Sleep-deprivation aggravates cortical gamma dysregulation in juvenile Syngap1$^{+/-}$ mice.

Yun, Hwang[1]; Kripi, Singapuri[1]; Sullivan, Brennan J.[1]; Preeti, Vyas.[1,2]; Anjali, Devireddy[1]; Kadam, Shilpa D.[1, 2]

[1]Neuroscience Laboratory, Hugo Moser Research Institute at Kennedy Krieger; [2] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, 21205

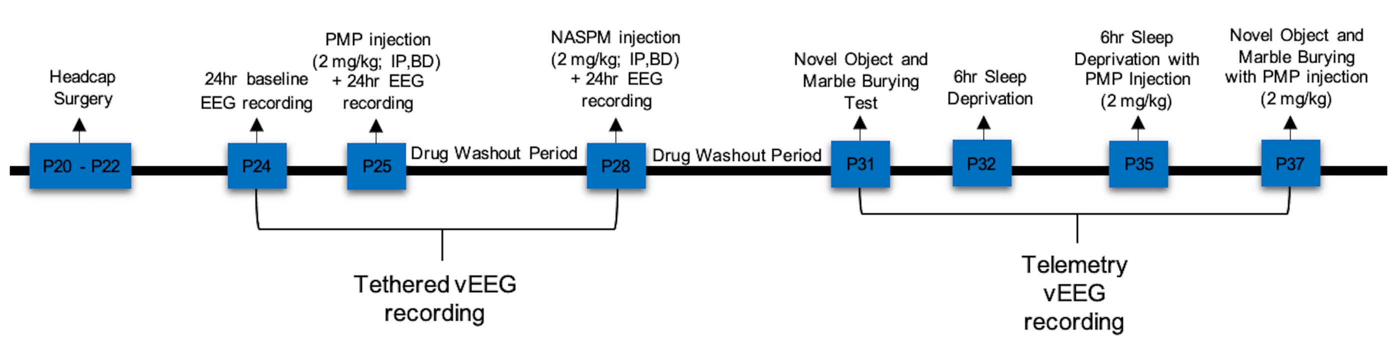
Introduction

SYNGAP1 codes for SYNGAP1, a Ras-GTPase activating protein that negatively regulates Ras-ERK signaling and tightly controls AMPA receptor (AMPAR) recruitment to the post synaptic density. Autosomal mental retardation type 5 (MRD5), one of the most prevalent intellectual disabilities (IDs) with epilepsy, is caused by heterozygous loss-of-function SYNGAP1 variants. Patients with MRD5 demonstrate abnormal spiking during EEG recordings as well as atonic, myoclonic, absence, and generalized tonic-clonic seizures (Hamdan et al., 2009; Mignot et al., 2016). MRD5 patients also report sleep complications, supporting the relationship between epilepsy and dysfunctional sleep (Parker et al., 2015; Prchalova et al., 2017). Perampanel (PMP) is an FDA approved non-competitive AMPAR antagonist. To investigate the natural history of the epilepsy associated with heterozygous loss-of-function *SYNGAP1* mutations and the anti-seizure efficacy of PMP, a *Syngap1* heterozygous loss-of-function mouse model (deletions of exon 7 and 8; clinically refractory) underwent 24h vEEG/EMG recordings at juvenile ages (P25-P40).

Methods

24h tethered vEEG recording was performed for Het$^{+/-}$ mice and their age- and sex matched WT littermates at P21-P30, followed by telemetric vEEG for 24h with 6h sleep deprivation at P35. Quantitative EEG (qEEG) analysis included the frequency bands: delta (0.5-4.0 Hz), theta (5.5-8.0 Hz), alpha (8.0-13.0 Hz), beta (13.0-30 Hz), and gamma (35-50 Hz). Specifically, linear regression of gamma frequency power during transition states from wake to sleep was quantified. Spike frequency over 24h EEG was scored by a blind reviewer based on previously published parameters. The effect of low-dose perampanel (PMP; 2mg/kg, IP, BD), an AMPA receptor antagonist, on these potential qEEG biomarkers was investigated. Movement analysis was performed at 5am – 7am using infrared cameras to trace fast-active movements. Additionally, 24h telemetric vEEG was recorded during novel object and marble burying behavioral tests.

