PHENOTYPING OF A MOUSE MODEL OF CDKL5

Study Plan No.: IFCR001

Sponsor: International Foundation on CDKL5 Research

Testing Facility: PsychoGenics Inc.
765 Old Saw Mill River Road
Tarrytown, NY 10591, USA

PHENOTYPING OF A MOUSE MODEL OF CDKL5

The above-named study was conducted at PsychoGenics, Inc., an AAALAC accredited facility. Procedures were approved by the Institutional Animal Care and Use Committee in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Integrity of the data was ensured through a quality control process.

I, the undersigned, hereby declare that the work described in this report was performed under my supervision and that the report provides a true and accurate record of the results obtained. The study was performed in accordance with the agreed protocol unless otherwise stated. All eventual modifications to the original protocol have been clearly indicated. The present study was performed in accordance with PsychoGenics' Standard Operating Procedures.

Study Director

Taleen Hanania, Ph.D.

Name & Title	**Signature**	**Date**

Quality Assurance

Lucinda Thiede, M.S.

Name & Title	**Signature**	**Date**

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Coordinator and that the interpretations and conclusions drawn from the data are consistent with the results obtained.

President and CEO

Emer Leahy, Ph.D., MBA

Name & Title	**Signature**	**Date**

CONTENTS

1 OBJECTIVE

This study aimed to behaviorally phenotype a mouse model of CDKL5 and to identify behavioral tests that show the most robust differences between wildtype (WT) and mutant mice that can potentially be used for future drug screening.

2 MATERIAL AND METHODS

2.1 Animals

Male and female WT and B6.129 (FVB)-Cdkl5<tm1.1joez>/J mice were provided by Sponsor and received from the Jackson laboratory at ~ 5 weeks of age. Upon receipt, mice were group housed in OPTI rat ventilated cages with 8 mice (gender and genotype matched) per cage. All animals were acclimated to the colony room for at least 1 week prior to implanting with RFID chip. Mice were allowed to recover for a week prior to testing. Animals were examined on a regular basis, handled, and weighed to assure adequate health and suitability. Animals were maintained on a 12 /12 light/dark cycle. Room temperature was maintained between 20 and 23°C with a relative humidity maintained between 30% and 70%. Chow and water were provided *ad libitum* unless otherwise indicated. Testing was performed during the animal's light cycle phase.

2.2 Methods

2.2.1 Body Weights

Body weights were measured weekly throughout the study.

2.2.2 NeuroCube

The NeuroCube® system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain & neuropathies. This platform is unique for gait testing for the following reasons:

- It is completely automated and thus removes any bias or subjectivity
- This system captures both gait geometry and gait dynamics (stance, swing, propulsion, etc)

Mice were allowed to freely walk for 5 minutes in the NeuroCube® System. Digital videos of each mouse were captured and processed through computer segmentation algorithms. The resulting fitted parameters are then analyzed to extract clips of locomotor behavior from which gait geometry (stride length, step length and basewidth) and gait dynamics (stride duration, step duration and swing duration) are analyzed. The most dominant of the features collected that define the disease phenotype (symptom descriptors) was identified and ranked. Complex bioinformatic algorithms were employed to calculate the discrimination probability between the WT and CDKL5 mice. In addition, the following measures are analyzed;

1) **Average Speed:** measurement of average speed to travel the length of the NeuroCube

2) **Body Position**: using paw imaging parameters (below), measures X and Y body coordinates, X and Y paw coordinates, and paw directional vectors as they pertain to movement of the subject's body
3) **Gait:** measurements of geometry (e.g. Stride Length, Step Length, Base Width) and dynamics (e.g. Stride Duration, Step Duration, Swing Duration) of gait
4) **Imaging:** measurements of the paw contact area, perimeter of contact zone, and paw diameter (horizontal/vertical)
5) **Paw Position:** the position of each paw print relative to the center of the body is registered. The overlay of all recorded relative positions of the four paws creates four clusters of points (one for each paw). For each paw, the coordinate of the cluster center, its size, the number of paw prints, and relative geometry of clusters positioning are measured.
6) **Rhithmicity:** 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; (F – forelimb; H – hindlimb; R – right; L – left)

Data analysis from NeuroCube: The output of NeuroCube is a set of dozens of behavioral features that are submitted for analysis with machine learning techniques used in bioinformatics. Many of these features are correlated (e.g. rearing counts and supported rearing counts). Therefore, PGI forms statistically independent combinations of the original features (further referred to as de-correlated features) that discriminate between the two groups more effectively. Each de-correlated feature extracts information from the whole cluster of the original features, so the new feature space has lower dimensionality. Next, PGI applies a proprietary feature ranking algorithm to score each feature's discrimination power (ability to separate the two groups, e.g. control and disease). Ranking is an important part of the analyses because it weighs each feature change by its relevance: if there is a significant change in some irrelevant feature measured for a particular phenotype, the low rank of this feature will automatically reduce the effect of such change in the analyses, so we don't have to resort to the conventional "feature selection" approach and discard information buried in the less informative features. Ranking algorithm can be applied to either original or the new features to gain insight about the key control-disease differences (see Figure A).

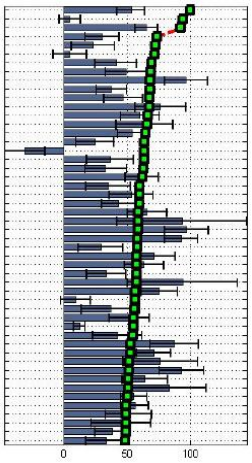

Figure A: *Difference in feature values and feature ranks (red curve with green squares). Relative difference (%) between feature values in two different sets is calculated and plotted in the order corresponding to feature ranks together with their ranks varying from 0 to 100%.*

Feature analysis: quantitative assessment of Disease Phenotype

In the new feature space, the overlap between the "clouds" (Gaussian distributions approximating the groups of mice in the ranked de-correlated features space) serves as a quantitative measure of separability ("distinguishability") between the two groups (see Figure B). For visualization purposes, we plot each cloud with its semi-axes equal to the one standard deviation along the corresponding dimensions.

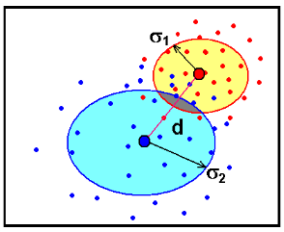

Figure B: *Visualization of binary discrimination in the ranked de-correlated feature space. The two highest ranked decorrelated features are chosen to form the 2D coordinate plane for visualization purposes. Each dot represents a mouse. Mice from the control group are shown as blue dots and mice from the disease group are plotted in red. The other convenient (from a scale perspective) but equivalent measure derived from the cloud overlap is discrimination probability = 1-overlap, which measures how reliably a classifier can be trained to discriminate between groups A and B above the chance level, zero corresponding to 100% overlap and no ability to distinguish the two groups above the chance level, whereas 100% meaning error free discrimination.*

2.2.3 PhenoCube

Apparatus: The PhenoCube® system consists of extensively customized Intellicage boxes (New Behavior AG) fitted with proprietary video analysis equipment.

Protocol: Animals were evaluated in a 72 hour test session. After a 16 hour water deprivation period in the home cage, mice were placed in the PhenoCube cages with their home cage-mates. The PhenoCube cages were maintained on a 12:12 light:dark cycle, with white light during the day and red light during the night, maintaining a low subjective light level for the subjects during the night period. While inside the cage, water was only available from within the PhenoCube corners, while food is freely available on the cage floor at all times. Mice were left undisturbed during the course of experimental sessions. Eight mice were tested in each box

In test sessions, the mice initially underwent a Habituation protocol, allowing them to freely retrieve water from the PhenoCube corners. In the Habituation phase all four of the PhenoCube corners are open, with both doors to water opening as soon as any mouse enters and remaining open until the mouse leaves the corner. This phase of the experiment is essentially what is typically described as "magazine training" and allows the mice to learn that water reinforcement is available in the corners of the PhenoCube; this type of preliminary training is widely employed in reinforcement-based animal behavioral studies

Prior to lights-out on day 1, after 6 hours in the cage, the protocol was switched to a training protocol described as 'Alternation', requiring the animals to visit specific locations to retrieve water and to alternate between potentially reinforced locations. For each subject, visits to two adjacent corners (active corners) along one of the shorter sides of the rectangular cage are contingently reinforced, while visits to the other two (exploratory corners) are never reinforced. The Alternation protocol trained the animals to switch between the two active corners, only receiving reinforcement for correctly nosepoking while alternating corners. For example, if Corners 1 & 2 were active, an initial visit to Corner 1 would be a correct visit and reinforced with a correct nosepoke. To obtain further reinforcement, the mouse was then required to visit Corner 2 and nosepoke correctly. Repeat visits to Corner 1 are classified as incorrect, and mice would not receive a reward. Following a visit to Corner 2, the corners would switch again, such that reinforcement would be available only in Corner 1 and so on. If a mouse incorrectly visits the same active corner twice or visits an exploratory corner after leaving an active corner, the identity of the target correct corner does not change and a visit to that corner will result in available reinforcement; the only event leading to a switch in the correct

corner identity is a visit to the currently correct corner, in which reinforcement will be available. The following was captured

- Computer vision summary data includes
  - *Activity measures:* locomotion, supported rearing, climbing
  - *Inactivity measures:* immobile, overall immobility
  - *Social measures:* approaches, interactions, and chasing/following behaviors
- Overall visit frequency: Overall measurement of activity
- Percentage of visits to active corners: Measurement of "water seeking"
- Percentage of overall repeat visits: Measurement of perseverative behavior
- Percentage of alternations made

2.2.4 Rotarod

Mice were taken to the experimental room and placed on the rotarod apparatus. The rod rotates at a constant or variable and accelerating speed of 4 rpm. Once a mouse lost its balance and fell onto an underlying platform the timer automatically stopped. The latency time in sec that it took for an animal to fall or the endurance time for each mouse was automatically recorded by the equipment. Mice were exposed to the apparatus for 5 min training each time tested at a constant speed and placed back on the rod after each fall. After a rest period of at least 1 hr animals were placed back on the rotarod apparatus for testing. Once all animals in a test session were loaded on the rod, the rotarod apparatus was placed on accelerating speed (0-40 rpm) over 5 min and the time until the first fall was recorded. The test was repeated three times for each mouse.

2.2.5 Clasping

Clasping is used to assess muscular strength in limb muscles. Mice were held by the tail and gently lifted until the front paws just lift off the counter surface. The experimenter observed the legs and determined clasping or splaying of limbs. After testing, animals were placed back into the test or home cage. Percent of mice showing clasping of the hindlimbs was determined.

2.2.6 Optokinetic Response

Rationale: This method depends on the automatic visual response of head-tracking to a moving vertical stripe pattern presented on a rotating drum to an animal placed at its center. The movement of the head is measured in synchrony with the stripe movement. Although the test measures 'relative acuity', it has considerable utility as a repeatable, non-stressful and reproducible measure of visual function.

Apparatus: This device consists of a stationary holding platform that is surrounded by a rotatable cylinder with interchangeable panels covered with a vertical stripe pattern. The mouse tracks the rotation of the grating with reflexive head movements recorded via a camera mounted above the apparatus. Visual sensitivity can be quantified by increasing the spatial frequency of the grating.

Protocol: Mice were 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 was 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). Maximum testing time was set at 30 minutes per session.

Measures: Number of responses in the direction of the drum and opposite to the direction of the drum at each speed was recorded.

2.2.7 Respiration

Mice were tested using a specialized whole body plethysmographs for the measurement of ventilation in conscious animals (Data Sciences International item 601-1425-001). Using this technique, the animal was unrestrained and able to freely move about the chamber. The chamber was also continually monitored for temperature and humidity measures. The flooring of the chamber is perforated for the passage of urine and feces. Mice were habituated to the chamber for ~15 min and then evaluated for differences in breathing patterns using a whole body plethysmograph for 30 minutes.

2.2.8 Fear Conditioning

Mice were placed into the conditioning chambers (Coulbourn Instruments, PA, USA) for 120 sec where they were exposed to a 6kHz, 75 dB tone (conditioned stimulus, CS) for 10 sec. During the last two seconds of the tone, mice received a foot shock (0.6mA), the unconditioned stimulus (US). Pairing of the CS and US was repeated for a total of 3 times, with random interval of >90s. The mouse is removed from the test chamber 2 minutes after the final foot shock. The test was conducted 24 hr following training session. The mouse was placed into the same training chamber it was trained in for a period of 6 min without shock or any other interference.

2.2.9 Audiogenic Seizures

Half the mice were tested in audiogenic seizures. Mice were individually placed in a Plexiglas chamber and allowed to explore for 15 sec following which they were exposed to a 125 dB tone for 2 minutes, followed by 1 minute of no sound, and then a further 2 minute tone. The mice were scored based on their response, latency, and seizure intensity:

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

The following endpoints were reported. Animals exhibiting no response were given latency scores of 300 sec for data analysis purposes:

1. Latency to seize (max 300 sec if no seizure)
2. Latency to respiratory arrest (max 300 sec if survival)
3. Seizure score
4. Percent survival

5. Percent seized

2.2.10 PTZ-induced Seizures

The remaining half of the mice were tested in PTZ-induced seizures Mice were brought into the experimental room and allowed to acclimate for a minimum of 1 hr prior to testing. Mice were injected with PTZ (60 mg/kg) followed by the observation of seizure profile and latency times to twitches and seizures. The latency time to first twitch, latency to first clonic/tonic seizure greater than 5 sec duration with loss of the righting reflex, latency to first full tonic seizure (animal is on its side with hind limbs pulled up then quickly stretched backwards in a tonic extension), and latency time to death were recorded.
Seizure profiles are described through 60 min post PTZ treatment, evaluated every 10 min and assessed and ranked for severity of seizure:

- Rank 0 = No motor seizure.
- Rank 1 = Staring, move into prone posture, ears flattened, mouth of facial movement, slight tremor, small movements, slight intermittent twitch, tail elevated, increased respiration and ataxia.
- Rank 2 = Prone, immobility, hind limb splay, increased tremor intensity, absence seizure, ataxia, head not/bow, isolated body twitches, straub or elevated tail and tail wag.
- Rank 3 = Slight clonic/tonic seizures less than 5 sec duration, muscle fasciculation, straub tail, tonic praying seizure and salivation.
- Rank 4 = Rearing, pawing, chomping, head bow, head arch, salivation, absence seizure, praying seizure, trmp0et seizure, pop seizure, clonic/tonic seizure with increased frequency and duration.
- Rank 5 = Clonic/tonic seizure greater than 5 sec duration with loss of righting reflex.
- Rank 6 = Crazy seizure, tonic seizure with extension of hind limbs and death.

Mice were euthanized after the test was completed.

2.2.11 Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) or t-test followed by post-hoc comparisons when appropriate. An effect was considered significant if $p < 0.05$.

3 RESULTS

3.1 Body Weight

The body weight of the mice throughout the study is shown in Figure 1. Repeated measures ANOVA found a significant genotype and gender effects as well as significant age effects. All mice steadily gained weight throughout the study. Post hoc analysis showed that male mice (WT or CDKL5) had significantly higher BW compared to female mice throughout the study. Within female mice, WT mice had significantly higher BW than the CDKL5 Homo mice only at 5.5 weeks of age. Within male mice, WT mice had significantly higher BW than the CDKL Homo mice at the early age of 5.5 weeks and then later on at 12.5 and 13.5 weeks of age.

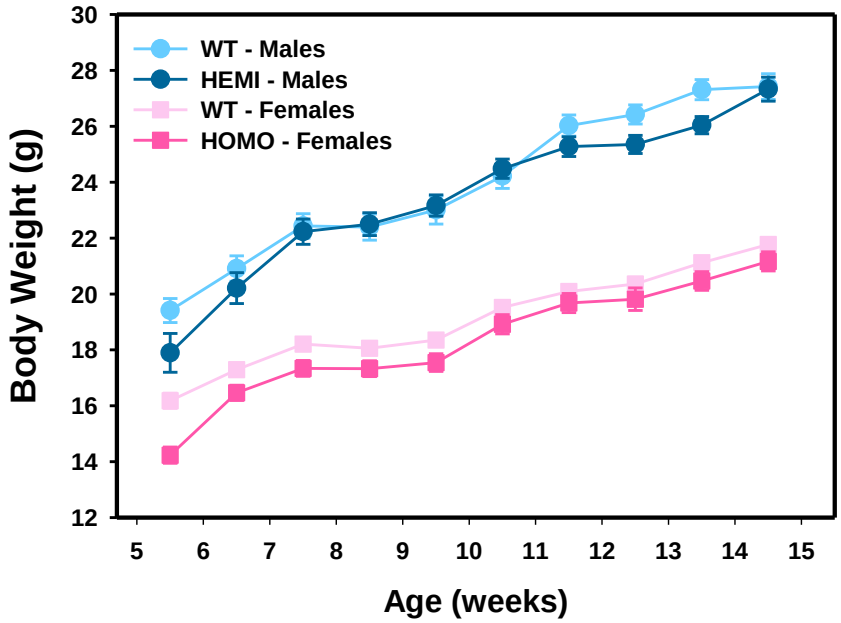

Figure 1**:** *Body Weights of mice prior to testing. Data are presented as mean ± SEM.*

3.2 Rotarod

Performance of all the mice on the rotarod is shown in Figure 2. Two-way ANOVA found a significant gender difference but no significant genotype difference in rotarod performance. Female mice stayed longer on the rotarod and fell at higher speeds compared to male mice.

