Research Square

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Abnormal Electrophysiological Phenotypes and Sleep Deficits in a Mouse Model of Angelman Syndrome

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Abstract

Background: Angelman Syndrome (AS) is a rare genetic disorder characterized by impaired communication, motor and balance deficits, intellectual disabilities, recurring seizures and abnormal sleep patterns. The genetic cause of AS is neuronal specific loss of expression of UBE3A (ubiquitin-protein ligase E6-AP), an imprinted gene. Seizure and sleep disorders are highly prevalent (>80%) in the AS population. The present experiments were designed to identify translational, neurophysiological outcome measures in a model of AS.

Methods: We used the exon-2 deletion mouse (Ube3a-del) on a C57BL/6J background to assess seizure, sleep and electrophysiological phenotypes. Seizure susceptibility has been reported in Ube3a-del mice with a variety of seizure induction methods. Here, we provoked seizures by a single high-dose injection of 80 mg/kg pentylenetetrazole. Novel experiments included the utilization of wireless telemetry devices to acquire global electroencephalogram (EEG) and neurophysiological data on electrographic seizures, power spectra, light/dark cycles, sleep stages and sleep spindles in Ube3a-del and WT mice.

Results: Ube3a-del mice exhibited reduced seizure threshold compared to WT. EEG illustrated that Ube3a-del mice had increased epileptiform spiking activity and delta power, which corroborates findings from other laboratories and recapitulates clinical reports in AS. This is the first report to use a cortical surface-based recording by a wireless telemetry device over tethered/fixed head-mount depth recordings. Less time in both paradoxical and slow-wave sleep, longer latencies to paradoxical sleep stages, and total less sleep time in Ube3a-del mice were observed compared to WT. For the first time, we detected fewer sleep spindles in the AS mouse model.

Limitations: This study was limited to the exon 2 deletion mouse model, future work will investigate the rat model of AS, containing a complete Ube3a deletion and pair EEG with behavior.

Conclusions: Our data enhance rigor and translatability as our study provides important corroboration of previous reports on epileptiform and elevated delta power. For the first-time we report neurophysiological phenotypes collected via translational methodology. Furthermore, this is the first report of reduced sleep spindles, a critical marker of memory consolidation during sleep, in an AS model. Our results are useful outcomes for therapeutic testing.

Full Text

This preprint is available for download as a PDF.

