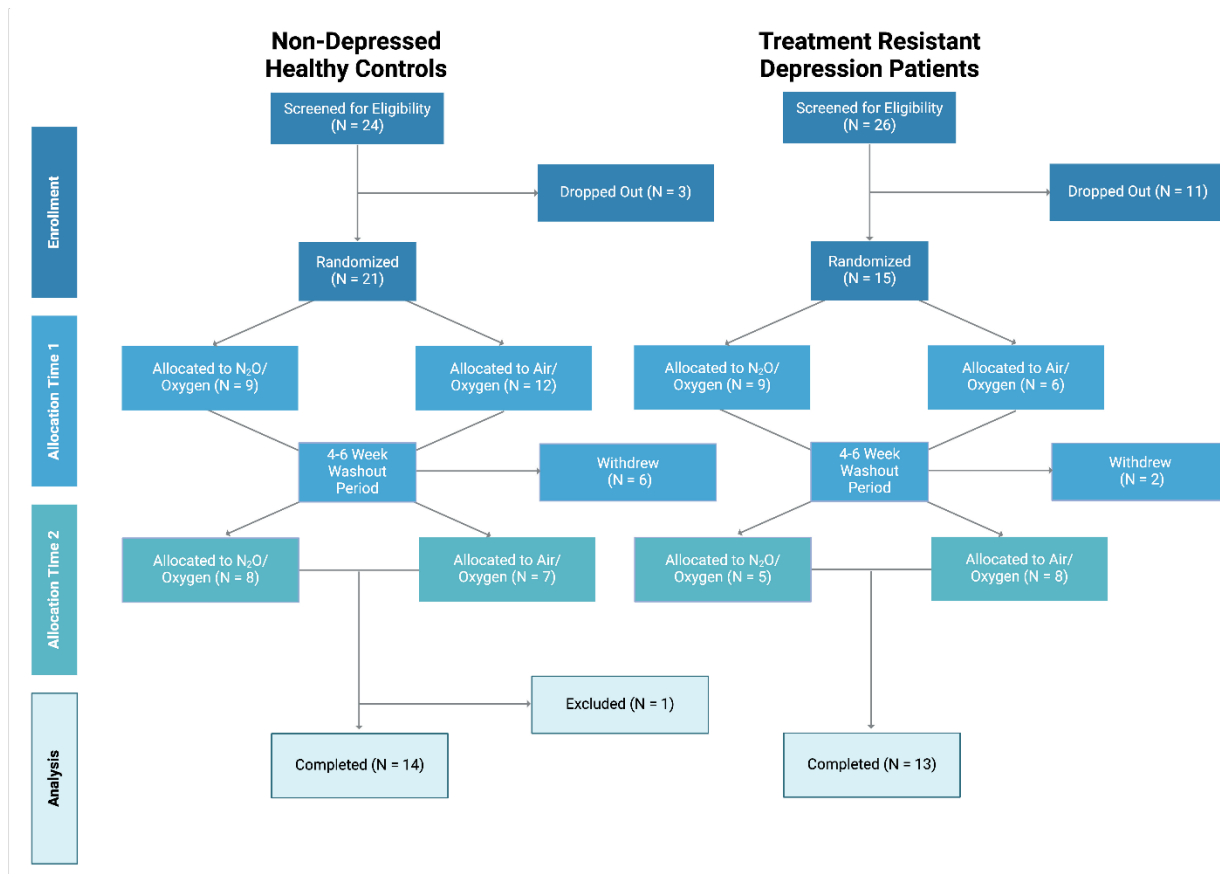


Supplemental Materials

Additional Results

Figure 1S. Consort Diagram



Participant Selection Process

Following eligibility screening, 24 CNTL and 26 TRD patients were consented via recruitment from an existing TRD database (gathered over the past 15 years at Washington University), existing psychiatric clinics at Washington University Department of Psychiatry, and through potential participant registries at Washington University School of Medicine and trial advertisement through the greater St. Louis community. Three CNTL and eleven TRD patients did not pass screening or voluntarily withdrew prior to randomization. Two TRD patients (one lost to follow-up, one terminated early due to Covid-19) and six CNTL (one lost to follow-up, one

