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Excessive laughter-like vocalizations, microcephaly, and translational outcomes in the
Ube3a deletion rat model of Angelman Syndrome

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Abstract

Angelman Syndrome (AS) is a rare genetic neurodevelopmental disorder characterized by intellectual disabilities, motor and balance deficits, impaired communication, and a happy, excitable demeanor with frequent laughter. We sought to elucidate a preclinical outcome measure in male and female rats that addressed communication abnormalities of AS and other neurodevelopmental disorders in which communication is atypical and/or lack of speech is a core feature. We discovered, and herein report for the first time, excessive laughter-like 50-kHz ultrasonic emissions in the \textit{Ube3a}^{mat+/pat} rat model of AS, which suggests an excitable, playful demeanor and elevated positive affect, similar to the demeanor of individuals with AS. Also in line with the AS phenotype, \textit{Ube3a}^{mat+/pat} rats demonstrated aberrant social interactions with a novel partner, distinctive gait abnormalities, impaired cognition, an underlying long-term potentiation deficit, and profound reductions in brain volume. These unique, robust phenotypes provide advantages compared to currently available mouse models and will be highly valuable as outcome measures in the evaluation of therapies for AS.

Significance Statement

Angelman Syndrome (AS) is a severe neurogenetic disorder for which there is no cure, despite decades of research using mouse models. This study utilized a recently developed rat model of AS to delineate disease-relevant outcome measures in order to facilitate therapeutic development. We found the rat to be a strong model of AS, offering several advantages over mouse models by exhibiting numerous AS-relevant phenotypes including overabundant laughter-like vocalizations, reduced hippocampal long-term potentiation, and volumetric anomalies across the brain. These findings are unconfounded by detrimental motor abilities and background strain, issues plaguing mouse models. This rat model represents an important advancement in the field of AS and the outcome metrics reported herein will be central to the therapeutic pipeline.
Introduction

Angelman Syndrome (AS) is a rare neurodevelopmental disorder (NDD) characterized by intellectual disability, impaired communication, ataxia, seizures, as well as a happy disposition with a high degree of excitability, smiling, and easily provoked laughter (Williams and Franco, 2010). AS is caused by dysfunction of maternal ubiquitin protein ligase E3A (UBE3A), typically from a de novo deletion in the 15q11-q13 region (Albrecht et al., 1997). Restoring functional UBE3A is seemingly possible by innovative gene therapy approaches including antisense oligonucleotides (Meng et al., 2015), viral vector delivery (Daily et al., 2011), artificial transcription factors (Bailus et al., 2016), stem cell mediated therapies (Adhikari et al., 2021), and the cutting edge Cas9 (Wolter et al., 2020). Gene replacement therapy is therefore on the horizon for AS and, indeed, two clinical trials using “gene therapy-like” antisense oligonucleotide interventions began recruitment in 2020 (GeneTx NCT04259281; Roche NCT04428281).

Indispensable to such a strategy of therapeutic development are in vivo studies utilizing preclinical model systems with rigorous translational outcomes. One domain that is critically impaired in AS and other NDDs but difficult to study in preclinical models due to their lack of human-interpretable language is communication. The increasing availability of rat models of NDDs opens up new opportunities to develop preclinical outcome measures of social communication. While the mouse has been the preferred model species in recent decades due to the genetic technologies available, there are complex behaviors and physiological processes difficult or impossible to investigate in mice that are easily observable in rats (Ellenbroek and Youn, 2016; Hofer et al., 2002; Portfors, 2007; Hammerschmidt et al., 2012; Wöhr and Schwarting, 2013; Portfors and Perkel, 2014).

One prominent example is the greater sophistication and complexity in the rat acoustic communication system. While both mice and rats emit ultrasonic vocalizations (USV), rats emit USV that serve as situation-dependent, evolved signals which accomplish important communicative functions that
are not observed as functions of mouse USV, such as low-frequency 22-kHz “alarm calls” which rats use to warn of potential threats (Brudzynski, 2013; Wöhr and Schwarting, 2013; Blanchard et al., 1991; Sadananda et al., 2008; Fendt et al., 2018; Wöhr and Schwarting, 2007; Kisko et al., 2017). The recent generation of the first rat model of AS therefore provides the unique opportunity to utilize a greater diversity of social and communication behaviors as compared to those previously available in mouse models (Jiang et al., 2010; Huang et al., 2013; Dutta and Crawley, 2020; Kondrakiewicz et al., 2019; Netser et al., 2020; Parker et al., 2014; Ellenbroek and Youn, 2016; Homberg et al., 2017; Reppucci et al., 2020).

To build upon the initial reports describing the Ube3a deletion rat model of AS, which revealed deficits in motor, cognition, social approach, and pup vocalizations (Berg et al., 2020c; Dodge et al., 2020), we sought to investigate nuanced social behaviors and further characterize vocalization patterns. With numerous novel therapies being assessed in clinical trials and at the investigational drug discovery level, AS-relevant outcome measures are vital for demonstrating functional efficacy of the varied intervention approaches. Leveraging the rat’s social communication system, we discovered that the Ube3a maternal deletion rat (Ube3a\textsuperscript{mat-\textsubscript{pat}}) produces excessive signals of positive affect characteristic of AS. Several other AS-relevant phenotypes were evident, including atypical social interactions and maladaptive impairments in gait and cognition. We also identified reduced hippocampal long-term potentiation, observed in mouse models of AS but not yet in rats, as a putative cellular mechanism underlying the learning and memory deficits apparent in the model. Finally, our neuroimaging analysis revealed decreased brain volume and pronounced increasing severity with age.

Materials and Methods

Subjects. Subjects were male and female Sprague Dawley Ube3a\textsuperscript{mat-\textsubscript{pat}} rats and their wildtype littermates (Ube3a\textsuperscript{mat+\textsubscript{pat}}) generated from breeding pairs of paternal Ube3a deletion females and wildtype males purchased from Envigo (Indianapolis, IN). The initial generation of Ube3a deletion rats using
CRISPR/Cas9 was described previously (Berg et al., 2020c). Genotyping was performed using a small sample of tail tissue collected at postnatal day (PND) 2, REDExtract-N-Amp (Sigma Aldrich, St. Louis, MO, USA), and primers Rube1123 TAGTGCTGAGGCACCTGTTCCAGAGC, Rube1606r TGCAAGGTTAGCTTACTCATGC, Ub3aDelSpcfcF6 ACCTAGCCAAAGCCATCTC, and Ub3aDelR2 GGGAACAGCAAAAGACATGG. All animals were socially housed in a temperature-controlled vivarium maintained on a 12:12 light-dark cycle with testing occurring during the light phase. All procedures were conducted in compliance with the NIH Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of California Davis. To minimize the carry-over effects from repeated testing and handling, seven mixed-sex cohorts of rats were tested and behavioral tests were carried out in order of least to most stressful with at least 48 hrs break between tests. Each cohort was comprised of four to nine litters and subjects were sampled as followed: subjects for 50-kHz ultrasonic (USV) playback were sampled from Cohort 1; contextual and cued fear conditioning from Cohort 2; gait analysis, heterospecific play, and social play from Cohort 3; acoustic startle and long-term potentiation from Cohort 4; spontaneous exploratory USV from Cohort 5; spontaneous alternation from Cohort 6; and olfactory discrimination from Cohort 7. Following behavioral testing, rats from Cohort 3 were perfused for magnetic resonance imaging.

