

Behavioral Evaluation of Angelman Syndrome Mice at Older Ages

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Abstract—Angelman syndrome is a neurodevelopmental disorder presenting with severe deficits in motor, speech, and cognitive abilities. The primary genetic cause of Angelman syndrome is a maternally transmitted mutation in the *Ube3a* gene, which has been successfully modeled in *Ube3a* mutant mice. Phenotypes have been extensively reported in young adult *Ube3a* mice. Because symptoms continue throughout life in Angelman syndrome, we tested multiple behavioral phenotypes of male *Ube3a* mice and WT littermate controls at older adult ages. Social behaviors on both the 3-chambered social approach and male–female social interaction tests showed impairments in *Ube3a* at 12 months of age. Anxiety-related scores on both the elevated plus-maze and the light ↔ dark transitions assays indicated anxiety-like phenotypes in 12 month old *Ube3a* mice. Open field locomotion parameters were consistently lower at 12 months. Reduced general exploratory locomotion at this age prevented the interpretation of an anxiety-like phenotype, and likely impacted social tasks. Robust phenotypes in middle-aged *Ube3a* mice appear to result from continued motor decline. Motor deficits may provide the best outcome measures for preclinical testing of pharmacological targets, towards reductions of symptoms in adults with Angelman syndrome.

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Key words: Angelman, *Ube3a* mice, 12 month old, social behavior, anxiety-related, exploration.

INTRODUCTION

Angelman syndrome is a rare genetic neurodevelopmental disorder characterized by severe intellectual disabilities, impaired speech, developmental delays, microcephaly, seizures, anxiety, motor dysfunctions, ataxic gait, social communication deficits, and a happy demeanor with excessive laughter (Angelman, 1965; Williams et al., 2010; Bird, 2014; Wheeler et al., 2017; den Bakker et al., 2018; www.angelman.org). The primary genetic cause of Angelman syndrome resides in the deletion of a sequence of imprinted genes at chromosomal locus 15q11-q13. Maternal transmission of the deletion, particularly the reduced expression of the ubiquitin ligase *UBE3A* gene within this locus, results in Angelman syndrome, whereas paternal transmission results in another distinct neurodevelopmental disorder, Prader-Willi syndrome (Knoll et al., 1989; Nicholls, 1993; Buiting et al., 2016). No medical treatments currently exist for the underlying causes of Angelman syndrome (Wheeler et al., 2017).

Mouse models incorporating the loss of maternal *Ube3a* have been generated and well-characterized for several behavioral features relevant to the symptoms of Angelman syndrome, including motor and cognitive deficits (Jiang et al., 1999, 2010; Heck et al., 2008; Mabb et al., 2011; Baudry et al., 2012; Jana, 2012; Kaphzan et al., 2012; Huang et al., 2013; Santini et al., 2015; Leach and Crawley, 2018; Sonzogni et al., 2018). *Ube3a* mutant mouse models provide a preclinical research tool for the discovery of effective therapeutics for Angelman syndrome (van Woerden et al., 2007; Huang et al., 2011; Egawa et al., 2012; Margolis et al., 2015; Beaudet and Meng, 2016; Bi et al., 2016; Tan and Bird, 2016; Ciarlone et al., 2017; Stoppel and Anderson, 2017; Guzzetti et al., 2018; Lee et al., 2018; Rotaru et al., 2018).

Here we focus on a relatively unexplored aspect of *Ube3a* mice. Although diagnosed in childhood, Angelman syndrome is a lifetime disorder. Adults with Angelman syndrome continue to display severe symptoms (Smith, 2001; Larson et al., 2015; Prasad et al., 2018). In contrast, most behavioral characterizations of *Ube3a* mutant mouse phenotypes have employed young mice, in the 8–14 week old range, paralleling the early stages of this neurodevelopmental disorder. At these younger ages, deficits have been consistently reported on motor assays including rotarod (Miura et al., 2002; Heck et al., 2008; Jiang et al., 2010; Daily et al.,

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2011; Egawa et al., 2012; Huang et al., 2013; Ciarlone et al., 2017; Leach and Crawley, 2018; Sonzogni et al., 2018) and open field exploratory locomotion (Allensworth et al., 2011; Huang et al., 2013; Ciarlone et al., 2017; Sonzogni et al., 2018). Higher anxiety-related behaviors (Jiang et al., 2010; Ciarlone et al., 2017 and others), impaired water maze spatial learning (Miura et al., 2002; Jiang et al., 2010; Huang et al., 2013; Leach and Crawley, 2018), and impaired fear conditioned learning and memory (Miura et al., 2002; Jiang et al., 2010; Huang et al., 2013) have been reported for *Ube3a* mice at younger ages. To our knowledge, the oldest ages of *Ube3a* mice tested behaviorally have been 17–23 weeks for water maze and at 31 weeks for rotarod (Huang et al., 2013). The present studies evaluated behavioral phenotypes of older *Ube3a* mice as compared to their wildtype littermates (WT) on two anxiety-related tests and two social tests, conducted primarily at age 12 months. In addition, open field locomotion was quantified to detect decreases or increases in activity at the older age, which could confound the interpretations of anxiety-related and social assay results.