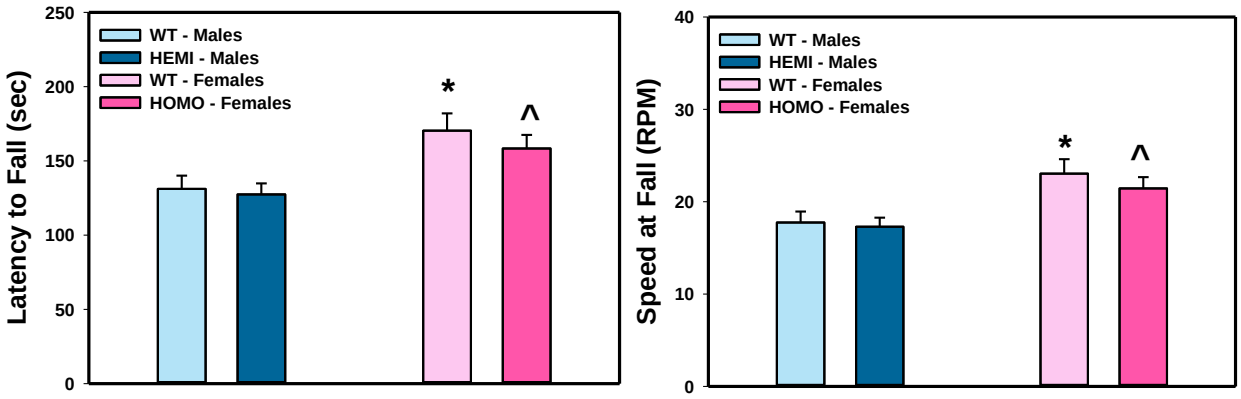

Figure 2**:** *Left: Latency to fall from the ratorad. Right: Speed of the rotarod at fall. Data are presented as mean ± SEM. *p<0.05 compared to WT males; ^p<0.05 compared to HEMI males*

3.3 Clasping

Mice were evaluated for clasping at 7.5, 11.5 and 13.5 weeks of age (Figure 3). WT mice (either male or female) showed no clasping at both ages. Male and female CDKL5 mice showed significant clasping behavior compared to their WT counterparts at 11.5 and 13.5 weeks of age. In addition, female CDKL5 mice showed significant clasping behavior compared to female WT mice at 7.5 weeks of age.

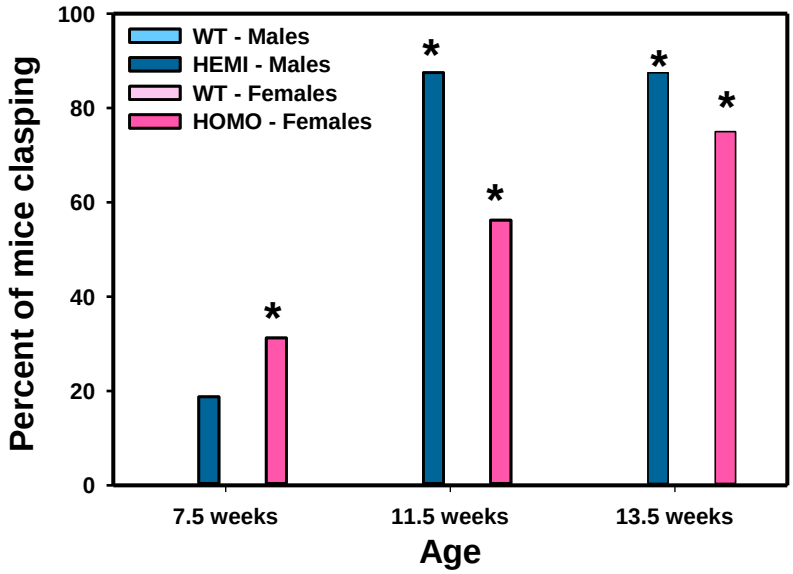

Figure 3**:** *Percent of mice showing clasping behavior at 7.5, 11.5 and 13.5 weeks of age. *p<0.05 compared to WT mice.*

3.4 Respiration

3.4.1 Average Breath Duration

Average breath duration of full breaths is shown in Figure 4. Two-way ANOVA found a significant gender effect and a gender x genotype interaction. Compared to WT males, WT female mice had significantly higher breath duration. In addition Hemi male mice showed a strong trend to having shorter breath duration (p=0.056).

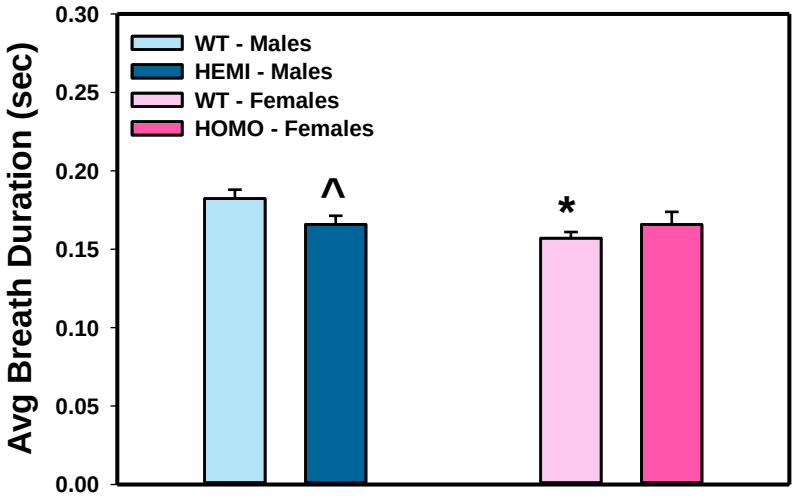

Figure 4**:** *Average breath duration of WT and CDKL5 mice. Data are presented as mean ± SEM. *p<0.05 compared to WT male mice; ^p=0.056 compared to WT male mice.*

3.4.2 Breathing Rate

Breathing rate of the mice is shown in Figure 5. Two-way ANOVA found a significant genotype effect and a gender x genotype interaction. Male and female CDKL5 mice showed significantly lower breathing rate compared to their WT counterparts.

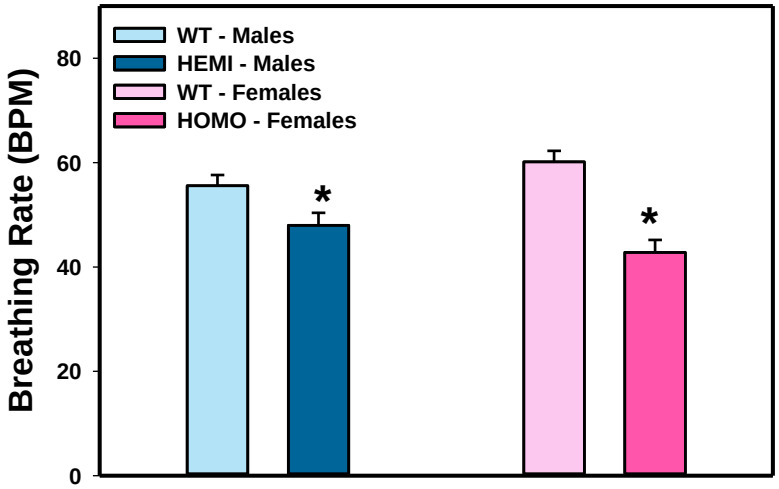

Figure 5*: *Breathing rate of WT and CDKL5 mice. Data are presented as mean ± SEM. *p<0.05 compared to WT counterparts.

3.4.3 Enhanced Pauses

Enhanced pauses, a measure of bronchoconstriction, are shown in Figure 6. Two-way ANOVA found a significant genotype effect and a significant gender effect. Male and female CDKL5 mice showed significantly lower enhanced pauses compared to WT mice. In addition, female mice (WT and CDKL5) had significantly less enhanced pauses compared to their male counterparts.

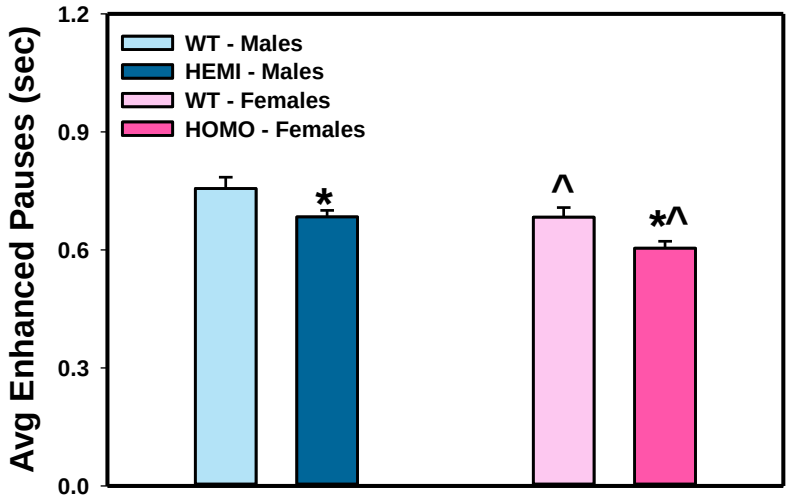

Figure 6*: *Average enhanced pauses of WT and CDKL5 mice. Data are presented as mean ± SEM. *p<0.05 compared to WT counterparts, ^p<0.05 compared to male mice.

3.4.4 Minute Volume

The minute volume which measured ventilation rats is shown in Figure 7. Two-way ANOVA found no significant genotype or gender effects/

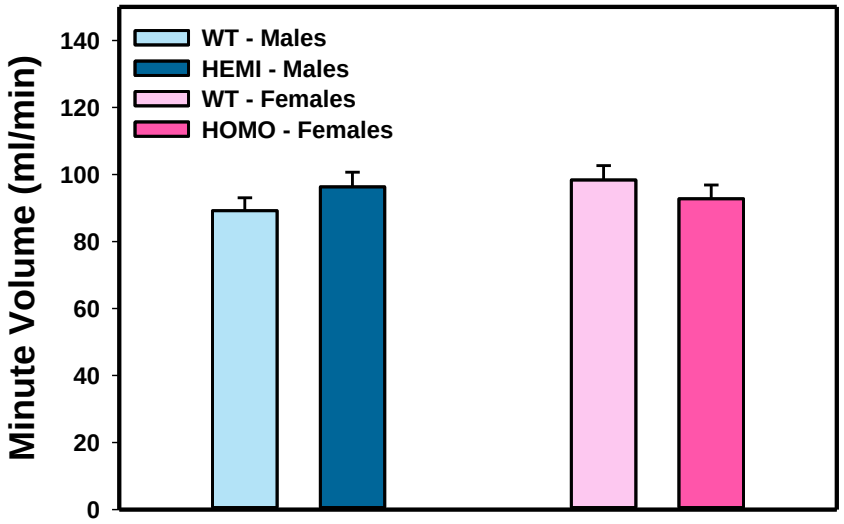

Figure 7*: *Average enhanced pauses of WT and CDKL5 mice. Data are presented as mean ± SEM.

3.5 Gait Analysis using the NeuroCube® System

The NeuroCube® System is a platform that assesses motor performance. Using proprietary computer vision and machine learning algorithms, it automatically tracks every locomotion detail and measures parameters of gait geometry, gait dynamics as well as non-locomotion behaviors.

The discrimination plots of CDKL5 male and female mice versus WT mice are shown in Figure 8 A and B, respectively. WT and CDKL5 female mice showed 95 % discrimination and WT and CDKL5 male mice showed 89% discrimination.

Analysis of highest ranking gait feature indicated that male CDKL5 mice have differences in gait dynamic measures including shorter stance duration, swing duration and stride duration compared to WT mice. On the other hand, analysis of the highest ranking gait features found that female CDKL5 have differences in gait geometry (shorter step length, stride length and base width) and to a lesser extent gait dynamics (shorter swing duration and stride duration) compared to WT mice. Additionally male CDKL5 mice showed increased average speed compared to WT mice.

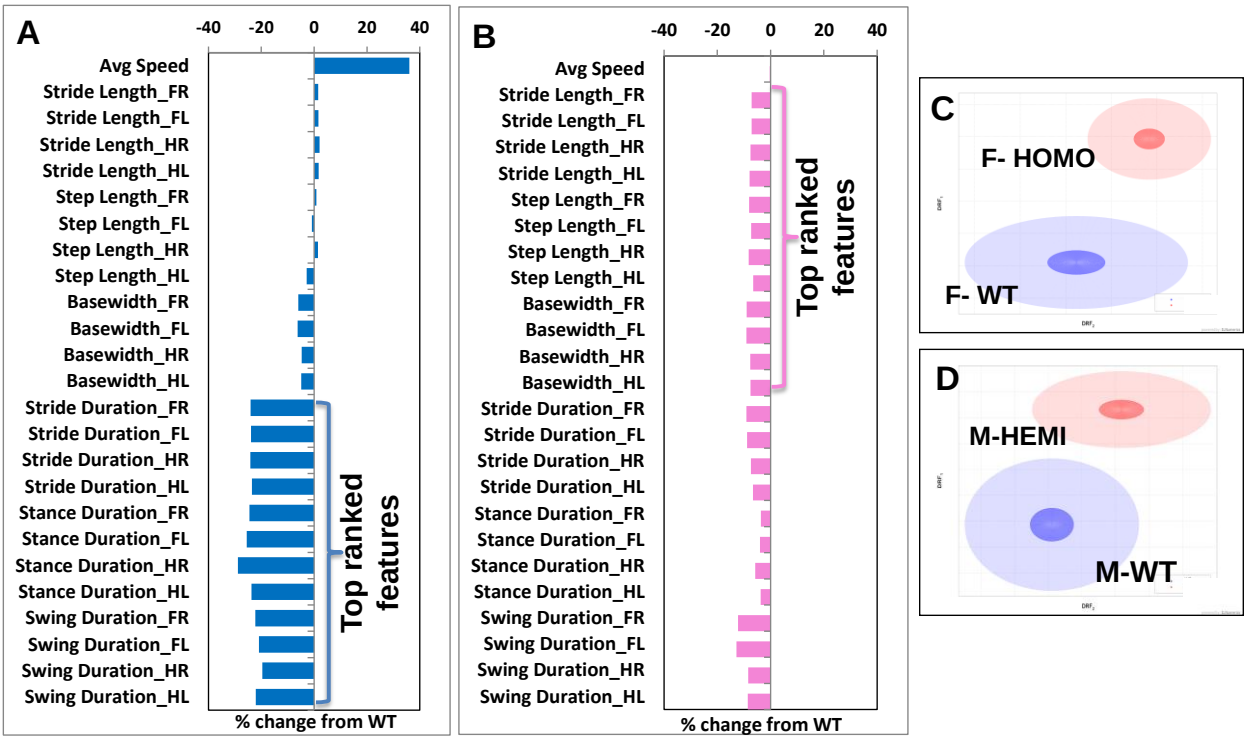

***Figure 8*: *A & B**: percent change of the various gait features in CDKL5 male and female mice compared to WT, respectively. **C &D:** cloud plots showing the degree of separation between female and male WT and CDKL5 mice, respectively, in gait measures.*

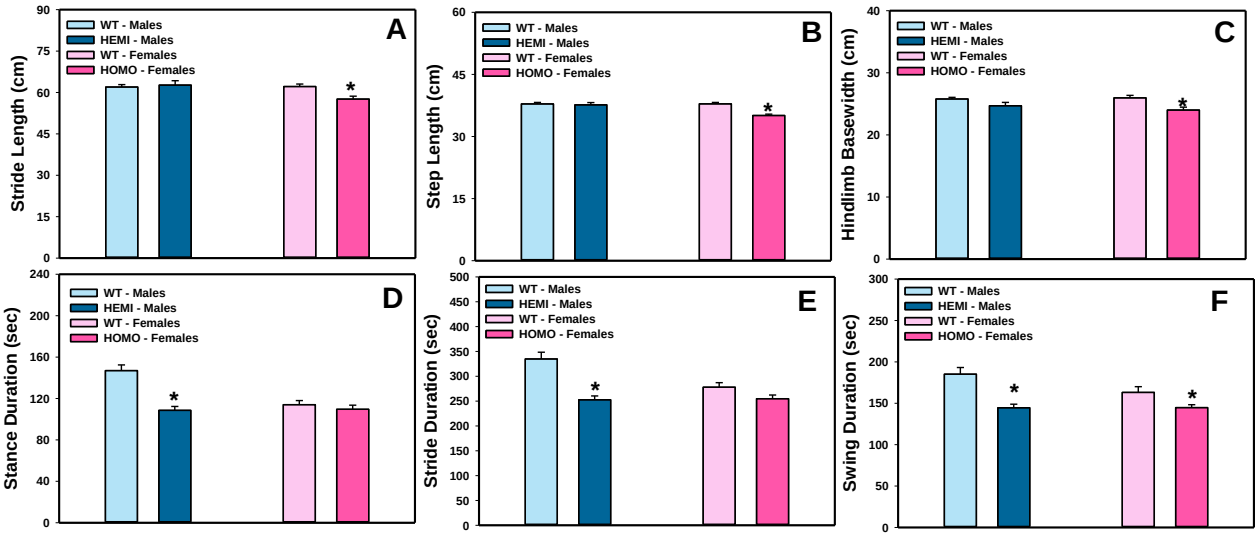

Figure 9*: *gait geometry: stride length (A), step length (B) and basewidth (C) and gait dynamic stance duration (D), stride duration (E) swing duration (F) measures. Data are presented as mean ± SEM.*P<0.05 compared the respective WT mice.

3.6 Optokinetic Response

The optokinetic responses for the WT and CDKL5 mice are shown in Figure 10. WT male mice showed significantly higher responses at both drum speeds compared to female mice and compared to CDKL5 male mice regardless of direction of drum rotation. WT female mice showed significantly higher clockwise responses at both drum speeds compared to CDKL5 mice when drum rotation was clockwise only. All mice showed higher responses in the direction of the drum rotation (i.e, if drum rotation was clockwise then clockwise responses where higher than counterclockwise responses and vice versa)

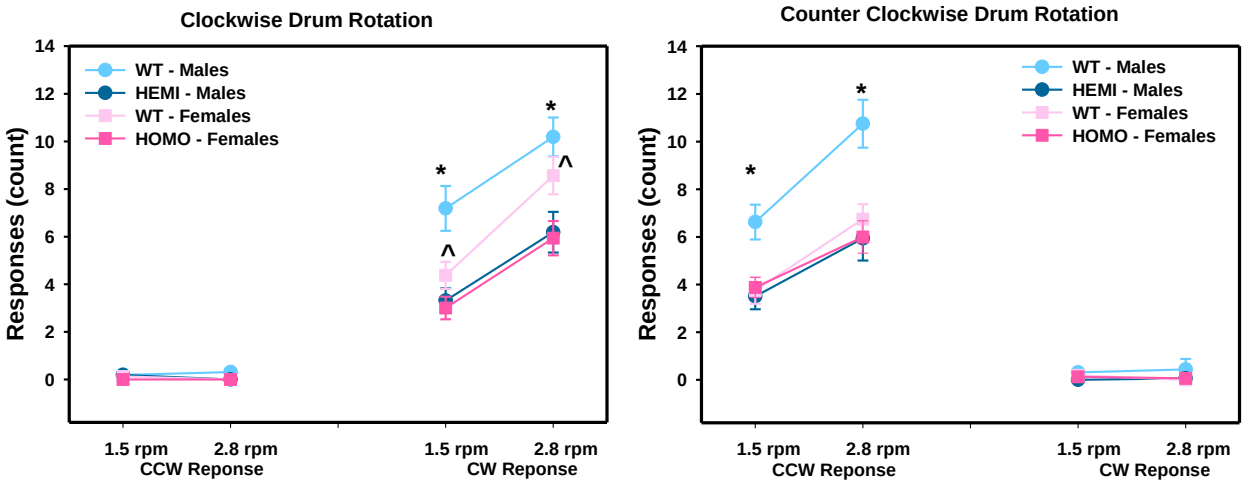

Figure 9*: *OKR responses in WT and CDKL5 mice in the direction of and opposite of drum rotation when rotation was clockwise (Left) or counter clockwise (Right). *P<0.05 compared to all mice. ^P<0.05 compared to female and male CDKL5 mice.

