Negative Allosteric Modulation of $GABA_A$ Receptors at α5 Subunit-Containing Benzodiazepine Sites Reverses Stress-Induced Anhedonia and Weakened Synaptic Function in Mice

SUPPLEMENTAL INFORMATION

Supplemental Methods

Animals and housing: Mice were group-housed in the University of Maryland Baltimore's vivarium on 12-hour light/dark cycle (light on at 7AM) with ad libitum access to water and standard rodent chow. Mice were 8 weeks old at the start of CMMS and were transferred to clean caging, where they remained singly housed through the duration of the experiment. We used male mice as stress-sensitive hedonic behaviors have been well characterized. Unfortunately, reliable stress-sensitive changes in a reward behavior in female mice are not well established.

$GABA_AR$ α5 knockout (KO) Mice: Genotyping was done by automated real-time PCR genotyping (Transnetyx). Tail snips obtained from juvenile mice were digested overnight in proteinase-K solution held at 50°C. Samples were spun down and the DNA-containing supernatant was amplified using the following PCR primers: $GABA_AR$ α5 WT (forward: TTTGCCACTCTAATTCTGACTTCAGTTAAT, reverse: GGAAACTGAAAGTTAGGAAGGACTCT) and $GABA_AR$ α5 EX (forward: AGTCAGGATCCGTCGAGGAATT, reverse: GGAAGGACTCTTGCTCTTTGATGAA).

Behavioral Protocols

Sucrose preference test (SPT): Two feeders, each with food and a bottle containing tap water, were introduced at either end of each animal's home cage one hour prior to the beginning of the animal's dark cycle. Bottles were weighed 12-16 hours later, and the bottle from which the least water was consumed was replaced with a bottle containing 2% sucrose solution to habituate mice to the presence of sucrose. One day later, the bottles were replaced with bottles containing either 1% sucrose solution or tap water, with the sucrose bottle placed on the opposite side of the 2% training bottle. Mice were given ad libitum access to both bottles overnight, starting one hour prior to the onset of the dark cycle. Bottles were weighed prior to placement in the cage and again 12-16 hours later. Sucrose preference was calculated by dividing the weight of 1% sucrose solution consumed by the total weight consumed from both bottles. This was repeated the following night with the placement of the two bottles reversed to minimize potential side preference. The preferences were then averaged to calculate a total sucrose preference. Only mice displaying a sucrose preference at baseline that decreases to ≤66% following stress were considered stress-susceptible and included in further sucrose preference tests.

Female urine sniff test (FUST): Sexually mature, stress-naïve male mice display a preference for sniffing the urine of female mice in estrous compared to the urine of males. Cages of 5 female and 5 male C57Bl/6J mice were exchanged daily for 5 days to induce female mice into estrous prior to urine collection. Individual experimental mice were habituated for an hour in clean cages containing a single cotton swab affixed to the rim of the cage and within reach of each mouse. Following habituation, the swab was removed and exchanged with two cotton swabs individually soaked in male and female urine affixed at opposite corners of one end of the cage. Mice were given 3 minutes to freely interact with the swabs, and the time spent sniffing each swab was recorded by a trained observer blinded to treatment and position of each swab. Time spent biting the swabs was excluded. Female urine preference was calculated as the total time a mouse spent sniffing the female urine swab over the total time sniffing both swabs. The positions of the male and female swabs were reversed between experimental timepoints (baseline, post-stress, post-treatment) to minimize any potential side preference. Mice must display a female urine preference of greater than 50% at baseline that decreases by at least 10% following stress to be considered stress-susceptible and used in the FUST arm of the study.

