PsychoGenics

Redefining Drug Discovery Through Innovation

Behavioral Phenotyping of CDKL5 mice

Study code: IFCR001

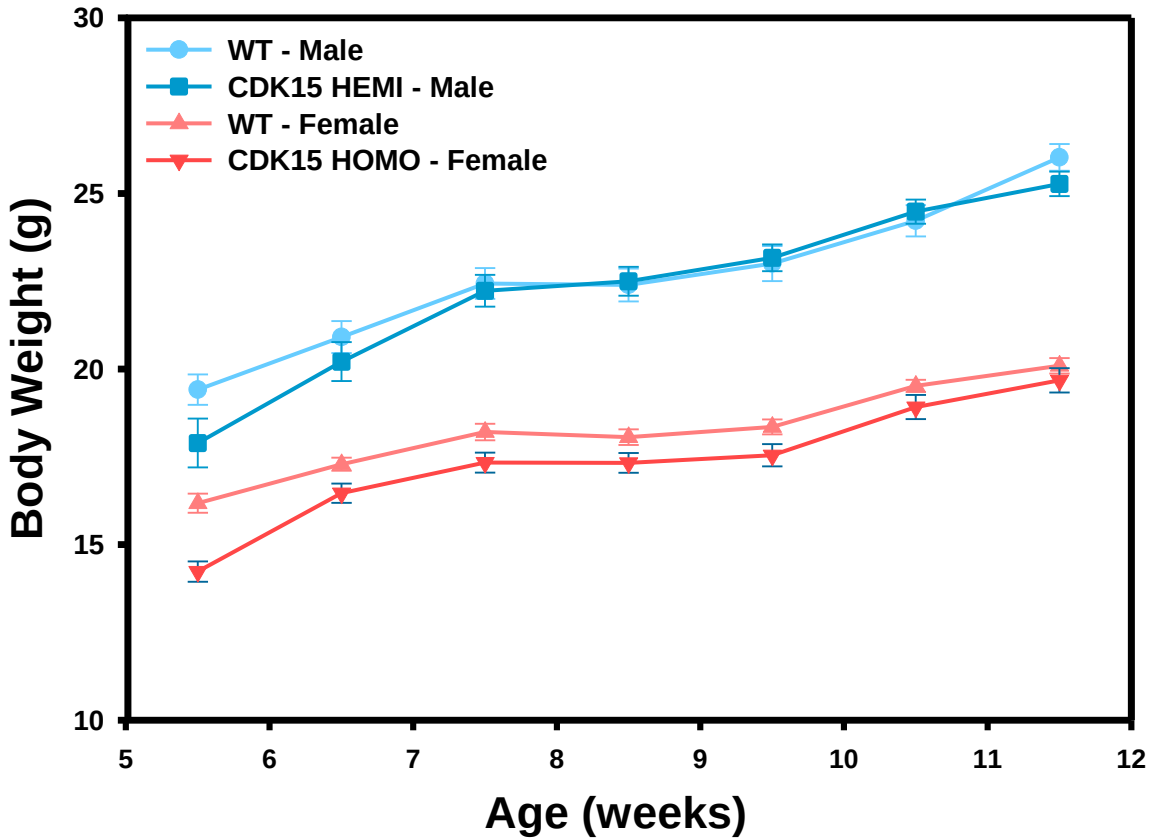
8/11/2016

IFCR001 Behavioral Phenotyping Study plan

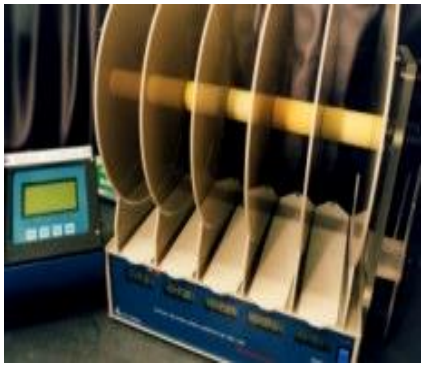
- Groups:
  - C57BL/6J WT (n=16 Males, 16 Females)
  - CDKL5 HOMO (n=16 Females)
  - CDKL5 HEMI (n=16 Males)
- Behavioral Tests Performed:
  - Body Weight
  - NeuroCube ®
  - PhenoCube ®
  - Optokinetic Response (OKR)
  - Clasping
  - Rotarod
  - Fear Conditioning -scheduled
  - AUX/PTZ- -scheduled

Behavioral Phenotyping: Body Weight

No significant genotype effect, however significant gender effect, whereby males weighed more than the females.