for mask discomfort, one determined to have pre-existing psychotic disorder, one could not attend sessions, one terminated early due to Covid-19, one due to nausea/dissociative experience) withdrew from the study following the first inhalation. Despite completing all inhalation and imaging sessions, 1 CNTL participant was excluded due to use of a different scanner system and imaging protocol.

Table 1S. Treatment-Resistant Depressed Participant Demographics

Subject ID	Sex	Race	No. of trials, current episode	No. of failed trials, lifetime	Years reported with MDD
1	Male	White/Caucasian	1	3	14
2	Female	Asian	1	3	7
3	Male	White/Caucasian	1	3	33
4	Female	Black	2	3	12
5	Female	White/Caucasian	1	9	38
6	Male	White/Caucasian	1	4	3
7	Female	White/Caucasian	2	19	39
8	Female	White/Caucasian	2	15	30
9	Female	Asian	1	3	3
10	Female	White/Caucasian	1	9	27
11	Male	White/Caucasian	1	4	19
12	Female	White/Caucasian	1	15	18
13	Female	White/Caucasian	2	14	36
14	Female	White/Caucasian	1	4	14

Table 2S. Non-depressed Healthy Control Participant demographics

Subject ID	Sex	Race	Ethnicity
1	Female	White/Caucasian	Not Hispanic/Latino
2	Male	White/Caucasian	Not Hispanic/Latino
3	Male	Asian	Not Hispanic/Latino
4	Male	Asian	Not Hispanic/Latino
5	Female	Black	Not Hispanic/Latino
6	Female	White/Caucasian	Not Hispanic/Latino
7	Male	White/Caucasian	Hispanic/Latino
8	Female	Black	Not Hispanic/Latino
9	Male	Black	Not Hispanic/Latino
10	Female	White/Caucasian	Not Hispanic/Latino
11	Male	Black	Not Hispanic/Latino
12	Male	White/Caucasian	Not Hispanic/Latino
13	Female	White/Caucasian	Not Hispanic/Latino
14	Female	Black	Not Hispanic/Latino
15	Female	Black	Not Hispanic/Latino
16	Female	White/Caucasian	Not Hispanic/Latino

Table 3S. Differences in Network Connectivity in TRD and Healthy Controls

Seed and Associated Network	Seed Coordinates (MNI)	Cluster Size (# voxels) Cluster Peak Coordinates (MNI)
BA 25 Subgenual Cingulate Cortex Reward Network seed (Figure 4; Row 1)	-5,25,-10	664; L DLPFC (BA 9); -16,+54,+30 299; L Paracingulate Gyrus (BA 32); -8,+48,+10 282; L Orbitofrontal Cortex; (BA 47); -38,+26,-8 274; R Paracingulate Gyrus (BA 32); +8,+50,+8 252; R DLPFC (BA 9); +6,+56,+10
BA 10/14 Ventromedial Prefrontal Cortex Anterior Default Mode Network seed (Figure 4; Row 2)	-1,55,-3	452; L temporal pole (BA 38); -32,10,-30 312; L middle temporal gyrus, posterior division (BA 21); -56,-26,-6 186; R parahippocampal gyrus, posterior division (BA 36); 24,-28,-18 168; R hippocampus; 26,-20,-16 167; L frontal orbital cortex (BA 47); -28,22,-22
BA 24 Ventral Anterior Cingulate Cortex Executive Control Network seed (Figure 4, Row 3)	-5,0,30	436; R paracingulate cortex/anterior PFC (BA 10); 6,48,10 327; R anterior frontal pole (BA 10); 20,58,4 312; R dorsal ACC (BA32); 0,38,10 298; L dorsal ACC (BA 32); -6,46,14 264; L anterior frontal pole (BA 10); -18,58,-2
BA 23/31 Posterior Cingulate Cortex Posterior Default Mode Network seed (Figure 4; Row 4)	-1,-61,38	684; L Precuneous Cortex (BA 31); -4,-54,48 590; R superior parietal lobule (BA 7); 28,-48,58 390; L superior parietal lobule (BA 7); -28,-46,58 259; L postcentral gyrus (BA 40); -42,-30,42 186; L lateral occipital cortex (BA 7); -22,-68,40
BA 32	-1,22,35	1868; R frontal pole (BA 10); 28,48,18. 1311; R middle frontal gyrus (BA 8); 36,16,46