EXPERIMENTAL PROCEDURES

Mice

Conventional *Ube3a* knockout mice, derived from maternal transmission and generated on a C57BL/6J background, were purchased from The Jackson Laboratory (JAX) in Bar Harbor, Maine (JAX catalog #016590), along with their WT littermates. Males were used for the present experiments, to achieve the full Ns required for evaluation of male–female social interactions in mice, while ensuring that all subject mice had identical past experience in the other behavioral assays. Attrition across the aging period reduced the numbers of originally purchased mice to N = 8 WT and N = 13 *Ube3a*. Female target mice used for the social tests were sex-matched 129/SvImJ (JAX #2448) for 3-chambered social approach, and C57BL/6J (B6, JAX #000664) for male–female interactions, purchased from JAX and housed by strain. Mice were housed in ventilated Tecniplast cages in an AAALAC approved temperature-controlled vivarium on a 12:12 circadian cycle with lights on at 7 AM. All husbandry, breeding, behavioral testing, and drug treatment procedures were approved by the University of California Davis Institutional Animal Care and Use Committee, and were conducted in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Methodological considerations

Behavioral testing was conducted during the light phase of the circadian cycle, between 8:30 AM and 5:30 PM. On the day of each experiment, mice in their home cages were habituated to the testing room for one hour before the start of the behavioral test. Order of testing

was (1) elevated plus-maze, (2) light ↔ dark transitions, (3) open field, (4) 3-chambered social approach, (5) male–female social interactions. For all assays, surfaces of the testing chamber were cleaned with 70% ethanol after each subject mouse was tested, with sufficient time for the ethanol odor to dissipate before the start of the next test session.

Mice were coded by ear notch pattern to ensure that investigators remained uninformed of genotype and treatment condition. Automated equipment was used for most behavioral assays. For the male–female social interaction assay, in which human scoring of videos was required, investigators remained blind to genotype through the use of numerically coded videos and coded mouse identification numbers.

Behavioral assays

Elevated plus-maze. The elevated plus-maze task was employed to assess anxiety-like conflict behavior in rodents, by titrating the tendency of mice to explore a novel environment versus the tendency of mice to avoid open, raised, brightly lit areas. The four arms are elevated one meter from the floor, with two closed arms surrounded by walls and two open arms in which the drop-off is detectable. Methods used were previously described (Bailey et al., 2007; Silverman et al., 2011; Brielmaier et al., 2012; Kazdoba et al., 2016; Rhine et al., 2019). The photocell-equipped automated plus-maze (Med Associates model ENV-560A, St. Albans, VT, United States) consisted of two open arms (35.5 cm × 6 cm) and two closed arms (35.5 cm × 6 cm). An 0.5 cm high lip surrounded the edges of the open arms. The two closed arms were surrounded by black Plexiglas walls 20 cm high. Room illumination was 300 lux. Each subject mouse was gently placed into the central start area (6 cm × 6 cm), facing an open arm. The mouse was allowed to freely explore for a 5 min test session. Time spent in the open arms and in the closed arms, number of entries into the open arms, and total number of entries into all four arms, were quantified by the Med Associates software.

Light dark transitions. The light ↔ dark transitions test, also termed the light/dark box, assesses anxiety-like conflict behavior in mice by evaluating the tendency of mice to explore a novel environment versus the tendency of mice to avoid brightly lit open areas. The light ↔ dark transitions test was performed as previously described (Silverman et al., 2011; Brielmaier et al., 2012; Rhine et al., 2019). The photocell-equipped automated 2-chambered apparatus and software were developed by George Dold and coworkers, Research Services Branch, National Institute of Mental Health, Bethesda, MD (Crawley and Goodwin, 1980). The test began by placing the mouse in the light chamber (28 cm × 27.5 cm × 27 cm, 300 lux), made of clear Plexiglas walls and open at the top. The enclosed dark chamber (28 cm × 27.5 cm × 19 cm, ~5 lux), made of black Plexiglas walls with a black Plexiglas ceiling, was reached by traversing the photocell beam in

the opening in the partition between the two chambers. The mouse was allowed to explore freely for 10 min. Time in the dark chamber and total number of transitions between the light and dark chambers were automatically recorded by Labview 8.5.1 software (National Instruments, Austin, TX, United States).

Open field. Open field activity served as a control assay to detect motor abnormalities that could introduce artifacts into the interpretations of social and/or anxiety-related abnormalities. Methods used were previously described (Silverman et al., 2011; Brielmaier et al., 2012; Rhine et al., 2019). Exploratory locomotion was assessed for 30 min in individual mice placed in a novel open field arena, 42 cm × 42 cm × 31 cm high, 30 lux. The open field arenas were equipped with photocell detectors in the x and y (horizontal), and z (vertical) dimensions, interfaced with beam break detection software (AccuScan, Omnitech Electronics, Columbus, OH, United States). Total distance traveled, horizontal movements, vertical movements, and time spent in the center of the arena were automatically recorded and quantified with Accuscan VersaMax 400 software.