Rotarod

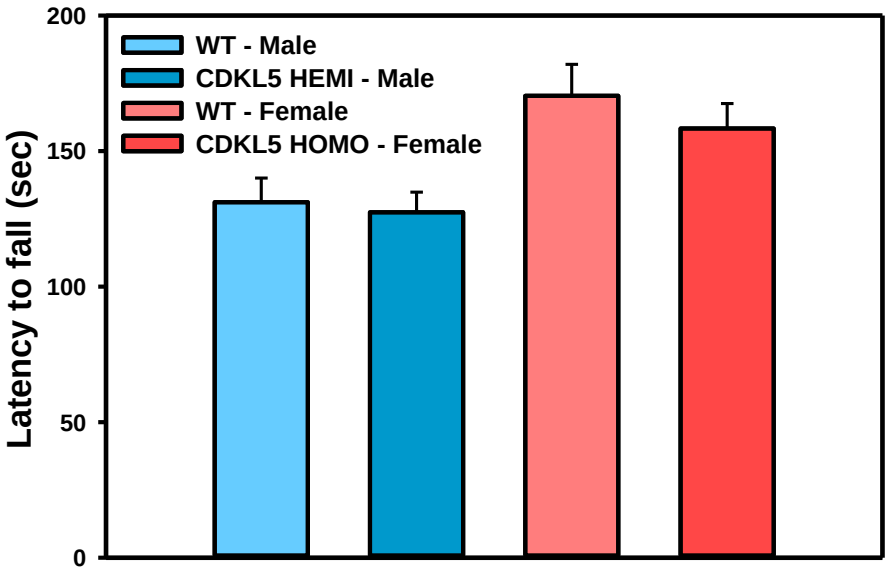


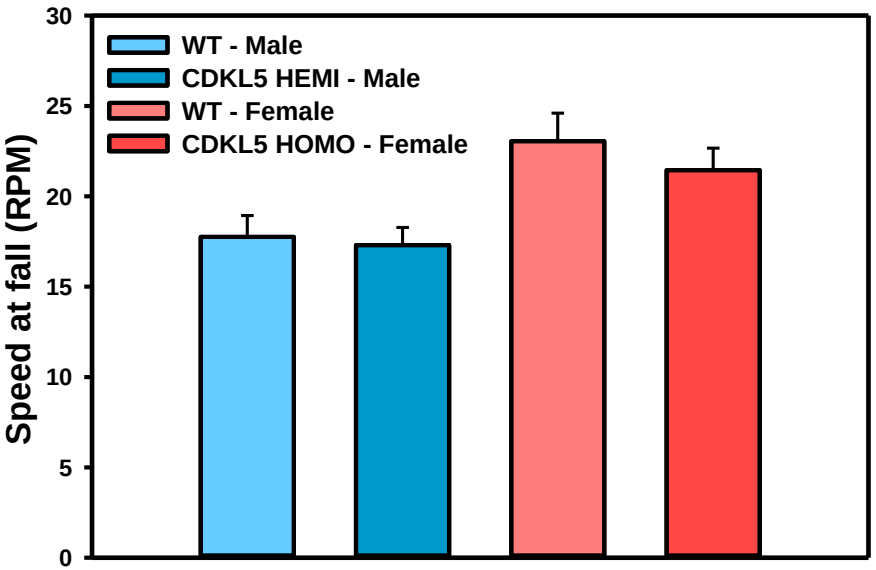
- Mice are placed on the rotarod apparatus consisting of a rod that rotates at a constant or variable and accelerating speed of 4 rpm. Once a mouse loses its balance and falls onto an underlying platform the timer automatically stops.
- Mice are exposed to the apparatus for 5 min training at a constant speed and placed back on the rod after each fall.
- After a rest period of at least 1 hr animals are placed back on the rotarod apparatus for testing. Once all animals in a test session are loaded on the rod, the rotarod apparatus is placed on accelerating speed (0-40 rpm) over 5 min and the time until the first fall is recorded. The test is repeated three consecutive times per animal.
- For each test session the RPM score at time of fall off the rod are recorded.

Behavioral Phenotyping: Rotarod

No significant genotype effect, however significant gender effect, whereby females stayed on rotarod longer and at higher speed than the males.