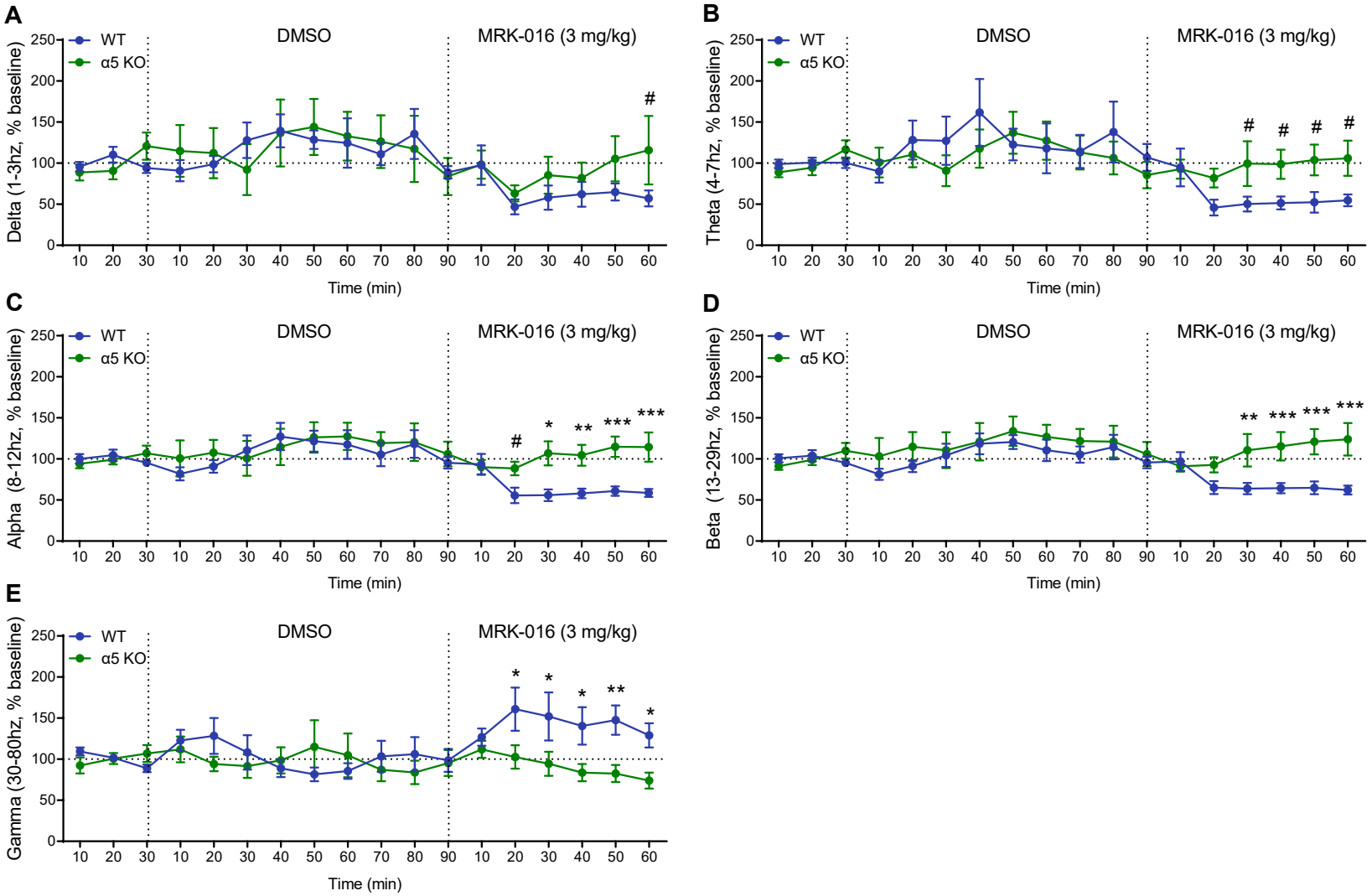

Figure S1. Effect of 3mg/kg (i.p) MRK-016 administration across power bands normalized to the last half hour of baseline activity. **(A)** Delta power in both WT (n=9) and $GABA_AR$ α5 KO (n=7) mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 3.478, p<0.0001) but not time × genotype ($F_{17,238}$ = 0.8442, p=0.6408). **(B)** Theta power is reduced in WT but not α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 3.935, p<0.0001) and time × genotype ($F_{17,238}$ = 2.083, p=0.0083). **(C)** Alpha power is reduced in WT but not α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 4.741, p<0.0001) and time × genotype ($F_{17,238}$ = 2.550, p=0.0009). **(D)** Beta power is reduced in WT but not α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 3.808, p<0.0001) and time × genotype ($F_{17,238}$ = 2.572, p=0.0008). **(E)** Gamma power is increased in WT but not α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 1.574, p=0.0719) and time × genotype ($F_{17,238}$ = 2.678, p=0.0005). p<0.10 #, p<0.05 *, p<0.01 **, p<0.005 ***.

