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RESEARCH ARTICLE

Figures and figure supplements

Re-expression of SynGAP protein in adulthood improves translatable measures of brain function and behavior

Thomas K Creson *et al*

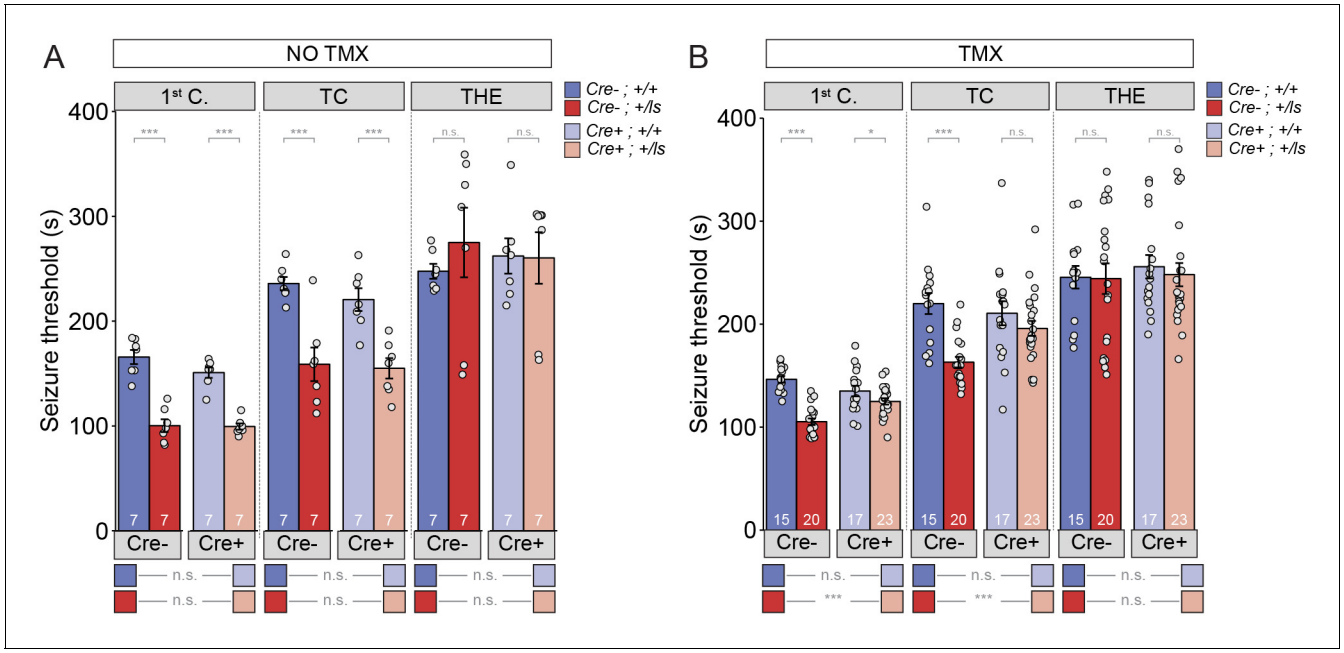

Figure 1. Seizure threshold is improved after adult restoration of SynGAP expression. (**A**) *Syngap*$^{Cre-;+/ls}$ and *Syngap*$^{Cre+;+/ls}$ mice exhibit hyperexcitability in two of the three events without Cre activation (No TMX) Main effects-1st clonus: Cre F(1,24)=2.13, p=0.157, Genotype F = 117.73, p=9.75E-11, Interaction F(1,24)=1.69, p=0.206); Cre- Cohen's d = 3.855, Cre +Cohen's d = 4.737. TC: Cre F(1,24)=722, p=0.404, Genotype F(1,24)=40.05, p=1.53E-6), Interaction F(1,24)=.257, p=0.617); Cre- Cohen's d = 2.396, Cre+ Cohen's d = 2.405. THE: Cre F(1,24)=9.99E-6, p=0.998, Genotype F(1,24) =.320, p=0.577), Interaction F(1,24)=.420, p=0.523. (**B**) *Syngap*$^{Cre+;+/ls}$ mice exhibit thresholds comparable to those of *Syngap*$^{Cre-;+/ls}$ mice after Cre activation (TMX-treated) in two of the three events Main effects-1st clonus: Cre F(1,71)=2.59, p=0.112; Genotype F(1,71)=58.328, p=7.86E-11, Interaction F = 1 (1,71)=18.84 p=4.62E-5; Cre- Cohen's d = 3.329, Cre+ Cohen's d = 0.674; TC: Cre F(1,71)=4.53, p=0.037, Genotype F(1,71)=26.15, p=2.57E-6, Interaction F(1,71)=6.50, p=0.013; Cre- Cohen's d = 2.040; Cre+ Cohen's d = 0.540; THE: Cre F(1,71)=.037, p=0.847, Genotype F(1,71)=1.15E-5, p=0.997, Interaction F(1,71)=.049, p=0.826. Data points (and numbers) in bars represent biological replicates (animals). Data from panel B are pooled from two separate experiments.

