Integrative behavioral, molecular and electrophysiological analyses of female Mecp2$^{tm1.1\ Bird}$ Rett Syndrome model

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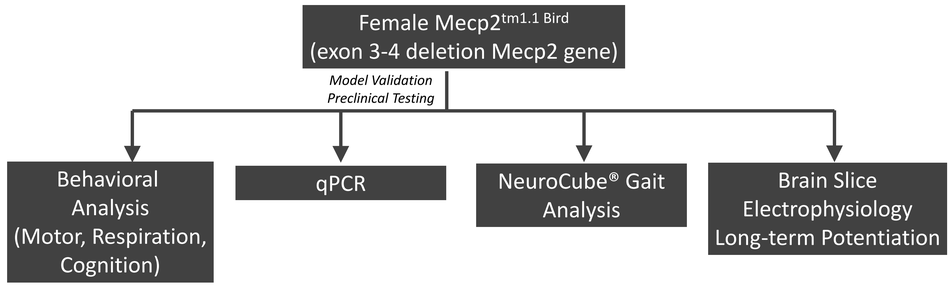
INTRODUCTION

Rett (RTT) syndrome is an X-linked neurodevelopmental disorders caused by de novo mutations in the methyl-CpG binding protein 2 (MeCP2). Female patients represent the majority of the clinical population of this disorder with classic symptoms including loss of hand skills, impaired speech, hand stereotypies, motor abnormalities, seizures, anxiety, and dysautonomia. Psychogenics, Inc has employed a combination of behavioral, molecular, and electrophysiological techniques to characterize the female Mecp2$^{tm1.1Bird}$ generated by Adrian Bird. This model was generated using a Cre-Lox recombination strategy to delete exon 3 and exon 4 of the Mecp2 gene. While most studies have analyzed male *Mecp2* mice, analysis of female mice is clinically relevant to the female population of Rett syndrome patients.

Behavioral studies in female *Mecp2$^{tm1.1Bird}$* mice show that the heterozygous mice have motor imbalance, gait deficits, breathing abnormalities, and impaired cognitive function. Extracellular field recordings in hippocampal slices from 6-month old female *Mecp2* mice displayed a reduction in long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse. Given that MeCP2 protein regulates gene expression, quantitative polymerase chain reaction (qPCR) analysis was employed here using hippocampal tissue from 4 and 10-month old female *Mecp2$^{tm1.1Bird}$*. qPCR analysis revealed a reduction in three known genes regulated by MeCP2: *Bdnf*, *Sapap3*, and *Kir4.1*. A reduction in mRNA coding for synaptic markers *Psd95* and *synaptophysin* was also detected along with upregulated mRNA levels for glutamate receptors (*Glur1*, *Glur2*, *Nr2a*, and *Nr2b*). Altogether, this integrative analysis suggests that female *Mecp2* mice displayed significant behavioral and synaptic plasticity deficits, along with robust alterations in gene expression that can be utilized as disease readouts for preclinical testing.



Behavioral motor, respiratory, and cognitive abnormalities in female Mecp2 Het mice