Figures

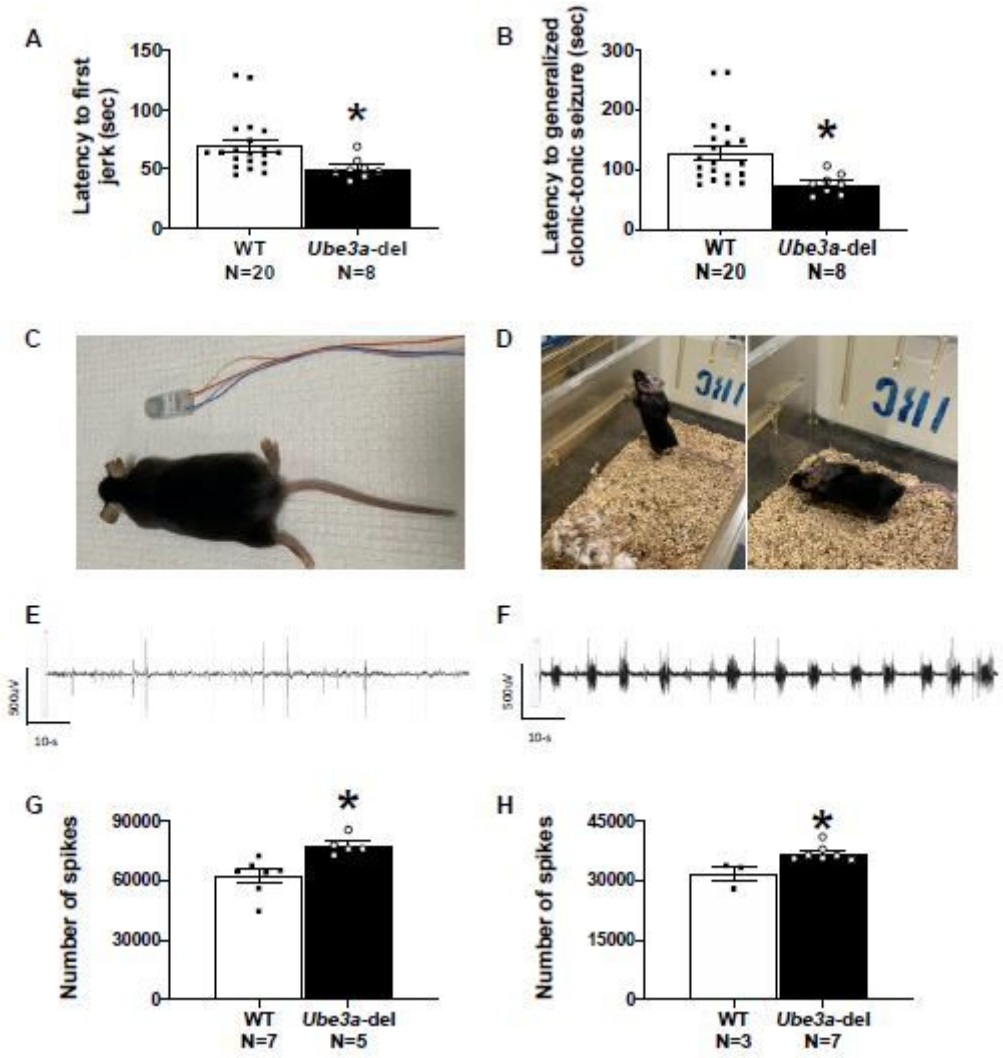

Figure 1

Ube3a-del mice exhibited seizure susceptibility and increased spiking compared to WT during baseline EEG recordings. Seizure susceptibility measures were observed for 30-min after an i.p. injection of 80 mg/kg PTZ. (A) Reduced latencies to first jerk were observed in the Ube3a-del mice compared to WT. (B) Faster onset to generalized tonic-clonic seizure was observed in Ube3a-del versus WT. To assess hyperexcitability, (C) mice were implanted with a telemetric device that collected both EEG and EMG data and (D) was small enough to allow for untethered, unrestrained data collection from the home cage of the test animals. (E-F) Representative EEG traces of both WT and Ube3a-del animals sampled after convulsant administration. Quantification of spiking activity during baseline data acquisition in mice recorded for (G) 72-hours and (H) 24-hours indicated more spiking events in Ube3a-del animals compared to their WT littermate controls. *p<0.05, Student's t-test between genotype.

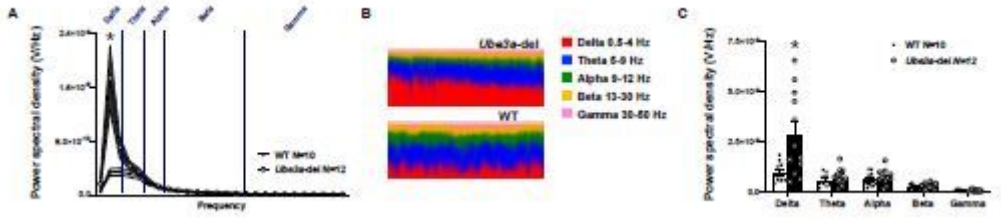

Figure 2

Ube3a-del mice exhibited elevated delta power. Surface EEG was collected in mice via a wireless telemetric device and analyzed for spectral power dissimilarities in delta (0.5-4 Hz), theta (5-9 Hz), alpha (9-12 Hz), beta (13-30 Hz) and gamma (30-50 Hz) frequencies during baseline EEG recordings. (A, C) Power spectral density collected from Ube3a-del mice differed from WT, specifically in the delta frequency range where Ube3a-del mice had higher delta power. No significant differences were detected at theta, alpha, beta, or gamma frequencies by genotype. (B) Representative 10-min spectrograms of Ube3a-del and WT mice illustrated the elevated delta phenotype observed in Ube3a-del mice. *p<0.05, Two-way RM ANOVA comparing genotypes across frequency and Student's t-test within frequency bands.