DOI: https://doi.org/10.7554/eLife.46752.002

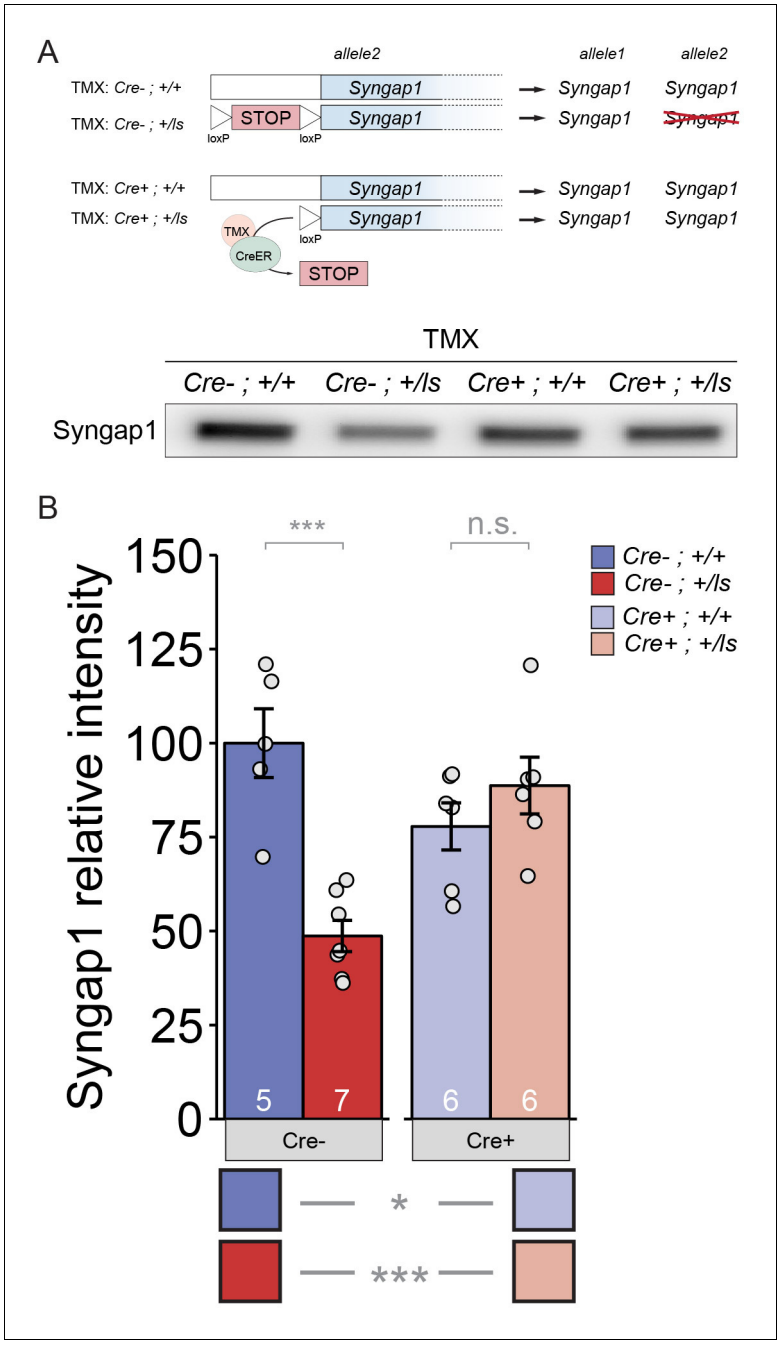

Figure 1—figure supplement 1. TMX-induced restoration of SynGAP protein levels in adult *Syngap1*$^{Cre+;+/ls}$ mice. (**A**) Western blot demonstrating expression levels of total SynGAP in Cre(-) or Cre(+) heterozygous Lox-Stop mice and WT littermates. (**B**) Densitometric analysis of SynGAP. Band intensities were normalized to total protein levels and transformed to % of the *Syngap1*$^{Cre-;+/+}$ group mean. Two-factor ANOVA. Main effects: Cre p=0.198, Genotype p=0.007, Interaction p=1.554E-4.=0.=0. Pairwise comparisons from posthoc tests can be found in ***Supplementary file 1***. Data points (and numbers) in bars represent biological replicates (animals).
DOI: https://doi.org/10.7554/eLife.46752.003

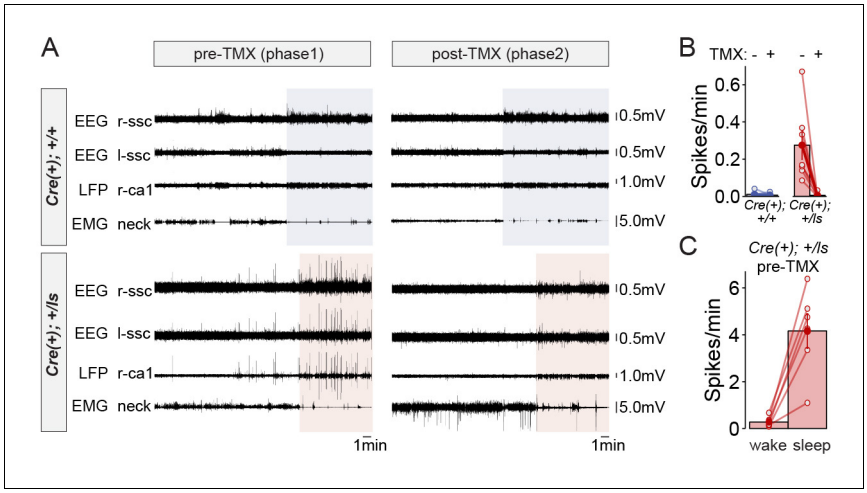

Figure 2. Rectification of state-dependent paroxysmal spiking events in *Syngap1* mutants after adult-initiated gene therapy. (**A**) Representative EEG/LFP traces from a WT [Cre(+); +/+] and *Syngap1* heterozygous mutant mouse [Cre(+); +/ls]. After initial recordings (pre-TMX), all animals were injected with TMX. Post-TMX recordings were acquired 30 days after the last TMX injection. TMX rescued low levels of SynGAP protein in +/ls animals (see ***Figure 1—figure supplement 1***). Highlighted areas correspond to periods of sleep (see Materials and methods). Phase I and Phase II recordings are from the same animals. (**B**) Frequency of spiking events observed in the hippocampal LFP channel during the wake phase (i.e. non-highlighted areas in panel A) from both pre- and post-TMX recording sessions in each animal. Two-way repeated measures ANOVA.:Main genotype effects: $F(1,11) = 10.1$, $p=0.00879$, Main TMX effects: $F(1,11)=12.088$, $p=0.00518$. Interaction between genotype and TMX: $F(1,11) = 9.777$, $p=0.00963$. Cre(+);+/+n = 6, Cre(+);+/ls n = 7. (**C**) Comparison of the spiking frequency from the *h*LFP channel in Cre(+);+/ls mice during wake and sleep before TMX injections, paired-*t* test $t(5)=-5.6007$, $p=0.002507$ (n = 5). Data points in plots represent biological replicates (animals).