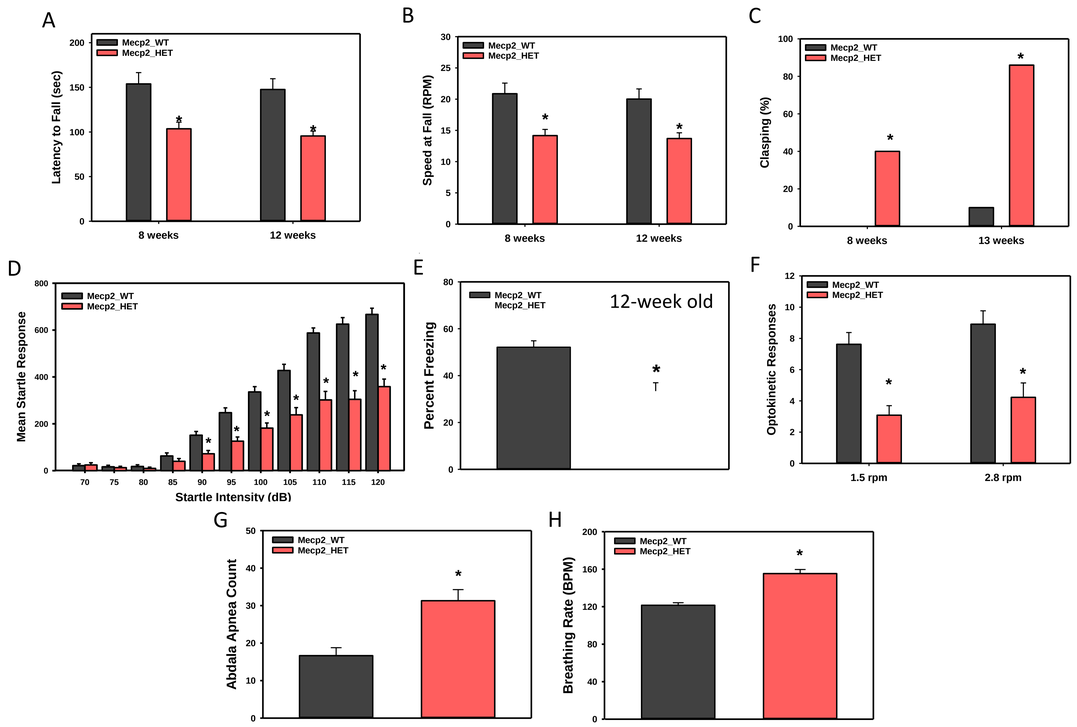


Figure 1. (A-B) Decreased performance in the rotarod behavioral test in female Mecp2 Het mice, displaying reduced latency to fall and falling at lower rotating speeds. Mice were placed on the continuous rotating rod and were given a 5-min training period at a slow speed (4 RPM). For the three accelerating 5-minute trials, mice are placed back on the rotarod and the speed is gradually and uniformly increased (0-40) RPM). C) Mecp2 Het mice show increased hindlimb clasping compared to wild-type controls. Mice were held by the tail and gently lifted until the front paws just lift off the counter surface. The experimenter observes the legs and determines clasping or splay of limbs. A score of 0 or 1 to designated absence or presence of clasping is assigned to each mouse. Percent of mice in each treatment group that show clasping was then calculated. D) Reduction in startle responses in response to graded increase in acoustic startle intensity in Mecp2 Het mice. Mice were placed in the startle enclosures and secured in the sound-attenuated startle chamber (*Med Associates Inc., St Albans, VT*) on top of a force transducer plate that measures the force of the movements made by the mouse for a 5min habituation period of white noise (70 dB). Subsequent test sessions consisted of 10 blocks of eleven trials each. Within each block, stimuli of 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, and 120dB were presented in a random order with a variable inter-trial interval of mean 15 sec (10-20sec). The duration of the stimulus was 40 ms. Responses were recorded for 150 ms from startle onset and sampled every ms. E) Mecp2 Het mice displayed impaired freezing behavior in contextual fear conditioning. F) Visual performance when tracking a moving object is impaired in Mecp2 Het mice as measured by a reduction in the number of correct optokinetic responses. Mice are placed on an elevated platform in the stationary chamber in the center of the cylinder, and habituated to the apparatus for 5 minutes the day before the test. The day of testing, the cylinder is rotated for one minute in both clockwise and counterclockwise directions, testing left and right eye sensitivities at 3 different speeds: 0 rev/min (0 cycles per degree), 1.5 rev/min (0.07 cycles per degree), and 2.8 rev/min (0.26 cycles per degree). G-H) Plethysmograph recordings revealed an increase in apnea count and increased breathing rate in Mecp2 Het mice (n=12-15 mice per genotype). Specialized whole body plethysmographs for the measurement of ventilation in conscious animals were procured from a commercial vendor (Data Sciences International item 601-1425-001). Using this technique, the animal is unrestrained and able to freely move about the chamber.

