PsychoGenics

Redefining Drug Discovery Through Innovation

# Fragile X Syndrome

# Background

- Caused by a CGG expansion in the FMR1 gene
- Males severely affected, females are mosaic
- Fragile X syndrome can be a cause of autism or related disorders, although not all children with fragile X syndrome have these conditions
- Symptoms include
  - Developmental delays: crawling, walking
  - Hand clapping or hand biting
  - Hyperactive or impulsive behavior
  - Mental retardation
  - Speech and language delay
  - Tendency to avoid eye contact
  - Physical signs: Flat feet, flexible joints and low muscle tone, large body size, large forehead or ears with a prominent jaw, long face, large testicles, soft skin

# FMR1 KO mice

- These mice have a knockout allele of the fragile X mental retardation syndrome 1 gene (*Fmr1*) on the X chromosome and exhibit many phenotypic characteristics of the Fragile X Syndrome in humans including hyperactivity, repetitive behavior and seizures.
- Absence of the Fragile X Mental Retardation protein (FMRP) in the mice causes activation of RAC1 protein resulting in abnormalities in dendritic spines in various regions of the brain. and altered synaptic function.
- The absence of FMRP also alters synaptic plasticity which results in an impairment of long-term potentiation in the cortex and hippocampus, as well as an augmentation of long-term depression in the hippocampus and cerebellum.
- Male FMR1 KO mice on FVB/n background bred at PsychoGenics are used in all studies

Bakker et al., 2004; Han et al;. 2015; Huber et al. 2002; Krueger et al., 2011; Koga et al., 2015

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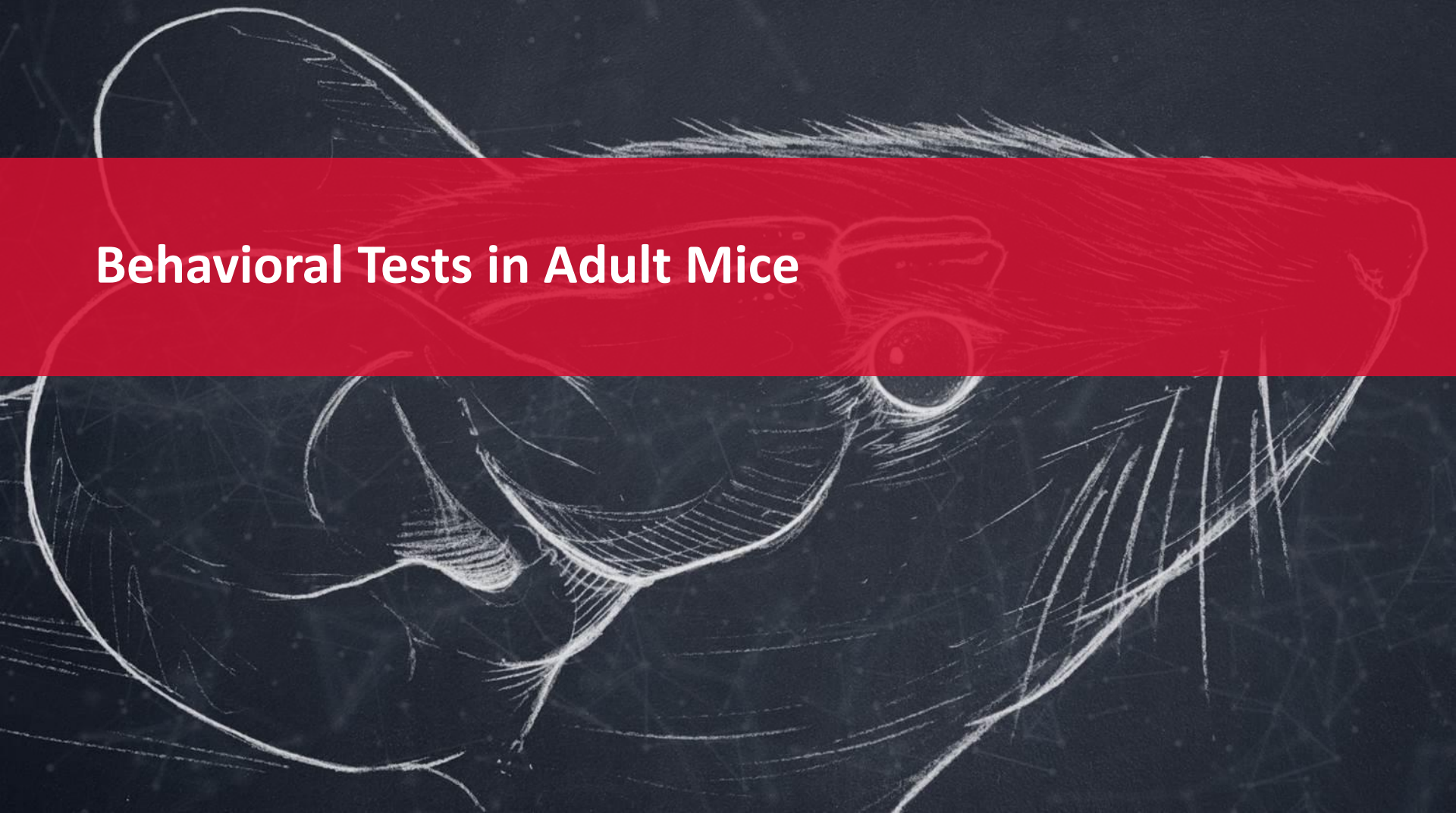
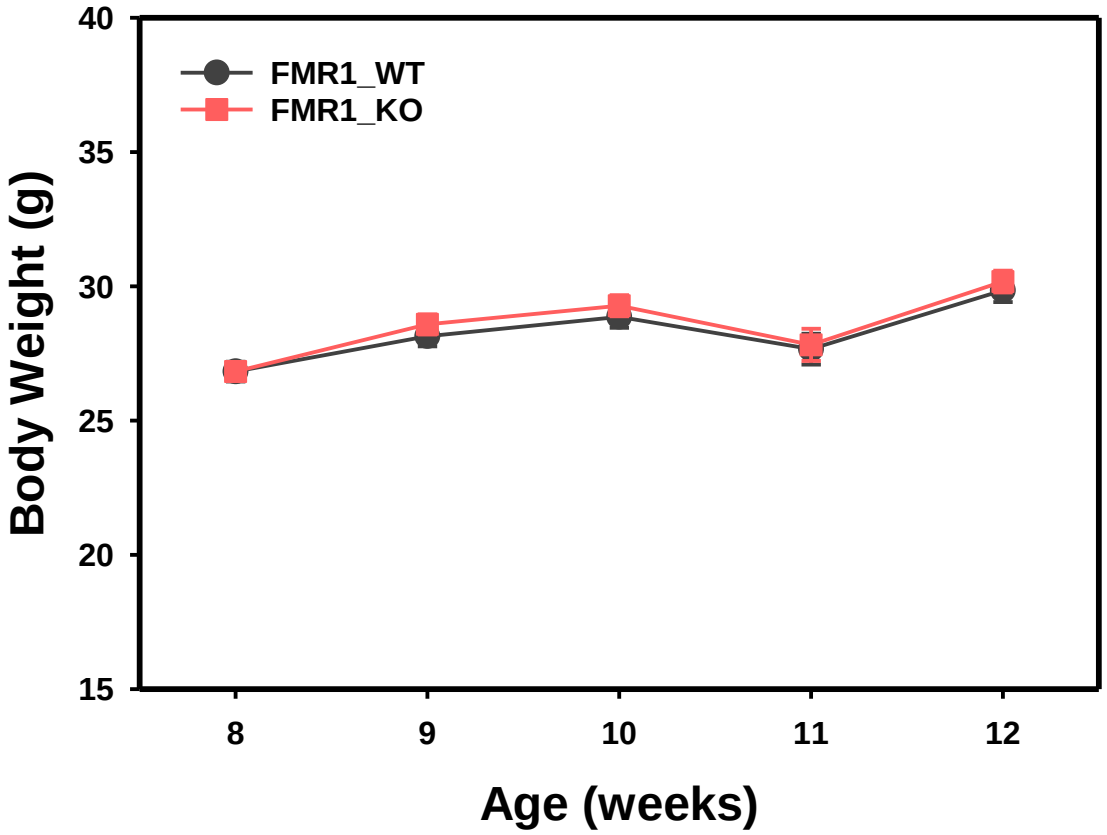
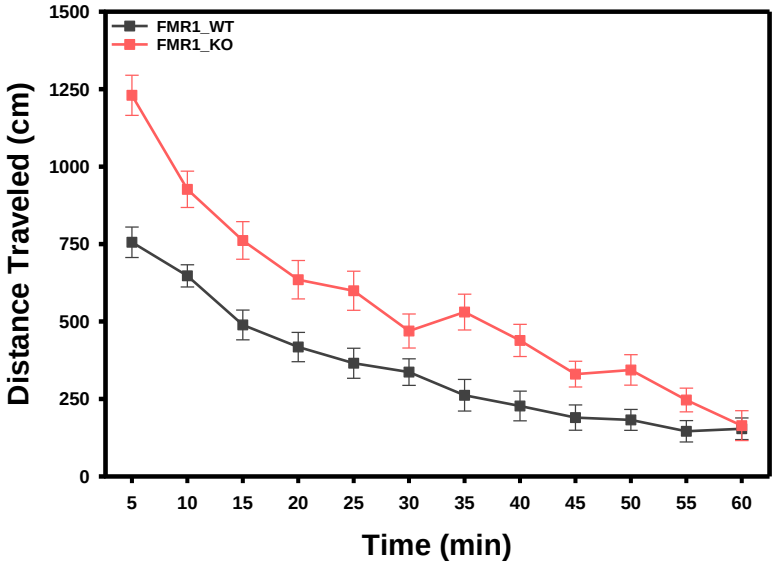
# Behavioral Tests in Adult Mice