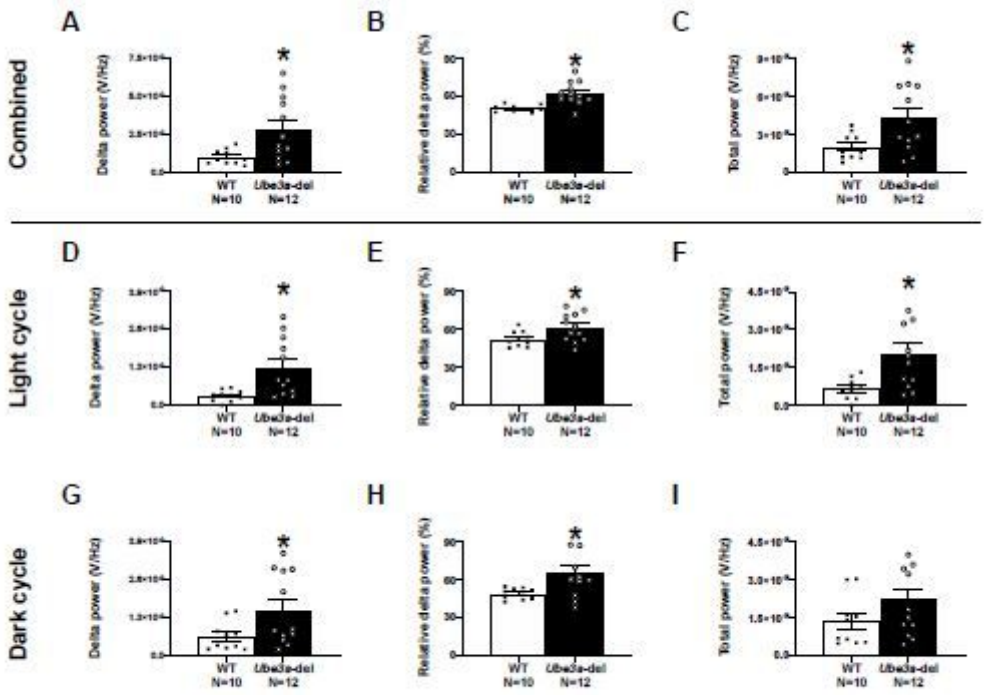

Figure 3

Elevation in delta power is persistent across light and dark cycles in Ube3a-del mice. Power spectral analysis was evaluated by total delta power (summation of power spectral densities across the delta frequency per animal), relative delta power (total delta power of a given animal divided by that animal's total power), and total power (summation of power spectral densities across all frequencies per animal)

between genotype. Quantification of (A) delta power and (B) relative delta power was significantly increased in Ube3a-del mice compared to WT littermate controls across light and dark cycles. (C) Total power was also significantly higher in Ube3a-del animals due to their elevated delta power. When analyzed by light and dark cycle separately, (D, E) total delta power and (E, H) relative delta power were also significantly increased in Ube3a-del mice, consistent with the findings seen across cycles. Additionally, total power is higher in Ube3a-del mice during the light cycle and trending towards significance during the dark cycle (p = 0.125). *p<0.05, Student's t-test between genotype.

