Abnormal electrophysiological phenotypes and sleep deficits in a mouse model of Angelman Syndrome

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Research

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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 pentylentetrazole. 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.

Introduction

Angelman Syndrome (AS) is a rare (~1:15,000) neurodevelopmental disorder (NDD) characterized by impaired expressive communication skills, ataxia, motor and balance deficits, severe intellectual disabilities, recurring seizures and poor sleep [1, 2]. Among the most frequent (>80%) clinical features of AS are seizures and abnormal encephalogram (EEG) patterns, where seizures often start early in life and are largely resistant to classic anti-epileptic drugs and distinct EEG signatures commonly precede most clinical features and are persistent throughout an AS individual's lifetime [3, 4]. Seizures occur frequently and typically present across multiple seizure types including, but not limited to, absence, myoclonic, and
generalized clonic-tonic seizures and, while they are generally diagnosed early in life, they are consistent throughout an individual’s lifetime, contributing to a significantly higher burden of care [5-7]. Abnormal encephalogram (EEG) patterns are widespread in AS, and include epileptiform discharges, increased rhythmic delta wave activity, and intermittent elevated theta activity [8, 9]. In addition, sleep deficits are also common in AS (20-80%) and are one of the most difficult symptoms to manage, as reported by parents and caretakers [3, 10].

AS results from the loss-of-expression and function of the ubiquitin-protein ligase E6-AP (UBE3A) gene in neuronal cells [11]. UBE3A is maternally imprinted, and as such, is only expressed from the maternal allele in neuronal but not glial cells in the brain albeit biallelically expressed in all other cell types throughout the body. UBE3A is located on chromosome 15q11.2-13 and its protein product, Ube3a, mainly functions as a ligase responsible for polyubiquitinating chains to substrates, targeting them for degradation by the proteasome [12]. A number of studies indicate a role for Ube3a in synaptic and neural plasticity, which may underlie the imbalance of excitatory/inhibitory homeostasis and contribute to seizure phenotypes and irregularities in sleep [13-15]. Despite a broad understanding of the genetic etiology and some of the basic mechanistic function(s) of Ube3a, there is an unmet need for therapies for individuals with AS.

While we and others have previously described behavioral deficits in rodent models of AS [16, 17] and other neurodevelopmental disorders (NDDs) [18-26], an effort has been made to incorporate in vivo electrophysiology, expanding clinically analogous phenotypes that can be provided as proof of in vivo efficacy. Pursuant to this goal we sought to identify relevant functional phenotypes, including seizures, EEG signature, sleep patterns and sleep spindles using the most rigorously characterized mouse model of AS, with a deletion of Ube3a (Ube3a-del) inherited from the maternal allele resulting in Ube3am+/p+ on the C57BL/6J background generated from breeding Ube3am+/p- females with Ube3am+/p+ males [11].

Many laboratories have used this AS mouse model of Jiang and Beaduet [11] and reported numerous characteristics that resemble core features of AS including seizure susceptibility, increased epileptiform activity, elevated delta and sleep deficits, though they vary widely on seizure induction methods, background strain and EEG collection techniques [15, 27-33]. In corroboration and extension, we quantified seizure susceptibility in Ube3a-del mice on the seizure resistant C57BL/6J background strain instead of the 129S1/SvImJ often used for seizure studies in AS, since the C57BL/6J’s are resistant to seizures [34]. We also utilized a novel high dose, 80 mg/kg, of pentylenetetrazole, as it has not been extensively reported in Angelman Syndrome, despite a large amount of seizure investigation in AS. We quantified seizure susceptibility in Ube3a-del mice on the seizure resistant C57BL/6J background strain [35]. Additionally, we investigated baseline epileptiform activity and its indication in hyperexcitability metrics. We compared spectral power signatures using our wireless telemetry devices that employed the use of surface electrodes to quantify cortical EEG while allowing free movement of the animal in its home cage. Finally, we extended earlier reports of sleep deficits, defined sleep stages, and, for the first time, quantified sleep spindles in the Ube3a-del mouse model on the C57BL/6J background.
Materials And Methods