Juvenile USV in response to heterospecific play. At postnatal day (PND) 30 through 34, rats were provided daily heterospecific play sessions involving manual stimulation using a slightly abbreviated procedure from those described previously (Burgdorf and Panksepp, 2001; Schwarting et al., 2007; Wöhr et al., 2009). For 5 min on 5 consecutive days, rats were individually manipulated by a familiar experimenter using a single clean hand within a clean, empty version of the home cage with fresh bedding (37.2 cm l x 30.8 cm w x 18.7 cm h; illuminated to ~30 lux) while vocalizations were recorded with an overhead ultrasonic microphone (Avisoft Bioacoustics, Glienicke, Germany) for later scoring by a trained observer blinded to genotype. The number of calls emitted during each 30-sec interval were counted and classified as either high (50-kHz) or low (short 22-kHz) frequency using a threshold of 33
kHz. Calls emitted during the minute immediately preceding the heterospecific play sessions on days 2-4 (“anticipation”) were also counted and classified.

All rats were handled by the experimenter in a standardized fashion (5 min on 3 days) prior to the first heterospecific play session. The physical manipulations performed were tickling the subject’s neck (2x), tickling the subject’s belly (1x), pushing into their shoulders (“push and drill”; 1x), and flipping the subject onto their back and momentarily pinning them down (“flip over”; 3x). Each manipulation lasted 30 sec with three 30-sec breaks interspersed at 0, 60, and 150 sec, during which the experimenter did not initiate touching the subject but moved their hand around the cage to encourage following or chasing. In an effort to provide a standardized experience, a single experimenter carried out the procedure for all subjects and the experimenter remained unaware of USV being emitted during the test, performing the manipulations in an equivalent manner for all rats. To mitigate any potential effect of order, the sequence of manipulations was re-ordered each day but remained consistent across all animals. The testing order of the subjects was also changed from day to day.

Juvenile spontaneous exploratory USV. At PND 30, rats were individually placed in a clean, empty version of the home cage (illuminated to ~30 lux) with clean bedding for 5 min similarly to methods described previously (Schwarting et al., 2007; Wöhr et al., 2008). Recording of ultrasonic vocalizations began immediately following the subject being placed into the cage and no other animals or any experimenter were present in the room during recording. Calls were classified by a trained observer blinded to genotype as either high (50-kHz) or low (short 22-kHz) frequency using a threshold of 33 kHz.

Juvenile USV in response to playback of 50-kHz USV. At PND 30±4, subjects were individually presented with 1-min of natural pro-social 50-kHz USV while on a radial maze illuminated to ~8 lux as described previously (Berg et al., 2018; Berg et al., 2020c). USV were presented to individual subjects using an established playback paradigm (Berg et al., 2018; Wöhr et al., 2016), including the USV stimulus previously demonstrated to elicit social approach (behavior shown in Berg et al., 2020c).
USV stimulus consisted of 221 natural 50-kHz USV recorded from a naïve male rat during exploration of a cage containing a recently separated cage mate. A 3.5-sec sequence of 13 calls was repeated 17 times such that 221 50-kHz calls were presented within 1 min. Response vocalizations were recorded with an overhead ultrasonic microphone (Avisoft Bioacoustics) and the number of calls emitted during the minute of playback were counted by a trained observer blinded to genotype and classified as high (50-kHz) or low (short 22-kHz) frequency using a threshold of 33 kHz.

Juvenile social play. At PND 38±1, social play behavior was assessed following a protocol described previously (Berg et al., 2018; Berg et al., 2020a; Berg et al., 2020b). Each subject rat was placed with a freely moving, unfamiliar, strain-, sex-, and age-matched wildtype stimulus rat for 10 min in a clean, empty test arena (illuminated to ~30 lux) containing a thin layer of clean bedding. In order to facilitate social play, each subject and stimulus animal was socially isolated in a separate holding room for 30 min prior to the test. Stimulus animals were generated from wildtype Sprague-Dawley breeders (Envigo, Indianapolis, IN) and handled in a standardized manner (5 min on 3 days) prior to the assay. The interaction was video-recorded, and behaviors were later scored by a trained observer blinded to genotype as follows: Social sniffing: sniffing the stimulus rat’s face, body, or tail; Anogenital sniffing: sniffing the stimulus rat’s anogenital region; Self-grooming: subject grooming itself; Exploring: sitting, walking, rearing, or sniffing the ground or wall; Following or chasing: following (walking pace) or chasing (running pace) the stimulus rat; Rough-and-tumble playing: accelerated movement involving chasing, pouncing, pinning, tumbling, and/or boxing which requires the stimulus rat’s participation (i.e., reciprocity); Push past: directed movement toward the stimulus rat to get next to, or move closely past, without sniffing or otherwise engaging; Push under or crawl over: head dip under the stimulus rat’s belly or completely stepping over the stimulus rat; Pounce: both paws placed via leap or directed movement onto the stimulus rat’s back. Blind scoring was possible since Ube3a<sup>mut−/pat+</sup> rats have normal body weight and are physically indistinguishable from their wildtype littermates (Berg et al., 2020c).
**Olfactory discrimination.** At 42±3, the ability of rats to discriminate between a social and non-social odor was tested by measuring the time spent investigating odor-saturated cotton swabs. Subjects were individually tested in clean chambers (40.6 cm l x 40.6 cm w x 28 cm h) dimly illuminated to ~30 lux. On the day before the test, rats were habituated to the test chamber containing a clean dry cotton swab (15.2 cm l) for 20 min. The tip of the swab was secured 3 cm above the floor in the center of the arena by being attached to the top of a clean weighted glass dome (7.6 cm d x 10 cm h) and angled downward. On the day of the test, rats were again habituated to the arena containing a clean dry cotton swab for 10 min, followed by a swab soaked in water, then vanilla (1:100 dilution; McCormick, Hunt Valley, MD), and then a social scent. The social scent was collected by wiping a cotton swab in a zig zag pattern along the bottom of a cage of same sex but unfamiliar Sprague Dawley rats (Envigo, Indianapolis, IN). Each saturated swab was presented for 2 min and the order of odor presentation was consistent across all animals. Time spent sniffing the swab soaked with vanilla scent and the swab soaked with social scent (defined as the nose within 2 cm of the cotton swab tip) was measured using videotracking software (EthoVision XT, Noldus Information Technology, Leesburg, VA), which was subsequently validated manually.

**Juvenile gait.** At PND 25, gait metrics were collected using the DigiGait automated treadmill system and analysis software (Mouse Specifics, Inc., Framingham, MA). Subjects were placed individually into the enclosed treadmill chamber and allowed to acclimate before the belt was turned on. The belt speed was slowly increased to a constant speed of 20 cm/sec, at which each rat was recorded making clearly visible consecutive strides for 3-6 sec.

**Juvenile contextual and cued fear conditioning.** At PND 43±1, learning and memory were assessed using an automated fear conditioning chamber (Med Associates, Inc., Fairfax, VT) following methods previously described (Copping et al., 2017; Adhikari et al., 2018; Berg et al., 2020b). On day one, rats were trained via exposure to a series of three noise-shock (conditioned stimulus-unconditioned
stimulus; CS-US; 80 dB white noise, 0.7 mA foot shock) pairings inside a sound-attenuated chamber with specific visual, tactile, and odor cues. On day two, contextual memory was tested by placing each subject back inside the training environment (no noise or foot shock occurred). On day three, cued memory was evaluated by placing subjects into a novel context with altered visual, tactile, and odor cues. Following a period of exploration, the white noise CS was presented for 3 min. Time spent freezing was measured using VideoFreeze software (Med Associates, Inc.).

