Title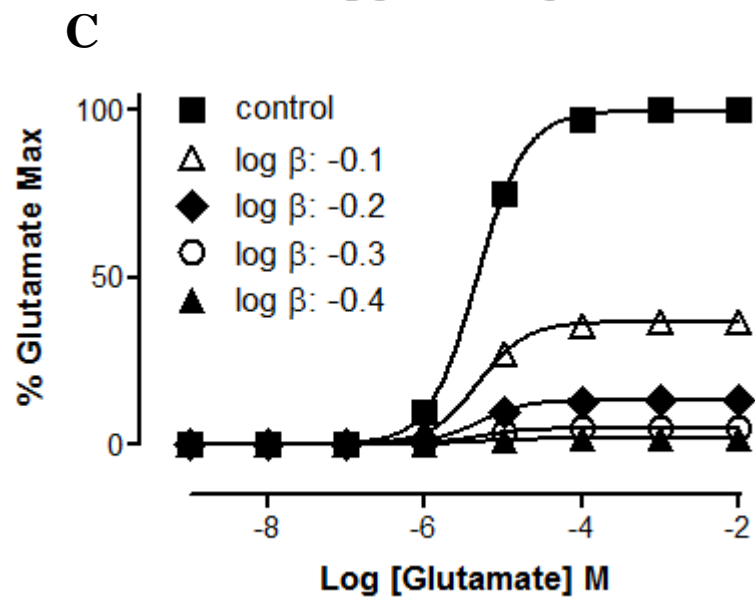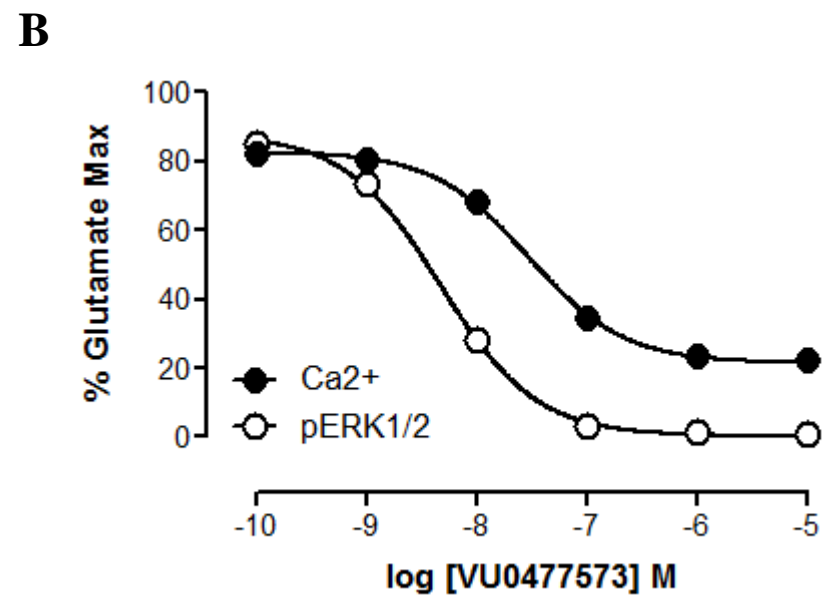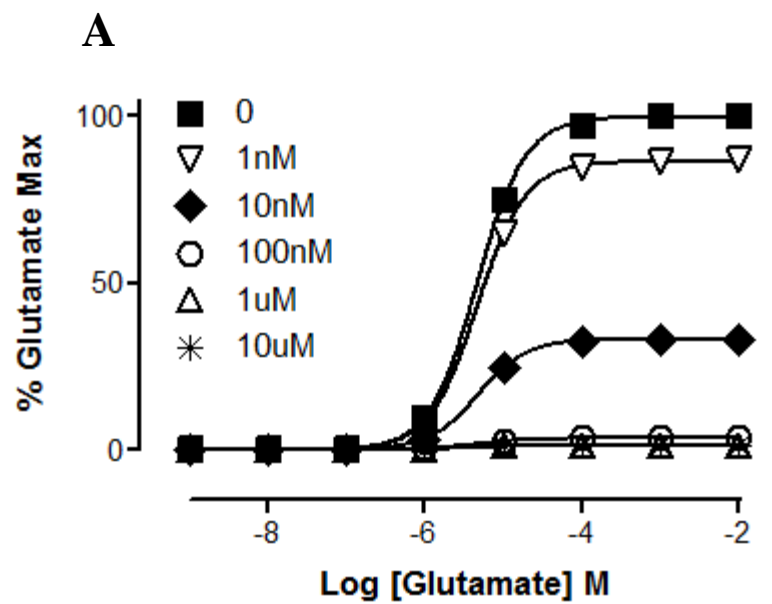: VU0477573: Partial negative allosteric modulator of the subtype 5 metabotropic glutamate receptor with *in vivo* efficacy

Authors: Hilary Highfield Nickols, Joannes P. Yuh, Karen J. Gregory, Ryan D. Morrison, Brittney S. Bates, Shaun R. Stauffer, Kyle A. Emmitte, Michael Bubser, Weimin Peng, Michael T. Nedelcovych, Analisa Thompson, Xiaohui Lv, Zixiu Xiang, J. Scott Daniels, Colleen M. Niswender, Craig W. Lindsley, Carrie K. Jones, P. Jeffrey Conn

