# Longitudinal Profiling of Vocalizations and the Effect of an ASO on the Ube3a Phenotype

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## ABSTRACT

Angelman Syndrome (AS) is a rare genetic neurodevelopmental disorder characterized, in part, by developmental delays, movement impairments, and difficulties with communication and language, the effects of which impact children and adults living with AS. The B6.129S7-Ube3atm1Alb/J (B6-E6-AP) mouse line displays characteristics of Angelman syndrome by knocking out the Ube3a allele in neurons. Here we characterized the phenotypic profile of this model by assessing behavioral performance in both neonatal and adult Ube3a gender mixed mice (wild-type litter mates served as controls). Animals were tested in a battery of behavioral tests starting at birth, including geotaxis (data not show) and ultrasonic vocalizations (USV) wherein Ube3a AS animals exhibited abnormalities. In adulthood, animals were again evaluated in a behavioral battery including open field, NeuroCube® gait and grip strength (data not shown) and ultrasonic vocalizations. Decreases in the diversity of the USV repertoire were observed in the Ube3a AS animals in adulthood. Evaluation of protein markers in the Ube3a AS mouse is in progress and results are pending. Finally, the extent to which the aforementioned behavioral phenotype in the Ube3a AS mouse can be rescued by postnatal (P2-3) intervention with a single injection of an antisense oligonucleotide (ASO) was examined with results indicating a partial rescue of the Ube3a phenotype in adulthood on measurements of USV, gait, and exploratory behaviors. The ultimate goal of this work is to identify robust early onset and non-invasive readouts that can be used to determine the efficacy of disease modifying therapies, such as ASOs, for Angelman Syndrome.

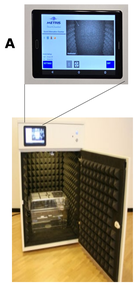
## METHODS

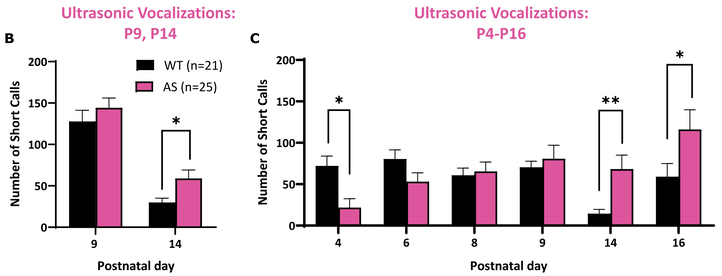
**Animals**: Time pregnant (E12-E14) mice were received at PsychoGenics (PGI) from the Jackson Laboratory. For this timed pregnancy project, JAX crossed HET B6.129S7-Ube3atm1Alb/J (E6-AP-, Stock #016590) female mice with C57Bl/6J (Stock #000664) male mice to generate 14-21 timed pregnant (E12-E15) female mice. Mice were assigned unique identification numbers (ear notched) at reception and housed in polycarbonate OptiMICE cages. At birth (postnatal day P0), pups were tattooed using non-toxic ink applied under the skin and a tail snip sample was taken for genotyping. Genotyping was performed by PGI's in vitro laboratory after assessing PCR methodology.

**Ultrasonic Vocalization Profiling Studies:** The **isolation-induced USV** assessments occurred at P4 through P16. USV assessments occurred using the Metris Sonotrack and Smartchamber system (Hoofddorp, Netherlands). The Metris Call Classification Software categorized the type of each call vocalized. On the day of the assessment, the cage with the dam and litter was taken to the testing room (room temperature 24°C). The dams were removed from the cage at least 30 minutes prior test and returned to the colony room. Pups remained in the home cage with familiar bedding and nest material. During the test, one pup at a time was placed in a standard cage within the USV chamber. The pup remained in the chamber a 5-minute trial. Vocalizations were measured via built-in ultrasound microphone and recorded using Metris software. After testing was completed, dams and pups were reunited in the home cage. **Sexual priming-induced USV** assessments occurred at 8 and 13 weeks of age using the same Metris system and software described above. In brief, freshly collected adult female urine was used to elicit vocalizations in adult males during a 5-minute trial.