Prepulse inhibition of an acoustic startle response. At 9-10 weeks of age, prepulse inhibition was measured using an SR-Lab System (San Diego Instruments, San Diego, CA). Subjects were placed in a clear plastic cylinder, which was mounted onto a platform connected to piezoelectric transducers inside a sound-attenuating chamber with internal speakers. The background noise level in the chamber was 70 decibel (dB) white noise. Each session consisted of a 5-min acclimation period followed by a pseudo-randomized presentation of 50 trials of five different trial types: one trial type was a 40-ms 120-dB startle stimulus, three trial types involved an acoustic prepulse (74, 82, or 90 dB) presented 120 ms prior to the 120-dB startle stimulus, and there were also trials with no startle stimulus in order to measure baseline movement inside the cylinder. Each trial type was presented in 10 blocks and was randomized within blocks. The intertrial interval varied randomly between 10 sec and 20 sec. Percent PPI was calculated using the equation: \( \% \text{PPI} = \left[1 - \left( \text{Prepulse}/\text{Max Startle} \right) \right] \times 100. \)

Spontaneous alternation. At 10 weeks of age, spontaneous alternation was measured by allowing rats to freely explore a novel Y-maze (black, opaque; arms: 21” l x 4.5” w x 11” h; illuminated to ~30 lux) for 8 min. An overhead camera connected to videotracking software (EthoVision XT; Noldus Information Technology, Wageningen, Netherlands) was used to quantify the number of arm entries, the number of errors (defined as the sum of direct and indirect revisits to an arm), the number of spontaneous alternations, and the maximum number of possible alternations for the entire session.
Long-term potentiation (LTP). Acute slice preparation. At 12-13 weeks of age, subjects were deeply anesthetized with isoflurane and, following decapitation, the brain was rapidly removed and submerged in ice-cold, oxygenated (95% O₂/5% CO₂) ACSF containing (in mM) as follows: 124 NaCl, 4 KCl, 25 NaHCO₃, 1 NaH₂PO₄, 2 CaCl₂, 1.2 MgSO₄, and 10 glucose. On an ice-cold plate, the brain hemispheres were separated, blocked, and the hippocampi removed. The 400-μm-thick slices were then cut using a McIlwain tissue chopper (Brinkman, Westbury, NY). Slices from the dorsal thirds of the hippocampus were used. Slices were incubated (at 33°C) for 20 min and then maintained in submerged-type chambers that were continuously perfused (2-3 mL/min) with ACSF and allowed to recover for at least 1.5-2 hr before recordings. Just prior to start of experiments slices were transferred to a submersion chamber on an upright Olympus microscope, perfused with 30.4°C normal ACSF saturated with 95% O₂/5% CO₂.

Electrophysiological recordings. A bipolar, nichrome wire stimulating electrode (MicroProbes) was placed in stratum radiatum of the CA1 region and used to activate Schaffer collateral/commissural fiber synapses. Evoked fEPSPs (basal stimulation rate = 0.033 Hz) were recorded in stratum radiatum using borosilicate pipettes (Sutter Instruments, Novato, CA) filled with ACSF (resistance 5-10 MΩ). Submerged-type recording chambers were used for all recordings. All recordings were obtained with a MultiClamp 700B amplifier (Molecular Devices, San Jose, CA), filtered at 2 kHz, digitized at 10 Hz. To determine response parameters of excitatory synapses, basal synaptic strength was determined by comparing the amplitudes of presynaptic fiber volleys and postsynaptic fEPSP slopes for responses elicited by different intensities of SC fiber stimulation. Presynaptic neurotransmitter release probability was compared by paired pulse facilitation (PPF) experiments, performed at 25, 50, 100 and 250 msec stimulation intervals. LTP was induced by high frequency stimulation (HFS) using a 2x tetanus (1-s-long train of 100 Hz stimulation) with a 10 sec inter-tetanus interval. At the start of each experiment, the maximal fEPSP amplitude was determined and the intensity of presynaptic fiber stimulation was adjusted to evoke fEPSPs with an amplitude ~40-50% of the maximal amplitude. The average slope of EPSPs elicited 55-60 min after HFS (normalized to baseline) was used for statistical comparisons.
Magnetic resonance imaging (MRI). At 6.5 months of age, ex vivo neuroimaging was carried out by following a protocol previously described (Berg et al., 2018; Berg et al., 2020c). Brains were flushed and fixed via transcardial perfusion with 50 mL phosphate-buffered saline (PBS) containing 10 U/mL heparin and 2 mM ProHance (gadolinium contrast agent; Bracco Diagnostics Inc., Monroe Township, NJ) followed by 50 mL 4% paraformaldehyde in PBS containing 2 mM ProHance. Brains were incubated in the 4% PFA solution at 4°C for 24 hrs, transferred to a 0.02% sodium azide PBS solution, and then incubated at 4°C for at least one month before being scanned. Magnetic resonance imaging (MRI) of the brains within their skulls was carried out using a multi-channel 7.0 Tesla scanner (Agilent Inc., Palo Alto, CA). Seven custom millipede coils were used to image the brains in parallel (Bock et al., 2005; Lerch et al., 2011). Parameters used in the anatomical MRI scans: T2 weighted 3D fast spin echo sequence, with a cylindrical acquisition of k-space, and with a TR of 350 ms, and TEs of 10.5 ms per echo for 12 echoes, field of view 36 x 36 x 40 mm³ and a matrix size of 456 x 456 x 504 giving an image with 0.079 mm isotropic voxels (Noakes et al., 2017). The current scan time for this sequence is ~3 hours.

To visualize and compare any changes in the rat brains the images were linearly and non-linearly registered together using the pydpiper framework. Registrations were performed using a combination of mni_autoreg tools (Collins et al., 1994) and ANTS (advanced normalization tools) (Avants et al., 2011).

Following registration, a population atlas was created representing the average anatomy of the study sample. At the end of the registration process all the scans were deformed into alignment with one another in an unbiased fashion. This allows for analysis of the deformations required to register the brains together, which can be used to assess the volume of the individual brains and compared them to one another (Bishop et al., 2006; Lerch et al., 2008c; Lerch et al., 2008a; Lerch et al., 2008b; Nieman et al., 2010; Nieman et al., 2018). For comparisons to the juvenile brains, a separate registration pipeline was used that included all the brains from this study as well as the previous Berg et al. (2020) study.

Volumetric differences were calculated on a regional and a voxelwise basis. An in-house manually
segmented hierarchical rat brain atlas was used to calculate the volumes of 52 different segmented
structures. These structures were derived from multiple atlases (Dorr et al., 2008; Steadman et al., 2014)
and then modified for use in the rat brain.

Experimental design and statistical analyses. Statistical analyses were performed using
GraphPad Prism 8 statistical software (GraphPad Software, San Diego, CA). Clampex 10.6 software suite
(Molecular Devices, San Jose, CA) was used for analyzing electrophysiological data. Congruent with
previous studies, no significant sex differences were detected so the results herein include both males and
females. Effect sizes and power were determined using Cohen’s $d$.

