Assessing the Mecp2 (Bird) Model of Rett Syndrome Across Species, Sex, and Age

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Introduction

Background:

- X-linked methyl CpG binding protein 2 (MECP2) gene mutations in humans have been shown to result in Rett syndrome, a leading cause of intellectual disabilities in girls and associated with embryonic lethality in males.
- Symptoms include the loss of voluntary movements, including speech and hand movements, gait disturbances, autism, and disorganized breathing patterns.

Mouse Models:

- Male MECP2 homozygous mice, used in preclinical studies, show a distinct phenotype but typically do not survive past 3 months of age and cannot perform many behavioral tests due to their compromised state.
- The more medically-relevant female MECP2 Bird mouse (Mecp2$^{tm1.1Bird}$) lacks one copy of the Mecp2 excised with Cre-loxP technology, has normal survival, and appears quite healthy.

Rat Model:

- A novel model, the female MeCP2 heterozygous knockout rat on a Sprague-Dawley background (SAGE Labs), exhibits complete loss of target protein and also shows normal survival and health.

The purpose of this study was to find optimal models and testing time points to screen promising compounds for the treatment of Rett syndrome.

Methods

Animals: Mice were bred at PsychoGenics, Inc. using ovarian transplanted dams to ensure optimal maternal care and health. Rats were supplied by SAGE Labs.

Behavioral Assessments:

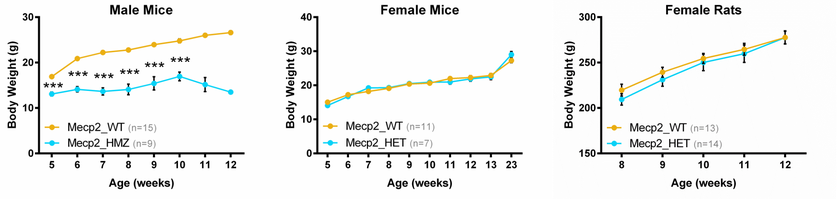
- HEALTH: Survival and body weight were assessed.
- NEUROCUBE: Mice were tested for 5 min in PGI's proprietary platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain & neuropathies.
- GRIP STRENGTH: Used to assess muscular strength in limb muscles, mice grab a mesh grip piece on a push-pull gauge with both front or both hind paws and gently pulled with consistent force until they release their grip. Force is recorded from the strain gauge.
- STARTLE/PPI: Mice are exposed to white noise for 5 min, then a block of 6 presentations of the startle stimulus alone, followed by 10 PPI blocks of 6 different types of trials presented at random within each block. The amount of inhibition of the normal startle is determined and expressed as a percentage of the basic startle response (from startle alone trials), excluding the startle response of the first habituation block.
- APNEA: Mice were acclimated to the procedure and head-out plethysmograph (DSI) before the 60-min testing period.
- ROTAROD: Mice are placed on a rod rotating at constant speed for a 5 min training session. After at least 1 hr rest, mice are placed back on the rod rotating at accelerating speed until the mice falls for three trials. Time that mouse remained on the rod and speed at fall are recorded and averaged across trials.
- CLASPING: Mice are gently lifted by the base of the tail and clasping of the hindlimbs is recorded.
- OPTOKINETIC RESPONSE: Mice are placed inside a rotating drum with vertical stripes that move bi-directionally and at differing frequencies. Number and direction of head movements are recorded.

Biomarker Assessment:

- Expression profiles of brain-derived neurotrophic factor (BDNF) and BDNF isoforms in the cortex were assessed in both mice (hemi brain) and rat (cortex) models.
- Reverse transcription (RT) reactions were carried out for each RNA sample.
- Each RT reaction was assayed in triplicate qPCR.
- Expression of BDNF isoforms were normalized to the geometric mean of two(rat) or three(mouse) housekeeping transcripts.

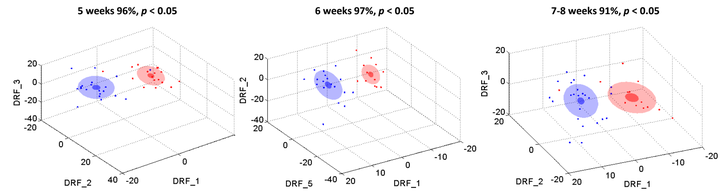
Results

HEALTH: No survival of male Mecp2 mice after 3 months of age. Mecp2 male but not female mice or rats have lower body weight than controls.

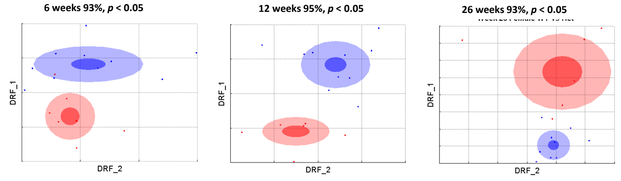


NEUROCUBE: Gait deficits were found across all models at all ages tested. Models were characterized by increased number of steps, shorter stride length, decreased body posture variability, and shortened vertical posture compared to controls.

