



Redefining Drug Discovery Through Innovation

Rett Syndrome

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.
- Adrian Bird's MECP2 heterozygous knockout mouse (*MECP2^{tm1.1Bird}*) can be used as preclinical models of Rett Syndrome.
- Whereas male MECP2 mice show poor health and die by 8-9 weeks of age, female MECP2 mice show normal survival and health and thus can be used to screen novel therapeutics.
- Female MECP2 Het mice bred on C57Bl6/J background at Jackson Laboratories are used in these studies.



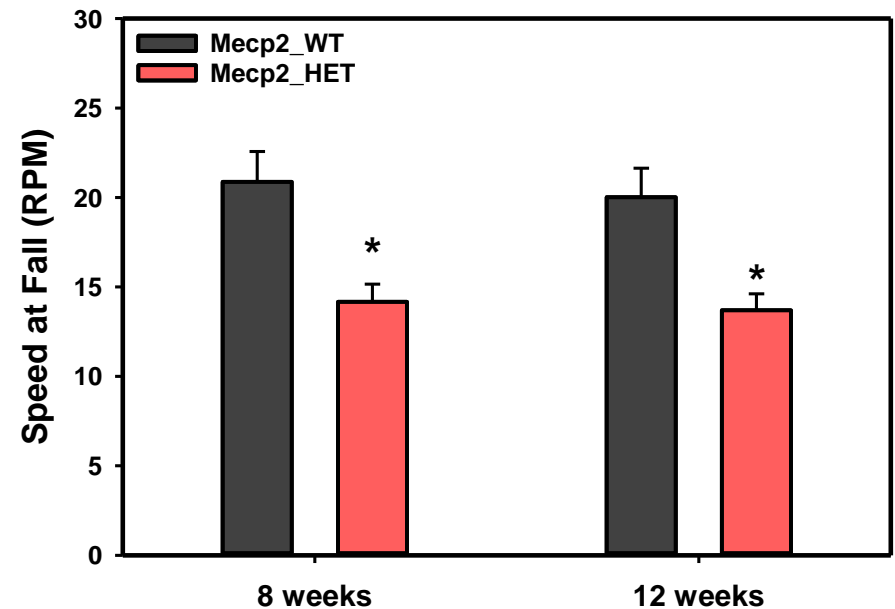
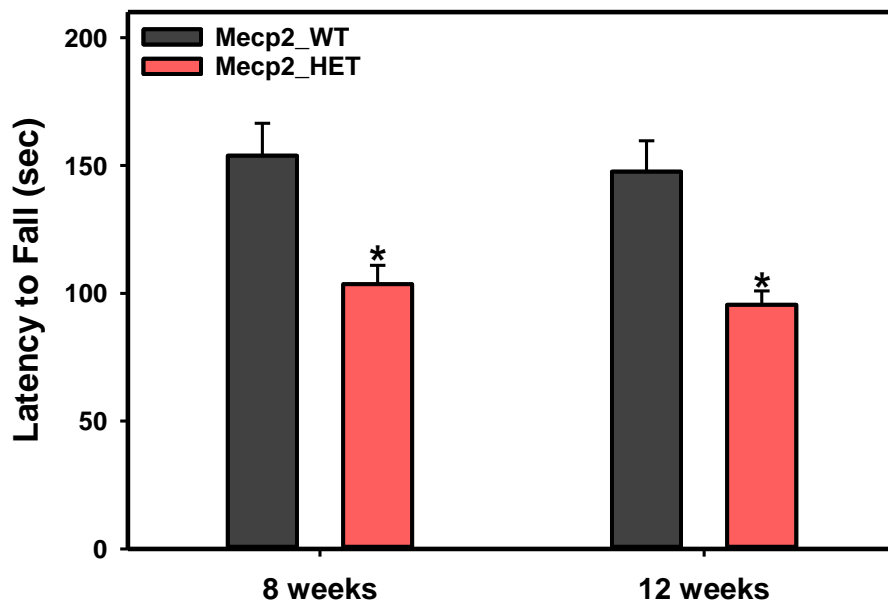


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Motor, Gait, Startle in 8-12 week old mice