Analysis of behavior and LTP. For single comparisons between two groups, either a Student’s $t$-
test or Mann-Whitney $U$ test was used. Data that passed distribution normality tests, were collected using
continuous variables, and had similar variances across groups were analyzed via Student’s $t$-test.
Alternatively, a Mann-Whitney $U$ test was used. Either a two-way analysis of variance (ANOVA) or two-
way repeated measures ANOVA was used to analyze the effects of genotype and a second factor. In
repeated measures ANOVA, genotype was the between-group factor and time, limb set, test phase, scent,
or prepulse intensity was the within-group factor. Post-hoc comparisons were performed following a
significant main effect or interaction and were carried out using Holm-Sidak’s multiple comparisons test
controlling for multiple comparisons. Data points within two standard deviations of the mean were
included, all significance tests were two-tailed, and a $p$-value of $< 0.05$ was considered significant.

Analysis of MRI. Statistical analyses were used to compare both the absolute and relative volumes
voxelwise as well as across the 52 different hierarchical structures in the rat brains. Absolute volume was
calculated as mm$^3$ and relative volume was assessed as a measure of % total brain volume. Voxelwise and
regional differences were assessed using linear models. All image analysis tools and software is available
on Github (https://github.com/Mouse-Imaging-Centre). Multiple comparisons were controlled for using
the False Discovery Rate (Genovese et al., 2002).
Results

Overabundant emission of laughter-like 50-kHz calls in juvenile $Ube3a^{mat-/pat+}$ rats. Since deficient expressive communication and elevated rates of positive affect are key clinical features of AS, we sought to quantify these characteristics in $Ube3a^{mat-/pat+}$ and $Ube3a^{mat+/pat+}$ (wildtype) rats. While vocalizations are readily collected during social play, recording USV from multiple interacting animals makes it difficult to determine which animal made each call. We therefore took advantage of the fact that rats emit laughter-like 50-kHz calls when social play is simulated by an experimenter via tickling and other physical maneuverings (Burgdorf and Panksepp, 2001; Burgdorf et al., 2005; Burgdorf et al., 2008; Ishiyama and Brecht, 2016). We implemented a standardized heterospecific play procedure (Figure 1A) to elicit USV (Figure 1B) while maintaining full confidence in the identity of the caller and controlling for the level of physical interaction across subjects.

We discovered that while both groups increased 50-kHz USV emission across consecutive sessions, $Ube3a^{mat-/pat+}$ emitted a substantially elevated level of 50-kHz USV (Figure 1C; $F_{Genotype(1,48)}=7.351, p=0.009; F_{Day(3,007,144.3)}=10.82, p<0.0001; F_{Dcf(4,192)}=1.052, p>0.05$). In total, $Ube3a^{mat-/pat+}$ emitted an average of 33 ± 5 USV per minute (mean ± S.E.M.), more than twice the rate of controls, which produced an average of 15 ± 3 calls per minute (Figure 1D; $U=175, p=0.007, d=0.77$). A closer examination revealed that 50-kHz USV were elevated during the break and belly tickle phases (Figure 1E; $F_c(1,48)=6.927, p=0.011; F_{Phase(1,722,82.64)}=27.83, p<0.0001; F_{Pcf(4,192)}=2.075, p>0.05$; post-hoc: break, $p=0.023, d=0.85$; belly tickle, $p=0.023, d=0.84$), although calling during the other phases also trended higher, providing strong evidence of elevated positive affect and a high hedonic impact of the assay (neck tickle, $p=0.057, d=0.62$; push and drill, $p=0.057, d=0.65$; flip over, $p=0.057, d=0.69$). There was no effect of sex, nor an interaction with sex, ($p>0.05$) for any parameter.

Additionally, 50-kHz USV were more frequently emitted during the anticipation period immediately prior to the play sessions (Figure 1F; $U=146.5, p=0.001, d=1.04$). In total, across all four
anticipation timepoints (days 2-5), $Ube3a^{mat/pat^+}$ emitted an average of 9 ± 2 USV per minute (mean ± S.E.M.), more than four times the rate of wildtypes, which produced an average of 2 ± 0.4 calls per minute. This indicates that $Ube3a^{mat/pat^+}$ predicted the impending onset of play and that the interaction had a high degree of incentive salience.

Excessive vocalization by $Ube3a^{mat/pat^+}$ rats was specific to 50-kHz USV. Production of short 22-kHz USV, which are emitted in modest amounts during play, was low and did not differ between genotypes (Figure 1G; $F_D(1.48)=1.771, p>0.05$; $F_D(1.825,87.62)=3.160, p>0.05$; $F_D<4.192)=1.330, p>0.05$). Elevated 50-kHz calling by $Ube3a^{mat/pat^+}$ was also specific to being provoked by heterospecific play, as 50-kHz and short 22-kHz USV production was normal during exploration of an empty cage (albeit a slight trend toward greater 50-kHz USV; Figure 1H; 50-kHz, $U=374.5, p>0.05$; 22-kHz, $U=433, p>0.05$; 22-kHz, $U=44.50, p>0.05$). No gross abnormalities in call structure were observed. Specifically, 50-kHz calls were of normal duration and peak frequency (Figure 1J: $U=205, p>0.05$; Figure 1K: $U=240, p>0.05$; Figure 1N: $U=52, p>0.05$; Figure 1O: $t(19)=0.3179, p>0.05$), suggesting that increased heterospecific play 50-kHz call numbers were not inflated by shorter or broken calls. Duration and peak frequency of 22-kHz USV were also comparable between genotypes (Figure 1L: $U=12, p>0.05$; Figure 1M: $t(11)=1.699, p>0.05$; Figure 1P: $U=1, p>0.05$; Figure 1Q: $U=1, p>0.05$). Since the average duration of the juvenile 22-kHz USV fell short of the usual durations of adult “typical 22-kHz” USV, we herein refer to them as “short 22-kHz” USV.

Intact social interest but deficient expression of key social interaction behaviors in juvenile $Ube3a^{mat/pat^+}$ rats. We sought to investigate whether elevated 50-kHz USV emission in $Ube3a^{mat/pat^+}$ rats was associated with greater social engagement with a conspecific. Starting around two weeks of age, rats play fight with each other by chasing, pouncing, pinning, and wrestling in a manner similar to cats and dogs. Through developmental experience, they learn how to appropriately initiate, engage in, and terminate play bouts with others. In order to more closely examine social behavior and the nuanced
reciprocal interactions of social play, we gave juvenile subjects the opportunity to freely interact with a
conspecific (Panksepp, 1981). Despite greater 50-kHz calling during heterospecific play, Ube3a_{mat-/pat+} rats
showed a normal degree of interest in the stimulus animal, demonstrated by the amounts of time spent
social sniffing (Figure 2A; t(20)=1.646, p>0.05) and anogenital sniffing (Figure 2B; t(20)=0.4457,
p>0.05). Putting forth a similar level of investigative effort suggested that Ube3a_{mat-/pat+} are just as
motivated for social interaction as controls. Levels of self-grooming (Figure 2C; U=38, p>0.05) and arena
exploration (Figure 2D; U=30, p>0.05) were also normal but Ube3a_{mat-/pat+} spent markedly less time
following or chasing the stimulus rat (Figure 2E; U=29, p=0.041, d=1.11). The key observation was the
reduced time spent rough-and-tumble playing (Figure 2F; U=33.50, p=0.029, d=0.89) compared to
wildtypes. In an attempt to reconcile the near lack of play with intact levels of social interest, we
quantified specific components of rough-and-tumble play. While the number of side-to-side social
contacts via push pasts were similar across genotypes (Figure 2G; t(20)=0.3852, p>0.05), there was a
trending reduction in the number of push under or crawl overs (Figure 2H; U=31.5, p=0.061, d=0.89) and
almost a complete lack of pouncing in Ube3a_{mat-/pat+} (Figure 2I; U=24, p=0.008, d=1.14). A separate test
of olfaction was used to rule out an olfactory deficit as a confounder of social investigation (Figure 2J;
F_{Genotype}(1,12)=0.0066, p=0.937; F_{Scent}(1,12)=14.20, p=0.003; F_{S×G}(1,12)=0.0165, p=0.900; post-hoc:
mat+/pat+, p=0.035; mat-/pat+, p=0.035).