3-Chambered social approach. Sociability was tested in four identical 3-chambered social approach chambers, using methods previously described (Silverman et al., 2011, 2012; Brielmaier et al., 2012; Yang et al., 2013; Kazdoba et al., 2016; Rhine et al., 2019). Each apparatus included a side chamber into which an empty inverted wire pencil cup (novel object) was placed, an empty middle chamber, and a side chamber into which an inverted wire pencil cup containing an age- and sexed-matched novel 129/SvImJ mouse (novel mouse) was placed. The target 129/SvImJ mice were previously habituated to the wire cup for 15 min/day during the two days preceding testing, to enhance their inactivity during the test session. Testing was conducted under low light, 30 lux. An initial 10 min habituation session confined to the center chamber was followed by a 10 min habituation session in all three empty chambers. Time in the two empty side chambers was similar, confirming absence of an innate side bias. After the two 10 min habituation sessions, the 10 min social approach session was conducted. Locations of the novel object wire cup and the novel 129/SvImJ target mouse were randomized between the two side chambers across test sessions. Time spent in each chamber (chamber time parameter), time spent within a 2 cm radius of each wire cup (sniff time parameter), and number of entries between compartments (control for exploratory locomotion), were videotracked and quantified by Noldus Ethovision software. Three body point detection tracking was employed to confirm that the subject mouse was facing the target mouse, to maximize accurate scoring of time spent sniffing the novel object and time spent sniffing the novel mouse.

Male-female social interactions. Male female reciprocal social interaction testing was conducted as previously described (Silverman et al., 2011, 2012;

Brielmaier et al., 2012; Kazdoba et al., 2016; Stoppel et al., 2018; Rhine et al., 2019). Male subject mice were group housed and sexually naïve at the time of testing. B6 females in proestrus or estrus (open vagina with pink or reddish pink surrounding tissue) were used as partner mice. Approximately synchronous estrus was induced by placing male urine and bedding from male cages into the cages of the partner B6 females on each of the three days preceding testing. A 5 min interaction session was conducted in a clean empty mouse cage within a sound attenuating chamber (ENV-018 V; Med Associations, St. Albans, VT, United States), with interior walls covered with convoluted foam sheets (Uline, Pleasant Prairie, WI, United States), under dim red lighting conditions (10 lux). Each female partner was given at least 30 min of rest time between re-use in another testing session. Male-female interactions were videorecorded with a digital closed-circuit television camera (Panasonic, Secaucus, NJ, United States). Parameters scored in the male subject mice included nose-to-nose sniffing, nose-to-anogenital sniffing, and following. Total duration and number of bouts of each parameter were quantified from numerically coded videos by a well-trained investigator uninformed of genotype, using Noldus Observer event recording software (Noldus Information Technology, Leesburg, VA, United States). Ultrasonic vocalization calls in the 40–120 Hz range were recorded during the test session using an Avisoft ultrasonic microphone and software (Avisoft Bioacoustics, Avisoft SASLabPro version 4.40). Previous work indicates that almost all calls during male–female interactions in laboratory mice are emitted by the male (Whitney et al., 1973; White et al., 1998; Wang et al., 2008; Sugimoto et al., 2011). Total number of calls were counted as previously described (Brielmaier et al., 2012; Yang et al., 2013, 2015).

Statistics

GraphPad Prism version 7 was used to conduct statistical analyses of the data and to generate graphs. Elevated plus-maze, light ↔ dark transitions, and male–female interactions were analyzed for genotype differences using unpaired Student's t-test statistics. Open field exploratory activity was analyzed with a Two-Way ANOVA, using time and genotype as factors, for each parameter (total distance, horizontal activity, vertical activity, and center distance). In cases of a significant ANOVA, a Sidak's multiple comparison test was used to compare open field activity between genotypes for specific 5 min time bins within the 30 min open field session. 3-chambered social approach, which provides a simple binary, yes-or-no measure of sociability within each genotype (Yang et al., 2011; Kazdoba et al., 2016; Rhine et al., 2019), was defined as significantly more time spent in the chamber with the novel mouse than in the chamber with the novel object, and/or significantly more time spent sniffing the novel mouse than sniffing the novel object. Unpaired Student's t-test was used to compare time spent in the chamber containing the novel mouse versus time in the chamber containing the novel object, and time spent sniffing the novel mouse versus time spent sniffing the novel object.

RESULTS

Anxiety-related behaviors in 12 month old *Ube3a* mice

Fig. 1 displays genotype differences in elevated plus-maze scores between *Ube3a* versus WT mice at age 12 months. A) Time spent in the open arms was not significantly different ($t_{1,19} = 1.702$, $p = 0.105$, NS). B) Number of entries into the open arms was significantly less in the *Ube3a* mice as compared to WT ($t_{1,19} = 3.45$, $p < 0.01$). C) Total entries into all four open and closed arms was significantly less in *Ube3a* as compared to WT ($t_{1,19} = 3.81$, $p < 0.01$). Fewer total entries indicate significantly less exploratory activity in the *Ube3a* group. Less overall exploratory locomotion in these older mice represents a confound for this assay. The likely interpretation of these data is that the apparent anxiety-related genotype difference was actually the consequence of less general exploration.

Fig. 2 displays a genotype difference in light ↔ dark transitions between 12 month old *Ube3a* and WT. A) Time spent in the light chamber did not differ between genotypes ($t_{1,19} = 0.460$, $p = 0.651$, NS). B) *Ube3a* made significantly fewer transitions between chambers ($t_{1,19} = 2.37$, $p < 0.05$). Fewer movements between compartments could indicate an anxiety-like component, but is consistent with lower general exploratory locomotion in the older mice, as detected in fewer total arm entries in the elevated plus-maze assay, as shown in Fig. 1C, and open field assay, shown in Fig. 3.