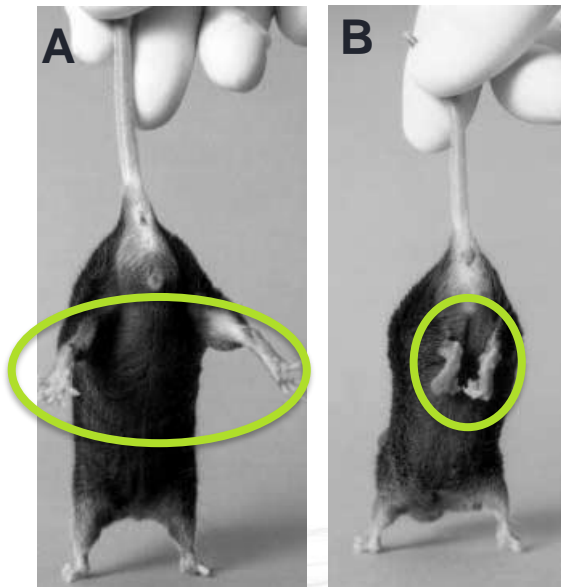
Female HET mice show deficits in rotarod performance

- Mice are placed on the continuous rotating rod [Columbus Rotamex Treadmill Apparatus) and are given a 5-min training period at a slow speed (4 RPM).
- After a 1 hour ITI, mice are exposed to three 5-minute trials. During each trial, the mice are placed back on the rotarod and the speed is gradually and uniformly increased (0-40 RPM). The time that each mouse remains on the rotating rod before falling as well as the speed of the rotarod when the mouse falls is recorded.



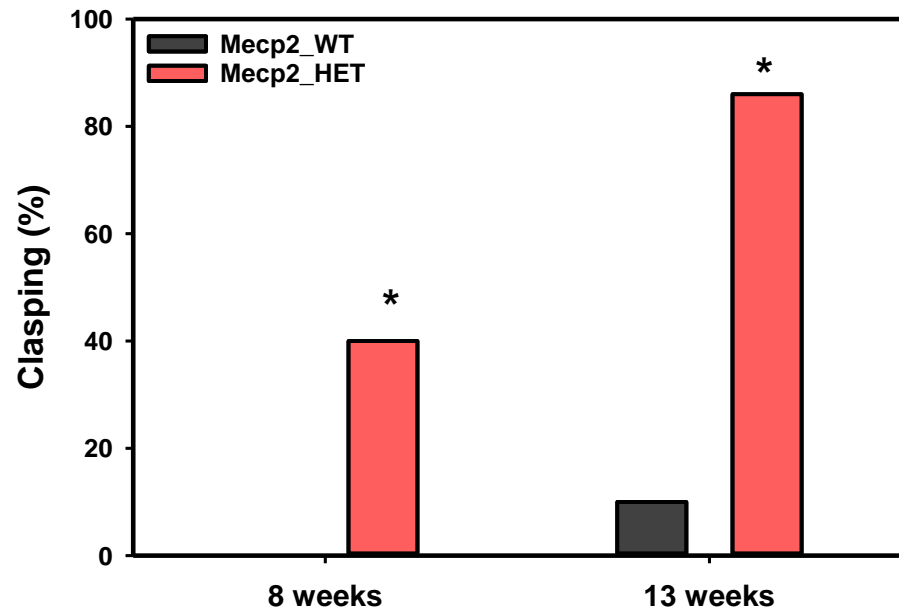
Female Het mice show increased hindlimb clasp

- Mice are lifted gently by the tail with front limbs remaining on surface. Normal response is a spread in the hind limbs (A)
- The percent of mice that show hindlimb clasp (B) is recorded