Abnormal gait in Ube3a_{mat-/pat+} rats. In an effort to assess the potential contribution of motor
defects to social play behavior, we explored motor dysfunction, which is a core clinical feature of AS
prevalent in mouse models (Huang et al., 2013; Leach and Crawley, 2018; Copping and Silverman, 2020)
and hypothesized by our group to underlie the open field, rotarod, and marble burying phenotypes of AS
mouse models. Previously, we discovered lower open field vertical activity in Ube3a_{mat-/pat+} rats while
other activity indices were typical (Berg et al., 2020c). Using the DigiGait automated treadmill system,
we found that juvenile Ube3a_{mat-/pat+} rats displayed robust abnormalities in limb propulsion time,
indicating reduced limb strength and less force produced per unit time compared to wildtypes (Figure 3A;
Impaired learning and memory in $Ube3a^{mat-/pat+}$ rats. Learning and memory impairments, which are characteristic of AS, may hinder the ability of $Ube3a^{mat-/pat+}$ rats to learn via developmental experience how to appropriately engage in social interactions. We therefore probed for a juvenile learning and memory deficit using a fear conditioning assay previously used to detect a deficit in adulthood (Dodge et al., 2020). Following successful fear conditioning (Figure 4A; $F_{\text{phase}(1,30)}=48.47$, $p<0.0001$; $F_{\text{gene} \times \text{phase}(1,30)}=0.2203$, $p>0.05$; $F_{\text{phase}(1,30)}=0.0613$, $p>0.05$; post-hoc: mat+/pat+, $p<0.0001$; mat-/pat+, $p<0.0001$), juvenile $Ube3a^{mat-/pat+}$ displayed normal levels of freezing in response to the training context...
but a robust deficit in cued fear memory 48 hrs after training (Figure 4C; \( F_{(1,30)}=7.395, \ p=0.011; \ F_{(1,30)}=42.36, \ p<0.0001; \ F_{p<c}(1,30)=8.699, \ p=0.006; \) post-hoc: pre-cue, \( p>0.05; \) cue, \( p<0.001, \ d=1.10).\) We assessed the potentially confounding variable of impaired sensorimotor processing by measuring the startle response to an intense acoustic stimulus and quantifying the reduction in startle response following prepulses of varying intensities. Both baseline activity (Figure 4D; \( t(22)=1.735, \ p>0.05)\) and the acoustic startle response of \( Ube3a^{mat-pat}\) rats were normal (Figure 4E; \( t(22)=1.157, \ p>0.05)\), illustrating intact hearing abilities. While there was a significant main effect of genotype on prepulse inhibition, indicative of a sensorimotor gating deficit (\( F_{\text{Genotype}(1,22)=4.740, \ p=0.041, \ d=0.88; \ F_{\text{Prepulse}(1,41.75)=20.64, \ p<0.0001; \ F_{p<c}(2,44)=2.127, \ p=0.1312), \) post-hoc testing revealed no significant difference between groups at any individual prepulse level (Figure 4F; 74 dB, \( p>0.05, \ d=0.25; \) 82 dB, \( p>0.05, \ d=0.73; \) 90 dB, \( p>0.05, \ d=1.05).\) As an additional assessment of cognitive functioning, we quantified spontaneous alternation during exploration of a Y-maze and found that \( Ube3a^{mat-pat}\) rats displayed reduced spontaneous alternation compared to wildtypes (Figure 4G; \( t(46)=3.115, \ p<0.01, \ d=0.90).\) \( Ube3a^{mat-pat}\) rats made 40% more errors (Figure 4H; \( t(46)=3.827, \ p<0.001, \ d=1.10)\) and more arm entries (Figure 4I; \( t(46)=3.620, \ p>0.05, \ d=1.04)\) despite no difference in the total distance moved (data not shown; Student’s \( t\)-test: \( t(46)=1.721, \ p>0.05).\) Taken together, these metrics indicate additional cognitive deficits in the \( Ube3a^{mat-pat}\) rats that were not confounded by a locomotor deficiency.

**Reduced hippocampal long-term potentiation (LTP) in \( Ube3a^{mat-pat}\) rats.** To elucidate the neurobiology underpinning the learning and memory deficits of \( Ube3a^{mat-pat}\) rats, we quantified long-term potentiation (LTP). Previous studies in mouse models of AS have shown that LTP, a major cellular mechanism underlying learning and memory (Collingridge and Isaac, 2003), is impaired (Jiang et al., 1998; van Woerden et al., 2007; Daily et al., 2011) Here, we examined hippocampal LTP in adult \( Ube3a^{mat-pat}\) rats compared to wildtype littermate controls. Since we found hippocampal-dependent contextual fear memory intact at the juvenile age, but a previous report detected a clear deficit in adults...
(Dodge et al., 2020), we measured hippocampal LTP in adulthood. Basal synaptic strength (Figure 5A; $F_{\text{Genotype}}(1,92)=0.2013$, $p>0.05$; $F_{\text{Amplitude}}(5,111)=94.04$, $p<0.0001$; $F_{\text{GxA}}(5,92)=0.4107$, $p>0.05$) and paired-pulse ratio (Figure 5B; $F_0(1,56)=0.065$, $p>0.05$; $F_{\text{Interval}}(3,116)=20.96$, $p<0.0001$; $F_{\text{GxI}}(3,56)=0.0758$, $p>0.05$) were unaltered in $Ube3a^{\text{mat-}}$ rats, suggesting no change in baseline excitatory transmission. However, consistent with the mouse models of AS (Jiang et al., 1998; van Woerden et al., 2007; Daily et al., 2011), we found that the magnitude of LTP was reduced in $Ube3a^{\text{mat-}}$ rats (Figure 5C and 5D; $t(25)=4.641$, $p<0.0001$, $d=1.78$), suggesting a putative mechanism underlying impairment of learning and memory (Zucker, 1989; Jiang et al., 1998; Zucker and Regehr, 2002; van Woerden et al., 2007; Daily et al., 2011).

Neuroanatomical pathology in $Ube3a^{\text{mat-}}$ rats revealed by high-resolution magnetic resonance imaging (MRI). MRI revealed striking differences in total brain volume at 6.5 months of age, which was decreased by 6.0% in $Ube3a^{\text{mat-}}$ rats ($q=0.04$, Figure 6, Figure 6-1). The overall brain volume difference was driven by decreases in the hippocampal region (-6.3%, $q=0.04$), brain stem (-5.6%, $q=0.04$), thalamus (-7.7%, $q=0.01$), cerebellum (-9.0%, $q=0.02$), and deep cerebellar nuclei (-12.3%, $q=0.0001$). Additional differences were found throughout the white matter fiber tracts (-7.6%, $q=0.02$), including but not limited to the cerebral peduncle (-7.6%, $q=0.02$), internal capsule (-8.4%, $q=0.02$), and arbor vitae of the cerebellum (-11.7%, $q=0.0004$). Moreover, trends were seen in other large white matter structures including the corpus callosum (-6.7%, $q=0.06$) and fornix system (-6.0%, $q=0.09$). A complete list of the regional structural differences in both absolute (mm$^3$) and relative (% total brain) volume is provided in Figure 6-1.