The total number of correct response at both drum speeds combined or at each individual speed is shown in Figure 10. Two-way ANOVA found a significant genotype and gender effect and a genotype x gender interactions. Compared to female mice (WT and CDKL5), WT male mice had significantly higher correct responses. Similarly WT mice had significantly higher correct responses compared to CDKL5 male mice. Among the female mice no significant differences were seen between WT and CDKL5 mice.

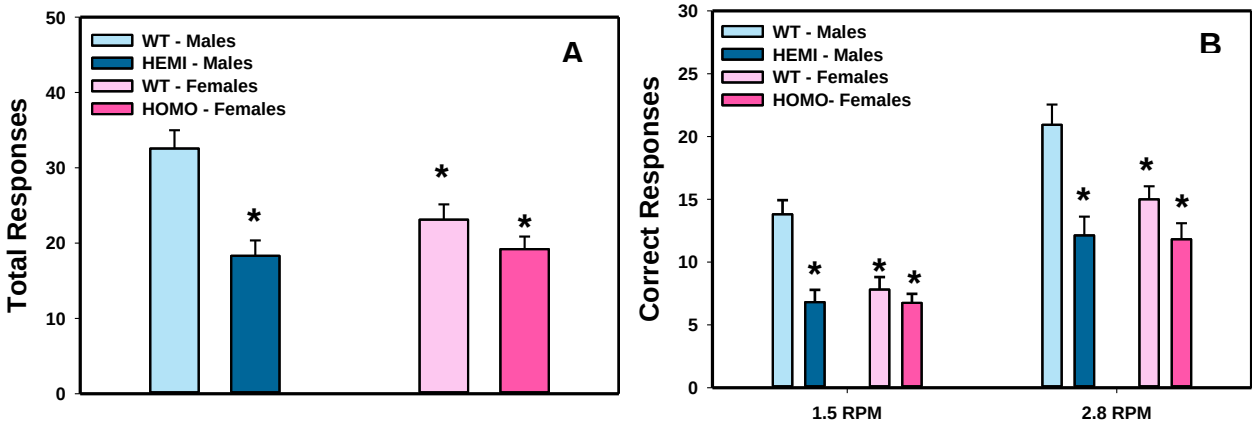

Figure 10**:** *Total correct OKR responses of WT and CDKL5 mice.* ***A:*** *combined responses at both drum speeds.* ***B:*** *correct responses at each drum speed. Data are presented as mean ± SEM.*P<0.05 compared to WT male mice.*

3.7 Fear Conditioning

3.8 Cued Fear Conditioning

Percent freezing during the cued fear condition test is shown in Figure 11. Repeated measures ANOVA a significant genotype effect. Prior to the presentation of the conditioned stimulus (CS), the freezing behavior of all the mice was low and no significant differences were find between the WT and CDKL5 mice. During the tone presentation freezing increased in all mice. WT mice had significantly higher freezing response compared to CDKL5 mice. Similarly female WT mice showed a trend to higher freezing compared to the CDKL5 female mice. Post CS, freezing was decreased but WT mice (males and females) maintained a higher freezing response compared to CDKL5 mice.

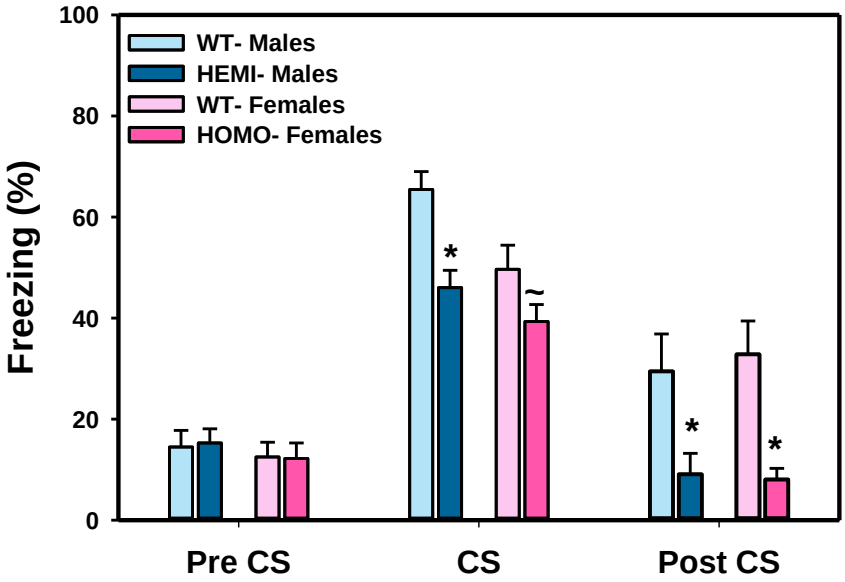

Figure 11**:** *Percent freezing during the cued fear conditioning test prio to, druing and post presentation of the conditioned stimulus. Data are presented as mean ± SEM.*P<0.05 compared to WT mice; ~p=0.06 compared to WT female mice.*

3.9 Contextual Fear Conditioning

The time course for % freezing is shown in Figure 12A and the average percentage of freezing during the 6 minutes test is shown in Figure 12B. Two-way ANOVA found a significant gender and genotype effects. Compared to WT mice, CDKL5 mice showed significantly less freezing behavior. Among the male mice the differences between WT and Hemi mice was mostly seen during minutes 3 and 4 of the test and among the female mice the differences between WT and Homo mice was mostly seen during minutes 3 and 5 of the test.

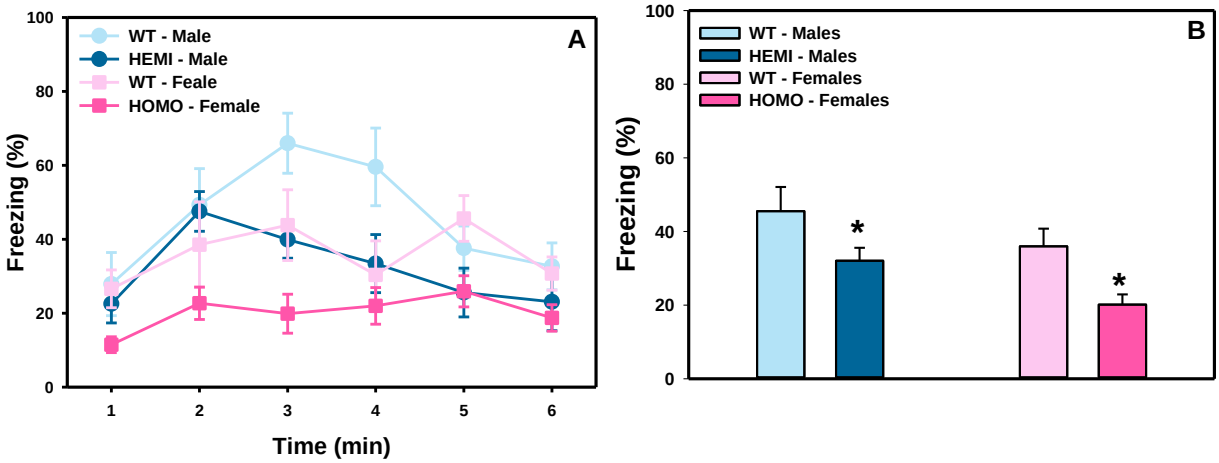

Figure 12A**:** *Time course for freezing in the contextual fear conditioning test.* ***B:*** *percent freezing averaged over the 6 minute test. Data are presented as mean ± SEM.*P<0.05 compared to WT mice*

3.10 PTZ-Induced Seizures
3.10.1 Latency to First Twitch

The latency to first twitch following PTZ injection is shown in Figure 11. Two-way ANOVA found no significant genotype or gender effect on this measure

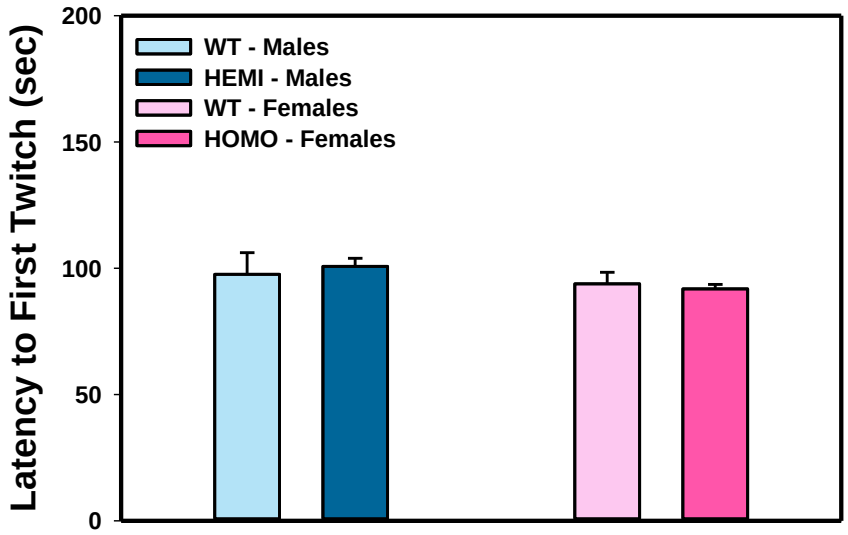

Figure 11*: *Latency to first twitch following PTZ injection. Data are presented as mean ± SEM.

3.10.2 Latency to Clonic Tonic Seizures

The latency to clonic tonic seizures following PTZ injection is shown in Figure 12. Two-way ANOVA found a significant genotype but no gender effect. Compared to WT mice, CDKL5 mice had significantly longer time to develop clonic/tonic seizures.

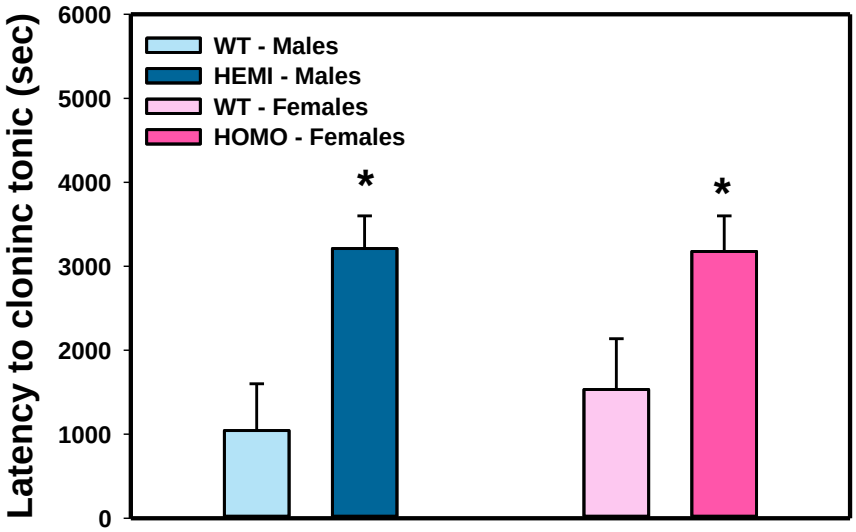

***Figure 12*:** *Latency to clonic tonic seizures following PTZ (60 mg/kg) injection. Data are presented as mean ± SEM.*P<0.05 compared the WT mice.*

3.10.3 Latency to Tonic Seizures

The latency to tonic seizures following PTZ injection is shown in Figure 13. Two-way ANOVA found no significant genotype or gender effect on this measure

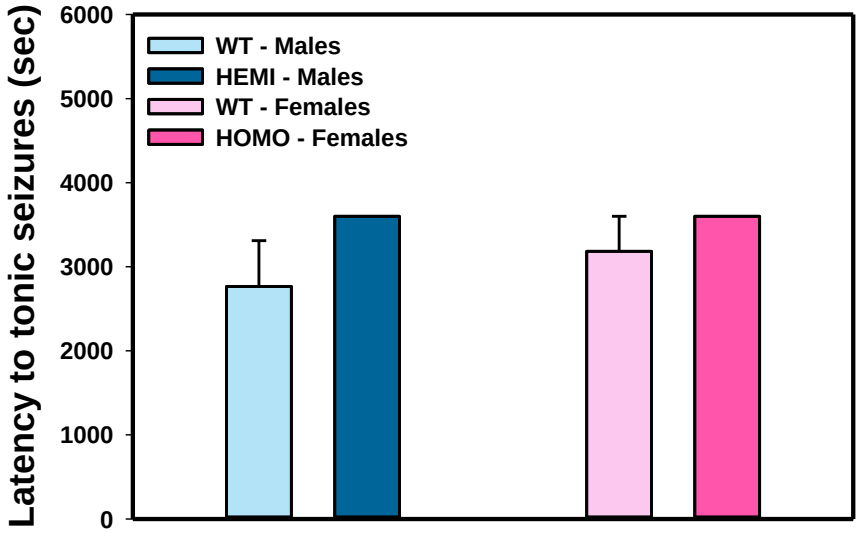

***Figure 13*:** *Latency to clonic/tonic seizures following PTZ (60 mg/kg) injection. Data are presented as mean ± SEM.*

3.10.4 Latency to Death

The latency to death following PTZ injection is shown in Figure 14. Two-way ANOVA found no significant genotype or gender effect on this measure

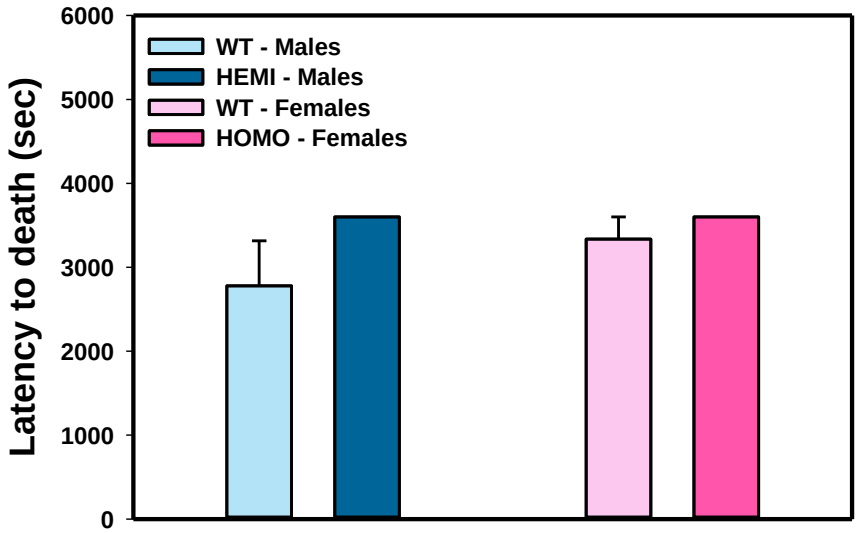

Figure 14**:** *Latency to death following PTZ (60 mg/kg) injection. Data are presented as mean ± SEM.*

3.10.5 Seizure Rank

Seizure rank during the 60 minutes is shown in Figure 15. Repeated measures ANOVA found no significant genotype effect, not significant gender effect and no interaction

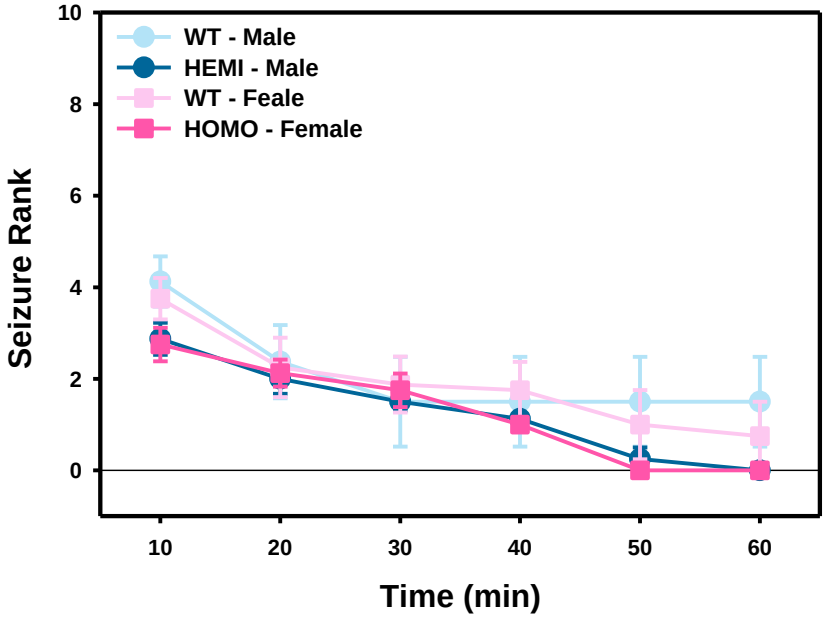

Figure 15**:** *Time course of PTZ-induced seizure rank. Data are presented as mean ± SEM.*

3.10.6 Survival

The survival charts of the mice are shown in Figure 16. Male and Female CDKL5 mice showed 100% survival following PTZ injection. Among the WT mice, 75% of the male mice survived (25% death) and 87.5% of the female mice survived (12.5% death).

