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Novel Behavioral Markers in the *Ppt1-/-* Mouse Model of Infantile Neuronal Ceroid Lipofuscinosis

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A dissertation submitted to The Graduate School of Arts and Sciences at the University of Missouri – St. Louis in fulfillment of the requirements for the degree Doctor of Philosophy in Psychology with an emphasis in Behavioral Neuroscience

July 2013

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Abstract

Infantile neuronal ceroid lipofuscinosis (INCL) is a lysosomal storage disease that is debilitating and fatal to children before their teenage years. The palmitoyl protein thioesterase 1 (PPT1) knockout (*Ppt1-/-*) mouse is an appropriate animal model that mimics much of the symptomology and brain pathology of the human disease. *Ppt1-/-* mice display blindness, seizures, and motor deficits and die well before wild-type mice. However, little is known about the cognitive and behavioral abilities of the *Ppt1-/-* mouse.

This study was conducted to examine further the behavioral phenotype of the *Ppt1-/-* mouse model of INCL by evaluating the animals' abilities in such domains as learning and memory, sensorimotor/motor coordination, and vision. To evaluate when behavioral symptoms become detectable, two ages of mice were included in the study. One cohort was tested beginning in the juvenile period (27 days old), and another cohort was tested beginning in adulthood (147 days old).

Young *Ppt1-/-* mice showed no deficits in locomotor behavior, learning and memory, or vision compared to WT mice. However, *Ppt1-/-* juveniles may experience slight deficits in sensorimotor ability and motor coordination as indicated by decreased distance traveled in the running wheel test and slower swimming speeds during the Morris Water Maze. Adult *Ppt1-/-* mice exhibited more robust performance deficits, including decreased locomotor activity, worse performance during Morris Water Maze cued trials, decreased running wheel ability, and altered reactivity to fear conditioning. These older animals appeared to maintain normal vision and spatial learning ability.

The results of this study expand our knowledge of the *Ppt1-/-* mouse model of INCL and provide novel information about the age of onset of behavioral symptoms. While the adult *Ppt1-/-* mice showed extensive behavioral deficits, some disease symptomatology was present even in the younger cohort. These results provide the grounds for examining *Ppt1-/-* mice at various ages on various domains, with the purpose of establishing solid behavioral markers to serve as benchmarks for disease progression and treatment efficacy.

Introduction

Lysosomal storage diseases (LSDs) are a group of more than 40 distinct disorders characterized by abnormal intracellular storage of undegraded substrates. The function of the lysosome is to convert unwanted intracellular substances into usable ones through enzymatic metabolism. Lysosomal storage diseases are the results of defective lysosomal function, often due to the reduced presence or efficacy of a specific enzyme. The neuronal ceroid lipofuscinoses (NCLs) are a group of at least eight distinct disorders that comprise a subset of LSDs typified by intracellular accumulation of autofluorescent (light-emitting) storage material (ceroid lipofuscins) throughout the brain and body. Individual NCLs are defined first by the age at onset of symptoms, and then by the mutated gene associated with the disorder. Adult-onset NCL (ANCL) is the most variant of the forms, with an age of onset ranging from 15-50 years and the mutation of one of a number of responsible genes. Juvenile NCL (JNCL) patients first present symptoms between 4 and 10 years of age and suffer from a mutation of the *Cln3* gene. Late infantile NCL (LINCL) is associated with mutations of the *Cln2, Cln5, Cln6, Cln7,* or *Cln8* gene and usually presents between 2 and 4 years of age. The most rapidly progressive of the NCL subtypes is infantile neuronal ceroid lipofuscionosis (INCL), with patients first showing symptoms between 6 months and 2 years of age. This condition occurs at a rate of approximately 1 per 10,000-12,500 births worldwide. (Galvin et al., 2008; Miao et al., 2009; Munasinghe, Zhang, Kong, Heffer, & Mukherjee, 2012; Shacka, 2012).

Infantile NCL is associated with an autosomal recessive mutation in the *Cln1* gene which encodes for palmitoyl-protein thioesterase-1 (PPT1), a lysosomal enzyme responsible for breaking long-chain fatty acids from proteins (Cooper, 2003). When this

gene is mutated such that DNA translation and transcription for the protein is disrupted, a deficiency in PPT1 occurs. Undegraded storage material begins to accumulate in many cell types throughout the body, including in the central nervous system (CNS). This storage material is made up of granular osmiophilic deposits, or GRODs, and its accumulation accompanies the progression of behavioral symptoms experienced by patients with INCL. Interestingly, while the NCLs are neurodegenerative in nature, there is no evidence for a direct link between GRODs and subsequent cell death (Mitchison, Ming, & Cooper, 2004). Still, the two occur simultaneously throughout disease progression, and it is clear that cell death in the CNS results in INCL symptomology. Although GROD accumulation occurs in virtually all cell types throughout the body, it is assumed that the effects of the disease are confined to the CNS, although functional significance of peripheral cell damage may not be apparent due to patients' shortened life spans (Galvin et al., 2008; Mitchison et al., 2004).

Patients with INCL appear unaffected at birth and show normal CNS development until the age of 6-12 months. By 1 to 1.5 years, they exhibit a progression of symptoms including visual loss to the point of blindness as well as motor deficiencies. Untreatable seizures appear between 16 and 24 months, and death occurs as early as 6 years, although some live into their teenage years (Macauley et al., 2009). The dearth of literature on cognition and behavior in humans with INCL is likely due to this shortened lifespan and early mental deterioration. In many cases, patients fall into a coma between 3 and 4 years of age after having suffered rapid declines in speech and coordination and an increase in irritability (Gupta et al., 2001; Miao et al., 2009). At such a young age with serious decrement in overall ability, there are few tests that can be administered to

measure cognitive capacity in these children. It is difficult, then, to speculate on the abilities of these children in specific behavioral and cognitive domains.

INCL is invariably fatal in humans, and there is currently no treatment available for the disease (Roberts et al., 2012). Cranial magnetic resonance imaging (MRI) of patients with INCL reveals severe atrophy in the cerebral hemispheres and less severe atrophy in the cerebellum (Kamate & Hattiholi, 2012). These images reveal that cerebral white matter, basal ganglia and, especially, the thalamus show compromised tissue integrity. Neuronal loss is profound in both the cerebral cortex and subcortical structures and is accompanied by astrogliosis, microglial activation (a consistent marker of brain injury and disease), and macrophage infiltration. At autopsy, the cerebellum shows loss of both Purkinje and granule cells, and demyelination is evident throughout white matter (Macauley et al., 2009).

In recent years an appropriate animal model of INCL has been developed by creating a PPT1-knockout mouse (*Ppt1-/-*) (Gupta et al., 2001). These mice show a disruption of the PPT1 enzyme and exhibit progressive motor abnormalities, myoclonic seizures, and shortened life spans, a phenotype similar to human INCL, although at older ages relative to the human ontogeny (Cooper, 2003). Additionally, this animal model recapitulates the GROD accumulation, microglial activation, loss of gamma-aminobutyric acid (GABA) neurons, apoptosis, astrocytosis, and cortical atrophy seen in the human condition (Galvin et al., 2008). The mice mimic human INCL pathology and exhibit a behavioral phenotype typical of the disease.

Mice show normal CNS development at a young age until about 2 months, which in rodents is the human equivalent of late adolescence and young adulthood. Then, the

mice begin to show a progression of symptoms including retinal dysfunction and accompanying loss of vision, loss of motor coordination, development of seizures, and premature death (Shacka, 2012). Retinal dysfunction progresses in severity such that, by 7 months of age, 60% of rod and cone function is lost (Griffey, Macauley, Ogilvie, & Sands, 2005). Deficits in motor coordination, as measured by rotarod performance, are evident, first, at 5 months and are severe by 7 months of age (Griffey et al., 2006; Macauley et al., 2009). Myoclonic jerks are present beginning at 3-4 months and are mostly brief upper body contractions that halt forward progress, appearing similar to a violent sneeze (Gupta et al., 2001). Full-blown, "popcorn-like" seizures, wherein the animals' limbs propel their rigid bodies into the air repeatedly for up to a min at a time, occur with increasing frequency beginning between 6.5 and 7 months of age and affects all animals 7.5 months and older (Galvin et al., 2008). Untreated *Ppt1-/-* mice invariably die before reaching 10 months, often living only to 8 months of age, considered approximately "middle-age" for rodents. (Gupta et al., 2001; Macauley et al., 2012).

Sufficient research has accrued to confirm this knockout mouse as a useful animal model for human INCL. It is currently the standard murine model upon which treatments are evaluated. However, much remains unknown regarding the *Ppt1-/-* mouse, not the least of which is a more complete behavioral profile beyond simply motor abilities. We know that the mice exhibit blindness, seizures, and altered rotarod performance as adults, beginning as early as 5 months, but other motor abilities and potential cognitive abnormalities at the same age are unknowns.

Other questions include what is the behavioral phenotype of this animal model during the juvenile and young adult stages? The *Ppt1-/-* mouse recapitulates many facets

of human INCL, but these mice experience symptoms at ages that are not analogous to the human condition. While humans with INCL do not live into their teens, *Ppt1-/-* mice live well into adulthood, and the current literature suggests that they may not experience symptoms until they reach full maturity at about 60 days old. This model provides us a unique window of opportunity to examine behavioral consequences of the condition. Are there cognitive or motor abnormalities that are detectable at a younger age? Recent pathological findings provide a basis for exploring these research questions.

Galvin et al. (2008) investigated areas of GROD accumulation over the short lifespan of the *Ppt1-/-* mouse and found small- to moderate-sized storage deposits in cortical neurons, hippocampal neurons, and cerebellar Purkinje cells as early as 1 month.

These cell-types showed progressive fat and protein build-up, called lipopigment, accumulating through 7 months of age at which point the deposits were described as frequent and large-sized. By 3 months of age, GROD accumulation was significant in glial and endothelial cells. Neuronal loss and resulting astrocytosis were apparent in the CNS at 5 months, affecting the cerebral neocortex and hippocampal pyramidal cells. In addition to these areas, astrocytosis was present in the cerebral and cerebellar white matter beginning at 5 months (Galvin et al., 2008). Kielar et al. (2009) discovered synaptic and axonal pathology in the *Ppt1-/-* thalamus as early as 3 months that progressively worsened as the animal aged (Kielar et al., 2009). The same lab described in detail the neuropathology of this mouse with regard to specific thalamic nuclei and cortical regions. As young as 3 months of age, *Ppt1-/-* mice show increased lysosomal storage material in the somatosensory cortex (S1) and thalamus as well as significant astrocytosis in S1 and primary motor cortex (M1).

Thalamic regions displaying astrocytosis at 3 months of age included the central posterior, dorsal lateral geniculate, mediodorsal, central medial, and reticular thalamic nuclei. Astrocytosis spread throughout the thalamus as the animals aged, but the aforementioned nuclei showed it most often. Microglial activation, a marker of neuroinflammation and neurodegeneration, was scattered across gray matter at 5 months of age but showed a significant presence specifically in M1, primary visual cortex (V1), S1, and various thalamic nuclei (Kielar et al., 2007).

The neuroanatomical studies, when considered alongside the existing research documenting motor behavior abnormalities in late stages of the disease, lead back to the fundamental questions for the *Ppt1-/-* mouse model of INCL. First and foremost, does the specific damage to the brains of young mutant mice lead to behaviors characteristic of INCL symptomatology? More specifically, given the areas of neuropathology and neurodegeneration from 5 months onward, do mice exhibit motor deficits beyond the rotarod, the standard behavioral test for this model? Also, given the various brain regions and nuclei affected at this age, what are the cognitive abilities of *Ppt1-/-* animals? Does brain pathology at 5 months and older translate to an abnormal behavioral phenotype beyond just the rotarod test? If so, how early are these symptoms detectable? It is also clear that parts of the brain show disease pathology as early as 1 month of age. The question is, does this damage in the brains of young mutant mice lead to motor or cognitive INCL symptomatology?

These are all questions that have yet to be addressed in the literature. The answers would help complete the profile of this animal model and provide useful behavioral benchmarks for disease progression and treatment efficacy. In this study, I sought to

answer these questions and, perhaps, uncover novel behavioral markers in the *Ppt1-/-* animal model of human INCL.

Materials and Methods

Animals

The method for developing PPT1-deficient mice is described in detail elsewhere (Gupta et al., 2001). Briefly, the strain was created through a targeted disruption strategy that eliminates the last exon of the murine PPT1 gene. This targeted mutation was backcrossed to C57BL/6 mice for at least 10 generations. Animals for the experiment were obtained from breeding pairs of mutant/mutant mice, and wild-type (WT) came from a colony of pedigreed C57BL/6 mice maintained by Dr. Mark Sands. The genotype of each animal was be determined by a three primer PCR assay explained in detail previously (Gupta et al., 2001). Male and female mice of each genotype (N=45 for each genotype) served as subjects in the experiment. Each gender was represented in each genotype. All animals were housed in an animal facility at the Washington University School of Medicine (St. Louis, MO) under a 12 h light/dark cycle with lights on at 06:00 and off at 18:00 and were provided food and water *ad libitum.* Behavioral tests were conducted during the light-on part of the day. Animal procedures were approved by the Institutional Animal Care and Use Committee at Washington University and were in accordance with the guidelines of the National Institutes of Health.

Experimental Design

A cross-sectional design was used which involved two cohorts of *Ppt1-/-* and WT mice and a total of N = 52 mice. In one cohort (n = 27) behavioral testing was initiated at a mean age of post-natal day (PND) 27, and testing continued through PND 65. Although

testing occurred up until early adulthood, this cohort will be referred to as the “juvenile” cohort for the sake of convenience. In the juvenile cohort, the sample size for the *Ppt1-/-* group was n = 16 (4 females; 12 males), while for the WT mice it was n = 11, (6 females, 5 males). In a second cohort of mice, behavioral testing began at PND 147 and continued through to PND 185. In this second, adult cohort, the sample sizes were n = 14 for the *Ppt1-/-* group (5 females; 9 males) and n = 11 for the WT control mice (7 females; 4 males). The mice were euthanized at the end of testing for each cohort.

	Mean Age at Testing (postnatal days; PND)	
Behavioral Tests	**Cohort 1**	**Cohort 2**
1 hr Locomotor Activity	PND 27	PND 147
Sensorimotor Battery	PND 28-29	PND 148-149
Morris Water Maze	PND 30-38	PND 150-158
Rotarod	PND39, 43, and 47	PND 159, 163, and 167
Visual Acuity	PND 40-42	PND 160-162
Actometer	PND44-46	PND164-166
Normal Running Wheel	PND 48-52	PND 168-172
Complex Running Wheel	PND 53-62	PND 173-182
Conditioned Fear	PND 63-65	PND 183-185

Table 1. Mean age of the cohorts for each behavioral test is shown.

Behavioral Testing

1-hr locomotor activity. To examine general activity levels and possible differences in emotionality, mice were evaluated over a 1-hr period in transparent polystyrene enclosures measuring 47.6 x 25.4 x 20.6 cm high. This testing has been described previously (Wozniak et al., 2004). Each enclosure was surrounded by pairs of photobeams which were monitored by computer software (MotorMonitor, Kinder

Scientific LLC, Poway, CA). General activity variables included total ambulations, rearing count, and time spent resting. Emotionality measures included time spent in, distance travelled in, and number of entries into both a 33 x 11 cm central zone and the surrounding peripheral area.

Sensorimotor battery. To evaluate possible effects of PPT1-deficiency on balance, strength, and coordination, mice were assessed on a battery of sensorimotor tests including walking initiation, ledge, platform, pole, 60° and 90° inclined screens, and inverted screen. All tests have been described previously (Wozniak et al., 2004). Mice were tested twice on each apparatus, and a mean of the two scores was used for analysis.

Walking initiation. Each mouse was placed in the middle of a square outlined with white tape measuring 21 x 21 cm on a smooth surface. The time it took each mouse to leave the square (all four paws outside of the tape) was recorded with a maximum time of 60 s.

Ledge test. Each mouse was timed for how long it remained on a 0.75 cm Plexiglas ledge without falling off with a maximum time of 60 sec.

Platform test. Each mouse was timed for how long it remained on an elevated circular platform (3.0 cm in diameter, 47 cm off the floor). Animals received a maximum score of 60 sec if they remained on the platform for the entire trial or if they successfully climbed down the pole supporting the platform without falling in less than 60 sec.

Pole test. Each mouse was placed head upward on top of a vertical metal rod with a finely textured surface (8 cm diameter, 55 cm height). The animals were tested for two behaviors: how long it took to completely invert their bodies 180°, and how long it took

to climb down the pole without falling. A maximum score of 120 sec was given if the animal fell before reaching the floor or if it remained on the pole for the entire trial.

60° and 90° inclined screen and inverted screen tests. For the inclined screen tests, mice were placed, head oriented toward the floor, in the middle of a wire mesh grid (0.5 cm squares) tilted at either 60 or 90 degrees. The time it took the animal to climb to the top of the screen was recorded, as well as the time the animal remained on the screen with a maximum score of 60 sec. If the animal successfully climbed to the top of the screen, a maximum score of 60 sec was recorded for the amount of time the animal remained on the screen. For the inverted screen, the animal was placed as described above and the screen was inverted. Time remaining on the screen was recorded with a maximum score of 60 sec.

Morris water maze. To evaluate spatial learning and memory, mice were evaluated on three different Morris water navigation trials (Morris, 1984). The protocol included cued, place, and probe trials. Testing took place in a round pool (118 cm diameter) containing water made opaque with non-toxic white tempura paint. All trials were monitored through a live video feed by computer software (Any-maze, Stoelting Co., Wood Dale, IL) which calculated swim speed, escape path length, escape latency, and time and distance spent in each of the four quadrants of the pool. The maximum score for all water maze trials was 60 sec.

