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Research report

Behavioral deficits in an Angelman syndrome model: Effects of genetic background and age

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HIGHLIGHTS

- *Ube3a*$^{m-/p+}$ mice had impaired reversal learning in the Morris water maze.
- Deficient acquisition of spatial learning varied across background strain and age.
- Aberrant phenotypes included deficits in rearing, rotarod ability, and marble-burying.
- The C57BL/6J background conferred susceptibility to a range of abnormal behaviors.

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ABSTRACT

Angelman syndrome (AS) is a severe neurodevelopmental disorder associated with disruption of maternally inherited *UBE3A* (ubiquitin protein ligase E3A) expression. At the present time, there is no effective treatment for AS. Mouse lines with loss of maternal *Ube3a* (*Ube3a*$^{m-/p+}$) recapitulate multiple aspects of the clinical AS profile, including impaired motor coordination, learning deficits, and seizures. Thus, these genetic mouse models could serve as behavioral screens for preclinical efficacy testing, a critical component of drug discovery for AS intervention. However, the severity and consistency of abnormal phenotypes reported in *Ube3a*$^{m-/p+}$ mice can vary, dependent upon age and background strain, which is problematic for the detection of beneficial drug effects. As part of an ongoing AS drug discovery initiative, we characterized *Ube3a*$^{m-/p+}$ mice on either a 129S7/SvEvBrd-*Hprt*$^{b-m2}$ (129) or C57BL/6J (B6) background across a range of functional domains and ages to identify reproducible and sufficiently large phenotypes suitable for screening therapeutic compounds. The results from the study showed that *Ube3a*$^{m-/p+}$ mice have significant deficits in acquisition and reversal learning in the Morris water maze. The findings also demonstrated that *Ube3a*$^{m-/p+}$ mice exhibit motor impairment in a rotarod task, hypoactivity, reduced rearing and marble-burying, and deficient fear conditioning. Overall, these profiles of abnormal phenotypes can provide behavioral targets for evaluating effects of novel therapeutic strategies relevant to AS.

1. Introduction

Angelman syndrome (AS) is a severe genomic imprinting disorder with phenotypes that typically manifest early in childhood. AS is characterized by intellectual disability, speech impairment, motor dysfunction, sleep disturbances, epilepsy, inappropriate laughter, and an unusually happy demeanor [1,2]. In most cases, AS arises from the deletion or mutation of maternal *UBE3A* [3,4], which encodes ubiquitin protein ligase E3A. In neurons, only the maternal copy of *UBE3A* is active, while the paternally inherited *UBE3A* allele is silenced [5,6]. Evidence from mouse lines with targeted disruption of the maternal *Ube3a* allele supports an important role for UBE3A in neuronal morphology, synaptic function, and the maturation of neocortical circuits in the brain [7–10]. Maternal *Ube3a*-deficient mice (*Ube3a*$^{m-/p+}$ mice) have abnormal phenotypes that resemble many of the clinical symptoms observed in AS, including motor dysfunction, cognitive deficits, and enhanced

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susceptibility to seizures [11]. *Ube3a*$^{m-/p+}$ mice also have overt deficiencies in hippocampal long-term potentiation and dendritic spine density, in line with deficits in behavioral tasks, such as contextual fear conditioning and spatial learning in the Morris water maze, which are mediated by the hippocampus [9,11–14].

Although no effective treatment currently exists for AS, recent findings utilizing genetic mouse models for the disorder have suggested that abnormal phenotypes can be rescued [12,14,15]. For example, van Woerden et al. [14] were able to genetically rescue the deficits in rotarod performance, quadrant selectivity in the water maze, and contextual fear conditioning that characterize *Ube3a*$^{m-/p+}$ mice. Our research group recently demonstrated that topoisomerase inhibitors can unsilence paternally inherited *Ube3a* [16], but the possible beneficial effects of these drugs on abnormal behaviors relevant to AS have not yet been established. While the *Ube3a*$^{m-/p+}$ mice provide a well-validated model for preclinical efficacy testing, there is a critical need to identify the optimal phenotypes to target for reversal in drug discovery studies. Importantly, there are known strain-specific differences in behavioral phenotypes [11]. Moreover, even in the most carefully controlled studies, it can be difficult to find behavioral phenotypes sufficiently penetrant for inter-species, inter-laboratory, and intra-laboratory reproducibility [17]. We therefore sought to identify AS phenotypes of sufficient magnitude and consistency to be suitable for screening potential therapeutics. Toward this goal, the present studies evaluated *Ube3a*$^{m-/p+}$ mice on two different genetic backgrounds, either 129S7/SvEvBrd-*Hprt*$^{b-m2}$ (129) or C57BL/6J (B6), using multi-component phenotyping regimens and testing at different ages. Because clinical studies have linked genotype to differential developmental trajectories in AS [18], separate cohorts of B6 mice were evaluated, beginning from either adolescence or adulthood, to examine both phenotypic trajectories and reproducibility.