Design

Three cohorts of mice were used in this study. All subjects were bred in our facility with a deletion of *Ube3a* (*Ube3a-del*) inherited from the maternal allele resulting in *Ube3a*/*p*+ mice generated from breeding *Ube3a*/*p*+ females with *Ube3a*/*p*+ males, termed *Ube3a-del* throughout the study and Figures. Cohort 1 consisted of 20 WT and 8 *Ube3a-*del mice (5 *Ube3a-*del/9 WT males and 3 *Ube3a-*del/11 WT females) that were observed for 30 minutes following administration of pentylenetetrazole (PTZ; 80 mg/kg; i.p.) for behavioral seizure characterization (SKU: P6500, Sigma Aldrich, St. Louis, MO, USA). EEG data was acquired in Cohorts 2 and 3 where animals were anesthetized and implanted with a wireless telemetry device designed to measure electroencephalogram (EEG) and electromyogram (EMG) in freely moving animals (Data Science International, New Brighton, MN). Cohort 2 consisted of 7 *Ube3a-*del animals (4 males, 3 females) and 3 WT (2 males, 1 females) that were recorded for 24 hours before administration of a lethal dose of pentylenetetrazole (80 mg/kg; i.p.) to observe EEG before and after seizure induction. Cohort 3 consisted of 5 *Ube3a-*del mice (4 males, 1 females) and 7 WT (3 males, 4 females) that were recorded for 72 hours to collect sleep data. All animals were between 8-12 weeks old and experimenters were blind to genotype. Subjects were implanted with the EEG device 7 days prior to data acquisition then, on day 8, began testing. All EEG recordings were collected in the subject animal's home cage in a temperature-controlled testing room maintained on a 12:12 light-dark cycle. All animals were littermates and singly housed after EEG implantation to avoid possible device displacement due to cage-mate interactions.

PTZ Administration

Seizure induction studies were conducted using 80 mg/kg pentylenetetrazole delivered intraperitoneally. Before administration, subjects were observed for 30-min, and weighed to determine the appropriate solution volume. For those that were previously implanted, the weight of the implant (4.0g) was subtracted from their total weight. Dosing was conducted in the morning (10:00-11:00) in a dim (~30 lux) holding room. Directly after administration of the convulsant, subjects were placed in a clean, empty cage where subsequent seizure stages were live-scored for 30-min. Seizure stages were scored using latencies to (1) first jerk/Straub’s tail, (2) loss of righting, (3) generalized clonic-tonic seizure, and (4) death. First jerk/Straub’s tail, previously described by Straub et al. (1911), was identified as a tonic dorsal extension of the tail usually accompanied by a jerk or jump of the animal’s entire body. Loss of righting was defined by the absence of both fore- and hindlimb paws from the surface of the cage bottom for >1-sec. Generalized clonic-tonic seizures were identified as loss of righting followed by phases of rigidity and forelimb/hindlimb spasms. Time to each stage was taken in seconds and analyzed across genotype.

EEG Implantation
Wireless EEG transmitters were implanted in anesthetized test animals using continuous isoflurane (2-4%). A 2-3 cm midline incision was made over the skull and trapezius muscles, then expanded to expose the subcutaneous space. Implants were placed in the subcutaneous pocket lateral to the spine to avoid discomfort of the animal and displacement due to movement. Attached to the implant were 4 biopotential leads made of a Nickel-Colbalt based alloy insulated in medical-grade silicone, making up two channels that included a signal and reference lead. These leads were threaded towards the cranial part of the incisions for EEG and EMG placement. The periosteum was cleaned from the skull using a sterile cotton-tip applicator and scalpel then two 1mm diameter burr holes were drilled (1.0mm anterior and 1.0mm lateral; -3.0mm posterior and 1.0mm lateral) relative to bregma. This lead placement allowed for measurement of EEG activity across the frontal cortical area. Steel surgical screws were placed in the burr holes and the biopotential leads were attached by removing the end of the silicone covering and tying the lead to its respective screw. Once in place, the skull screws and lead connections were secured using dental cement. For EMG lead placement, the trapezius muscles of the animal were exposed, and each lead was looped through and sutured to prevent displacement. Finally, the incision was sutured using non-resorbable suture material and the animals were placed in a heated recovery cage where they received Carprofen (5mg/kg; i.p.) directly after surgery and 24 hours post-surgery as an analgesic. Subjects were individually caged with ad libitum access to food and water for 1-week before EEG acquisition and monitored daily to ensure proper incision healing and recovery. Each implantation surgery took <45-min and no fatalities were observed.

**EEG Data Acquisition, Processing and Analysis**

After a 1-week recovery from surgical implantation, individually housed mice were assigned to PhysioTel RPC receiver plates that transmitted data from the EEG implants to a computer via the data exchange matrix using Ponemah software (Data Sciences International, St. Paul, MN). EEG and EMG data were collected at a sampling rate of 500 Hz with a 0.1 Hz high-pass and 100 Hz low-pass bandpass filter. Activity, temperature and signal strength were collected at a sampling rate of 200 Hz. Data acquired in Ponemah was read into Python and further processed with a bandpass filter from 0-50 Hz to focus on our frequencies of interest.