Cued trials were conducted first to evaluate nonassociative learning factors such as sensorimotor ability and motivational disturbances. Mice received 4 cued trials per day for a total of 4 days in a room with very few items on the walls and ceiling so as to limit spatial learning. On days 1 and 2, animals were placed in the water directly opposite a

submerged platform marked with a visible pole and red tennis ball. In order to limit spatial learning in the cued condition, the platform was moved to a different location for each trial within the day and no extra-maze cues were placed on the walls or ceiling. After each trial, mice were allowed to remain on the platform for 30 sec. Intertrial intervals (ITIs) were approximately 45 min. During days 3 and 4 of cued trials, mice were placed in the water directly opposite the submerged platform marked with only the visible pole; the tennis ball having been removed. Again, the platform was moved to a different location for each trial within the day, and ITIs were approximately 45 min.

Place trials took place the day after the final cued trials. In the presence of salient distal cues to facilitate spatial learning, mice were evaluated on their ability to learn the location of a submerged, unmarked platform. Large geometric shapes (a plus sign, a triangle, a circle) cut from black poster board served as cues and were placed on the white walls surrounding the maze. Cues remained in the same configuration on the walls for the entirety of place (and probe) trials. Four place trials per day were administered for 5 consecutive days during which the platform remained in the same location with the mice being released at 4 different locations per day. The daily protocol was to administer 2 sets of 2 trials each, with sets separated by approximately 1 hr.

A single *probe trial* was administered approximately 1 hr after completion of place trials on the fifth day to evaluate retention of the platform location. During the probe trial, the platform was removed from the pool and the animals placed in the quadrant directly opposite the former platform location. Mice were allowed to explore the water maze for 60 sec during which swim speed, path length, time spent and distance travelled in each quadrant, and platform crossings were recorded.

Rotarod. The rotarod test (Economex, Columbus Instruments, Columbus, OH) was administered to evaluate motor coordination and balance. The protocol was similar to one described previously (Ho et al., 2000) and involved three conditions: stationary rod (60 sec maximum), constant rotation (2.5 rotations per min, 60 sec maximum), and accelerating rotation (2.5-10.5rotations per min, 180 sec maximum). Trials were administered on 3 test days, each separated by 4 days in an attempt to minimize confounding with motor learning. Each test day included, in the following order, one stationary trial, two constant trials, and two accelerating trials with trials separated by approximately 30 min. Time spent on the rod for each trial was recorded.

Visual acuity. Mice were evaluated for visual acuity using the Virtual Optomotor System (VOS) as described previously (Brown et al., 2010; Prusky, Alam, Beekman, & Douglas, 2004). Briefly, the apparatus consists of a virtual cylinder comprising a vertical sine wave grating projected in three-dimensional (3-D) space on computer monitors. The 4 monitors are arranged in a quadrangle around a central circular platform, forming a square arena (46 cm x 46 cm). The floor of the arena is a square mirror and the ceiling is the same but with a large central access hole. A camera (FireWire iSight; Apple Computer Corp., Mountain View, CA, USA) is positioned directly above the platform to allow visual access to the mouse's behavior. After a mouse was placed on the central platform, light and dark bars were projected on the monitors to give the appearance of a cylinder rotating around the mouse. The virtual rotational motion of these bars induced optokinetic head/body tracking movements. Thresholds for visuospatial acuity were generated by increasing the frequency of the sine waves until the optokinetic response was no longer observed, indicating that the animal no longer distinguished the individual

lines rotating around them. An observer without knowledge of the direction of grating rotation judged the presence or absence of head/body tracking movements by watching live video of the mouse on a computer screen. The speed of rotation and geometry of the projected cylinder was controlled by the system software. The dependent variable was the highest grating (in cycles per degree; cyc/deg) at which the animal could discriminate between light and dark rotating lines before failing to display optokinetic responses. All testing was conducted under photopic conditions (1.8 log cd/m^2).

Actometer. To assess exploratory behavior and gait, each animal was tested in the force plate actometer. The design and detailed description of the actometer is similar to a previous description but on a slightly larger scale (S. C. Fowler et al., 2001). Briefly, a square load plate (42 x 42 cm) sits atop 4 load cell transducers (Honeywell/Sensotec, Columbus, OH) and is surrounded by 4 Plexiglas walls measuring 43 x 30.5 cm. The apparatus quantifies the movements of the animal within the chamber by measuring the displacement of the center of force or center of pressures along the plate bottom. Information from the 4 transducers is sent through the LabMaster (Scientific Solutions, Mentor, OH) which converts analogue signals to digital form which can then be processed through data acquisition software. To reduce outside movement, the chamber is set atop a large slate slab (90 x 55.7 x 3 cm) that is stabilized by 4 rubber legs. The animals were placed in the center of the chamber and allowed to explore for 20 min. Dependent variables included distance travelled, low mobility bouts (times during which the animal moved fewer than 15 mm in a span of 10 s), and gait analysis. For gait analysis, for each 20-min recording session, a computer algorithm identified all episodes of lateral movement on the actometer load plate that constituted ambulations or “runs”

that covered a distance of 17.5 cm or more in a period of 1.5 s. In addition to providing information on the mice's capacities to sustain locomotion over a multi-body-length distance, these runs were subjected to Fourier analysis as described previously (Fowler, Miller, Gaither, Johnson, & Rebec, 2009). For a run to qualify for inclusion in the analysis it had to have a nearly straight-line trajectory between its starting and ending point. Dependent variables included stride length (in mm) and stride rate (in mm/s).

Normal and complex running wheel. Mice were evaluated for voluntary wheel running activity as well as fine motor coordination between fore- and hindlimbs utilizing a motor skill sequence (MOSS) protocol described previously (Maloney, Noguchi, Wozniak, Fowler, & Farber, 2011).

Normal running wheel. Mice were tested on a normal mouse activity wheel (Mouse Motor Skill Sequences Activity Wheel, Lafayette Instrument, Lafayette, IN) for 1 h on 5 consecutive days to establish baseline voluntary wheel running activity. Each mouse was placed in a polycarbonate activity wheel chamber measuring 31.5 x 19.5 x 19.5 cm. The chamber floors were covered with a thin layer of bedding, and a conventional aluminum running wheel was suspended from the chamber top. Running wheel data was collected by an optical rotation sensor mounted 0.5 cm from the wheel rungs and was transmitted to the Activity Wheel Monitoring System (Lafayette Instrument, Lafayette, IN) on a nearby computer. The normal activity wheel contained 38 consecutive rungs (0.4 cm in diameter) that were spaced 0.614 cm apart. Mice were allowed to explore the wheel and activity chamber for 1 h on 5 consecutive days. Dependent variables were tracked by the software system and included average speed, maximum speed, time spent running on the wheel (sum of 10 sec intervals in which the

wheel rotated at least 6 times, converted to min), distance travelled (where 1 revolution of the wheel equals 0.40 meters of distance travelled) and time spent resting (sum of 10 sec intervals in which the wheel rotated 0 times, converted to min).

Complex running wheel. To assess fine motor coordination between the fore- and hind-limbs, the mice were tested on the complex activity wheel. Spacing between wheel rungs was made irregular by removing rungs to create gaps of 0.614, 1.6, or 2.6 cm. This protocol allowed for more sensitivity in evaluating fine motor coordination than is allowed by the normal activity wheel test (Maloney et al., 2011). Besides the "customized" wheel, all other parameters of the apparatus remained the same as in the normal activity wheel phase. Again, mice were allowed to explore the wheel and activity chamber for 1 h each day, this time for 10 consecutive days. Dependent variables were the same as those measured in the normal activity wheel.

Conditioned fear. The conditioned fear test was the final test administered because of the emotional component involved and the possibility that this experience could alter the animals' reactivity to novel stimuli presented in subsequent testing. Mice were evaluated for fear conditioning over 3 test days using a previously described protocol (Wozniak, Xiao, Xu, Yamada, & Ornitz, 2007). Testing took place in two Plexiglas conditioning chambers, each differing in terms of visual, tactile, and olfactory cues. On the first day, mice were placed in the first Plexiglas chamber for one 5-min trial during which freezing behavior was quantified by computer software (FreezeFrame, Coulborn Instruments, Whitehall, PA). The first 2 min served as a baseline period, after which an 80 dB tone (conditioned stimulus; CS) of white noise was presented for 20 sec. During the last sec of CS, a 1.0 mA continuous foot shock (unconditioned stimulus; US)

was administered. This pairing was repeated for each of the next 2 min. Twenty-four hr later, each mouse was placed in the same chamber as the first day for an 8-min trial during which no CS or US was presented. Again, freezing behavior was quantified, this time as a measure of the hippocampal-related contextual fear conditioning. Twenty-four hr after this trial, mice were evaluated on the auditory cue test. The animals were placed in the other Plexiglas chamber and freezing behavior was quantified for 10 min in this "altered context." The first 2 min again served as a baseline period, followed by 8 min during which the auditory tone (CS) was continuously presented. The dependent variable for each trial was the percent of time the animal spent freezing. The FreezeFrame software adjusts a freezing threshold for each animal and categorizes behavior as freezing or not freezing during 0.75 sec intervals.

Statistical Analyses

All statistical analyses were conducted using PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL). Means and standard errors were computed for each variable, and tables and figures are used to report findings. Analyses included, where appropriate, factorial ANOVAs, including repeated measures ANOVAs (rmANOVAs), and one-way ANOVAs. All ANOVA models contained Genotype as a between-subjects variable. The rmANOVA models also typically contained either one within-subjects variable (e.g., Blocks of Trials) or two within-subjects variables (e.g., trials and sessions). Sex was not included in the ANOVA models due to an unequal distribution of sexes across groups. Simple main effects were calculated in the case of a significant interaction. In the event of a violation of sphericity as measured by Mauchly's sphericity test, the F statistic, degrees of freedom, and *p*-value were all corrected via the

Greenhouse-Geisser or Huynh-Feldt method in accordance with accepted guidelines (Girden, 1992) For a significant F value for main and simple effects, pairwise comparisons were used to compare means over the repeated measure. Probability value for all analyses was $p < .05$, and multiple comparisons were Bonferroni adjusted.

Research Questions and Hypotheses

Research question 1: Do *Ppt1-/-* mice exhibit cognitive and motor deficits, compared to wild-type mice, during adolescence: comparisons of *Ppt1-/-* and WT mice at 1-2 months of age.

The current study expanded the behavioral profile of the *Ppt1-/-* mouse by employing a comprehensive battery of tests. The rotarod, a standard test for this animal model, was used, along with other measures of motor ability and cognitive measures. Since these animals had not yet been tested in many behavioral paradigms, it was difficult to speculate how they would perform at any age, much less an age younger than has been evaluated on any test. Still, the body of research detailing neuropathology at various ages provided some clues. Hypotheses regarding behavioral testing at 1-2 months of age included:

Hypothesis 1A: My hypothesis was that there would be no differences between experimental and WT control groups on horizontal ambulations, vertical rears, or time spent in center or peripheral zones of the activity chamber. This prediction was based on informal observations in our lab and others.

Hypothesis 1B: The researchers who developed the mouse model of INCL noted a "strongly abnormal clasping behavior" when the animal was suspended by its tail as early as 50 days of age (Gupta et al., 2001). However, in my first cohort, mice

were 3 weeks younger than those that displayed the abnormal clasping behavior. Moreover, no changes in Purkinje cell numbers were observed in the cerebellum at this age (Macauley et al., 2009). Although Purkinje cells show GROD accumulation at this age, there may be no direct link between this accumulation and behavioral disruptions (Galvin et al., 2008). Consequently, the hypothesis was for no differences between groups on any of the sensorimotor tasks.

Hypothesis 1C: Since there is no detectable hippocampal pathology in the *Ppt1-/-* mouse at this age, I expected no deficits in the Morris Water Maze. No differences between groups were expected on latency to find the escape platform, distance travelled to the platform, or average swim speed.

Hypothesis 1D: In accordance with previous findings (Macauley et al., 2009), *Ppt1-/-* mice were expected to perform similarly to WT mice on the stationary and constant rotarod conditions. On the accelerating rotarod, however, the hypothesis was that the *Ppt1-/-* group would spend significantly less time balanced on the apparatus. I expected significant changes over days of testing as the mutant animals experience increased neuropathology in S1, M1, and various thalamic nuclei.

Hypothesis 1E: Previous research has shown that untreated *Ppt1-/-* mice experience an 18% decrease in retinal function as early as 60 days of age (Griffey et al., 2005). The animals in the current study were approximately 41 to 43 days old, and therefore no differences were expected in visual acuity, measured by the highest grating at which they could discriminate between light and dark rotating lines before failing to display an optokinetic response, between the mutants and WTs.

Hypothesis 1F: There would be no differences in measures of low mobility bouts or distance traveled between *Ppt1-/-* and WT mice. Because mutants at this age show increasing neuropathology in S1, M1, and various thalamic nuclei, the hypothesis was that *Ppt1-/-* mice would have altered gaits as revealed by abnormal foot-strike patterns on the force-plate actometer.

Hypothesis 1G: No differences were expected between groups on any of the variables measured by the normal activity wheel test.

Hypothesis 1H: By the end of complex activity wheel testing, groups were approximately 63 days old. Pathology will have been nearing significance in various thalamic nuclei as well as S1 and M1 areas. The complex activity wheel is a sensitive measure of motor coordination and learning, and it was expected that *Ppt1-/-* mice would show deficits. Specifically, mutants were expected to run a shorter distance, run at a slower average speed and have a lower maximum speed than WT mice. No differences were expected in time spent interacting with the wheel.

Hypothesis 1I: Although a different animal model of NCL (the *mnd* mouse with Cln8 mutation) than the one used here has shown deficits in fear conditioning possibly associated with amygdalar neuropathology (Bolivar, Ganus, & Messer, 2002), no differences were expected in time spent freezing on any of the conditioned fear test days. Significant pathology in the amygdala-hippocampal circuit has not been found in 1-2 month old *Ppt1-/-* mice.

Research question 2: Do adult *Ppt1-/-* mice exhibit cognitive and/or motor deficits: comparisons of experimental and wild type mice at 5-6 months of age.

Hypothesis 2A: Previous research with 7-month-old *Ppt1-/-* animals shows reduced general exploratory behavior as measured by total horizontal ambulations. Mutants also exhibited fewer entries into the central area of the activity chamber, indicating increased anxiety-like behavior (Griffey et al., 2006). Because 5 month and 7 month old *Ppt1-/-* mice show similar pathologies, similar results were expected. *Ppt1-/-* were predicted to exhibit decreased horizontal ambulations, vertical rears, and entries into the central zone, and time spent in the central zone compared to WT controls.

Hypothesis 2B: It was revealed in the same study (Griffey et al., 2006) that *Ppt1-/-* mice 7 months of age showed increased time to climb down the pole and decreased time balancing on the ledge, tests included in the sensorimotor battery in the current study. This hypothesis was that similar differences would be found in mutants at 5 months of age. *Ppt1-/-* mice were expected to show increased time to leave the square, decreased time on the ledge, decreased time on the platform, increased time to climb down the pole, increased time to climb to the top of the 60- and 90-degree screens, and decreased time hanging on the inverted screen compared to control mice. No group differences were expected in time remaining on the 60- and 90-degree screens.

Hypothesis 2C: Due to the motor deficits expected at this stage of life, as well as emerging neuropathology in the hippocampus and decreased retinal function, it was expected that *Ppt1-/-* animals would show deficits in the Morris Water Maze.

Specifically, mutants were predicted to take longer to find the platform, travel a farther distance to the platform, and swim slower than controls during the cued and place trials. During the probe trial, *Ppt1-/-* animals were expected to swim slower than controls and would exhibit less time spent in the target quadrant as well as fewer platform crossings.

Hypothesis 2D: In accordance with previous findings (Macauley et al., 2009; Roberts et al., 2012), it was predicted that *Ppt1-/-* mice would spend less time on the rotarod apparatus than WT controls during both the continuous and accelerating conditions. No differences were expected between groups on the stationary trials.

Hypothesis 2E: With the significant decrease in retinal function at 5 months of age (Griffey et al., 2005), this hypothesis was that mutants would exhibit decreased visual acuity compared to WT control mice.

Hypothesis 2F: Mutants were expected to show more low mobility bouts, decreased distance traveled, and an abnormal gait compared to controls.

Hypothesis 2G: It was expected that *Ppt1-/-* mice would show decreased levels of activity in the normal running wheel as indicated by shorter distance, slower average speed and slower maximum speed compared to WT mice. However, I hypothesized that *Ppt1-/-* mice would spend a similar amount of time interacting with the wheel as controls.

Hypothesis 2H: Similarly, 6 month old *Ppt1-/-* mice were predicted to travel a shorter distance, run slower on average and have a slower maximum speed than WT mice. Again, no group differences were expected in time spent interacting with the wheel.

Hypothesis 2I: Hippocampal pathology suggests that *Ppt1-/-* mice would show decreased freezing activity compared with control mice on all 3 trials of the conditioned fear test.

Results

1-h Locomotor Activity

Juveniles. To assess habituation and exploratory behaviors over the 1 hr testing period, rmANOVAs were conducted on total ambulations and vertical rearing frequency over six 10-min blocks with Genotype (*Ppt1-/-* versus WT) as the main factor and Time Blocks as the within-subjects repeated measure. No significant main or interaction effects involving Genotype were found for either total ambulations or rearing frequency. To evaluate habituation, the performance on Block 1 versus Block 6 was evaluated within each group for total ambulations and rearing frequency. The results of these comparisons showed that *Ppt1-/-* mice exhibited significant decreases indicating habituation from Block 1 compared to Block 6 for total ambulations, $p = .000$, and rearing frequency, $p = .000$, while the WT group demonstrated the same effects, $p = .000$ for both. One-way ANOVAs conducted on time spent in center zone, distance traveled in center zone, entries made into center zone, distance traveled in peripheral zone, and total rest time did not yield any significant main or interaction effects involving Genotype, indicating that the two groups performed similarly on these variables (Figure 1A-F).