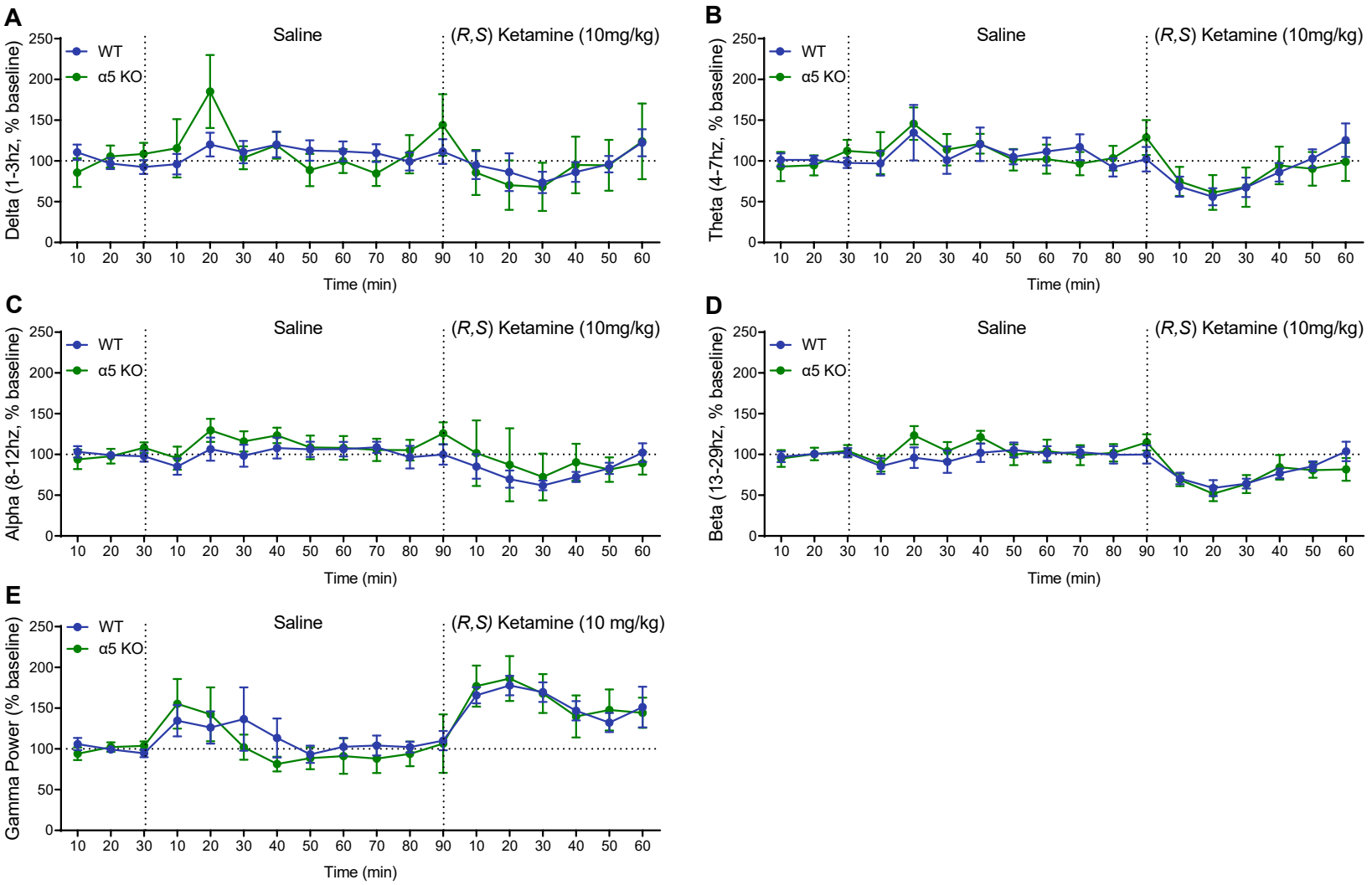

Figure S2. Effect of 10mg/kg (i.p) (*R,S*) ketamine administration across power bands normalized to the last half hour of baseline activity. **(A)** Delta power is reduced in both WT (n=9) and $GABA_AR$ α5 KO (n=7) mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 2.285, p=0.0033) but not time × genotype ($F_{17,238}$ = 0.8256, p=0.6627). **(B)** Theta power is reduced in both WT and α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 3.495, p<0.0001) but not time × genotype ($F_{17,238}$ = 0.4278, p=0.9780). **(C)** Alpha power is reduced in both WT and α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 2.334, p=0.0026) but not time × genotype ($F_{17,238}$ = 0.3730, p=0.9896). **(D)** Beta power is reduced in both WT and α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 6.657, p<0.0001) but not time × genotype ($F_{17,238}$ = 0.7838, p=0.7112). **(E)** Gamma power is increased in both WT and α5 KO mice. Two-way ANOVA yields effect of time ($F_{17,238}$ = 8.569, p<0.0001) but not time × genotype ($F_{17,238}$ = 0.5589, p=0.9193).