Figure 1: Experimental Scheme



Sleep Bout Analysis

Juvenile HET$^{+/-}$ mice displayed altered sleep architecture

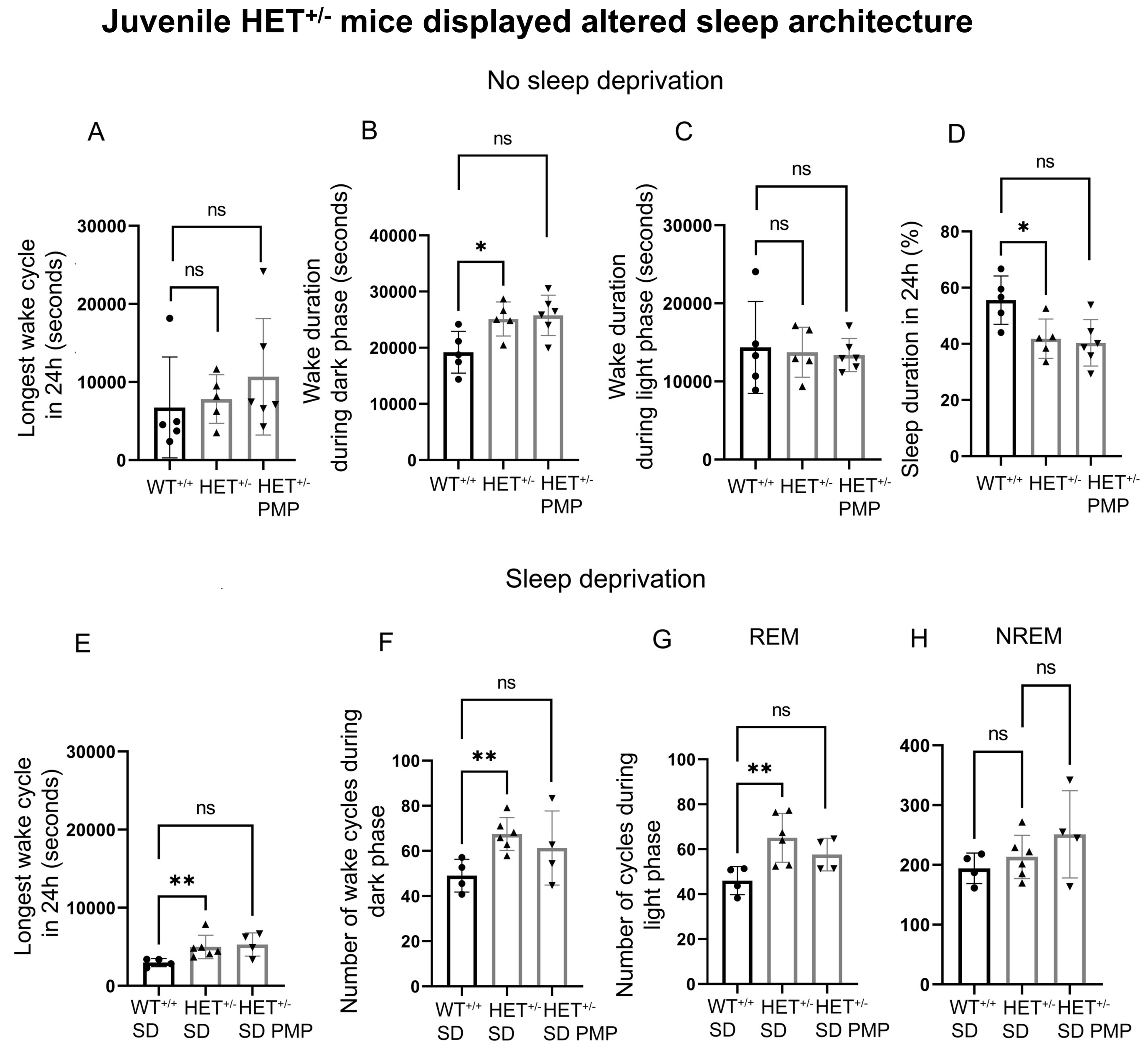


Figure 2: (A) Prior to sleep deprivation, the longest wake cycle remains constant between groups. **(B)** HET$^{+/-}$ displayed longer wake duration during the dark phase (WT$^{+/+}$ vs. HET$^{+/-}$: unpaired t-test; p <0.01) **(C)** while the relation was absent during the light phase. **(D)** HET$^{+/-}$ had shorter duration of sleep than WT$^{+/+}$ in a 24h recording (WT$^{+/+}$ vs. HET$^{+/-}$: unpaired t-test; p <0.05). **(E)** Unlike prior to 6h sleep deprivation, HET$^{+/-}$ SD presented with higher longest wake cycle (WT$^{+/+}$ SD vs. HET$^{+/-}$ SD: unpaired t-test; p <0.01) **(F)** and number of wake cycles during the dark phase (WT$^{+/+}$ SD vs. HET$^{+/-}$ SD: unpaired t-test; p <0.01). **(G-H)** Additionally, HET$^{+/-}$ SD had increased frequency of REM cycles during the light phase (WT$^{+/+}$ SD vs. HET$^{+/-}$ SD: unpaired t-test; p <0.01), while the frequency of NREM cycles was constant.

