Photopharmacological manipulation of amygdala metabotropic glutamate receptor mGlu4 alleviates neuropathic pain

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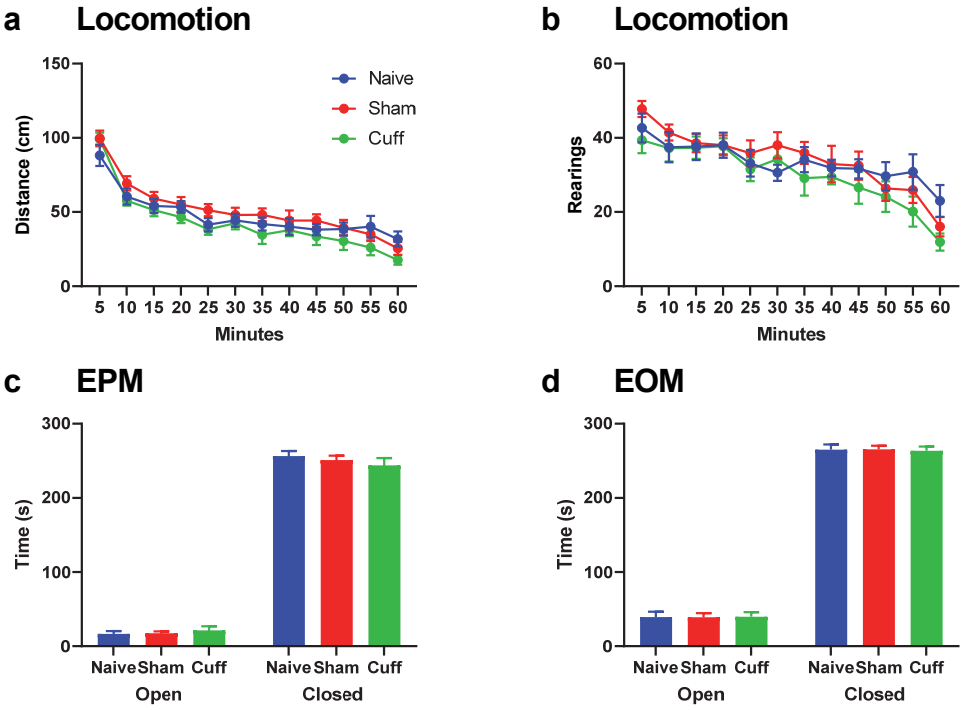
SUPPLEMENTAL FIGURES, TABLES AND LEGENDS