Dorsal Anterior Cingulate Cortex Salience Network seed (Figure 4, Row 5)		622; R anterior cingulate gyrus (BA 24); 2,34,14 506; R precentral gyrus; 40,4,48 278; R paracingulate gyrus (BA 10); 12,42,16
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Supplementary Table 4S Behavioral Assessments Following N₂O/Placebo Inhalation

	Nitrous Oxide Timepoints			Placebo Timepoints		
	0 (baseline)	2 hour post	24 hour post	0 (baseline)	2 hour post	24 hour post
n						
controls	16	16	16	17	17	17
TRD	14	14	14	14	14	14
Treatment = N2O (%)						
controls	16 (100.0)	16 (100.0)	16 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
TRD	14 (100.0)	14 (100.0)	14 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sex = Female (%)						
controls	7 (43.8)	7 (43.8)	7 (43.8)	7 (41.2)	7 (41.2)	7 (41.2)
TRD	4 (28.6)	4 (28.6)	4 (28.6)	4 (28.6)	4 (28.6)	4 (28.6)
MADRS (mean, SD)						
controls	0.21 (0.58)	0.29 (0.47)	0.43 (1.09)	1.24 (2.36)	1.47 (2.58)	0.88 (2.55)
TRD	27.64 (15.18)	16.21 (12.39)	18.00 (13.36)	30.21 (7.90)	17.93 (10.62)	16.29 (10.31)
HAMD-17 (mean, SD)						
controls	0.43 (0.76)	0.21 (0.58)	0.71 (1.33)	1.24 (1.99)	1.71 (2.39)	1.12 (2.39)
TRD	17.14 (8.87)	11.23 (6.67)	11.64 (7.35)	19.07 (4.25)	11.93 (5.77)	11.71 (5.89)
QIDS (mean, SD)						
controls	2.36 (2.62)	1.71 (1.54)	1.50 (1.51)	2.47 (1.50)	1.94 (1.20)	1.65 (1.32)
TRD	13.43 (6.32)	8.93 (6.16)	9.93 (5.97)	14.64 (4.22)	9.64 (5.46)	9.29 (5.88)
CADSS (mean, SD)						
controls	0.14 (0.36)	0.07 (0.27)	0.00 (0.00)	0.29 (1.21)	0.76 (2.91)	0.76 (2.66)
TRD	1.91 (4.30)	1.00 (3.46)	0.93 (2.64)	0.93 (2.92)	1.38 (3.48)	0.50 (1.87)
POMS (mean, SD)						
controls	30.79 (9.82)	28.69 (10.61)	27.43 (9.98)	31.80 (8.28)	27.50 (7.70)	26.75 (7.09)
TRD	55.00 (23.61)	34.00 (18.00)	37.64 (18.25)	55.38 (18.50)	35.15 (16.00)	36.15 (15.77)
BPRS (mean, SD)						
controls	24.21 (0.58)	24.14 (0.53)	24.29 (0.73)	25.06 (2.28)	24.94 (2.01)	25.12 (2.12)
TRD	37.08 (8.61)	31.14 (6.97)	32.38 (7.16)	38.29 (5.37)	31.14 (4.83)	31.93 (5.47)