General exploratory locomotion in 12 month old *Ube3a* mice

Fig. 3 displays open field exploratory locomotion in 12 month old *Ube3a* and WT mice. A) Horizontal activity was significantly lower in *Ube3a* as compared to WT throughout the test session (Two-Way ANOVA: Time: $F_{5,95} = 19.9$, $p < 0.001$; Genotype: $F_{1,19} = 44.2$, $p < 0.001$; Interaction: $F_{5,95} = 1.58$, $p < 0.05$; Sidak's multiple comparison test $p < 0.001$ at all time points).

B) Vertical activity was significantly lower in *Ube3a* than WT at most time points (Two-Way ANOVA: Time: $F_{5,95} = 1.66$, NS; Genotype: $F_{1,19} = 14.7$, $p < 0.001$; Interaction: $F_{5,95} = 2.54$, $p < 0.054$; Sidak's $p < 0.05$ at all time points except 11–15 min). C) Center time was lower in *Ube3a* as compared to WT (Two-Way ANOVA: Time: $F_{5,95} = 4.48$, $p < 0.001$; Genotype: $F_{1,19} = 9.44$, $p < 0.001$; Interaction: $F_{5,95} = 1.10$, $p = 0.364$; Sidak's $p < 0.05$ at 6–10 and 11–15 min). D) Total distance traveled was lower in *Ube3a* than WT during the first five minute time bin (Two-Way ANOVA: Time: $F_{5,95} = 44.0$, $p < 0.001$; Genotype: $F_{1,19} = 8.23$, $p < 0.01$; Interaction: $F_{5,95} = 2.68$, $p < 0.05$; Sidak's $p < 0.001$ at 1–5 min). E) Total distance summed across the 30 min test session was lower in *Ube3a* than WT ($t_{1,19} = 2.87$, $p < 0.01$).

Social behavior assays in 12 month old *Ube3a* mice

Fig. 4 presents evidence for a partial absence of sociability in 12 month old *Ube3a* and WT mice on the 3-chambered social approach assay, which may have been caused by less overall exploration of the three chambers. A) WT displayed normal sociability on the chamber time parameter, spending significantly more time in the side chamber with the novel mouse than in the side chamber with the novel object ($t_{1,14} = 2.61$, $p < 0.05$). *Ube3a* displayed significant sociability on the chamber time parameter, spending significantly more time in the side chamber with the novel mouse than in the side chamber with the novel object ($t_{1,24} = 3.31$, $p < 0.01$). B) WT displayed normal sociability in the 3-chambered social approach task on the sniff time parameter, spending significantly more time sniffing the novel mouse than sniffing the novel object ($t_{1,14} = 2.59$, $p < 0.05$). *Ube3a* failed to display sociability on the sniff time parameter ($t_{1,24} = 0.501$, NS). C) Number of entries into the side chambers, the internal control measure for general exploration, showed fewer total entries by *Ube3a* than WT ($F_{1,38} = 5.269$, $p < 0.05$), indicating lower exploratory locomotion. D) During the

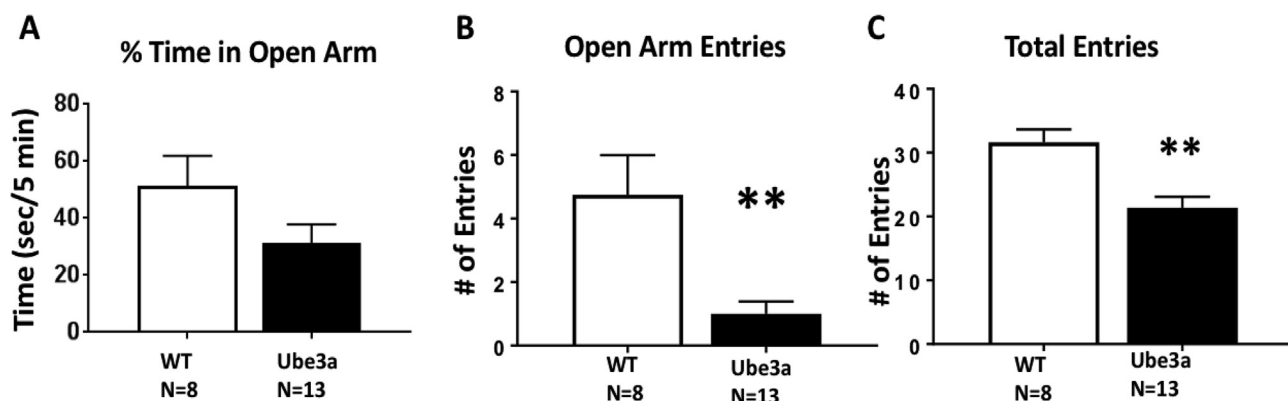


Fig. 1. Elevated plus-maze behavior in 12 month old *Ube3a* mice and WT littermate controls. (A) Time spent in the open arms was not significantly different. (B) Number of entries into the open arms was significantly less in the *Ube3a* mice as compared to the WT mice (** $p < 0.01$). (C) Total entries into all four open and closed arms was significantly less in *Ube3a* than WT (* $p < 0.01$), indicating less overall exploratory locomotion at the older age, rather than a specific anxiety-like phenotype. For all figures, data are expressed as mean ± standard error of the mean, numbers of mice per group are displayed under the x-axis, and statistical results are more fully described in the Results text.