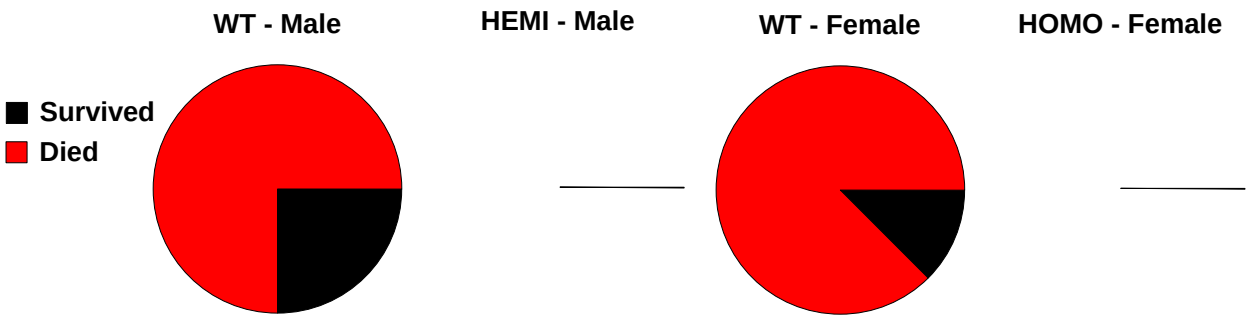

Figure 16**:** *Survival charts of WT and CDKL4 mice following PTZ injection.*

3.11 Audiogenic Seizures

No audiogenic seizures were observed in either the WT or the CDKL5 mice.

3.12 PhenoCube

3.13 Social Interaction

3.13.1 Number of Approaches

The number of approaches to the front or back are shown in Figure 17 A and B, respectively. Repeated measures ANOVA found a significant genotype effect and a time of day effect and significant interactions. Number of approaches was higher during the dark cycle compared to the light cycle. During the light cycle, WT male mice had significantly higher number of front approaches compares to CDKL5 male mice. No genotypic differences were found in this measure among the female mice or among the CDKL5 mice. During the dark cycle no differences were seen between WT male and CDKL5 male mice or between WT female and CDKL5 female mice. In general male mice had higher interactions compared to WT female mice.

During the light cycle, WT male mice also had significantly higher number of approaches to the back compared to the CDKL5 male mice. Similarly WT female mice had higher number of approaches to the back compared to the CDKL5 female mice, this effect just missed significance, p = 0.056. During the dark cycle CDKL5 male mice had significantly less interactions compared WT male mice. Female mice had significantly less interactions compared to WT male mice

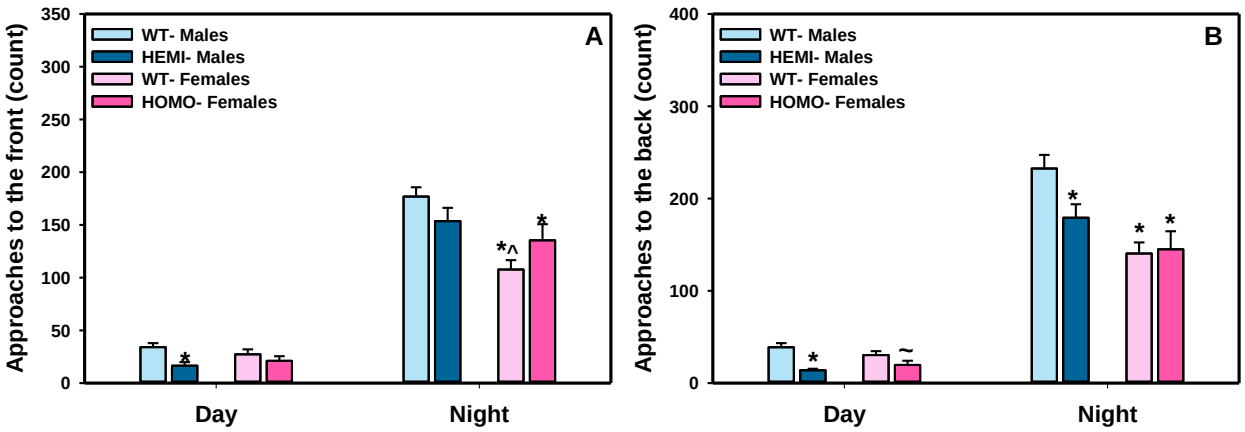

Figure 17**:** *Number of approaches to the front (A) or to the back of the mice. Data are presented as mean ± SEM.*p<0.05 compared to WT male mice; ~p=0.056 compared to WT female mice; ^p,0.05 compared to Hemi male mice*

3.13.2 Duration of Interaction

Duration of interactions to the front or back are shown in Figure18 A and B, respectively. Repeated measures ANOVA found no significant genotype or time of day effects.

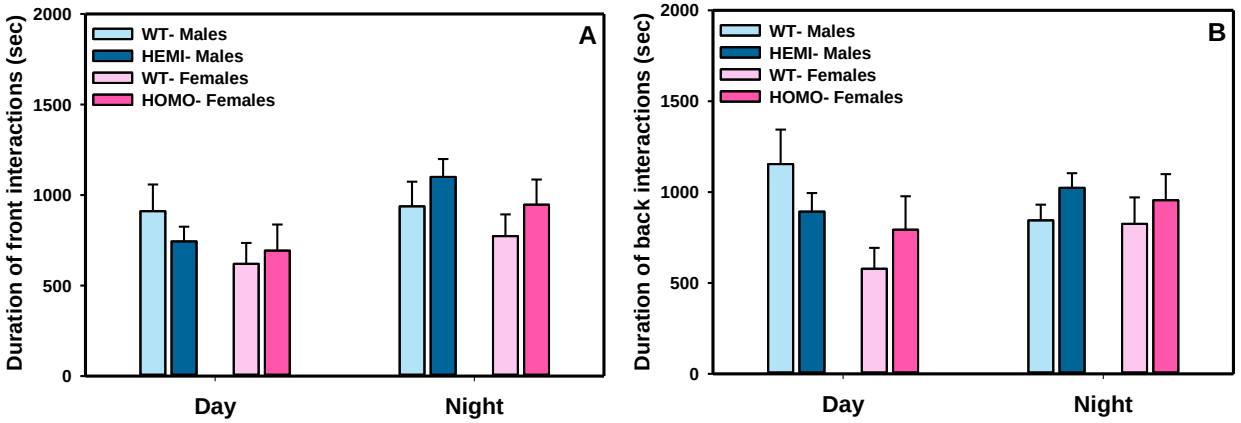

Figure 18*: *Duration of interactions to the front (A) or to the back of the mice. Data are presented as mean ± SEM.

3.14 Activity

3.14.1 Locomotion

Distance traveled and duration of locomotion during the 24 hour time period are shown in Figure 19. Repeated measures ANOVA found a significant genotype effect, time effect and a significant interaction. Activity during the dark cycle was significantly higher than in the light cycle. WT mice (males and females) showed higher locomotor activity during the day compared to CDKL5 mice. During the dark cycle, WT male mice showed higher locomotor activity compared to CDKL5 male mice and compared to female mice (both WT and CDKL5). No differences were found in distance traveled between male and female CDKL5 mice. The duration of locomotion was also higher in WT mice (males and females) compared to CDKL5 mice during the light and dark cycles. In female mice this effect just missed significance p = 0.057 during the dark cycle. No differences were found in the duration of locomotion between male and female CDKL5 mice.

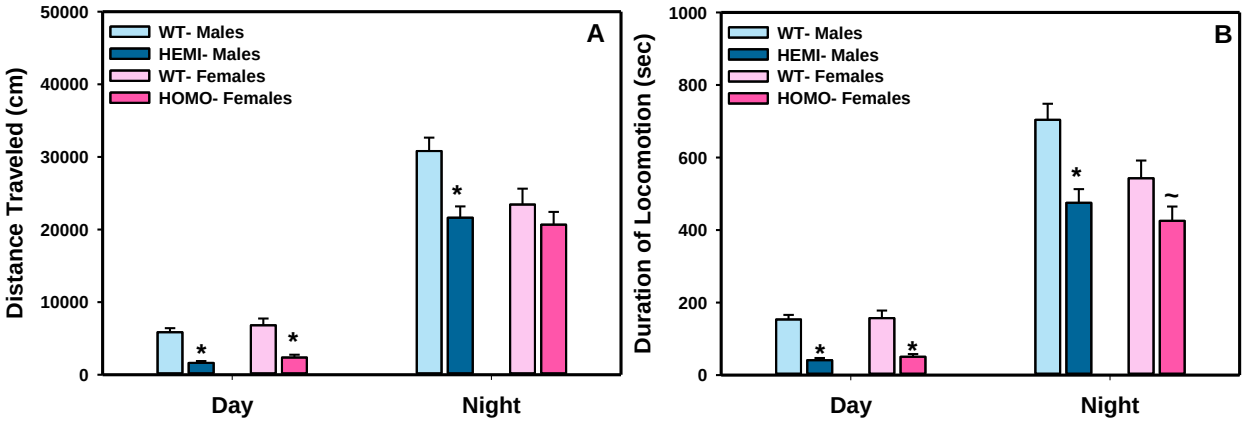

Figure 19*: *A:* *Distance traveled during the light and dark cycles during a 24 hour period. ***B: *Duration of locomotion during a 24 hour period. Data are presented as mean ± SEM. *p<0.05 compared to WT mice. ~p=0.057 compared to WT female mice.***

3.14.2 Supported Rearing

Supported Rearing behavior and duration of rearing during the 24 hour time period are shown in Figure 20. Repeated measures ANOVA found a significant genotype effect, time effect and a significant interaction. Rearing activity during the dark cycle was significantly higher than in the light cycle. WT male mice had higher rearing activity during the day and during the night compared to CDKL5 male mice. WT female mice showed trends to having higher rearing activity during the day compared to the CDKL5 female mice but this effect could not be seen in the dark cycle. No differences were found in rearing activity between male and female CDKL5 mice.

The duration of supported rearing was also higher in WT males mice compared to CDKL5 male mice during both cycles. No differences were seen between WT and CDKL5 female mice or between male and female CDKL5 mice on this measure.

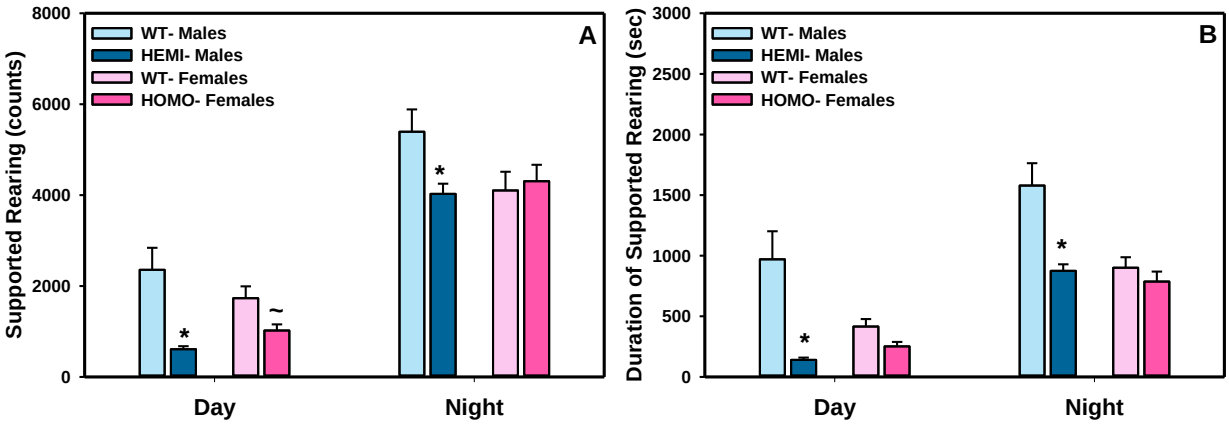

Figure 20*: *A: *Supported rearing counts during the light and dark cycles during a 24 hour period.* ***B:*** *Duration of supported rearing during a 24 hour period. Data are presented as mean ± SEM. *p<0.05 compared to WT male mice. ~p=0.08 compared to WT female mice.*

3.14.3 Climbing

Climbing behavior and duration of climbing during the 24 hour time period are shown in Figure 21. Repeated measures ANOVA found a significant genotype effect, time effect and a significant interaction. Climbing during the dark cycle was significantly higher than in the light cycle. WT mice (males and females) had higher climbing activity during their light cycle compared to their CDKL5 counterparts. In addition, Female mice (WT and CDKL5) showed more climbing activit compared to WT male mice. During the dark cycle, no genotypic differences were found in climbing activity between female mice. WT male still had higher climbing activity compared to CDKL5 male mice. Interestingly comparison between male and female CDKL5 mice showed that during the dark cycle, female mice had higher climbing behavior compared to e male mice.

The duration of climbing was higher in female WT compared to CDKL5 female mice and WT male mice during the light cycle. No differences were found in the duration of climbing between male and female CDKL5 mice during the light cycle. During the dark cycle, WT females spent more time climbing compared to male mice. In addition, comparison between male and female CDKL5 mice showed that during the dark cycle, female mice spent more time climbing compared to male mice.

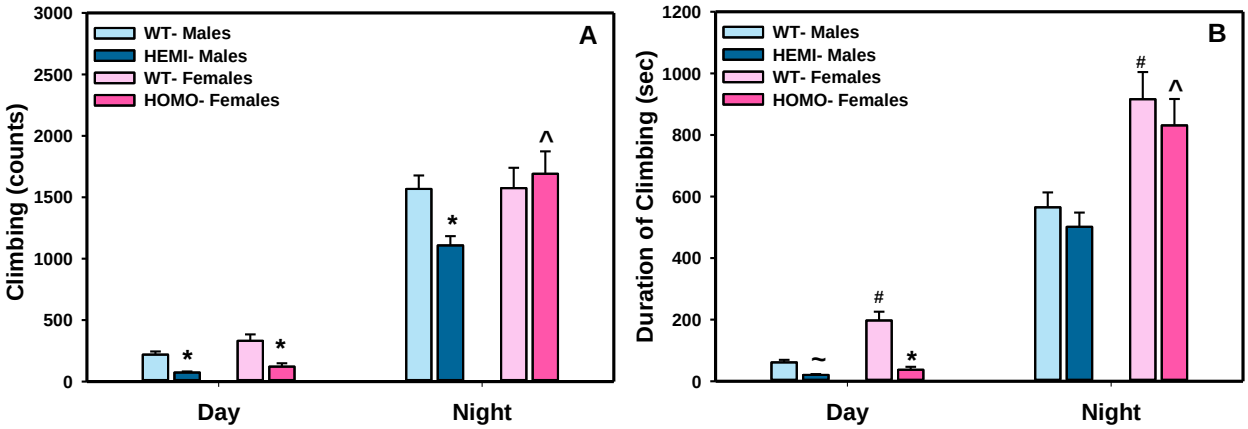

Figure 20*: *A: *Climbing counts during the light and dark cycles during a 24 hour period.* ***B:*** *Duration of climbing during a 24 hour period. Data are presented as mean ± SEM. *p<0.05 compared to respective WT mice. ~p=0.08 compared to male WT mice. ^p<0.05 compared to Hemi male mice; #p<0.05 compared to WT male mice*

3.15 Cognitive Behavior

3.15.1 Overall Visit Frequency

Figure 21 illustrates genotypic differences in total visit frequency in the. Visits to the corners were more frequent during the night for all groups. WT male and female mice had increased visits compared to CDKL5 male and female mice specially during the day time. In additiona female mice had more visits compared to male mice. This was also seen during the light cycle

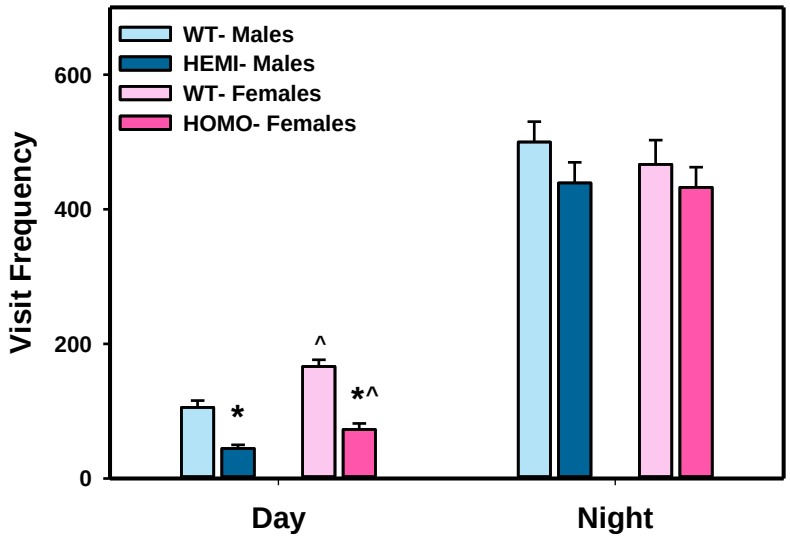

Figure 21*: *Total number of visits to the corners of PC. Data are presented as mean ± SEM. *p<0.05 compared to respective WT mice; ^ p<0.05 compared to respective male mice.

3.15.2 Percent Alternation

Alternation behavior during the light and dark cycles is shown in Figure 22. No genotypic differences were found. In general more alternations were done during the light cycle.

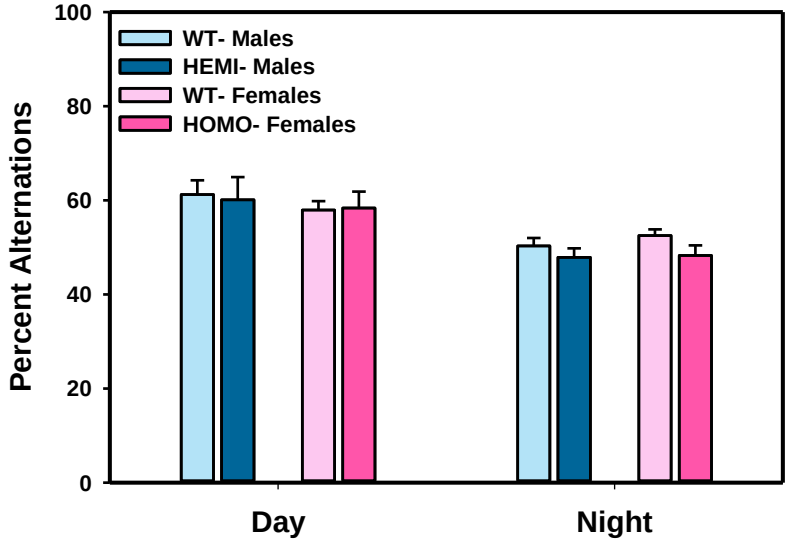

Figure 22*: *Percent alternations in PC. Data are presented as mean ± SEM.

