

Figure S1: Chemical synthesis and spectroscopic characterization of (4-[[2-(2-Methyl-2-propanyl)oxy]carbonyl]-2-phenylpiperazinyl){4-[(4-trifluoromethoxy)phenyl]-1H-1,2,3-triazol-1-yl}metanone (DO53).

Synthetic Procedure: Diisopropylethylamine (275 mg, 2 mmol) followed by triphosgene (100 mg, 0.34 mmol) was added to a solution of 1-Boc-3-phenylpiperazine (175 mg, 0.65 mmol) dissolved in anhydrous tetrahydrofuran (5 mL), and the resultant reaction mixture was stirred 1 hour on ice. The reaction mixture was poured into water (5 mL) and extracted with ethyl acetate, and the organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was kept under vacuum overnight, then dissolved in anhydrous tetrahydrofuran (10 mL), to which diisopropylethylamine (275 mg, 2 mmol), 4-(dimethylamino)pyridine (80 mg, 0.65 mmol) and 4-(4-trifluoromethoxyphenyl)-1H-1,2,3-triazole (150 mg, 0.65 mmol) were added chronologically. The reaction mixture was warmed and stirred for 2 hours at 50 °C. The reaction mixture was cooled to ambient temperature and poured into saturated aqueous ammonium chloride solution and extracted with ethyl acetate. Combined organic layer was washed with water and brine, dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by silica gel column chromatography (ethyl acetate : hexane = 1:8, v/v) to afford (4-[[2-(2-methyl-2-propanyl)oxy]carbonyl]-2-phenylpiperazinyl){4-[(4-trifluoromethoxy)phenyl]-1H-1,2,3-triazol-1-yl}metanone (DO53) (119 mg, 34%).

Spectroscopic Characterization: ^1H NMR (DMSO- d_6 , 600 MHz): 1.33 (s, 9H), 3.03-3.20 (m, 1H), 3.21-3.42 (m, 1H), 3.45-3.59 (m, 1H), 3.69-3.89 (m, 1H), 3.95-4.10 (m, 1H), 4.45-4.55 (m, 1H), 5.58 (s, 1H), 7.25-7.35 (m, 1H), 7.36-7.42 (m, 4H), 7.48 (d, 2H, $J = 8.6$ Hz), 8.08 (d, 2H, $J = 8.6$ Hz), 8.19 (s, 1H). ESI MS calcd. $\text{C}_{25}\text{H}_{26}\text{F}_3\text{N}_5\text{O}_4$ $[\text{M}+\text{H}]^+$ 518.20, MS found 518.08.