**Power spectral density analysis**

Spectral analysis was performed in Python using MEG and EEG Analysis and Visualization (MNE) open-source software. Frequency bands were defined as delta 0.5-4 Hz, theta 5-9 Hz, alpha 9-12 Hz, beta 13-30 Hz, and gamma 30-50 Hz. Spectral power was analyzed using the Welch's Method which windows over the signal and averages across spectral samples. For power spectral densities (PSD) investigated in Cohort 2, analysis started 3 hours into recording and finished 3 hours prior to the end of recording and PTZ administration, resulting in an 18-hour sampling window. PSD analysis in Cohort 3 also began 3 hours into recording but continued over the three-day recording resulting in a 69-hour sampling window. No statistical difference was detected in PSD within genotype between samples, therefore both cohorts were combined. Total delta power was determined by adding the density data detected in the 0.5-4 Hz
frequency range while total power summed all the power spectral density data in the 0.5-50 Hz frequency range. Relative delta frequencies were calculated by dividing total delta power by total power per animal and averaging across genotype.

**Spiking analysis**

For spiking analysis, baseline EEG data was segmented into 30-second windows where the mean amplitude was calculated per window. Spiking analysis was conducted in data collected in the 24 hours prior to PTZ administration in Cohort 2 and all of the data collected in Cohort 3. In a first pass assessment, potential spike data was demarcated as any point 2.5 standard deviations above or below the mean amplitude of a given window. To determine true spike events, the data was then filtered for peaks which were defined as points where the three data points prior to and following the peak were increasing and decreasing in amplitude, respectively to the potential peak of interest. If activity was detected during a 30-sec window, that data was not included in the spike count to avoid possible movement artifact. Similar to PSD analyses, the first and final three hours were removed from the spiking data for both cohorts. Spiking activity could not be combined between cohorts 2 and 3 as the difference in recording time (24 versus 72 hours) greatly contributed to the number of spikes detected.

**Sleep analysis**

Sleep in mice was assessed using EEG/EMG signals and automatically binned with Neuroscore software (Data Sciences International, St. Paul, MN) into active wake, wake, slow-wave sleep, or paradoxical sleep states. 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. EEG data was segmented into 1-sec windows and the sleep stage was determined by Neuroscore. We defined a scoring epoch of 30-sec and, if at least 50% of the epoch was predominantly one type of sleep stage, that epoch was marked with the majority stage. If a 50% criterion was not reached, then that epoch was not included in analysis. Sleep/wake stages were evaluated in Cohort 3 for the entirety of the acquisition period as this cohort did not conclude with seizure induction. Mean time in a sleep state was calculated by averaging the time spent in each bout of that state. Sleep latency was defined as the average latency to a sleep state from either active wake or wake. Total sleep time was summed across the entirety of the recording from sleep state bouts.

For sleep parameter analysis across light-dark cycles, the first 24-hr time period was sectioned into 2-hr time bins starting at 12:00 am (0-2 time of day). Paradoxical sleep and slow-wave sleep were evaluated separately, while active wake and wake stages were combined into awake readouts. To determine frequency of each sleep stage across time bins, sleep stage scorings were summed for awake,
paradoxical sleep, and slow-wave sleep then divided by total scored stages per bin. To further quantify by across light-dark cycles, percent time across 0-7 and 19-24 time bins was summed and considered “dark cycle.” The sum of the percent time across time bins 7-19 was considered the “light cycle.” Time was summed for awake, paradoxical sleep, and slow-wave sleep, this is the duration metric. Similarly, duration was quantified across light-dark cycles where time bins 0-6 and 18-24 were summed and considered the “dark cycle” and time bins 6-18 were considered the “light cycle.”

Sleep spindle analysis

To identify and analyze sleep spindles, we developed a custom Python script modified from a study designed to validate automated sleep spindle detection [36]. Briefly, a bandpass filter with cutoff frequencies of 10 and 15 Hz was applied to include the mouse spindle peak frequency of 11 Hz [37]. 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. 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. Finally, a spindle had to be longer than 0.5 sec and less than 10 sec for detection. Spindle detection was analyzed for the entirety of the acquisition period of 72 hours in Cohort 3.

Statistical analysis

All statistical analyses were performed in Prism (Version 8, GraphPad Software, San Diego, CA, USA) and data is shown as mean ± standard error. All data sets were tested for outliers using the Rout test with Q=1%. For seizure susceptibility (Figure 1A-B, Supplemental Figure 1A-B), spiking activity (Figure 1G-H), power spectral comparisons (Figure 2D-F), light-dark power spectral comparisons (Figure 3B-D and 3F-H), sleep parameters (Figure 4B-E), and spindles (Figure 6A) Student’s t-tests were used to test significance and t, degrees of freedom and p-values are reported. Two-way ANOVAs were used to analyze power spectral density differences between genotypes and the Holm-Sidak multiple comparison posthoc test was used for each frequency band (Figure 2A, C and Figure 3A, E). Additionally, two-way ANOVAs were used to analyze percent time and duration in sleep stages across time bins and between genotypes (Figure 5A, C, E, G, I, K). F, degrees of freedom, and p-values are reported. Mixed effects models were used to analyze percent time and duration in sleep stages between genotypes and light-dark cycles (Figure 5B, D, F, H, J, L) and Tukey’s multiple comparison posthoc test was used for post hoc analysis. F, degrees of freedom, and p-values are reported for mixed effects models and p-values are reported for multiple comparisons. Finally, simple linear regression was used for all correlation data (Figure 6B-F, and Supplemental Figure 2A-J). F, degrees of freedom, and p-values are reported in the text and R² values are provided in the figures. All statistics are provided in the text and “*” indicates p < 0.05.