3.15.3 Visits to Active Corners (Water seeking behavior)

Active visits, presented as a percentage of total visits in the Phenocube testing environement is shown in Figure 23. Repeated measures ANOVA found no significant genoytpe or gender differences in this measure.

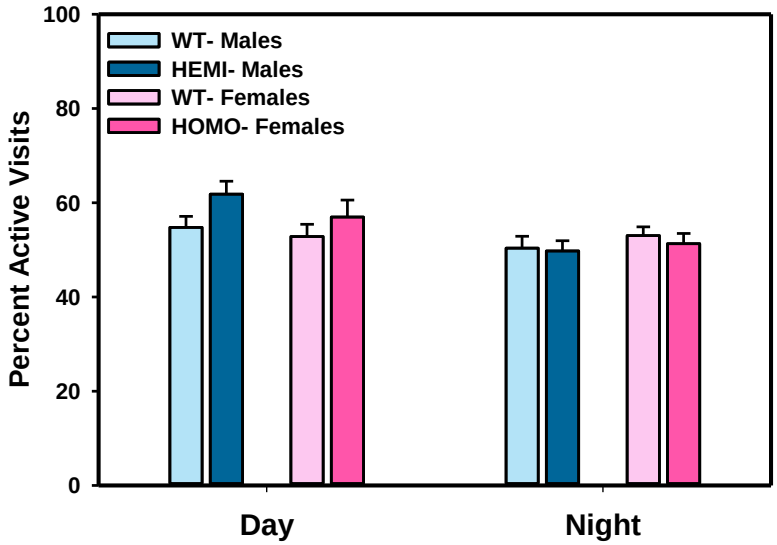

Figure 23*: *Percent visits to the active corners. Data are presented as mean ± SEM.

3.15.4 Repeat Visits (Preservative Behavior)

Repeat visits to the same corner as a percent of total number of visits is shown in Figure 24. Repeated measures ANOVA found a genotype effect and time of day effect. Female CDKL5 mice showed higher number of repeat visits compared to WT female mice during light and dark cycles. This effect was not seen in the male mice.

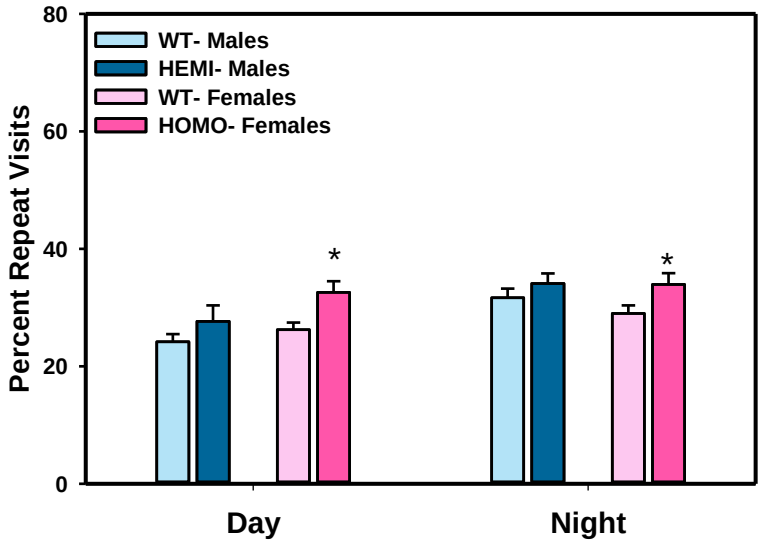

Figure 23**:** *Percent repeat visits to the same corner**.** Data are presented as mean ± SEM.*p<0.05 compared to female WT mice*

4 SUMMARY

Significant genotype differences were found in several of the tests. Below is a summary table for all the tests.

Test	Male	Female
Body Weight	Genotype differences early on, CDKL5 had lower BW compared to WT mice	Genotype differences early on, CDKL5 had lower BW compared to WT mice
Rotarod	No genotype differences	No genotype differences
	Female CDKL5 mice had longer latency to fall compared to hemi male mice. Female WT mice had longer latency to fall compared to WT male mice	
Clasping	At all ages CDKL5 mice showed clasping	At all ages CDKL5 mice showed clasping
	More clasping seen in male CDKL5 mice at older ages	
NeuroCube Gait	Significant genotype differences	Significant genotype differences
Optokinetic Responses	Significant genotype differences, deficits in CDKL5 mice compared to WT	No genotype differences
Fear conditioning	Significant genotype differences in context and cued FC - less freezing in CDKL5 mice	Significant genotype differences in context and post cue FC - less freezing in CDKL5 mice
Audiogenic Seizure	No genotype differences	No genotype differences
PTZ-induced Seizures	Increased latency to clonic seizure in CDKL5 mice	Increased latency to clonic seizure in CDKL5 mice
Respiration	Significant genotype differences in breathing rate and apnea	Significant genotype differences in breathing rate and apnea
Activity in PhenoCube		
Locomotion	Decreased activity of CDKL5 mice during light and dark cycles	Decreased activity of CDKL5 mice during light cycle only
Supported Rearing	Decreased rearing behavior in CDKL5 mice during light and dark cycles	No genotype differences
Climbing	Decreased climbing behavior in CDKL5 mice during light and dark cycles	No genotype differences
Cognition in PhenoCube		
Percent Alternation	No genotype differences	No genotype differences
Water seeking behavior	No genotype differences	No genotype differences
Overall Visit frequency	CDKL5 mice showed overall less visits compared to WT mice only during light cycle	CDKL5 mice showed overall less visits compared to WT mice only during light cycle
	Female CDKL5 and WT mice showed more visits compared to male mice during light cycle	
Persevative Behavior	No genotype differences	CDKL5 female mice showed higher number of repeat visits during light and dark cycles compared to WT mice
Social in Phenocube	Small but significant genotype differences in social approaches	No genotype differences

5 STATISTICAL TABLES

5.1 Body Weight

ANOVA Table for Age

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotypes	3	4086.688	1362.229	112.185	<.0001	336.554	1.000
Subject(Group)	60	728.564	12.143				
Category for Age	9	3218.895	357.655	352.198	<.0001	3169.786	1.000
Category for Age * Genotypes	27	158.731	5.879	5.789	<.0001	156.309	1.000
Category for Age * Subject(Group)	540	548.366	1.015				

Means Table for Age
Effect: Category for Age * Genotypes

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, 5.5 wks	16	17.891	2.779	.695
HEMI - Males, 6.5 wks	16	20.212	2.219	.555
HEMI - Males, 7.5 wks	16	22.231	1.816	.454
HEMI - Males, 8.5 wks	16	22.501	1.636	.409
HEMI - Males, 9.5 wks	16	23.169	1.517	.379
HEMI - Males, 10.5 wks	16	24.484	1.370	.343
HEMI - Males, 11.5 wks	16	25.274	1.404	.351
HEMI - Males, 12.5 wks	16	25.353	1.292	.323
HEMI - Males, 13.5 wks	16	26.041	1.235	.309
HEMI - Males, 14.5 wks	16	27.331	1.718	.430
HOMO - Females, 5.5 wks	16	14.231	1.161	.290
HOMO - Females, 6.5 wks	16	16.463	1.095	.274
HOMO - Females, 7.5 wks	16	17.335	1.138	.285
HOMO - Females, 8.5 wks	16	17.324	1.130	.283
HOMO - Females, 9.5 wks	16	17.544	1.263	.316
HOMO - Females, 10.5 wks	16	18.915	1.378	.345
HOMO - Females, 11.5 wks	16	19.675	1.383	.346
HOMO - Females, 12.5 wks	16	19.814	1.618	.405
HOMO - Females, 13.5 wks	16	20.470	1.336	.334
HOMO - Females, 14.5 wks	16	21.169	1.371	.343
WT - Females, 5.5 wks	16	16.179	1.085	.271
WT - Females, 6.5 wks	16	17.287	.746	.187
WT - Females, 7.5 wks	16	18.205	.937	.234
WT - Females, 8.5 wks	16	18.056	.893	.223
WT - Females, 9.5 wks	16	18.350	.846	.212
WT - Females, 10.5 wks	16	19.518	.692	.173
WT - Females, 11.5 wks	16	20.091	.891	.223
WT - Females, 12.5 wks	16	20.360	.959	.240
WT - Females, 13.5 wks	16	21.121	.867	.217
WT - Females, 14.5 wks	16	21.775	.795	.199
WT - Males, 5.5 wks	16	19.411	1.729	.432
WT - Males, 6.5 wks	16	20.913	1.820	.455
WT - Males, 7.5 wks	16	22.439	1.750	.438
WT - Males, 8.5 wks	16	22.395	1.869	.467
WT - Males, 9.5 wks	16	23.006	2.005	.501
WT - Males, 10.5 wks	16	24.224	1.785	.446
WT - Males, 11.5 wks	16	26.029	1.517	.379
WT - Males, 12.5 wks	16	26.424	1.366	.342
WT - Males, 13.5 wks	16	27.311	1.436	.359
WT - Males, 14.5 wks	16	27.425	1.844	.461

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	5.155	.779	<.0001	S
HEMI - Males, WT - Females	4.354	.779	<.0001	S
HEMI - Males, WT - Males	-.509	.779	.1964	
HOMO - Females, WT - Females	-.800	.779	.0443	S
HOMO - Females, WT - Males	-5.664	.779	<.0001	S
WT - Females, WT - Males	-4.863	.779	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 5.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	3.661	1.286	<.0001	S
HEMI - Males, WT - Females	1.713	1.286	.0099	S
HEMI - Males, WT - Males	-1.519	1.286	.0214	S
HOMO - Females, WT - Females	-1.948	1.286	.0036	S
HOMO - Females, WT - Males	-5.180	1.286	<.0001	S
WT - Females, WT - Males	-3.232	1.286	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 6.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	3.750	1.118	<.0001	S
HEMI - Males, WT - Females	2.925	1.118	<.0001	S
HEMI - Males, WT - Males	-.700	1.118	.2152	
HOMO - Females, WT - Females	-.825	1.118	.1451	
HOMO - Females, WT - Males	-4.450	1.118	<.0001	S
WT - Females, WT - Males	-3.625	1.118	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 7.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	4.896	1.033	<.0001	S
HEMI - Males, WT - Females	4.026	1.033	<.0001	S
HEMI - Males, WT - Males	-.209	1.033	.6875	
HOMO - Females, WT - Females	-.870	1.033	.0972	
HOMO - Females, WT - Males	-5.104	1.033	<.0001	S
WT - Females, WT - Males	-4.234	1.033	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 8.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	5.177	1.015	<.0001	S
HEMI - Males, WT - Females	4.444	1.015	<.0001	S
HEMI - Males, WT - Males	.106	1.015	.8359	
HOMO - Females, WT - Females	-.732	1.015	.1542	
HOMO - Females, WT - Males	-5.071	1.015	<.0001	S
WT - Females, WT - Males	-4.339	1.015	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 9.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	5.625	1.039	<.0001	S
HEMI - Males, WT - Females	4.819	1.039	<.0001	S
HEMI - Males, WT - Males	.162	1.039	.7555	
HOMO - Females, WT - Females	-.806	1.039	.1259	
HOMO - Females, WT - Males	-5.463	1.039	<.0001	S
WT - Females, WT - Males	-4.656	1.039	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 10.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	5.569	.965	<.0001	S
HEMI - Males, WT - Females	4.966	.965	<.0001	S
HEMI - Males, WT - Males	.260	.965	.5918	
HOMO - Females, WT - Females	-.603	.965	.2159	
HOMO - Females, WT - Males	-5.309	.965	<.0001	S
WT - Females, WT - Males	-4.706	.965	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 11.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	5.599	.934	<.0001	S
HEMI - Males, WT - Females	5.184	.934	<.0001	S
HEMI - Males, WT - Males	-.755	.934	.1112	
HOMO - Females, WT - Females	-.416	.934	.3770	
HOMO - Females, WT - Males	-6.354	.934	<.0001	S
WT - Females, WT - Males	-5.939	.934	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 12.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	5.539	.941	<.0001	S
HEMI - Males, WT - Females	4.993	.941	<.0001	S
HEMI - Males, WT - Males	-1.071	.941	.0264	S
HOMO - Females, WT - Females	-.546	.941	.2499	
HOMO - Females, WT - Males	-6.610	.941	<.0001	S
WT - Females, WT - Males	-6.064	.941	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 13.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	5.571	.875	<.0001	S
HEMI - Males, WT - Females	4.920	.875	<.0001	S
HEMI - Males, WT - Males	-1.271	.875	.0051	S
HOMO - Females, WT - Females	-.651	.875	.1422	
HOMO - Females, WT - Males	-6.841	.875	<.0001	S
WT - Females, WT - Males	-6.191	.875	<.0001	S

Fisher's PLSD for Age
Effect: Genotypes
Significance Level: 5 %
Split By: Category for Age
Cell: 14.5 wks

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	6.162	1.053	<.0001	S
HEMI - Males, WT - Females	5.556	1.053	<.0001	S
HEMI - Males, WT - Males	-.094	1.053	.8592	
HOMO - Females, WT - Females	-.606	1.053	.2540	
HOMO - Females, WT - Males	-6.256	1.053	<.0001	S
WT - Females, WT - Males	-5.650	1.053	<.0001	S

5.2 Rotarod

5.2.1 Latency to Fall

Two Way Analysis of Variance

Source of Variation	DF	SS	MS	F	P
Genotype	2	1272.354	636.177	0.450	0.640
Gender	1	12307.550	12307.550	8.707	0.005
Residual	60	84816.048	1413.601		
Total	63	105758.570	1678.707		

ANOVA Table for Latency To fall

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	20942.410	6980.803	4.938	.0039	14.815	.903
Residual	60	84816.302	1413.605				

Means Table for Latency To fall

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males	16	127.463	29.595	7.399
HOMO - Females	16	158.365	36.744	9.186
WT - Females	16	170.412	46.441	11.610
WT - Males	16	131.190	35.660	8.915

Fisher's PLSD for Latency To fall
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-30.902	26.590	.0235	S
HEMI - Males, WT - Females	-42.950	26.590	.0020	S
HEMI - Males, WT - Males	-3.727	26.590	.7801	
HOMO - Females, WT - Females	-12.048	26.590	.3684	
HOMO - Females, WT - Males	27.175	26.590	.0453	S
WT - Females, WT - Males	39.223	26.590	.0045	S

5.2.2 Speed at Fall

Two Way Analysis of Variance

Source of Variation	DF	SS	MS	F	P
Genotype	2	22.156	11.078	0.443	0.644
Gender	1	224.016	224.016	8.959	0.004
Residual	60	1500.261	25.004		
Total	63	1879.013	29.826		

ANOVA Table for Speed at Fall

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	378.741	126.247	5.049	.0035	15.147	.910
Residual	60	1500.283	25.005				

Means Table for Speed at Fall

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males	16	17.300	3.899	.975
HOMO - Females	16	21.450	4.864	1.216
WT - Females	16	23.050	6.224	1.556
WT - Males	16	17.758	4.735	1.184

Fisher's PLSD for Speed at Fall
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-4.150	3.536	.0222	S
HEMI - Males, WT - Females	-5.750	3.536	.0019	S
HEMI - Males, WT - Males	-.458	3.536	.7963	
HOMO - Females, WT - Females	-1.600	3.536	.3691	
HOMO - Females, WT - Males	3.692	3.536	.0410	S
WT - Females, WT - Males	5.292	3.536	.0040	S

5.3 Clasping

http://www.measuringu.com/ab-calc.php

7.5 weeks	11.5 weeks	13.5 weeks
Genotype (Male)	**Genotype (Male)**	**Genotype (Male)**
Two Tailed p-value: **0.0733286**	Two Tailed p-value: **9.0E-7**	Two Tailed p-value: **9.0E-7**
Genotype (Female)	**Genotype (Female)**	**Genotype (Female)**
Two Tailed p-value: **0.0165758**	Two Tailed p-value: **0.0004962**	Two Tailed p-value: **1.63E-5**

5.4 Respiration

5.4.1 Breathing Rate

ANOVA Table for Breathing rate

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	2485.853	2485.853	30.714	<.0001	30.714	1.000
Gender	1	1.470	1.470	.018	.8932	.018	.052
Genotype * Gender	1	380.413	380.413	4.700	.0341	4.700	.560
Residual	60	4856.099	80.935				

Means Table for Breathing rate

	Count	Mean	Std. Dev.	Std. Err.
Hemi Males	16	47.985	9.605	2.401
Homo Females	16	42.806	9.554	2.388
WT Females	16	60.147	8.492	2.123
WT Males	16	55.574	8.252	2.063

Fisher's PLSD for Breathing rate
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi Males, Homo Females	5.179	6.362	.1087	
Hemi Males, WT Females	-12.161	6.362	.0003	S
Hemi Males, WT Males	-7.589	6.362	.0202	S
Homo Females, WT Females	-17.341	6.362	<.0001	S
Homo Females, WT Males	-12.768	6.362	.0002	S
WT Females, WT Males	4.573	6.362	.1557	

5.4.2 Average Breath Duration

ANOVA Table for Breath Duration sec

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	2.380E-4	2.380E-4	.418	.5202	.418	.095
Gender	1	.003	.003	4.488	.0383	4.488	.539
Genotype * Gender	1	.003	.003	4.463	.0388	4.463	.537
Residual	60	.034	.001				

Means Table for Breath Duration sec

	Count	Mean	Std. Dev.	Std. Err.
Hemi Males	16	.166	.022	.006
Homo Females	16	.166	.032	.008
WT Females	16	.157	.016	.004
WT Males	16	.182	.023	.006

Fisher's PLSD for Breath Duration sec
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi Males, Homo Females	3.470E-5	.017	.9967	
Hemi Males, WT Females	.009	.017	.3022	
Hemi Males, WT Males	-.016	.017	.0557	
Homo Females, WT Females	.009	.017	.3041	
Homo Females, WT Males	-.016	.017	.0552	
WT Females, WT Males	-.025	.017	.0040	S