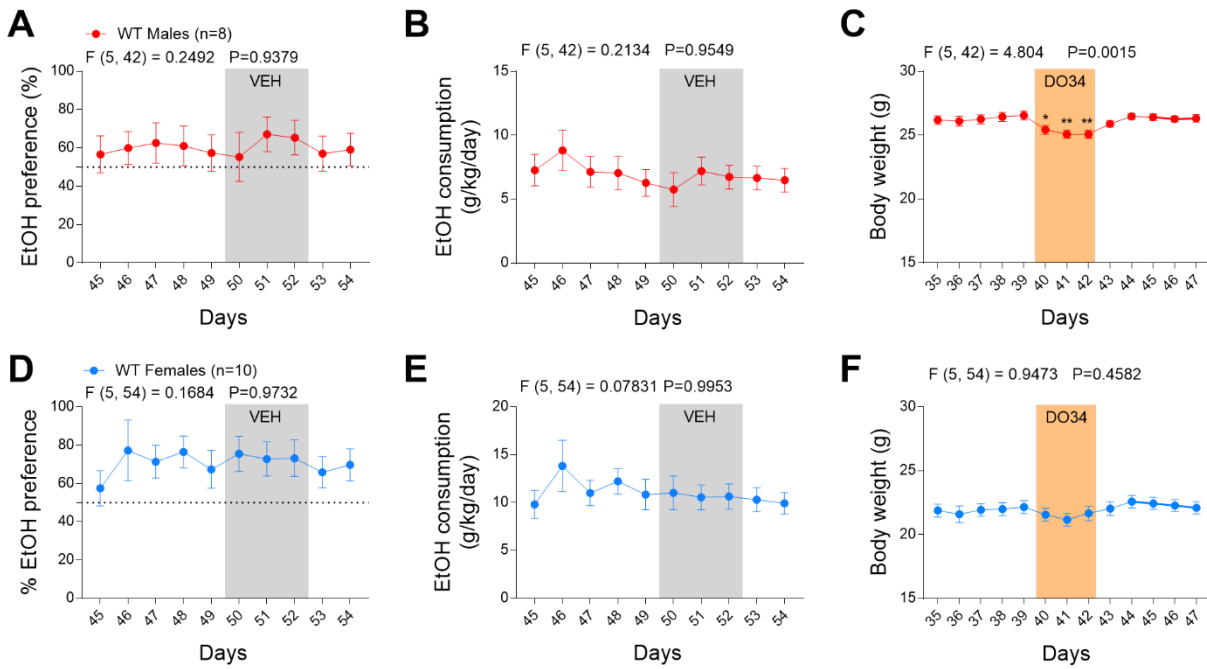


Figure S2: Vehicle treatment does not affect EtOH preference or consumption and DO34 reduces body weight only in male mice. Vehicle treatment had no effect on EtOH (A, D) preference or (B, E) consumption in either sex. (c) DO34 decreased body weight in male mice. Body weight recovered after cessation of DO34 treatment. (F) DO34 had no effect on body weight in female mice. All DO34 treatments were dosed at 50 mg/kg. Data were analyzed by one-way ANOVA on time points 49-54 (A-B, D-E) or 39-44 (C, F) (to include baseline, drug treatment, and two recovery points) followed by a Holm-Sidak test for multiple comparisons to baseline control. Sample size *n*, *P*, and *F* values for main effects of drug treatment reported on graphs. Significance for post-hoc multiple comparisons reported on graphs (**P*<.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001). Data are mean ± SEM.

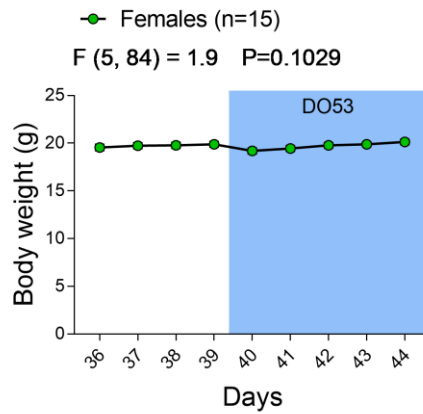


Figure S3: DO53 treatment has no effect on body weight in female mice. Treatment with the control compound DO53 (5 days) had no effect on body weight. Baseline data were averaged for analyses. Data analyzed by one-way ANOVA on averaged baseline (Days 36-39) and individual treatment days followed by a Holm-Sidak test for multiple comparisons to averaged baseline. Sample size n and P and F values for main effects of drug treatment reported on graph. All DO53 treatments were dosed at 50 mg/kg. Female mice were used for these experiments. Significance for post-hoc multiple comparisons reported on graphs (* $P < .05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Data are mean \pm SEM.

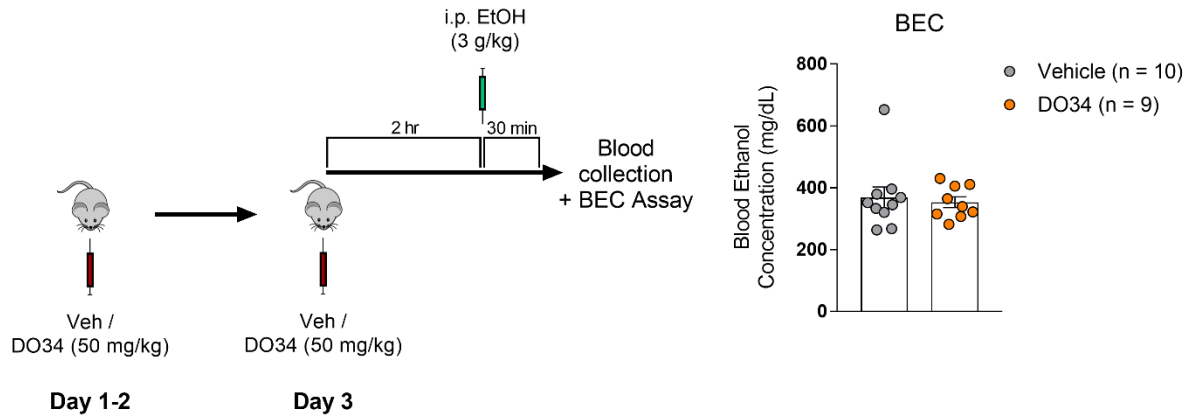


Figure S4: DO34 does not alter blood ethanol concentrations. (*left*) Schematic of experimental design for BEC measurements. (*right*) DO34 treatment had no effect on BEC 30 min after 3 g/kg i.p. EtOH injections. Sample size n reported on graph. Data analyzed by two-tailed student's t-test ($P=0.7145$). Data are mean \pm SEM. BEC, blood ethanol concentration.