Subjects
All animals were housed in a temperature-controlled vivarium maintained on a 12:12 light-dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California Davis and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were performed on B6.129S7-Ube3a<sup>tm1Alb</sup>/J (Ube3a) mice obtained from The Jackson Laboratory (Stock number 016590; Bar Harbor, ME, USA) and housed in a 24-h light-dark cycle (7am–7pm), temperature controlled room and fed a standard diet of Teklad global 18% protein rodent diets (Envigo, Hayward, CA, USA). To maintain the colony, Ube3a<sup>m+/p-</sup> male mice were paired with C57BL/6J wildtype females resulting in paternal transmission of the mutant allele that is silenced due to imprinting and litters with normal Ube3a expression. To create mice with maternal transmission of the mutant allele, Ube3a<sup>m+/p+</sup> (WT) male mice are paired with Ube3a<sup>m-/p+</sup> females resulting in Ube3a-del mice and their WT littermate controls. To identify mice, pups were labelled by paw tattoo on postnatal day 2-3 using non-toxic animal tattoo ink (Ketchum Manufacturing Inc., Brockville, ON, Canada). At postnatal day 2-7, tails of pups were clipped (1-2 mm) for genotyping, following the UC Davis IACUC policy regarding tissue collection. Genotyping was performed with REDExtract-N-Amp (Sigma Aldrich, St. Louis, MO, USA) using primers Wildtype Forward: TCA ATG ATA GGG AGA TAA AAC A, Common: GAA AAC ACT AAC ATG GAG CTC, and Mutant Forward CTT GTG TAG CGC CAA GTG C.

**Design**

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and \( t \), degrees of freedom and \( p \)-values are reported. Two-way ANOVAs were used to analyze power spectral density differences between genotypes and the Holm-Sidak multiple comparison posthoc test was used for each frequency band (Figure 2A, C and Figure 3A, E). Additionally, two-way ANOVAs were used to analyze percent time and duration in sleep stages across time bins and between genotypes (Figure 5A, C, E, G, I, K). \( F \), degrees of freedom, and \( p \)-values are reported. Mixed effects models were used to analyze percent time and duration in sleep stages between genotypes and light-dark cycles (Figure 5B, D, F, H, J, L) and Tukey’s multiple comparison posthoc test was used for post hoc analysis. \( F \), degrees of freedom, and \( p \)-values are reported for mixed effects models and \( p \)-values are reported for multiple comparisons. Finally, simple linear regression was used for all correlation data (Figure 6B-F, and Supplemental Figure 2A-J). \( F \), degrees of freedom, and \( p \)-values are reported in the text and \( R^2 \) values are provided in the figures. All statistics are provided in the text and * indicates \( p < 0.05 \).

**Results**

**Increased seizure susceptibility and spiking activity in Ube3a-del mice.**

Seizure susceptibility was evaluated in Cohort 1 by latency to (1) first myoclonic jerk and/or Straub’s tail, (2) loss of righting reflex, (3) generalized clonic-tonic seizure, and (4) death after administration of PTZ (80 mg/kg; i.p.)[35]. A reduction in latency, indicated susceptibility, while an increase in latency, indicated resistance. Ube3a-del animals exhibited seizure susceptibility via reduced latency to first jerk and generalized clonic-tonic seizure (Figure 1(A-B): \( t_{(26)} = 2.287, p = 0.031; t_{(26)} = 2.627, p = 0.014 \)), compared to WT. No differences were detected in loss of righting reflex or death (Supplemental Figure 1(A-B): \( t_{(26)} = 1.461, p = 0.156; t_{(26)} = 1.333, p = 0.194 \)). Hyperexcitability and seizure susceptibility was further analyzed by implanting Cohort 2 subjects with a wireless telemetric device that captured continuous EEG (Figure 1(C-D)). Epileptiform activity, such as spiking, is sufficient in the detection and diagnosis of epilepsy. As expected, Ube3a-del mice in Cohort 2 exhibited increased spiking activity during baseline recording of 24 hours compared to WT controls (Figure 1(G-H): \( t_{(10)} = 3.435, p = 0.006; t_{(8)} = 3.132, p = 0.014 \)).