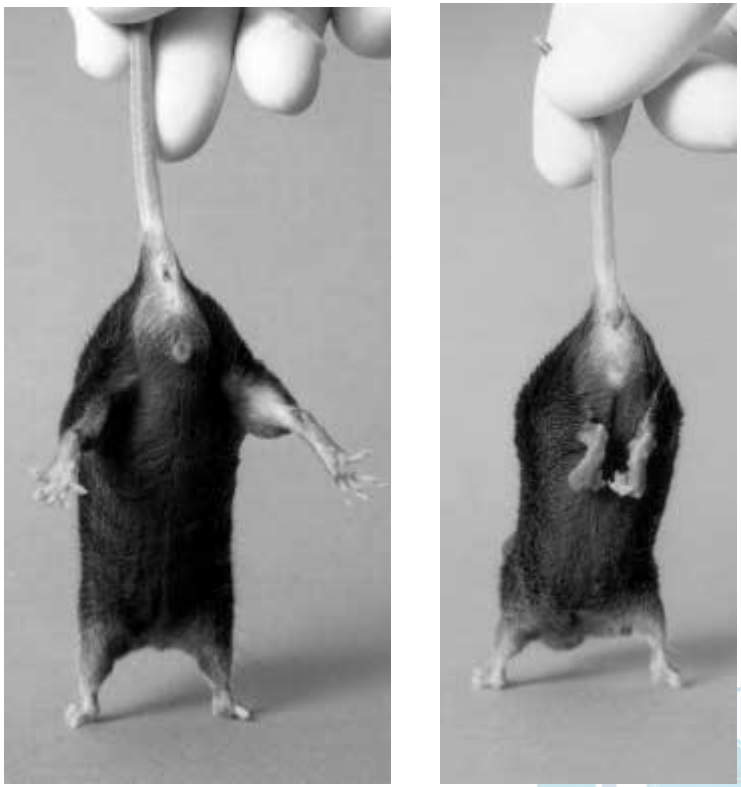
Age: 7.5 weeks old





Clasping

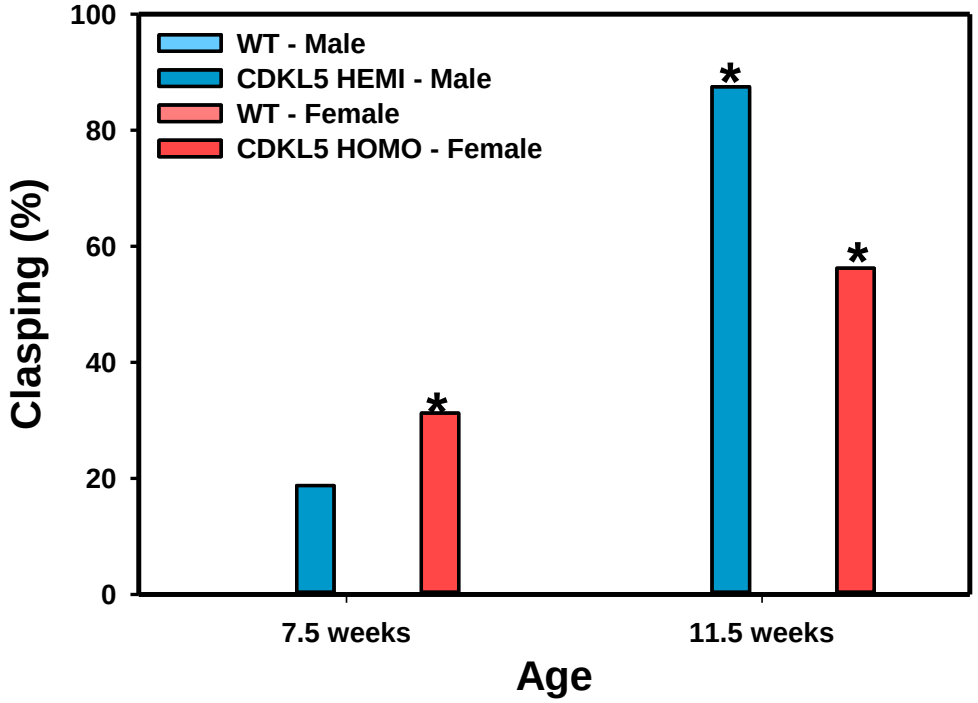
- Mice are lifted gently by the tail with front limbs placed just above surface.
- Clasping of hind legs is noted (normal is a spread in the hind legs)-



Example of splayed (left) and clasping (right) of hind legs

Clasping

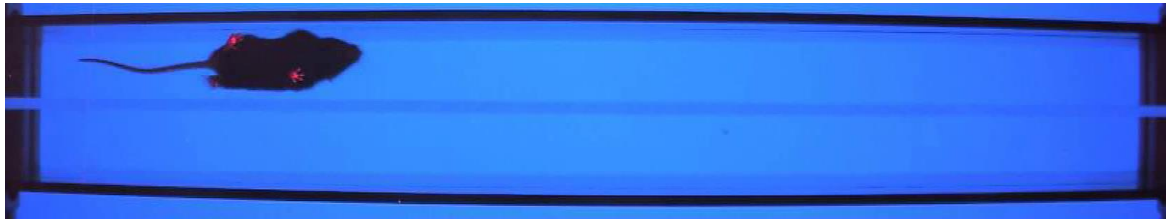
- CDKL5 mice show significantly higher clasping compared to WT mice for both gender at 11.5 weeks of age.
- At 7.5 weeks of age, female CDKL5 mice showed higher clasping compared to WT mice. The males at this age appeared to show higher clasping but this measure failed to reach significance ($p<0.075$)

