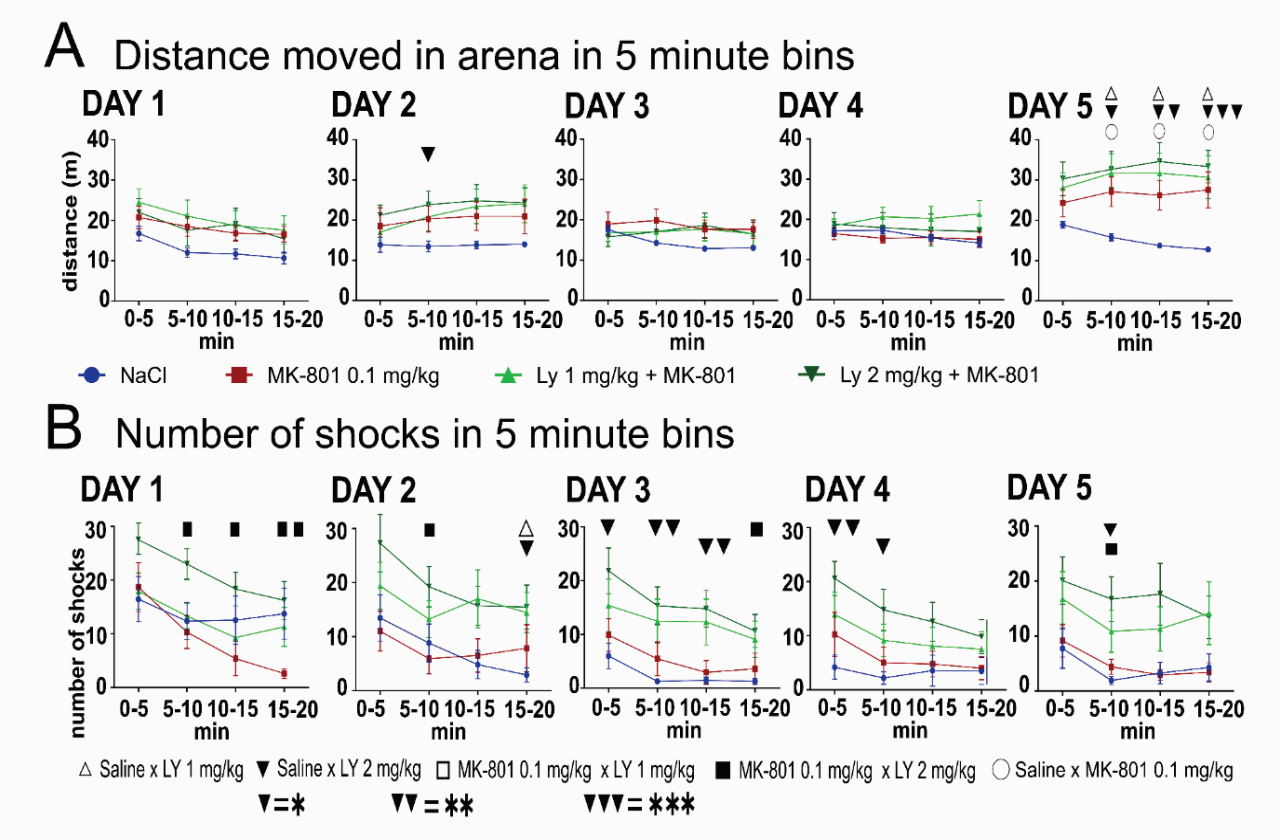
Supplementary Results: Within-Session Analysis of Locomotor Activity and Avoidance Behavior