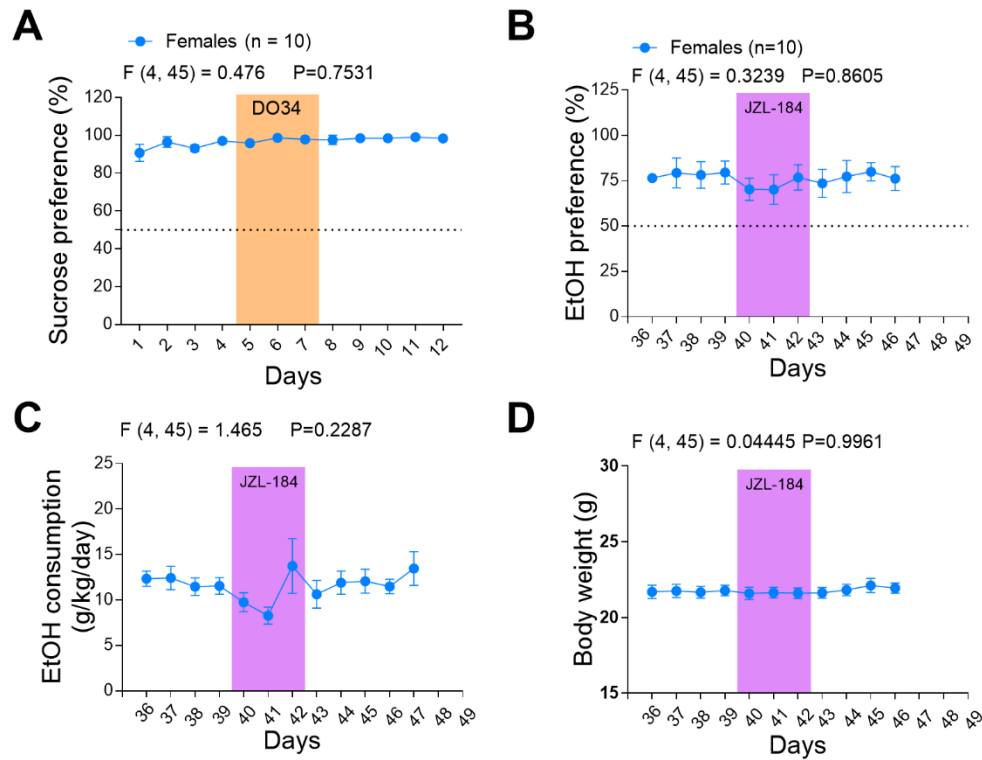


Figure S5: DO34 does not alter sucrose preference and 2-AG augmentation has no effect on EtOH drinking or body weight. (A) DO34 treatment had no effect on 2BC sucrose preference. (B) Treatment with the monoacylglycerol lipase inhibitor JZL-185 had no effect on EtOH preference, (C) EtOH consumption, or (D) body weight. Data were analyzed by one-way ANOVA on time points (A) 4-8 or (B-D) 39-41 (to include baseline, drug treatment, and one recovery point) followed by a Holm-Sidak test for multiple comparisons to baseline control. Sample size n , P , and F values for main effects of drug treatment reported on graphs. All DO34 treatments were dosed at 50 mg/kg. All JZL-184 treatments were dosed at 10 mg/kg. Female mice were used for these experiments. Data are mean \pm SEM.