**Elevated delta power in Ube3a-del mice.**

Rhythmic delta activity is a consistent spectral signature of AS [38, 39] and has been previously reported in mouse models ([31, 32]), but not via a single channel electrode placed in the skull via a wireless telemetric system. To test whether our methodology was sensitive to capture EEG signal that would also detect the clinically reported increased delta activity, we evaluated spectral power dynamics of WT and Ube3a-del mice. When comparing power spectral densities across 0-50 Hz frequencies, Ube3a-del mice displayed robust elevated delta power (Figure 2(A): \( F_{(1,20)} = 6.432, p = 0.020; t_{(20)} = 2.763, p = 0.012 \) for delta). When densities were binned into delta, theta, alpha, beta and gamma frequency groups, delta power in Ube3a-del mice was significantly higher than WT littermate controls (Figure 2(C): \( F_{(1,20)} = 5.862, p = 0.025; t_{(20)} = 2.763, p = 0.012 \) for delta).
Delta-specific dynamics were further examined by looking at total delta, relative delta and total power across the entire recording. *Ube3a*-del mice exhibited higher total and relative delta power compared to WT across the entire recording (*Figure 2(D-E)*: $t_{(20)} = 2.763, p = 0.012$; $t_{(20)} = 4.089, p = 0.0001$). Interestingly, total power was also higher in *Ube3a*-del animals, likely a result of the robust increase in delta (*Figure 2(F)*: $t_{(20)} = 2.564, p = 0.019$).

As delta activity is elevated in sleep stages, particularly slow-wave sleep, delta activity was also analyzed across light-dark cycle to control for their possible influence. In the light cycle, elevated delta activity was detected across all 0-50 Hz power spectral densities (*Figure 3(A)*: $F_{(1, 20)} = 8.263, p = 0.009$; $t_{(20)} = 3.020, p = 0.007$ for delta). Furthermore, both relative and total delta power were significantly higher in *Ube3a*-del as well as total power compared to WT (*Figure 3(B-D)*: $t_{(20)} = 3.042, p = 0.006$; $t_{(20)} = 2.278, p = 0.034$; $t_{(20)} = 2.989, p = 0.007$). Similarly, in the dark cycle, *Ube3a*-del mice displayed elevated delta activity (*Figure 3(E)*: $t_{(20)} = 2.178, p = 0.042$ for delta), with trends towards significantly increased PSDs across the 0-50 Hz frequency bins (*Figure 3(E)*: $F_{(1, 20)} = 4.027, p = 0.059$). Relative delta power was observed in *Ube3a*-del animals, however no change in total power was detected (*Figure 3(F-H)*: $t_{(20)} = 2.191, p = 0.041$; $t_{(20)} = 2.823, p = 0.011$; $t_{(20)} = 1.60, p = 0.125$), likely a result of increased activity in the dark cycle, as mice are nocturnal.

**Sleep deficits and abnormal sleep-wake cycles detected in *Ube3a*-del mice.**

Sleep data was acquired in Cohort 3 animals and subsequently parsed into 4 distinct sleep-wake stages: Active Wake, Wake, Slow-Wave Sleep, and Paradoxical Sleep. 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. *Ube3a*-del mice displayed reduced mean time in paradoxical sleep (*Figure 4(B)*: $t_{(10)} = 2.91, p = 0.016$) and took longer to reach paradoxical sleep stages compared to WT (*Figure 4(D)*: $t_{(10)} = 3.691, p = 0.004$). Furthermore, a trend toward reduced mean time in slow-wave sleep was detected in *Ube3a*-del mice (*Figure 4(D)*: $t_{(10)} = 1.931, p = 0.0823$), contributing to a significantly reduced total sleep time compared to WT littermate controls (*Figure 4(E)*: $t_{(10)} = 2.741, p = 0.021$).