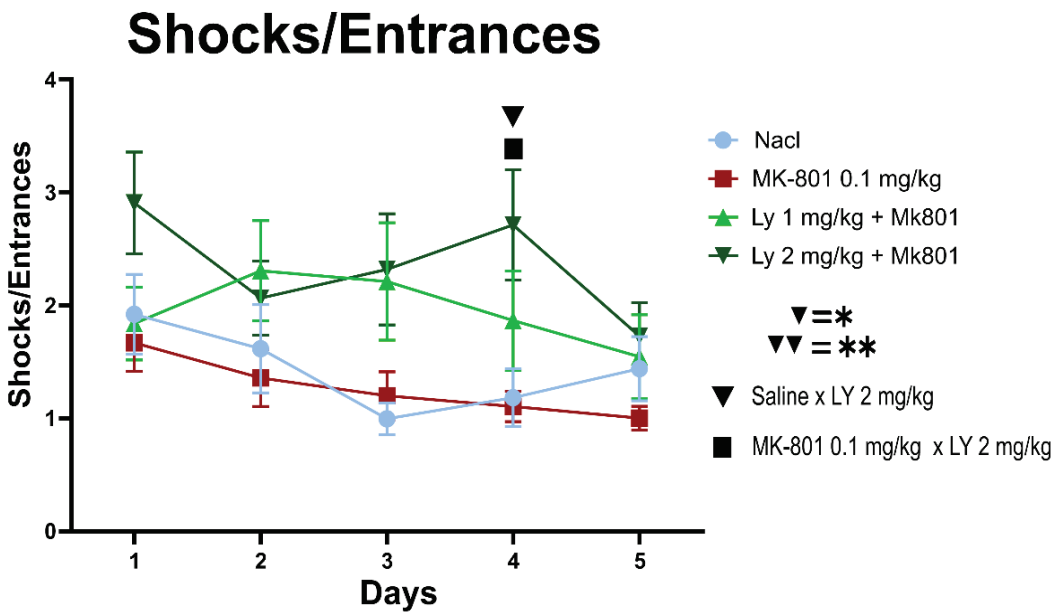
Supplementary Fig. 1. In the rotating arena, within-session analysis revealed no early differences, but significant increases in locomotor activity emerged on day 5 in MK-801 and LY379268 + MK-801 groups compared to saline: *(A) Distance moved in 5-minute intervals across the five days of learning. Group differences are indicated as follows: saline vs. 1 mg/kg LY379268 (△), saline vs. 2 mg/kg LY379268 (▼), MK-801 vs. 1 mg/kg LY379268 (□), MK-801 vs. 2 mg/kg LY379268 (■), and saline vs. MK-801 (○) The level of significance is indicated by the number of symbols: one symbol represents p≤0.05, two symbols represent p≤0.01, and three symbols represent p≤0.001.* **Within-session analysis of shocks received revealed that LY379268 (2 mg/kg) combined with MK-801 consistently led to higher shock counts across multiple days and time bins, indicating worsened avoidance behavior compared to both saline and MK-801 groups.** *(B) The number of shocks received across training days was presented in 5-minute time bins.*

To explore the dynamic effects of pharmacological treatments during each session, we divided the training periods into 5-minute bins and analyzed locomotor activity across the five days of learning (presented in 5-minute time bins; Suppl. Fig. 1A). Distance moved was analyzed for each day using a two-way repeated-measures ANOVA with Group as a between-subject factor and Time bin as a within-subject factor (Day 1: time × group, $F(9,150)=0.5561$, $p=0.8311$; time, $F(2.181,109.0)=15.39$, $p<0.0001$; group, $F(3,50)=1.040$, $p=0.3831$; Day 2: time × group, $F(9,153)=0.6151$, $p=0.7829$; time, $F(1.759,89.71)=3.234$, $p=0.0502$; group, $F(3,51)=1.582$, $p=0.2050$; Day 3: time × group, $F(9,150)=1.295$, $p=0.2440$; time, $F(1.772,88.60) = 0.8583$, $p=0.4155$; group, $F(3,50)=0.4990$, $p=0.6846$; Day 4: time × group, $F(9,147)=0.8720$, $p = 0.5518$; time, $F(2.096,102.7)=0.5231$, $p=0.6027$; group, $F(3,49)=0.5916$, $p=0.6235$; Day 5: time × group, $F(9,150)=1.482$, $p=0.1592$; time, $F(1.557,77.84)=0.5380$, $p=0.5421$; group, $F(3,50)=3.822$, $p=0.0153$). On day 1, no *post hoc* group differences were detected. On day 2, a single significant difference was observed in the 5-10 min bin between the saline and LY 2 mg/kg + MK-801 groups (*p=0.0427), with no other pairwise differences. On days 3 and 4, no significant *post hoc* group differences were found in any bin. In contrast, on day 5, robust differences emerged across the later bins: in the 5-10 min bin, the saline group differed from MK-801 (*p=0.0449), LY 1 mg/kg + MK-801 (*p=0.0297) and LY 2 mg/kg + MK-801 (*p=0.0103); in the 10-15 min bin, saline differed from MK-801 (*p=0.0238), LY 1 mg/kg + MK-801 (*p=0.0176) and LY 2 mg/kg + MK-801 (**p=0.0020); and in the

