Supporting Information

Walker et al. 10.1073/pnas.1416196112

SI Materials and Methods

Drugs. (1*R*,4*R*,5*S*,6*R*)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268), 2*S*)-2-Amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495), and D-(-)-2-Amino-5-phosphonopentanoic acid (AP5) were purchased from R&D Systems. See schemes in *MRK-8-29 Chemical Synthesis* section for details on the synthesis of MRK-8-29. Selective NAMs of $mGlu_3$, VU0469942, and VU0477950 were synthesized as previously described (1). For electrophysiology experiments, stock solutions of LY379268 and LY341495 were made in water containing 1.0 Meq and 1.2 Meq of NaOH, respectively, AP5 in water, and $mGlu_3$ NAMs in DMSO. All compounds were diluted to their final concentrations in aCSF and bath applied as described in the text. For behavioral studies, VU0477950 was maintained in suspension in 10% (vol/vol) Tween 80 with sonication and heating (37–45 °C). This formulation was vortexed for 30 s immediately before i.p. injection at a volume of 0.10 mL/10 g body weight.

Ca^{2+} Imaging. Imaging experiments were conducted only on cells exhibiting stereotypical properties of pyramidal cells. Experiments commenced after at least a 15-min dialysis time and were conducted in the presence of tetrodotoxin (TTX) (1 μM) to isolate Ca^{2+} signals due to direct activation of postsynaptic receptors and to exclude modulation of presynaptic neurotransmitter release. Agonist application occurred for 10 min after fluorescence readings were stable for at least 5 min. Application of $mGlu_3$ NAMs began 5 min before the beginning of the baseline recording and coterminated with the agonist application. Images were collected with a Cool Snap HQ camera (Photometrics) and MetaFluor software (Molecular Devices). An external shutter was mounted to a mercury light source (Olympus Instruments), connected to the microscope with a liquid light guide tube, and controlled with a Lambda 10-2 (Sutter Instruments) interfaced with the imaging software. Cellular fluorescence was measured from a region within the soma of the labeled neuron and was background subtracted using a fluorescence reading taken from a region near the cell, but not containing any processes. Changes in fluorescence in the background-subtracted signal were calculated as ΔF/F.

Statistical Analysis. All statistical analyses were performed in GraphPad Prism 5. The effects of various pharmacological and genetic conditions on LTD were compared using a one-way analysis of variance (ANOVA) with Tukey post hoc tests. An unpaired *t* test was used to compare the effects of VU0469942 to control conditions in mean ΔF/F Ca^{2+} imaging experiments. A two-way, repeated-measures ANOVA was used to compare freezing during conditioning (trial × dose) and extinction (block × dose). When a significant interaction was detected ($P \leq 0.05$), Bonferroni post hoc tests were used to compare individual means within each trial or block of trials, as appropriate. The effects of VU0477950 on initial CS memory, the average number of trials to criterion, and the first block of extinction retrieval testing were compared with vehicle, using a one-way ANOVA with Tukey post hoc analysis. The effects of vehicle and VU0477950 on locomotor activity were analyzed with a two-way repeated-measures ANOVA and Bonferroni post hoc tests.