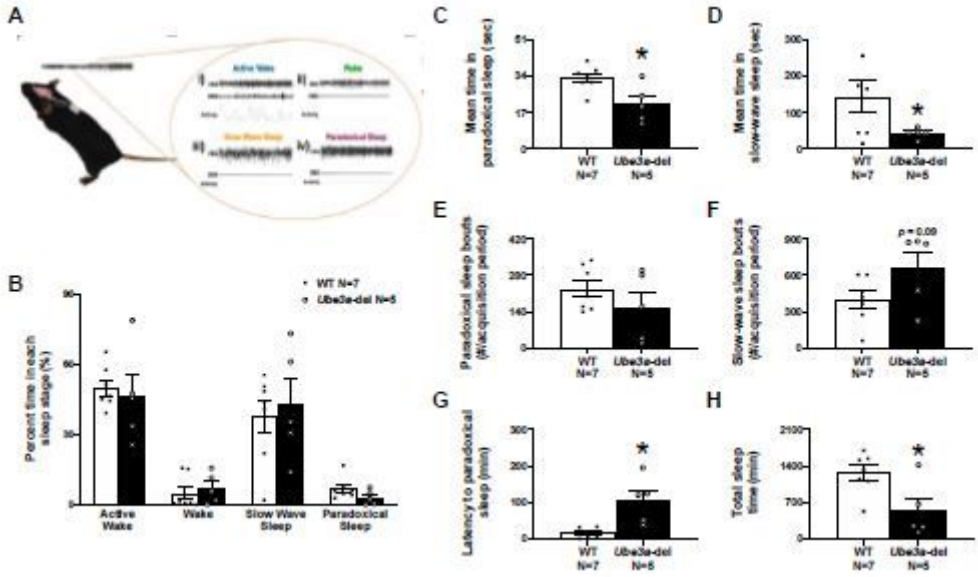

Figure 4

Sleep-wake cycles are altered in Ube3a-del mice. (A) A schematic detailing how each sleep state is binned using representative samples. A wake state was characterized by a low-amplitude, high-frequency signal with low-EMG tone while an active wake state was distinguished by high-EMG tone. Sleep was divided into either a slow-wave sleep state or a paradoxical sleep state. Slow-wave sleep was defined by having a high-amplitude, low-frequency signal with elevated delta power and low-EMG tone while paradoxical sleep had a low amplitude, low frequency signal with elevated theta power and low-EMG tone. (B) A significant genotype effect was detected across percent time spent in each sleep state though this was not specific to any one state using a Sidak's multiple comparison test. Sleep parameters, such as mean time in (C) paradoxical sleep and (D) slow-wave sleep were significantly reduced in Ube3a-del mice compared to their wildtype littermate controls. Additionally, (E, G) Ube3a-del mice had fewer paradoxical sleep bouts and longer latencies to paradoxical sleep states with (F) trends towards fewer slow-wave sleep bouts (p = 0.09). Finally, (H) total sleep time was significantly lower in Ube3a-del mice compared to WT. *p<0.05, Two-way ANOVA comparing genotypes across sleep stages and Student's t-tests for specific sleep parameters

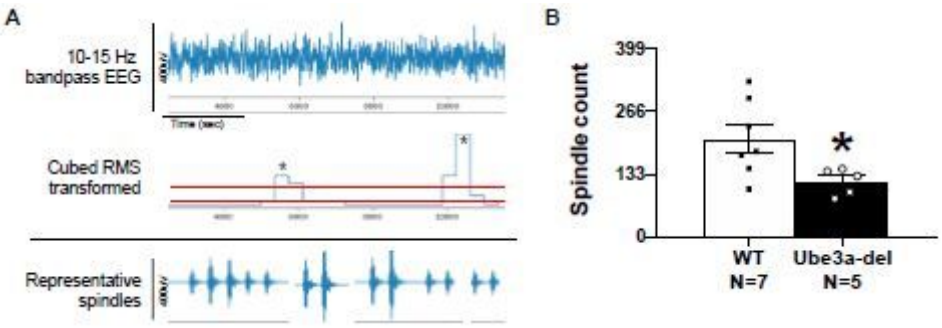

Figure 5

Ube3a-del mice exhibited reduced sleep spindle production. Sleep spindles were automatically detected using a custom written Python script. (A) A schematic of the levels of data processing imposed on the EEG signal to detect sleep spindles. A 10-15 Hz bandpass filter was first applied as depicted in the top EEG trace. Additionally, a Butterworth filter (3 Hz first stopband, 10 Hz first passband, 15 Hz second passband, 22 Hz second stopband, 24 dB attenuation level) was used to further filter for the frequency bands of interest. Next, the root-mean square (RMS) of the filtered signal was calculated with a 750 ms window to smooth the EEG trace before cubing the entire signal to amplify the signal-noise ratio as seen with the middle EEG trace. To detect spindles, a lower threshold (1.2 x mean-cubed RMS) was used to determine the start and end of a spindle while an upper threshold (3.5 x mean-cubed RMS) was used to identify the peak of a spindle. The red lines on the middle EEG trace indicate the upper and lower thresholds and the “*” denotes where the code identified a spindle. (B) Ube3a-del mice had significantly less sleep spindles when compared to their wildtype littermate controls. *$p<0.05$, Student’s t-test between genotype.