15-20 min bin, saline again differed from MK-801 (*p=0.0279), LY 1 mg/kg + MK-801 (*p=0.0252) and LY 2 mg/kg + MK-801 (***p=0.0006), indicating persistently higher locomotor activity in all MK-801–treated groups at the end of training.

For shocks received in the avoided sector, within-session effects were analyzed using a two-way repeated-measures ANOVA with Group and Time bin (0-5, 5-10, 10-15, 15-20 min) as factors (Suppl. Fig. 1B). For day 1, the analysis showed a significant main effect of time ($F(1.766,88.30)=19.71$, $p<0.0001$) and group ($F(3,50)=3.252$, *p=0.0293), but no time × group interaction ($F(9,150)=1.741$, $p=0.0845$). On day 2, there were again significant main effects of time ($F(1.690,86.21)=7.643$, **p=0.0016) and group ($F(3,51)=3.306$, *p=0.0274), without a significant time × group interaction ($F(9,153)=0.9411$, $p=0.4915$). On day 3, both time ($F(1.671,85.20)=11.28$, ***p=0.0001) and group ($F(3,51)=4.192$, **p=0.0100) were significant, whereas the time × group interaction was not ($F(9,153)=0.6577$, $p=0.7460$). On day 4, the ANOVA revealed significant main effects of time ($F(2.108, 105.4)=10.61$, $p<0.0001$) and group ($F(3,50)=2.837$, *p=0.0473), but again no time × group interaction ($F(9,150)=1.249$, $p=0.2694$). Finally, on day 5, significant main effects of time ($F(2.043,102.2)=7.296$, **p=0.0010) and group ($F(3,50)=3.138$, *p=0.0334) were observed, with no significant time × group interaction ($F(9,150)=0.8202$, $p=0.5984$).