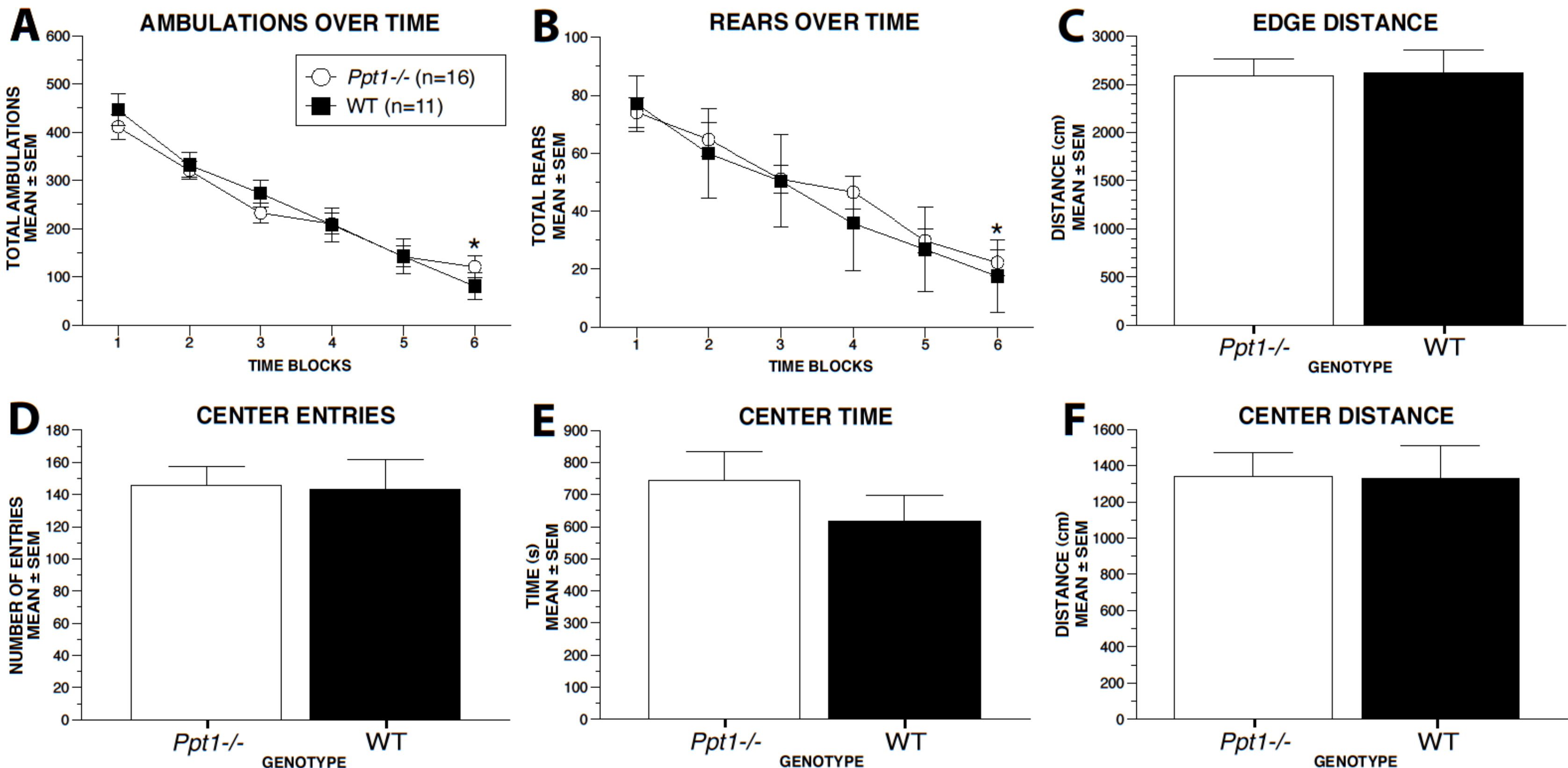

Figure 1. Juvenile 1-h locomotor activity. Juvenile WT and *Ppt1-/-* mice performed similarly on all measures during the 1 h locomotor activity test, including (A) total ambulations, and (B) total number of rears. Both groups showed habituation over time, exhibiting fewer ambulations and rears during Time Block 6 compared to Time Block 1, $*p = .000$. WT and *Ppt1-/-* mice also performed similarly on (C) distance traveled in the edge of the chamber, (D) entries into the center of the chamber, (E) time spent in the center zone, and (F) distance traveled in the center zone.

Adults. In contrast to the juvenile results, ANOVAs conducted on the variables from the 1 h activity test in the adult mice showed that groups differed on every performance variable. Specifically, rmANOVAs conducted on the total ambulations and rearing data produced significant main effects of Genotype for each variable, $F(1,23)=15.11$, $p=.001$ and $F(1,23)=5.10$, $p=.034$, respectively, indicating that the WT mice exhibited significantly increased levels for both variables over time (Figure 2A and B). WT mice showed increased total ambulations, compared to *Ppt1-/-* mice, during Time Blocks 3, 4, 5, and 6, $p < .002$ for all. Comparisons of performance during Block 1 versus Block 6 demonstrated that the WT and *Ppt1-/-* groups each significantly decreased their total ambulations, $p = .000$ for both, but not rearings, over the test session. Significant Genotype effects were found following one-way ANOVAs conducted on each of the remaining variables (Figure 2C-F). For example, WT mice traveled a farther distance in both the peripheral and center zones, $F(1,24)=6.95$, $p=.015$ and $F(1,24)=22.62$, $p=.000$, respectively, compared to the *Ppt1-/-* group. In addition, WT animals entered the center zone more times than the *Ppt1-/-* animals and also spent more time in the center zone, $F(1,24)=26.63$, $p=.000$ and $F(1,24)=4.49$, $p=.045$ respectively. Also, adult *Ppt1-/-* mice spent more time resting during the 1 h test than the WT mice, $F(1,24)=19.77$, $p=.000$.

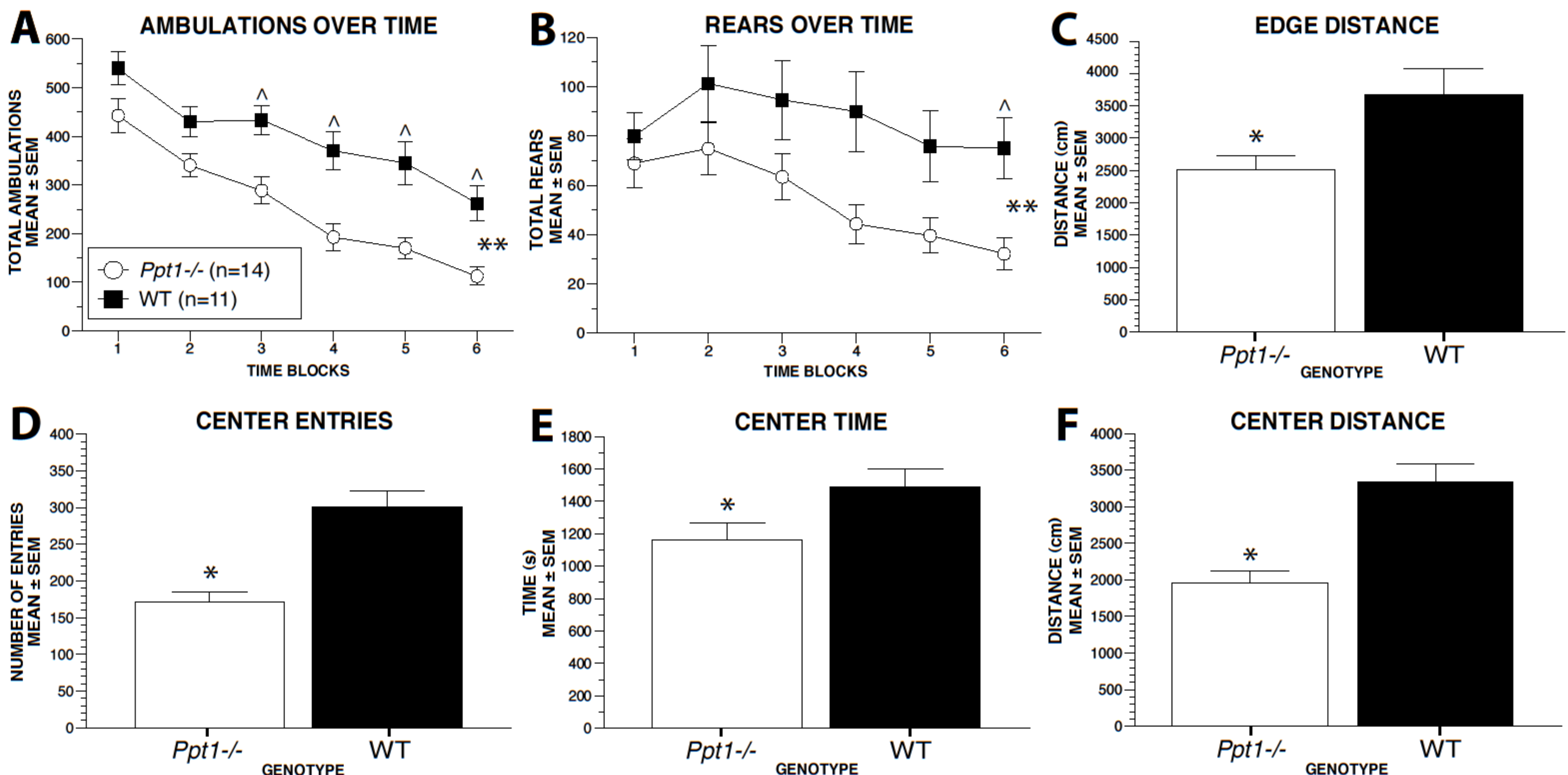

Figure 2. Adult 1-h locomotor activity. Adult *Ppt1-/-* mice exhibited fewer (A) total ambulations, p = .001 and (B) fewer total rears, p = .034, compared to WT mice during the 1 h locomotor activity test. *Ppt1-/-* mice also (C) traveled a shorter distance in the edge of the chamber, p = .015, (D) entered the center zone fewer times, p = .000 (E) spent less time in the center zone, p = .045 and (F) traveled a shorter distance in the center zone, p = .000, compared to WTs. ** Denotes a significant main effect of Genotype across intervals. * Denotes a significant main effect of Genotype. ^ Denotes group differences at a single Time Block.

Sensorimotor Battery

Sensorimotor data were analyzed by a one-way ANOVA for each of the tests in the battery, where Genotype (*Ppt1-/-* versus WT) served as the main factor and scores averaged across two trials served as the dependent variables.

Juveniles. One-way ANOVAs revealed no effect of Genotype for the ledge, platform, pole, 90° inclined screen, or inverted screen tests. However, *Ppt1-/-* mice required significantly more time to move out of the square during the walking initiation test compared to the WT group, $F(1, 25)=5.86$, $p=.023$. In addition, a significant Genotype effect was found for the 60° inclined screen data, with *Ppt1-/-* mice taking longer to climb to the top of the screen than their WT counterparts, $F(1,25)=14.16$, $p=.001$, (Figure 3).

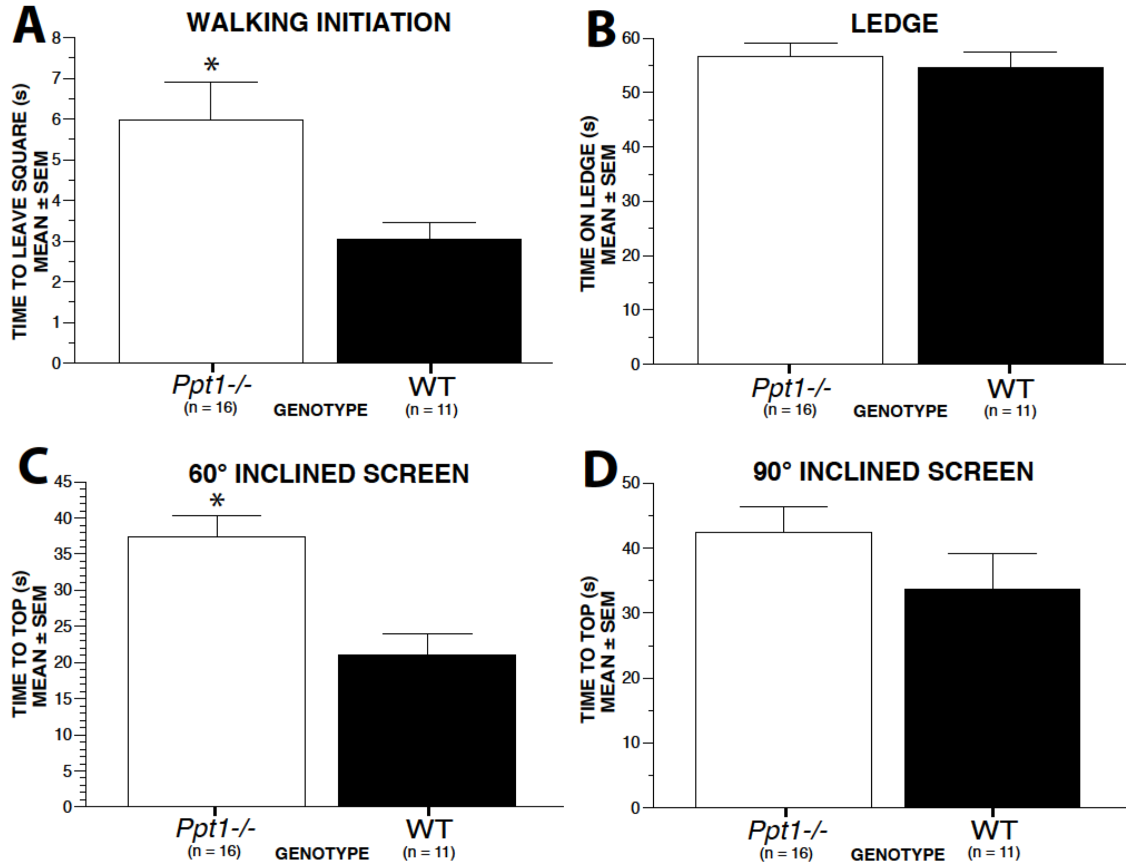

Figure 3. Juvenile sensorimotor battery. Juvenile *Ppt1-/-* mice (A) required more time to leave the walking initiation square, p = .023, and (C) more time to climb to the top of the 60° inclined screen, p = .001, compared to WT mice. Genotypes performed similarly on the (B) ledge and (D) 90° inclined screen test. * Denotes a significant main effect of Genotype.

Adults. One-way ANOVAs yielded significant Genotype effects for the ledge, $F(1,24)=7.70$, $p=.011$, and the 90° inclined screen, $F(1,24)=5.20$, $p=.032$, tests showing that *Ppt1*-/- mice spent less time balancing on the ledge and required more time to climb to the top of the 90° screen compared to WT mice (Figure 4). There were no differences between groups on any of the other sensorimotor measures.

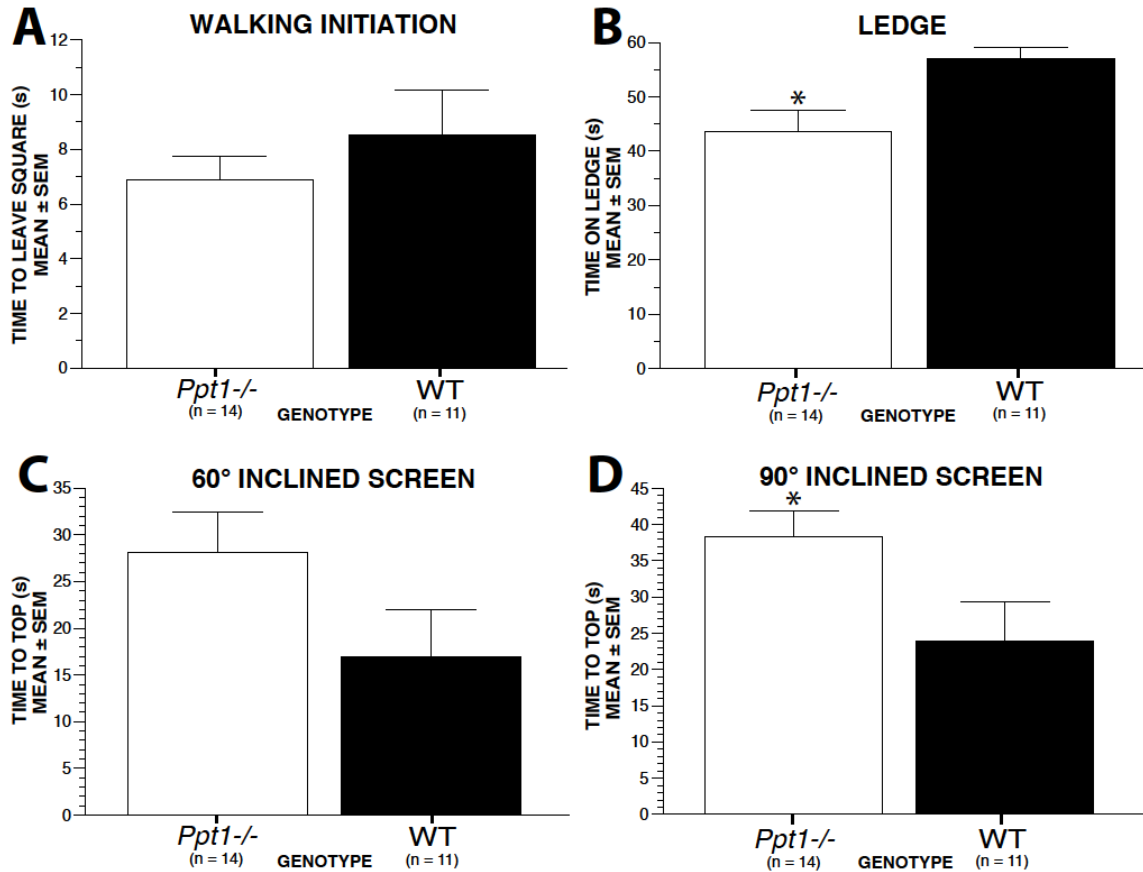

Figure 4. Adult sensorimotor battery. Adult *Ppt1-/-* mice performed similarly to WT mice on the (A) walking initiation and (C) 60° inclined screen tests. However, *Ppt1-/-* mice (B) balanced on the ledge for less time, $p = .011$, and (D) required more time to climb to the top of the 60° inclined screen, $p = .032$, compared to WT mice. * Denotes a significant main effect of Genotype.