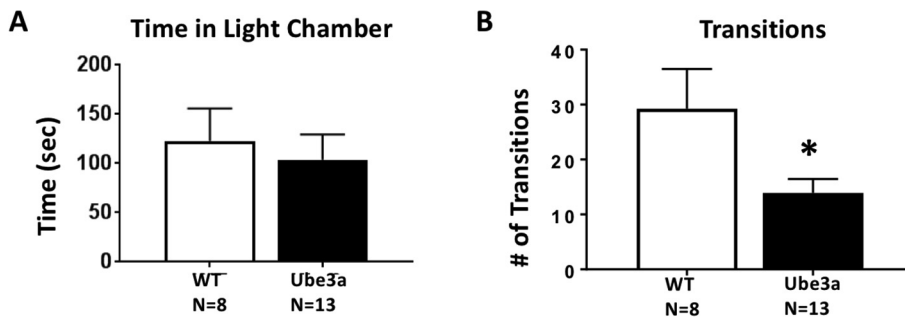


Fig. 2. Light ↔ dark transitions in 12 month old *Ube3a* and WT mice. **(A)** Time spent in the light chamber did not differ between genotypes. **(B)** *Ube3a* made significantly fewer transitions between chambers as compared to WT ($*p < 0.05$). Lower number of transitions is likely to be the result of reduced overall locomotion, as seen in the elevated plus-maze and open field assays, rather than a specific anxiety-like phenotype.

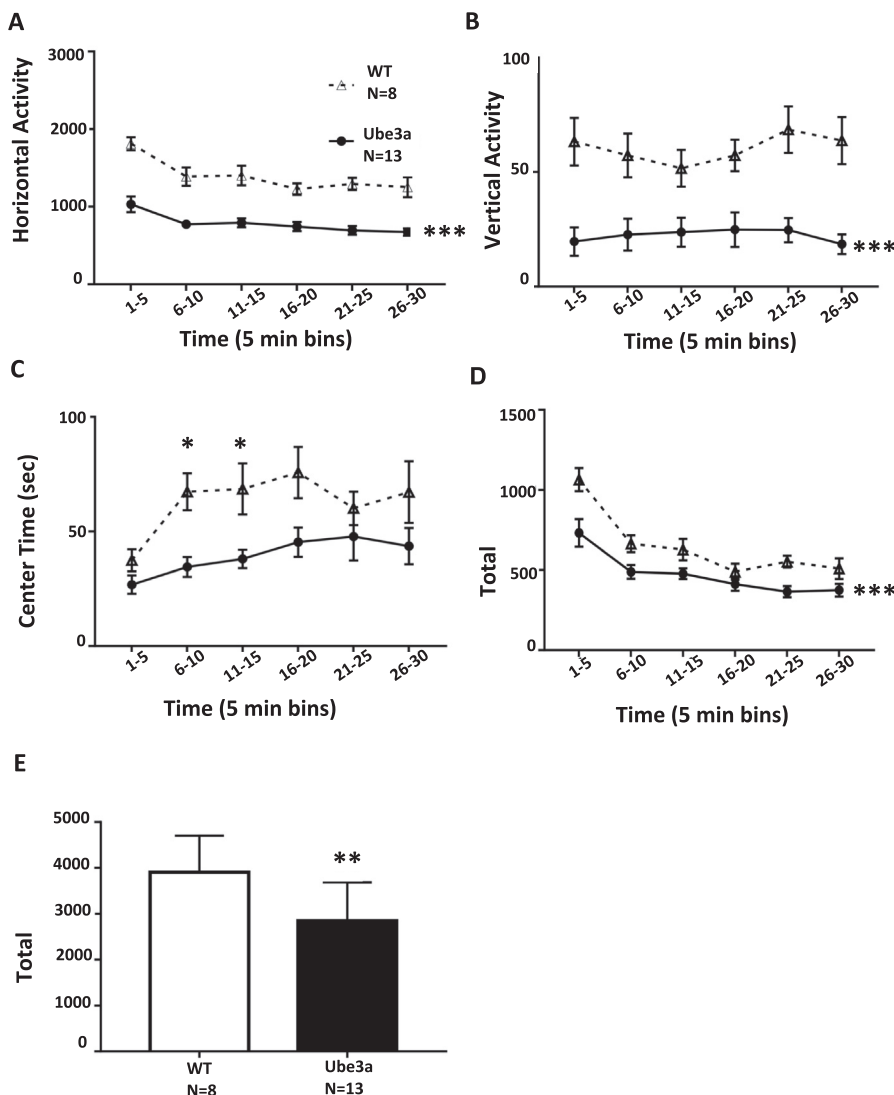


Fig. 3. Open field exploratory locomotion in 12 month old *Ube3a* and WT mice. **(A)** Horizontal activity was lower in *Ube3a* than WT ($***p < 0.001$). **(B)** Vertical activity was lower in *Ube3a* mice at most time points ($*p = 0.05$). **(C)** Center time was lower in *Ube3a* ($*p < 0.05$). **(D)** Total distance traveled was lower in *Ube3a* ($***p < 0.001$). **(E)** Summed total distance was lower in *Ube3a* ($**p < 0.001$), supporting the interpretation of reduced general exploratory locomotion in the older 12 month old mice.

familiarization phase before the start of the sociability session, time spent in the two side chambers was similar, confirming lack of innate side bias, for both genotypes ($F_{1,38} = 0.256$, NS). **E)** During the familiarization session, the total number of entries into the two side chambers was lower in *Ube3a* than WT, again indicating lower general exploratory locomotion at this age ($F_{1,38} = 9.202$, $p < 0.01$), which creates a potential confound for interpreting the results of this assay.