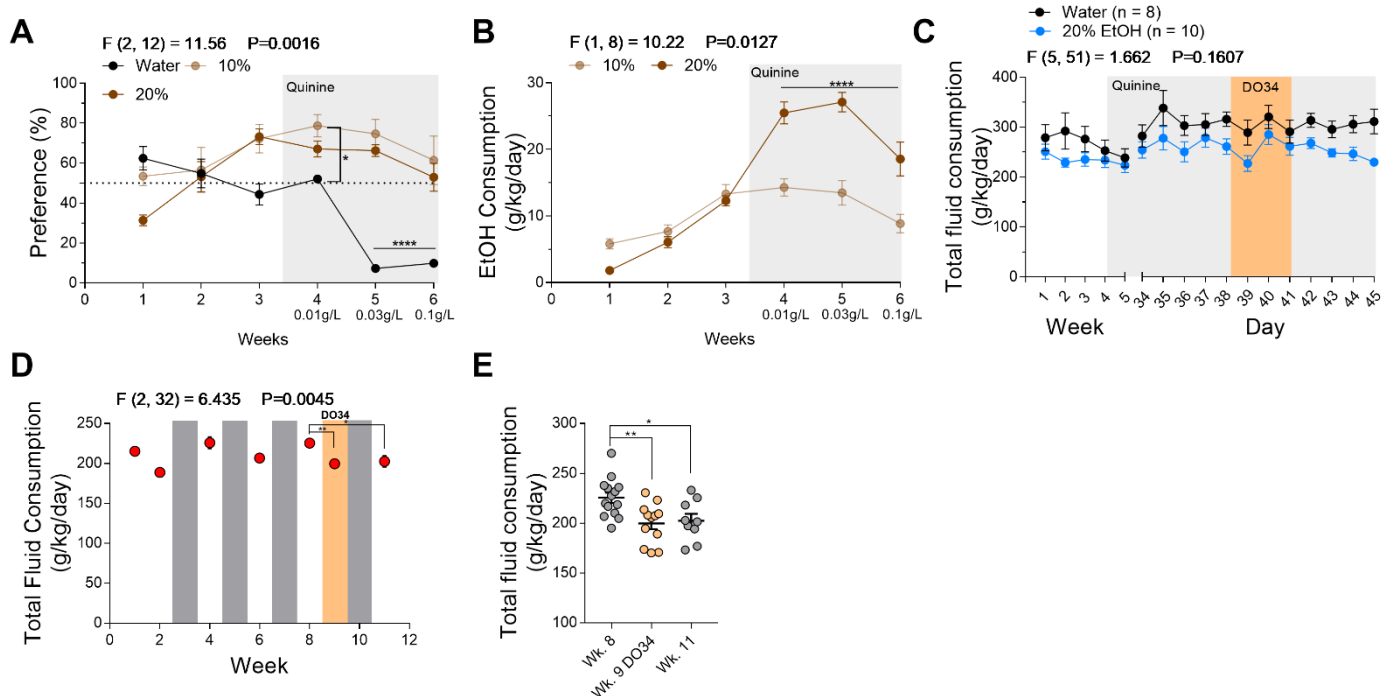


Figure S6: Mice reliably drink quinine-adulterated EtOH and DO34 has variable but minimal effects on total fluid consumption across EtOH drinking models. (A) Mice showed significantly higher preference for EtOH + quinine compared to water + quinine at 0.03 and 0.1 g/L quinine. (B) Mice drank significantly higher levels of 20% EtOH + quinine compared to 10% EtOH + quinine. (C) DO34 treatment had no effect on total fluid consumption in the aversion-resistant drinking model. (D) DO34 treatment reduced total fluid intake in the chronic intermittent ethanol model compared to the baseline week and fluid consumption remained decreased during the recovery week. (E) Graph depicting individual mouse total fluid consumption during baseline, treatment, and recovery weeks. Fluid consumption is lower in treatment and recovery weeks compared to baseline, but treatment and recovery consumption levels are not significantly different. (A-C) Data were analyzed by repeated measures two-way ANOVA during (A-B) quinine exposure or (C) time points 38-43 (to include baseline, drug treatment, and two recovery points), followed by a Holm-Sidak test for multiple comparisons. (D-E) Data were analyzed by one-way ANOVA on time points 8, 9, and 11, followed by a Holm-Sidak test for multiple comparisons between these 3 time points. *P* and *F* values for main effects of drug or quinine treatment reported on graphs. *n* = 5 mice per group in (A-B). Sample size *n* reported on graph in (C). *n* = 9-14 mice in (D-E). Significance for post-hoc multiple comparisons reported on graphs (**P*<.05, ***P*<.01, *****P*<.0001). Data are mean ± SEM.