Morris Water Maze

For the MWM cued condition, trials were grouped into blocks consisting of 2 trials each, resulting in 8 total blocks. The data for each block was the mean score of those two trials. The first 4 blocks were analyzed with a rmANOVA, with Genotype (*Ppt1-/-* versus WT) as the main factor and Blocks of Trials as the repeated measure, to assess cued learning when the platform was marked by both a pole and a ball. A second rmANOVA was used to analyze the final 4 Blocks of Trials to assess the same behavior when the platform was only marked by a thin pole. For the place condition, this same blocking technique was applied but with blocks of 4 trials instead of 2. Again, data was

analyzed with a rmANOVA with Genotype as the main factor with Blocks of Trials as the repeated measure. Data from the single probe trial consisted of time spent in each of the 4 pool quadrants (the target quadrant and each of the quadrants to the left, right, and opposite the target) and number of entries into the platform zone. A one-way ANOVA was performed separately for the number of platform entries and time spent in the target quadrant, with Genotype serving as the main factor for both. Additionally, a rmANOVA was used to compare the amount of time the animals spent in each of the 4 quadrants during the probe trial

Juveniles. No significant main or interaction effects involving Genotype were found for escape path length, latency, or swimming speeds during the first 4 Blocks of Trials in the cued MWM (Figure 5A-C), thus documenting similar performances by the WT and *Ppt1-/-* mice on these variables when the platform was marked by a visible pole and ball. A significant effect of Blocks of Trials for latency, $F(3, 75)=41.11$, $p=.000$, and path length, $F(3, 75)=43.43$, $p=.000$, plus significant differences from comparisons made between the first and last block of trials within each group which showed significant decreases in the levels of both variables, $p = .000$ for all, demonstrated significant acquisition of cued learning in both groups.

However, when the platform was marked only by a visible pole, a main effect of Genotype was found for both latency, $F(1,25) = 4.39$, $p = .046$, and swim speed, $F(1,25) = 4.89$, $p = .036$, indicating that *Ppt1-/-* mice required more time to find the escape platform and swam slower across blocks of trials compared to WT mice (Figure 5A-C). A significant Genotype x Blocks of Trials interaction effect was found for swim speed, $F(3,75) = 4.320$, $p = .007$, with pairwise comparisons indicating that while WT animals

showed an increase in swim speed between the first and the last blocks of trials, $p = .001$, *Ppt1-/-* mice did not. In addition, WT mice swam significantly faster than *Ppt1-/-* during the final block of trials, $p = .006$. A significant effect of Blocks of Trials was revealed for latency, $F(3,75)=4.37$, $p=.007$, path length, $F(3,75)=3.56$, $p=.018$, and swim speed, $F(3,75)=7.09$, $p=.000$. Across genotypes, mice required less time ($p = .005$) and traveled less distance ($p = .006$) to find the escape platform..

No significant main or interaction effects involving Genotype were found for path length or latency during acquisition training in the place condition, indicating that the two groups performed similarly concerning these two variables (Figure 6A and B). However, there was a main effect of Genotype with regard to swimming speeds, $F(1,25) = 6.36$, $p = .018$, indicating that the WT mice swam significantly faster, on average, than *Ppt1-/-* mice during place trials (Figure 6C). Evidence that both groups displayed significant place (spatial) learning was supported by a significant effect of Blocks of Trials for latency, $F(3.02, 75.57) = 14.70$, $p = .000$, and path length, $F(3.13, 78.30) = 18.96$, $p = .000$. Further evidence was provided by a significant decrease in *Ppt1-/-* latency ($p = .000$) and path length ($p = .000$) as well as WT latency ($p = .004$) and path length ($p = .003$) from trial block 1 to trial block 5. The two groups also performed similarly on the retention variables during the probe trial. Specifically, no significant overall effects involving Genotype were found for platform crossings, time spent in the target quadrant, or spatial bias. Both groups showed spatial bias for the target quadrant, $F(2.32, 57.90) = 59.86$, $p = .000$, in that they spent significantly more time in the target quadrant compared to the times spent in the quadrant to the right ($p = .000$), left ($p = .000$), and opposite ($p = .000$) the target quadrant (Figure 7A and B).

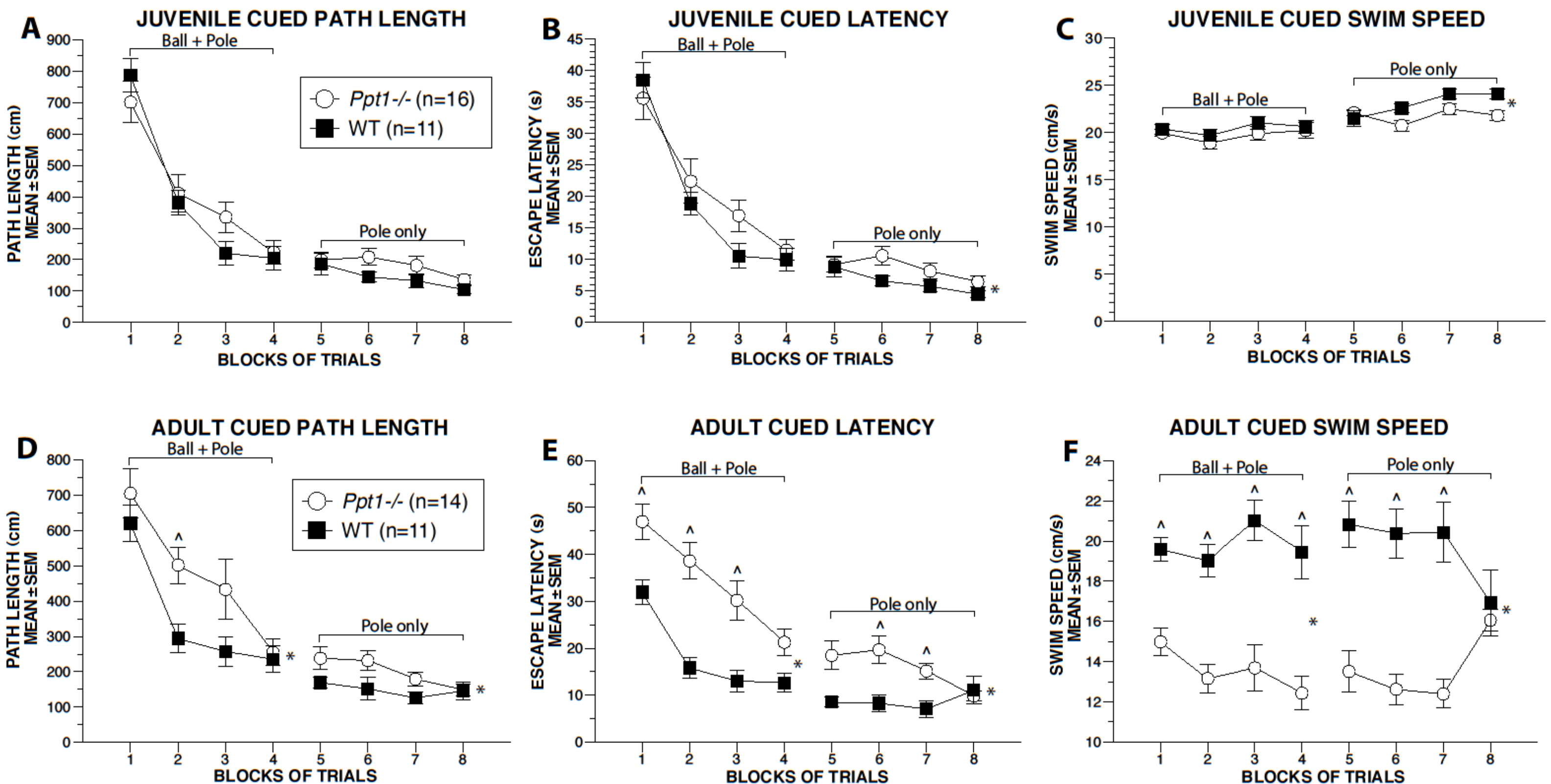

Figure 5. Morris Water Maze, cued trials. In the juvenile cohort (A-C), genotypes performed similarly on all variables when the platform was visibly marked with a pole and tennis ball (Ball + Pole). When the ball was removed from the pole (Pole only), *Ppt1-/-* mice (B) required more time to find the escape platform, $p = .046$, and (C) swam significantly slower than WT mice, $p = .036$. In the adult cohort, *Ppt1-/-* mice (D) required more time to find the platform, $p = .000$, (E) swam a longer distance, $p = .011$ and (F) swam significantly slower than WTs, $p = .000$ during Ball + Pole blocks of trials. During Pole only trial blocks, the differences in (D) path length, $p = .006$, (E) latency, $p = .000$, and (F) swim speed, $p = .000$, persisted. * Denotes a significant main effect of Genotype. ^ Denotes group differences at a single trial block.

Adults. Multiple rmANOVAs on the data from the first 4 Blocks of Trials during cued MWM produced a significant main effect of Genotype on latency, $F(1, 23)=36.62$, $p=.000$, path length, $F(1, 23)=7.56$, $p=.011$, and swimming speeds, $F(1, 23)=33.00$, $p=.000$, demonstrating that *Ppt1-/-* mice required more time, $p = .000$, swam a longer distance, $p = .000$, and swam at a slower speed, $p = .000$, to find the escape platform (Figure 5D-F). A significant effect of Blocks of Trials for latency, $F(3, 69)=19.30$, $p=.000$, path length, $F(3, 69)=20.22$, $p=.000$, and swimming speeds, $F(3, 69) = 3.18$, $p = .029$, as well as both Genotypes showing improvement on latency and path length from trial block 1 to trial block 4, $p = .000$ for all, provide evidence of cued learning in both groups.

During the second 4 Blocks of Trials, significant main effects of Genotype on latency, $F(1, 23)=12.07$, $p=.000$, path length, $F(1, 23)=8.97$, $p=.006$, and swimming speeds, $F(1, 23)=23.88$, $p=.000$ were found. Again, *Ppt1-/-* mice required more time, $p = .002$, swam a longer distance, $p = .006$, and swam at a slower speed, $p = .000$, to find the escape platform (Figure 5D-F). There was no main effect of Blocks of Trials on any of the 3 variables, but a significant Genotype x Blocks of Trials interaction was found for latency, $F(2.54, 58.50) = 4.04$, $p = .015$, as well as swimming speeds, $F(3,69) = 11.53$, $p = .000$. Specifically, WT mice required less time to find the escape platform during trial blocks 1, 2, and 3, $p \leq .010$, but not block 4, compared to *Ppt1-/-* mice. Mutants swam slower than WTs during blocks 1, 2, and 3, $p = .000$, but not block 4.

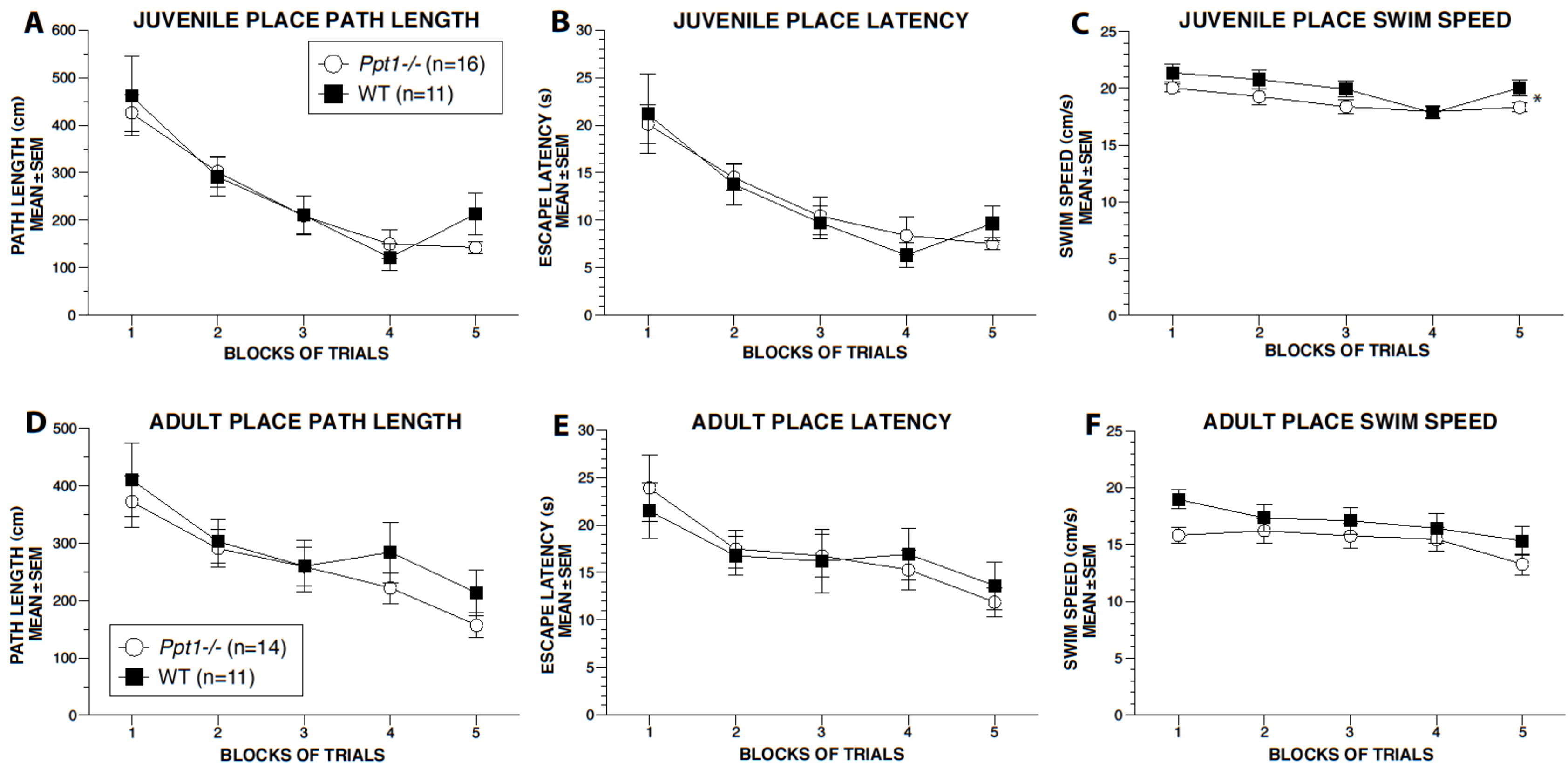

Figure 6. Morris Water Maze, place trials. Juvenile groups performed similarly with regard to length (A) escape path and (B) latency during the place trials. However, *Ppt1-/-* mice (C) showed significantly decreased swimming speeds averaged across blocks of trials (p = .018). No differences were observed between the adult groups in terms of (D) path length, (E) latency, or (F) swimming speeds. *Denotes a significant main effect of Genotype.

During place trials, rmANOVAs failed to produce any significant main or interaction effects involving Genotype for escape path length, latency, or swimming speeds (Figure 6D-F). A significant effect of Blocks of Trials for latency, $F(4, 92)=5.22$, $p=.001$, and path length, $F(4,92)=8.16$, $p=.000$, followed by comparisons showing significant decreases in the levels of both variables between the first and last block of trials, documented spatial learning acquisition in both groups. No significant overall effects involving Genotype were found concerning swimming speeds although all mice swam significantly slower during block 5 compared to all other blocks, $p < .001$ for all comparisons. The retention data from the probe trial measures were consistent with the lack of differences during acquisition training in the place condition. Specifically, no overall effects involving Genotype were found with regard to platform crossings or time spent in the target quadrant. Each group showed spatial bias for the target quadrant by spending significantly more time in it compared to the times spent in quadrant to the right, $p = .000$, left, $p = .000$, and opposite the pool, $p = .000$ (Figure 7C and D).