Cortical Gamma Dysregulation

PMP rescued gamma dysregulation

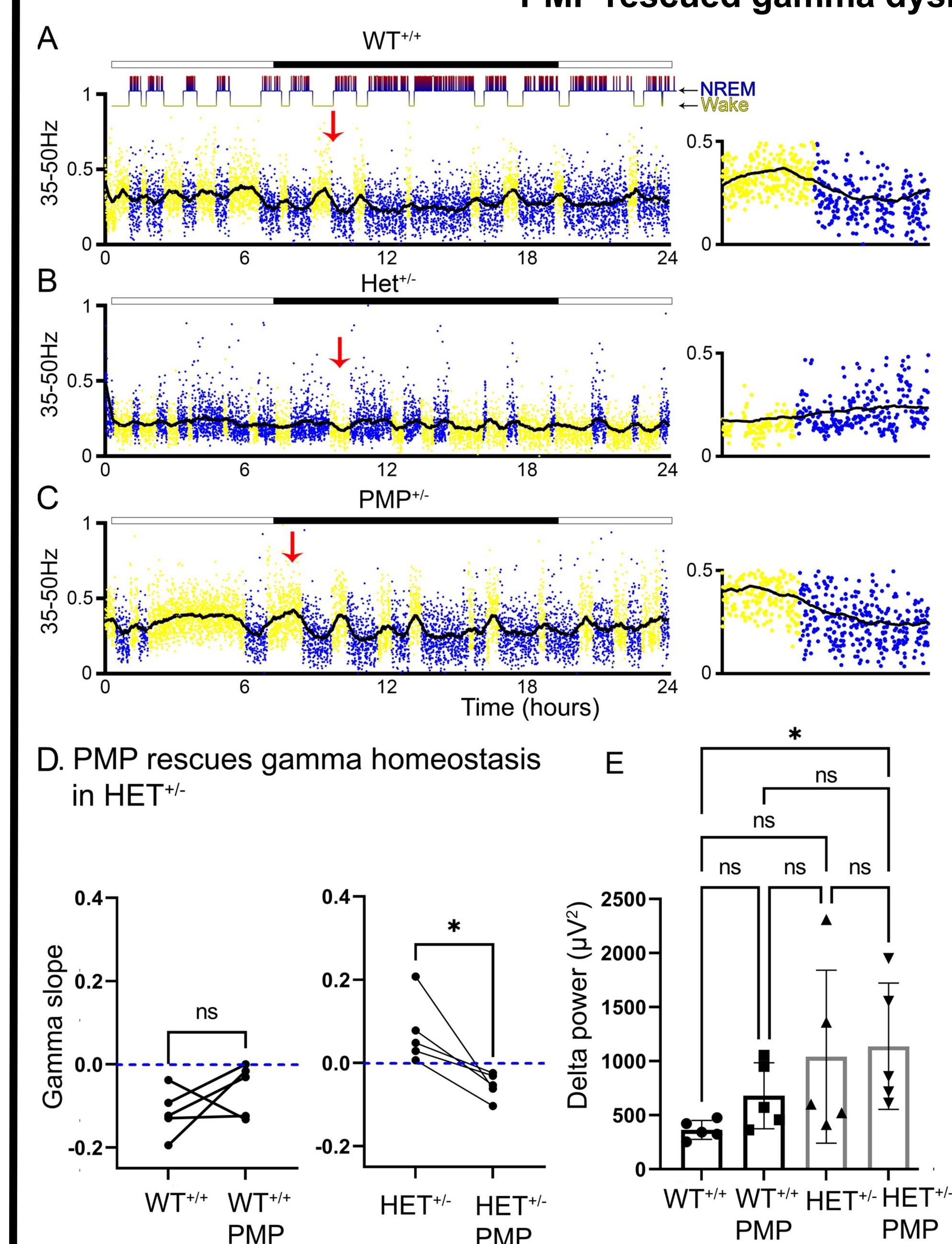


Figure 3: (A) Representative 24h hypnograms and WT$^{+/+}$ 10 second epoch of a 24h baseline vEEG with a loess trendline in black. On the right is a representative homeostatic gamma slope that decreased from wake to sleep at a transition point noted with a red arrow. The light/dark phase was noted on top with a white/black bar. **(B)** Representative gamma dysregulation in HET$^{+/-}$ where the gamma power did not decrease from wake to NREM sleep. **(C)** Representation of low-dose PMP (2mg/kg, IP, BD) rescuing the gamma homeostasis in HET$^{+/-}$. **(D)** PMP significantly reduced the gamma slope and rescued the gamma homeostasis in behavioral transitioning points (HET$^{+/+}$ vs. HET PMP$^{+/-}$: paired t-test; p < 0.05). **(E)** Delta power during NREM did not show notable statistical significance across all groups (One-way ANOVA).