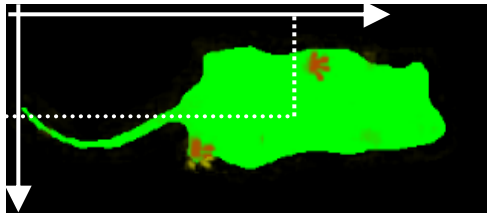


**p<0.05 compared to WT mice*

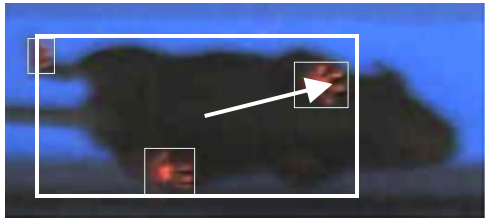
NeuroCube: Gait analysis system

- Measurements include
  - Gait geometry
  - Gait dynamics
  - Rhythmicity
  - Paw placement
  - Body motion
  - Non-gait behaviors

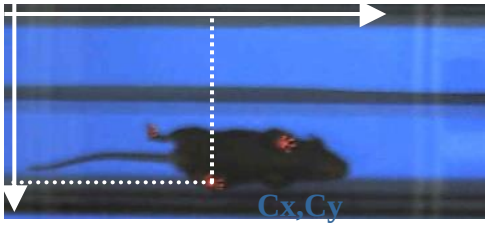




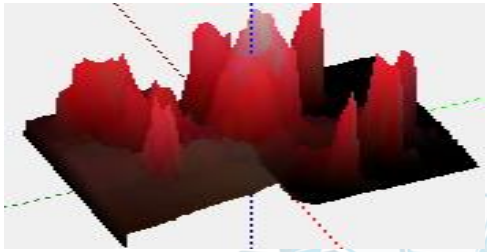
X & Y body coordinates



Paw directional vectors



X & Y paw coordinates



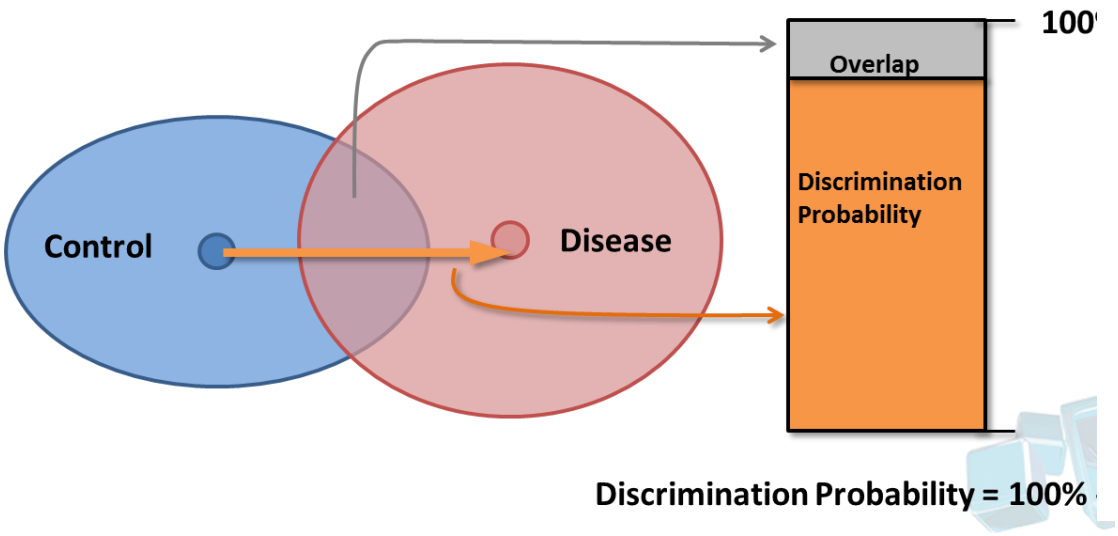
Paw pressure

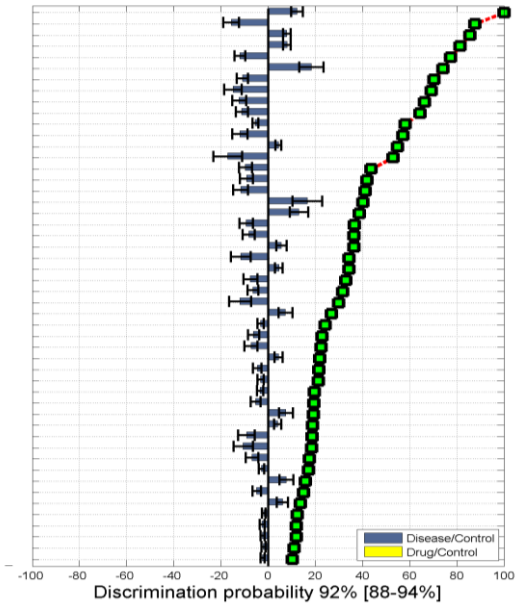
Additional Gait Features

- Gait Rhythmicity
  - Correlation coefficients between gait signals of each paw and all others: RF-LF, RF-LH, RF-RH, LH-RH, LH-RF, LF-RH, LH-RH.
- Paw positioning
  - Cluster coordinates relative to the center of the body,
  - Cluster shapes
  - Relative number of paw prints in each cluster
  - Relative clusters positioning
- Paw Features
  - Area, Perimeter, Semi-Axes, etc
- Body Motion Features
  - Various temporal characteristics of body contour changes