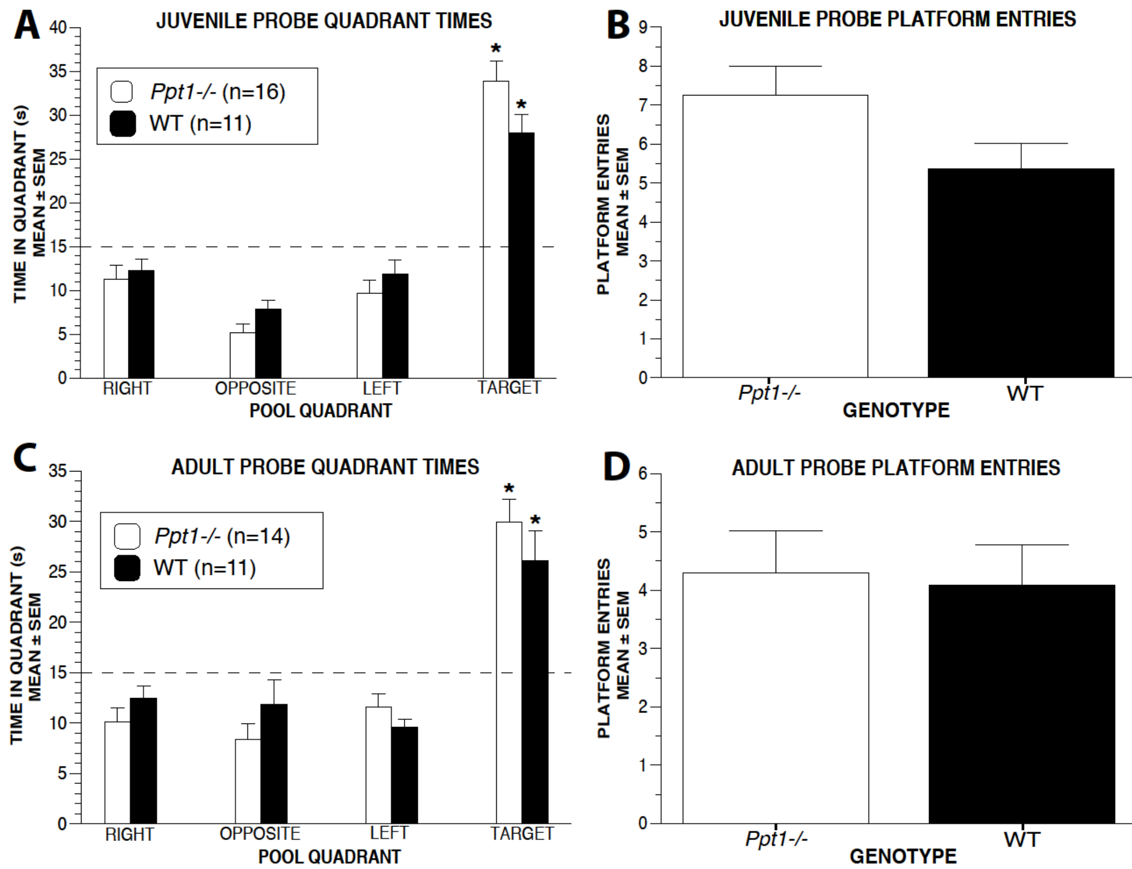

Figure 7. Morris Water Maze, probe trials. Juvenile *Ppt1-/-* and WT mice (A) showed significant spatial bias for the target quadrant compared to each of the other three quadrants, although no differences were observed between groups with regard to time spent in the target quadrant. In addition, *Ppt1-/-* mice (B) exhibited a similar number of platform crossings during the probe trial. The same pattern of results was found for the adult cohort (C and D). The dashed lines at 15 s in A and C indicate the time expected in each quadrant based on chance alone. * Denotes a significant difference from each of the other quadrants, $p \leq .005$.

Rotarod

The dependent variable for the rotarod test was time spent, in sec, on the rod for each trial.

Juveniles. Analyses included a rmANOVA for stationary trials (1-3), with Genotype (*Ppt1-/-* versus WT) as the main factor and Trial as the repeated measure. Results indicated no effect of Genotype (data not shown). A 2 x 2 x 3 rmANOVA was performed on time on the constant speed rotarod with Genotype as the between-subjects

factor and test Session (1-3) and Trial (1 and 2) as the within-subjects repeated measures (Figure 8A). No significant main or interaction effects involving Genotype were found. There was a significant main effect of Session, as well as a significant Session-by-Trial interaction, $F(1.15, 28.69) = 5.01$, $p = .029$ and $F(1.12, 27.93) = 4.65$, $p = .036$, respectively. Mice performed better on the constant speed trials of session 2, $p = .044$, and session 3, $p = .024$, than on constant speed trials of session 1. Within session 1, animals performed better on trial 2 than on trial 1, $p = .033$.

Another 2 x 2 x 3 rmANOVA was performed on accelerating rotarod time with Genotype as the between-subjects factor and test Session (1-3) and Trial (1 and 2) as the within-subjects repeated measures (Figure 8B). Results indicated no effect of Genotype, but a significant effect of Trial, $F(1,25)=15.82$, $p=.001$, and a Session x Trial x Genotype interaction, $F(2,50)=5.58$, $p=.007$. In session 1, but not session 2 or 3, WT mice performed better on trial 2 than on trial 1, $p = .001$. However, *Ppt1-/-* mice performed better on trial 2 versus trial 1 only during session 3, and not session 1 or 2, $p = .008$.

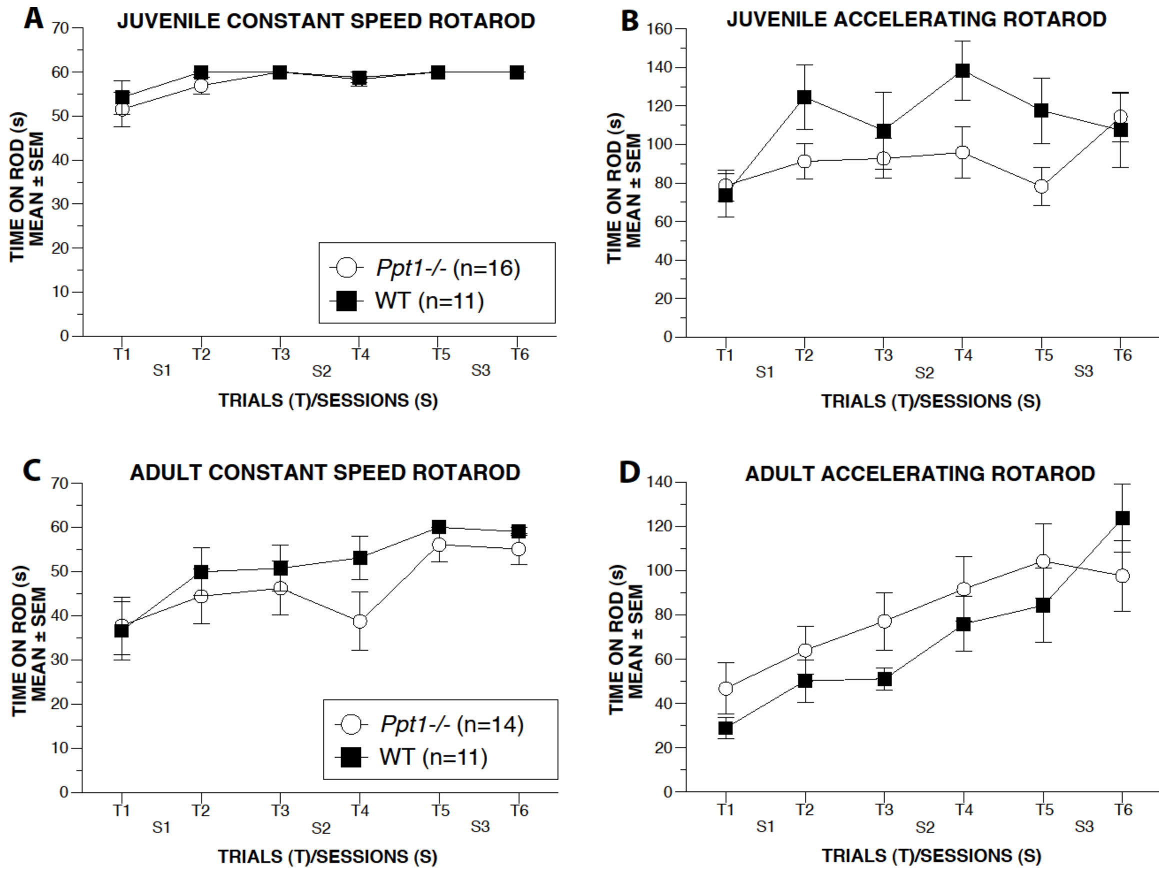

Figure 8. Constant speed and accelerating rotarod. WT and *Ppt1-/-* mice performed similarly on constant speed and accelerating rotarod trials as both (A and B) juveniles and (C and D) adults.

Adults. Analyses revealed no main or interaction effects of Genotype on stationary, constant speed, or accelerating rotarod trials (Figure 8C and D). During constant speed rotarod trials, a main effect of Session was found, $F(2,46) = 10.23$, $p = .000$, with all animals balancing longer on the rod in session 3 than session 1, $p = .000$, and session 2, $p = .004$. All mice also performed differently over test sessions, $F(2,46) = 23.52$, $p = .000$, and between trials, $F(1,23) = 6.64$, $p = .017$, during accelerating rotarod testing. Over sessions, animals remained on the rod significantly longer during trial 2 than trial 1; over trials, animals remained on the rod longer during session 3 than sessions 1 and 2, $p = .000$ and .001 respectively.

Visual Acuity

Analyses for the visual acuity test included a one-way ANOVA to compare means for each genotype (*Ppt1-/-* versus WT) calculated for the highest grating (in cyc/deg) at which the animals discriminated between light and dark rotating lines before failing to display an optokinetic response.

Juveniles. Results indicated that *Ppt1-/-* mice displayed a higher threshold for optokinetic response compared to WT mice, $F(1,25)=5.72$, $p=.025$ (Figure 9A).

Adults. In contrast to the younger cohort, adult WT and *Ppt1-/-* mice performed similarly on this test of visual acuity (Figure 9B).

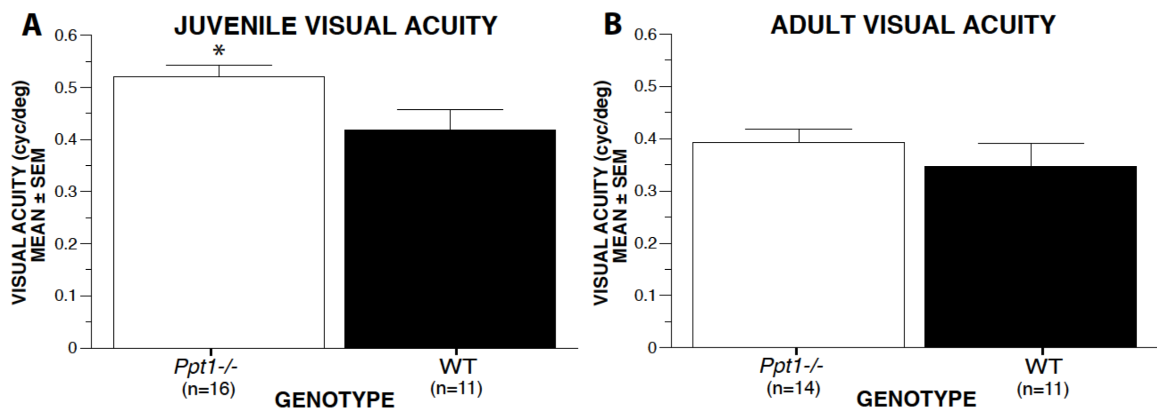

Figure 9. Visual Acuity. Juvenile *Ppt1-/-* (A) mice exhibited a greater visual acuity threshold compared to WT mice, while the adult cohort (B) did not exhibit this difference. * Denotes a significant main effect of Genotype, $p = .025$.

Actometer

Distance, low mobility bouts, stride length, and stride rate were analyzed using a one-way ANOVA with genotype (*Ppt1-/-* versus WT) as the main factor. For gait analysis, data was sent to Dr. Stephen Fowler at the University of Kansas, Department of Pharmacology and Toxicology. His analyses included the Fourier transformations

previously described (Fowler et al., 2009) to determine genotypic differences in force plate power spectra.

Juveniles. A main effect of Genotype was found on distance traveled, $F(1,26) = 6.53$, $p = .017$, and stride rate, $F(1, 25) = 8.95$, $p = .006$, revealing that WT mice traveled a significantly farther distance during the 20-min trial and took more strides per s during long runs compared to *Ppt1-/-* mice (10B). There was no effect of Genotype on number of low mobility bouts or stride length (10A).

Adults. There was no main effect of Genotype with regard to distance traveled or low mobility bouts during the actometer test. However, analyses revealed a significant Genotype effect on stride length, $F(1,23) = 37.635$, $p = .000$, showing that WT mice ran around the test chamber using a longer stride length compared to *Ppt1-/-* mice (Figure 10C). In contrast to the juveniles, adult WT and *Ppt1-/-* mice ran at a similar number of strides per s during the actometer trial (Figure 10D).

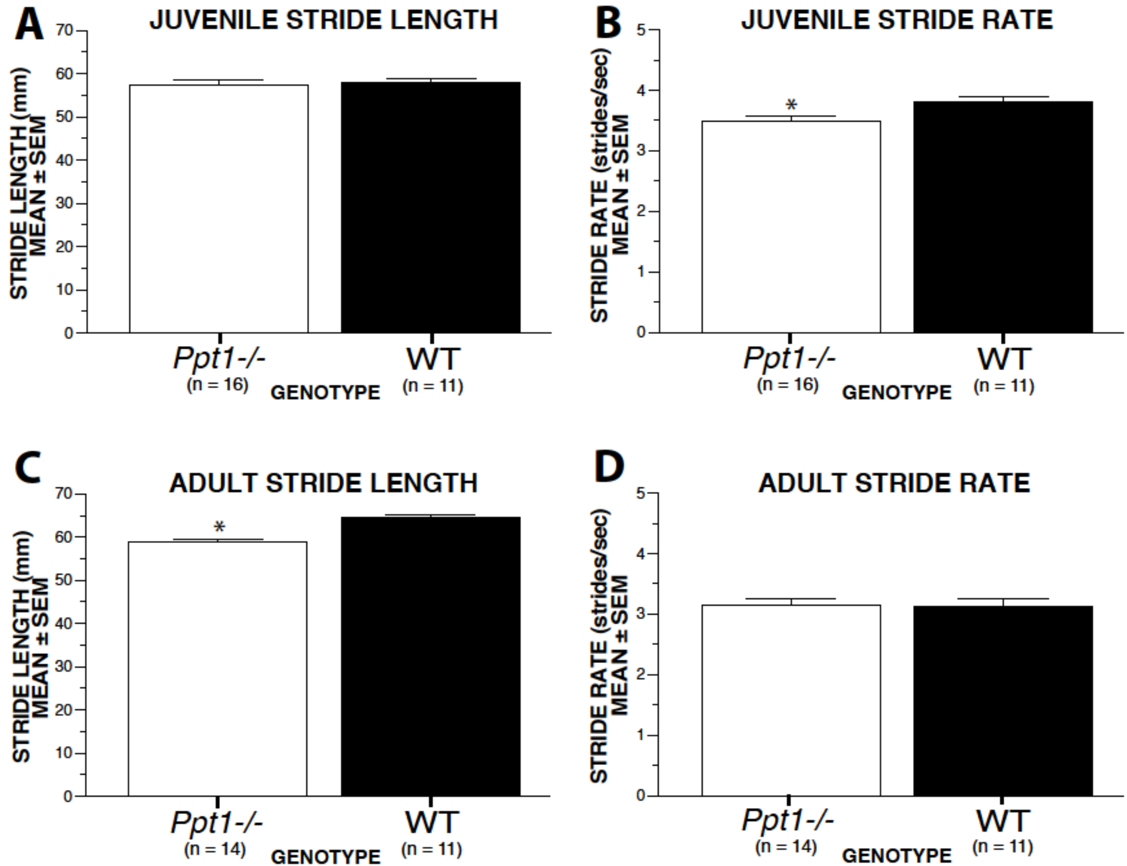

Figure 10. Actometer. During the 20 min actometer session, juvenile *Ppt1-/-* and WT mice exhibited (A) similar stride lengths, but (B) WT mice traveled at a higher stride rate. In the adult cohort, (C) *Ppt1-/-* mice exhibited a shorter stride length but (D) stride rate did not differ between genotypes. * Denotes a significant main effect of Genotype, $p < .05$.

Normal and Complex Running Wheel

Three separate rmANOVAs were used to analyze the running wheel d WT) as the main factor and day (1-5) as the repeated measure. Dependent variables included average speed, maximum speed, distance travelled, and time spent resting.

Juveniles. During baseline testing (week 1), analyses revealed a significant main effect of Genotype on distance traveled, $F(1,25)=7.00$, $p=.014$, and time spent away from the wheel (resting), $F(1,25)=7.83$, p=.010, with WT mice running farther (Figure 11A) and spending less time resting (Figure 14A) compared to *Ppt1-/-* mice. There was no main effect of Genotype on average speed (Figure 12A) or maximum speed (Figure

13A). A main effect of Trial was found for distance traveled, $F(3.26, 81.56)=73.47$, $p=.000$, average speed, 3.17, 60.18)=53.54, $p=.000$, and maximum speed, $F(3.28, 82.02)=104.33$, $p=.000$. For each of these measures, the value for trial 5 was significantly higher than the value for trial 1, $p = .000$. No effect of Trial was found for time spent resting, and all interactions were nonsignificant (data not shown).

Results for all variables during acquisition testing (week 2) reflect the differences found during baseline testing. Again, analyses revealed a main effect of Genotype on distance traveled, $F(1,25)=4.32$, $p=.048$ and time spent resting, $F(1,25)=10.52$, p=.003, with WT mice running farther (Figure 11B) and spending less time resting (Figure 14B) compared to mutants. No effect of Genotype was found for average speed (Figure 12B) or maximum speed (13B). A significant effect of Trial was found for distance traveled, $F(3.28, 82.11)=66.55$, $p=.000$, average speed, $F(1.82, 45.25)=126.89$, $p=.000$, and maximum speed, $F(2.05, 51.25)=59.29$, $p=.000$, and comparisons of values between trial 1 and trial 5 indicated that all mice improved performance on these measures over trials, $p = .000$.