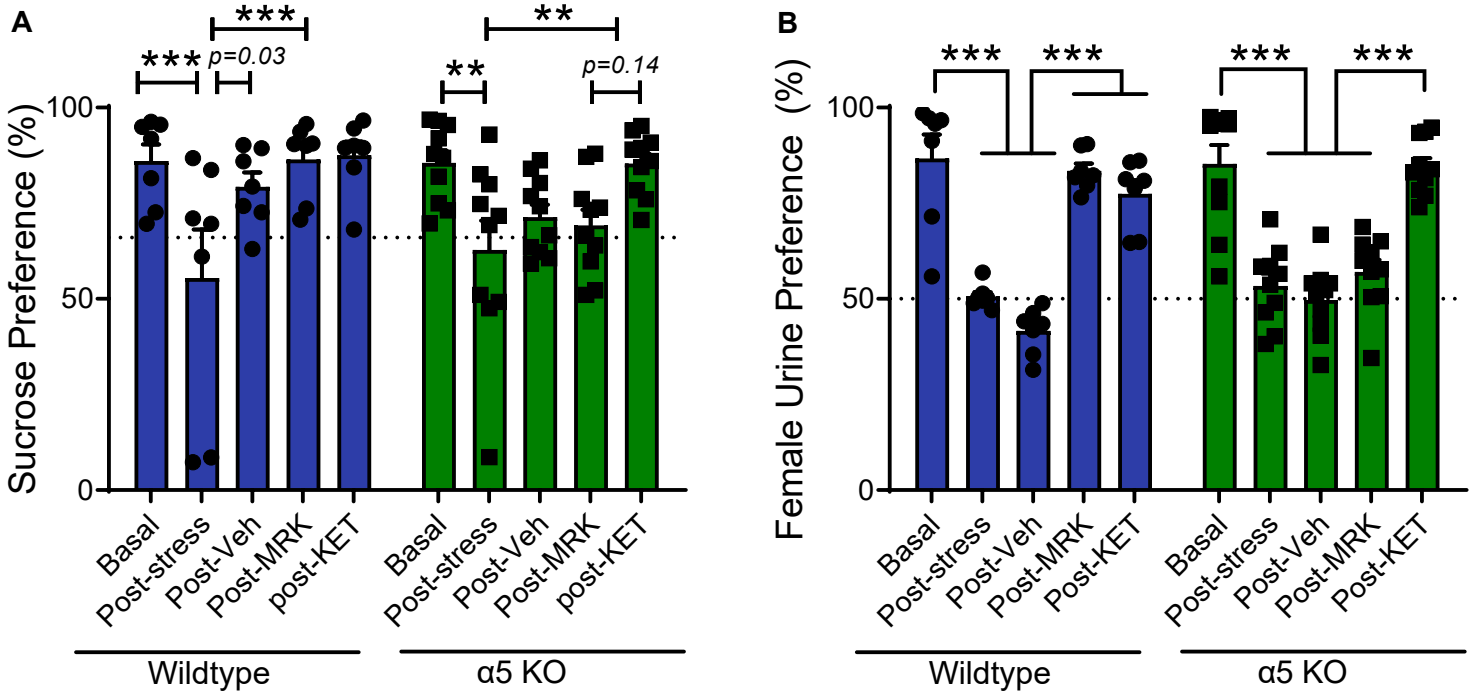

Figure S3. (A) CMMS significantly decreases sucrose preference in both wildtype (n=7, p=0.0020) and α5 KO animals (n=10, p=0.0083). All animals in cohort are shown. 3mg/kg MRK-016 significantly increased sucrose preferences over post-stress values in wildtype (p=0.0017), but not α5 KO (p=0.9801) groups. 10 mg/kg (*R,S*) ketamine significantly increased sucrose preference over post-stress values (p=0.0088) in the α5 KO group. Two-way ANOVA indicated phase × genotype ($F_{4,60}$ = 1.638, P=0.1764). **(B)** CMMS significantly lowers female urine preferences in both wildtype (p<0.0001) and α5 KO (p<0.0001) groups. MRK-016 increases female urine preference in wildtype animals over post-stress (p<0.0001) and post DMSO preferences (p<0.0001), but not in α5 KO