Sleep Deprivation

Sleep deprivation aggravated gamma dysregulation

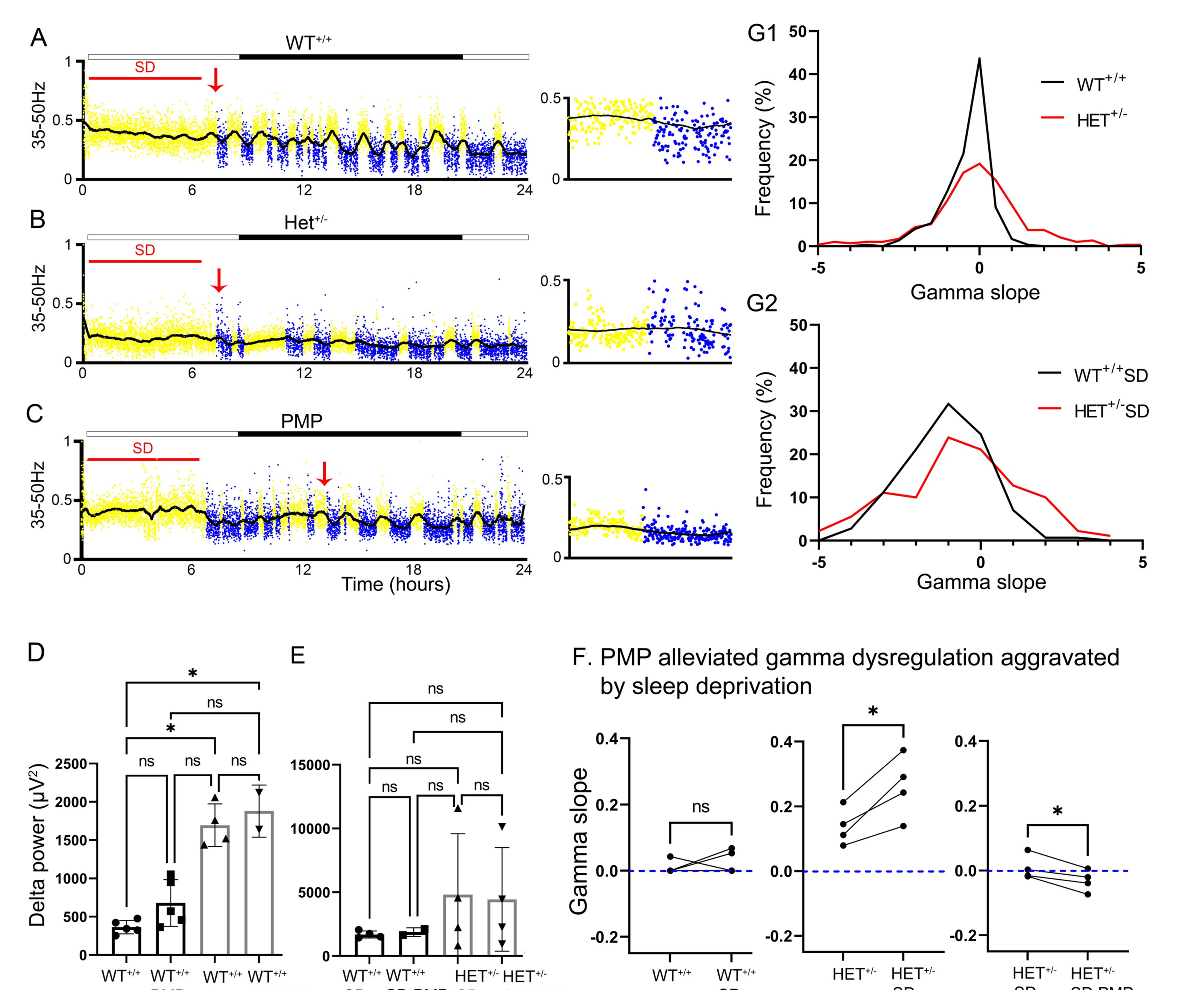


Figure 4: (A) WT$^{+/+}$ 10 second epoch of a 24h vEEG with 6h sleep deprivation. On the right is a representative gamma homeostasis that decreases from wake to sleep at a transition point noted with a red arrow. The light/dark phase is noted on top with white/black bar, and the sleep deprivation segment is marked in red. **(B)** HET$^{+/-}$ displayed increased gamma slope from wake to sleep after sleep deprivation. **(C)** Low-dose PMP (2mg/kg, IP) rescued gamma dysregulation during sleep transition points in HET$^{+/-}$ after 6h sleep deprivation. **(D)** The effect of 6h sleep deprivation was confirmed by comparing the delta frequency power before and after sleep deprivation in WT$^{+/+}$. As expected, the delta frequency power increased with 6h sleep deprivation (WT$^{+/+}$ vs. WT$^{+/+}$ PMP: One-way ANOVA; p < 0.05). **(E)** Delta power during NREM did not show notable statistical significance across all groups (One-way ANOVA, multiple comparison). **(F)** Gamma dysregulation was aggravated by 6h sleep deprivation as the magnitude of abnormal positive gamma slope further increased (HET$^{+/-}$ vs. HET$^{+/-}$ SD: paired t-test; p < 0.05). PMP rescued gamma homeostasis by decreasing the most dominant slope in sleep deprived HET$^{+/-}$ mice (HET$^{+/-}$ SD vs. HET$^{+/-}$ SD PMP$^{+/-}$: paired t-test; p < 0.05). **(G1-2)** Histogram of gamma slope from REM to NREM before and after sleep deprivation in WT$^{+/+}$ and HET$^{+/-}$. Following a similar trend, HET$^{+/-}$ had broader positive tail in gamma slope in with and without sleep deprivation (WT$^{+/+}$ vs. HET$^{+/-}$: one-way ANOVA; p < 0.05. WT$^{+/+}$ SD vs. HET$^{+/-}$ SD: one-way ANOVA; p < 0.05).