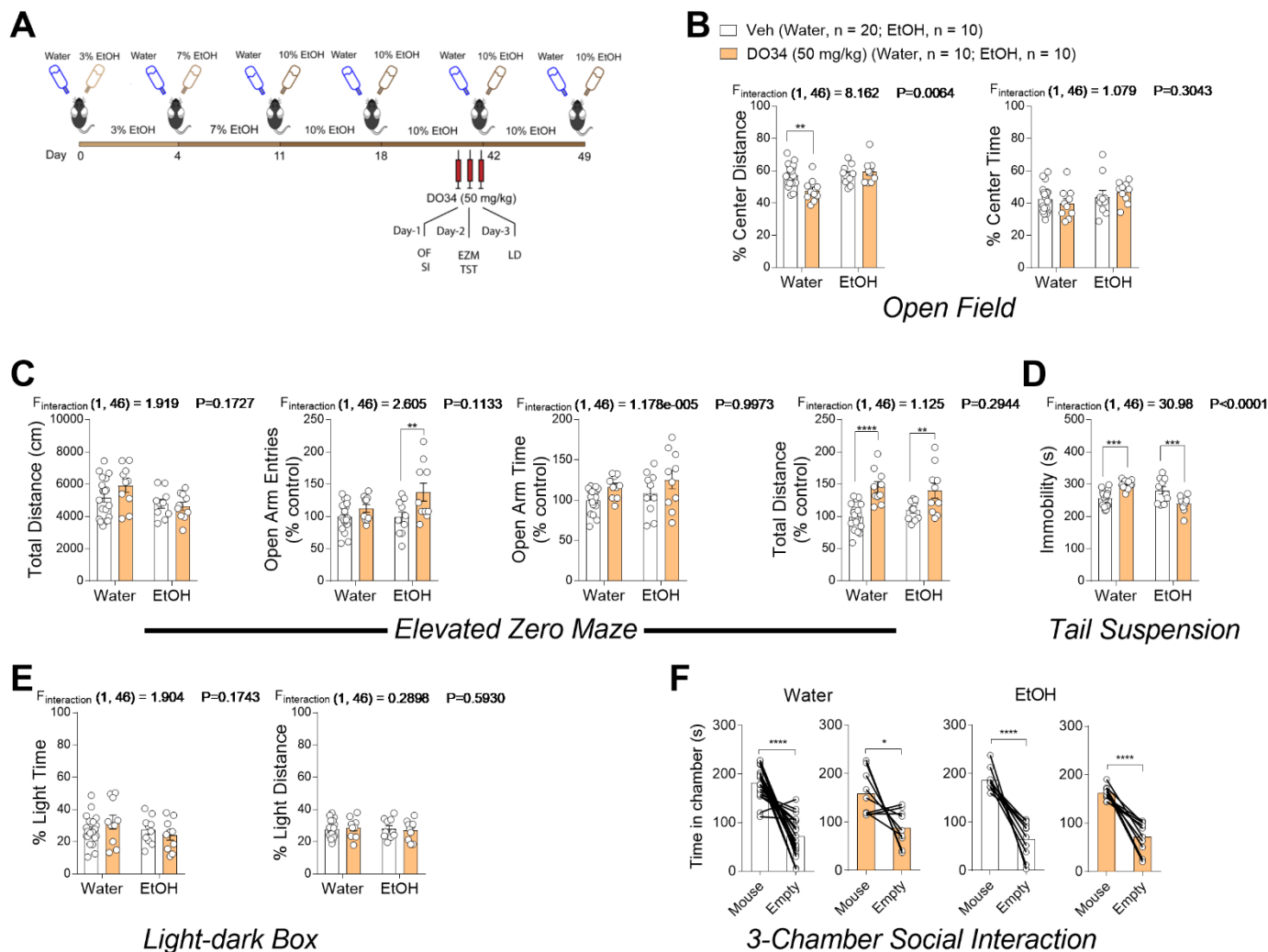


Figure S7: DO34 treatment does not precipitate negative affective phenotypes after chronic drinking at 10% EtOH. (A) Schematic depicting 2BC drinking paradigm and time course of behavioral testing. (B) DO34 treatment decreased open field test % center time in water control mice but not in EtOH drinking mice. (C) DO34 treatment increased % open arm entries in EtOH mice but not water control mice and increased total distance travelled in both water and EtOH drinking mice. (D) DO34 increased immobility time in the tail suspension test in water control mice but decreased immobility time in EtOH mice. (E) DO34 treatment had no effect on anxiety-like behaviors in the light-dark box assay. (F) DO34 treatment had no effect on social behaviors in the 3-chamber social interaction test. (B-E) Data were analyzed by two-way ANOVA or (F) paired t-test followed by a Holm-Sidak test for multiple comparisons between all groups. Female mice were used in all experiments. *P* and *F* values for EtOH x drug interaction reported on graphs. Significance for post-hoc multiple comparisons reported on graphs (**P*<.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001). Data are mean ± SEM.