2. Materials and methods

2.1. Animals

Subjects were heterozygous mice with maternal deficiency of *Ube3a* (*Ube3a*$^{m-/p+}$) and wild type (*Ube3a*$^{m+/p+}$) littermates, on two different background strains: 129S7/SvEvBrd-*Hprt*$^{b-m2}$ (129) and C57BL/6J (B6) [11]. The *Ube3a*$^{m+/p-}$ mice on a 129 strain background were developed by the Beaudet laboratory [11] and were obtained from Jackson Laboratory (Bar Harbor, ME). The *Ube3a*$^{m-/p+}$ mice on a B6 background were originally developed by the Beaudet laboratory [11] and were backcrossed at least 10 generations onto the B6 strain by Dr. Yong-hui Jiang. All mice in the B6 groups for the present study were offspring from breeding pairs obtained from Dr. Yong-hui Jiang. One group of 129-background mice and four separate cohorts of B6-background mice were tested for behavior (described below). Mice were group-housed in ventilated cages, with free access to water and Prolab RMH 3000 chow. The housing room had a 12-h light/dark cycle (lights off at 7:00 p.m.). Genotyping was conducted by PCR from tail tissue samples. All procedures were conducted in strict compliance with the policies on animal welfare of the National Institutes of Health and the University of North Carolina (stated in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, 1996 edition).

2.2. Test groups

129-background mice. Subjects were 10 *Ube3a*$^{m+/p+}$ mice (5 males and 5 females) and 11 *Ube3a*$^{m-/p+}$ mice (4 males and 7 females), derived from 7 litters. Testing began when mice were between 7 and 8 weeks in age.

B6-background mice (cohort 1). Subjects were 14 *Ube3a*$^{m+/p+}$ mice (8 male and 6 female) and 11 *Ube3a*$^{m-/p+}$ mice (4 male and 7 female), derived from 5 litters. Testing began during the adolescent period, when mice were 4–5 weeks in age.

B6-background mice (cohort 2). Subjects were 19 *Ube3a*$^{m+/p+}$ and 16 *Ube3a*$^{m-/p+}$ mice, all male, derived from 9 litters. Testing began in adulthood, when mice were 12–13 weeks in age.

B6-background mice (cohort 3). One set of male subjects (10 *Ube3a*$^{m+/p+}$ and 7 *Ube3a*$^{m-/p+}$ mice, derived from 6 litters) was used to confirm results from the marble-burying assay. Mice were given two tests, one at age 18–20 weeks, and a second test at age 19–21 weeks.

B6-background mice (cohort 4). Subjects in the conditioned fear group were 13 *Ube3a*$^{m+/p+}$ and 12 *Ube3a*$^{m-/p+}$ mice, all male, derived from 6 litters. Testing began when mice were between 11 and 13 weeks of age.

129- and B6-background mice for body weight evaluation. Data on body weight were compiled for offspring from the same breeding colony that provided mice for the behavioral studies, with one weight measure per mouse. Subjects on the 129-background were 157 *Ube3a*$^{m+/p+}$ mice (82 males and 75 females) and 101 *Ube3a*$^{m-/p+}$ mice (53 males and 48 females). Subjects on the B6-background were 337 *Ube3a*$^{m+/p+}$ mice (159 males and 178 females) and 244 *Ube3a*$^{m-/p+}$ mice (139 males and 105 females).