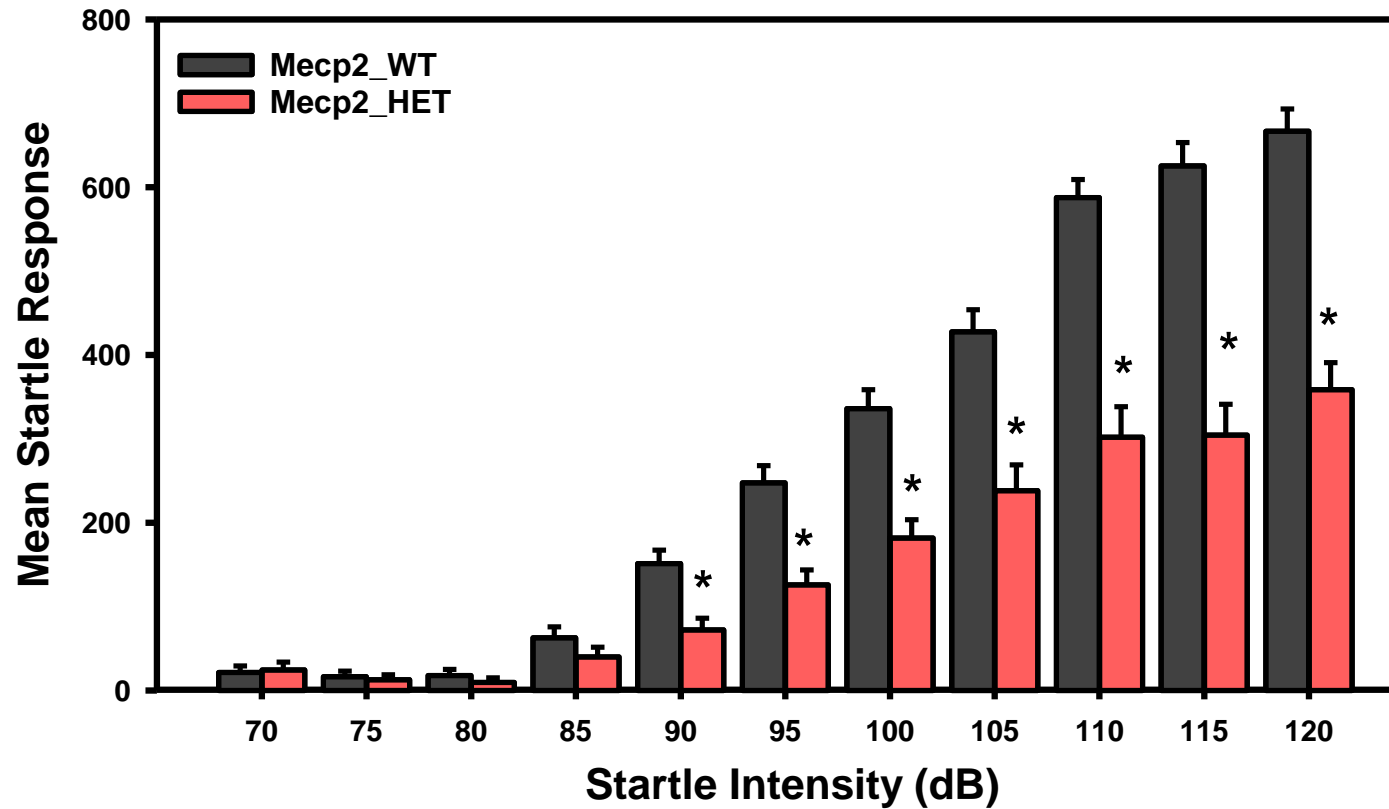


Normal spread of hind limbs

Clasping of hind limbs



Female HET mice show reduced startle response



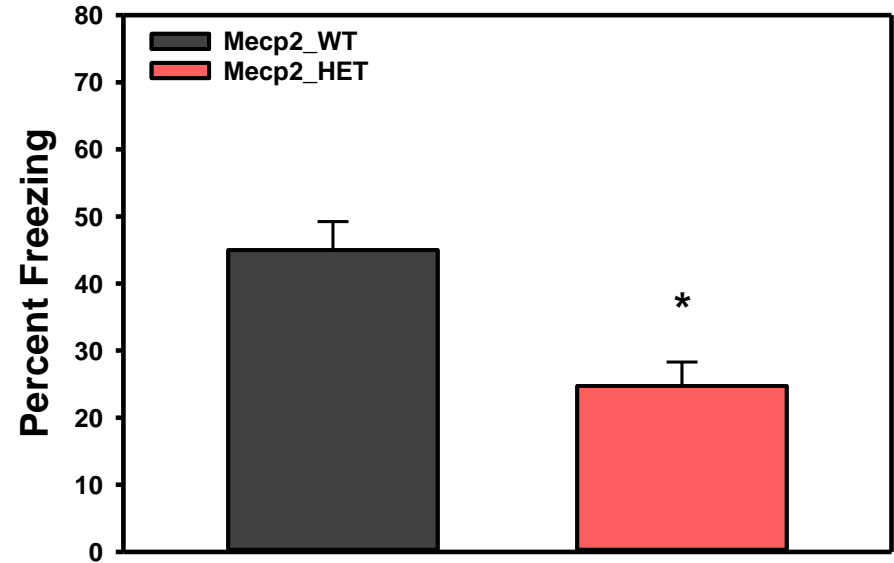
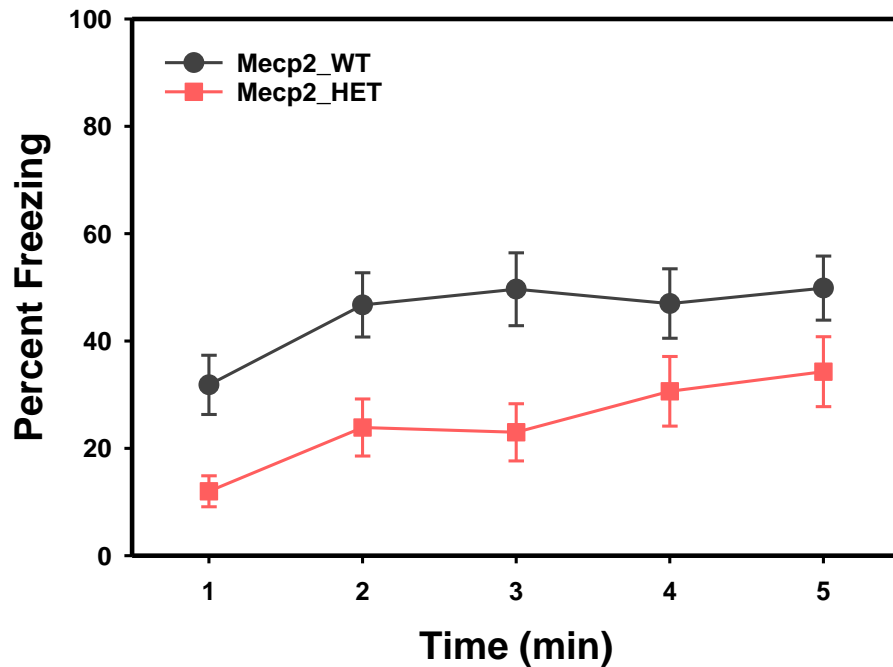