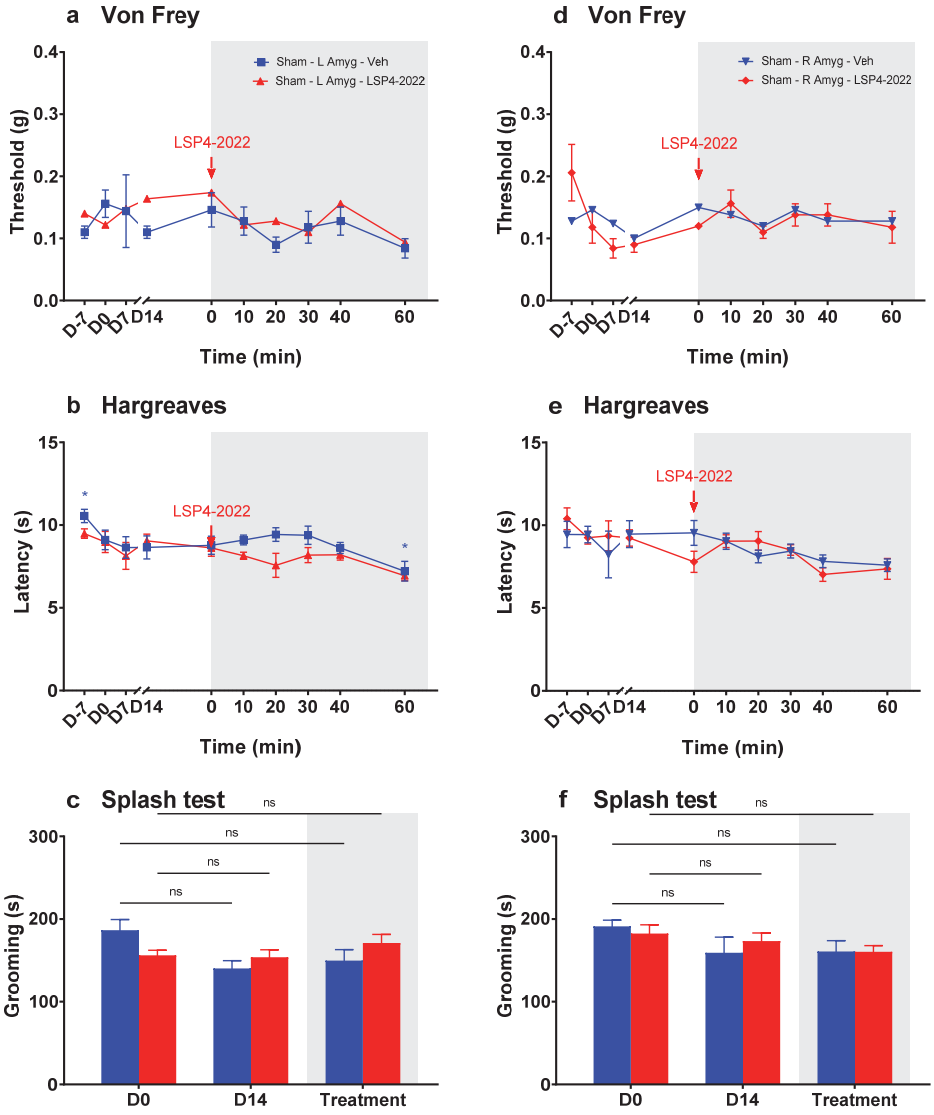


Supplemental figure 1: Cuff-induced mononeuropathic pain model does not significantly impair locomotion or elicit anxiety-like behavior in male mice. a-b: Mice locomotion was assessed using cyclotron/circular corridor. No significant difference in the numbers of ¼ turns or number of rearing was observed between Naïve, Sham or Cuff mice 7 days following surgery. **c-d:** Anxiety–like behavior was assessed in the elevated plus maze (EPM) or elevated O-maze (EOM). No significant difference in time spent in open arms or closed arm between Naïve, Sham or Cuff mice 17 (EPM) and 18 days (EOM) following surgery. (male mice, Naïve n=10, Sham n=10, Cuff n=9, two-way ANOVA, Tukey post-hoc test).