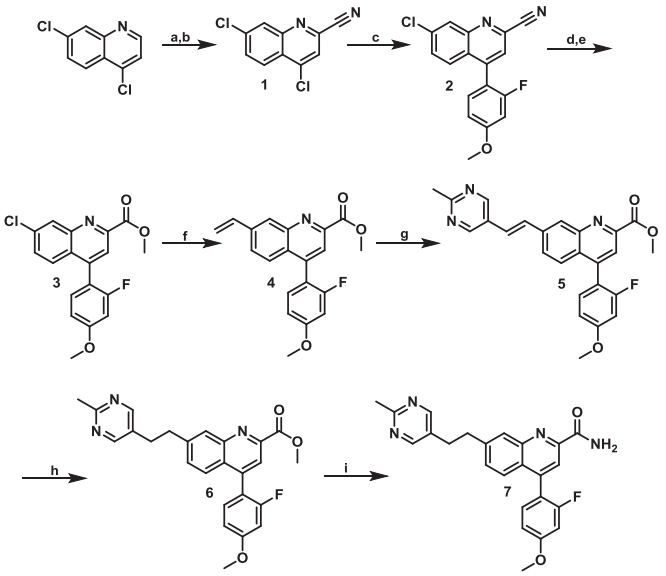
Chemical Analysis. NMR spectra were recorded on a Bruker-DRX 400 (400 MHz) spectrophotometer. ^1H chemical shifts are reported in parts per million relative to the residual $CDCl_3$ peak as an internal standard set to 7.26 ppm. ^{13}C chemical shifts are reported in parts per million with the residual $CDCl_3$ carbon peak set to 77.16 ppm. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet, dd = doublet of doublets), coupling constant (Hz), integration. Low-resolution mass spectra were obtained on an Agilent 6130 Quadrupole LC/MS with electrospray ionization. Analytical TLC was performed on 0.25-mm silica plates from Sorbent Technologies and visualized with UV light. Analytical HPLC was performed on an Agilent 1200 with UV detection at 214 nm and 254 nm along with evaporating light scattering detector (ELSD) detection. Preparative purification was performed on a Gilson chromatograph, using a Luna 5u C18 (2) 100A AXIA column (30 × 50 mm), using a water/acetonitrile gradient. Purities of compounds were in all cases greater than 98%, as determined by reverse-phase HPLC analysis.

Cell Culture. Rat $mGlu_2$($mGlu_2$)/HEK-293 cells stably transfected expressing the chimeric G-protein $G_{\alpha 15}$ were cultured in 90% Dulbecco's modified Eagle media (DMEM), 10% dialyzed FBS, 100 units/mL penicillin/streptomycin, 20 mM Hepes (pH 7.3), 1 mM sodium pyruvate, 2 mM glutamine, 0.1 mM nonessential amino acids, 700 μg/mL G418 sulfate (Mediatech), and 600 ng/mL puromycin at 37 °C in the presence of 5% CO_2. Rat $mGlu_3$($mGlu_3$)/TReX cells transfected expressing the chimeric G-protein $G_{\alpha 15}$ under the control of a tetracycline inducible promoter were cultured in 90% DMEM, 10% dialyzed FBS, 100 units/mL penicillin/streptomycin, 20 mM Hepes (pH 7.3), 1 mM sodium pyruvate, 2 mM glutamine, 0.1 mM nonessential amino acids, 700 μg/mL G418 sulfate (Mediatech), 100 μg/mL hygromycin, and 5 μg/mL Blasticidin S at 37 °C in the presence of 5% CO_2. All cell culture reagents were purchased from Invitrogen unless otherwise noted.

Calcium Mobilization Assays. Rat $mGlu_2$/$G_{\alpha 15}$/HEK-293 or $mGlu_3$/$G_{\alpha 15}$/TReX cells (15,000 cells/20 μL per well) were plated in black-walled, clear-bottomed, poly-d-lysine–coated, 384-well plates (Greiner Bio-One) in DMEM containing 10% dialyzed FBS, 20 mM Hepes, 100 units/mL penicillin/streptomycin, and 1 mM sodium pyruvate (Assay Media). The cells were grown overnight at 37 °C in the presence of 5% CO_2. During the day of assay, the medium was replaced with 20 μL of 1 μM S3 Fluo-4, AM (Invitrogen) prepared as a 10-mM stock in DMSO, mixed in a 1:1 ratio with 10% (wt/vol) pluronic acid F-127, and diluted in assay buffer [HBSS, 20 mM Hepes and 2.5 mM Probenecid (Sigma-Aldrich)] for 1 h at 37 °C. Dye was removed and replaced with 20 μL of assay buffer. Test compounds were transferred to daughter plates, using an Echo acoustic plate reformatter (Labcyte), and then diluted into assay buffer. Ca^{2+} flux was measured using the Functional Drug Screening System 7000 (FDSS 7000; Hamamatsu). Baseline readings were taken (10 images at 1 Hz; excitation, 470 ± 20 nm; emission, 540 ± 30 nm) and then 20 μL/well test compounds were added using the FDSS's integrated pipettor. Cells were incubated with compounds for ∼2.5 min and then an EC_{80} concentration of glutamate was applied. For concentration-response curve experiments, compounds were serially diluted 1:3 into 10-point concentration-response curves and were transferred to daughter plates, using the Echo. For fold-shift experiments, compounds were added at 2× their final concentration and then increasing concentrations of glutamate were added in the presence of vehicle or the appropriate concentration of test compound. Curves were fitted using a 4-point logistical equation,