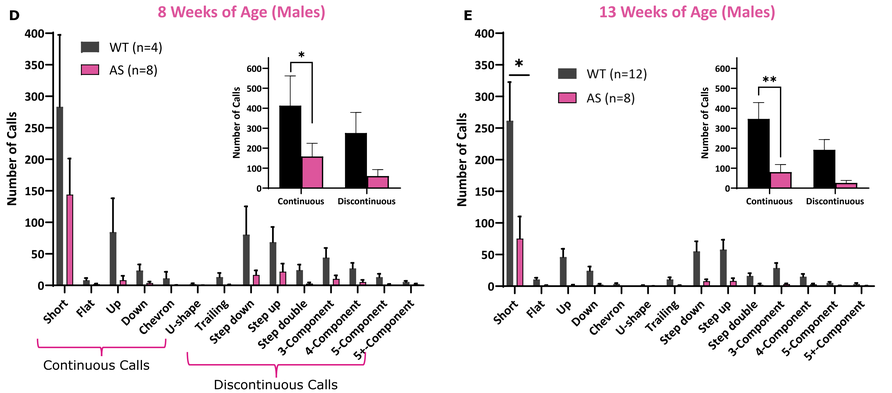
**Acute Antisense Oligomer Tolerability and Efficacy Studies**: The following **ASO sequence** was used: 5'-GATCCATTTGTGTTAAGCTG-3' as in Meng et al., 2015. The ASO was synthesized by Integrated DNA Technologies (IDT); **Neonatal ICV injection** occurred at P2-P3 or at P6. Animals were anesthetized via cryoanesthesia and dosed via bilateral ICV injection (dose volume of 3 µl per side)) Control ASO or ASO (10-100 ug) were administered using 10 µl Hamilton syringes and custom Hamilton 30 ½ g needles. Animals were placed on a warm (~36°C) heating pad immediately following the ICV injection. Once all animals from the litter are dosed and warmed, they will be returned to the dam. The **open field** assessment occurred at 4 and 8 weeks of age. The open field chambers are plexiglass square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Incs., St Albans, VT) surrounded by infrared photobeam sources (16 x 16 x 16). Animals were placed in the OF chambers for 30 minutes. Horizontal activity (distance traveled) and vertical activity (rearing) were measured from consecutive beam breaks. **Neurocube®** assessment occurred at 6 weeks of age. NeuroCube® is an automated behavioral platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain, and neuropathies and extracts gait and non-gait features (Dave et al. *Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. Neurobiology of Disease*, 2014). Mice were placed into the NeuroCube® and given 5 minutes to move freely inside the apparatus for automated gait measures recording. Digital videos were analyzed through computer assisted segmentation algorithms. Fitted parameters were then used to extract clips of motor behavior that were used to extract information about gait geometry and dynamics. Bioinformatics-driven procedures then guide the discrimination probability between treatment groups to determine behavior phenotypes. **Sexual priming-induced USV** assessment occurred at 8 weeks of age using the same methodology described above.

## Trajectory of USV Calls in Development and Diversity of Calls in Adulthood are Altered in UBE3A Mice






### USV Classifications




*(A) Image of Metris Smartchamber/Sonotrack USV system. (B) Number of short USV calls in WT and AS mice at P9 and P14. (C) Number of short USV calls in WT and AS mice at P4 through P16. (D, E) Classification of USV call types in WT and AS male mice at 8 weeks of age (D) and 13 weeks of age (E). (D and E Insets) Number of continuous versus discontinuous call types. Data expressed as mean ± sem. **p<0.01; *p<0.05*

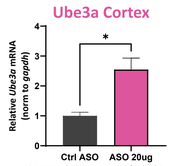
## Tolerability of Acute ASO in Neonatal WT and UBE3A Mice

**Percent Survival in WT Following Single ASO Injection at P6**

| Genotype | ASO Dose (ug) | Survival (%) |
|---|---|---|
| WT | 100 | 0 |
| WT | 50 | 25 |
| WT | 25 | 75 |