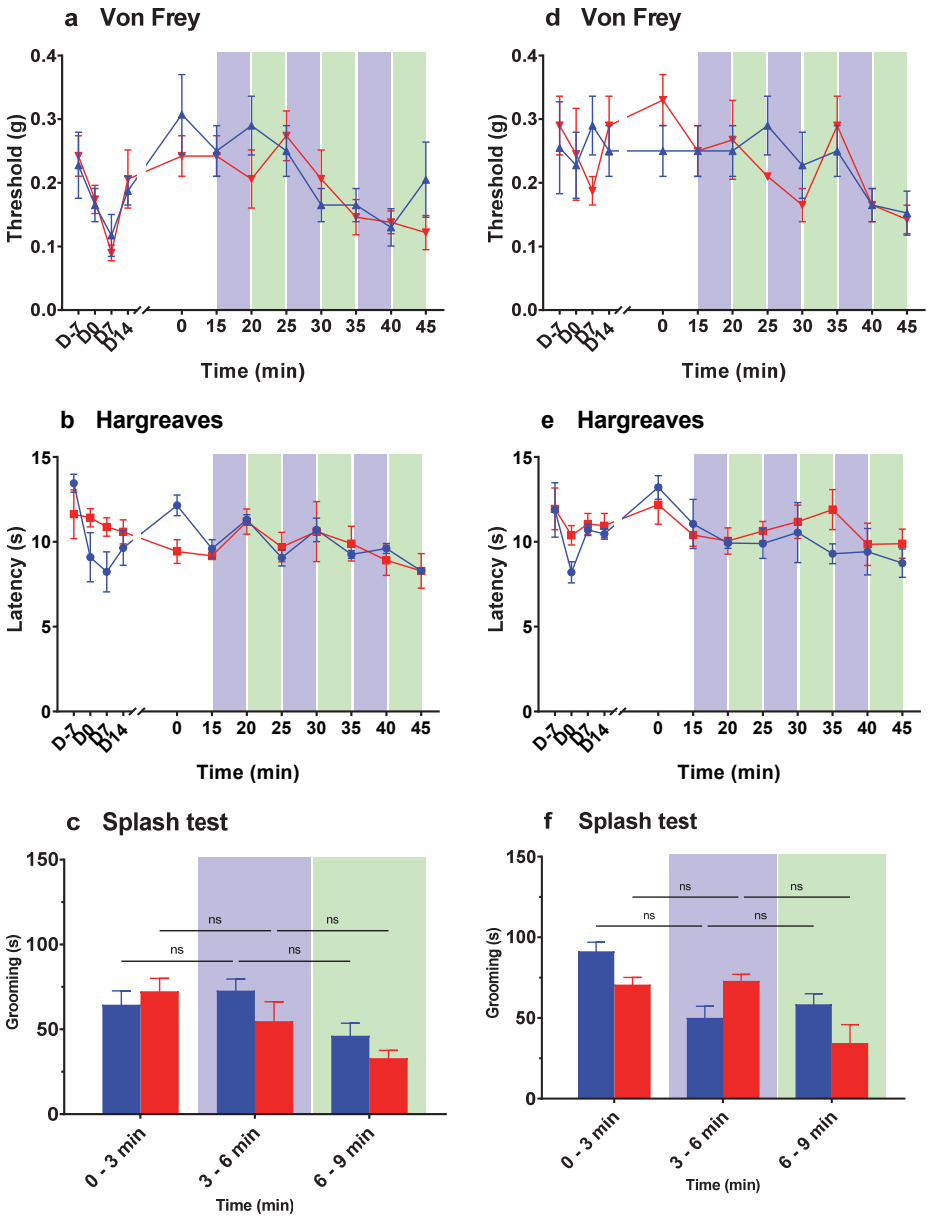
Figure 1	**Time (D_0 vs D_{14})**		
1b (males, VF)	F (1, 18) = 83,16; P<0,0001		
1c (males, HG)	F (1, 15) = 247,4; P<0,0001		
1d (males, ST)	F (1, 18) = 153,1; P<0,0001		
1e (fem, VF)	F (1, 15) = 64,04; P<0,0001		
1f (fem, HG)	F (1, 17) = 52,04; P<0,0001		
1g (fem, ST)	F (1, 17) = 356,4; P<0,0001		
Figure 2	**Time x LSP4-2022 (ipsi or contro)**	**Time (t_0 vs t_x)**	**LSP4-2022 (ipsi or contro)**
2a (males, VF)	F (8, 128) = 11,54; P<0,0001	F (3,131, 50,10) = 74,64; P<0,0001	F (1, 16) = 15,94; P=0,0010
2b (males, HG)	F (7, 112) = 5,538; P<0,0001	F (4,015, 64,24) = 91,56; P<0,0001	F (1, 16) = 20,00; P=0,0004
2c (males, ST)	F (2, 32) = 0,7724; P=0,4703	F (1,657, 26,51) = 122,7; P<0,0001	F (1, 16) = 1,150; P=0,2995
2d (fem, VF)	F (9, 72) = 7,386; P<0,0001	F (9, 72) = 11,19; P<0,0001	F (1, 8) = 4,312; P=0,0715
2e (fem, HG)	F (9, 72) = 1,727; P=0,0983	F (3,184, 25,48) = 19,34; P<0,0001	F (1, 8) = 12,69; P=0,0074
2f (fem, ST)	F (2, 16) = 0,1997; P=0,8210	F (1,801, 14,41) = 142,0; P<0,0001	F (1, 8) = 3,354; P=0,1044
Figure 3	**Time x Vehicle (ipsi or contro)**	**Time (t_0 vs t_x)**	**Vehicle (ipsi or contro)**
3a (males, VF)	F (11, 88) = 1,766; P=0,0723	F (3,557, 28,46) = 12,92; P<0,0001	F (1, 8) = 16,21; P=0,0038
3b (males, HG)	F (11, 88) = 5,563; P<0,0001	F (3,298, 26,39) = 13,24; P<0,0001	F (1, 8) = 193,5; P<0,0001
3c (males, ST)	F (2, 16) = 1,613; P=0,2301	F (1,625, 13,00) = 7,441; P=0,0094	F (1, 8) = 0,4377; P=0,5268
	Time x Optogluram (ipsi or contro)	**Time (t_0 vs t_x)**	**Optogluram (ipsi or contro)**
3d (male, VF)	F (11, 88) = 3,784; P=0,0002	F (3,725, 29,80) = 11,54; P<0,0001	F (1, 8) = 12,12; P=0,0083
3e (male, HG)	F (11, 88) = 8,762; P<0,0001	F (4,181, 33,44) = 18,23; P<0,0001	F (1, 8) = 51,53; P<0,0001
3f (male, ST)	F (2, 16) = 0,2507; P=0,7813	F (1,882, 15,06) = 107,7; P<0,0001	F (1, 8) = 0,7228; P=0,4199
Figure 4	**Conditioning (Veh or optogluram)**		
4e,f (male, aCPP, time in violet or green area)	F (3, 36) = 4,769; P=0,0067		