Fig. 5 displays lower scores on all three parameters of male–female social interactions in 12 month old male *Ube3a* mice as compared to male WT. **A)** Number of bouts of nose-to-nose sniffing was fewer in *Ube3a* than WT ($t_{1,19} = 3.73$, $p < 0.01$). **B)** Time spent in nose-to-nose sniffing was less in *Ube3a* than WT ($t_{1,19} = 4.72$, $p < 0.001$). **C)** Number of bouts of nose-to-anogenital sniffing were fewer in *Ube3a* than WT ($t_{1,19} = 2.90$, $p < 0.01$). **D)** Time spent in nose-to-anogenital sniffing was less in *Ube3a* than WT ($t_{1,19} = 3.72$, $p < 0.01$). **E)** Number of bouts of following were fewer in *Ube3a* than WT ($t_{1,19} = 3.08$, $p < 0.01$), which may be the result of less general exploratory locomotion. **F)** Time spent following showed a trend for less following in *Ube3a* than WT ($t_{1,19} = 1.94$, $p = 0.0674$). **G)** Total number of ultrasonic vocalizations was lower in *Ube3a* than WT ($t_{1,19} = 2.78$, $p < 0.05$). Essentially no calls were emitted by *Ube3a* males during the 5 min session with an estrous female.

DISCUSSION

Adults with Angelman syndrome present with a range of symptoms, including impaired locomotion and mobility, continuing severe cognitive impairments, limited speech, anxiety, sleep dysfunction, seizures, obesity, and gastrointestinal disruption (Smith, 2001; Larson et al., 2015; Prasad et al., 2018). The present studies employed the *Ube3a* mutant mouse

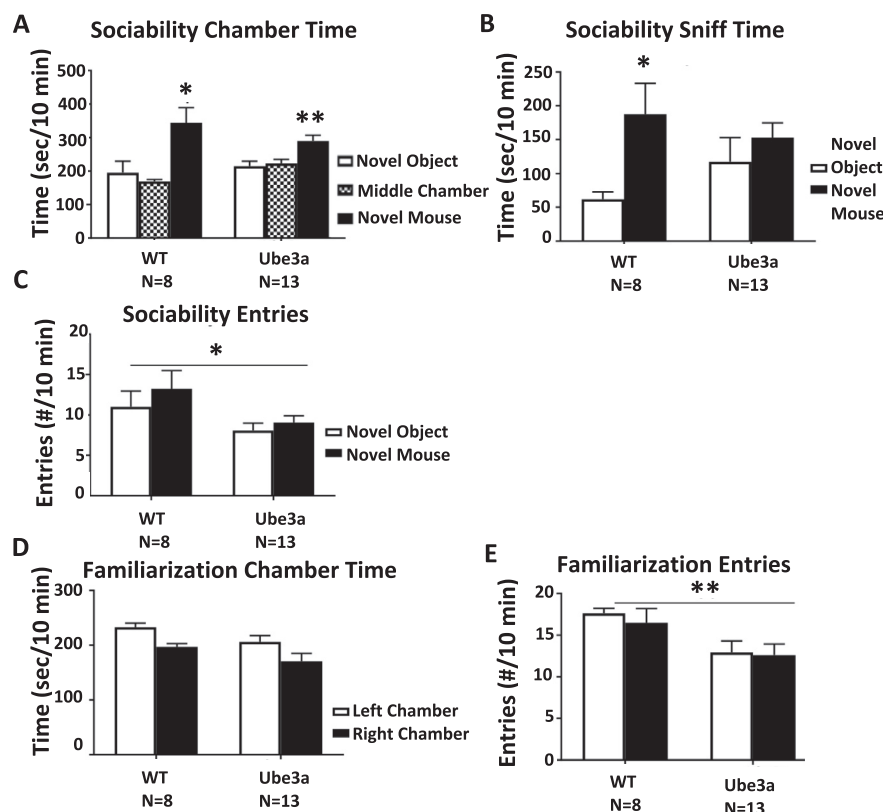


Fig. 4. Sociability in 12 month old *Ube3a* and WT mice. **(A)** WT displayed normal sociability in the 3-chambered social approach task on the chamber time parameter, spending significantly more time in the side chamber with the novel mouse than in the side chamber with the novel object (* $p < 0.05$). *Ube3a* displayed significant sociability on the chamber time parameter, spending significantly more time in the side chamber with the novel mouse than in the side chamber with the novel object (** $p < 0.01$). **(B)** WT displayed normal sociability in the 3-chambered social approach task on the sniff time parameter, spending significantly more time sniffing the novel mouse than sniffing the novel object (* $p < 0.05$). *Ube3a* failed to display sociability on the sniff time parameter. **(C)** Number of entries into the side chambers, the internal control measure for general exploration, was lower for *Ube3a* than WT (* $p < 0.05$). **(D)** During the familiarization phase before the start of the sociability session, time spent in the two side chambers was similar, confirming lack of innate side bias. **(E)** During the familiarization session, number of entries into the two side chambers was lower for *Ube3a* than WT (** $p < 0.01$), again indicating less exploratory locomotion at the 12 month age, which likely explains the apparent social deficit.

model of Angelman syndrome to explore anxiety-related, social, and motor phenotypes of older adult male *Ube3a* mice as compared to their wild-type littermates. Establishing phenotypic abnormalities across the lifespan of *Ube3a* mice could enable preclinical discovery of interventions to ameliorate symptoms in adults with Angelman syndrome.