Sleep parameters were further analyzed by light-dark cycles. The first 24-hr time period was sectioned into 2-hr time bins starting at 12:00 am (0-2 time of day) where frequency of sleep stage scores were represented as the percent of a time bin and duration spent in sleep stages was measured in seconds. Wake and active wake stages were combined for awake analysis. A trend towards increased awake score frequency was detected in *Ube3a*-del mice compared to WT littermate controls (*Figure 5(A)*: $F_{(1, 10)} = 3.470, p = 0.092$). *Ube3a*-del mice had significantly higher percent awake frequencies in the dark cycle compared to WT littermates controls and a trend of higher percent awake frequency in the light cycle (*Figure 5(B)*: Genotype-effect $F_{(1, 6)} = 17.490, p = 0.006$; WT and *Ube3a*-del by dark cycle $p = 0.033$; WT
and Ube3a-del by light cycle \( p = 0.118 \). No change in awake duration was observed across time bins or light-dark cycles between genotypes (Figure 5(C): \( F_{(1, 10)} = 0.075, p = 0.789 \)), however more time was spent in awake stages during the dark cycle for WT animals, as typically expected since mice are diurnal, and more time awake during the dark cycle is anticipated. Interestingly, Ube3a-del mice did not exhibit awake duration differences between the light-dark cycles (Figure 5(D): Light-Dark WT \( p = 0.029 \); Light-Dark Ube3a-del \( p = 0.935 \)) emphasizing abnormal sleep across stages. Ube3a-del animals had a strong trend of reduced duration of paradoxical sleep across time bins (Figure 5(G): \( F_{(1, 10)} = 4.784, p = 0.054 \)), compared to WT littermate controls (Figure 5(H): Genotype-effect \( F_{(1, 6)} = 5.716, p = 0.027 \); WT and Ube3a-del by light cycle \( p = 0.011 \)). Ube3a-del mice showed decreased paradoxical sleep frequency across time bins (Figure 5(E): \( F_{(1, 10)} = 12.64, p = 0.005 \)), reduced in both light and dark cycles compared to WT (Figure 5(F): Genotype-effect \( F_{(1, 6)} = 25.85, p = 0.002 \); WT and Ube3a-del by dark cycle \( p = 0.015 \); WT and Ube3a-del by light cycle \( p = 0.008 \)). Again, WT animals demonstrated normal reductions in the percent paradoxical sleep frequency between light and dark cycles (Figure 5(F): \( p = 0.044 \)), while Ube3a-del mice did not (Figure 5(F): \( p = 0.164 \)), suggesting that paradoxical sleep deficits in Ube3a-del were sustained across cycles. Slow-wave sleep frequencies were not significantly different between genotypes (Figure 5(I): \( F_{(1, 10)} = 0.032, p = 0.861 \)), across light-dark cycle (Figure 5(J): Genotype-effect \( F_{(1, 6)} = 1.265, p = 0.304 \)). Although no changes in slow-wave sleep frequency were observed, Ube3a-del mice did have a trend of reduced slow-wave sleep duration (Figure 5(K): \( F_{(1, 10)} = 4.047, p = 0.072 \)), specifically in the light cycle (Figure 5(L): \( F_{(1, 6)} = 4.047, p = 0.091 \); WT and Ube3a-del by light cycle \( p = 0.135 \)).

Sleep spindles are reduced in Ube3a-del mice and negatively correlated with the elevated delta phenotype.

We wanted to identify, validate and quantify sleep spindles in the Ube3a-del mouse, adding another clinically relevant functional phenotype for therapeutic testing. First, a 10-15 Hz bandpass filter was first applied, followed by a Butterworth filter (3 Hz first stopband, 10 Hz first passband, 15 Hz second passband, 22 Hz second stopband, 24 dB attenuation level) 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. 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. Ube3a-del mice exhibited less sleep spindles compared to WT littermate controls (Figure 6(A): \( t_{(10)} = 2.357, p = 0.04 \)). This is the first report of altered sleep spindle production in a preclinical model of Angelman Syndrome. To ensure the reduction in sleep spindles was not confounded by sleep deficits, spindle count was correlated with mean time in paradoxical sleep (Figure 6(C): \( F_{(1, 3)} = 5.422, p = 0.102 \), mean time in slow-wave sleep (Figure 6(D): \( F_{(1, 3)} = 4.723, p = 0.118 \)), and total sleep time (Figure 6(E): \( F_{(1, 3)} = 1.322, p = 0.334 \)) in Ube3a-del animals. No significant correlations were detected between spindle count and sleep metrics, suggesting that the spindle count phenotype observed in Ube3a-del mice was separate from reported sleep deficits. Spindle count and spiking count correlations were also analyzed, though no significant correlation was detected (Figure 6(F): \( F_{(1, 3)} = 0.077, p = 0.800 \)). Interestingly, spindle count was negatively correlated with elevated delta power, where animals with higher delta power had lower spindle
counts (Figure 6(A): $F_{(1, 3)} = 59.44, p = 0.005$). Additional correlation studies showed no significant relationship between spiking, delta, and sleep phenotypes (Supplemental Figure 2A-J).