Altered gait in female Mecp2 Het mice using NeuroCube®

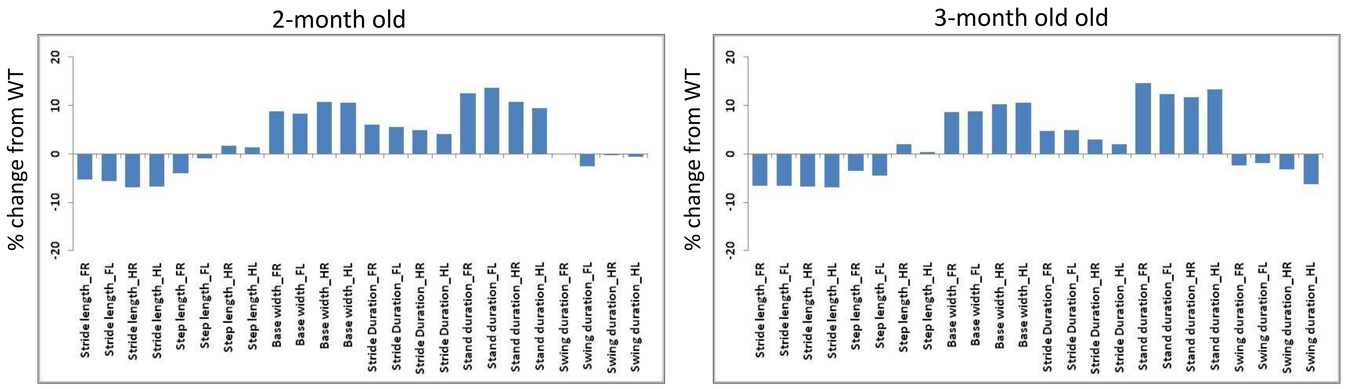


Figure 2. NeuroCube® assessment of Mecp2 mice revealed significant gait abnormalities based on analysis of gait geometry, dynamics, rhythmicity, body motion, and paw placement. NeuroCube® system is PsychoGenics' proprietary gait analysis system. The platform employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain & neuropathies Mice are placed in the NeuroCube for a 5 min test. The most dominant features that define the disease phenotype (symptom descriptors) are identified and ranked. Complex bioinformatics algorithms are employed to calculate the discrimination probability between WT and HET mice and can thus detect a test compound's ability to reverse the disease phenotype.

Decreased hippocampal expression of direct molecular targets of Mecp2: BDNF, Sapap3, and Kir4.1