**Percent Survival in WT and AS Following Single ASO Injection at P2-P3**

| Genotype | ASO Dose (ug) | Survival (%) |
|---|---|---|
| WT | 20 | 100 |
| AS | 20 | 17 |
| WT | 10 | 100 |
| AS | 10 | 100 |



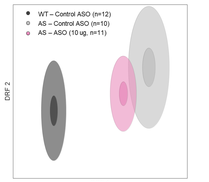

*Restored expression of Ube3a in the cortex of adult AS mice following a neonatal injection of ASO (20 ug). *p<0.05*

## Neonatal Acute ASO Treatment Partially Rescues UBE3A Behavioral Phenotype in Adulthood

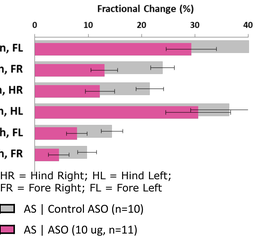
**Design of ASO Efficacy Study**

| Genotype | Group | Dose (ug) | Age at Dose | N | Behavior Assessments (Age) |
|---|---|---|---|---|---|
| WT | Control ASO | 0 | P2-P3 | 12 | • USV (P9, P14)<br>• OF (4 weeks, 8 weeks)<br>• NeuroCube (6 weeks)<br>• Adult USV (8 weeks) |
| AS | Control ASO | 0 | P2-P3 | 10 | |
| AS | ASO | 10 | P2-P3 | 11 | |

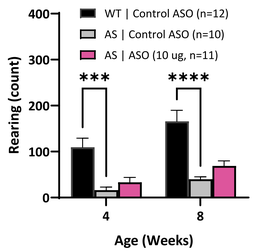
### NeuroCube®: 6 Weeks of Age



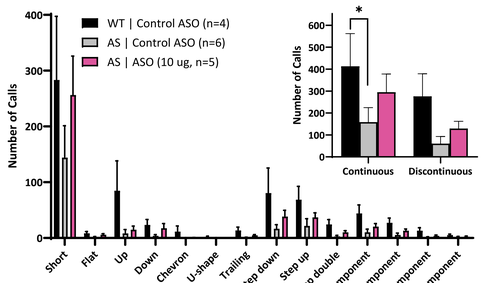

Discrimination (p<0.0001)
WT-Control ASO vs. AS-Control ASO: 99.7%
Rescue (p=0.0071)
AS-ASO (10 ug) vs. AS-Control ASO: 27.3%




### Open Field Rearing Frequency: 4 and 8 Weeks of Age




### USV: 8 Weeks of Age (Males)




*(A) Image of the mouse NeuroCube®, a fully automated platform that employs computer vision to detect changes in gait geometry and dynamics. (B) Cloud analysis of all gait features in WT-Control ASO, AS-Control ASO, and AS-ASO (10 ug) groups at 6 weeks of age. (C) The top gait features from the NeuroCube® analysis contributing to the overall difference between WT and AS at 6 weeks of age. (D) Rearing frequency in an open field in WT-Control ASO, AS-Control ASO, and AS-ASO (10 ug) at 4 and 8 weeks of age. (E) Classification of USV call types in male WT-Control ASO, AS-Control ASO, and AS-ASO (10 ug) at 8 weeks of age; (E Inset) Number of continuous versus discontinuous call types. Data expressed as mean ± sem. ****p<0.0001; ***p<0.001; *p<0.05*

## SUMMARY

- During the neonatal period, AS mice displayed a reduced number of short call vocalizations at P4 and an elevated number of short calls at P14 and P16 compared to their WT littermates.
- In adulthood (8 and 13 weeks of age), the diversity of the vocalization repertoire in AS animals was decreased compared to WT littermates.
- A single neonatal ICV injection of an ASO (10ug) at P2-P3 partially rescued the gait, rearing, and ultrasonic vocalization phenotype of AS mice. This partial phenotypic rescue was observed as early as 6 week of age.
- Overall, this phenotypic characterization of the Ube3a mouse model of Angelman Syndrome may offer robust, early onset, and non-invasive readouts which may ultimately have utility in determining the efficacy of disease modifying therapies, such as ASOs, for Angelman Syndrome.