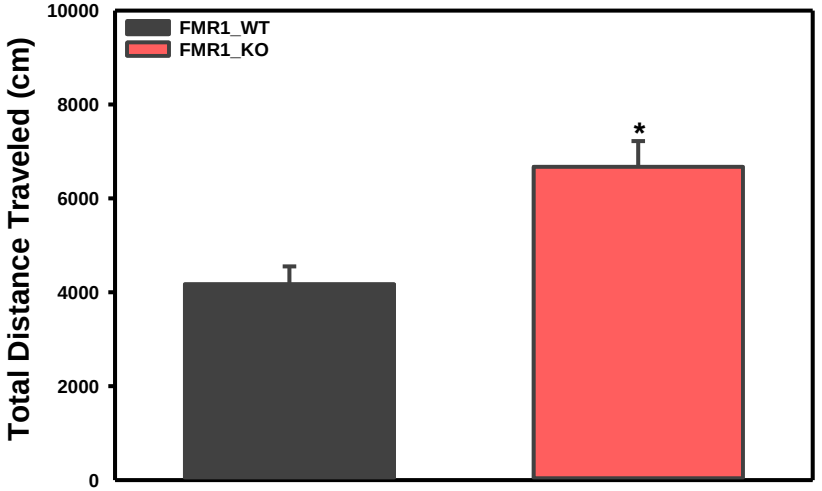


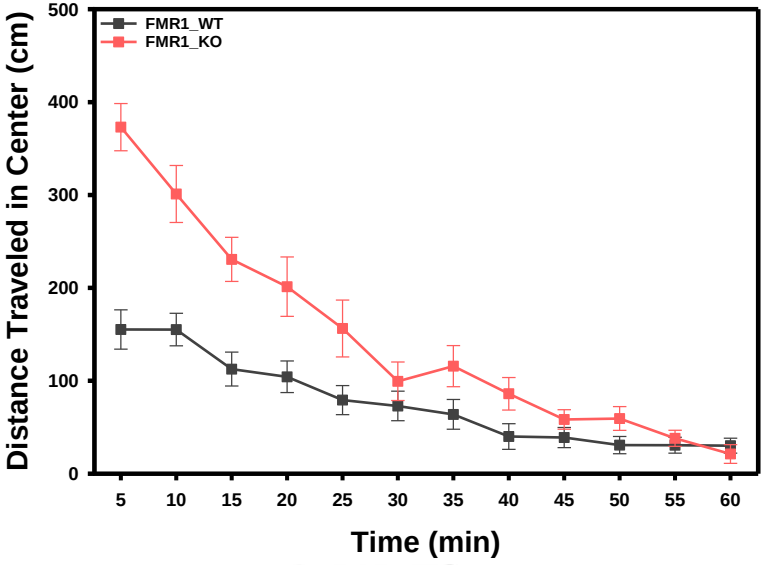
# Male KO mice show similar BW compared to WT mice