Unlike results from the preceding 2 weeks of testing, no main effect of Genotype was found for any of the running wheel variables during performance testing (week 3). However, the rmANOVA revealed a significant Genotype x Trial interaction for distance traveled, $F(3.29, 82.29)=2.70$, $p=.046$. Within-groups, the *Ppt1-/-* mice increased their distance traveled from trial 1 to trial 5, a difference that the WTs did not exhibit. Pairwise comparisons did not reveal differences between groups with regard to distance traveled on any trial. A main effect of Trial was revealed for distance traveled, $F(3.29, 82.29)=8.94$, $p=.000$, average speed, $F(3.19, 79.70)=10.51$, $p=.000$, and time spent

resting, $F(4,100)=6.39$, $p=.000$. Pairwise comparisons indicated that all mice increased distance traveled (Figure 11C) and average speed from trial 1 to trial 5, $p = .000$ (Figure 12C), and all mice rested more during trial 1 than trial 5, $p = .005$ (Figure 14C).

Adults. During baseline testing, rmANOVAs revealed a main effect of Genotype on distance traveled, $F(1, 23)=44.44$, $p=.000$, average speed, $F(1, 16)=5.73$, $p=.029$, maximum speed, $F(1, 23)=17.27$, $p=.000$, and time resting, $F(1, 23)=32.93$, $p=.000$, indicating that WT mice traveled a farther distance (Figure 11D) at a faster average speed (Figure 12D) than *Ppt1-/-* mice. WT mice also ran at a higher maximum speed (Figure 13D) and spent less time resting compared to *Ppt1-/-* mice (Figure 14D). On each of these measures, a significant effect of Trials was found. All mice ran farther, $F(4,92) = 10.66$, $p = .000$, during trial 5 than trial 1, $p=.000$. Average running speed differed over trials, $F(4,92) = 13.51$, $p = .000$, with animals averaging a faster pace during trial 5 than trial 1, $p=.000$. Maximum speed was also different over trials, $F(4,92) = 20.85$, $p = .000$, with all animals reaching a higher maximum speed during trial 5 than during all other trials, $p < .002$. Finally, animals spent more time at rest, $F(4,92) = 3.95$, $p = .005$, during trial 2 compared to trial 1, $p=.001$, and trial 3, $p=.005$.

Main effects of Genotype on distance traveled, $F(1, 23) = 30.89$, $p = .000$, average speed, $F(1, 14) = 5.143$, $p = .040$, maximum speed, $F(1, 23) = 15.070$, $p = .001$, and time resting, $F(1, 23) = 20.61$, $p = .000$, were found again during acquisition testing. WT mice exhibited higher values, compared to mutants, on distance (Figure 11E), average speed (Figure 12E), and maximum speed (Figure 13E), while *Ppt1-/-* mice rested for more time than WTs (Figure 14E). Results of rmANOVAs also revealed a significant Genotype x Trial interaction for distance traveled, $F(3.08, 70.92)=5.97$, $p=.001$, and

average speed, $F(1.88, 26.23)=5.501$, $p=.011$. WT mice traveled farther than *Ppt1-/-* mice at every trial, $p = .000$. Within-groups, both *Ppt1-/-* and WT mice showed an increase in distance traveled from trial 1 to trial 5, $p < .001$. Genotypes ran at similar average speeds during every trial, but only WT mice exhibited an increase in average speed from trial 1 to trial 5, $p = .000$. Analyses revealed a significant effect of Trials on all of these variables as well; distance traveled, $F(3.08, 70.92) = 34.29$, $p=.000$, average speed, $F(1.88, 26.32) = 28.77$, $p=.000$, maximum speed, $F(1.82, 41.85) = 39.10$, $p=.000$, and time resting, $F(2.01, 46.23) = 3.50$, $p=.038$, all differed over trials. Pairwise comparisons revealed that all mice increased scores on distance traveled, average speed, and maximum speed from trial 1 to trial 5, $p = .000$. For time resting, none of the pairwise comparisons for trials was significant following Bonferroni correction.

The pattern of between-group differences was essentially repeated during performance testing. Analyses revealed a significant Genotype x Trial interaction for average speed, $F(3.07, 52.22) = 4.92$, $p = .004$, and maximum speed, $F(4, 92)$ 5.46, $p =$ .001.. Wild-type mice ran at a faster average speed than *Ppt1-/-* mice during trials 1, 4, and 5, $p < .005$, and also reached a faster maximum speed than *Ppt1-/-* mice during all trials, $p < .005$. Within-groups, and unlike in previous weeks, neither group showed a reliable increase in average speed or maximum speed from trial 1 to trial 5 (data not shown). Multiple rmANOVAs revealed a main effect of Genotype on distance $F(1, 23) = 36.92$, $p = .000$, average speed $F(1, 17) = 11.99$, $p = .003$, maximum speed $F(1, 23) = 25.11$, $p = .000$, and time resting, $F(1, 23) = 25.51$, $p = .000$. WT mice traveled farther (Figure 11F), ran at a higher average speed (Figure 12F), and reached a higher maximum speed (Figure 13F) compared to mutants, and mutants rested for more time compared to

WTs (Figure 14F). As before, a main effect of Trial was found for distance $F(3.16, 72.73) = 8.65$, $p = .000$, average speed $F(3.07, 52.22) = 10.29$, $p = .000$, maximum speed $F(4, 92) = 7.64$, $p = .000$, and time resting, $F(2.01, 46.28) = 4.18$, $p = .021$. All mice ran farther during trials 2 and 3 compared to trial 4, $p < .005$, but no pairwise comparisons on time resting remained significant following Bonferroni correction.

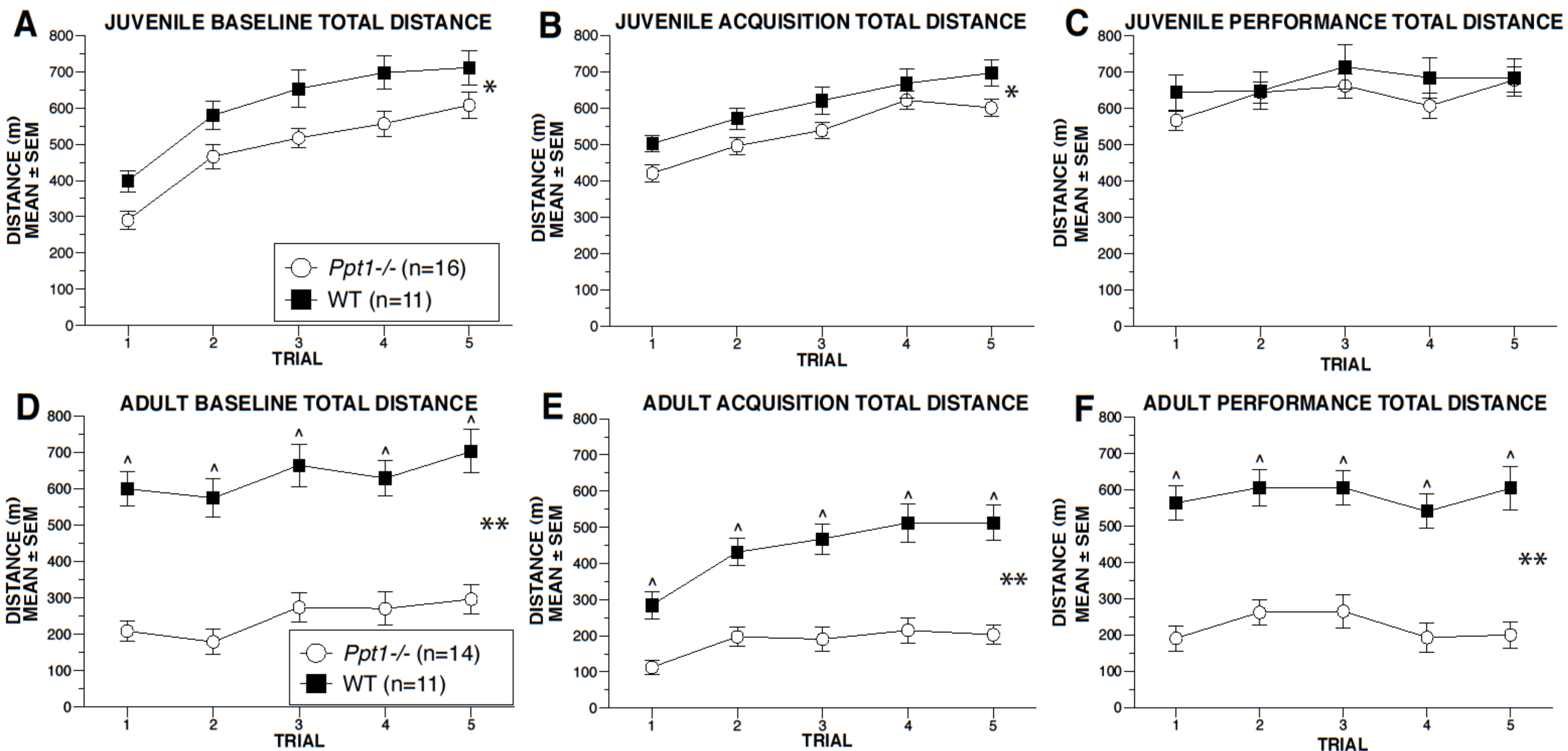

Figure 11. Running wheel, total distance traveled. Juvenile WT mice ran significantly farther than *Ppt1-/-* mice during (A) baseline and (B) acquisition running wheel testing. No effect of genotype was found on distance traveled during (C) performance testing. In the adult cohort, WT mice ran farther than mutants over all three phases of testing (D, E, and F). * Denotes a significant main effect of Genotype, $p < .05$. ** Denotes a significant main effect of Genotype, $p = .000$. ^ Denotes group differences at a single trial.

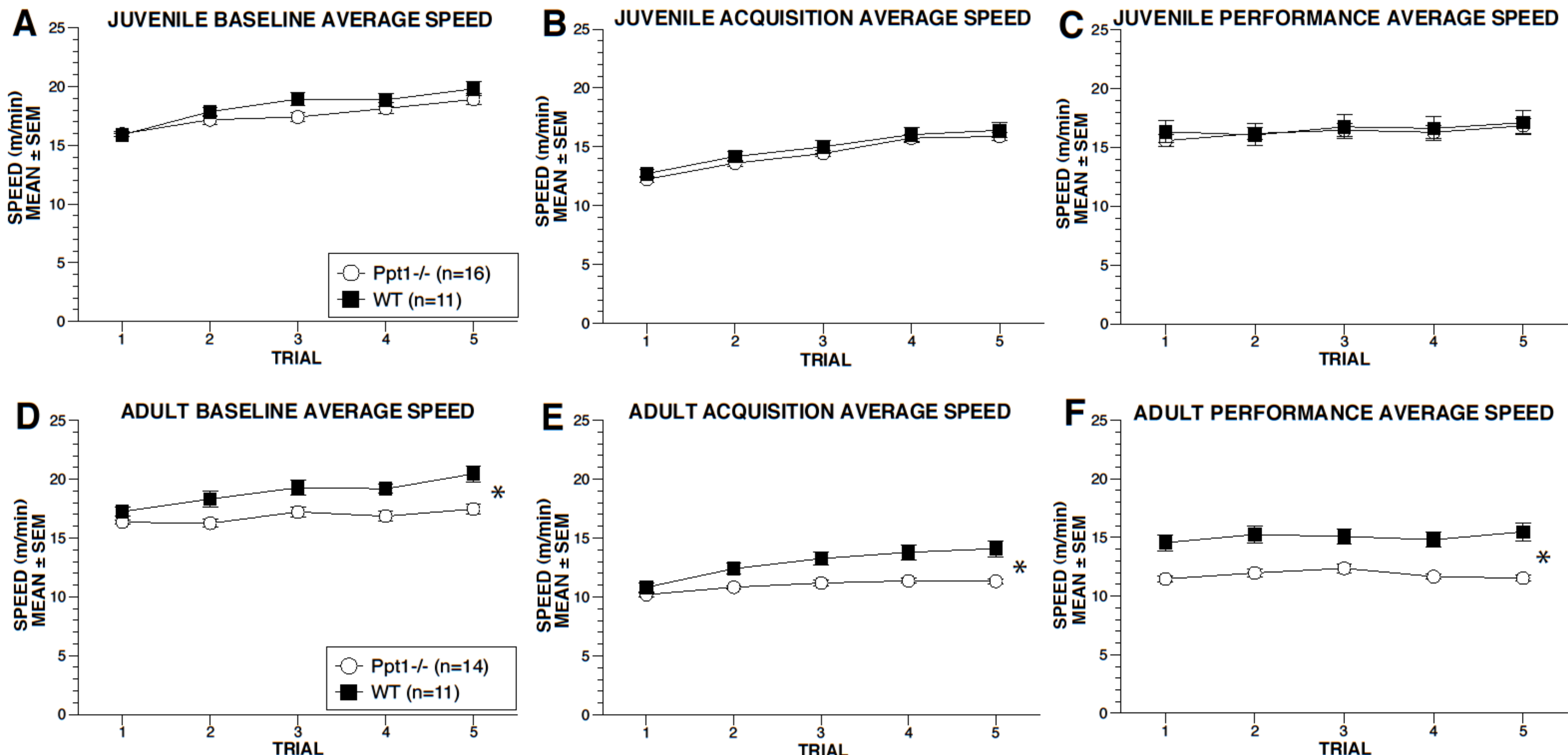

Figure 12. Running wheel, average speed. WT and *Ppt1-/-* juvenile mice ran at similar average speeds during (A) baseline, (B) acquisition, and (C) performance running wheel testing. In the adult cohort, WT mice ran faster, on average, than *Ppt1-/-* mice during (D) baseline, (E) acquisition, and (F) performance testing.. * Denotes a significant main effect of Genotype, $p < .05$. ^ Denotes group differences at a single trial.

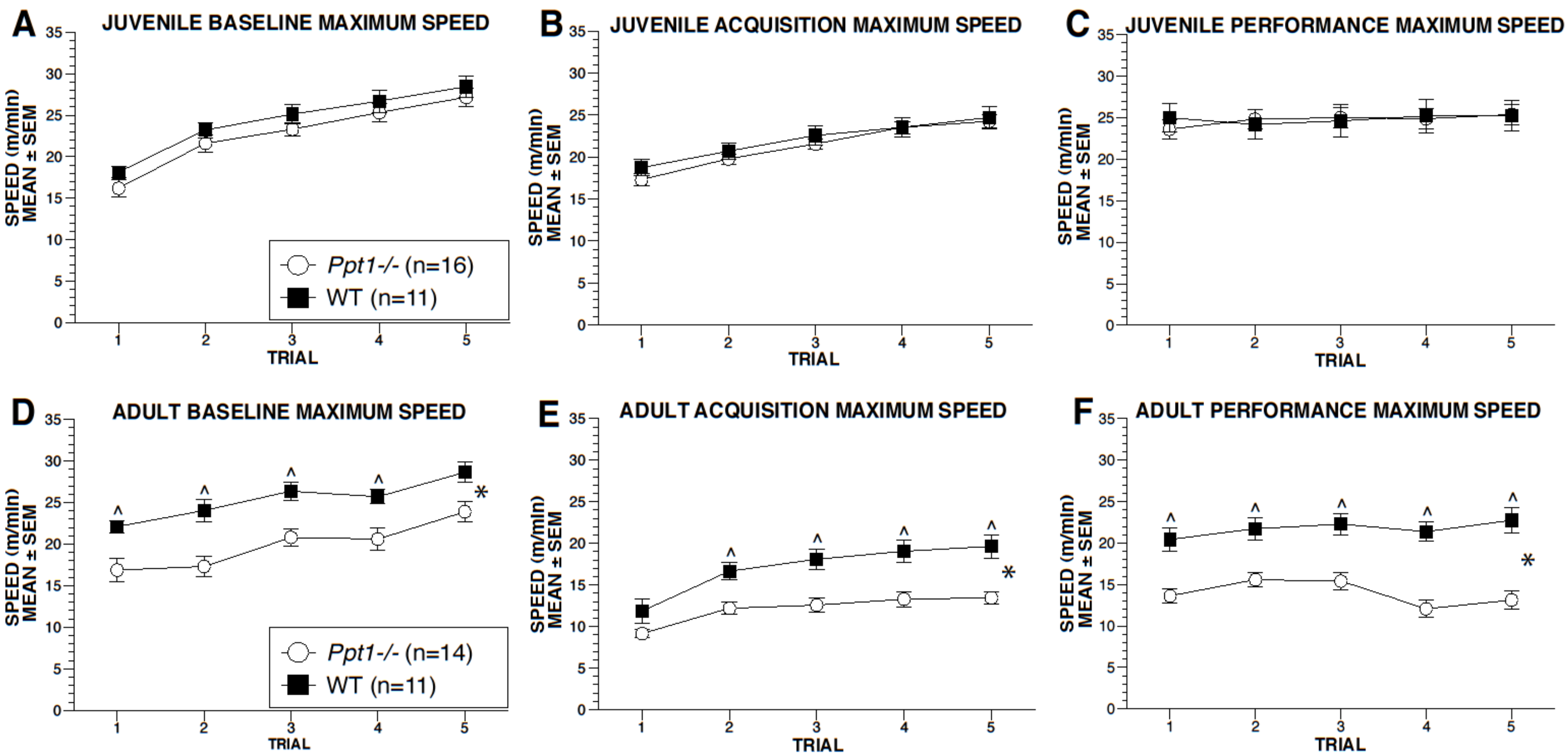

Figure 13. Running wheel, maximum speed. Juveniles of both genotype attained similar maximum running speeds during (A) baseline, (B) acquisition, and (C) performance testing. In the adult cohort, WT mice consistently reached higher maximum running speeds compared to *Ppt1-/-* mice over (D) baseline, (E) acquisition, and (F) performance testing. * Denotes a significant main effect of Genotype, $p \leq .001$. ^ Denotes group differences at a single trial.