DOI: https://doi.org/10.7554/eLife.46752.007

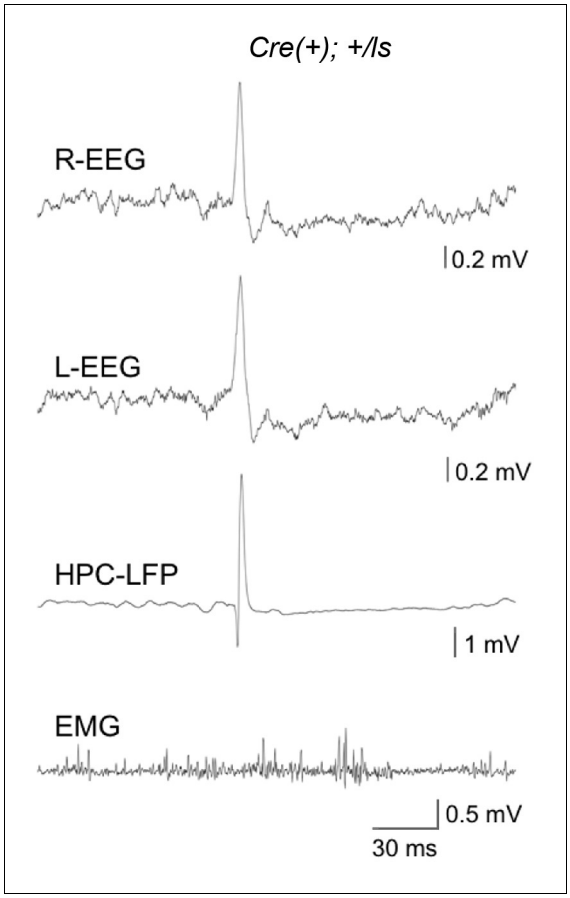

Figure 2—figure supplement 1. Generalization of high-amplitude spikes across the forebrain. Representative traces from all channels during a Phase I recording from a Cre(+) Lox-Stop mouse.
DOI: https://doi.org/10.7554/eLife.46752.008

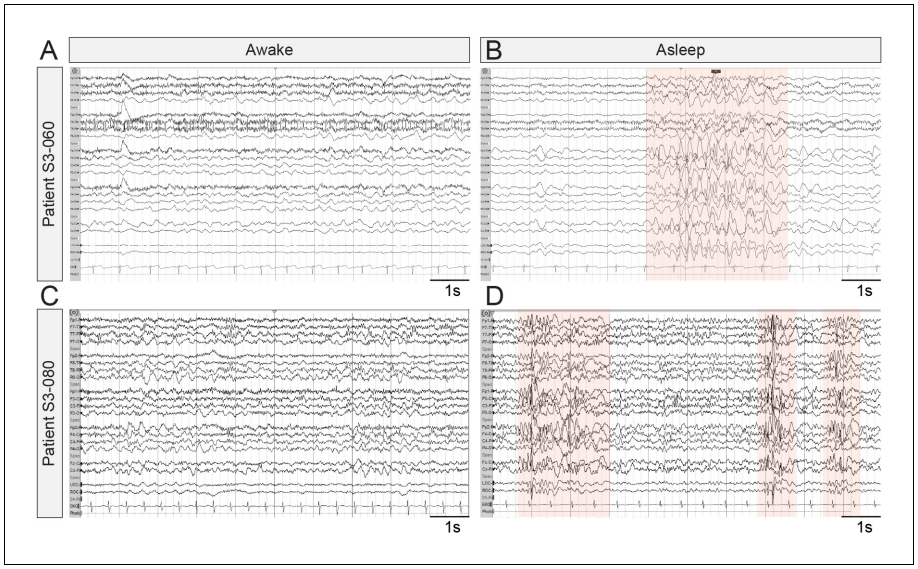

Figure 3. Representative EEG recordings taken from *SYNGAP1* patients during wake and sleep. Ten second epochs of electroencephalograms from patients with SYNGAP1 pathogenic variants. (**A**) Patient S3-060 while awake (**B**) Patient S3-060 while asleep (**C**) Patient S3-080 while awake (**D**) Patient S3-080 while asleep. Shaded areas indicate bursts of generalized epileptiform activity.