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Cognitive function



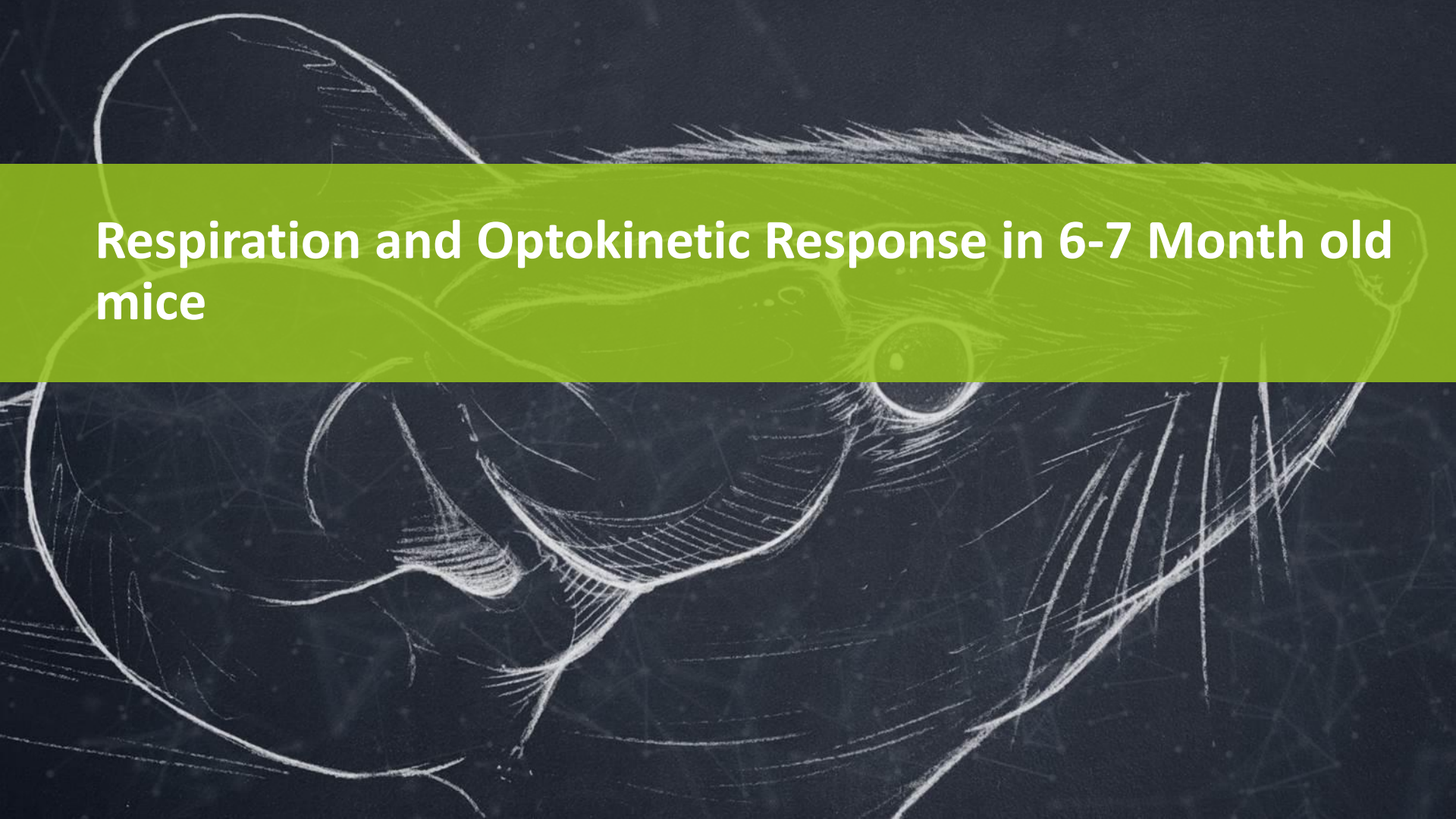
Female HET mice show deficits in Fear Conditioning