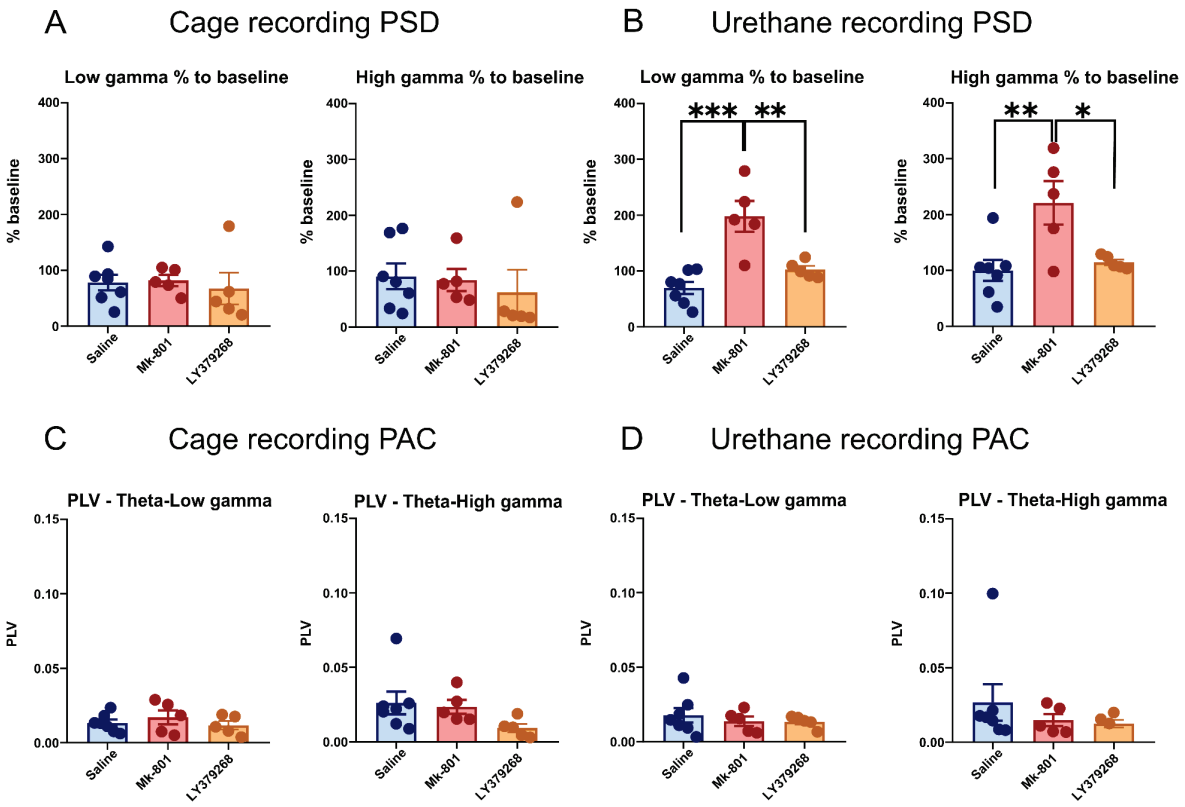
Several within-session *post hoc* differences were detected. On day 1, significant differences emerged between MK-801 and LY 2 mg/kg + MK-801 in the 5-10 minute bin (*p=0.0252), 10-15 minute bin (*p=0.0348), and 15-20 minute bin (**p=0.0075). On day 2, differences were found between saline and LY 2 mg/kg + MK-801 in the 5-10 minute bin (*p=0.0394), and between saline and both LY 1 mg/kg + MK-801 (*p=0.0449) and LY 2 mg/kg + MK-801 (*p=0.0386) in the 15-20 minute bin. On day 3, group differences emerged early: saline vs. LY 2 mg/kg + MK-801 in the 0-5 minute bin (*p=0.0173), 5-10 minute bin (**p=0.0059), and 10-15 minute bin (**p=0.0072); additionally, MK-801 vs. LY 2 mg/kg + MK-801 showed significance at 10-15 minutes (*p=0.0354). On day 4, only the first two bins showed significant differences between saline and LY 2 mg/kg + MK-801 (**p=0.0014 and *p=0.0258, respectively). On the final day (day 5), significant differences were again observed: MK-801 vs. LY 2 mg/kg + MK-801 in the 0-5 minute bin (*p=0.0445), and in the 5-10 minute bin, saline vs. LY 2 mg/kg + MK-801 (*p=0.0236), MK-801 vs. LY 1 mg/kg + MK-801 (*p=0.0492), and MK-801 vs. LY 2 mg/kg + MK-801 (**p=0.0053). In the 10-15 minute bin, both saline vs. LY 2 mg/kg + MK-801 (*p=0.0121) and MK-801 vs. LY 2 mg/kg + MK-801 (*p=0.0129) were significant. In the final 15-20 minute interval, a difference was observed between saline and LY 2 mg/kg + MK-801 (**p=0.0015).



Supplementary Fig. 2. The *shocks-per-entrance* parameter revealed an effect of LY379268 (2 mg/kg) administration compared to the saline and MK-801 groups on Day 4 in the rotating arena.

To examine procedural learning in the arena, we calculated the number of shocks per entrance (formula: shocks / (entrances + 1) to avoid division by zero). A two-way ANOVA revealed an effect of days

(F(3.449,175.0)=2.907, p=0.0296) and an effect of group (F(3,51)=3.709, p=0.0173), but no interaction (F(10.35,175.0)=1.275, p=0.2461). Tukey's *post hoc* test showed a significant difference between the LY379268 (2 mg/kg) and saline groups, and between the LY379268 (2 mg/kg) and MK-801 (0.1 mg/kg) groups, indicating that the slightly worsened procedural learning observed in the LY379268 (2 mg/kg) group reached statistical significance only on this particular day.