using Microsoft XLfit (IDBS). Subsequent confirmations of concentration-response parameters were performed using independent serial dilutions of source compounds and data from multiple-day experiments were integrated and fitted using a 4-point logistical equation in GraphPad Prism. Calcium assays were used to assess activity of compounds at $mGlu_2$ and $mGlu_3$.

MRK-8-29 Chemical Synthesis.



Reagents and conditions: (a) mCPBA, DCM, 23 °C, 1 h; (b) TMSCN, Me_2NOCl, DCM, 23 °C, 16 h; (c) 10 mol% $Pd(PPh_3)_4$, Na_2CO_3, (2-F-4-OMe)PhB$(OH)_2$, Dioxane/H_2O, 75 °C, 18 h; (d) NaOH, EtOH, Reflux, 4 h; (e) H_2SO_4, MeOH, Reflux, 4 h; (f) 5 mol% $Pd(OAc)_2$, 10 mol% Sphos, Cs_2CO_3, BF_3KCHCH_2, Dioxane/H_2O, 85 °C, 1 h; (g) 5-Br-2-MePyr, Et_3N, $Pd(OAc)_2$, DMF, Reflux, 2 h; (h) Pd/C, H_2, MeOH, 23 °C, 1 h; (i) NH_3/MeOH, 60 °C, 8 h.

1) 4,7-Dichloroquinoline-2-Carbonitrile; $C_{10}H_4Cl_2N_2$. To a solution of 4,7-dichloroquinoline (9.90 g, 50 mmol) in dichloromethane (500 mL) was added 3-chloroperoxybenzoic acid (17.26 g, 100 mmol) in five portions, such that the temperature of the reaction did not rise above 34 °C. The resulting suspension was stirred for 1 h. The reaction was then quenched with an aqueous solution of NaOH (1 M, 500 mL) and extracted into dichloromethane (500 mL). The organic layer was separated, dried with $MgSO_4$, and filtered to yield a solution of 4,7-dichloro-1-oxido-quinolin-1-ium in dichloromethane, which was carried forward without purification. To this solution was added trimethylsilyl cyanide (13.00 mL, 100 mmol), followed by dimethylcarbamoyl chloride (9.50 mL, 100 mmol). The resulting solution was then heated to reflux for 48 h. The reaction was then quenched with a saturated aqueous solution of $NaHCO_3$ (500 mL), diluted with H_2O (500 mL), and extracted into dichloromethane (1 L). The organic layer was then separated, dried with $MgSO_4$, and filtered, and the solvent was removed under vacuum. Compound **1** (6.50 g, 58%) was isolated following recrystallization in methanol. LC (254 nm) 1.167 min (>99%); MS (ESI) m/z = 222.9; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)]: 8.22 (d, J = 9.0 Hz, 1H); 8.17 (d, J = 2.0 Hz, 1H); 7.77 (s, 1H); 7.74 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H) ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 149.3; 144.6; 138.9; 134.7; 131.9; 129.5; 126.0; 125.9; 123.8;116.6.