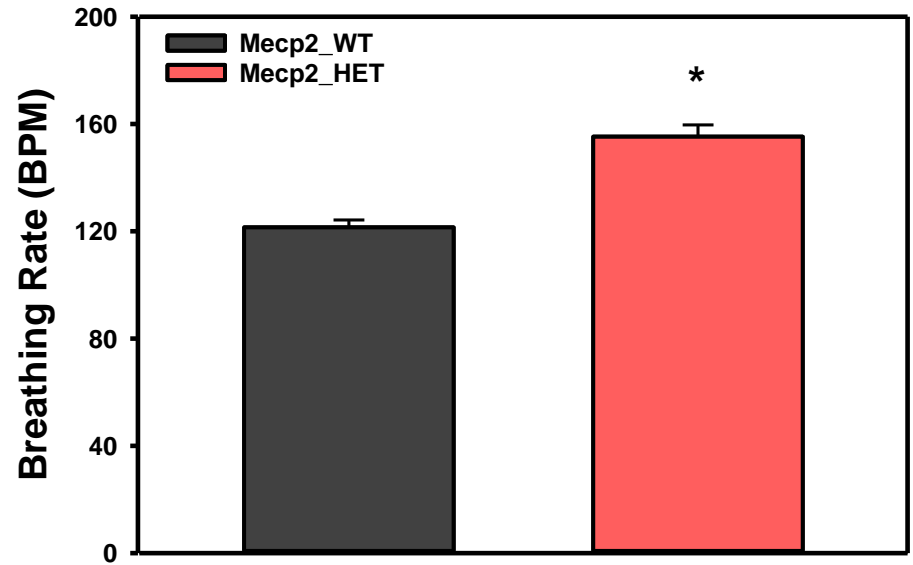
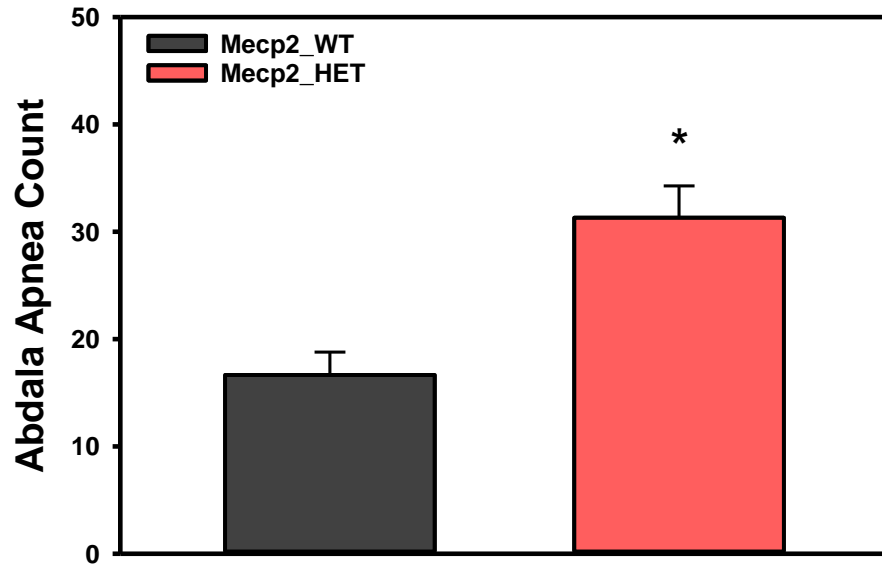
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Respiration and Optokinetic Response in 6-7 Month old mice