DOI: https://doi.org/10.7554/eLife.46752.011

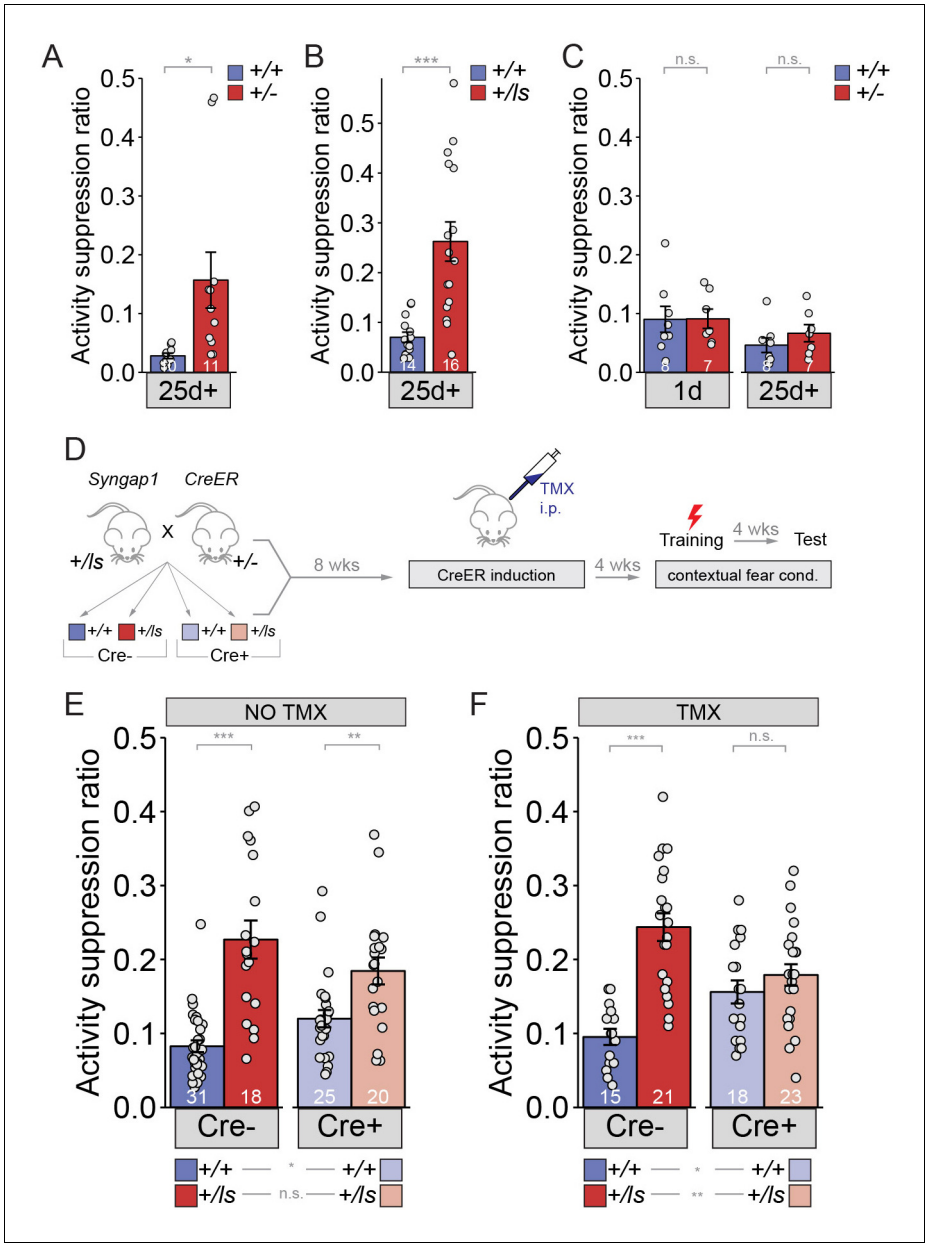

Figure 4. Long-term memory can be improved in adult mice with *Syngap1* pathogenicity. (**A**) *Syngap1*$^{+/+}$ and *Syngap1*$^{+/-}$ ± were trained in the remote contextual fear conditioning paradigm and tested one month later for activity suppression levels. Activity of the *Syngap1*$^{+/-}$ was suppressed significantly less than that of the *Syngap1*$^{+/+}$ group indicating compromised remote memory for the mutant group. Unpaired *t* test (t(19)=-2.567, p=0.019). Cohen's d = 1.150. (**B**) *Syngap1*$^{+/+}$ and *Syngap1*$^{+/ls}$ mice were trained in the contextual fear conditioning paradigm and tested one month later for activity suppression levels. Activity of the *Syngap1*$^{+/ls}$ group was suppressed significantly less than that of the *Syngap1*$^{+/+}$ group indicating compromised remote memory for the mutant group. Wilcoxon rank sum test W = 19, p=2.82E-5, Cohen's d = 1.676. (**C**) *Syngap1*$^{+/+}$ and *Syngap1*$^{+/-}$ were tested, firstly, 1d after training, followed by another testing one month later. Activity suppression levels were not significantly different between the groups for either testing (unpaired t test,1-day t(13)=-0.033, p=0.974; 26 days t(13)=-1.068, p=0.305). (**D**) Experimental schematic depicting the breeding strategy for generation of Cre-inducible *Syngap1*$^{Cre+;+/ls}$ mice and Cre induction with TMX treatment for restoration of *Syngap1* expression and subsequent remote fear conditioning testing. (**E–F**) *Syngap1*$^{Cre-;+/+}$, *Syngap1*$^{Cre-;+/ls}$, *Syngap1*$^{Cre+;+/+}$, and *Syngap1*$^{Cre+;+/ls}$ mice were run in the remote contextual fear conditioning paradigm without (**E**) and with (**F**) TMX administration. Activity suppression values from mice without TMX administration (**No TMX**) were assessed (2-factor ANOVA: Main Effects-Cre F(1,90)=0.030, p=0.864, Genotype F(1,91)=46.78, p=9.28E-10, Interaction F(1,91)

Figure 4 continued on next page

Figure 4 continued

=6.81, p=0.011; Cre- Cohen's d = 1.725, Cre+ Cohen's d = 0.910. **With TMX administration** (2-factor ANOVA: Main Effects- Cre F=(1,73)=0.019, p=0.891, Genotype F(1,73)=27.49, p=1.48E-6, Interaction F(1,73)=14.75, p=2.59E-4; Cre- Cohen's d = 2.167). Data points (and numbers) in bars represent biological replicates (animals). Data from panels E-F are pooled from at least two separate experiments.