Supplemental Table 1. Summary of statistical analysis of data presented in figure panels

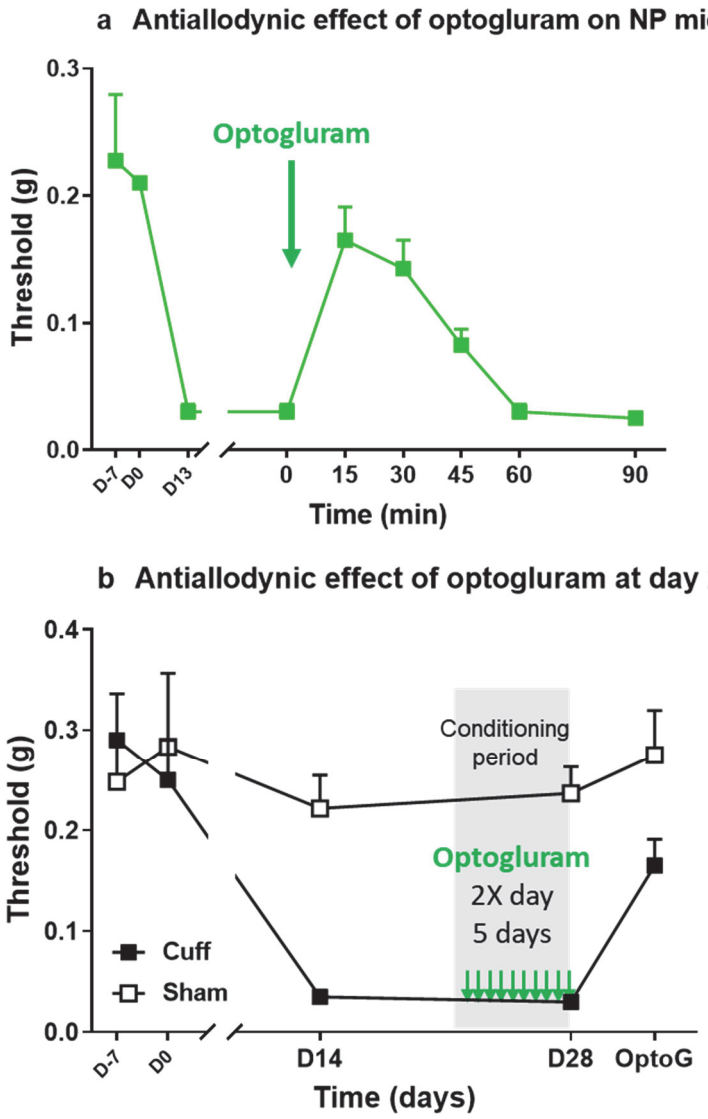
Abbreviations: fem = females; VF = Von Frey; HG = Hargreaves; ST = Splash test, aCPP = analgesic conditioned place preference