Supplemental Figure 1. Simulating negative allosteric modulators of mGlu$_5$ with weak negative cooperativity. A) The operational model of allosterism was used to model the expected results from interactions between VU0477573 and glutamate using the phosphoERK1/2 assay. In this simulation τ_A, n, Em, basal and K_A values were taken from previously published data (Gregory et al., 2012), alpha (α) was assumed to equal 1. If VU0477573 has the same K_B and β constants as calculated from Figure 1C, Table1, then complete abolishment of the glutamate response is predicted. **B**) Plotting the predicted EC$_{80}$ inhibition curve from the pERK1/2 simulation in A as well as a simulation of the calcium mobilization data where the glutamate τ_A = 0.5677 as estimated from Figure 1C. Based the K_B and β constants derived for VU0477573, the inhibition curve for pERK1/2 (open circles) is predicted to be more potent (pEC$_{50}$: 8.3) and achieve complete blockade as compared with that observed in the calcium assay (closed circles; pEC$_{50}$: 7.52, bottom plateau: 22%). **C**) Exploring the relationship between negative cooperativity and inhibition of glutamate-mediated pERK1/2. In this simulation τ_A, n, Em, basal, K_B and K_A values are held constant as for panel A, the effect of 10 μM of NAM with varying degrees of cooperativity is demonstrated.
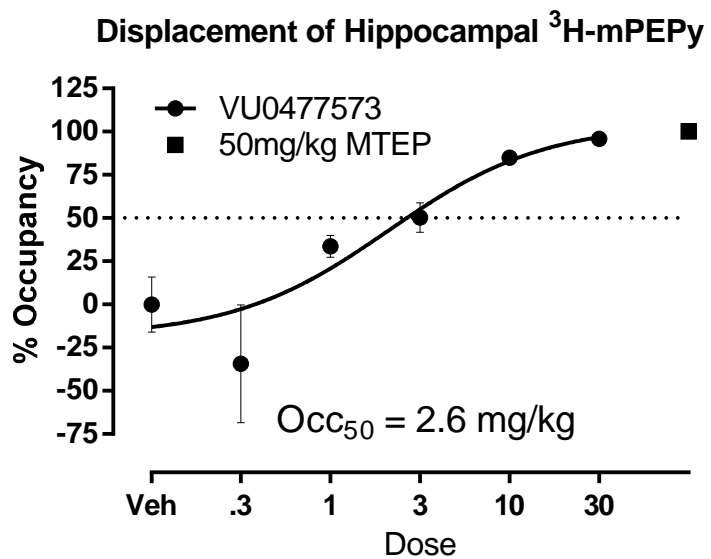
Supplemental Figure 2: Calculating [^{3}H]-mPEPy occupancy. A) Excluding bad tail vein injections (low to no radiation counts) and values >2 SD from the mean for each group; To obtain Occ_{50}, nonlinear regression was performed in Graphpad Prism using log(inhibitor) vs. response (three parameters); B) Excluding bad tail vein injections (low to no radiation counts) and values >2 SD from the mean for each group
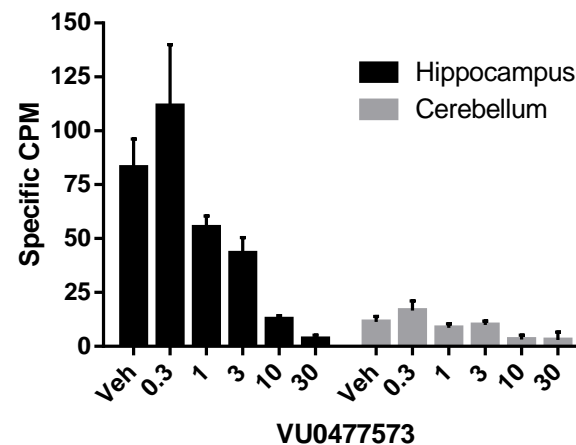
Supplemental Figure 1

Supplemental Figure 2

A

Displacement of Hippocampal ^{3}H-mPEPy

% Occupancy

- ● VU0477573
- ■ 50mg/kg MTEP

125
100
75
50
25
0
-25
-50
-75

Occ_{50} = 2.6 mg/kg

Veh .3 1 3 10 30

Dose

B

Specific CPM

150
125
100
75
50
25
0

■ Hippocampus
■ Cerebellum

Veh 0.3 1 3 10 30   Veh 0.3 1 3 10 30

VU0477573

	Hippocampus			Cerebellum		
	Mean	SEM	N	Mean	SEM	N
Veh	82.989	13.220	10	11.304	2.568	10
0.3	111.536	28.343	7	16.540	4.515	8
1	55.131	5.265	9	8.544	1.851	9
3	43.214	7.243	6	9.964	1.722	7
10	12.536	1.651	7	3.228	1.942	8
30	3.464	1.668	8	2.915	3.685	8