Cloud Analysis - Phenotype

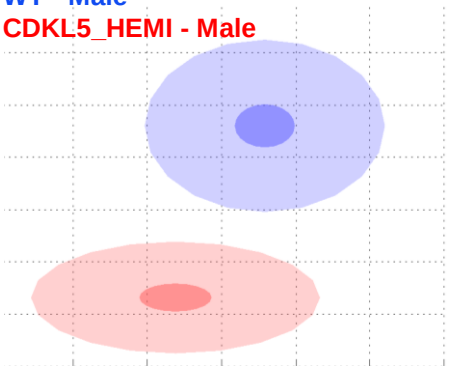
- Transform original feature set to the non-redundant de-correlated ranked features space
- Plot Control and Disease in the coordinate system formed by the two highest-ranked (best-discriminating between the two group's new features)
- Quality Measure of Disease Model = Overlap between the Control and Disease groups (*Discrimination Probability* = 100% - Overlap)



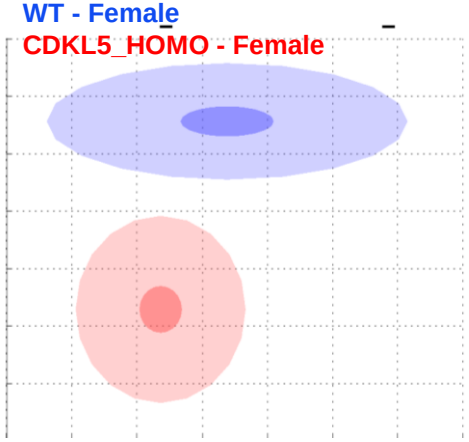


Gait changes using NeuroCube® System

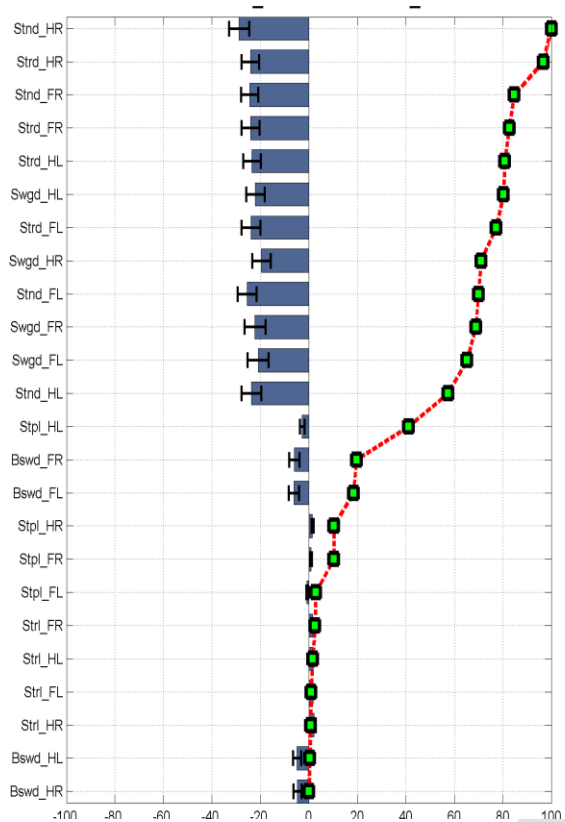
WT - Male
CDKL5_HEMI - Male



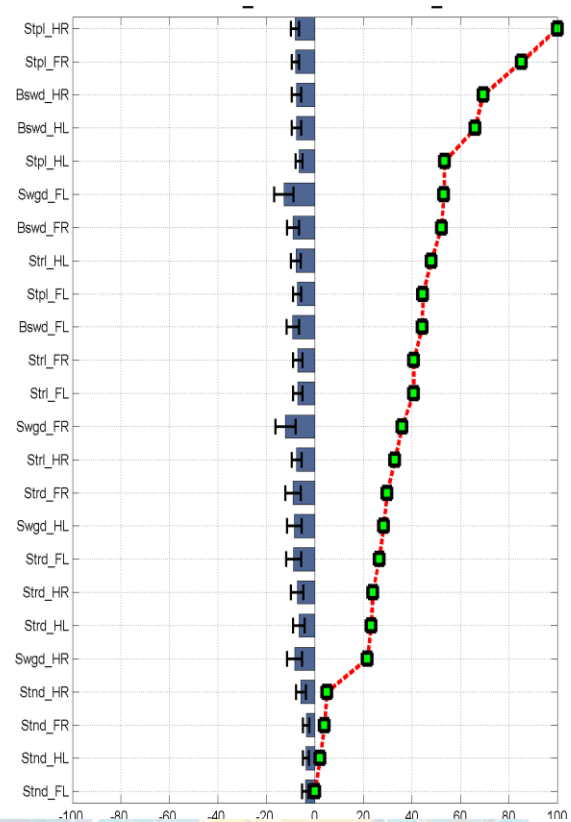
WT - Female
CDKL5_HOMO - Female



WT Male vs CDKL5 HEMI Male: 89% discrimination



WT Female vs CDKL5 HOMO Female 95% discrimination

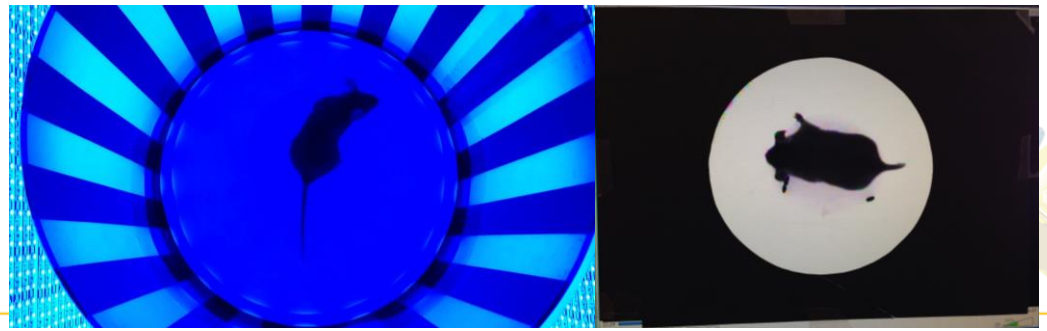


Discrimination between WT and KO mice in all gait measures

	WT v HOMO Female	*WT v HET Male*
Overall		
	93, *p*< 0	**91, p= 0**