Behavioral analysis

Juvenile HET$^{+/-}$ mice displayed hyperactivity in movement analysis

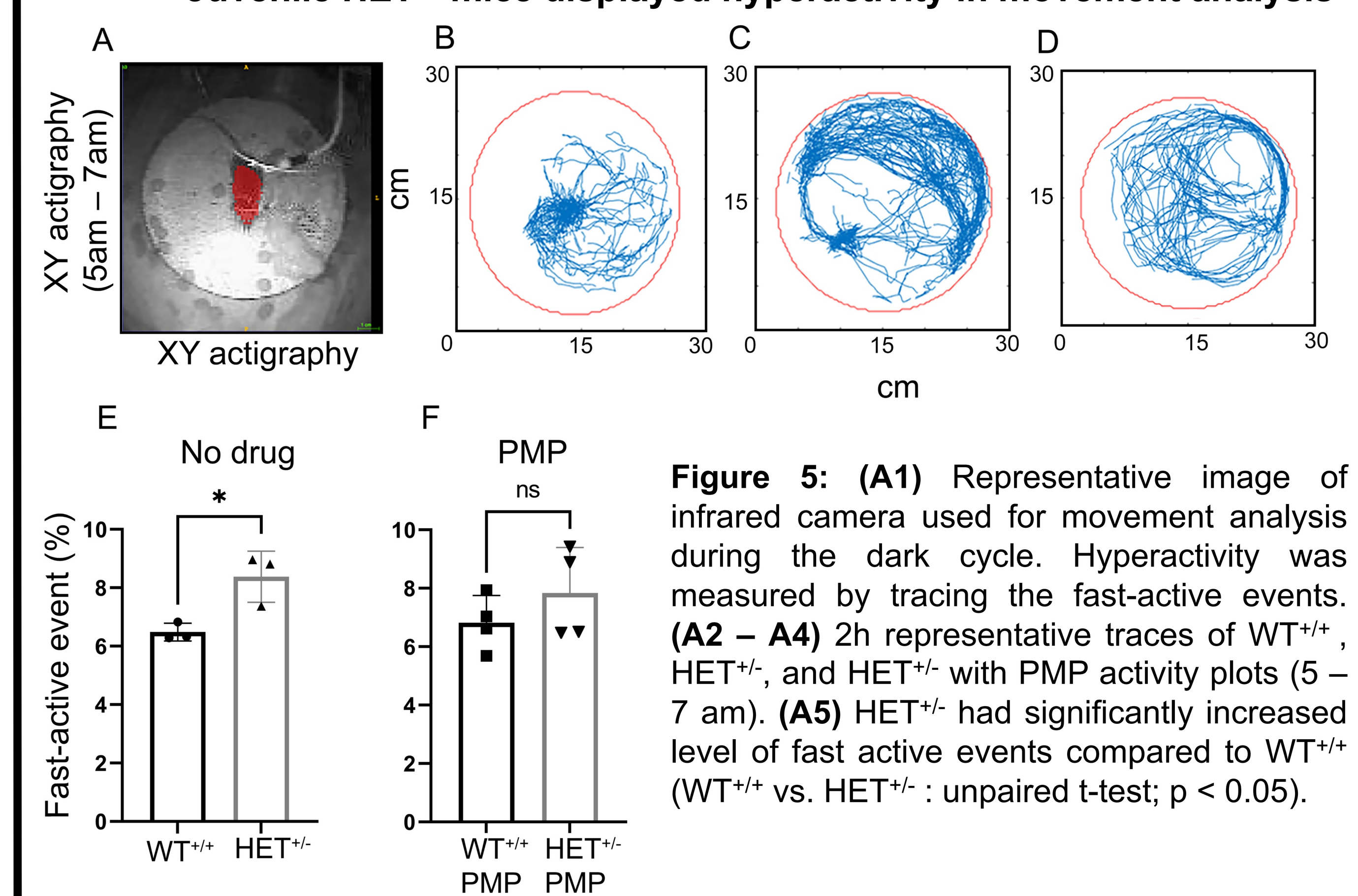


Figure 5: (A1) Representative image of infrared camera used for movement analysis during the dark cycle. Hyperactivity was measured by tracing the fast-active events. **(A2 – A4)** 2h representative traces of WT$^{+/+}$, HET$^{+/-}$, and HET$^{+/-}$ with PMP activity plots (5 – 7 am). **(A5)** HET$^{+/-}$ had significantly increased level of fast active events compared to WT$^{+/+}$ (WT$^{+/+}$ vs. HET$^{+/-}$: unpaired t-test; p < 0.05).