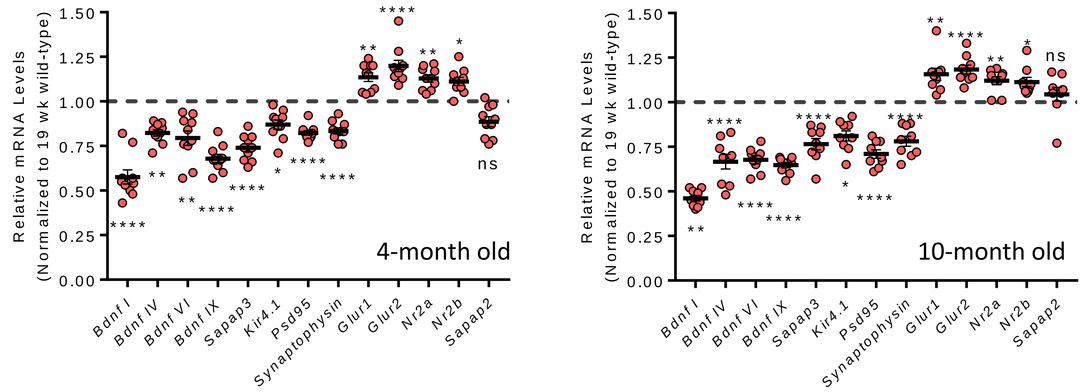


Figure 3. The direct molecular targets of Mecp2 (BDNF, Sapap3, and Kir4.1) were decreased in hippocampal tissue collected from Mecp2 Het mice. Analysis of synaptic mRNA markers revealed a significant down-regulation of Psd95 and synaptophysin, whereas genes encoding for AMPA receptors (Glur1, Glur2) and NMDA receptors (Nr2a, Nr2b) were significantly increased in Mecp2 Het mice. (n=10-12 mice per genotype, two-way ANOVA with Tukey's multiple comparison test). Relative mRNA levels were determined by normalizing to the geometric mean of housekeeping genes (Atp5b, Gapdh, and Elf4a2), and then normalized to the 4-month wild-type control.

Reduced LTP at Schaffer collateral-CA1 synapses in 6-month old female Mecp2 Het mice

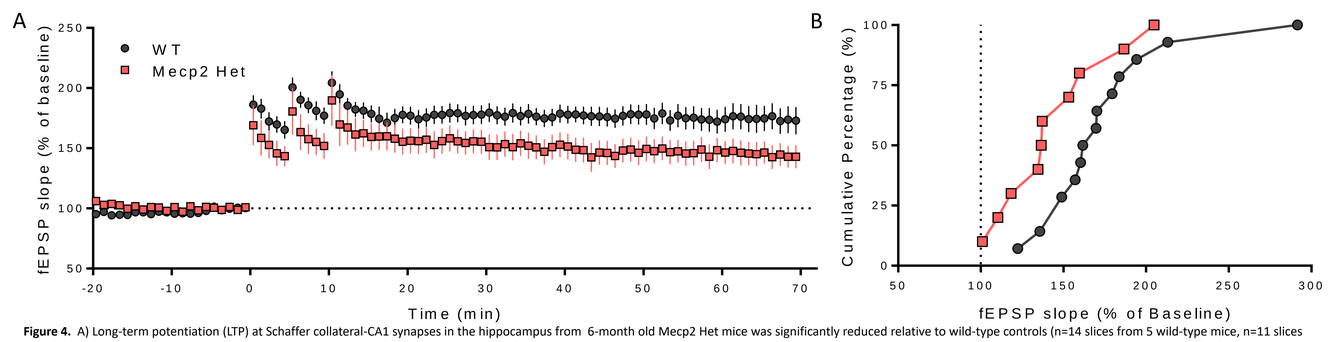


Figure 4. A) Long-term potentiation (LTP) at Schaffer collateral-CA1 synapses in the hippocampus from 6-month old Mecp2 Het mice was significantly reduced relative to wild-type controls (n=14 slices from 5 wild-type mice, n=11 slices from 5 Mecp2 mice; p=0.0420 two-way repeated measures ANOVA between time 40-70). Power analysis revealed this assay will require a sample size of 10 mice per genotype to achieve a power of 0.80 and alpha 0.05. B) Cumulative percentage plot with quantification of LTP magnitude (relative to baseline) during the last 5 minutes of the experiment (each point represents LTP magnitude per hippocampal slice). Input-output curve analysis revealed a trend towards increased slope of EPSP and Fiber Volley in Mecp2 Het mice (data not shown). LTP experiments using field potentials evoked with 40% of the maximum stimulus intensity. Paired pulse facilitation was not affected between wild-type and Mecp2 Het mice (data not shown).

SUMMARY

The female Mecp2 Het model of Rett syndrome displays significant alterations in motor function, breathing, and cognition. Further characterization of these mice revealed mRNA changes consistent with the function of Mecp2 as a regulator of transcription. Analysis of synaptic plasticity is a well known endpoint linked to learning and memory, and the LTP deficit correlates with a significant reduction in BDNF expression, providing evidence that one potential mechanism for the LTP impairment is consequential to impaired BDNF signaling in the hippocampus of Mecp2 mice. The identification of these markers is highly relevant as they can have a higher clinical impact to the female population of Rett syndrome patients. The combination of behavior, molecular, and electrophysiological analyses provides a powerful platform for testing therapeutic strategies in female Mecp2 mice.