To blind experimenters to genotype, all mice for behavioral testing were given new identification codes, and all genotype information was removed from cage cards. Mice in the first three groups, the 129 mice and first two cohorts of B6 mice, were evaluated for activity (1-h duration), grip strength in a wire-hang test, motor coordination on an accelerating rotarod, sensorimotor gating in an acoustic startle test, and acquisition/reversal learning in the Morris water maze. Only a subset of the second cohort of B6 mice was tested in the water maze (10 *Ube3a*$^{m+/p+}$ and 11 *Ube3a*$^{m-/p+}$). Following the initial battery of tests, the first two B6-background cohorts were given further activity, grip strength, rotarod, and acoustic startle tests, in order to evaluate changes in behavior across time (see Table 1 for age at each test).

The 129 and first cohort of B6 mice were also assessed for sociability in a 3-chamber choice task. The second cohort of B6 mice was assessed for digging behavior in a marble-burying assay as an index of repetitive responses.

2.3. Testing procedures

2.3.1. Activity

Exploratory activity in a novel environment was assessed by 1-h trials in a photocell-equipped automated chamber (41 cm × 41 cm × 30 cm; Versamax system, Accuscan Instruments). Measures were taken of total distance traveled, number of rearing movements, and time spent in the center of the field. Activity chambers were contained inside sound-attenuating boxes equipped with ceiling-mounted lights and fans.

2.3.2. Wire hang test for grip strength

Each mouse was placed on a large metal cage lid. The lid was gently shaken to induce the mouse to grip the metal grid. The cage top was then inverted, and latency for the mouse to fall from the lid was recorded. The maximum trial length was 60 s.

2.3.3. Rotarod performance

Mice were assessed for balance and motor coordination on an accelerating rotarod (Ugo-Basile, Stoelting Co., Wood Dale, Il). Revolutions per minute (rpm) were set at an initial value of 3, with a progressive increase to a maximum of 30 rpm across 5 min, the maximum trial length. Test sessions consisted of 2 or 3 trials, with 45 s between each trial. Latency to fall, or to rotate off the top of the turning barrel, was measured by the rotarod timer.

2.3.4. Acoustic startle procedure

The acoustic startle measure was based on the reflexive whole-body flinch, or startle response, following exposure to a sudden noise. Animals were tested with a San Diego Instruments SR-Lab system, using published methods [19]. Briefly, mice were placed in a small Plexiglas cylinder within a larger, sound-attenuating chamber (San Diego Instruments). The cylinder was seated upon a piezoelectric transducer, which allowed vibrations to be quantified and displayed on a computer. The chamber included a ceiling light, fan, and a loudspeaker for the acoustic stimuli (bursts of white noise). Background sound levels (70 dB) and calibration of the acoustic stimuli were confirmed with a digital sound level meter (San Diego Instruments). Each test session consisted of 42 trials, presented following a 5-min habituation period. There were 7 different types of trials: the no-stimulus trials, trials with the acoustic startle stimulus (40 ms; 120 dB) alone, and trials in which a prepulse stimulus (20 ms; either 74, 78, 82, 86, or 90 dB) had onset 100 ms before the onset of the startle stimulus. The different trial types were presented in blocks of 7, in randomized order within each block, with an average intertrial interval of 15 s (range: 10–20 s). Measures were taken of the startle amplitude for each trial, defined as the peak response during a 65-ms sampling window that began with the onset of the startle stimulus. Levels of PPI (prepulse inhibition) at each prepulse sound level were calculated as 100 − [(response amplitude for prepulse stimulus and startle stimulus together/response amplitude for startle stimulus alone) × 100].

2.3.5. Sociability and preference for social novelty

Mice were tested in an automated 3-chambered box, using published methods [20,21]. Dividing walls had retractable doorways allowing access into each chamber. The automated box had photocells embedded in each doorway to allow quantification of entries and duration in each chamber of the social test box. The chambers of the apparatus were cleaned with water and dried with paper towels between each trial. At the end of each test day, the apparatus was sprayed with 70% ethanol and wiped clean with paper towels.

The choice test had two 10-min phases: (1) *Habituation*. The test mouse was first placed in the middle chamber and allowed to explore, with the doorways into the two side chambers open. (2) *Sociability*. After the habituation period, the test mouse was enclosed in the center compartment of the social test box, and an unfamiliar mouse (the stranger; a sex-matched C57BL/6J adult) was enclosed in a wire cage