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Supplementary Information for

Harnessing psilocybin: antidepressant-like behavioral and synaptic actions of psilocybin are independent of 5-HT2R activation in mice

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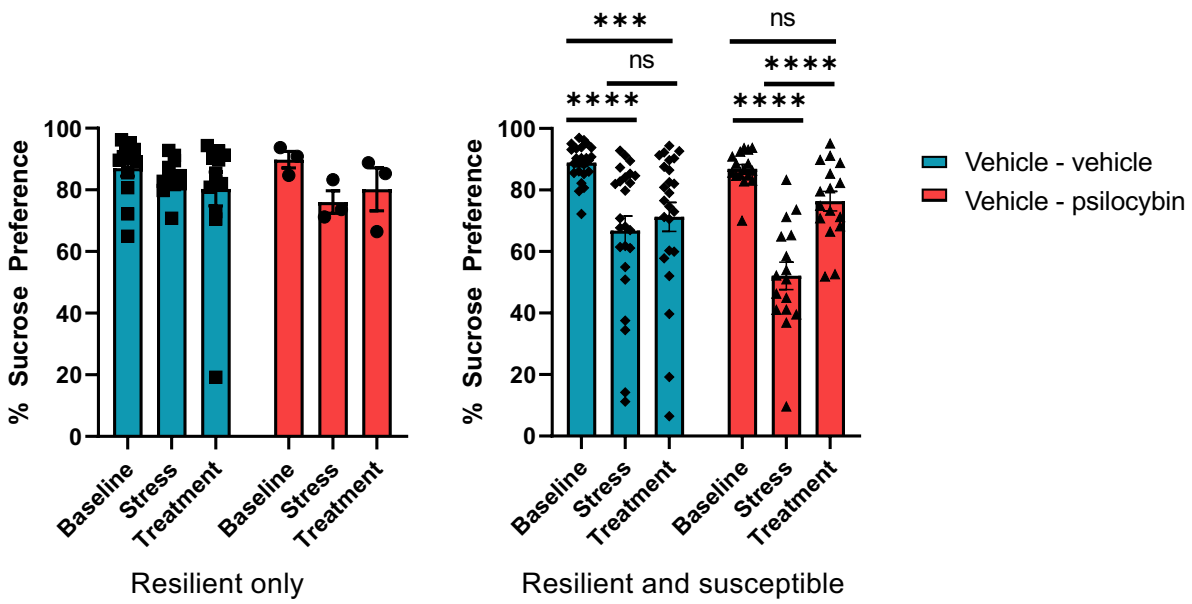
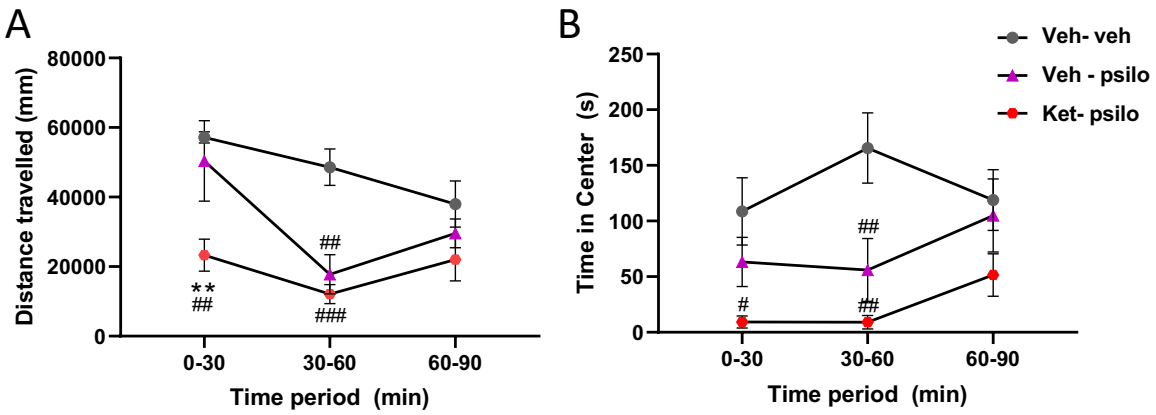
This PDF file includes:

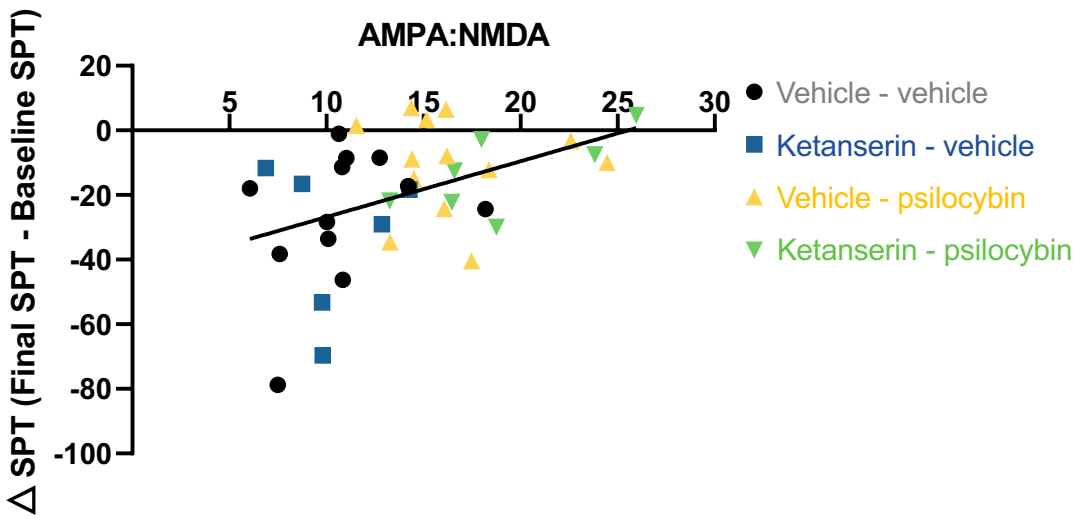
Supplemental Figures S1 to S4



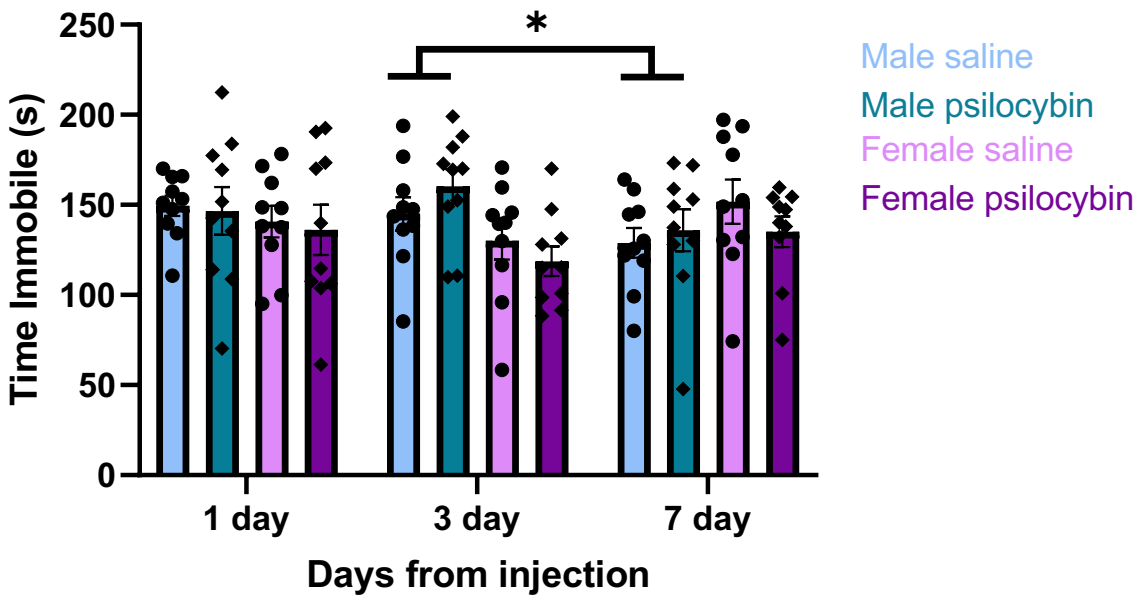
Supplemental Figure 1. Psilocybin has no effects on reward behavior in resilient animals. Resilient mice were defined as those exhibiting a high sucrose preference (>70%) following 14 days CMMS. A. Injection of psilocybin (1mg/kg, ip) had no effect on sucrose preference in resilient animals (n=3, red), nor did injection of vehicle (n=12, blue). Two-way repeated measures ANOVA: $F_{1,14} = 0.14$, $p = 0.72$. B. The effects of psilocybin remained significant when data from resilient and susceptible animals are combined. a two-way repeated measures ANOVA ($F_{2,76} = 4.16$, $p = 0.02$). A Holm-Sidak post-hoc test revealed a significant decrease in sucrose preference following CMMS in both vehicle-vehicle and vehicle-psilocybin treated mice that was only rescued by vehicle-psilocybin treatment. Sucrose preference is presented as the group means ± SEM with data from individual animals superimposed. ****, $p<0.0001$; ***, $p<0.001$; ns, not significant