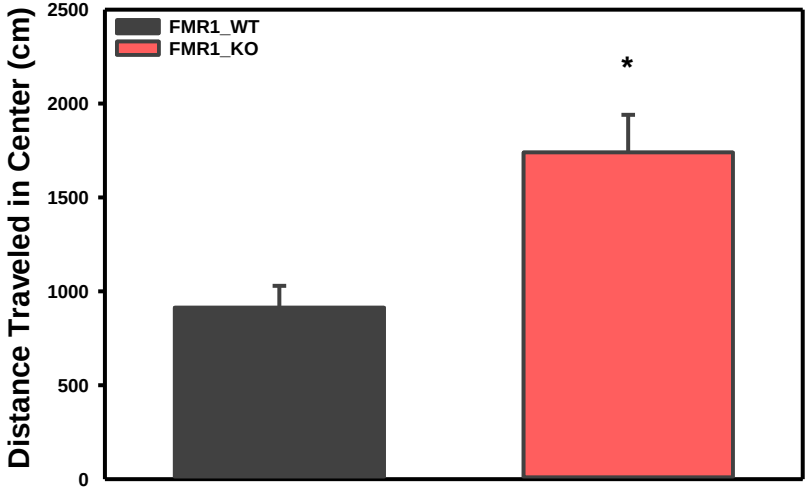


# FMR1 KO mice are hyperactive compared to WT mice



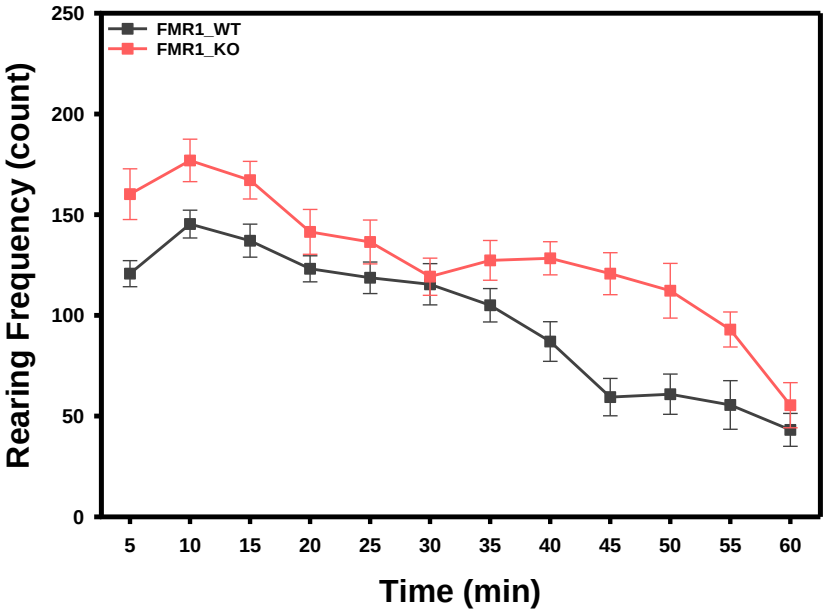



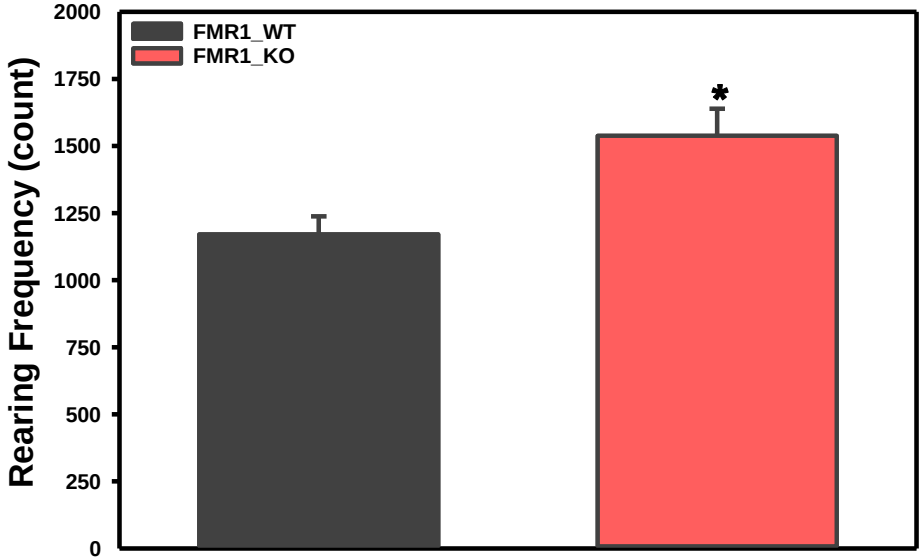




Male mice tested at 9 weeks of age

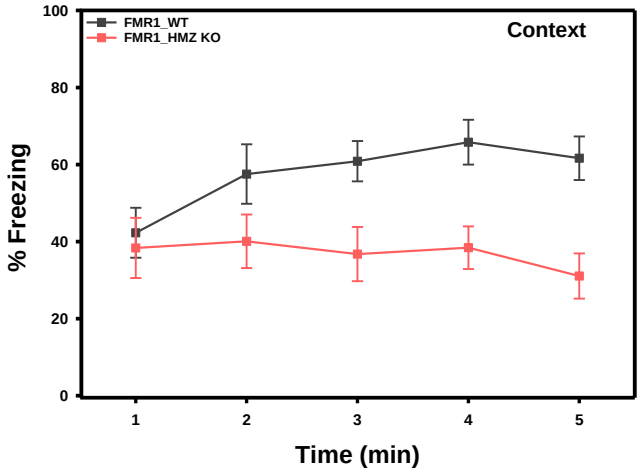
# Male FMR1 KO mice show increased rearing activity