Supplementary Fig. 3. We did not observe any effect of LY379268 administration on local field potentials (LFPs) in the mPFC. *(A) No significant effect of treatment on PSD was observed in the low and high gamma ranges compared to baseline activity in home cage recordings. (B) In urethane recordings, significant differences in power spectral density (PSD) were observed between the control (saline) group and the MK-801 group, as well as between the MK-801-treated group and the LY379268 group. However, no significant differences were found between the control group and the LY379268 group in both low and high gamma ranges. (C) In home cage recordings, there were no significant differences in phase-amplitude coupling (PAC) between theta and low gamma or between theta and high gamma, as indicated by phase-locking value (PLV). (D) Similarly, in urethane recordings, no significant differences in PAC were observed between theta and low gamma or between theta and high gamma, as indicated by PLV. Data are shown as mean ± SEM; saline n=7, MK-801 n=5, MK-801 + LY379268 n=5.*

We analyzed neural activity in rats under both home cage conditions and urethane anesthesia after the administration of LY379268 but before the administration of MK-801 (see Fig. 2, marked by a dashed line) to assess whether LY379268 alone induces differences in PSD or theta-gamma PAC compared to controls.

Under home cage conditions, ordinary one-way ANOVA showed no significant differences in PSD in the low gamma range (F(2,14)=0.1496; p=0.8624), and the Kruskal-Wallis test similarly showed no significant differences in the high gamma range (H(2)=3.227; p=0.2084). In urethane recordings, however, ordinary one-way ANOVA revealed significant differences in the low gamma range (F(2,14)=16.31; ***p=0.0002), with *post hoc* tests showing significant differences between the control group and the MK-801-treated group (***p=0.0002) and between the MK-801-treated group and the LY379268-treated group (p=0.0045). For high gamma under urethane anesthesia, ordinary one-way ANOVA also indicated significant differences (F(2,14)=7.298; **p=0.0067), with *post hoc* tests identifying significant differences between the control group and the MK-801-treated group (**p=0.0081) and between the MK-801-treated

group and the LY379268-treated group (*p=0.0206).

For theta-gamma PAC, in home cage recordings, ordinary one-way ANOVA showed no significant differences in PLV between theta and low gamma (F(2,14)=0.6312; p=0.5467). While the Kruskal-Wallis test displayed a significant difference in theta-high gamma PAC (H(2)=6.413; *p=0.0339), *post hoc* tests did not reveal any significant pairwise differences. In urethane conditions, ordinary one-way ANOVA showed no significant differences in PLV between theta and low gamma (F(2,14)=0.3888; p=0.6849). Similarly, the Kruskal-Wallis test showed no significant differences in theta-high gamma PAC (H(2)=1.250; p=0.5545). These findings indicate that LY379268 alone does not induce significant changes in PSD or PAC compared to controls under either condition.

A

Correlation of Distance moved and Power Spectral Density

Correlation: Distance moved/ Low gamma % to baseline			
	Saline	MK-801	LY + MK-801
Spearman r			
r	0.3571	0	-0.5
95% confidence interval			
P value			
P (two-tailed)	0.4444	>0.9999	0.45
P value summary	ns	ns	ns
Exact or approximate P value?	Exact	Exact	Exact
Significant? (alpha = 0.05)	No	No	No

Correlation: Distance moved/ High gamma % to baseline			
	Saline	MK-801	LY + MK-801
Spearman r			
r	-0.3214	0.6	-0.1
95% confidence interval			
P value			
P (two-tailed)	0.4976	0.35	0.95
P value summary	ns	ns	ns
Exact or approximate P value?	Exact	Exact	Exact
Significant? (alpha = 0.05)	No	No	No