DOI: https://doi.org/10.7554/eLife.46752.012

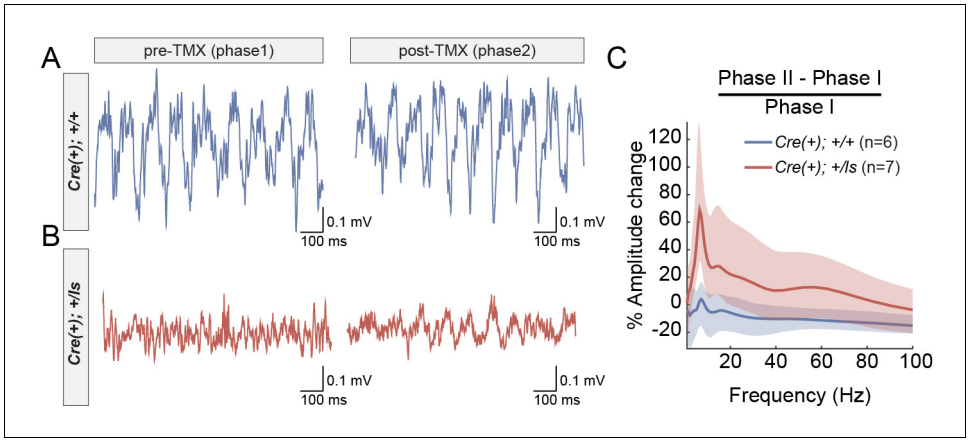

Figure 5. Increased amplitude of theta oscillations after SynGAP re-expression in adult *Syngap1* mutant mice. (**A–B**) CA1 LFP traces from a WT (**A**) and a *Syngap1* mutant (**B**) mouse during Phase I and Phase II sessions. (**C**) Grand average of within-subjects changes in signal amplitude across the full spectrum of hippocampal rhythms. The amplitude change was normalized by the average amplitude during Phase I sessions. The shaded areas represent 95% bootstrapped confidence intervals. Significant increases in amplitude in Phase II were detected in the 6–12 Hz theta range (Permutation test: p=0.0128, 5000 shuffles). N's are biological replicates (animals). Legends for Figure Supplements.