2) 7-Chloro-4-(2-Fluoro-4-Methoxyphenyl)Quinoline-2-Carbonitrile; $C_{17}H_{10}ClFN_2O$. To a stirred solution of compound **1** (6.50 g, 29.14 mmol) in 1,4-dioxanes:H_2O (9:1, 60 mL) was added (2-fluoro-4-methoxyphenyl)boronic acid (4.95 g, 29.14 mmol), Cs_2CO_3 (18.95 g, 58.30 mmol), and $Pd(PPh_3)_4$ (0.842 g, 0.728 mmol, 2.5 mol%) under argon. The reaction was stirred at 75 °C for 18 h. The reaction was then diluted with ethyl acetate (300 mL), washed with brine (300 mL), and filtered through a Celite plug. The organic layer was separated, dried with $MgSO_4$, and filtered, and the solvent was removed under vacuum. Compound **2** (7.27 g, 80%) was isolated following purification on reverse-phase HPLC. LC (254 nm) 1.250 min (>99%); MS (ESI) m/z =312.9; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)]: 8.20 (d, J = 2.0 Hz, 1H); 7.74(dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H); 7.63 (s, 1H); 7.59 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H); 7.29 (t, J = 8.5 Hz, 1H); 6.90 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 6.82 (dd, J_1 = 11.7 Hz, J_2 = 2.5 Hz, 1H); 3.91 (s, 3H) ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 162.6 (d, $^3J_{CF}$ = 11.3 Hz); 160.5 (d, $^1J_{CF}$ = 249.8 Hz); 149.2; 145.1; 137.6; 134.6; 132.1(d, $^3J_{CF}$ = 4.8 Hz); 130.7; 129.4; 127.7; 126.5; 124.9; 117.5; 115.5 (d, $^2J_{CF}$ = 15.4 Hz); 111.2 (d, $^4J_{CF}$ = 3.1 Hz); 102.6 (d, $^2J_{CF}$ = 25.2 Hz); 56.2.

3) Methyl-7-Chloro-4-(2-Fluoro-4-Methoxyphenyl)Quinoline-2-Carboxylate; $C_{18}H_{13}ClFNO_3$. To a stirred solution of compound **2** (7.27 g, 23.31 mmol) in ethanol (150 mL) was added an aqueous solution of NaOH (1 M, 225 mL). The reaction was heated to reflux for 14 h. After cooling to room temperature, the reaction was passed through a filter and the solid, 7-chloro-4-(2-fluoro-4-methoxyphenyl)quinoline-2-carboxylic acid, was retained. This solid was then suspended in methanol (180 mL), and concentrated H_2SO_4 (20 mL) was added dropwise with vigorous stirring. The reaction was heated to reflux for 2 h, then slowly neutralized to pH 7, using a saturated aqueous solution of $NaHCO_3$, and extracted into dichloromethane (250 mL). The organic layer was separated, dried with $MgSO_4$, and filtered, and the solvent was removed under vacuum. Compound **3** (6.16 g, 76%) was isolated following purification on reverse-phase HPLC. LC (254 nm) 1.218 min (>99%); MS (ESI) m/z = 345.9; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)]: 8.36 (d, J = 2.0 Hz, 1H); 8.31 (s, 1H); 7.73(dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H); 7.54 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H); 7.31 (t, J = 8.5 Hz, 1H); 6.88 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 6.81 (dd, J_1 = 11.7 Hz, J_2 = 2.5 Hz, 1H); 4.08 (s, 3H); 3.89 (s, 3H) ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.9; 162.3 (d, $^3J_{CF}$ = 11.3 Hz); 160.5 (d, $^1J_{CF}$ = 249.8 Hz); 148.7 (d, $^2J_{CF}$ = 12.1 Hz); 144.7; 136.6; 132.1(d, $^3J_{CF}$ = 4.8 Hz); 130.0; 129.4; 127.5; 127.4; 127.0; 122.8; 116.7 (d, $^2J_{CF}$ = 15.2 Hz); 111.0 (d, $^4J_{CF}$ = 3.0 Hz); 104.6 (d, $^2J_{CF}$ = 24.3 Hz); 56.2; 53.6.