The present results replicate and extend previous reports of motor behavioral phenotypes in *Ube3a* mice. Lower open field locomotor activity on all parameters was highly significant in *Ube3a* mice at 12 months of age, extending previous findings at younger ages (Allensworth et al., 2011; Huang et al., 2013; Ciarlone et al., 2017; Sonzogni et al., 2018). Interpretations of anxiety-related phenotypes appear to be confounded by the motor deficits in older *Ube3a* mice. At 12 months of age, *Ube3a* mice made significantly fewer entries into the open arms of the elevated plus-maze. However, because total entries into all four arms were also significantly lower, it is likely that the open arm entries were

impaired due to generally lower exploratory locomotion. Similarly, *Ube3a* mice at 12 months of age made significantly fewer transitions between the light and dark compartments of the light ↔ dark transitions apparatus. However, because time spent in the light did not differ between genotypes, it is possible that the fewer transitions between compartments were due to overall lower exploratory locomotion. A definitive interpretation of anxiety-related phenotypes in older *Ube3a* mice therefore cannot be made.

Evidence for impaired social behaviors was seen in 12 month old *Ube3a* mice in two social assays. On 3-chambered social approach, WT displayed normal sociability, i.e. time in the chamber with the novel mouse was significantly higher than time in the chamber with the novel object, and time spent sniffing the novel mouse was significantly higher than time spent sniffing the novel object, meeting the definition of sociability in this binary, yes-or-no assay (Yang et al., 2013; Rhine et al., 2019). The 12 month old *Ube3a* also met the definition for sociability on the chamber time parameter, spending significantly more time in the chamber with the novel mouse than in the chamber with the novel object, but failed to display significant sociability on the sniff time parameter. At this older age, the total number of entries into the side chamber was lower in *Ube3a* than WT, consistent with less exploratory locomotion in the open field,

introducing a motor artifact into the interpretation of a sociability deficit in *Ube3a* mice at 12 months of age. Therefore, the partial absence of sociability in the 12 month *Ube3a* could be due to lower general exploration of the side chambers.

Previous investigations of 3-chambered social approach behavior in *Ube3a* mutant mice at younger ages have reported normal sociability at age 2–3 months (Allensworth et al., 2011), absence of sociability at age 2–3 months (Jamal et al., 2017), partial sociability on one parameter (Huang et al., 2013), and normal sociability during the first 5 min of the 10 min test, with continuing sociability during the second 5 min whereas WT did not display continuing sociability (Stoppel and Anderson, 2017). These several studies were conducted at ages 6–13 weeks. It will be important to evaluate additional cohorts of older *Ube3a* and WT mice, to more fully under-