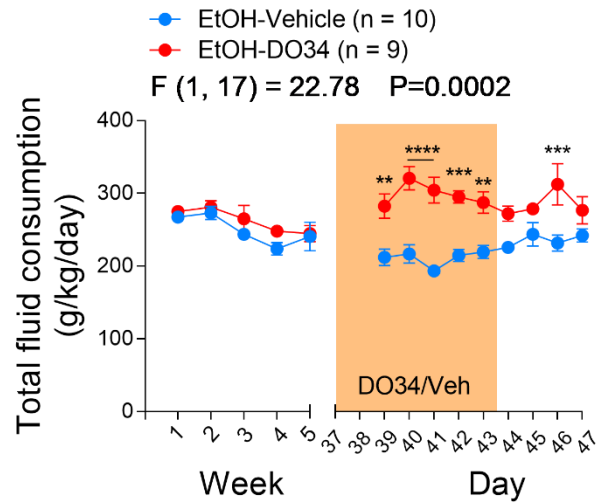


Figure S8: DO34 treatment does not precipitate negative affective phenotypes after chronic drinking at 10% EtOH. DO34 increased total fluid consumption in the relapse drinking model. Female mice were used in this experiment and DO34 was dosed at 50 mg/kg. Data analyzed by repeated measures two-way ANOVA followed by a Holm-Sidak test for multiple comparisons between treatment conditions. Sample size n , P and F values for main effects of drug treatment, significance for post-hoc multiple comparisons reported on graphs (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Data are mean \pm SEM.

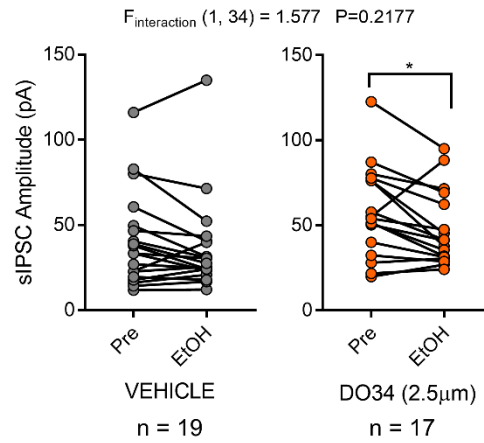


Figure S9: EtOH reduces sIPSC amplitude onto putative dopamine neurons in the posterior VTA of DO34-treated slices. Bath application of 100mM EtOH reduced sIPSC amplitude in DO34- but not vehicle-treated slices. All cells recorded were putative dopamine neurons visually-identified by their red fluorescence (see main text, **Fig. 5A**). Data were analyzed by repeated measures two-way followed by a Holm-Sidak test for multiple comparisons between baseline and EtOH treatment. Vehicle, $n = 19$ cells; DO34, $n = 17$ cells; from 13 mice. P and F value for EtOH x DO34 interaction reported on graph. Significance for post-hoc multiple comparisons reported on graph (* $P < .05$).

Midbrain Punches

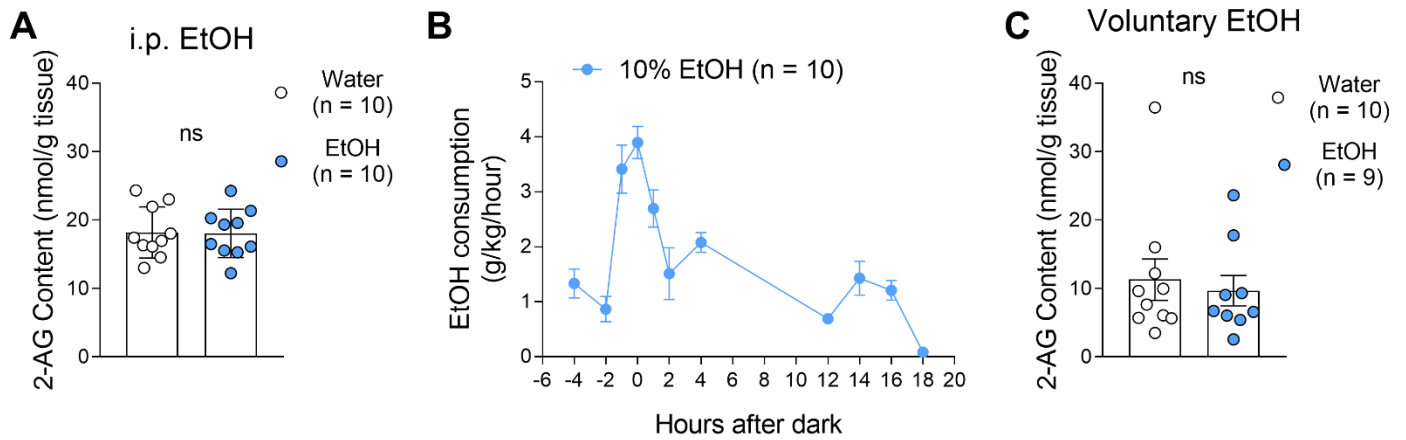


Figure S10: EtOH does not increase 2-AG levels measured by mass spectrometry in midbrain punches containing VTA. (A) Intraperitoneal (i.p.) EtOH injection (3 g/kg) had no effect on midbrain punch 2-AG levels 30 minutes after injection. (B) EtOH consumption time course of an independent cohort in our 2BC drinking paradigm. (C) Voluntary 2BC EtOH intake had no effect on midbrain punch 2-AG levels when collected between 0-2 hours after dark. (A, C) Data analyzed by two-tailed student's t-test. Sample size n reported on graphs. Data are mean \pm SEM.