**Discussion**

Novel therapies in development for genetic precision medicine for AS that could be “curative” have resulted in 1) unsilencing of paternal Ube3a; 2) molecular reversal of *Ube3a* expression levels; and 3) some degree of functional phenotypic rescue by a wide variety of molecular therapies. These include gene therapy by antisense oligonucleotides (ASO) [40], viral vector delivery [41], and Artificial Transcription Factors (ATFs) [42]. In fact, two ASO compounds are in Phase I clinical trials (GeneTx NCT04259281; Roche NCT044282881). Outcome measures are required to demonstrate the utility of these innovative therapeutic designs, as well as to validate other traditional medicinal therapies that may be in the drug discovery pipeline by biotechnology and pharmaceutical companies for AS. Previous research has comprehensively characterized the AS mouse model line of Jiang and Beaduet both behaviorally and biochemically and discovered motor abnormalities [43], learning and memory deficits in fear conditioning [40, 41], strain dependent seizure susceptibility [32] [15, 32], elevated delta spectral power [31] and abnormal sleep signatures [44]. This study rigorously reproduced findings on hyperexcitability and seizure susceptibility and extended studies using a unique chemoconvulsant mechanism, GABA$_B$ antagonist, pentylentetrazol. Innovation reported herein is the detection of similar effect sizes and phenotypes using a translational approach of acquisition of EEG in the home cage over several days via a skull screw and wireless telemetry over hippocampal depth recordings of local field potentials requiring a head mount and tethered system. Previous work from Sahin and Rotenberg have established this technology as useful for models of NDDs, by their extensive studies in Tubero Sclerosis Complex and Phelan McDermid Syndrome models [24, 45]. Both of these genetic NDDs share common phenotypes with AS, including motor difficulties, intellectual disabilities, recurring seizures, and sleep difficulties, in addition to diagnoses of autism spectrum disorders (ASD).

It is interesting, although not surprising, that no behavioral seizures were observed in either the mouse or rat model of AS, given it has been recognized by our laboratory and others that mouse models on C57BL6/J backgrounds are resistant to seizures [22, 35]. Since multiple genetic NDDs that are syndromic forms of ASD have high seizure co-morbidity, our laboratory adapted to uncovering subthreshold behavioral seizures with wireless, untethered, telemetry implants that acquire the EEG signal. This is the first report to identify with translationally relevant methods, global (non-hippocampal; non-depth local field potentials; freely moving non-tethered) neurophysiology and sleep in AS mice.

In addition to observations of epileptiform and spike trains via EEG in AS subjects, we observed elevated spectral power in the delta frequency band in mice with *Ube3a*$_{m^-/p^+}$ deletions. Our laboratory works closely with patient advocacy groups and gathers observations of clinical AS from leading epileptologists (Thibert, Anderson) providing us numerous deidentified clinical examples for which EEG signatures in individuals with mutations or excess expression of *Ube3a* are unique and distinguishable. For example, Drs. Thibert and Anderson have carefully explained their reports of elevated delta power in AS clinics [31,
Further, we reproduced Sidorov et al. earlier work [46] that highlighted delta power as translational biomarker [31, 33]. This phenotypic reliability paves the pathway to power spectral signatures as therapeutic windows for precision treatment within AS and beyond including other NDDs, such as Dup15q and genetic forms of ASD [47-50].

Sleep is greatly affected in individuals with AS [51-53]. Sleep disturbances reported include reduced overall time sleeping, higher number of nighttime awakenings and longer onset latencies to falling asleep are the most common [54, 55]. Sleep analysis is highly translational, since a majority of genetic NDDs, including AS, have clinical sleep disruption. This report defined rodent sleep cycles over a 36-hour acquisition period. We measured alterations by quantifying time in: a) wake, defined as high-frequency, low-amplitude signals with without EMG or video activity b) active wake, similar to wake but with detected activity; c) time in slow-wave sleep, defined as low-frequency, high-amplitude signal with elevated delta; d) time to sleep onset and time spent in paradoxical (~REM) sleep, characterized by a low-frequency, low-amplitude signal with elevated theta.

We reproduced Ehlen and Philpot’s earlier work [46] that maternal Ube3a loss had a striking effect on the architecture of sleep/wake cycles, as reflected by a reduced duration in NREM and REM (also termed paradoxical) sleep. As reported, Ube3a-del had short NREM bout durations throughout the dark cycle, unlike WT mice, where NREM bouts were short at the beginning of the cycle then increased over time. The number of NREM bouts also remained high during the early night in Ube3a-del mice. These results show that Ube3a-del mice had a “fragmented” NREM sleep in the dark cycle compared to WT mice. Similarly, we saw reduced duration in slow-wave sleep in Ube3a-del mice, but our results differed from Ehlen et al. in that we observed this phenotype during the light cycle. The authors suggest these findings reveal that maternal Ube3a loss disrupts mechanisms involved in initiating and maintaining NREM sleep during the night that may be applicable across both light and dark cycles. In addition to the NREM deficits, REM (paradoxical) sleep was also affected by reduced REM bouts and percent 2 hour time bins in Ube3a-del mice compared to WT controls, during the dark phase. We reproduced this data by reduced duration in paradoxical sleep and lower bout frequency of paradoxical sleep across light-dark cycles. Ube3a-del animals showed impairments in total sleep time, namely through reductions in mean time spent in paradoxical sleep, less percent time in Ube3a-del mice in paradoxical sleep, a strong trend (p = 0.054) of reduced duration in Ube3a-del mice in paradoxical sleep across light and dark cycles and overall compared to WT. Mean time in slow-wave sleep was reduced in Ube3a-del mice compared to WT. Furthermore, Ube3a-del mice took longer to reach paradoxical sleep compared to WT, which suggests an overall increased time awake, difficulty reaching the two deeper sleep stages and, once there, difficulty remaining in those stages, remarkably valid to what is observed clinically. We believe our data corroborate the “fragmented” sleep phenotype described by Ehlen and Philpot [46] although our signal collection methods differed substantially.