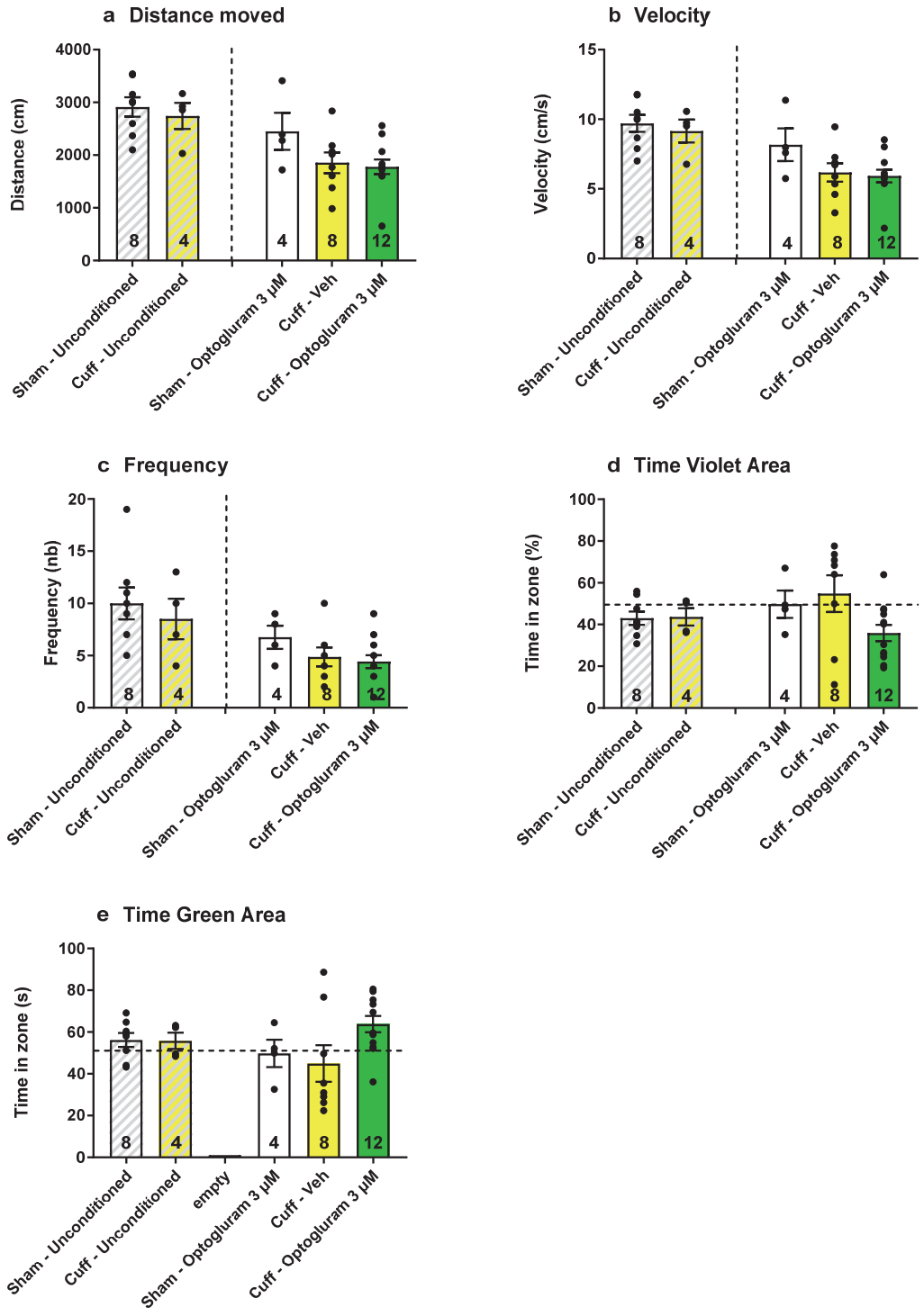
Supplemental figure 2: mGlu4 activation in right or left amygdala does not modify mechanical sensitivity, heat sensitivity or grooming in Sham animals. a-f: Females, sham left hindpaw, Vehicle (500 nL PBS, blue) or LSP4-2022 (5 µM, 500 nL in PBS, red) delivered unilaterally in ipsi or controlateral amygdala. **a, d**. Von Frey. Mean ± SEM, t_x vs t_0 (0 min), two-way ANOVA, Dunnett's post-hoc test. **b, e**. Hargreaves. Mean ± SEM, t_x vs t_0 (0 min), two-way ANOVA, Dunnett's post-hoc test.. **c, f**. Splash Test. # t_x vs t_0 (0 min), two-way ANOVA, Tukey's post-hoc test.