DOI: https://doi.org/10.7554/eLife.46752.018

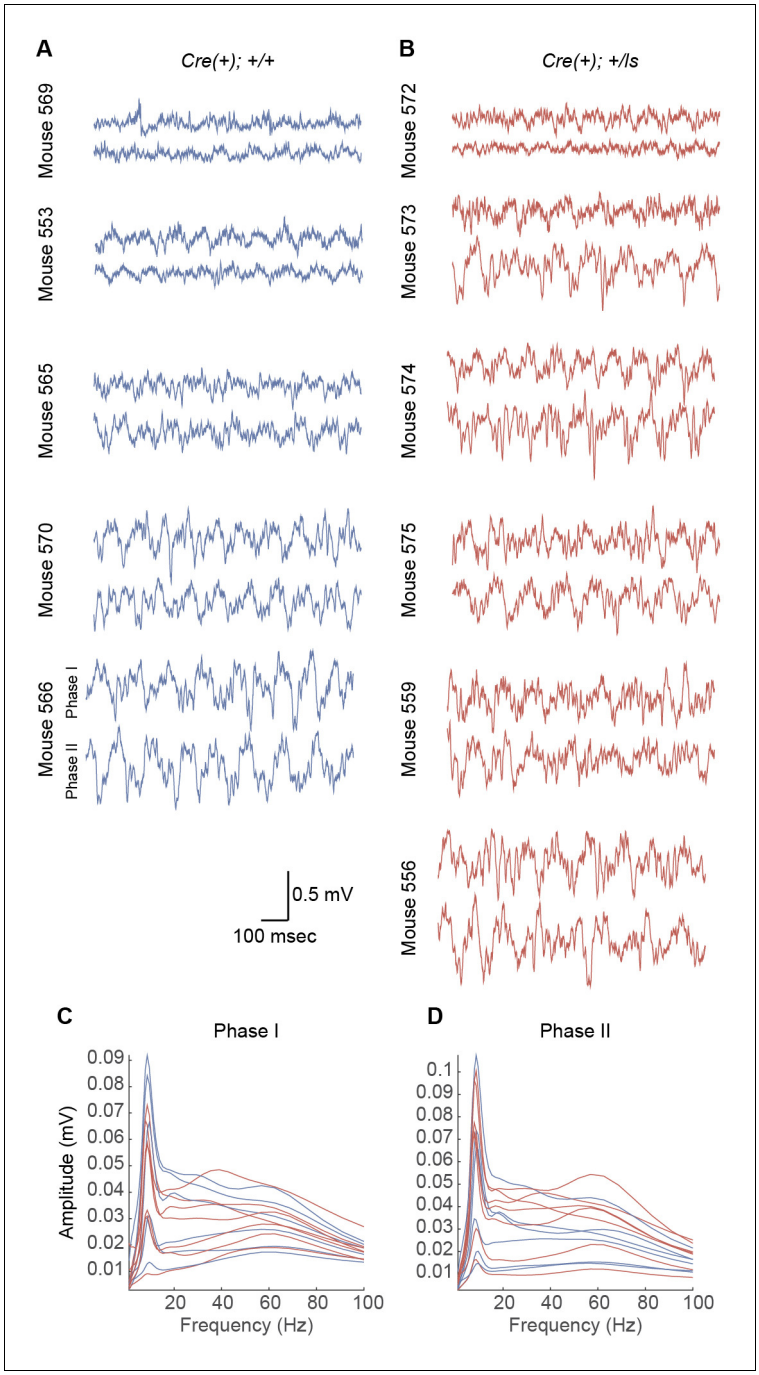

Figure 5—figure supplement 1. Amplitude of theta oscillations in each mouse during Phase I and Phase II recording sessions. (**A–B**) CA1 LFP recordings from WT (**A**) and *Syngap1* mutant (**B**) mice during Phase I and Phase II sessions. (**C–D**) Average amplitude spectra for each mouse during Phase I (**C**) and Phase II (**D**) sessions. Individual mice are indicated with individual lines, and WT and *Syngap1* mutant spectra are depicted in blue and red, respectively.

DOI: https://doi.org/10.7554/eLife.46752.019

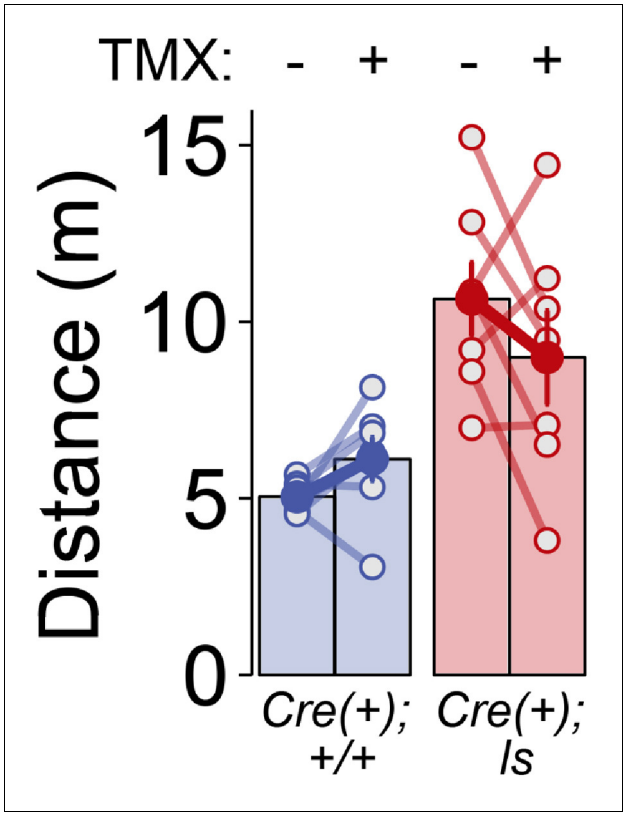

Figure 5—figure supplement 2. Effect of genotype, but not phase, on horizontal activity during neurophysiological recordings. Cre(+) WT and Cre(+) Lox-Stop mice were video tracked for distances traveled during the first ten minutes of recording during Phase I (TMX-) and Phase II (TMX+) sessions. RMANOVA- Group: $F(1,12)=16.527$, $p=0.002$; Phase: $F(1,12)=0.164$, $p=0.692$; Group x Phase: $F(1,12)=3.521$, $p=0.085$. Data points in bars represent biological replicates (animals). Legends for Supplementary Files.

DOI: https://doi.org/10.7554/eLife.46752.020