Nesting behavior, marble burying, and novel object tests showed hyperactivity

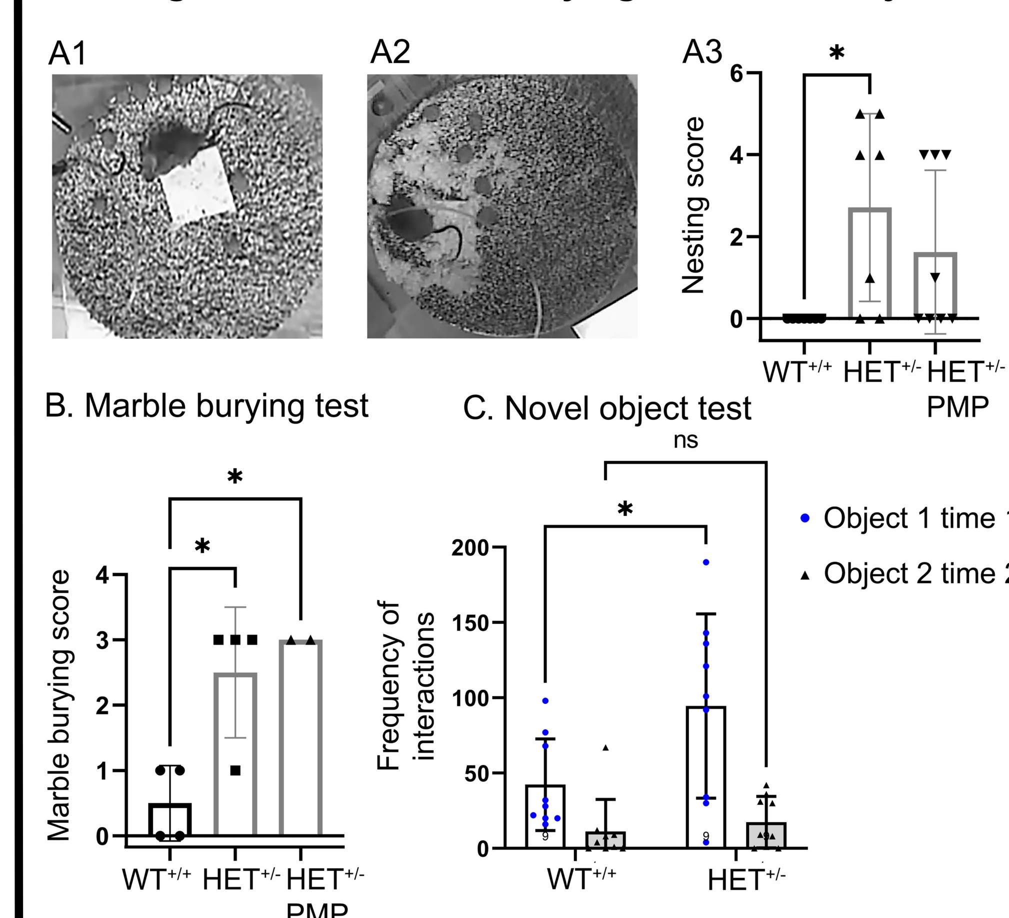


Figure 6: **(A1)** Representative image of a nest of WT$^{+/+}$ **(A2)** and HET$^{+/-}$ after 24h recording. **(A3)** Score was given based on the area of the nest. HET$^{+/-}$ had significantly higher score compared to WT$^{+/+}$ (WT$^{+/+}$ vs. HET$^{+/-}$: unpaired t-test; p < 0.05). **(C)** Additionally, marble burying testing showed hyperactivity in HET$^{+/-}$ (WT$^{+/+}$ vs. HET$^{+/-}$: unpaired t-test; p < 0.05). **(D)** HET$^{+/-}$ had more interaction with the initial novel object than WT$^{+/+}$ (WT$^{+/+}$ vs. HET$^{+/-}$ at object 1 time 1: two-way ANOVA; p < 0.05)

Interictal Spikes

Figure 7: (A) Representative EEG traces of a *Syngap1$^{+/-}$* mouse demonstrating abnormal cortical spikes in the frontal region (denoted by *) during wake and NREM sleep. **(B)**

Summary

- Sleep bout analysis suggested altered sleep architecture in juvenile *Syngap1$^{+/-}$* mice
- Gamma dysregulation from wake to sleep was present in juvenile *Syngap1$^{+/-}$* mice.
- Gamma dysregulation was aggravated after 6h sleep deprivation.
- Acute treatment with PMP, an AMPAR antagonist, rescued cortical gamma homeostasis.
- Behavior analyses on juvenile *Syngap1$^{+/-}$* mice suggest hyperactivity.
- Some juvenile *Syngap1$^{+/-}$* mice displayed abnormal cortical spikes predominantly during NREM sleep stage.

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