For the first time, in a preclinical model of AS, we have identified, defined and quantified a reduction in sleep spindles. We accomplished this via custom labeling with and custom built automation, that followed manually filtering data and processed these data via our custom machine learning algorithm.
Sleep spindles are thalamocortical oscillations ranging from 11-16 Hz and may “in theory” mediate memory consolidation. There have been reports of sleep spindle reductions in the AS population, but not in a mouse model of AS [44], to date.

*Ube3a*-del mice exhibited less spindles, as we hypothesized given the reduction in sleep spindles and difficult sleep patterns observed clinically in AS [44]. Spindle count was not influenced by sleep stage alterations as there was no significant correlations between spindles and mean paradoxical sleep time, mean slow-wave sleep time, or total sleep time. Fewer sleep spindles have been implicated in intellectual disabilities [56, 57] and for numerous genetic NDDs, such as Phelan-McDermid and Prader-Willi syndromes [58, 59] as well as pediatric epilepsies [60] [61] and ASDs [62, 63]. Sleep spindles were also altered by mGluR5 deficiency [64], mGluR5 dysregulation and have been postulated as an underlying mechanism of phenotypes in Fragile X Syndrome, another NDD with poor sleep regulation [65, 66]. Interestingly, there was a significant negative correlation between spindles and delta power, with subjects with higher/highest delta power tended to have the lowest/lower spindle counts. It is unclear if elevated delta power directly correlates with any clinical features of AS, but this data offers a promising link between delta rhythmicity and poor sleep regulation which probably relates to poor or impairments in cognitive outcome measures.

Limitations to this work, were our lack of inclusion of an analysis of the anatomical substrates involved in sleep either by gross MRI scan and DTI of white matter tracts or histopathological correlates of the neuronal integrity or lack thereof of the circuit underlying delta power and/or sleep spindles. While less regionally specific than typical depth recording EEG, we sought to use a more clinically analogous EEG recording method that collected signals from the surface of the skull and had the benefit of being wireless to allow for freely moving, behaving test subjects. We have long hypothesized that quantification of neurophysiology metrics in the home cage environment with wireless telemetry devices, would be innovative and multi-disciplinary allowing us to comprehensively assess behavioral seizures, and sub threshold seizures, sleep pattern irregularities and spectral band power biomarkers associated with NDDs as well as be applicable for evoked response potentials when paired with behavioral apparatuses and offer translational observation of global neuronal activity, as previously described [45]. One other limitation was failure to correlation the reduction of spindles with dysfunctional cognitive behavior observed by our laboratory and others in AS mice, a popular theory of function of spindles [56, 57].

In summary, our data are rigorous, reproducible and translatable EEG readouts that have substantial biomarker potential for preclinical testing of therapeutics. Our study provides important corroborations of earlier preclinical and clinical reports that used depth electrodes and field potentials to identify epileptiform in the *Ube3a*-del mice. This is the first report to use a cortical surface-based recording with wireless telemetry device over tethered/fixed head-mount depth recordings and address percent time and duration in 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, a critical marker of memory consolidation during sleep. This study was
limited to one mouse model and future work will evaluate other mouse, rat and pig models of AS, with various types of \textit{Ube3a} deletions.

\textbf{Declarations}

No humans were involved in this study.

Animal research was conducted under the UC Davis IACUC #21779 (PI Silverman).

\textbf{Consent for publication:}

N/A

\textbf{Availability of data and materials}

Data supporting our findings can be found in the UC Davis School of medicine Shared Network. Please contact author for data/custom algorithm requests.

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\textbf{Author Contributions}

Conceived and designed the experiments: NAC and JLS; Performed the experiments: NAC; Analyzed and interpreted the data: NAC and JLS; Provided reagents, tools and funding: JLS; Drafted the article: NAC and JLS; Revised the article: NAC and JLS.

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