRI scans were acquired at three time points: pre-inhalation, 2 hours post-inhalation, and 24 hours post-inhalation. For rs-fMRI functional connectivity analyses, five *a priori* seeds in medial limbic structures targeted cortical networks implicated in major depression – the salience, anterior and posterior default mode, reward, and cingulo-opercular networks – and a dorsal nexus in the dorsal paracingulate region previously identified in MDD. Depression, dissociation, and psychosis assessments were made before and after inhalations.

Results

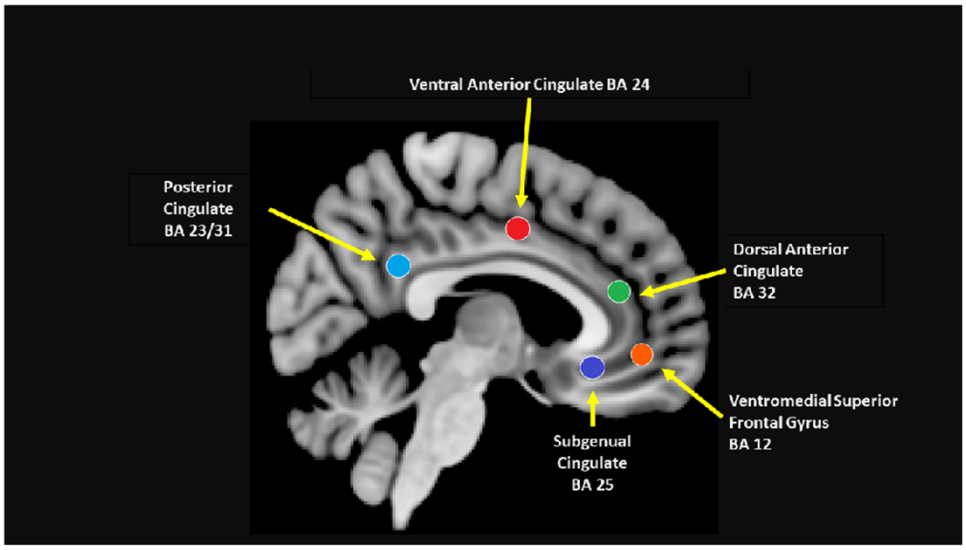
In TRMD patients, statistically significant functional connectivity *reductions* were observed in all seeded networks after N_2O exposure (**Fig 1**). N_2O progressively *decreased* connectivity in patients with TRMD but *increased* connectivity in healthy controls. In TRMD patients, each seeded network demonstrated post-exposure functional connectivity reductions in the dorsal paracingulate cortex ("dorsal nexus"). Of note, the subgenual cingulate seed in the TRMD cohort demonstrated considerably higher baseline connectivity vis-à-vis the CNTL cohort. However, following N_2O inhalation, this functional connectivity difference was reduced levels seen in the CNTLs. Further, a voxel-wise global analysis global correlation analysis (GCOR), which assessed functional connectivity changes across all brain regions, demonstrated changes in functional connectivity in medial prefrontal cortex, with exposure-related reductions in functional connectivity in TRMD participants and increases in CNTLs. There was a clinically significant greater reduction in depressive symptoms in the TRMD participants receiving N_2O, compared to placebo. Similar to other N_2O trials, significant placebo effects, as well as carry-over effects, were observed despite a minimal one-month separation between N_2O sessions.

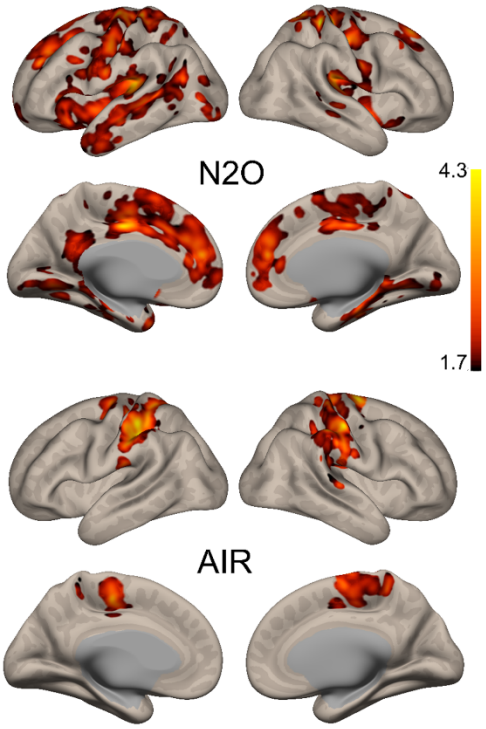
Conclusions

This study further elucidates neural mechanisms underlying the antidepressant properties of N_2O, supporting the notion that N_2O specifically alters mood-associated brain regions in the depressed brain state by reducing functional connectivity within these brain networks.

Figure 1. Seeds (top panel) and Changes in Functional Connectivity Observed Before and After N_2O (middle panel) and AIR (bottom panel).

Top Panel: *A priori* seeds were selected to compare pre-post exposure to N_2O and placebo. These five seeds targeting midline limbic structures probed networks known to be involved in mood regulation: the reward network (Brodmann's area BA 25), the cingulo-opercular executive network (midline dorsal cingulate, BA 24,red), the salience network (dorsal anterior cingulate, BA 32, green), and the anterior and posterior nodes of the default mode network (ventromedial prefrontal cortex, BA 12, orange, and posterior cingulate, BA31, cyan, respectively). Middle Panel: Changes in functional connectivity averaged across all 5 seeds comparing pre- to 2 and 24 hours post-N_2O exposure. There are notable changes in functional connectivity observed in the anterior and posterior portions of the cingulate cortex, hippocampal and parahippocampal regions, and insular cortex. Lower Panel: Changes in functional connectivity averaged across all 5 seeds comparing pre- to 2- and 24-hours post-placebo exposure. We observed markedly different spatial changes in functional connectivity, with changes limited to primarily parietal cortex and no involvement of the cingulate, hippocampus, insula or dorsal paracingulate gyrus.


Ventral Anterior Cingulate BA 24
Posterior Cingulate BA 23/31
Dorsal Anterior Cingulate BA 32
Ventromedial Superior Frontal Gyrus BA 12
Subgenual Cingulate BA 25


N2O
4.3
1.7
AIR