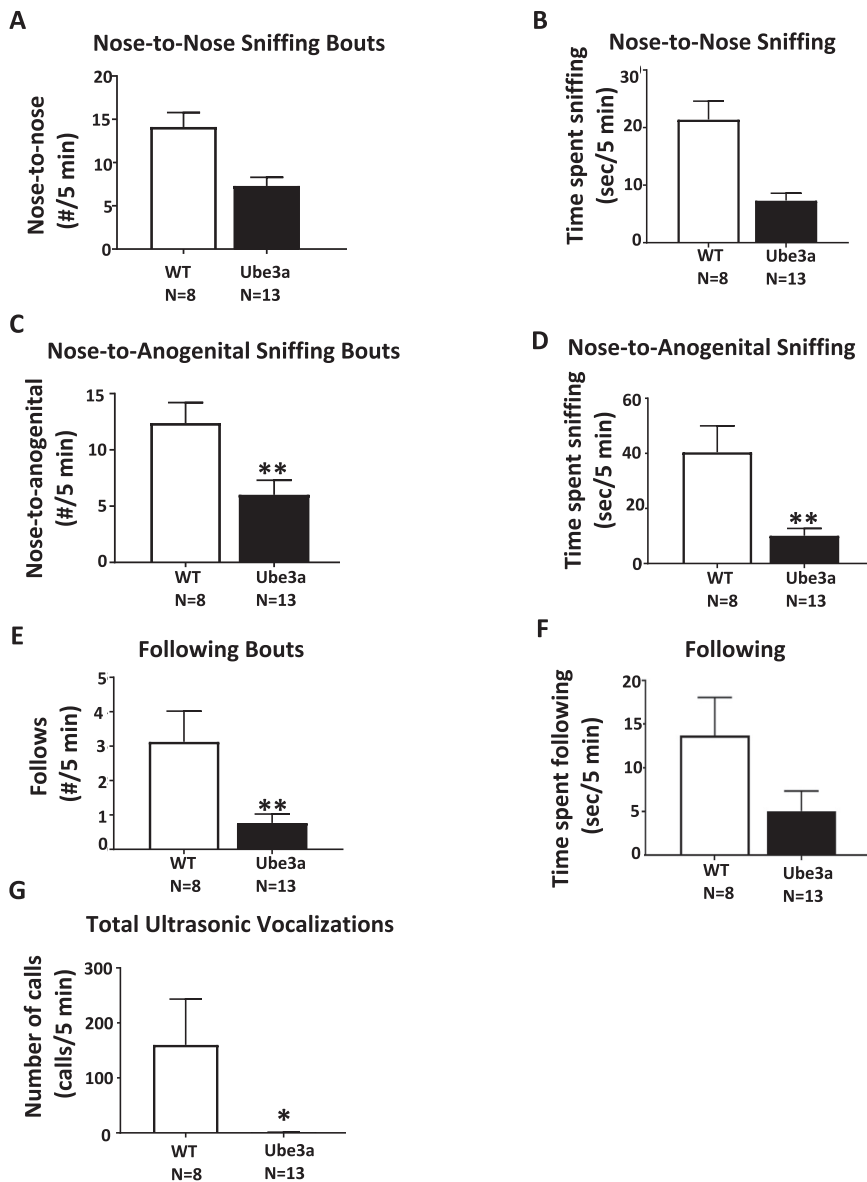


Fig. 5. Male-female social interactions in 12 month old male WT and male *Ube3a* mice. **(A)** Number of bouts of nose-to-nose sniffing were fewer in *Ube3a* than WT (** $p < 0.01$). **(B)** Time spent in nose-to-nose sniffing was less in *Ube3a* than WT (*** $p < 0.001$). **(C)** Number of bouts of nose-to-anogenital sniffing were fewer in *Ube3a* than WT (** $p < 0.01$). **(D)** Time spent in nose-to-anogenital sniffing was less in *Ube3a* than WT (** $p < 0.01$). **(E)** Number of bouts of following were fewer in *Ube3a* than WT (** $p < 0.01$). **(F)** Time spent following showed a trend for less following in *Ube3a* than WT ($p = 0.0674$), which may be the result of less general exploratory locomotion. **(G)** Number of ultrasonic vocalizations emitted was lower in *Ube3a* than WT (* $p < 0.05$).

stand the apparent social approach deficits and motor confounds.

Social interactions between a freely-moving subject male and an estrous female were significantly lower in 12 month old *Ube3a* than WT on all of the parameters measured, including time spent in nose-to-nose sniffing, number of bouts of nose-to-nose sniffing, time spent in nose-to-anogenital sniffing, number of bouts of nose-to-anogenital sniffing, time spent in following, number of bouts of following, and number of vocalizations emitted. However, given the lower exploratory locomotion scores

of 12 month old *Ube3a* on open field, on the exploratory locomotion components of elevated plus-maze, and on number of side chamber entries during 3-chambered social approach, a definitive interpretation of a social deficit in this assay cannot be made in the 12 month old group due to the likely confound of reduced general exploration.

Reporting artifacts and negative results is especially important in the preclinical phase of evaluating therapeutics for neurodevelopmental disorders. The present results indicate that motor deficits are prominent in 12 month old male *Ube3a* mice. The extent to which significantly lower general exploratory activity affected social behaviors is unclear. Particularly in the male-female social interaction test, the arena is an empty mouse cage, which is small enough to require minimal locomotion. While open field activity parameters were consistently lower in *Ube3a* mice, scores were within ranges generally displayed by many strains of mice. Further experiments will be needed, using additional motor assays and at intermediate ages, to determine the magnitude of motor deficits that artifactually affect performance on the social and anxiety-related assays in *Ube3a* mice.

Effective therapeutics for adults with Angelman syndrome could significantly improve quality of life. The remarkably diverse range of pharmacological interventions which have been evaluated preclinically in *Ube3a* mice (van Woerden et al., 2007; Huang et al., 2011; Baudry et al., 2012; Kaphzan et al., 2012; Meng et al., 2012, 2015; Powell et al., 2013; Godavarthi et al., 2014; Llewellyn et al., 2015; Mandel-Brehm et al., 2015; Silva-Santos et al., 2015; Sun et al., 2015, 2016; Bailus et al., 2016; Ciarlone et al., 2016, 2017; Mabb et al., 2016; Jamal et al., 2017; Guzzetti et al., 2018; Lee et al., 2018) employed juvenile and young adult ages of *Ube3a* mice, as appropriate for the discovery of cures early in life. Our findings suggest that robust motor phenotypes in *Ube3a* mice at older adult ages, including open field activity and entries into the side chambers during social

approach, and possibly some social and anxiety-related phenotypes, may offer additional preclinical opportunities to discover pharmacological interventions that significantly improve aspects of the severe symptomatology present in adults with Angelman syndrome.

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AUTHOR CONTRIBUTIONS

RD contributed to the experimental design, conducted the experiments, analyzed the data, prepared the figures, and contributed to sections of the manuscript text. JNC conceived the project, contributed to the experimental design, established protocols, supervised the conduct of experiments, and wrote the manuscript.

DECLARATION OF COMPETING INTEREST

All authors report no financial interests or conflicts of interest. No industry involvement or funding supported these studies.

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