As we had previously examined $Ube3a^{\text{mat-}}$ rats at a juvenile age (postnatal day (PND) 21) (Berg et al., 2020c), we felt an age by genotype comparison was warranted. Figure 6 highlights these changes in eight coronal slices, separately from both the previous work on juvenile rats and from the current data on adults. A combined dataset using both the juvenile and adult data was then used to examine a genotype by age interaction model, which revealed several regions to diverge with age and...
genotype: total brain volume ($q=0.048$), caudoputamen ($q=0.03$), white matter fiber tracts ($q=0.03$; Figure 491 6A; $F_{\text{Age}}(A)(1,97)=546.5$, $p<0.0001$; $F_{\text{Genotype}}(G)(1,97)=11.87$, $p<0.001$; $F_{\text{Age} \times \text{Genotype}}(A,G)(1,97)=10.68$, $p=0.002$; post-hoc: juvenile, $p>0.05$; adult, $p<0.0001$, $d=1.02$), hypothalamus ($q=0.046$; Figure 6B; $F_{\text{Age}}(A)(1,97)=460.2$, $p<0.0001$; $F_{\text{Genotype}}(G)(1,97)=5.081$, $p=0.026$; $F_{\text{Age} \times \text{Genotype}}(A,G)(1,97)=8.760$, $p=0.004$; post-hoc: juvenile, $p>0.05$; adult, $p=0.001$, $d=0.82$), hippocampal region ($q=0.046$; Figure 6C; $F_{\text{Age}}(A)(1,97)=434.4$, $p<0.0001$; $F_{\text{Genotype}}(G)(1,97)=10.89$, $p=0.001$; $F_{\text{Age} \times \text{Genotype}}(A,G)(1,97)=8.760$, $p=0.004$; post-hoc: juvenile, $p>0.05$; adult, $p<0.0001$, $d=1.02$), and thalamus ($q=0.02$; Figure 6D; $F_{\text{Age}}(A)(1,97)=430.2$, $p<0.0001$; $F_{\text{Genotype}}(G)(1,97)=11.14$, $p=0.001$; $F_{\text{Age} \times \text{Genotype}}(A,G)(1,97)=14.96$, $p<0.001$; post-hoc: juvenile, $p>0.05$; adult, $p<0.0001$, $d=1.22$). A full list of the regional genotype by age interactions is located in Figure 6-2. Voxelwise changes were also found throughout the brain of adult $\text{Ube3a}^{\text{mat-}/\text{pat}+}$ rats compared to the juvenile age. The changes in the adults were substantially larger, signaling a more severe neuroanatomical phenotype with age (Figure 6).

Discussion

Indispensable to therapeutic development are in vivo studies utilizing preclinical model systems. While mice have prevailed as the animal model of AS in recent decades (Jiang et al., 1998), the $\text{Ube3a}^{\text{mat-}/\text{pat}+}$ rat offers a unique and suitable system for investigating certain complexities of the human AS phenotype, particularly social communication and affect (Brudzynski, 2013; Wöhr and Schwarting, 2013; Burgdorf et al., 2020; Burke et al., 2017; Homberg et al., 2017; Netser et al., 2020). Our discovery of excessive laughter-like 50-kHz USV is the first report of this affective outcome measure in a model of AS, mirroring the affected population. Moreover, reduced social play, atypical gait, impaired cognition, and anatomical and cellular physiology anomalies were easily detected in this model.

We leveraged our model species to discover that $\text{Ube3a}^{\text{mat-}/\text{pat}+}$ rats produced an overabundance of 50-kHz vocalizations, which reflect a positive affective state and have been referred to as rat laughter (Panksepp and Burgdorf, 2000; Panksepp, 2005), as well as a trend of elevated laughter-like 50-kHz USV without provocation. Excessive 50-kHz USV, suggestive of enhanced ‘wanting’ and ‘liking’ the
interaction (Berridge, 2009; Berridge and Aldridge, 2009; Berridge et al., 2009; Okabe et al., 2021), closely aligns with the AS profile of a happy disposition and easily provoked laughter. To our knowledge, this is the first report of this method being used in a genetic rat model of a neurological disorder.

Exaggerated 50-kHz calling could suggest enhanced effort to elicit social interaction or may be unrelated to the social component of heterospecific play, potentially a neurobiological consequence of a disinhibited vocal production pathway. AS is typified by laughter that is easily provoked regardless of stimuli valence. The phenotype may also reflect enhanced sensitivity to tactile stimulation. Deriving greater reward from physical interactions could explain typical levels of social investigation in the reciprocal interaction test but reduced social approach in three-chambered and USV playback assays, as well as the disinhibition of social interactions in the clinical population. One limitation of our USV analysis was the lack of acoustic feature quantification for the calls evoked by heterospecific play. We did, however, subsequently carry out this analysis for all other USV assays and found no genotype effect on call features.

Juvenile social play is a critical way that rats develop social competence and learn how to appropriately engage and communicate with others, analogous to play in young children (Hofer and Shair, 1978; Panksepp and Beatty, 1980; Panksepp, 1981; Brudzynski, 2009, 2013; Argue and McCarthy, 2015). Ube3a\textsuperscript{mat-\textit{pat+}} rats were interested in a novel partner but did not engage in rough-and-tumble play behaviors characteristic of the species, albeit specific to sex and strain. Our finding of no sex difference in rough-and-tumble play aligns with previous reports which also used pre-test social isolation to motivate the subjects to play (Veenema et al., 2013; Bredewold et al., 2014; Bredewold et al., 2015; Reppucci et al., 2018; Kisko et al., 2020). In contrast with studies on mouse models of AS using the three-chambered social approach task (Jamal et al., 2017; Kumar et al., 2019; Perrino et al., 2020; Dutta and Crawley, 2020), which have reported contradictory social deficits and “hypersociability,” the rat model displayed a typical level of social investigation.

Movement disorders (Wheeler et al., 2017) are a hallmark feature of AS, with gait ataxia being one of the most common issues. While the deficits of Ube3a\textsuperscript{mat-\textit{pat+}} rats were not obvious to the eye, subtle
aberrations in stance and paw placement, paired with abnormal braking and propelling, reflect impaired motor coordination. All of this evidence suggests that altered postures affect motor dynamics which results in the gait patterns exhibited by AS individuals and $Ube3a^{mat-/pat+}$ rats. The limb weakness indicated by our gait analysis aligns with the reduced rearing previously observed (Berg et al., 2020c).

We discovered and report for the first time, to our knowledge, of long-term potentiation (LTP) deficits in this rat model (Jiang et al., 1998; Weeber et al., 2003; van Woerden et al., 2007; Filonova et al., 2014; Ciarlone et al., 2016), which provides a putative cellular signaling mechanism underlying the learning and memory impairments reported herein and previously (Berg et al., 2020c; Dodge et al., 2020). Juvenile $Ube3a^{mat-/pat+}$ rats exhibited deficits in cued fear memory 48 hours post-training, which extends the previous finding by Dodge et al. of deficient contextual and cued fear conditioning in adults 72 hours post-training (Dodge et al., 2020). We ruled out impaired sensorimotor abilities as a confounding variable since the acoustic startle response was unaffected.