4) Methyl-4-(2-Fluoro-4-Methoxyphenyl)-7-Vinylquinoline-2-Carboxylate; $C_{20}H_{16}FNO_3$. To a stirred solution of compound **3** (6.16 g, 17.82 mmol) in 1,4-dioxanes:H_2O (9:1, 90 mL) was added potassium vinyltrifluoroborate (4.78 g, 35.64 mmol), Cs_2CO_3 (5.67 g, 53.46 mmol), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (1.10 g, 2.67 mmol, 15 mol%), and $Pd(OAc)_2$ (0.30 g, 1.34 mmol, 7.5 mol%) under argon. The reaction was stirred at 85 °C for 1 h, diluted with ethyl acetate (250 mL), and then washed with brine (250 mL). The organic layer was separated, dried with $MgSO_4$, and filtered, and the solvent was removed under vacuum. Compound **4** (4.10 g, 68%) was isolated following purification on reverse-phase HPLC. LC (254 nm) 1.203 min (>99%); MS (ESI) m/z = 337.9; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)]: 8.36 (d, J = 2.0 Hz, 1H); 8.30 (s, 1H); 7.71(dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1H); 7.54 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H); 7.31 (t, J = 8.5 Hz, 1H); 7.03 (dd, J_1 = 17.6 Hz, J_2 = 11.0 Hz, 1H); 6.88 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 6.81 (dd, J_1 = 11.7 Hz, J_2 = 2.5 Hz, 1H); 5.97 (d, J = 17.6 Hz, 1H); 5.45 (d, J = 11.0 Hz, 1H); 4.08 (s, 3H); 3.89 (s, 3H) ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 165.8; 161.7 (d, $^3J_{CF}$ = 12.0 Hz); 160.2 (d, $^1J_{CF}$ = 249.6 Hz); 148.1; 147.6; 144.0; 139.4; 135.9; 131.9 (d, $^3J_{CF}$ = 4.8 Hz); 129.7; 129.5; 128.4; 126.4; 125.8; 122.7; 116.7(d, $^2J_{CF}$ = 15.2 Hz); 110.4; 101.9 (d, $^2J_{CF}$ = 28.0 Hz); 55.7; 53.2.

5) Methyl-4-(2-Fluoro-4-Methoxyphenyl)-7-(2-(2-Methylpyrimidin-5-yl)vinyl) quinoline-2-Carboxylate; $C_{25}H_{20}FN_3O_3$. To a stirred solution of compound **4** (3.76 g, 11.16 mmol) in *N*,*N*-dimethylformamide (100 mL) was added 5-bromo-2methylpyrimidine (1.93 g, 11.16

mmol), triethylamine (9.32 mL, 66.97 mmol), and $Pd(OAc)_2$ (0.25 g, 1.12 mmol, 10 mol%). The reaction was heated to reflux for 2.5 h, quenched with H_2O (100 mL), and then extracted into dichloromethane (250 mL).The organic layer was separated, dried with $MgSO_4$, and filtered, and the solvent was removed under vacuum. Compound **5** (1.32 g, 28%) was isolated following purification on reverse-phase HPLC. LC (254 nm) 1.074 min (>99%); MS (ESI) m/z = 429.9; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)]: 8.82 (s, 2H); 8.42 (s, 1H); 8.13 (s, 1H); 7.79 (s, 2H); 7.36 (d, J = 16.2 Hz, 1H), 7.35 (t, J = 8.5 Hz, 1H); 7.18 d, J = 16.2 Hz, 1H); 6.89 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 6.83 (dd, J_1 = 11.7 Hz, J_2 = 2.5 Hz, 1H); 4.10 (s, 3H); 3.91 (s, 3H); 2.78 (s, 3H) ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 167.2; 166.0; 162.1; 160.2 (d, $^1J_{CF}$ = 248.9 Hz); 157.1; 148.3 (d, $^2J_{CF}$ = 12.1 Hz); 144.2; 138.2; 132.3 (d, $^3J_{CF}$ = 5.3 Hz); 130.7; 129.5; 128.4; 127.6; 126.6; 124.2; 122.7; 117.0; 116.9(d, $^2J_{CF}$ = 15.8 Hz); 110.8 (d, $^4J_{CF}$ = 2.7 Hz); 102.3 (d, $^2J_{CF}$ = 25.3 Hz); 56.0; 53.4; 25.8.