GAIT		
	95, *p*= 0	**89, *p*< 0**
Paw Features		
	63, *p*> 0.21	58, *p*> 0.31
Correlation		
	81, *p*< 0.003	57, *p*> 0.44
Body Motion		
	82, *p*< 0.0006	**78, *p*< 0.005**
Paw Positioning		
	88, *p*< 0	**81, *p*< 0.0004**

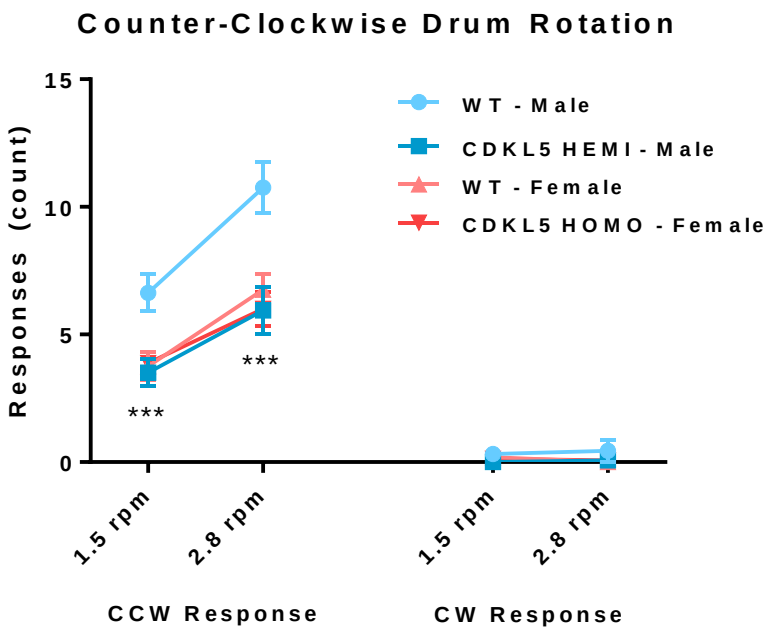
Optokinetic Response (OKR)

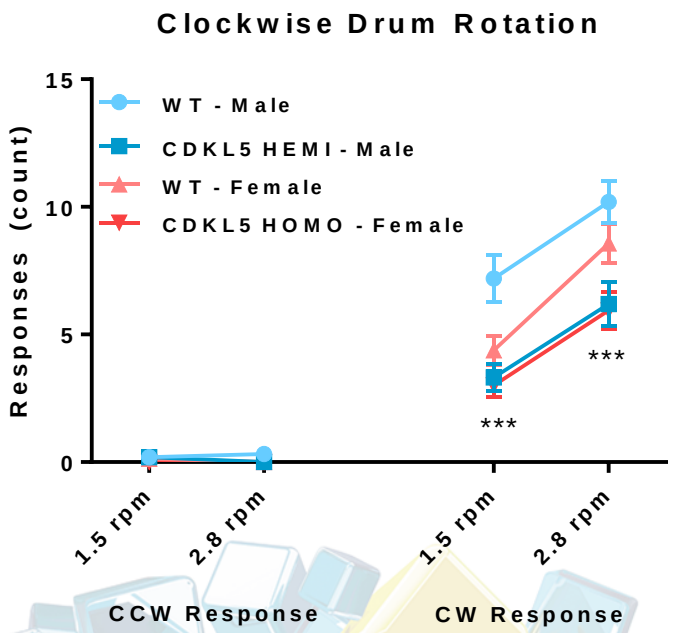
- The apparatus contains an elevated platform
- The day before testing, animals are habituated the to the testing apparatus for 5 minutes
- Mice are tested at 5 bar frequencies by changing the speed of drum rotation based on width of the stripes
  - 0 rev/min = 0 cycles per degree
  - 1.5 rev/min = 0.07 cycles per degree
  - 2.8 rev/min = 0.13 cycles per degree
- Mice are tested for 60 sec trials in clockwise and counter-clockwise directions for each bar frequency
- The optokinetic response and bar tracking are scored by an observer blind to the animal strain, bar rotation, and frequency.