5.4.3 Minute Volume

ANOVA Table for Minute volume

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	8.357	8.357	.030	.8630	.030	.053
Gender	1	126.889	126.889	.456	.5020	.456	.099
Genotype * Gender	1	647.399	647.399	2.327	.1324	2.327	.307
Residual	60	16692.313	278.205				

Means Table for Minute volume

	Count	Mean	Std. Dev.	Std. Err.
Hemi Males	16	96.304	17.560	4.390
Homo Females	16	92.759	16.500	4.125
WT Females	16	98.397	17.135	4.284
WT Males	16	89.220	15.447	3.862

5.4.4 Average Enhanced Pause

ANOVA Table for Avg Enhanced Pause

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	.091	.091	11.392	.0013	11.392	.932
Gender	1	.092	.092	11.566	.0012	11.566	.935
Genotype * Gender	1	2.028E-4	2.028E-4	.025	.8740	.025	.053
Residual	60	.480	.008				

Means Table for Avg Enhanced Pause

	Count	Mean	Std. Dev.	Std. Err.
Hemi Males	16	.684	.065	.016
Homo Females	16	.605	.071	.018
WT Females	16	.684	.097	.024
WT Males	16	.756	.115	.029

Fisher's PLSD for Avg Enhanced Pause
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi Males, Homo Females	.080	.063	.0145	S
Hemi Males, WT Females	.001	.063	.9855	
Hemi Males, WT Males	-.072	.063	.0266	S
Homo Females, WT Females	-.079	.063	.0152	S
Homo Females, WT Males	-.151	.063	<.0001	S
WT Females, WT Males	-.072	.063	.0254	S

5.5 Optokinetic Response

5.5.1 Counter Clockwise Drum Rotation

5.5.1.1 Counter Clockwise Responses

ANOVA Table for Counter Clockwise Drum Rotation

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotypes	3	336.461	112.154	9.700	<.0001	29.101	.998
Subject(Group)	60	693.719	11.562				
Category for Counter Clockwise Drum Rotation	1	273.195	273.195	59.451	<.0001	59.451	1.000
Category for Counter Clockwise Drum Rotation * Genotypes	3	18.586	6.195	1.348	.2673	4.045	.332
Category for Counter Clockwise Drum Rotation * Subject(Group)	60	275.719	4.595				

Means Table for Counter Clockwise Responses

	Count	Mean	Std. Dev.	Std. Err.
Hemi male, 1.5 rpm CCW	16	3.500	2.160	.540
Hemi male, 2.8 rpm CCW	16	5.938	3.714	.929
Homo Female, 1.5 rpm CCW	16	3.875	1.708	.427
Homo Female, 2.8 rpm CCW	16	6.000	2.708	.677
WT -Female, 1.5 rpm CCW	16	3.750	2.236	.559
WT -Female, 2.8 rpm CCW	16	6.750	2.490	.622
WT male, 1.5 rpm CCW	16	6.625	2.918	.730
WT male, 2.8 rpm CCW	16	10.750	4.025	1.006

Fisher's PLSD for Counter Clockwise Drum Rotation
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi male, Homo Female	-.219	1.700	.7978	
Hemi male, WT -Female	-.531	1.700	.5344	
Hemi male, WT male	-3.969	1.700	<.0001	S
Homo Female, WT -Female	-.313	1.700	.7145	
Homo Female, WT male	-3.750	1.700	<.0001	S
WT -Female, WT male	-3.438	1.700	.0002	S

5.5.1.2 Clockwise Responses

ANOVA Table for CW responses

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotypes	3	2.273	.758	1.659	.1854	4.977	.404
Subject(Group)	60	27.406	.457				
Category for CW responses	1	.008	.008	.015	.9041	.015	.052
Category for CW responses * Genotypes	3	.461	.154	.288	.8340	.863	.101
Category for CW responses * Subject(G...	60	32.031	.534				

Means Table for CW responses

	Count	Mean	Std. Dev.	Std. Err.
Hemi male, 1.5 rpm CW	16	0.000	0.000	0.000
Hemi male, 2.8 rpm CW	16	.063	.250	.063
Homo Female, 1.5 rpm CW	16	.125	.342	.085
Homo Female, 2.8 rpm CW	16	.063	.250	.063
WT -Female, 1.5 rpm CW	16	.188	.544	.136
WT -Female, 2.8 rpm CW	16	0.000	0.000	0.000
WT male, 1.5 rpm CW	16	.313	.602	.151
WT male, 2.8 rpm CW	16	.438	1.750	.438

5.5.2 Clockwise Drum Rotation

5.5.2.1 Counter Clockwise Responses

ANOVA Table for CW Rotation - CCW responses

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotypes	3	1.086	.362	2.683	.0547	8.050	.621
Subject(Group)	60	8.094	.135				
Category for CW Rotation - CCW respon...	1	.070	.070	1.063	.3067	1.063	.165
Category for CW Rotation - CCW respon...	3	.461	.154	2.323	.0841	6.969	.549
Category for CW Rotation - CCW respon...	60	3.969	.066				

Means Table for CW Rotation - CCW responses

	Count	Mean	Std. Dev.	Std. Err.
Hemi male, 1.5 rpm CCW.2	16	.188	.403	.101
Hemi male, 2.8 rpm CCW.2	16	0.000	0.000	0.000
Homo Female, 1.5 rpm CCW.2	16	0.000	0.000	0.000
Homo Female, 2.8 rpm CCW.2	16	0.000	0.000	0.000
WT -Female, 1.5 rpm CCW.2	16	.125	.342	.085
WT -Female, 2.8 rpm CCW.2	16	0.000	0.000	0.000
WT male, 1.5 rpm CCW.2	16	.188	.403	.101
WT male, 2.8 rpm CCW.2	16	.313	.602	.151

5.5.2.2 Clockwise Responses

ANOVA Table for CW rotation - CW responses

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotypes	3	362.063	120.688	10.991	<.0001	32.974	1.000
Subject(Group)	60	658.813	10.980				
Category for CW rotation - CW responses	1	338.000	338.000	57.197	<.0001	57.197	1.000
Category for CW rotation - CW response...	3	9.437	3.146	.532	.6618	1.597	.150
Category for CW rotation - CW response...	60	354.562	5.909				

Means Table for CW rotation - CW responses

	Count	Mean	Std. Dev.	Std. Err.
Hemi male, 1.5 rpm CW.2	16	3.313	2.056	.514
Hemi male, 2.8 rpm CW.2	16	6.188	3.410	.853
Homo Female, 1.5 rpm CW.2	16	3.000	1.897	.474
Homo Female, 2.8 rpm CW.2	16	5.938	2.886	.722
WT -Female, 1.5 rpm CW.2	16	4.375	2.277	.569
WT -Female, 2.8 rpm CW.2	16	8.563	3.119	.780
WT male, 1.5 rpm CW.2	16	7.188	3.763	.941
WT male, 2.8 rpm CW.2	16	10.188	3.270	.818

Fisher's PLSD for CW rotation - CW responses
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi male, Homo Female	.281	1.657	.7354	
Hemi male, WT -Female	-1.719	1.657	.0423	S
Hemi male, WT male	-3.938	1.657	<.0001	S
Homo Female, WT -Female	-2.000	1.657	.0188	S
Homo Female, WT male	-4.219	1.657	<.0001	S
WT -Female, WT male	-2.219	1.657	.0095	S

5.5.3 Total Responses

ANOVA Table for Total responses

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	1463.063	1463.063	23.090	<.0001	23.090	.999
Gender	1	473.063	473.063	7.466	.0082	7.466	.777
Genotype * Gender	1	441.000	441.000	6.960	.0106	6.960	.744
Residual	60	3801.875	63.365				

Means Table for Total responses

	Count	Mean	Std. Dev.	Std. Err.
KO, FEMALE	16	18.500	6.909	1.727
KO, MALE	16	18.688	8.292	2.073
WT, FEMALE	16	22.813	7.722	1.930
WT, MALE	16	33.500	8.794	2.198

Fisher's PLSD for Total responses
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi male, Homo Female	.188	5.630	.9471	
Hemi male, WT -Female	-4.125	5.630	.1480	
Hemi male, WT male	-14.813	5.630	<.0001	S
Homo Female, WT -Female	-4.313	5.630	.1307	
Homo Female, WT male	-15.000	5.630	<.0001	S
WT -Female, WT male	-10.688	5.630	.0003	S

5.5.4 Total Responses at each drum speed

ANOVA Table for Drum Speed

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotypes	3	1375.586	458.529	13.936	<.0001	41.808	1.000
Subject(Group)	60	1974.156	32.903				
Category for Drum Speed	1	1218.945	1218.945	98.663	<.0001	98.663	1.000
Category for Drum Speed * Genotypes	3	31.273	10.424	.844	.4753	2.531	.217
Category for Drum Speed * Subject(Grou...	60	741.281	12.355				

Means Table for correct responses

	Count	Mean	Std. Dev.	Std. Err.
Hemi male, 1.5 rpm correct	16	6.813	3.885	.971
Hemi male, 2.8 rpm correct	16	12.125	5.999	1.500
Homo Female, 1.5 rpm correct	16	6.750	2.864	.716
Homo Female, 2.8 rpm correct	16	11.813	5.167	1.292
WT -Female, 1.5 rpm correct	16	7.813	3.970	.993
WT -Female, 2.8 rpm correct	16	15.000	4.179	1.045
WT male, 1.5 rpm correct	16	13.813	4.490	1.123
WT male, 2.8 rpm correct	16	20.938	6.455	1.614

Fisher's PLSD for 1.5 rpm correct
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi male, Homo Female	.063	2.721	.9635	
Hemi male, WT -Female	-1.000	2.721	.4652	
Hemi male, WT male	-7.000	2.721	<.0001	S
Homo Female, WT -Female	-1.063	2.721	.4379	
Homo Female, WT male	-7.063	2.721	<.0001	S
WT -Female, WT male	-6.000	2.721	<.0001	S

Fisher's PLSD for 2.8 rpm correct
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi male, Homo Female	.313	3.903	.8733	
Hemi male, WT -Female	-2.875	3.903	.1458	
Hemi male, WT male	-8.813	3.903	<.0001	S
Homo Female, WT -Female	-3.188	3.903	.1075	
Homo Female, WT male	-9.125	3.903	<.0001	S
WT -Female, WT male	-5.938	3.903	.0035	S

Fisher's PLSD for Drum Speed
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Hemi male, Homo Female	.188	2.868	.8964	
Hemi male, WT -Female	-1.938	2.868	.1817	
Hemi male, WT male	-7.906	2.868	<.0001	S
Homo Female, WT -Female	-2.125	2.868	.1436	
Homo Female, WT male	-8.094	2.868	<.0001	S
WT -Female, WT male	-5.969	2.868	.0001	S

5.6 Fear Conditioning

5.6.1 Contextual FC

Average Freezing

ANOVA Table for Average Freezing

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotypes	1	1719.398	1719.398	9.974	.0038	9.974	.878
Gender	1	920.974	920.974	5.342	.0284	5.342	.602
Genotypes * Gender	1	10.969	10.969	.064	.8027	.064	.057
Residual	28	4827.071	172.395				

Means Table for Average Freezing

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males	8	32.034	9.927	3.510
HOMO - Females	8	20.134	7.831	2.769
WT - Females	8	35.965	13.569	4.797
WT - Males	8	45.524	18.590	6.573

ANOVA Table for Average Freezing

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	2651.341	883.780	5.126	.0059	15.379	.888
Residual	28	4827.071	172.395				

Fisher's PLSD for Average Freezing
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	11.900	13.448	.0806	
HEMI - Males, WT - Females	-3.931	13.448	.5541	
HEMI - Males, WT - Males	-13.489	13.448	.0493	S
HOMO - Females, WT - Females	-15.831	13.448	.0227	S
HOMO - Females, WT - Males	-25.390	13.448	.0006	S
WT - Females, WT - Males	-9.559	13.448	.1565	

Time course for % freezing during the 6 min test

ANOVA Table for Time

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	15908.044	5302.681	5.126	.0059	15.379	.888
Subject(Group)	28	28962.427	1034.372				
Category for Time	5	9749.375	1949.875	7.454	<.0001	37.268	1.000
Category for Time * Genotype	15	7165.639	477.709	1.826	.0365	27.391	.925
Category for Time * Subject(Group)	140	36624.274	261.602				

Means Table for % freezing

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Minute 1	8	22.545	14.621	5.169
HEMI - Males, Minute 2	8	47.555	15.227	5.384
HEMI - Males, Minute 3	8	39.942	14.264	5.043
HEMI - Males, Minute 4	8	33.445	22.195	7.847
HEMI - Males, Minute 5	8	25.615	18.689	6.608
HEMI - Males, Minute 6	8	23.102	22.089	7.810
HOMO - Females, Minute 1	8	11.495	6.078	2.149
HOMO - Females, Minute 2	8	22.720	12.393	4.382
HOMO - Females, Minute 3	8	19.889	14.881	5.261
HOMO - Females, Minute 4	8	22.000	14.054	4.969
HOMO - Females, Minute 5	8	25.949	11.923	4.216
HOMO - Females, Minute 6	8	18.750	10.142	3.586
WT - Females, Minute 1	8	26.619	14.465	5.114
WT - Females, Minute 2	8	38.556	32.784	11.591
WT - Females, Minute 3	8	43.831	27.071	9.571
WT - Females, Minute 4	8	30.389	26.035	9.205
WT - Females, Minute 5	8	45.648	17.500	6.187
WT - Females, Minute 6	8	30.747	12.751	4.508
WT - Males, Minute 1	8	27.903	24.170	8.545
WT - Males, Minute 2	8	49.334	27.705	9.795
WT - Males, Minute 3	8	66.000	22.971	8.122
WT - Males, Minute 4	8	59.594	29.709	10.504
WT - Males, Minute 5	8	37.611	16.995	6.009
WT - Males, Minute 6	8	32.700	17.935	6.341

Fisher's PLSD for Time
Effect: Genotype
Significance Level: 5 %
Split By: Category for Time
Cell: Minute 1

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	11.050	16.548	.1822	
HEMI - Males, WT - Females	-4.074	16.548	.6180	
HEMI - Males, WT - Males	-5.358	16.548	.5126	
HOMO - Females, WT - Females	-15.124	16.548	.0717	
HOMO - Females, WT - Males	-16.408	16.548	.0518	
WT - Females, WT - Males	-1.284	16.548	.8749	

Fisher's PLSD for Time
Effect: Genotype
Significance Level: 5 %
Split By: Category for Time
Cell: Minute 2

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	24.835	24.171	.0444	S
HEMI - Males, WT - Females	8.999	24.171	.4521	
HEMI - Males, WT - Males	-1.779	24.171	.8813	
HOMO - Females, WT - Females	-15.836	24.171	.1904	
HOMO - Females, WT - Males	-26.614	24.171	.0321	S
WT - Females, WT - Males	-10.777	24.171	.3689	

Fisher's PLSD for Time
Effect: Genotype
Significance Level: 5 %
Split By: Category for Time
Cell: Minute 3

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	20.054	21.024	.0608	
HEMI - Males, WT - Females	-3.889	21.024	.7076	
HEMI - Males, WT - Males	-26.057	21.024	.0170	S
HOMO - Females, WT - Females	-23.942	21.024	.0271	S
HOMO - Females, WT - Males	-46.111	21.024	.0001	S
WT - Females, WT - Males	-22.169	21.024	.0395	S

Fisher's PLSD for Time
Effect: Genotype
Significance Level: 5 %
Split By: Category for Time
Cell: Minute 4

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	11.445	24.294	.3428	
HEMI - Males, WT - Females	3.056	24.294	.7985	
HEMI - Males, WT - Males	-26.149	24.294	.0359	S
HOMO - Females, WT - Females	-8.389	24.294	.4852	
HOMO - Females, WT - Males	-37.594	24.294	.0037	S
WT - Females, WT - Males	-29.205	24.294	.0202	S

Fisher's PLSD for Time
Effect: Genotype
Significance Level: 5 %
Split By: Category for Time
Cell: Minute 5

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-.334	16.880	.9680	
HEMI - Males, WT - Females	-20.032	16.880	.0217	S
HEMI - Males, WT - Males	-11.996	16.880	.1566	
HOMO - Females, WT - Females	-19.699	16.880	.0238	S
HOMO - Females, WT - Males	-11.663	16.880	.1680	
WT - Females, WT - Males	8.036	16.880	.3378	

Fisher's PLSD for Time
Effect: Genotype
Significance Level: 5 %
Split By: Category for Time
Cell: Minute 6

	Mean Diff.	Crit. Diff.	P-Value
HEMI - Males, HOMO - Females	4.352	16.790	.5996
HEMI - Males, WT - Females	-7.645	16.790	.3590
HEMI - Males, WT - Males	-9.597	16.790	.2515
HOMO - Females, WT - Females	-11.997	16.790	.1544
HOMO - Females, WT - Males	-13.950	16.790	.0999
WT - Females, WT - Males	-1.952	16.790	.8135

5.6.2 Cued FC

ANOVA Table for Freezing

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	8263.416	2754.472	5.612	.0019	16.835	.940
Subject(Group)	60	29450.931	490.849				
Category for Freezing	2	48849.055	24424.527	131.265	<.0001	262.529	1.000
Category for Freezing * Genotype	6	6010.606	1001.768	5.384	<.0001	32.303	.997
Category for Freezing * Subject(Group)	120	22328.519	186.071				

Means Table for Freezing

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, PreCS	16	15.262	11.245	2.811
HEMI - Males, During CS	16	46.050	13.698	3.424
HEMI - Males, Post CS	16	9.067	16.642	4.160
HOMO - Females, PreCS	16	12.182	12.359	3.090
HOMO - Females, During CS	16	39.306	13.460	3.365
HOMO - Females, Post CS	16	8.036	8.880	2.220
WT - Females, PreCS	16	12.500	11.606	2.901
WT - Females, During CS	16	49.667	19.154	4.789
WT - Females, Post CS	16	32.812	26.410	6.603
WT - Males, PreCS	16	14.481	13.068	3.267
WT - Males, During CS	16	65.479	14.152	3.538
WT - Males, Post CS	16	29.438	29.632	7.408

Fisher's PLSD for Freezing
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	3.618	9.046	.4269	
HEMI - Males, WT - Females	-8.200	9.046	.0748	
HEMI - Males, WT - Males	-13.006	9.046	.0056	S
HOMO - Females, WT - Females	-11.818	9.046	.0113	S
HOMO - Females, WT - Males	-16.624	9.046	.0005	S
WT - Females, WT - Males	-4.806	9.046	.2921	