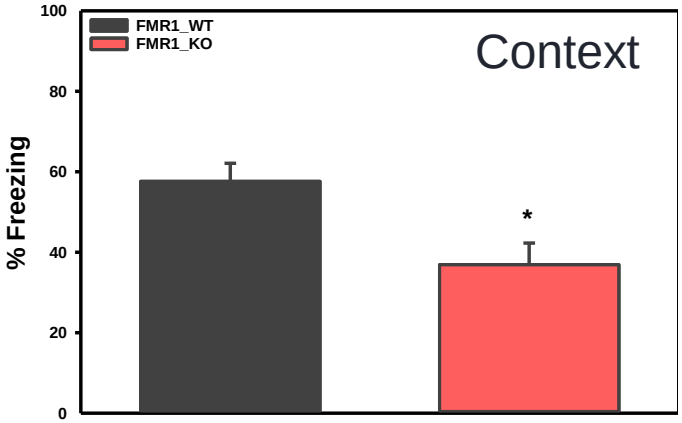


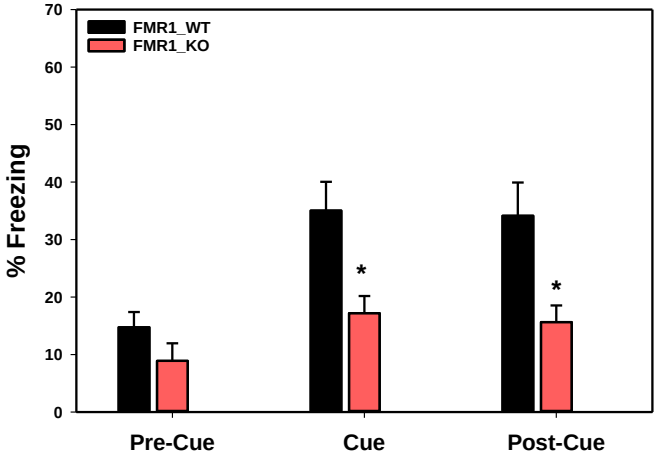




Male mice tested at 9 weeks of age

# KO mice show deficits in Contextual and Cued Fear Conditioning

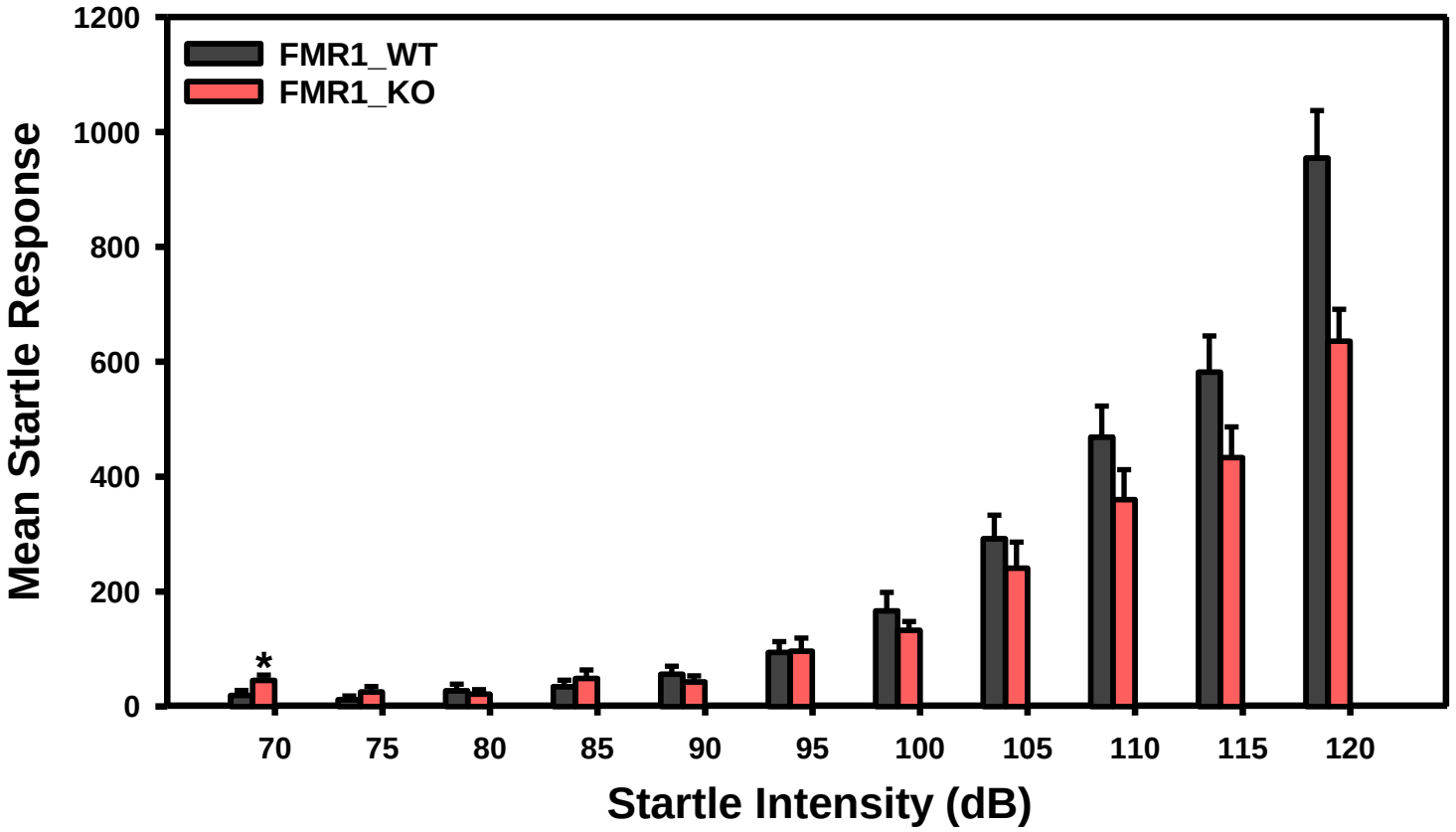



Male mice tested at 11 weeks of age

# FMR1 KO mice show reduced startle response




Male mice tested at 10 weeks of age

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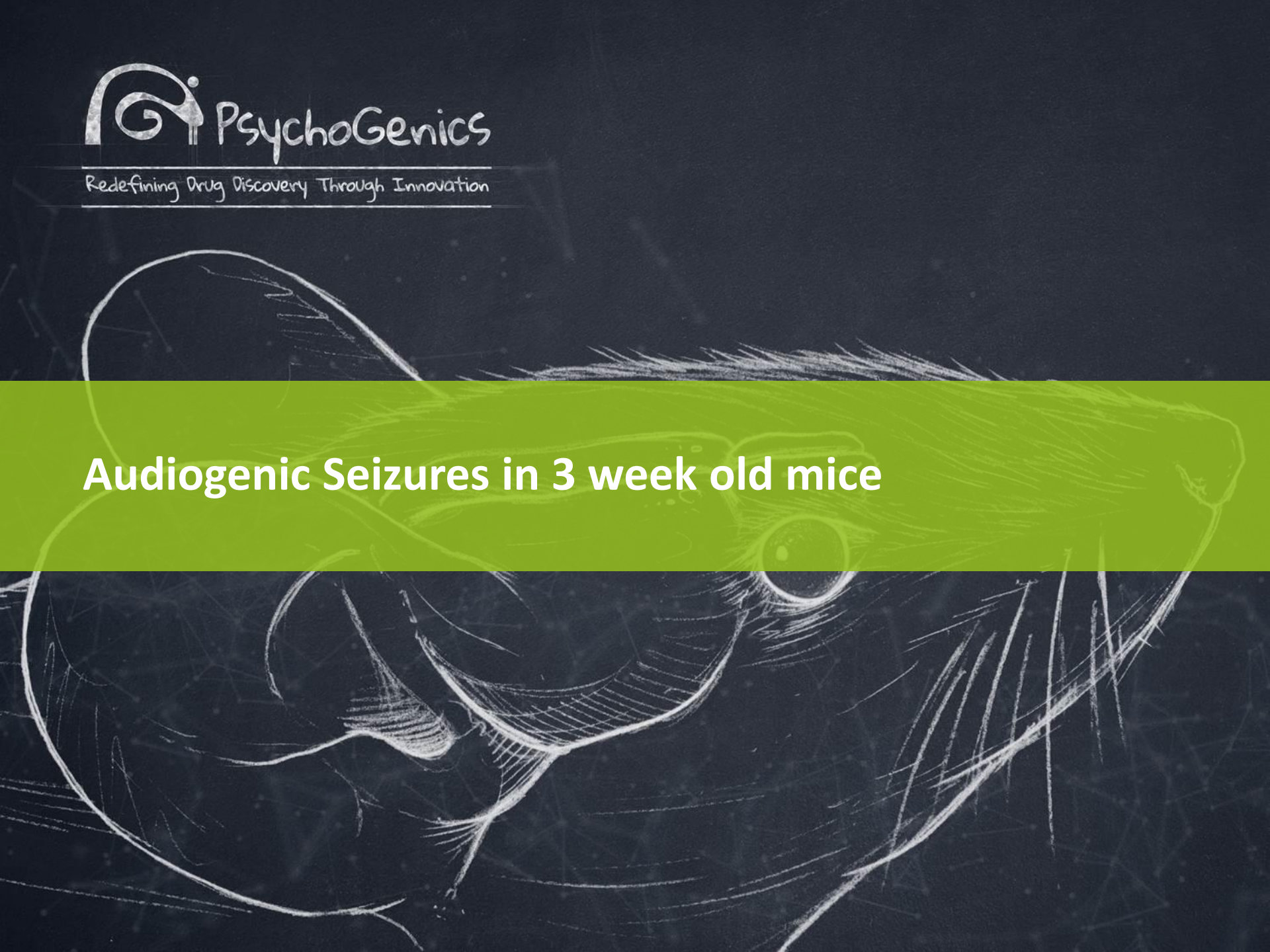