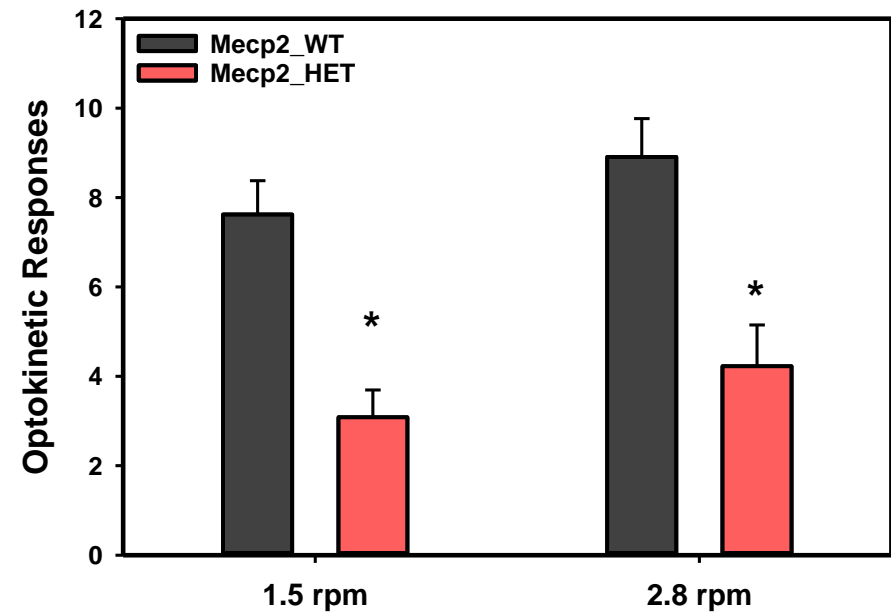
Female HET mice have higher breathing rates and apnea count

- Mice are tested in a whole body plethysmograph system for 1 hour.



Optokinetic Responses are impaired in female HET mice

- The optokinetic response and bar tracking are scored by an observer blind to the animal strain, bar rotation, and frequency.
- Visual performance when tracking a moving object is impaired in the HET mice resulting in fewer correct optokinetic responses compared to WT mice