6) Methyl-4-(2-Fluoro-4-Methoxyphenyl)-7-(2-(2-Methylpyrimidin-5-yl)ethyl) quinoline-2-Carboxylate; $C_{25}H_{22}FN_3O_3$. Compound **5** (1.25 g, 2.92 mmol) was placed in a flame-dried two-neck flask and dissolved in methanol (100 mL). Five percent Pd/C (636 mg, 0.3 mmol, 10 mol%) was added while stirring. The flask was purged and refilled with H_2 three times and then stirred at room temperature for 1 h. The reaction was then filtered through Celite, and compound **6** (0.85 g, 68%) was isolated after the solvent was removed under vacuum. LC (254 nm) 1.068 min (>99%); MS (ESI) m/z = 431.9; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)]: 8.39 (s, 2H); 8.11 (s,2H); 7.71(dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 7.39 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 7.32 (t, J = 8.5 Hz, 1H); 6.87 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 6.80 (dd, J_1 = 11.7 Hz, J_2 = 2.5 Hz, 1H); 4.07 (s, 3H); 3.89 (s, 3H); 3.14 (t, J = 8 Hz, 2H); 3.00 (t, J = 8 Hz, 2H); 2.67 (s, 3H) ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 166.5; 166.2; 162.0 (d, $^3J_{CF}$ = 11.3 Hz); 160.4 (d, $^1J_{CF}$ = 248.9 Hz); 157.1; 148.1; 147.9 144.2; 142.7; 132.3 (d, $^3J_{CF}$ = 5.9 Hz); 130.5; 130.0 (d, $^2J_{CF}$ = 13.6 Hz) ; 129.3; 127.1; 126.3; 122.3; 120.0; 117.1(d, $^2J_{CF}$ = 16.1 Hz); 110.7 (d, $^4J_{CF}$ = 2.7 Hz); 102.3 (d, $^2J_{CF}$ = 25.3 Hz); 56.0; 53.4; 37.2; 31.4; 25.8.

7) 4-(2-Fluoro-4-Methoxyphenyl)-7-(2-(2-Methylpyrimidin-5-yl)ethyl)quinoline-2-Carboxamide; $C_{24}H_{21}FN_4O_2$. Compound **6** (0.85 g, 1.98 mmol) was dissolved in methanol:ammonia (2 M, 5 mL) and stirred at 60 °C for 8 h. The solvent was removed under vacuum, and compound **7** (0.15 g, 20%) was isolated following purification on reverse-phase HPLC. LC (254 nm) 0.986 min (>99%); MS (ESI) m/z = 416.9 ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)]: 8.50 (s, 2H); 8.22 (s,1H); 8.12 (b, 1H); 7.94 (s, 1H) 7.73(dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 7.39 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 7.32 (t, J = 8.5 Hz, 1H); 6.87 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H); 6.80 (dd, J_1 = 11.7 Hz, J_2 = 2.5 Hz, 1H); 5.72 (b, 1h); 3.90 (s, 3H); 3.17 (t, J = 8 Hz, 2H); 3.06 (t, J = 8 Hz, 2H); 2.73 (s, 3H) ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 166.9; 165.9; 162.0 (d, $^3J_{CF}$ = 11.3 Hz); 160.4 (d, $^1J_{CF}$ = 248.9 Hz); 157.1; 148.1; 147.9 144.2; 142.7; 132.3 (d, $^3J_{CF}$ = 5.9 Hz); 130.5; 130.0 (d, $^2J_{CF}$ = 13.6 Hz); 129.3; 127.1; 126.3; 122.3; 120.0; 117.1(d, $^2J_{CF}$ = 16.1 Hz); 110.7 (d, $^4J_{CF}$ = 2.7 Hz); 102.3 (d, $^2J_{CF}$ = 25.3 Hz); 56.0; 37.2; 31.4; 25.8.