# Audiogenic Seizures in 3 week old mice

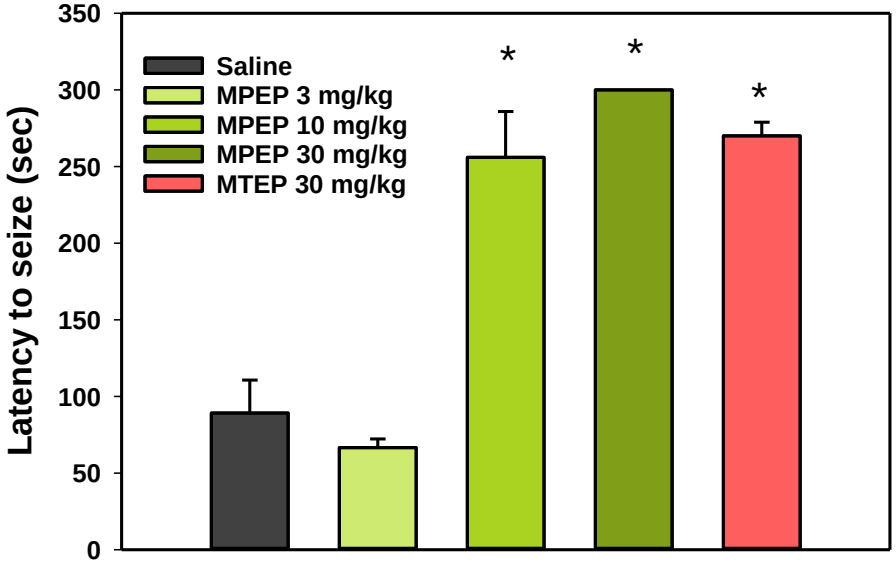


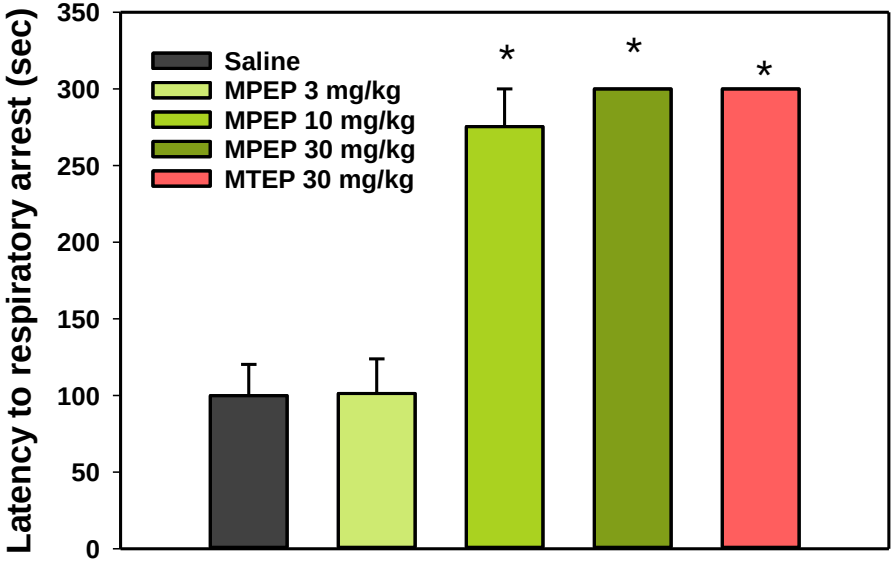
# Audiogenic seizures

- KO mice are tested at 3 weeks of age
- Mice are are individually placed in a Plexiglas chamber and allowed to explore for 15 sec. They are then exposed to a 125 dB tone for 2 minutes, followed by 1 minute of no sound, and then a repeat 2 minute tone. The mice are scored based on their response, latency, and seizure intensity:

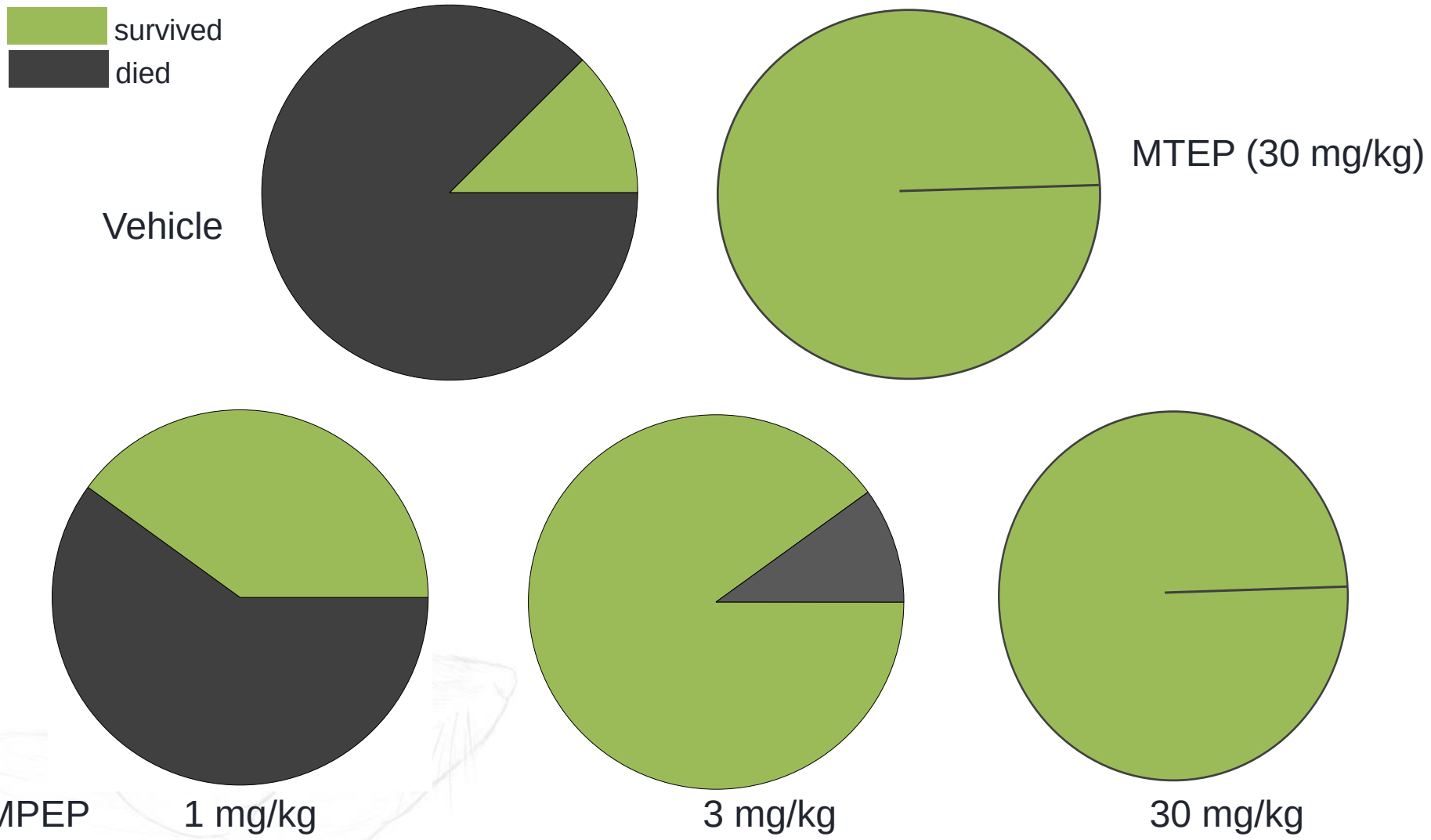
0: no response
1: wild running and jumping
2: clonic seizures
3: clonic/tonic seizures
4: tonic seizures
5: respiratory arrest