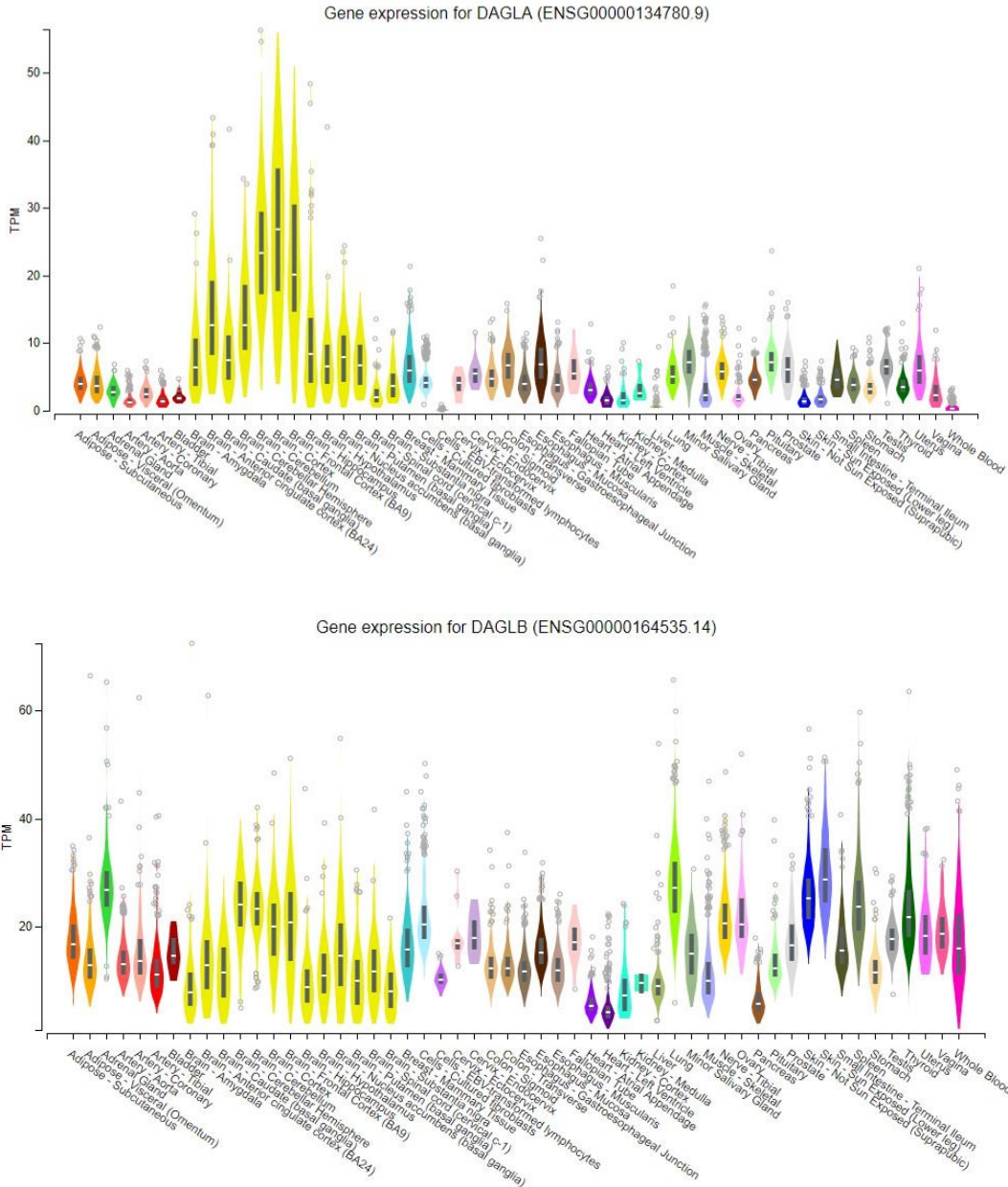


Figure S11: GTEx data on tissue-specific expression of *DAGLA* and *DAGLB* expression: (top) Expression profile for *DAGLA*, demonstrating highest expression in brain tissues (yellow). **(bottom)** Expression profile for *DAGLB*, demonstrating ubiquitous tissue distribution. Data are expressed as transcripts per million reads (TPM), and obtained from the GTEx Portal (dbGaP Accession phs000424.v8.p2; see <https://gtexportal.org/home>)