Pronounced deficits in adulthood are supported by neuroimaging. Previously, we discovered a variety of trending volumetric abnormalities at PN D 21 (Berg et al., 2020c), however, these new data show more substantial reductions in adults throughout the brain, highlighting a more severe neuroanatomical phenotype with age. Reduced total brain volume may indicate a loss of cellular volume or dendritic complexity over time, and the drastic volume loss in fiber tracts could indicate a loss in axonal numbers, axonal volume, or myelination. In a mouse model of AS, white matter loss was found to play a large role in the overall microcephaly observed (Judson et al., 2017), with an 11% loss in the corpus callosum making it the most affected white matter structure. $Ube3a^{mat-/pat+}$ rats showed a trend towards reduced corpus callosum volume (-6.7%), but the largest white matter deficits were cerebellar. In alignment with Judson et al.’s (2017) study in mice, the reduced fiber tracts volume was also disproportionate to the overall brain volume loss, confirming that white matter development plays the major role in the impaired brain growth in AS. Additionally, the 9% decrease in cerebellum size was consistent with cortical loss (-9%), but there was a disproportionate reduction in arbor vitae and deep cerebellar nuclei volume, indicating that the outputs of the cerebellum are impaired.
GABAergic neuron loss (Judson et al., 2016) and decreased tonic inhibition in cerebellar granule cells (Egawa et al., 2012) underlie the theory of brain dysfunction in AS, and hypotheses addressing the theory of reduced inhibitory tone are being pursued for small molecule development (Ciarlone et al., 2017). The present results are congruent with this overarching mechanism theory of AS, loss of inhibitory tone. Emission of 50-kHz USV induced by heterospecific play is associated with dopamine release in the nucleus accumbens/ventral striatum. These calls have been considered as “joy,” “euphoria,” and “laughter,” supported by behavioral pharmacology showing increased 50-kHz USV resulting from amphetamine administration (Brudzynski, 2013, 2015; Wöhr, 2021). Both vocalizations and gait require fine motor control and thus striatal and motivational components support aberrant frontal-striatal circuitry. Interestingly, the ventral striatum and “reward” associated substrates of the basal ganglia have inhibitory projections, in line with overall theories of AS regarding inhibitory loss.

While gross and fine movement are complicated multi-system physiological processes, AS individuals show ataxic movements in both upper and lower limbs and aberrant gait, suggesting the particular involvement of the cerebellum. This was corroborated here and in earlier work by the large reductions in cerebellar nuclei size, which is consistent with our overarching mechanistic hypothesis since Purkinje cell neurons to the deep cerebellar nuclei modulate excitation via inhibition and Egawa et al. (2012) highlighted decreased cerebellar granule cells in AS. Utilizing conditional Ube3a mouse models to identify the neural substrates of circuit hyperexcitability in AS, Judson et al. (2016) provided compelling evidence that GABAergic, but not glutamatergic, Ube3a loss is responsible for mediating the EEG abnormalities and seizures of AS. Previously, we reproduced and extended Judson et al.’s. (2016) data (Copping and Silverman, 2021) and our hypothesis is that this mechanism extends to social communication, cognitive phenotypes, and impaired gait outcomes, as each shares components of learning and motivation. The loss of brain volume in regions dense with inhibitory neurons seen herein provides further corroborative evidence that GABAergic tone underlies functional outcomes. Independent corroboration comes from ErbB inhibitors, which have been reported to reverse LTP deficits in AS model mice (Kaphzan et al., 2012). While glutamate receptor expression and function were unaltered in Ube3a
mice (Kaphzan et al., 2012; Judson et al., 2016), ErbB signaling was shown to rescue LTP impairments in
Ube3a mice via an increase in inhibitory synaptic transmission, corroborating our core overarching
mechanism of reduced inhibitory tone.

In conclusion, we discovered that Ube3a^{mat-pat} rats exhibited interest in a social partner but
expressed an atypically high level of laughter-like vocalizations. Deficits in other AS-relevant domains
were also discovered, including gait and cognition, and reduced hippocampal LTP. Future lines of
investigation will assess the circuitry and mechanisms underlying the excessive laughter-like USV and
social-cognitive anomalies in USV reception, in addition to pursuing other neurobiological endpoints.
Overall, our results indicate that the deletion of maternal Ube3a in the rat creates a sophisticated rodent
model with high face validity to the human AS phenotype. In the pursuit of effective therapeutics, it is
essential to be equipped with a diverse set of behavioral outcome measures and neurological biomarkers
by which to assess efficacy. Taken together, we demonstrate that the Ube3a^{mat-pat} rat offers numerous
potential outcome measures that are detectable throughout the lifespan.

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Data Availability

Data are available from the corresponding author upon request.
**Figure Legends**

**Figure 1.** Overabundant emission of laughter-like 50-kHz calls in juvenile $Ube3a^{mat-/pat^+}$ rats. (A) Example images of the manipulations used to mimic social play and elicit ultrasonic vocalizations (USV). (B) Example spectrograms of USV from a wildtype littermate control ($Ube3a^{mat+/pat^+}$; mat+/pat+; upper) and $Ube3a^{mat-/pat^+}$ rat (mat-/pat+; lower). (C) Across five days of heterospecific play sessions, 50-kHz USV emission increased with repeated testing in both mat-/pat+ ($n=25$) and controls ($n=25$), but the emission rate was substantially elevated in mat-/pat+. (D) On average, mat-/pat+ rats produced 50-kHz USV at more than twice the rate of controls. (E) Specifically, 50-kHz calling was abnormally high during the break and belly tickle phases, with trending increases during neck tickle, push and drill, and flip over. (F) Prior to the onset of play, mat-/pat+ rats emitted anticipatory 50-kHz USV at more than three times the rate of controls. (G) Production of short 22-kHz USV was low, did not differ between genotypes, and did not change over subsequent play sessions. (H) The rates of 50-kHz and short 22-kHz calling during empty cage exploration were comparable between genotypes (mat+/pat+, $n=32$; mat-/pat+, $n=29$), as were the (I) 50-kHz and short 22-kHz calling rates in response to hearing playback of conspecific 50-kHz USV (mat+/pat+, $n=9$; mat-/pat+, $n=12$). Call features did not differ by genotype: (J) The average duration and (K) peak frequency of spontaneous 50-kHz calls made during exploration of an empty cage was comparable between mat-/pat+ ($n=23$) and mat+/pat+ rats ($n=25$). (L) The average duration and (M) average peak frequency of short 22-kHz calls made within an empty cage were also similar between genotypes (mat+/pat+, $n=6$; mat-/pat+, $n=7$). (N) For 50-kHz USV emitted in response to hearing playback of natural pre-recorded 50-kHz rat USV, average duration and (O) average peak frequency were comparable between mat-/pat+ rats ($n=12$) and wildtype littermates ($n=9$). (P) There was no genotype effect on the average duration or (Q) average peak frequency of short 22-kHz calls made during USV playback (mat+/pat+, $n=3$; mat-/pat+, $n=2$). Of note, long 22-kHz USV known to function as “alarm
calls” were very rarely observed, indicating that our paradigms were not aversive. Data are depicted as mean ± S.E.M. C: **p<0.01, repeated measures ANOVA. D, F: ***p<0.001, **p<0.01, Mann-Whitney test. E: *p<0.05, #p<0.06, repeated measures ANOVA, Holm-Sidak’s post-hoc.