Fisher's PLSD for Freezing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Freezing
Cell: PreCS

	Mean Diff.	Crit. Diff.	P-Value
HEMI - Males, HOMO - Females	3.079	8.550	.4741
HEMI - Males, WT - Females	2.762	8.550	.5207
HEMI - Males, WT - Males	.781	8.550	.8557
HOMO - Females, WT - Females	-.317	8.550	.9410
HOMO - Females, WT - Males	-2.299	8.550	.5927
WT - Females, WT - Males	-1.981	8.550	.6447

Fisher's PLSD for Freezing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Freezing
Cell: During CS

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	6.744	10.818	.2173	
HEMI - Males, WT - Females	-3.617	10.818	.5062	
HEMI - Males, WT - Males	-19.429	10.818	.0007	S
HOMO - Females, WT - Females	-10.361	10.818	.0602	
HOMO - Females, WT - Males	-26.172	10.818	<.0001	S
WT - Females, WT - Males	-15.812	10.818	.0049	S

Fisher's PLSD for Freezing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Freezing
Cell: Post CS

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	1.031	15.540	.8949	
HEMI - Males, WT - Females	-23.745	15.540	.0033	S
HEMI - Males, WT - Males	-20.371	15.540	.0111	S
HOMO - Females, WT - Females	-24.776	15.540	.0023	S
HOMO - Females, WT - Males	-21.401	15.540	.0078	S
WT - Females, WT - Males	3.374	15.540	.6656	

5.7 PTZ-induced seizures

5.7.1 Latency to first twitch

ANOVA Table for Latency to first twitch (secs)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	2.531	2.531	.012	.9142	.012	.051
Gender	1	318.781	318.781	1.487	.2328	1.487	.206
Genotype * Gender	1	52.531	52.531	.245	.6244	.245	.076
Residual	28	6001.125	214.326				

Means Table for Latency to first twitch (secs)

	Count	Mean	Std. Dev.	Std. Err.
KO, Female	8	91.875	4.883	1.726
KO, Male	8	100.750	9.083	3.211
WT, Female	8	93.875	12.889	4.557
WT, Male	8	97.625	24.183	8.550

5.7.2 Latency to clonic tonic seizures

ANOVA Table for Latency to clonic/tonic (secs)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	29121804.031	29121804.031	14.449	.0007	14.449	.970
Gender	1	412913.281	412913.281	.205	.6543	.205	.071
Genotype * Gender	1	545751.281	545751.281	.271	.6069	.271	.078
Residual	28	56434758.125	2015527.076				

Means Table for Latency to clonic/tonic (secs)

	Count	Mean	Std. Dev.	Std. Err.
KO, Female	8	3177.875	1193.950	422.125
KO, Male	8	3211.875	1097.783	388.125
WT, Female	8	1531.125	1714.141	606.040
WT, Male	8	1042.750	1578.982	558.254

Fisher's PLSD for Latency to clonic/tonic (secs)
Effect: Genotypes
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	34.000	1454.054	.9621	
HEMI - Males, WT - Females	1680.750	1454.054	.0250	S
HEMI - Males, WT - Males	2169.125	1454.054	.0049	S
HOMO - Females, WT - Females	1646.750	1454.054	.0279	S
HOMO - Females, WT - Males	2135.125	1454.054	.0055	S
WT - Females, WT - Males	488.375	1454.054	.4971	

5.7.3 Latency to tonic seizure

ANOVA Table for Latency to tonic (secs)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	3125625.031	3125625.031	3.316	.0793	3.316	.405
Gender	1	349657.031	349657.031	.371	.5474	.371	.089
Genotype * Gender	1	349657.031	349657.031	.371	.5474	.371	.089
Residual	28	26392764.875	942598.746				

Means Table for Latency to tonic (sec)

	Count	Mean	Std. Dev.	Std. Err.
KO, Female	8	3600.000	0.000	0.000
KO, Male	8	3600.000	0.000	0.000
WT, Female	8	3184.000	1176.626	416.000
WT, Male	8	2765.875	1544.651	546.117

5.7.4 Latency to death

ANOVA Table for Latency to death (secs)

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	2353365.125	2353365.125	3.282	.0808	3.282	.402
Gender	1	619941.125	619941.125	.865	.3604	.865	.140
Genotype * Gender	1	619941.125	619941.125	.865	.3604	.865	.140
Residual	28	20076607.500	717021.696				

Means Table for Latency to death (secs)

	Count	Mean	Std. Dev.	Std. Err.
KO, Female	8	3600.000	0.000	0.000
KO, Male	8	3600.000	0.000	0.000
WT, Female	8	3336.000	746.705	264.000
WT, Male	8	2779.250	1520.039	537.415

5.7.5 Seizure Rank

ANOVA Table for Seizure Rank

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	1	24.083	24.083	1.805	.1899	1.805	.241
Gender	1	.521	.521	.039	.8448	.039	.054
Genotype * Gender	1	.333	.333	.025	.8755	.025	.053
Subject(Group)	28	373.542	13.341				
Category for Seizure Rank	5	174.917	34.983	65.121	<.0001	325.607	1.000
Category for Seizure Rank * Genotype	5	9.354	1.871	3.483	.0054	17.413	.913
Category for Seizure Rank * Gender	5	3.042	.608	1.132	.3461	5.662	.387
Category for Seizure Rank * Genotype * ...	5	1.479	.296	.551	.7376	2.753	.196
Category for Seizure Rank * Subject(Gro...	140	75.208	.537				

Means Table for Seizure Rank

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, 10 minutes	8	2.875	.991	.350
HEMI - Males, 20 minutes	8	2.000	.926	.327
HEMI - Males, 30 minutes	8	1.500	.535	.189
HEMI - Males, 40 minutes	8	1.125	.354	.125
HEMI - Males, 50 minutes	8	.250	.707	.250
HEMI - Males, 60 minutes	8	0.000	0.000	0.000
HOMO - Females, 10 minutes	8	2.750	1.035	.366
HOMO - Females, 20 minutes	8	2.125	.835	.295
HOMO - Females, 30 minutes	8	1.750	1.035	.366
HOMO - Females, 40 minutes	8	1.000	0.000	0.000
HOMO - Females, 50 minutes	8	0.000	0.000	0.000
HOMO - Females, 60 minutes	8	0.000	0.000	0.000
WT - Females, 10 minutes	8	3.750	1.282	.453
WT - Females, 20 minutes	8	2.250	1.832	.648
WT - Females, 30 minutes	8	1.875	1.727	.611
WT - Females, 40 minutes	8	1.750	1.753	.620
WT - Females, 50 minutes	8	1.000	2.138	.756
WT - Females, 60 minutes	8	.750	2.121	.750
WT - Males, 10 minutes	8	4.125	1.553	.549
WT - Males, 20 minutes	8	2.375	2.264	.800
WT - Males, 30 minutes	8	1.500	2.777	.982
WT - Males, 40 minutes	8	1.500	2.777	.982
WT - Males, 50 minutes	8	1.500	2.777	.982
WT - Males, 60 minutes	8	1.500	2.777	.982

5.8 PhenoCube

5.8.1 Social Interaction

5.8.1.1 Number of Approaches

Approaches to the Back

ANOVA Table for Approaches to back

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	58034.883	19344.961	7.084	.0004	21.251	.982
Subject(Group)	60	163854.859	2730.914				
Category for Approaches to back	1	706860.500	706860.500	527.402	<.0001	527.402	1.000
Category for Approaches to back * Gen...	3	34786.578	11595.526	8.652	<.0001	25.955	.995
Category for Approaches to back * Subj...	60	80416.172	1340.270				

Means Table for Approaches to back

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	13.844	6.710	1.677
HEMI - Males, Night	16	179.219	58.805	14.701
HOMO - Females, Day	16	19.656	17.855	4.464
HOMO - Females, Night	16	145.000	77.937	19.484
WT - Females, Day	16	30.375	16.982	4.245
WT - Females, Night	16	140.406	48.053	12.013
WT - Males, Day	16	38.844	17.731	4.433
WT - Males, Night	16	232.594	58.964	14.741

Fisher's PLSD for Approaches to back
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	14.203	26.133	.2813	
HEMI - Males, WT - Females	11.141	26.133	.3972	
HEMI - Males, WT - Males	-39.188	26.133	.0039	S
HOMO - Females, WT - Females	-3.063	26.133	.8155	
HOMO - Females, WT - Males	-53.391	26.133	.0001	S
WT - Females, WT - Males	-50.328	26.133	.0003	S

Fisher's PLSD for Approaches to back
Effect: Genotype
Significance Level: 5 %
Split By: Category for Approaches to back
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-5.813	10.994	.2945	
HEMI - Males, WT - Females	-16.531	10.994	.0038	S
HEMI - Males, WT - Males	-25.000	10.994	<.0001	S
HOMO - Females, WT - Females	-10.719	10.994	.0558	
HOMO - Females, WT - Males	-19.188	10.994	.0009	S
WT - Females, WT - Males	-8.469	10.994	.1286	

Fisher's PLSD for Approaches to back
Effect: Genotype
Significance Level: 5 %
Split By: Category for Approaches to back
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	34.219	43.765	.1231	
HEMI - Males, WT - Females	38.813	43.765	.0811	
HEMI - Males, WT - Males	-53.375	43.765	.0177	S
HOMO - Females, WT - Females	4.594	43.765	.8344	
HOMO - Females, WT - Males	-87.594	43.765	.0002	S
WT - Females, WT - Males	-92.188	43.765	<.0001	S

Approaches to the Front

ANOVA Table for Approaches to front

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	24649.047	8216.349	4.370	.0075	13.109	.857
Subject(Group)	60	112822.922	1880.382				
Category for Approaches to front	1	450656.445	450656.445	749.645	<.0001	749.645	1.000
Category for Approaches to front * Gen...	3	19344.195	6448.065	10.726	<.0001	32.178	.999
Category for Approaches to front * Subj...	60	36069.609	601.160				

Fisher's PLSD for Approaches to front
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	6.875	21.685	.5284	
HEMI - Males, WT - Females	17.609	21.685	.1095	
HEMI - Males, WT - Males	-20.422	21.685	.0644	
HOMO - Females, WT - Females	10.734	21.685	.3261	
HOMO - Females, WT - Males	-27.297	21.685	.0145	S
WT - Females, WT - Males	-38.031	21.685	.0009	S

Fisher's PLSD for Approaches to front
Effect: Genotype
Significance Level: 5 %
Split By: Category for Approaches to front
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-4.625	11.580	.4275	
HEMI - Males, WT - Females	-10.844	11.580	.0659	
HEMI - Males, WT - Males	-17.625	11.580	.0035	S
HOMO - Females, WT - Females	-6.219	11.580	.2870	
HOMO - Females, WT - Males	-13.000	11.580	.0284	S
WT - Females, WT - Males	-6.781	11.580	.2461	

Fisher's PLSD for Approaches to front
Effect: Genotype
Significance Level: 5 %
Split By: Category for Approaches to front
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	18.375	33.272	.2737	
HEMI - Males, WT - Females	46.063	33.272	.0075	S
HEMI - Males, WT - Males	-23.219	33.272	.1679	
HOMO - Females, WT - Females	27.688	33.272	.1012	
HOMO - Females, WT - Males	-41.594	33.272	.0151	S
WT - Females, WT - Males	-69.281	33.272	.0001	S

5.8.1.2 Duration of interaction

Interactions to the Front

ANOVA Table for Duration Front

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	1118353.646	372784.549	.965	.4153	2.895	.244
Subject(Group)	60	23180923.930	386348.732				
Category for Duration Front	1	1250669.971	1250669.971	11.261	.0014	11.261	.929
Category for Duration Front * Genotype	3	476732.037	158910.679	1.431	.2427	4.293	.351
Category for Duration Front * Subject(Gr...	60	6663596.367	111059.939				

Means Table for Duration Front (sec)

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	743.906	324.731	81.183
HEMI - Males, Night	16	1100.156	396.242	99.061
HOMO - Females, Day	16	692.844	575.496	143.874
HOMO - Females, Night	16	947.219	554.007	138.502
WT - Females, Day	16	619.438	462.354	115.589
WT - Females, Night	16	773.063	481.363	120.341
WT - Males, Day	16	911.344	588.408	147.102
WT - Males, Night	16	937.875	545.300	136.325

Interactions to the Back

ANOVA Table for Duration Back

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	1672637.977	557545.992	1.087	.3616	3.261	.272
Subject(Group)	60	30774920.453	512915.341				
Category for Duration Back	1	106548.820	106548.820	1.246	.2687	1.246	.185
Category for Duration Back * Genotype	3	1499225.727	499741.909	5.845	.0014	17.534	.950
Category for Duration Back * Subject(Gr...	60	5130156.703	85502.612				

Means Table for Duration Back (sec)

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	893.000	407.431	101.858
HEMI - Males, Night	16	1023.656	322.452	80.613
HOMO - Females, Day	16	792.719	738.607	184.652
HOMO - Females, Night	16	955.719	572.689	143.172
WT - Females, Day	16	577.906	459.889	114.972
WT - Females, Night	16	824.969	584.816	146.204
WT - Males, Day	16	1154.375	759.219	189.805
WT - Males, Night	16	844.469	346.790	86.697

5.8.2 Activity

5.8.2.1 Locomotion

Distance Traveled

ANOVA Table for Distance Traveled

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	1016914029.959	338971343.320	7.575	.0002	22.724	.988
Subject(Group)	60	2685046213.523	44750770.225				
Category for Distance Traveled	1	12770566939.502	12770566939.502	799.883	<.0001	799.883	1.000
Category for Distance Traveled * Genoty...	3	310721571.662	103573857.221	6.487	.0007	19.462	.970
Category for Distance Traveled * Subject...	60	957932734.461	15965545.574				

Means Table for Distance Traveled

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	1626.188	1020.361	255.090
HEMI - Males, Night	16	21629.719	6254.774	1563.693
HOMO - Females, Day	16	2366.594	1555.318	388.830
HOMO - Females, Night	16	20667.344	7080.701	1770.175
WT - Females, Day	16	6811.313	3690.115	922.529
WT - Females, Night	16	23449.688	8760.542	2190.136
WT - Males, Day	16	5853.469	2230.653	557.663
WT - Males, Night	16	30818.781	7403.157	1850.789

Fisher's PLSD for Distance Traveled
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	110.984	3345.299	.9473	
HEMI - Males, WT - Females	-3502.547	3345.299	.0405	S
HEMI - Males, WT - Males	-6708.172	3345.299	.0002	S
HOMO - Females, WT - Females	-3613.531	3345.299	.0347	S
HOMO - Females, WT - Males	-6819.156	3345.299	.0001	S
WT - Females, WT - Males	-3205.625	3345.299	.0600	

Fisher's PLSD for Distance Traveled
Effect: Category for Distance Traveled
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-19976.992	1412.900	<.0001	S

Fisher's PLSD for Distance Traveled
Effect: Genotype
Significance Level: 5 %
Split By: Category for Distance Traveled
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-740.406	1660.553	.3760	
HEMI - Males, WT - Females	-5185.125	1660.553	<.0001	S
HEMI - Males, WT - Males	-4227.281	1660.553	<.0001	S
HOMO - Females, WT - Females	-4444.719	1660.553	<.0001	S
HOMO - Females, WT - Males	-3486.875	1660.553	<.0001	S
WT - Females, WT - Males	957.844	1660.553	.2532	

Fisher's PLSD for Distance Traveled
Effect: Genotype
Significance Level: 5 %
Split By: Category for Distance Traveled
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	962.375	5254.500	.7154	
HEMI - Males, WT - Females	-1819.969	5254.500	.4911	
HEMI - Males, WT - Males	-9189.063	5254.500	.0009	S
HOMO - Females, WT - Females	-2782.344	5254.500	.2938	
HOMO - Females, WT - Males	-10151.438	5254.500	.0003	S
WT - Females, WT - Males	-7369.094	5254.500	.0068	S

Duration of locomotion

ANOVA Table for Duration Locomotion

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	745734.250	248578.083	10.830	<.0001	32.489	.999
Subject(Group)	60	1377217.000	22953.617				
Category for Duration Locomotion	1	6096595.508	6096595.508	663.577	<.0001	663.577	1.000
Category for Duration Locomotion * Gen...	3	154951.586	51650.529	5.622	.0018	16.866	.941
Category for Duration Locomotion * Subj...	60	551248.156	9187.469				

Means Table for Duration Locomotion (sec)

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	40.969	23.601	5.900
HEMI - Males, Night	16	475.156	151.184	37.796
HOMO - Females, Day	16	50.344	29.745	7.436
HOMO - Females, Night	16	425.406	158.296	39.574
WT - Females, Day	16	157.063	82.662	20.665
WT - Females, Night	16	543.063	195.718	48.929
WT - Males, Day	16	153.406	50.138	12.535
WT - Males, Night	16	704.094	177.640	44.410

Fisher's PLSD for Duration Locomotion
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	20.188	75.764	.5960	
HEMI - Males, WT - Females	-92.000	75.764	.0182	S
HEMI - Males, WT - Males	-170.688	75.764	<.0001	S
HOMO - Females, WT - Females	-112.188	75.764	.0044	S
HOMO - Females, WT - Males	-190.875	75.764	<.0001	S
WT - Females, WT - Males	-78.688	75.764	.0420	S

Fisher's PLSD for Duration Locomotion
Effect: Category for Duration Locomotion
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-436.484	33.894	<.0001	S

Fisher's PLSD for Duration Locomotion
Effect: Genotype
Significance Level: 5 %
Split By: Category for Duration Locomotion
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-9.375	36.728	.6115	
HEMI - Males, WT - Females	-116.094	36.728	<.0001	S
HEMI - Males, WT - Males	-112.438	36.728	<.0001	S
HOMO - Females, WT - Females	-106.719	36.728	<.0001	S
HOMO - Females, WT - Males	-103.063	36.728	<.0001	S
WT - Females, WT - Males	3.656	36.728	.8428	