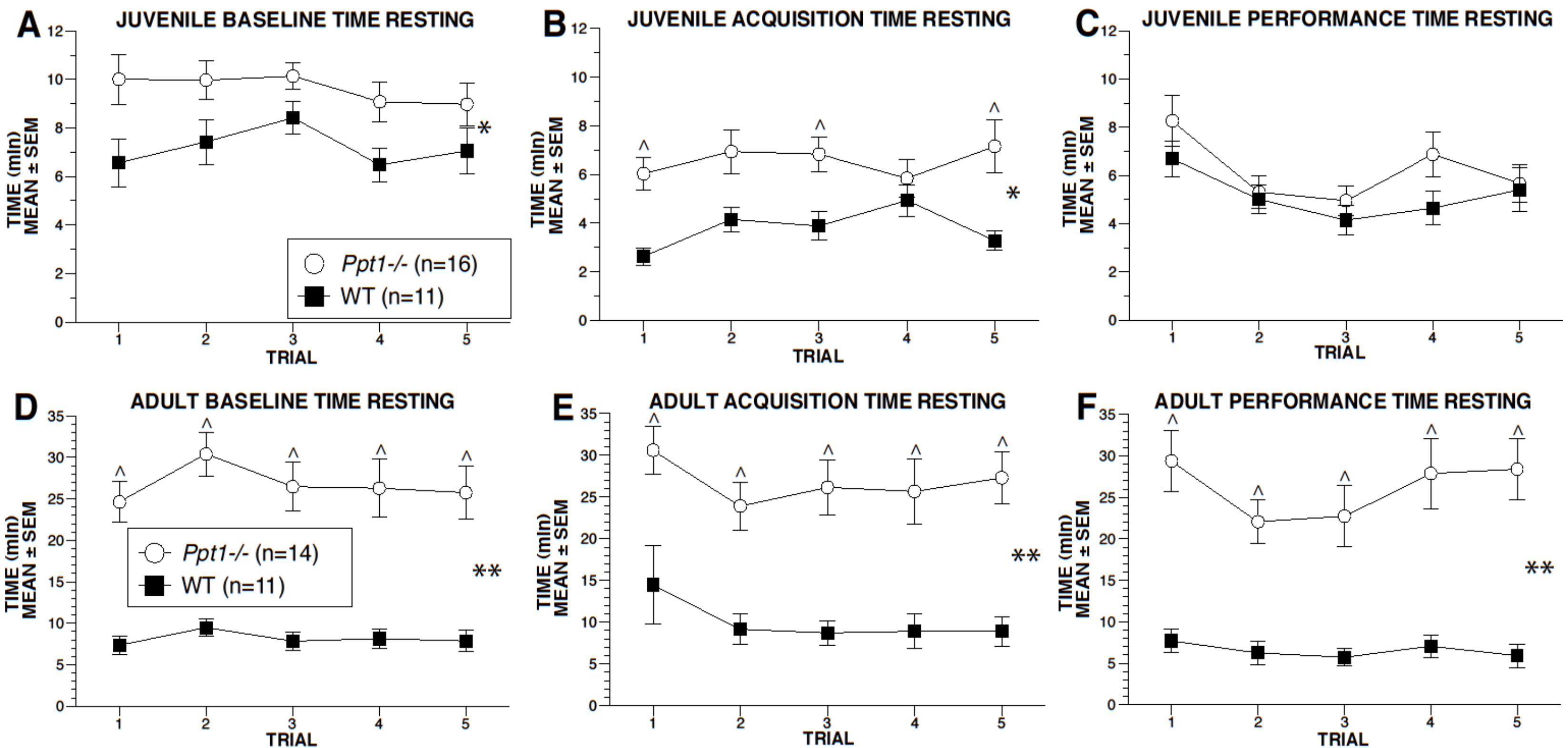

Figure 14. Running wheel, time resting. Juvenile *Ppt1-/-* mice spent more time away from the wheel (resting) compared to WT mice during (A) baseline and (B) acquisition testing. These groups did not differ during (C) performance testing. Adult *Ppt1-/-* mice spent more time away from the wheel compared to WT mice over all 3 phases of running wheel testing (D, E, and F). * Denotes a significant main effect of Genotype, $p \leq .01$. ** Denotes a significant main effect of Genotype, $p = .000$. ^ Denotes group differences at a single trial.

Conditioned Fear

For each min of a conditioned fear trial a percentage was recorded representing the proportion of that min the animal spent freezing. Data for each of the 3 trials (tone-shock training, contextual fear, and auditory cue) were analyzed with rmANOVAs with Genotype (*Ppt1-/-* versus WT) as the main factor and Minute as the repeated measure. For the tone-shock training and auditory cue trials, the first 2 min were analyzed apart from subsequent min to assess baseline freezing behavior.

Juveniles. A rmANOVA revealed no effect of Genotype, Minute, or interaction on baseline freezing behavior during tone-shock training. Analyses of subsequent min revealed no significant interaction and no main effect of Genotype. However, a main effect of Minute was revealed, $F(1.63, 40.75) = 34.91$, $p = .000$, indicating that all animals increased freezing behavior from min 3 to min 5, $p < .005$ (Figure 15A).

Analyses revealed no significant effect of Genotype or Minute during contextual fear trial (Figure 15B).

During the auditory cue trial, a main effect of Minute was discovered during the first 2 min (baseline), $F(1, 25) = 7.24$, $p = .013$, revealing that all animals froze more during min 2 than min 1. A rmANOVA over min 3-10 also revealed main effect of Minute, $F(3.36, 83.96) = 18.47$, $p = .000$, but no effect of Genotype or any interaction (Figure 15C). All animals froze more during min 3, 4, and 5 compared to min 8, 9, and 10, $p < .001$.

Adults. During the first 2 min of tone-shock training, a main effect of Genotype was revealed for time spent freezing, $F(1,23) = 5.49$, $p = .028$, with *Ppt1-/-* mice freezing significantly more than WT mice. During subsequent min, a main effect of Genotype was

discovered, $F(1,23) = 19.92$, $p = .000$, as well as a main effect of Minute, $F(2,46) = 55.79$, $p = .000$, but no significant interaction was found. *Ppt1-/-* mice froze more than WT mice overall and during min 3, $p = .000$, and min 5, $p = .012$. All mice froze significant more during min 4 and 5 compared to min 3, $p = .000$, and during min 5 compared to min 3 and 4, $p = .000$ (Figure 15D).

Analyses revealed no effect of Genotype, nor a significant interaction effect, on freezing behavior during the contextual fear test, but did reveal a significant effect of Minute, $F(3.44, 79.11) = 3.29$, $p = .020$, with all animals freezing more during min 2 and 3 compared to min 1, $p = .000$ (Figure 15E).

Main effects of Genotype, $F(1,23) = 11.96$, $p = .002$, and Minute, $F(1,23) = 17.08$, $p = .000$ were found during the first 2 min of the auditory cue test. Analyses revealed that *Ppt1-/-* mice froze more compared to WT mice overall and also during min 1, $p = .003$, and all animals froze more during min 2 compared to min 1. No interaction effect was found on baseline freezing behavior. A rmANOVA over minutes 3 through 10 revealed a main effect of Genotype, $F(1,23) = 28.07$, $p = .000$, and Minute, $F(5.60, 128.78) = 2.87$, $p = .014$, as well as a significant Genotype x Minute interaction, $F(5.60, 128.78) = 5.46$, $p = .000$. WT mice froze significantly more than *Ppt1-/-* mice during min 3, 4, 5, and 6, $p = .000$. Within-groups, WT mice froze more during min 3 compared to min 9 and 10, and during min 4 and 5 compared to min 9, $p = .000$ (Figure 15F).

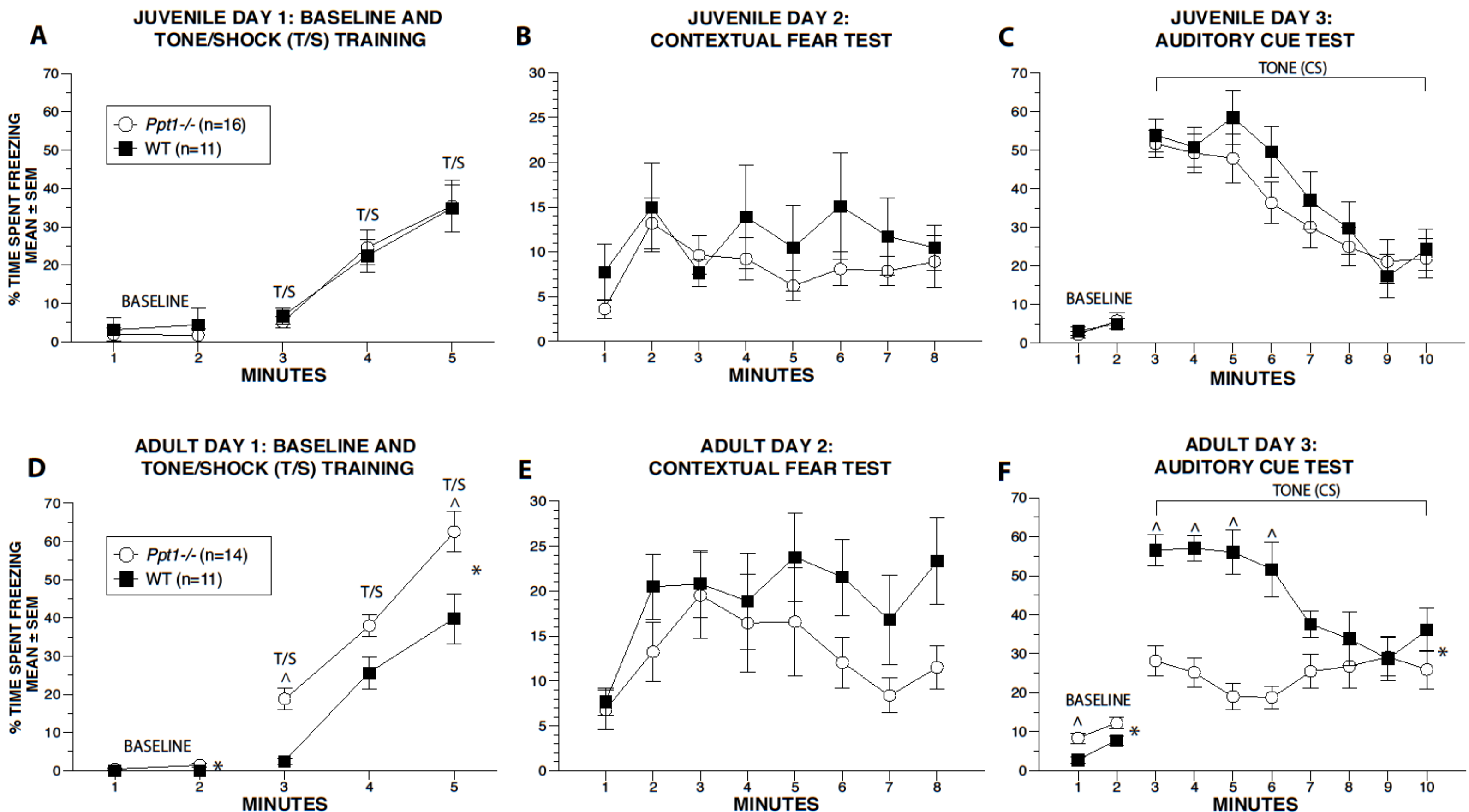

Figure 15. Conditioned fear. *Ppt1-/-* and WT juveniles performed similarly across all 3 days of conditioned fear testing, (A-C). There was no effect of Genotype on baseline freezing behavior, fear conditioning to the T/S pairing, or contextual fear. In adults, on day 1, there was a main effect of Genotype on baseline freezing behavior , with *Ppt1-/-* mice freezing more than WT mice, $p = .028$. Adult mice also showed a significant increase in freezing behavior in response to the T/S pairing, but *Ppt1-/-* mice froze significantly more than WT mice, $p = .000$ (D). Groups performed similarly as adults on day 2 (E). During the auditory cue test (F), *Ppt1-/-* mice froze more than WT mice during the first 2 min (baseline), $p = .002$. All mice exhibited increased freezing behavior in response to the CS (min 3-10), but WT mice froze significantly more than *Ppt1-/-* mice, $p = .000$. * Denotes a significant main effect of Genotype. ^ Denotes group differences at a single minute.

Discussion

Infantile neuronal ceroid lipofuscinosis is a rare neurodegenerative disorder of childhood that is devastating to those it afflicts. Underlying INCL is a mutation in the *Ppt1* gene. The result is a PPT enzyme deficiency which leads to the accumulation of intracellular waste material. The *Ppt1-/-* mouse mimics this human condition in many ways, including brain pathology, myoclonic seizures, and shortened life span. Little is known about the behavior of this animal model.

The goal of the current study was to evaluate a breadth of behaviors in the *Ppt1-/-* mouse model to further our understanding of INCL by providing useful behavioral benchmarks of disease progression and treatment efficacy. The logic of the experiment was to compare behaviors of juvenile *Ppt1-/-* mice, which have limited INCL-related neuropathology, with adult *Ppt1-/-* mice, which have widespread and severe INCL-related neuropathology. This was accomplished by examining performance of mutant and WT strains at two ages, 1-2 months (referred to in this study as the “juvenile cohort”) and 5-6 months (“adult cohort”).

Results, overall, were that there were robust locomotor performance and gross motor coordination differences between genotypes in adulthood, whereas juveniles displayed more subtle differences, if any, in motor ability.

Experimental tests and hypotheses were based on previous literature indicating a few behavioral deficits as well as the location and severity of disease pathology. Both cohorts (juveniles and adults) were tested on the same battery of tests and in the same order. Animals were first tested on the 1 h locomotor activity test with which differences in activity levels were evaluated by quantifying vertical and horizontal movements in a

novel environment. Then mice were administered a battery of sensorimotor tasks to measure strength, coordination, and balance. Spatial reference memory was evaluated through the MWM, where a longer escape path length or latency indicated impaired spatial memory acquisition during place trials. Platform crossings, time spent in the target quadrant, and spatial bias were used to assess spatial memory retention. The rotarod test followed as a measure of balance and fine motor coordination, and then the VOS test was administered to evaluate visual acuity. One 20 min trial in the force plate actometer was included as another measure of locomotor activity and to assess ambulatory-related variables like stride length which may be associated with ataxia. The next test involved a one-week exposure to a normal running wheel, followed by two consecutive weeks of access to a custom, complex running wheel, which were used to evaluate general ambulatory activity as well as fine motor coordination between hind- and fore-limbs. Lastly, the conditioned fear procedure was conducted to assess Pavlovian conditioning capabilities.

A sampling of the significant results revealed that *Ppt1-/-* mice during the juvenile stage, and well before full adulthood, may experience sensorimotor deficits that affect balance and/or coordination, and that it takes them numerous trials before they run the same amount of time and distance as WT mice on the normal and complex running wheels. Strangely, the *Ppt1-/-* mice in the juvenile cohort performed better on the visual acuity test, compared to WT mice.

The two groups in the adult cohort exhibited robust differences across various functions. The adult *Ppt1-/-* mice exhibited abnormal performance on all variables from the 1 h locomotor activity test, and showed performance deficits on the ledge test, and

took longer to learn to swim to the visible platform during the cued water maze trials. The adult *Ppt1-/-* mice also demonstrated inferior performance on all variables of the normal and complex activity wheels, and demonstrated impaired conditioning and/or retention on the auditory cue component of the conditioned fear test.

The results of the 1 h locomotor activity test support my hypothesis that juvenile groups would not differ on any variable, but that adult *Ppt1-/-* show performance deficits on all of them. The current results also support previous findings on older animals. For example, Griffey and colleagues (2006) reported that 7-month-old *Ppt1-/-* animals exhibited reduced general exploratory behavior utilizing the same procedures that were as used in the current experiment. Those 7-month-old *Ppt1-/-* mice also made fewer entries into the central area of the activity chamber, suggesting increased anxiety-like behavior. The same results were found in our 5-month-old mutants, which is not surprising given the similarities in neuropathological indices between 5- and 7-month-old *Ppt1-/-* mice. It is possible that this difference between *Ppt1-/-* and WT mice is due to disease-related pathology. Kielar et al. (2007) described significant microglial activation at 5 months of age in this animal model. Microglial activation is a reliable marker of neuroinflammation and neurodegeneration, and Kielar et al. specifically noted this activation in S1, M1, and various thalamic nuclei. With neuron loss in these loci accompanied by widespread GROD accumulation, it is plausible that INCL neuropathology directly accounts for decreased motor/sensorimotor capabilities or motivation to explore a novel environment. The lower levels of activity in the older *Ppt1-/-* mice may also reflect a general malaise caused by the disease, or a significant alteration in emotionality, e.g. increased anxiety-

like behavior as suggested by these mice making fewer entries into, spending less time in, and traveling a shorter distance in the center zone.

It is noteworthy in the current study that adult *Ppt1-/-* mice displayed deficits on the ledge test of the sensorimotor battery. This is again consistent with the results of Griffey et al. (2006) of 7-month-old mutants, although our older *Ppt1-/-* mice were not impaired on the pole test whereas their mice were. Also of note were findings that both the older and younger *Ppt1-/-* mice exhibited performance deficits on one of the screen tests; juvenile *Ppt-/-* mice took longer than WT mice to climb to the top of the 60° screen, while adult *Ppt-/-* mice showed a similar performance on the 90° screen. The impaired inclined screen test performance of the *Ppt1-/-* groups is probably not due to diminished strength since no differences were observed between groups on the inverted screen test at either age. Instead, differences between groups on the inclined screen tests may reflect slower movement or delays in initiation of movement on the part of the *Ppt1-/-* compared to WT controls. However, it is not clear whether slower movement of the *Ppt1-/-* mice reflects motor-sensorimotor disturbances, alterations in emotionality, or both.