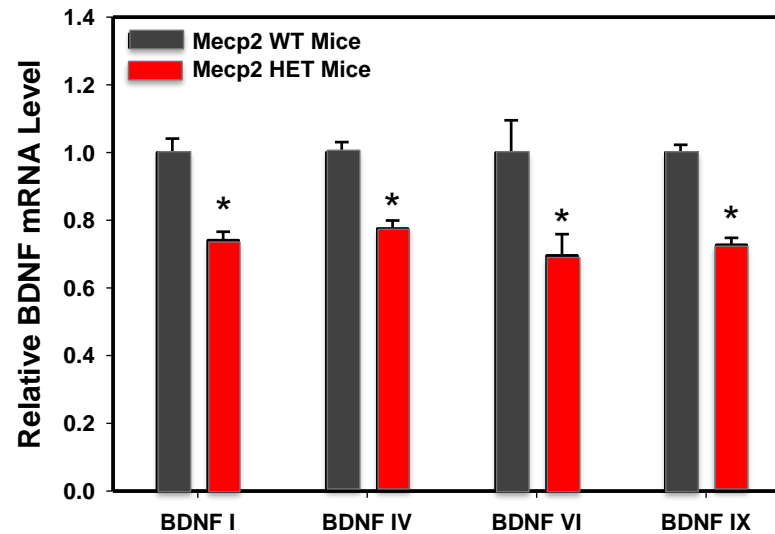


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Biomarker Analysis

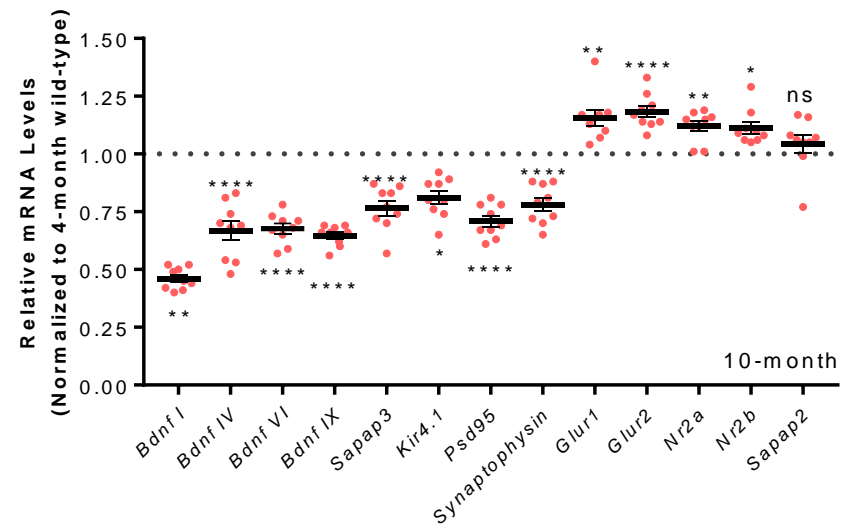
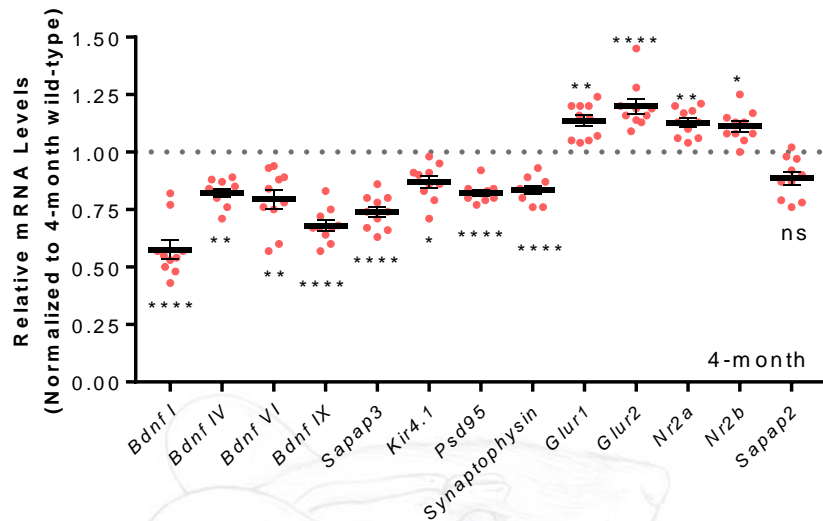