Fisher's PLSD for Duration Locomotion
Effect: Genotype
Significance Level: 5 %
Split By: Category for Duration Locomotion
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	49.750	121.352	.4154	
HEMI - Males, WT - Females	-67.906	121.352	.2675	
HEMI - Males, WT - Males	-228.938	121.352	.0004	S
HOMO - Females, WT - Females	-117.656	121.352	.0572	
HOMO - Females, WT - Males	-278.688	121.352	<.0001	S
WT - Females, WT - Males	-161.031	121.352	.0102	S

5.8.2.2 Supported Rearing

Supported Rearing Counts

ANOVA Table for Supported Rearing

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	42813071.453	14271023.818	4.269	.0085	12.806	.847
Subject(Group)	60	200593318.766	3343221.979				
Category for Supported Rearing	1	293573709.383	293573709.383	907.856	<.0001	907.856	1.000
Category for Supported Rearing * Genot...	3	5211755.008	1737251.669	5.372	.0024	16.117	.929
Category for Supported Rearing * Subje...	60	19402221.609	323370.360				

Means Table for Supported Rearing counts

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	608.844	268.757	67.189
HEMI - Males, Night	16	4029.563	887.991	221.998
HOMO - Females, Day	16	1021.656	537.352	134.338
HOMO - Females, Night	16	4306.344	1450.218	362.555
WT - Females, Day	16	1731.563	1039.342	259.836
WT - Females, Night	16	4102.875	1647.407	411.852
WT - Males, Day	16	2356.469	1935.471	483.868
WT - Males, Night	16	5395.313	1968.121	492.030

Fisher's PLSD for Supported Rearing
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-344.797	914.360	.4536	
HEMI - Males, WT - Females	-598.016	914.360	.1958	
HEMI - Males, WT - Males	-1556.688	914.360	.0012	S
HOMO - Females, WT - Females	-253.219	914.360	.5817	
HOMO - Females, WT - Males	-1211.891	914.360	.0102	S
WT - Females, WT - Males	-958.672	914.360	.0402	S

Fisher's PLSD for Supported Rearing
Effect: Category for Supported Rearing
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-3028.891	201.080	<.0001	S

Fisher's PLSD for Supported Rearing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Supported Rearing
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-412.813	805.357	.3093	
HEMI - Males, WT - Females	-1122.719	805.357	.0071	S
HEMI - Males, WT - Males	-1747.625	805.357	<.0001	S
HOMO - Females, WT - Females	-709.906	805.357	.0830	
HOMO - Females, WT - Males	-1334.813	805.357	.0016	S
WT - Females, WT - Males	-624.906	805.357	.1259	

Fisher's PLSD for Supported Rearing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Supported Rearing
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-276.781	1088.688	.6129	
HEMI - Males, WT - Females	-73.313	1088.688	.8933	
HEMI - Males, WT - Males	-1365.750	1088.688	.0148	S
HOMO - Females, WT - Females	203.469	1088.688	.7098	
HOMO - Females, WT - Males	-1088.969	1088.688	.0499	S
WT - Females, WT - Males	-1292.438	1088.688	.0208	S

Duration of Supported Rearing

ANOVA Table for Duration Supp Rearing

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	12652813.195	4217604.398	10.142	<.0001	30.426	.999
Subject(Group)	60	24951581.297	415859.688				
Category for Duration Supp Rearing	1	11175219.070	11175219.070	449.844	<.0001	449.844	1.000
Category for Duration Supp Rearing * Ge...	3	282907.758	94302.586	3.796	.0147	11.388	.795
Category for Duration Supp Rearing * Su...	60	1490544.922	24842.415				

Means Table for Duration Supp Rearing (sec)

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	140.031	74.254	18.563
HEMI - Males, Night	16	874.656	217.088	54.272
HOMO - Females, Day	16	251.563	147.223	36.806
HOMO - Females, Night	16	786.344	328.633	82.158
WT - Females, Day	16	415.219	245.672	61.418
WT - Females, Night	16	900.219	345.974	86.494
WT - Males, Day	16	969.750	926.437	231.609
WT - Males, Night	16	1579.156	736.312	184.078

Fisher's PLSD for Duration Supp Rearing
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-11.609	322.484	.9428	
HEMI - Males, WT - Females	-150.375	322.484	.3547	
HEMI - Males, WT - Males	-767.109	322.484	<.0001	S
HOMO - Females, WT - Females	-138.766	322.484	.3928	
HOMO - Females, WT - Males	-755.500	322.484	<.0001	S
WT - Females, WT - Males	-616.734	322.484	.0003	S

Fisher's PLSD for Duration Supp Rearing
Effect: Category for Duration Supp Rearing
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-590.953	55.734	<.0001	S

Fisher's PLSD for Duration Supp Rearing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Duration Supp Rearing
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-111.531	343.895	.5190	
HEMI - Males, WT - Females	-275.188	343.895	.1147	
HEMI - Males, WT - Males	-829.719	343.895	<.0001	S
HOMO - Females, WT - Females	-163.656	343.895	.3450	
HOMO - Females, WT - Males	-718.188	343.895	<.0001	S
WT - Females, WT - Males	-554.531	343.895	.0020	S

Fisher's PLSD for Duration Supp Rearing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Duration Supp Rearing
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	88.313	319.614	.5825	
HEMI - Males, WT - Females	-25.563	319.614	.8734	
HEMI - Males, WT - Males	-704.500	319.614	<.0001	S
HOMO - Females, WT - Females	-113.875	319.614	.4788	
HOMO - Females, WT - Males	-792.813	319.614	<.0001	S
WT - Females, WT - Males	-678.938	319.614	<.0001	S

5.8.2.3 Climbing

Climbing Counts

ANOVA Table for Climbing

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	2630133.334	876711.111	3.876	.0134	11.628	.805
Subject(Group)	60	13571388.414	226189.807				
Category for Climbing	1	54046855.080	54046855.080	518.951	<.0001	518.951	1.000
Category for Climbing * Genotype	3	1188681.131	396227.044	3.805	.0145	11.414	.796
Category for Climbing * Subject(Group)	60	6248786.164	104146.436				

Means Table for Climbing (counts)

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	73.063	36.372	9.093
HEMI - Males, Night	16	1108.063	299.445	74.861
HOMO - Females, Day	16	121.656	109.753	27.438
HOMO - Females, Night	16	1691.438	731.027	182.757
WT - Females, Day	16	330.906	210.071	52.518
WT - Females, Night	16	1575.000	660.014	165.004
WT - Males, Day	16	218.656	104.423	26.106
WT - Males, Night	16	1568.188	439.608	109.902

Fisher's PLSD for Climbing
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-315.984	237.832	.0101	S
HEMI - Males, WT - Females	-362.391	237.832	.0034	S
HEMI - Males, WT - Males	-302.859	237.832	.0134	S
HOMO - Females, WT - Females	-46.406	237.832	.6977	
HOMO - Females, WT - Males	13.125	237.832	.9125	
WT - Females, WT - Males	59.531	237.832	.6184	

Fisher's PLSD for Climbing
Effect: Category for Climbing
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-1299.602	114.115	<.0001	S

Fisher's PLSD for Climbing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Climbing
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-48.594	92.482	.2975	
HEMI - Males, WT - Females	-257.844	92.482	<.0001	S
HEMI - Males, WT - Males	-145.594	92.482	.0026	S
HOMO - Females, WT - Females	-209.250	92.482	<.0001	S
HOMO - Females, WT - Males	-97.000	92.482	.0401	S
WT - Females, WT - Males	112.250	92.482	.0182	S

Fisher's PLSD for Climbing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Climbing
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-583.375	395.809	.0045	S
HEMI - Males, WT - Females	-466.938	395.809	.0216	S
HEMI - Males, WT - Males	-460.125	395.809	.0235	S
HOMO - Females, WT - Females	116.438	395.809	.5584	
HOMO - Females, WT - Males	123.250	395.809	.5357	
WT - Females, WT - Males	6.813	395.809	.9726	

Duration of Climbing

ANOVA Table for Duration Climbing

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	1672865.352	557621.784	10.622	<.0001	31.866	.999
Subject(Group)	60	3149811.953	52496.866				
Category for Duration Climbing	1	12481257.031	12481257.031	418.744	<.0001	418.744	1.000
Category for Duration Climbing * Genotype	3	582807.266	194269.089	6.518	.0007	19.553	.971
Category for Duration Climbing * Subject(...	60	1788386.953	29806.449				

Means Table for Duration Climbing sec

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	20.563	10.564	2.641
HEMI - Males, Night	16	501.594	185.981	46.495
HOMO - Females, Day	16	37.031	39.753	9.938
HOMO - Females, Night	16	831.313	342.784	85.696
WT - Females, Day	16	197.375	113.542	28.385
WT - Females, Night	16	916.250	352.350	88.088
WT - Males, Day	16	61.313	32.649	8.162
WT - Males, Night	16	565.250	193.192	48.298

Fisher's PLSD for Duration Climbing
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-173.094	114.578	.0037	S
HEMI - Males, WT - Females	-295.734	114.578	<.0001	S
HEMI - Males, WT - Males	-52.203	114.578	.3658	
HOMO - Females, WT - Females	-122.641	114.578	.0363	S
HOMO - Females, WT - Males	120.891	114.578	.0390	S
WT - Females, WT - Males	243.531	114.578	<.0001	S

Fisher's PLSD for Duration Climbing
Effect: Category for Duration Climbing
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-624.531	61.048	<.0001	S

Fisher's PLSD for Duration Climbing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Duration Climbing
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-16.469	44.236	.4594	
HEMI - Males, WT - Females	-176.813	44.236	<.0001	S
HEMI - Males, WT - Males	-40.750	44.236	.0703	
HOMO - Females, WT - Females	-160.344	44.236	<.0001	S
HOMO - Females, WT - Males	-24.281	44.236	.2766	
WT - Females, WT - Males	136.063	44.236	<.0001	S

Fisher's PLSD for Duration Climbing
Effect: Genotype
Significance Level: 5 %
Split By: Category for Duration Climbing
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-329.719	198.008	.0015	S
HEMI - Males, WT - Females	-414.656	198.008	<.0001	S
HEMI - Males, WT - Males	-63.656	198.008	.5226	
HOMO - Females, WT - Females	-84.938	198.008	.3943	
HOMO - Females, WT - Males	266.063	198.008	.0093	S
WT - Females, WT - Males	351.000	198.008	.0008	S

5.8.3 Cognitive Behavior

5.8.3.1 Percent Alternation

ANOVA Table for Percent Alternation

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	118.252	39.417	.274	.8441	.821	.099
Subject(Group)	59	8495.075	143.984				
Category for Percent Alternation	1	2932.559	2932.559	28.545	<.0001	28.545	1.000
Category for Percent Alternation * Genot...	3	201.173	67.058	.653	.5844	1.958	.175
Category for Percent Alternation * Subje...	59	6061.309	102.734				

Means Table for Percent Alternation

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	60.135	19.331	4.833
HEMI - Males, Night	16	47.913	7.563	1.891
HOMO - Females, Day	16	58.372	13.944	3.486
HOMO - Females, Night	16	48.313	8.570	2.143
WT - Females, Day	15	57.939	7.748	2.000
WT - Females, Night	15	52.521	5.119	1.322
WT - Males, Day	16	61.244	12.073	3.018
WT - Males, Night	16	50.332	6.740	1.685

Fisher's PLSD for Percent Alternation
Effect: Category for Percent Alternation
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	9.720	3.614	<.0001	S

5.8.3.2 Total number of visits

ANOVA Table for Total visits

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	129696.414	43232.138	4.096	.0104	12.288	.830
Subject(Group)	60	633290.391	10554.840				
Category for Total visits	1	4204637.508	4204637.508	613.126	<.0001	613.126	1.000
Category for Total visits * Genotype	3	47234.914	15744.971	2.296	.0868	6.888	.544
Category for Total visits * Subject(Group)	60	411462.578	6857.710				

Means Table for Total visits

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	44.563	21.293	5.323
HEMI - Males, Night	16	439.219	122.891	30.723
HOMO - Females, Day	16	72.719	35.566	8.892
HOMO - Females, Night	16	432.781	119.539	29.885
WT - Females, Day	16	166.375	39.322	9.830
WT - Females, Night	16	466.969	143.845	35.961
WT - Males, Day	16	105.531	40.967	10.242
WT - Males, Night	16	500.156	120.930	30.232

Fisher's PLSD for Total visits
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-10.859	51.376	.6739	
HEMI - Males, WT - Females	-74.781	51.376	.0050	S
HEMI - Males, WT - Males	-60.953	51.376	.0209	S
HOMO - Females, WT - Females	-63.922	51.376	.0156	S
HOMO - Females, WT - Males	-50.094	51.376	.0558	
WT - Females, WT - Males	13.828	51.376	.5923	

Fisher's PLSD for Total visits
Effect: Category for Total visits
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-362.484	29.283	<.0001	S

Fisher's PLSD for Total visits
Effect: Genotype
Significance Level: 5 %
Split By: Category for Total visits
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-28.156	24.861	.0271	S
HEMI - Males, WT - Females	-121.813	24.861	<.0001	S
HEMI - Males, WT - Males	-60.969	24.861	<.0001	S
HOMO - Females, WT - Females	-93.656	24.861	<.0001	S
HOMO - Females, WT - Males	-32.813	24.861	.0105	S
WT - Females, WT - Males	60.844	24.861	<.0001	S

Fisher's PLSD for Total visits
Effect: Genotype
Significance Level: 5 %
Split By: Category for Total visits
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value
HEMI - Males, HOMO - Females	6.438	89.949	.8866
HEMI - Males, WT - Females	-27.750	89.949	.5395
HEMI - Males, WT - Males	-60.938	89.949	.1805
HOMO - Females, WT - Females	-34.188	89.949	.4501
HOMO - Females, WT - Males	-67.375	89.949	.1393
WT - Females, WT - Males	-33.188	89.949	.4634

5.8.3.3 Percent Active Visits

ANOVA Table for Active Visits

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	176.321	58.774	.403	.7514	1.209	.124
Subject(Group)	59	8605.524	145.856				
Category for Active Visits	1	1055.729	1055.729	18.483	<.0001	18.483	.995
Category for Active Visits * Genotype	3	500.231	166.744	2.919	.0414	8.758	.663
Category for Active Visits * Subject(Gro...	59	3370.017	57.119				

Means Table for Active Visits

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	61.848	10.984	2.746
HEMI - Males, Night	16	49.782	8.696	2.174
HOMO - Females, Day	16	56.990	14.361	3.590
HOMO - Females, Night	16	51.318	8.673	2.168
WT - Females, Day	15	54.092	9.496	2.452
WT - Females, Night	15	53.056	7.095	1.832
WT - Males, Day	16	54.772	9.378	2.344
WT - Males, Night	16	50.380	10.077	2.519

Fisher's PLSD for Active Visits
Effect: Category for Active Visits
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	5.867	2.695	<.0001	S

Fisher's PLSD for Active Visits
Effect: Genotype
Significance Level: 5 %
Split By: Category for Active Visits
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value
HEMI - Males, HOMO - Females	-1.536	6.172	.6203
HEMI - Males, WT - Females	-3.275	6.274	.3006
HEMI - Males, WT - Males	-.598	6.172	.8470
HOMO - Females, WT - Females	-1.739	6.274	.5814
HOMO - Females, WT - Males	.938	6.172	.7621
WT - Females, WT - Males	2.677	6.274	.3967

Fisher's PLSD for Active Visits
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value
HEMI - Males, HOMO - Females	1.661	6.042	.5843
HEMI - Males, WT - Females	2.241	6.141	.4682
HEMI - Males, WT - Males	3.239	6.042	.2878
HOMO - Females, WT - Females	.580	6.141	.8508
HOMO - Females, WT - Males	1.578	6.042	.6032
WT - Females, WT - Males	.998	6.141	.7462

5.8.3.4 Percent Repeat Visits

ANOVA Table for Repeat visits

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Genotype	3	676.145	225.382	3.079	.0343	9.236	.690
Subject(Group)	59	4319.022	73.204				
Category for Repeat visits	1	644.564	644.564	22.016	<.0001	22.016	.999
Category for Repeat visits * Genotype	3	201.596	67.199	2.295	.0871	6.886	.543
Category for Repeat visits * Subject(Gro...	59	1727.342	29.277				

Means Table for Repeat visits

	Count	Mean	Std. Dev.	Std. Err.
HEMI - Males, Day	16	27.655	10.880	2.720
HEMI - Males, Night	16	34.085	6.933	1.733
HOMO - Females, Day	16	32.575	7.732	1.933
HOMO - Females, Night	16	33.926	7.836	1.959
WT - Females, Day	15	26.166	5.026	1.298
WT - Females, Night	15	28.995	5.297	1.368
WT - Males, Day	16	24.173	5.186	1.297
WT - Males, Night	16	31.665	6.229	1.557

Fisher's PLSD for Repeat visits
Effect: Genotype
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-2.381	4.280	.2702	
HEMI - Males, WT - Females	3.290	4.351	.1356	
HEMI - Males, WT - Males	2.951	4.280	.1729	
HOMO - Females, WT - Females	5.670	4.351	.0115	S
HOMO - Females, WT - Males	5.332	4.280	.0155	S
WT - Females, WT - Males	-.339	4.351	.8767	

Fisher's PLSD for Repeat visits
Effect: Category for Repeat visits
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Day, Night	-4.552	1.929	<.0001	S

Fisher's PLSD for Repeat visits
Effect: Genotype
Significance Level: 5 %
Split By: Category for Repeat visits
Cell: Day

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	-4.920	5.347	.0706	
HEMI - Males, WT - Females	1.434	5.347	.5937	
HEMI - Males, WT - Males	3.482	5.347	.1978	
HOMO - Females, WT - Females	6.354	5.347	.0207	S
HOMO - Females, WT - Males	8.402	5.347	.0026	S
WT - Females, WT - Males	2.048	5.347	.4467	

Fisher's PLSD for Repeat visits
Effect: Genotype
Significance Level: 5 %
Split By: Category for Repeat visits
Cell: Night

	Mean Diff.	Crit. Diff.	P-Value	
HEMI - Males, HOMO - Females	.159	4.711	.9465	
HEMI - Males, WT - Females	5.090	4.789	.0376	S
HEMI - Males, WT - Males	2.420	4.711	.3083	
HOMO - Females, WT - Females	4.932	4.789	.0438	S
HOMO - Females, WT - Males	2.261	4.711	.3408	
WT - Females, WT - Males	-2.670	4.789	.2691	