1. Wenthur CJ, et al. (2013) Discovery of (R)-(2-fluoro-4-((-4-methoxyphenyl)ethynyl) phenyl) (3-hydroxypiperidin-1-yl)methanone (ML337), an mGlu3 selective and CNS penetrant negative allosteric modulator (NAM). *J Med Chem* 56(12):5208–5212.

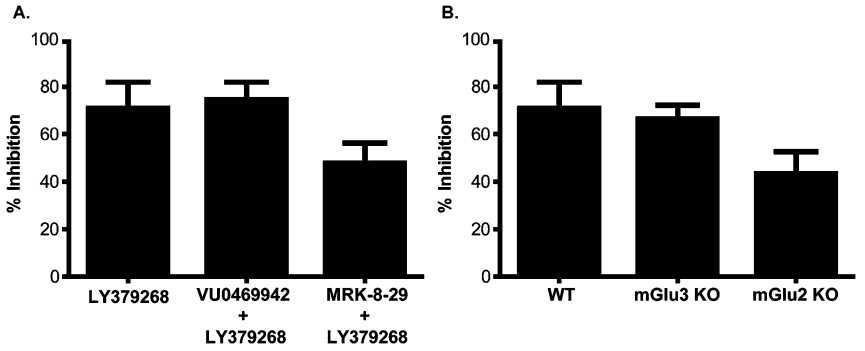


Fig. S1. Effects of group II mGlu subtype selective NAMs and receptor knockout on transient inhibition induced by LY379268. (*A*) The $mGlu_2$ NAM MRK-8-29 did not significantly reduce the transient depression relative to LY379268 alone or the $mGlu_3$ NAM VU0469942 ($P > 0.05$, one-way ANOVA). (*B*) Relative to WT and $mGlu_3$ KO mice, LY379268 did not induce a significant reduction in the magnitude of the transient depression in slice from $mGlu_2$ KO mice ($P > 0.05$, one-way ANOVA). Data are expressed as mean ± SEM.

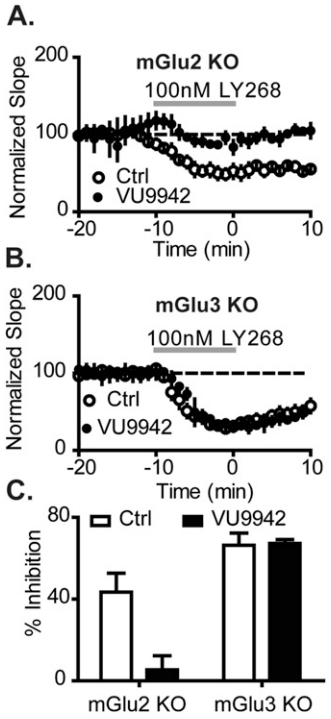


Fig. S2. (*A–C*) Selectivity of the $mGlu_3$ NAM VU0469942 was confirmed by measuring the maximal inhibition of layer V fEPSPs induced by LY379268 in mPFC slices from $mGlu_2$ and $mGlu_3$ KO mice. Open circles and bars are control experiments and solid plots are in the presence of VU0469942. Data are expressed as mean ± SEM.