BDNF levels are significantly lower in HET mice compared to WT



Molecular biomarkers

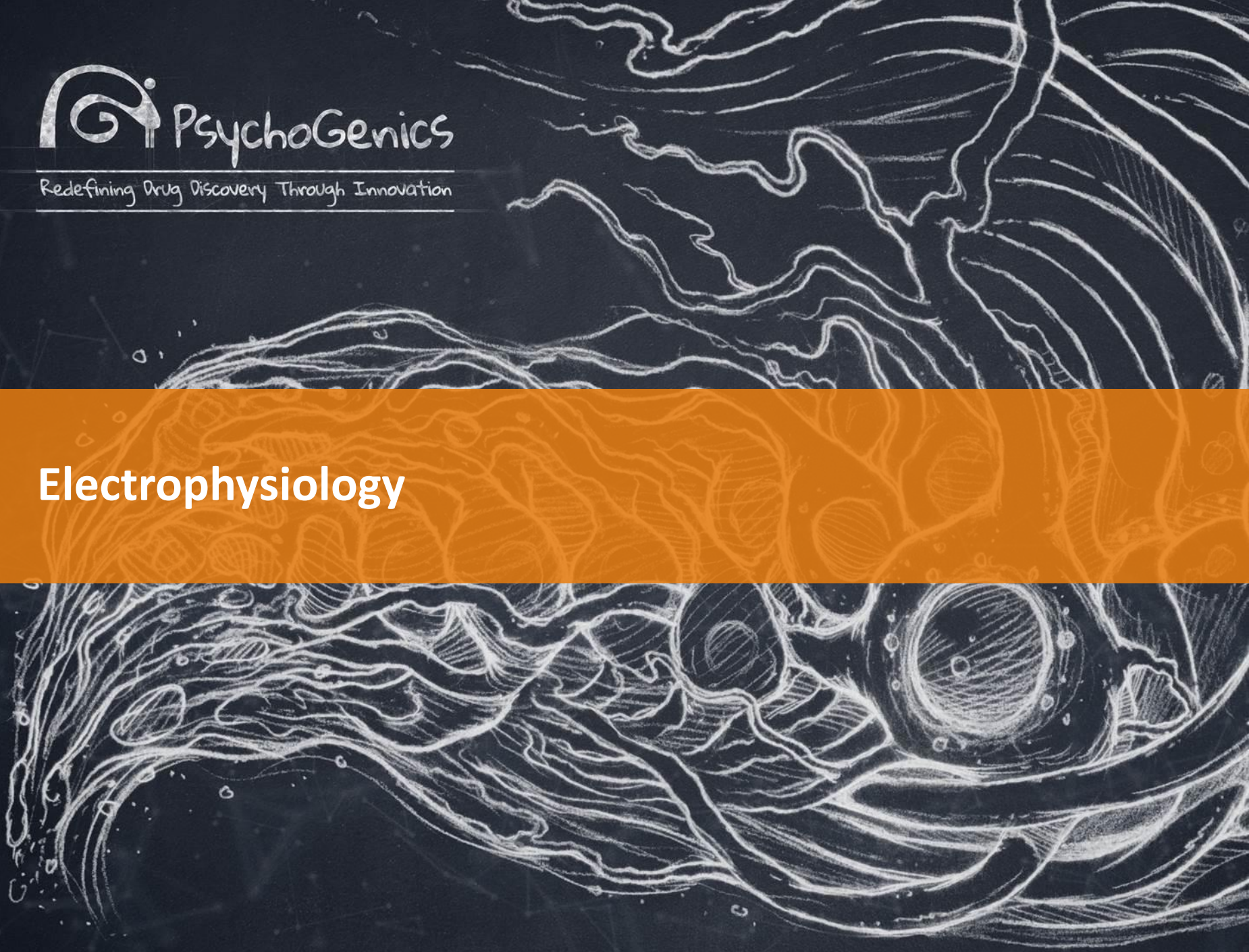
- Hippocampal markers in age-matched wild-type and MeCP2 mice at 4 and 10 months of age.
- BDNF, Sapap3, and Kir4.1 genes are direct molecular targets of MeCP2 and are decreased in the HET mice compared to WT mice
- MeCP2 mice show decreased levels of PSD95 and Synaptophysin and increased levels of glutamate receptor subunits including GluR1, GluR2, NR2A and NR2B.





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Electrophysiology



Impaired LTP in female HET mice

- Extracellular field excitatory postsynaptic potentials (fEPSP) were evoked in hippocampal slices at the Schaffer collateral-CA1 synapse from 6-month old female wild-type and MeCP2 mice.
- Stable baseline recordings were obtained with fEPSPs evoked using 40% of maximum stimulus intensity. LTP was induced by delivering three trains of 100 Hz high frequency stimulation, each train separated by five minutes inter-train interval. Synaptic responses were normalized and expressed as percent (%) from baseline