Figure 2. Intact social interest but deficient expression of key social interaction behaviors in juvenile Ube3a\textsuperscript{mat-/pat+} rats. (A) During a 10-min interaction session with a novel same-sex wildtype conspecific, Ube3a\textsuperscript{mat-/pat+} rats (mat-/pat+; n=12) spent similar amounts of time social sniffing, (B) anogenital sniffing, (C) self-grooming, and (D) exploring the arena compared to wildtype littermate controls (Ube3a\textsuperscript{mat+/pat+}; mat+/pat+; n=10). (E) Robust deficits, however, were discovered in the time spent following or chasing and (F) rough-and-tumble playing. (G) The number of push pasts were similar across genotypes but (H) there was a trend for mat-/pat+ to less frequently push under or crawl over and (I) mat-/pat+ rats did not perform nearly as many pounces as wildtype littermates. (J) A separate test of olfactory discrimination revealed normal sniff times of social and non-social scents. Time spent investigating novel odors was similar for mat-/pat+ (n=7) and mat+/pat+ rats (n=7) and both groups spent more time investigating a social scent compared to a non-social vanilla odor. Data are depicted as mean ± S.E.M. E-I: **p<0.01, *p<0.05, #p<0.065, Mann-Whitney test. J: *p<0.05, repeated measures ANOVA, Holm-Sidak’s post-hoc.

Figure 3. Abnormal gait in Ube3a\textsuperscript{mat-/pat+} rats. (A) While treadmill walking, Ube3a\textsuperscript{mat-/pat+} rats (mat-/pat+; n=23) displayed aberrant propulsion time (time from maximal paw contact with belt to just before liftoff) in both sets of limbs. Compared to wildtype littermates (Ube3a\textsuperscript{mat+/pat+}; mat+/pat+; n=23), propulsion time was decreased in the forelimbs and increased in hindlimbs. (B) Brake time (time from initial to maximal paw contact with belt) was significantly elevated in the forelimbs of mat-/pat+ while a trending reduction in hindlimb brake time was found (p=0.150). (C) Swing time (no paw contact with the belt) and (D) stride time (sum of swing and stance time) were similar across genotypes. (E) Example paw prints illustrating the spatial gait parameters depicted in panels F, G, and H. (F) Stride length did not differ between groups, but (G) forelimb stance width was narrower and (H) absolute paw angle for the forelimbs was greater.
indicating more external rotation in mat-/pat+ rats. (I) No significant difference in gait symmetry (ratio of forelimb to hindlimb stepping frequency) was detected. Data are depicted as mean ± S.E.M. **p<0.01, *p<0.05, repeated measures ANOVA, Holm-Sidak’s post-hoc.

Figure 4. Impaired learning and memory in Ube3a\textsuperscript{mat-/pat+} rats. (A) During fear conditioning training, juvenile Ube3a\textsuperscript{mat-/pat+} (mat-/pat+; n=12) and wildtype littermate controls (Ube3a\textsuperscript{mat+/pat+}; mat+/pat+; n=20) showed similar increases in freezing post-training. (B) When returned to the training context 24 hrs following training, mat-/pat+ rats exhibited a similar level of freezing to wildtypes. (C) When introduced to a novel context 48 hrs after training, no difference in freezing pre-cue was found but mat-/pat+ rats froze for less than half the time of wildtypes during presentation of the auditory cue. A separate sensorimotor test confirmed intact auditory sensitivity: (D) Baseline activity within the testing apparatus was comparable between mat-/pat+ (n=10) and mat+/pat+ rats (n=14). (E) There was no effect of genotype on the startle response to a 120 decibel (dB) startle stimulus. (F) Prepulse inhibition of the startle response was generally reduced in adult mat-/pat+ rats. (G) Spontaneous arm alternation during Y-maze exploration was significantly reduced in adult mat-/pat+ rats (n=24) compared to wildtype littersmates (n=24). (H) Mat-/pat+ rats made 40% more errors and (I) made more entries into the maze arms. Bars indicate mean ± S.E.M. A, C: ****p<0.0001, ***p<0.001, repeated measures ANOVA, Holm-Sidak’s post-hoc. F: *p<0.05, repeated measures ANOVA main effect. G-I: ***p<0.001, **p<0.01, Student’s t-test.

Figure 5. Reduced hippocampal long-term potentiation (LTP) in Ube3a\textsuperscript{mat-/pat+} rats. (A) Normal basal synaptic transmission as measured by presynaptic fiber volley amplitudes and postsynaptic fEPSP slopes for responses elicited by different intensities of SC fiber stimulation in Ube3a\textsuperscript{mat-/pat+} (mat-/pat+; n=16) and wildtype littermate (Ube3a\textsuperscript{mat+/pat+}; mat+/pat+; n=11) hippocampal slices. (B) Paired-pulse facilitation was unchanged at mat-/pat+ SC-CA1 synapses compared to mat+/pat+ (n=15 mat+/pat+ and n=20 mat-/pat+ slices). (Right) Traces represent fEPSPs evoked by stimulation pulses delivered with a 50 msec
interpulse interval. Scale bars: 0.5 mV, 25 msec. 

(C) High frequency stimulation (HFS)-induced LTP in mat+/pat+ (n=11) was significantly greater compared to mat-/pat+ (n=16). Traces at right represent superimposed fEPSPs recorded during baseline and 60 min after HFS. Scale bars: 1 mV, 5 msec. 

(D) Summary graph of average percentage potentiation relative to baseline demonstrating that mat+/pat+ exhibited significantly enhanced SC-CA1 LTP at 60 min after HFS (delivered at time = 0), fEPSPs were potentiated to 160 ± 7% of baseline in mat+/pat+ (n=11) and were 127 ± 5% of baseline in mat-/pat+ slices (n=16). Data were collected from two rats per genotype. ****p<0.0001, Student’s t-test. 

Figure 6. Neuroanatomical pathology in Ube3a mat-/pat+ rats revealed by high-resolution magnetic resonance imaging (MRI). (Left) Slice series comparing absolute volume (mm³) of juvenile and adult populations of Ube3a mat-/pat+ (mat-/pat+) rats and wildtype littermates (Ube3a mat+/pat+; mat+/pat+). Red to yellow coloration indicates increased volume compared to wildtype whereas dark blue to light blue indicates decreased volume. The leftmost column is data on juvenile mat-/pat+ rats from Berg et al. (2020) and the center column illustrates the same slices on the adult dataset presented here. Most notably, total brain volume was 6.0% smaller in mat-/pat+ rats compared to wildtype. Additionally, the third column shows the genotype by age interaction highlighting several regions of interest, (right) four of which are shown in panels A-D. Namely, (A) fiber tracts, (B) the hypothalamus, (C) the hippocampal region, and (D) the thalamus. Full detail of regional findings for adult animals and the interaction effect are described in Figures 6-1 and 6-2. Group sizes: juvenile mat+/pat, n=29; juvenile mat-/pat+, n=25; adult mat+/pat+, n=23; adult mat-/pat+, n=24. Bars indicate mean ± S.E.M. ****p<0.0001, **p<0.01, two-way ANOVA, Holm-Sidak’s post-hoc. 

Extended Data Figure Legends

Figure 6-1. Brain volumes for adult Ube3a mat-/pat+ rats and wildtype littermates.
Figure 6-2. Age by genotype interaction for absolute brain volumes of juvenile and adult $Ube3a^{mat-\text{pat}+}$ rats and wildtype littermates.
Volume Differences
Ube3a^{mat+/pat+} vs. Ube3a^{mat+/-pat+}
Absolute Volume (mm$^3$)

A. Fiber tracts
B. Hypothalamus
C. Hippocampal region
D. Thalamus

- mat+/pat+
- mat-/-pat+

Statistical Significance:
- **: p < 0.05
- ****: p < 0.0001

Genotype * Age Interaction

- Larger
- Smaller
- False Discovery Rate

Juvenile
Adult