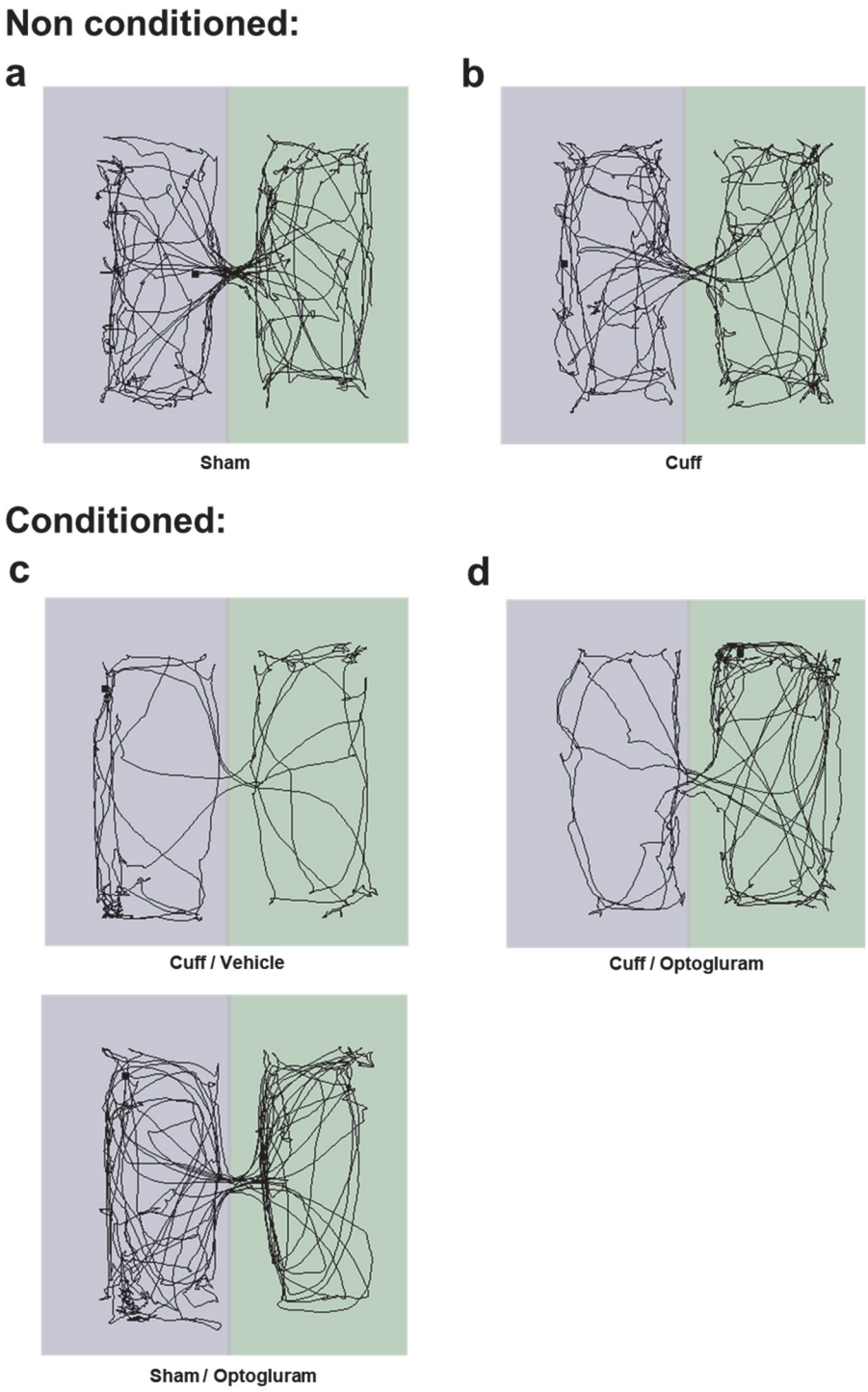
Supplemental figure 3: Photopharmacological manipulation of mGlu4 in right or left amygdala does not modify mechanical sensitivity, thermal sensitivity or grooming in Sham animals. a-f: Females, sham left hindpaw, Vehicle (500 nL PBS, blue) or LSP4-2022 (5 µM, 500 nL in PBS, red) delivered unilaterally in ipsi or controlateral amygdala. **a, d**. Von Frey. Mean ± SEM, t_x vs t_0 (0 min), two-way ANOVA, Dunnett's post-hoc test. **b, e**. Hargreaves. Mean ± SEM, t_x vs t_0 (0 min), two-way ANOVA, Dunnett's post-hoc test.. **c, f**. Splash Test. # t_x vs t_0 (0 min), two-way ANOVA, Tukey's post-hoc test.



Supplemental figure 4: No tolerance following 5 days of chronic treatment (twice daily) of optogluram (30µM) during the conditioning period. Testing of aCPP 15 minutes following intra-amygdala injection of optogluram on day 6



Supplemental figure 5: aCPP: different behavioural parameters on non-conditioned and conditioned Sham or neuropathic mice



Supplemental figure 6: aCPP: Examples of videotracks on non-conditioned and conditioned Sham or neuropathic mice