animals (p=0.9925 and p=0.5862, respectively). Ketamine increased female urine preference over post MRK-016 values in all α5 KO animals (p<0.0001). Two-way ANOVA indicated a significant phase × genotype interaction ($F_{4,60}$ = 9.354, P<0.0001). p<0.05 *, p<0.01 **, p<0.005 ***.

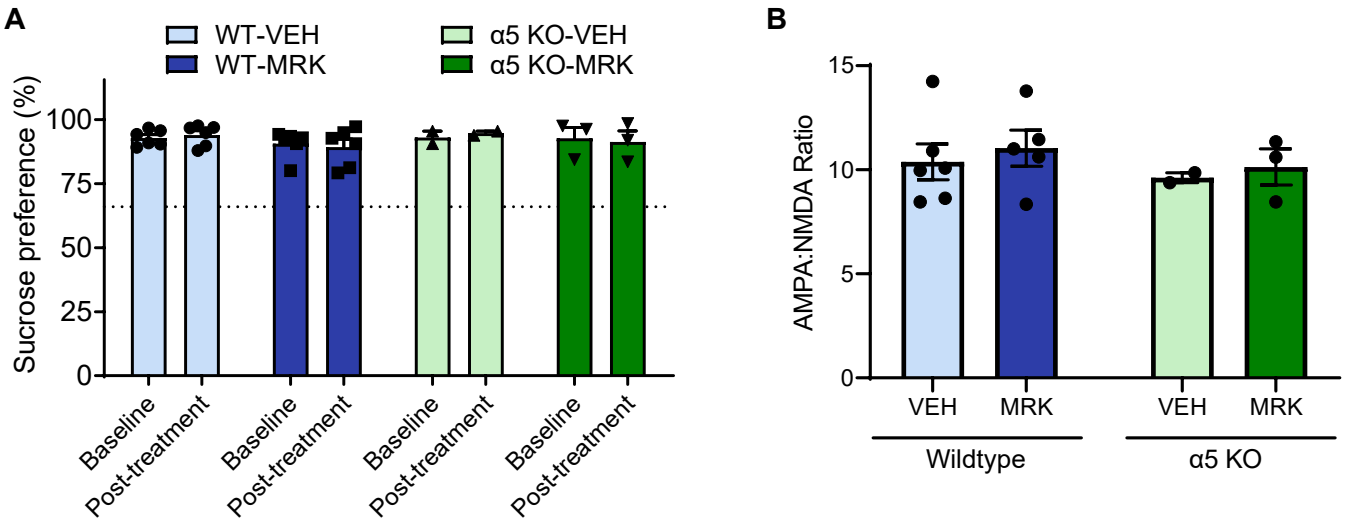

Figure S4. (A) Sucrose preferences in a cohort of unstressed α5 KO and wildtype littermates before and after MRK-016 administration. Three-way ANOVA yields no effect of time × treatment ($F_{1,13}$ = 0.6025, p=0.4515) or genotype × treatment ($F_{1,13}$ = 0.1168, p=0.7380). **(B)** TA-CA1 fEPSP AMPA:NMDA ratios in unstressed animals are comparable between genotypes. Two-way ANOVA yields no effect of genotype ($F_{1,12}$ = 0.6612, p=0.4320) or treatment ($F_{1,12}$ = 0.3316, p=0.5753).