# Effects of MPEP and MTEP on audiogenic seizures

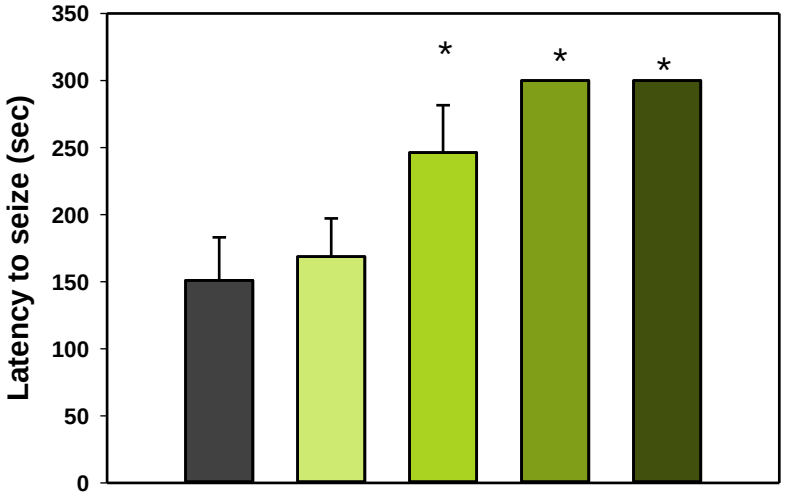


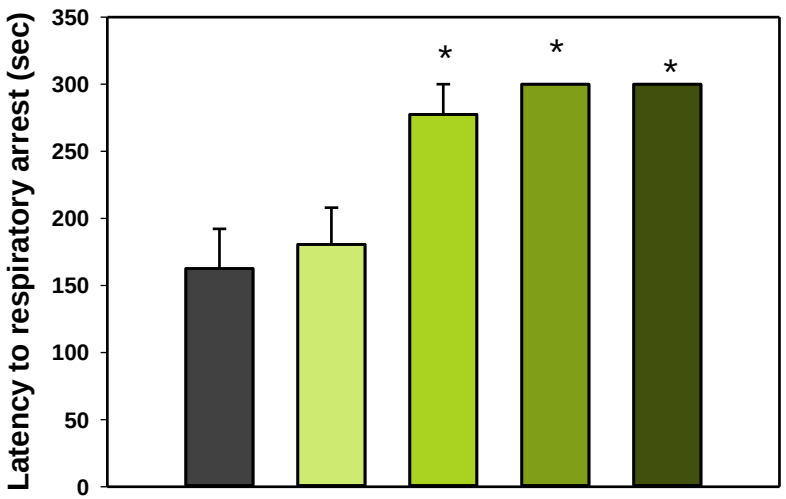



# Effects of MPEP and MTEP on survival



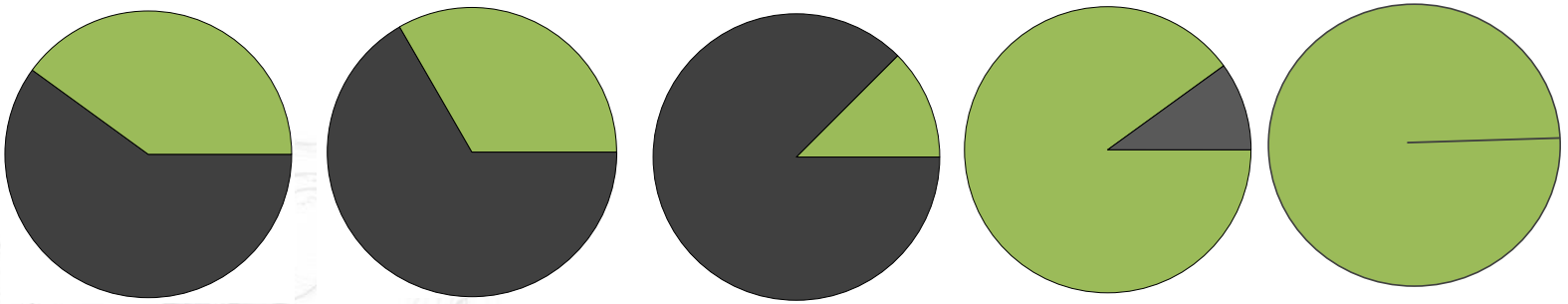
# Effects of diazepam on audiogenic seizures



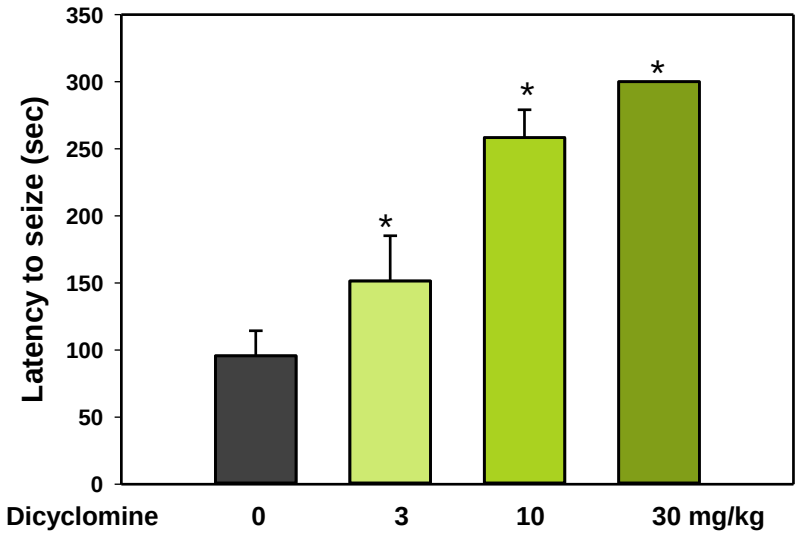

Vehicle
Diazepam 0.03 mg/kg
Diazepam 0.1 mg/kg
Diazepam 0.3 mg/kg
Diazepam 1 mg/kg