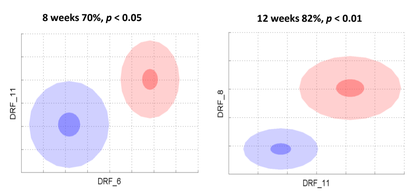
Male Mice:



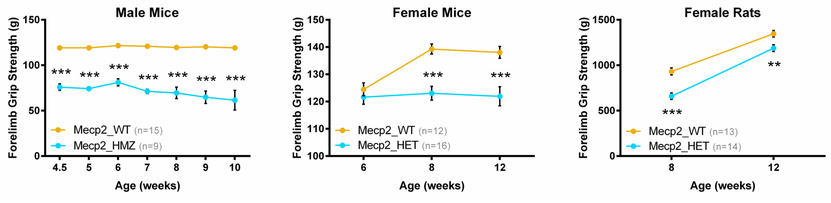
Female Mice:



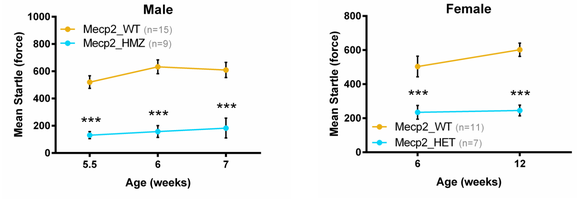
Female Rats:



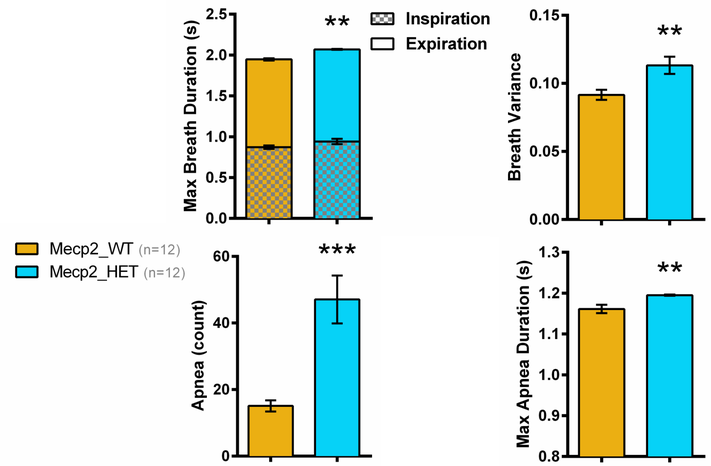
GRIP STRENGTH: All Mecp2 models showed weaker forelimb grip strength compared to controls.



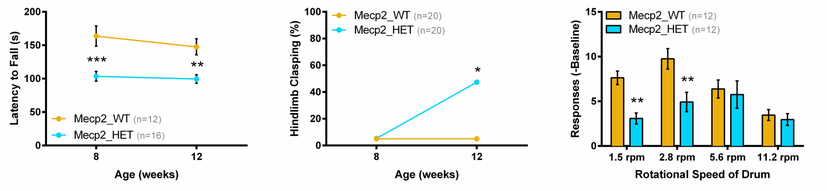
STARTLE/PPI (mice only): Both male and female Mecp 2 mice startled less than WT mice.



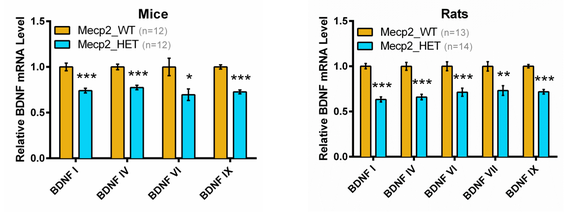
RESPIRATION (female mice only): Mecp2 female mice had longer maximum breath durations as a function of longer expiration times compared to controls at ~15 weeks of age. They also showed more variance in their breathing, more incidences of apnea, and longer maximum apnea durations.



ROTAROD, CLASPING, OPTOKINETIC RESPONSE (female mice only): Mecp2 female mice fall more quickly and at lower speeds on the rotarod, showed more clasping, and fewer optokinetic responses compared to WT mice.



BIOMARKER ASSESSMENT: BDNF was reduced in female Mecp2 mice and rats at 16 weeks of age.



Conclusions

- Our results suggest that both female Mecp2 mice and rats show robust deficits amenable to drug screening of compounds that could be beneficial in the treatment of Rett Syndrome.
- These deficits appear as early as 6 weeks of age (earliest age tested) and appear to reach a plateau consistent with clinical observations.
- Importantly, the behavioral and molecular characteristics of these models recapitulate the pathology and functional deficits found in Rett syndrome.
- Considering the strong construct and etiological validity of the models and the robustness of the deficits, we believe that a drug screen using female Mecp2 rodents will provide strong translation to the clinic.