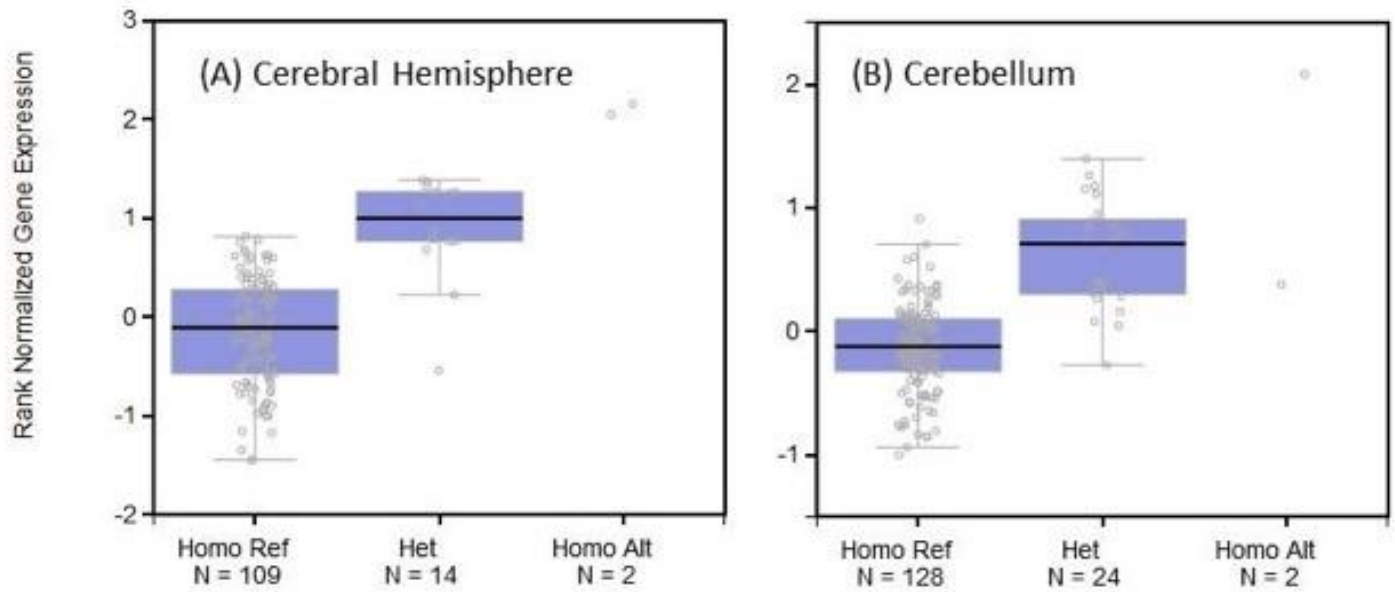


Figure S12: GTEx data on the effect of *DAGLα* genetic variant rs11604261 on relative gene expression: The effect of the common variant rs11604261 on *DAGLα* expression in two brain regions. Normalized expression values are plotted for the three genotypes: homozygous reference, heterozygous, and homozygous alternative allele. Data obtained from the GTEx Portal (dbGaP Accession phs000424.v8.p2; see <https://gtexportal.org/home>).

Analytes were detected by selected reaction monitoring (SRM) as $[M + H]^+$ complexes in positive ion mode except for AA which was detected by SRM as in negative ion mode (as the $[M - H]^-$ ion). The table below gives the Q1 and Q3 values for each analyte (mass in parentheses is the mass of the deuterated internal standard):

<u>Analyte</u>	<u>Q1 (m/z)</u>	<u>Q3 (m/z)</u>
2AG (-d5)	379.2 (384.2)	287.2 (287.1)
AEA (-d4)	348.2 (352.2)	62 (66)
AA (-d8)	303.2 (311.2)	259.1 (267.1)
PGE2_D2 (-d4)	351.2 (355.2)	271.1 (275.1)

The samples were chromatographed on a Acquity C18 column with a gradient elution scheme. The gradient profile was as follows: 0-0.25 min, 62%B, %B was then increased to 99% over 3 minutes, held at 99% for 2 more 5 minutes, and then returned to initial conditions at 5.25 minutes. Mobile phase A was water with 0.1% Formic Acid and 3%B and Mobile phase B was 4:1 acetonitrile:methanol with 0.1% formic acid and 1% of A.

Table S1: Detection methods for LC-MS/MS analysis of analytes and deuterated internal standards. 2-AG, 2-arachidonoylglycerol; AEA, anandamide or N-arachidonylethanolamine; AA, arachidonic acid; PGE2_D2, prostaglandins E2 and D2.