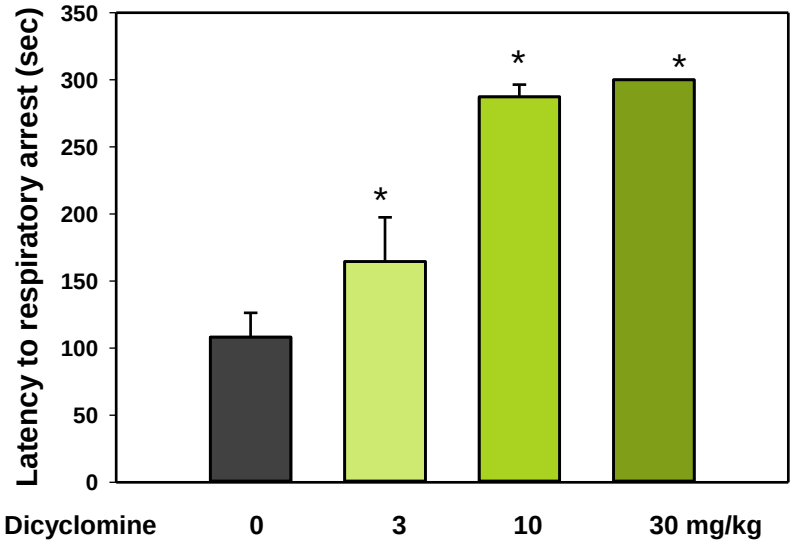
survived
died

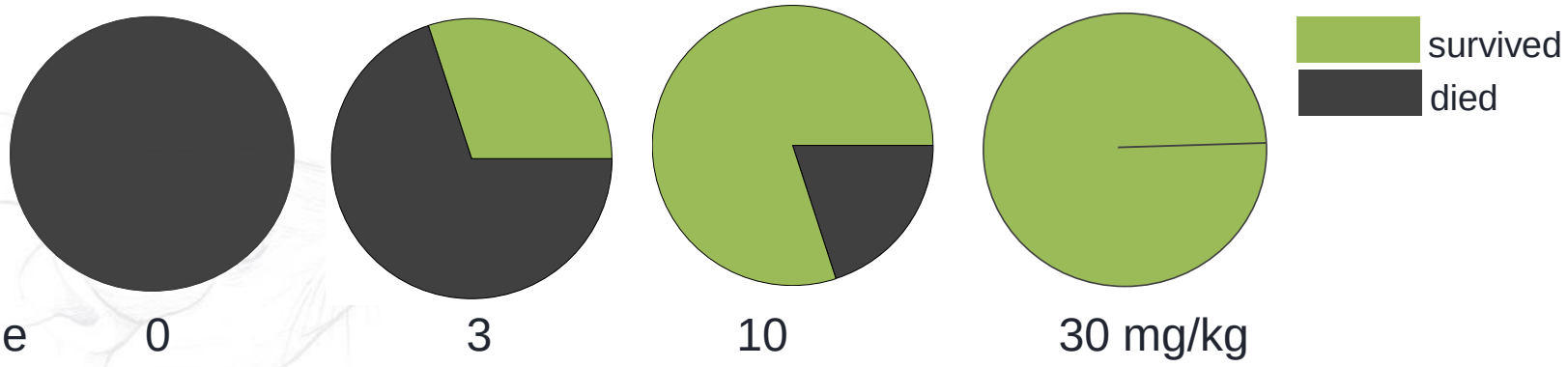


Diazepam 0 0.03 0.1 0.3 1 mg/kg

# Effects of dicyclomine on audiogenic seizures



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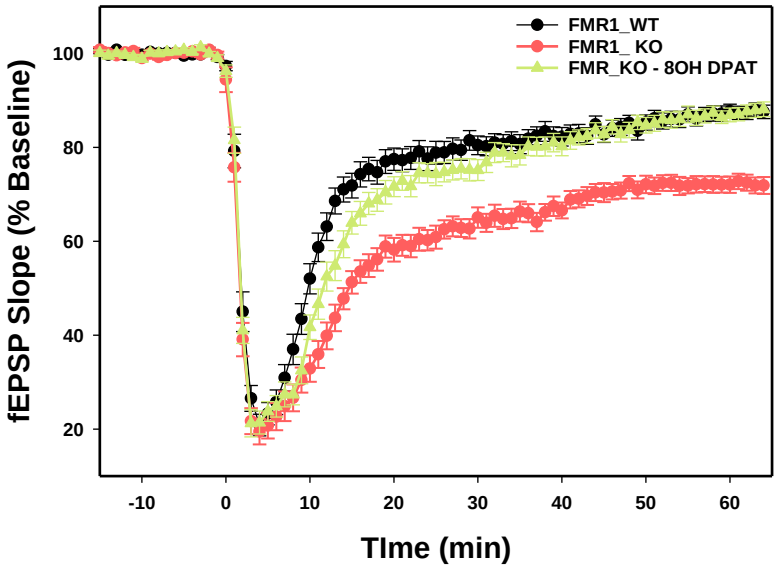
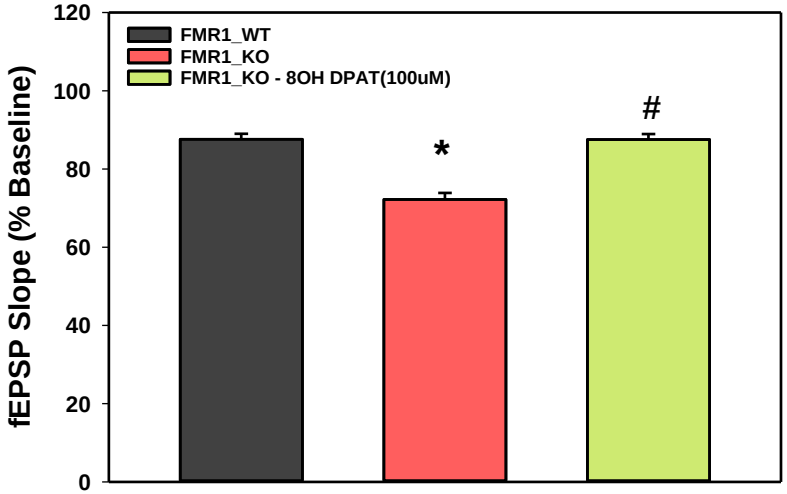
# Electrophysiology

# Fmr1 mice exhibit enhanced hippocampal mGluR-dependent long-term potentiation (LTD), which is reversed by mGluR antagonist 8-OH-DPAT



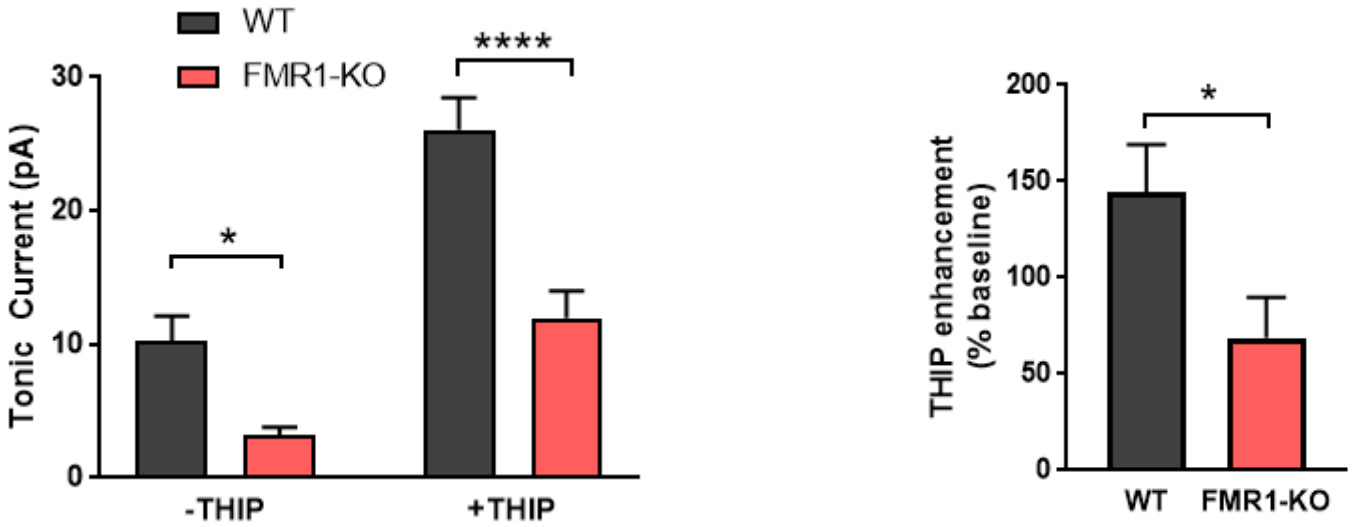

Time course of the changes in responses recorded in CA1 area of hippocampus induced with an application of an mGluR agonist (S)-DHPG (100µM). DHPG was applied from 0 to 5 min and the subsequent mGluR antagonist 8-OH-DPAT (100nM) was applied from 10 to 15 minutes




Summary of the data for the last 5 min of experiment. *p<0.05 compared to WT; #p<0.05 compared to KO

# Reduced tonic inhibition in dentate gyrus observed in 2-month old male Fragile X mice

A significant reduction in tonic inhibitory currents, a critical factor modulating neuronal excitability, was observed in dentate granule cells of 2 month-old male FMR1-KO mice in agreement with prior observations (Zhang, N. et al (2017). Exp. Neurol., PMID: 28822839).




***Tonic inhibitory currents are significantly reduced in dentate granule cells of FMR1-KO mice.*** *Whole-cell patch clamp recordings were made in dentate granule cells of hippocampal slices from 2-month old male wild-type (WT) (n=22 cells, 6 mice) and FMR1-KO mice (n=24 cells, 5 mice),* $V_{hold}$*=-70 mV.* ***Left*** *Following a stable baseline (-THIP) and subsequent enhancement of tonic GABA currents by the d subunit-selective agonist THIP (gaboxadol, 1 mM; +THIP), tonic currents were unmasked by blocking* $GABA_A$ *receptors with 100 mM picrotoxin. *p<0.05, ****p<0.0001, two-way repeated measures ANOVA, Bonferroni's multiple comparisons test.* ***Right*** *Enhancement of tonic current by application of THIP normalized to baseline current (as measured prior to THIP application, -THIP). n=21 cells (WT), 17 (FMR1-KO), *p<0.05, Mann-Whitney test.*