Supplemental Figure 2. Ketanserin pretreatment enhances the hypolocomotive effects of psilocybin. A separate cohort of animals were pretreated with saline vehicle (n = 9) or ketanserin (n = 9; 2 mg/kg) 60 minutes prior to psilocybin (5 mg/kg) administration. Following injection with psilocybin at time 0, mice were immediately placed in open field arenas and video recorded for 90 minutes. A) Ketanserin-psilocybin mice travelled less at 0-30 minutes compared to vehicle-vehicle controls (n = 6; p = 0.0019) and vehicle-psilocybin mice (p = 0.0059), and at 30-60 minutes (p = 0.0008) compared to controls. Vehicle-psilocybin mice displayed hypolocomotion compared to controls at 30-60 minutes timepoint (p = 0.005). There was a significant effect of treatment ($F_{2,21}$ = 7.56, p = 0.0034), time ($F_{2, 42}$ = 10.69, p = 0.0002) and interaction of time x treatment ($F_{4, 42}$ = 3.17, p = 0.023). B) Mice treated with ketanserin-psilocybin spent less time in the center of the arena compared to control mice at 0-30 minutes (p = 0.017) and 30-60 minutes (p = 0.0001), while vehicle-psilocybin mice displayed less center time compared to controls at 30-60 minute timepoint (p = 0.0077). There was a significant effect of treatment on time in center ($F_{2, 21}$ = 7.085, p = 0.0045) but no effect of time ($F_{2, 42}$ = 2.58, p = 0.088). Individual points represent the mean +/- SEM for treatment groups at each timepoint. ## v. control, p<0.01; ** v. veh-psil, p<0.01; ### v. control, p<0.001.



Supplemental Figure 3. Synaptic strength is correlated with changes in sucrose preference. Changes in sucrose preference in individual mice between baseline and post-treatment measurements were significantly correlated with the average AMPA:NMDA ratio, a measure of TA-CA1 synaptic strength ($y = 1.725x – 44.06$, $R^2 = 0.18$, $p = 0.0072$).



Supplemental Figure 4. Psilocybin has no effect on immobility time in the forced swim test. A cohort of unstressed C57Bl/6J were injected with psilocybin (n = 10 males, n = 10 females; 1 mg/kg) or saline (n = 10 males, n = 10 females). Time spent immobile during the forced swim test was measured 1, 3, and 7 days post-injection. There was no effect of psilocybin ($F_{1, 18} = 2.28$, p = 0.15) or time ($F_{2, 36} = 1.53$, p = 0.23) in the female cohort. In the male cohort, there was a significant effect of time ($F_{2, 36} = 3.85$, p = 0.031) but no effect of psilocybin ($F_{1, 18} = 0.36$, p = 0.56). Post-hoc comparisons in the male cohort revealed a significant difference in immobility time between days 3 and 7 (p = 0.031), but no differences between saline and psilocybin treated animals at any timepoint. The height of the bars represents the mean +/- SEM. *, p<0.05