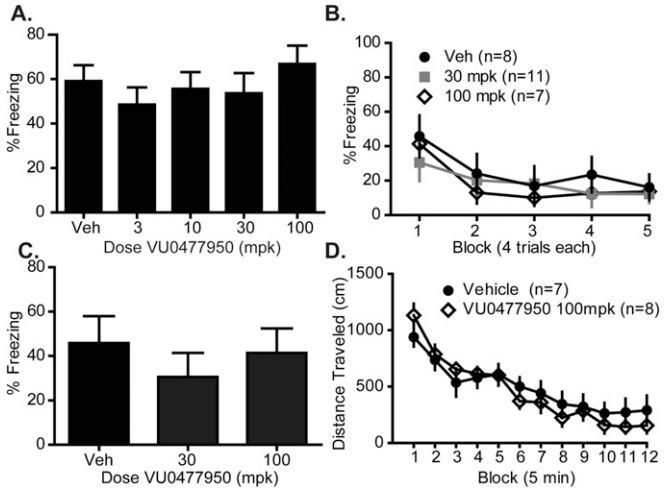


Fig. S3. The $mGlu_3$ NAM VU0477950 does not affect initial CS retrieval, extinction retrieval, or general locomotor activity. (*A*) Freezing behavior during the first four CS presentations during extinction training was comparable for all dosage groups, indicating the compound did not alter memory for the CS. (*B*) A subset of mice was tested on a second extinction session to evaluate extinction memory retrieval. (*C*) Comparison of freezing during the first block of trials presented in *B*. There were no significant group differences. (*D*) The effects of VU0477950 on motor activity were tested in a separate group of mice. There was no difference in the distance traveled between vehicle-treated and VU0477950-treated mice.

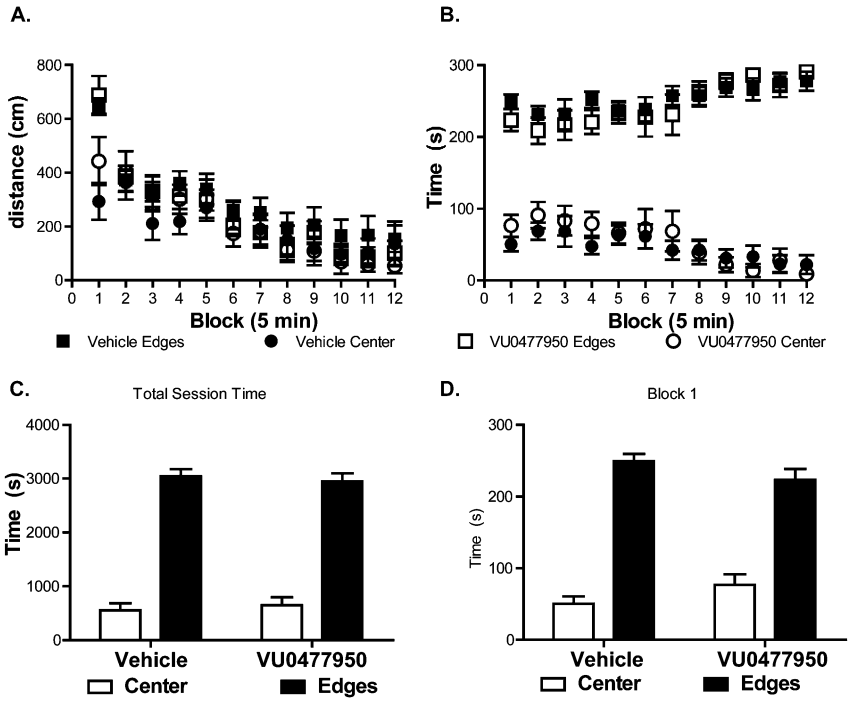


Fig. S4. The $mGlu_3$ NAM VU0477950 is not anxiogenic in an open-field activity chamber. (*A* and *B*) VU0477950 (open symbols, $n = 8$) does not significantly alter the amount of distance traveled (*A*) or time (*B*) spent in the center (circles) vs. the edges (squares) relative to vehicle-treated mice (solid symbols, $n = 7$). (*C*) Total time spent in the center (open bars) and edges (solid bars) during the 60-min session. (*D*) Total time spent in the center (open bars) and edges (solid bars) during the first 5-min block.