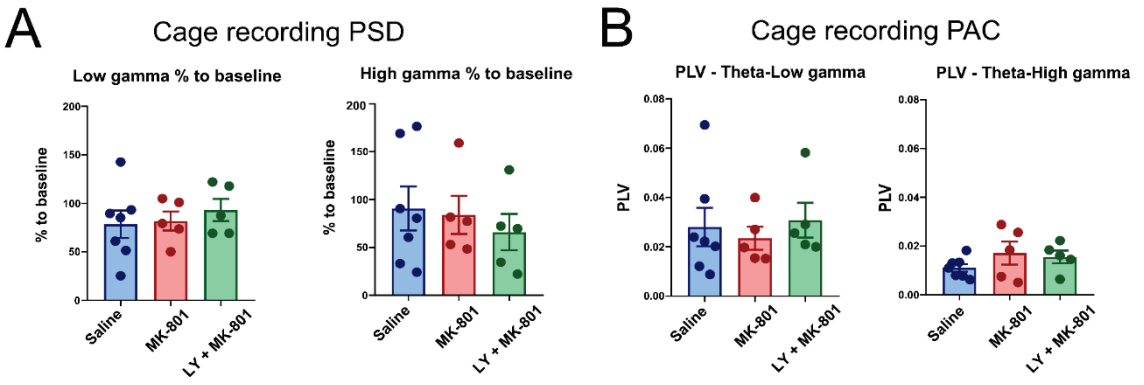
B

Correlation of Distance moved and Phase-Amplitude Coupling

Correlation: Distance moved/ PLV Theta-Low gamma			
	Saline	MK-801	LY + MK-801
Pearson r			
r	-0.9234	-0.4493	-0.2854
95% confidence interval	-0.9888 to -0.5594	-0.9536 to 0.7173	-0.9328 to 0.7977
R squared	0.8527	0.2019	0.08145
P value			
P (two-tailed)	0.003	0.4478	0.6416
P value summary	**	ns	ns
Significant? (alpha = 0.05)	Yes	No	No

Correlation: Distance moved/ PLV Theta-High gamma			
	Saline	MK-801	LY + MK-801
Pearson r			
r	0.2731	0.3201	0.0341
95% confidence interval	-0.6042 to 0.8511	-0.7834 to 0.9376	-0.8745 to 0.8896
R squared	0.07459	0.1024	0.001163
P value			
P (two-tailed)	0.5534	0.5995	0.9566
P value summary	ns	ns	ns
Significant? (alpha = 0.05)	No	No	No

Supplementary Fig. 4. Distance moved correlates with theta-low Gamma PAC in controls, but not with PSD in any group. *(A) No significant correlation was observed between distance moved and power spectral density (PSD) in either the low gamma or high gamma frequency ranges. (B) A significant correlation between distance moved and phase-amplitude coupling (PAC) was found only in the control group for theta-low gamma frequencies. No significant correlations were observed in the other groups or for theta-high gamma coupling.*



Supplementary Fig. 5. In home cage recordings, there was no effect of drug treatments on PSD and PAC: Power Spectral Density (PSD) and Phase-Amplitude Coupling (PAC) in the home cage. *(A) No significant effect of treatment on PSD was observed in both low and high gamma ranges compared to baseline activity. (B) There were no observable differences in PAC between theta and lower gamma or between theta and higher gamma, as indicated by phase-locking value (PLV). Data are shown as mean ± SEM; saline n=7, MK-801 n=5, MK-801 + LY379268 n=5.*

Home cage recording

We compared measured power to baseline activity for electrophysiological recordings in the home cage. Baseline activity was defined using a 10-minute period from 20 to 30 min after connecting the animal to the electrophysiological setup. This time frame was chosen to minimize stress-related activity from the initial head stage connection and to provide the animal with adequate time to acclimate to the setup. The baseline period was then compared to a 10-minute period recorded 30 to 40 min after the injection of saline, MK-801, or MK-801 following a prior injection of LY379268 (1 mg/kg). See Fig. 2 for a detailed experimental scheme.

Electrophysiological recordings in the home cage showed no significant differences in PSD or PLV across treatment groups. Ordinary one-way ANOVA revealed that the application of MK-801 or the combination of LY379268 (1 mg/kg) and MK-801 had no significant effect on PSD in the low gamma band ($F(2,14)=0.3557$; $p=0.7069$) or high gamma band ($F(2,14)=0.3429$; $p=0.7155$) as shown in Fig. 5A. Similarly, no significant differences were observed in PLV for theta-low gamma PAC ($F(2,14)=0.3750$; $p=0.694$) or theta-high gamma PAC ($F(2,14)=0.2486$; $p=0.7833$) (Fig. 5C). These findings indicate that, under home cage conditions, neither MK-801 nor the combination of LY379268 + MK-801 significantly altered the neural activity in the mPFC, suggesting that the treatments did not disrupt baseline oscillatory dynamics or PAC in this setting.

In addition, no differences were observed in PSD or PLV between the control saline, the MK-801 group, and the single administration of LY379268, which preceded MK-801 administration, in home cage recordings (Suppl. Fig. 5).