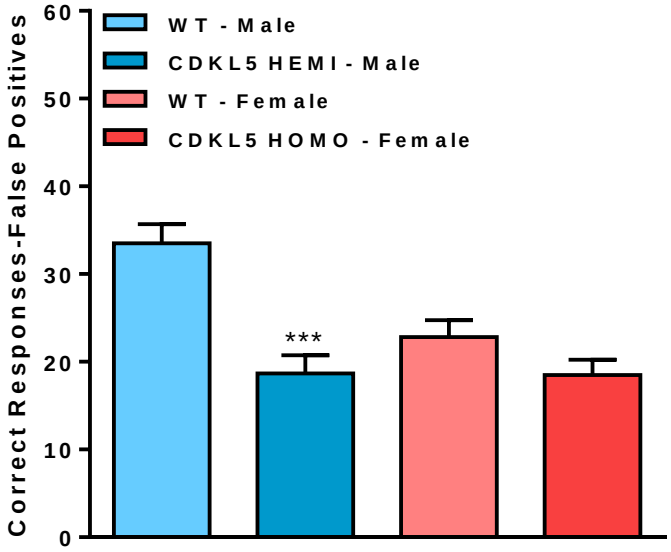
OKR Data

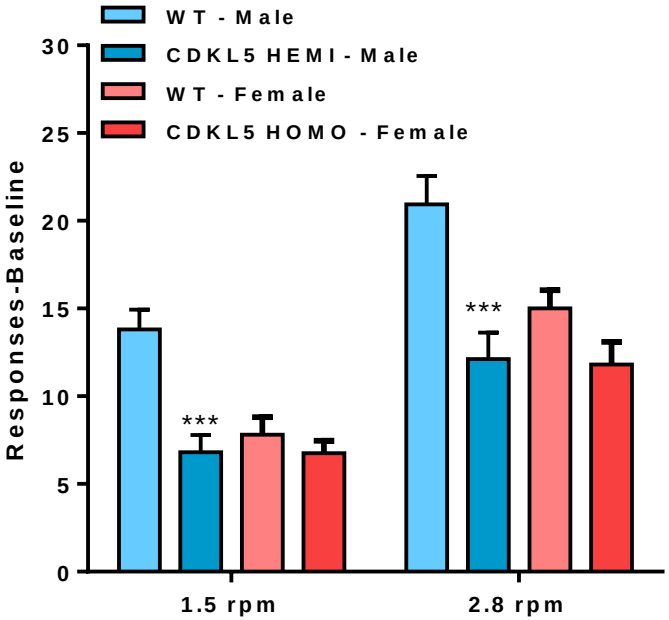
- *Among the WT group, there was a significant gender difference, whereby females showed fewer responses than male WT.*
- *Male CDKL5 KO mice, showed fewer responses than male WT mice at both drum speeds.*
- *Female CDKL5 KO mice, responded similar to female WT mice.*





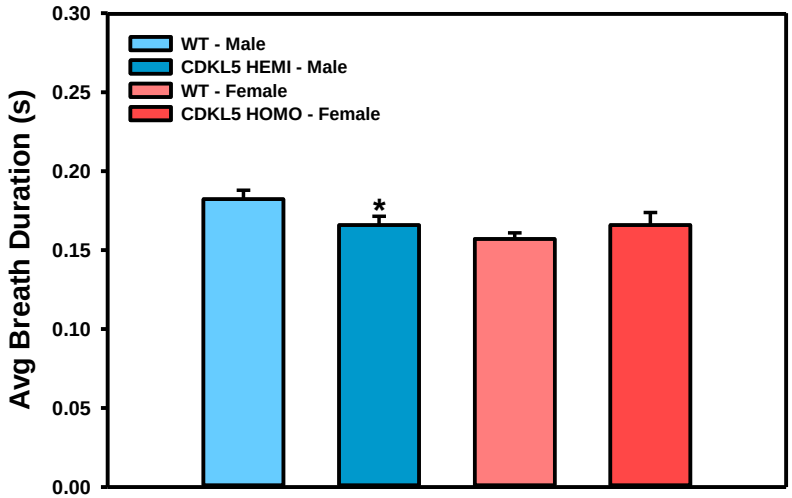
OKR Data

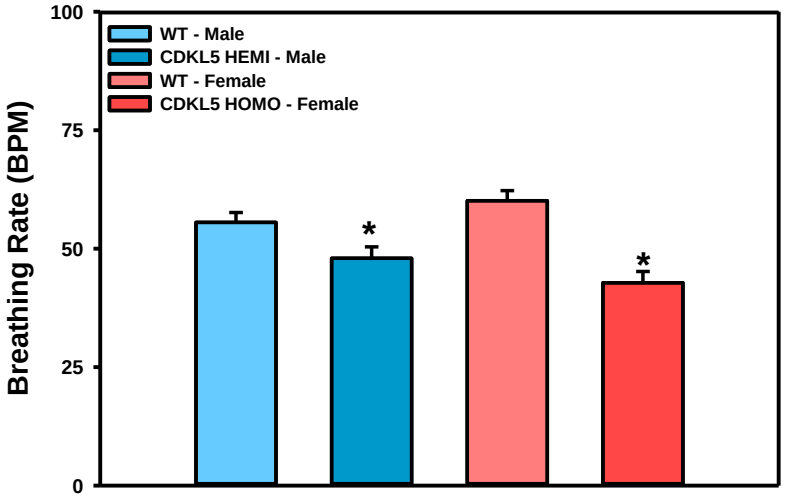

Correct Responses-False Positives
60
50
40
30
20
10
0
WT - Male
CDKL5 HEMI - Male
WT - Female
CDKL5 HOMO - Female
***

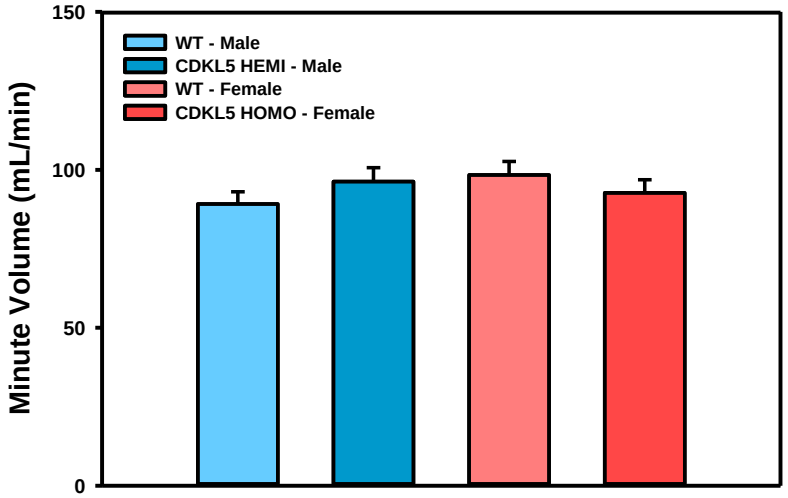

Responses-Baseline
30
25
20
15
10
5
0
WT - Male
CDKL5 HEMI - Male
WT - Female
CDKL5 HOMO - Female
***
***
1.5 rpm
2.8 rpm

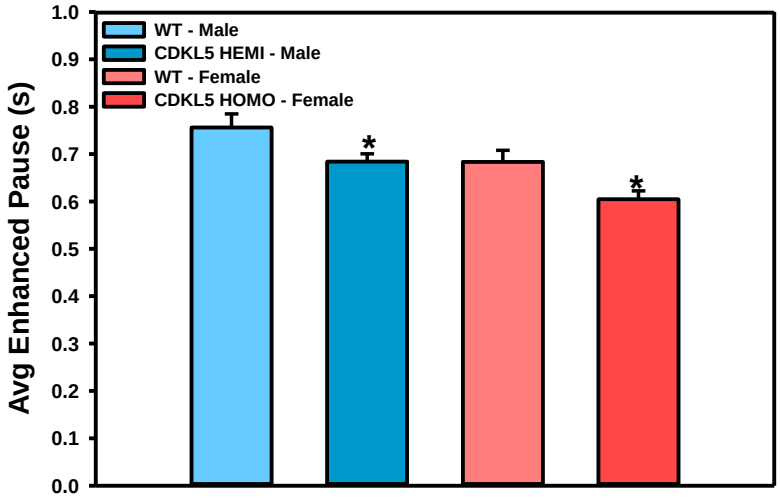
Respiration/Apnea:

CDKL5 KO mice, both male and females, showed decrease in respiration rate and enhanced pauses. In addition, Male CDKL5 mice had shorter average breath duration compared to male WT.









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Summary to date

- KO mice show clasping behavior compared to WT mice
- Gait differences in both male and female CDLK5 mice seen compared to WT mice. Differences in gait geometry, gait dynamics, rhythmicity, body motion and paw positioning .
- Deficits in Optokinetic response seen in male but no female KO mice
- No differences in rotarod performance between WT and CDLK5 mice.
- No differences in body weight between WT and CDLK5 mice. Male mice weighed more than female mice regardless of genotype
- Male and Female CDLK5 mice showed a decrease in respiration rate compared to WT mice.