The finding that juvenile *Ppt-/-* mice took longer than the WT mice to move out of the square during the walking initiation test may be viewed as being consistent with the inclined screen results. However, since the groups did not differ on the walking initiation test as adults, it seems unlikely that the results from this test reflect a meaningful difference related to disease progression. Some of the following discussion concerning the impairment of the *Ppt1-/-* mice on other behavioral tests has relevance for interpreting the possibly slowed movement of the INCL-model mice.

There is little information in the literature documenting the cognitive abilities of human patients with INCL. This surely is related to the early age of symptom onset and rapid progression of the disease. There is a small window between birth and the advent of symptoms. After onset, aggressiveness of the disease often results in the child living only for a few years, during which time many functions have deteriorated due to progressive neuropathology. It is simply not practical or even possible to test learning and memory on such young children with already compromised brain function. It is not surprising, then, that the learning capabilities of INCL patients are virtually unknown.

The *Ppt1-/-* mouse provides a unique window within which we assess changes in learning, memory, and other cognitive domains. It was predicted that juvenile *Ppt1-/-* mice would perform similarly to WTs regarding learning and memory functions since only minor neuropathology was likely to be present at this age. In contrast, it was expected that adult *Ppt1-/-* mice would exhibit impaired learning and memory functions given the presence of significant GROD accumulation and astrocytosis in the brain, as well as neuronal loss in the neocortex and hippocampus at this age.

The results from the juvenile water maze testing were consistent with the above stated hypothesis. Specifically, no differences were observed between groups in terms of escape path length or latency during the cued or place (spatial learning) trials. Retention performance during the probe trial was also similar between groups with regard to all of the relevant dependent variables. However, the *Ppt1-/-* mice swam more slowly than the WT control group during the cued condition, although this did not seem to have a significant impact on the path length and latency results. Moreover, the slower swimming

speeds of the *Ppt1-/-* mice during the cued trials are consistent with the inclined screen data describe above.

I also hypothesized that adult *Ppt1-/-* mice would display performance impairment relative to WTs on all variables of the MWM because of likely accumulating hippocampal pathology during full adulthood. The expected decrease in visual acuity and impaired sensorimotor abilities would also predict poorer performance in the MWM paradigm. The predicted visual deficit in these older animals was not found although this result may be due to inadequate test sensitivity of the VOS procedure. The suggestion is that the MWM results were free from this confound.

As was found with the younger animals, the adult *Ppt1-/-* mice swam more slowly than the WT controls during the cued trials, but at this age the *Ppt1-/-* mice were also found to be impaired in terms of escape path length and latency. Escape latency is directly affected by swimming speeds so this measure is not a reliable index of acquisition performance. In contrast, path length is probably minimally affected by slower swimming speeds and thus longer path lengths of the *Ppt1-/-* mice suggest some disturbance in cued learning. However, the nature of and mechanisms underlying the cued learning deficit is not clear.

A remarkable finding form the water maze experiments in the present study is the finding that adult *Ppt1-/-* and WT mice performed similarly on all place and probe variables, including swim speeds after showing robust performance impairments during the cued trials. This suggests that spatial learning and memory are intact in *Ppt1-/-* mice, although it may be inferred from the cued trials data that cognitive functions related to procedural memory may be compromised in these adult mutant mice and that these

deficits may be overcome with increased practice. It should be noted that an extended cued trials period was used in the present study in an effort to thoroughly assess the possibility that visually-guided behaviors were compromised in the *Ppt1-/-* mice. The extended training during the cued condition was likely important for providing adequate practice and familiarity with the procedural aspects of the water of the water maze tank such that the *Ppt1-/-* mice were able to perform normally during the subsequent place trials.

Contrary to the hypothesis, genotype did not seem to affect performance on the stationary, constant speed, or accelerating rotarod trials. No differences in performance were observed between *Ppt1-/-* mice and the WT group in terms of their ability to remain (balance) on the rod, whether stationary or rotating during either the juvenile or adult testing.. The results from the juvenile cohort are similar to those found in a previous study (Macauley et al., 2009) suggesting that at 1-month-old *Ppt1-/-* mice do not have demonstrable performance deficits on the rotarod using standard protocols. However, it was reported in the same study that 5-month-old *Ppt1-/-* mice performed significantly worse on the constant speed (continuous) rotarod, compared to a WT group of the same age, which is in contrast to our findings that there were no differences between the two groups on any component of the rotarod procedure at 5 months of age, including the accelerating rotarod.

There are major differences in the experimental design and rotarod protocols between the present study and that of Macauley et al. that may account for the disparate results between the two studies. First, the rotarod test was the only behavioral measure used in the Macauley et al. study, whereas there were several tests in the present study

that were conducted before the rotarod, and some involved relatively complex motor-sensorimotor responding (e.g. sensorimotor battery, water maze). As discussed above, the 5-month-old *Ppt1-/-* mice in the present study exhibited fairly prominent sensorimotor disturbances (e.g., decreased swimming speeds in the water maze), but this impaired performance was resolved by continued swimming experience during testing such that the *Ppt1-/-* mice eventually swam as fast as the WT group during the place trials. The extensive handling of the mice in the current study also may have had a positive effect on behavioral performance in general. In addition, there were important procedural differences between the studies in that Macauley et al. evaluated their animals on 3 trials (one stationary and two continuous) but only used the score on the final trial for analysis. This is in contrast to the protocol used in the present study where mice were administered 2 constant speed and 2 accelerating rotarod trials on 3 separate days with all scores being used for statistical analyses. Nevertheless, the general conclusion from both studies is that *Ppt1-/-* mice have demonstrable motor/sensorimotor deficits at 5 months of age. In future studies similar to the one presented here, replication of the rotarod using the currently described protocol will help to solidify whether or not adult mutants show a deficit.

There is a consensus in the INCL literature that visual deficits progressing to complete blindness are a reliable pattern of the disease, often occurring as the first noticeable symptoms in patients (Griffey et al., 2005). Previous studies with the *Ppt1-/-* mouse have revealed progressive retinal dysfunction beginning as early as 2 months of age. One study in particular measured rod and cone function by electroretinography (ERG) beginning at 1 month of age and retesting once a month through 7 months of age (Griffey et al., 2005). Significant differences between mutants and WTs were found at 2

months for dark-adapted rod/cone ERGs, and 3 months for light-adapted ERGs. Retinal function continued to worsen over months such that, by 7 months of age, *Ppt1-/-* mice showed a 60% decrease in rod and cone function.

The hypothesis for the current experiment with the same animal model was that adult, but not juvenile, *Ppt1-/-* mice would exhibit a decrease in visual acuity as measured by the VOS test. However, the data from the present study do not support this. Present results showed that no differences were observed between older *Ppt1-/-* mice and the WT group in terms of visual acuity (grating) thresholds derived from the VOS measure, suggesting that the *Ppt1-/-* mice were not impaired on this parameter. In addition, *Ppt1-/-* mice showed a higher acuity threshold compared to the WT control group when tested on the VOS when the mice were 1-month-old, which was also unexpected. It is unknown how much of a decrease in ERG is required before it may be documented by a behavioral test of visually-guided behavior. The results from the cued trials in the water maze suggest the possibility of compromised visual function in the *Ppt1-/-* mice, although they are clearly not blind since they are able to perform at control-like levels during the place trials which implies that their vision may be good enough to use distal cues to learn the location of the submerged platform. The pattern of the VOS results and the suspiciously low thresholds found in the 5-month-old mice, along with the well-documented neuropathology in the visual processing areas of the *Ppt1-/-* mouse brain (Kielar et al., 2007; Shacka, 2012), lead us to suspect that our VOS data is unreliable. Thus, further VOS testing is required in new cohorts of mice before we can draw any conclusions about the degree to which visually-guided behavior is affected by progressive visual system neuropathology in the *Ppt1-/-* mouse.

It was hypothesized that juvenile *Ppt1-/-* and WT mice would perform similarly with regard to distance traveled and the frequency of low mobility bouts during the 20 min actometer test session. However, *Ppt1-/-* animals traveled a significantly shorter distance during the session than WTs, although the number of low mobility bouts was not different between the two groups. It is not clear why the *Ppt1-/-* group traveled a shorter distance in the actometer considering that the groups exhibited similar levels locomotor activity (e.g., in distance traveled), during the preceding 1 h activity test. While the number of low mobility bouts was similar between the two groups of mice, the distance traveled when walking was greater in the WT group compared to the *Ppt1-/-* mice. It is possible that shorter distance traveled by the *Ppt1-/-* group may reflect a subtle alteration in stride length or rate potentially attributable to INCL neuropathology in relevant brain regions. Indeed, while stride length was similar between genotypes at this age, stride rate differed. WT mice had a faster stride rate compared to *Ppt1-/-* mice during the juvenile stage. In this context the difference between genotypes in distance traveled becomes clearer. WT mice take more strides per s than *Ppt1-/-* mice at this age. This early changes in stride rate could be important in that it may presage the onset of motor-sensorimotor disturbances. Alternatively, motivational factors may be responsible for the decreased distance traveled by the *Ppt1-/-* mice.

Given that there is significant pathology in cerebellar white matter at 5 months of age in *Ppt1-/-* mice, including heightened GROD accumulation in the thalamus and S1, increased astrocytosis in S1 and M1, and microglial activation in M1, S1, and various thalamic nuclei (Galvin et al., 2008; Kielar et al., 2007; Kielar et al., 2009), it was expected that they would travel a shorter distance and exhibit increased low mobility

bouts compared to WT mice. The data did not support this hypothesis in that there were no differences between groups in either variable. It is interesting to note that although the *Ppt1-/-* mice showed generally decreased ambulatory activity and rearing during the 1 h locomotor activity test, differences were smallest during the first 20 min of the test, after which differences became quite substantial. One possible explanation for the actometer findings is that the session was only 20 m in length, and that the *Ppt1-/-* mice can match the ambulatory levels of the WTs for a short duration like 20 min, but are unable to sustain it over longer periods. Alternatively, the equivalent levels of locomotion in the actometer may reflect similar responses to a novel environment which habituation and allow for differences in activity to become apparent. The test chamber used for the 1 h activity test is much more similar to the home cages of the mice compared to the actometer, and thus the latter may have more salient novel features.

Another possible explanation comes from the measurement of individual stride length and stride rate. While stride rate was similar between genotypes, stride length was significantly different. WT mice took significantly longer strides when compared to *Ppt1-/-* mice. At the juvenile stage, groups ran with a similar stride length, but WT mice took faster individual strides. At the adult stage, groups ran at the same rate, but WT mice took longer individual strides. It appears that there is a complex relationship between INCL neuropathology and stride length and rate in this mouse model; juvenile *Ppt1-/-* mice may experience disturbances in gross motor ability, while adult *Ppt1-/-* mice may show a deficit in fine motor coordination between hind- and fore-limbs,

I hypothesized that there would be no differences between groups, during the juvenile stage, on any of the variables during the normal (baseline) running wheel test.

This was true for some variables but not others. For example, the juvenile *Ppt1-/-* mice had similar average and maximum speeds compared to the WT group, although they ran for shorter distances compared to the controls. Additionally, the *Ppt1-/-* mice spent more time not running in the wheel compared to the WT group. Similar results were found in the juvenile cohort during the complex wheel acquisition phase. While all animals tended to increase their scores on variables associated with running over trials, the *Ppt1-/-* mice traveled a significantly shorter distance compared to WT mice. It is possible that these results stem from an overall inability of *Ppt1-/-* animals to match the stamina of WT animals, even at a young age. Although the *Ppt1-/-* and WT groups show similar levels of ambulatory/exploratory activity during the 1 h locomotor activity test, the running wheel is a qualitatively different task that requires a higher level of coordination between fore- and hind-limbs, particularly during the complex wheel trials. While the *Ppt1-/-* mice show the ability to run as fast as the WT group, they may tire and/or become less motivated more quickly. Similar to results from a previous study involving neonatal dexamethasone treatment in mice when this same protocol was used, the results here suggest that juvenile *Ppt1-/-* mice are capable of running at WT-like speeds, but that subtle sensorimotor deficits may directly result in the mutants strain being unable to maintain these speeds for long periods of time (Maloney et al., 2011). An alternative explanation is that the running wheel was more difficult for *Ppt1-/-* mice and therefore less reinforcing to them.

However, these differences between groups in the juvenile cohort disappeared during the complex wheel performance trials: there was no main effect of genotype on any variable, and all mice tended to increase performance over time. This result may be

similar to the practice effect observed with regard to swimming speeds in the water maze where swimming also involves complex coordination between fore- and hind-limbs. In other words, continued practice on the normal activity wheel may have improved the general coordination of the *Ppt1-/-* mice such that they were able to perform as well as the WT control group during the complex wheel trials.

Results from the adult cohort showed a much more robust performance impairment in the *Ppt1-/-* mice on the normal and complex running wheel trials, which was observed across all variables and all trials. Not only did the *Ppt1-/-* mice travel a shorter distance during all three phases, they also ran at slower average speeds and reached slower maximum speeds compared to WT mice. These results partially support my hypotheses; adult *Ppt1-/-* mice ran slower and a shorter distance than WT mice. However, unlike my predictions, *Ppt1-/-* actually spent more time not running, indicating either a loss of interest because of difficulty, or reflecting the lower levels of general activity seen during the 1 h locomotor activity test. The aforementioned pathology present in adult *Ppt1-/-* mice would seem to indicate both influences are possible: it is likely that the *Ppt1-/-* mice were simply less active in general during running wheel trials, but because of progressive neuropathology to M1, S1, and thalamus, even the normal running wheel was likely more difficult for them than it was for WT mice.

The between-group differences found in the juvenile cohort during the baseline and acquisition phases of running wheel testing were replicated in the adult cohort. It is possible that the normal and complex running wheel protocol used in the current experiment is sensitive enough to detect certain subtle sensorimotor disturbances in the younger *Ppt1-/-* mice that are then exacerbated by older age and consequently appears

more pervasive and are more easily detected. The consistent difference across ages for total distance traveled, time spent running, and time spent resting during baseline and acquisition is particularly notable because of disparate age-related results in other behavioral tests used here. For instance, juvenile *Ppt1-/-* mice performed similar to WT mice across all variables of the 1 h locomotor activity test, whereas adult *Ppt1-/-* performed uniformly worse. In contrast, swim speeds during place and probe MWM trials were similar between genotypes at both ages. Further, *Ppt1-/-* juveniles traveled a shorter distance than WTs during the actometer test, whereas no difference between genotypes was found in the adults. A complex profile of *Ppt1-/-* sensorimotor impairment, across ages, becomes apparent when considering all of these results together. The normal-complex activity wheel protocol may be sensitive enough to detect a deficit in the *Ppt1-/-* mice at any age while other tests cannot. This has been found for other mouse models of neurological disorders that include compromised sensorimotor function (e.g. Liebetanz et al., 2007).

As was hypothesized, WT and *Ppt1-/-* juvenile mice performed similarly across baseline and tone-shock training on day 1 as well as during contextual fear, and cued fear trials. Both genotypes exhibited a significant response to the tone/shock pairing, increasing freezing behavior over min 3, 4, and 5 during conditioning training. Groups performed similarly on the contextual fear trial, suggesting similar levels of conditioning to the contextual cues present during training in the absence of the tone-shock pairing. Lastly, the *Ppt1-/-* and WT showed similar levels of freezing in response to the auditory cue (CS) suggesting equivalent levels of conditioning or sensitization. This was

predicated as the result because of the lack of hippocampal-amygdalar pathology at this age.

I hypothesized that widespread neuropathology in adult *Ppt1-/-* mice would impair their ability to associate a conditioned stimulus (tone) or contextual cues with an unconditioned stimulus (shock), thereby resulting in decreased freezing behavior in mutants over all three trials. The data only partially supported this hypothesis; *Ppt1-/-* adults in fact froze less in response to CS-exposure in a novel environment (cued fear). Surprisingly, the *Ppt1-/-* mice showed an exaggerated freezing response to the initial tone/shock pairing during fear conditioning training, compared to controls. This exaggerated fear response is difficult to explain in light of their reduced cued fear response during the third day, unless compromised auditory function affected cued conditioning/sensitization. It is perhaps not surprising that the *Ppt1-/-* mice performed similarly to the WT group during the contextual fear trial given that their spatial learning and memory seems to be intact, as indicated by their performance on the MWM. The conditioned fear results raise the possibility that some forms of conditioning or conditioning-like processes may be disturbed in *Ppt1-/-* mice.

The current study has several notable limitations. First and foremost, it would be ideal to include a third cohort with animals aged 3-4 months to better attain a complete behavioral phenotype of the *Ppt1-/-*mouse. The current study provides previously unavailable information on the behavior of this animal model of INCL during adulthood, and also lays the groundwork for evaluating this mutant during younger ages to appreciate just how early disease symptomology is detectable. Repeating the current protocol on a group of animals aged 3-4 months would fill in the space between the

cohorts presented here, thus helping to pinpoint the temporal presentation of symptoms and potentially allowing for the comparison of symptom severity at various ages. One of the greatest benefits of this study is that it establishes a more complete behavioral phenotype of an established animal model of a rare human disorder, thus providing useful benchmarks of disease progression and creating markers of specific symptoms by which treatments can be evaluated. A third cohort would help to complete the picture regarding the onset of each symptom.

Another consideration when interpreting the results of this study is that there was an uneven distribution of sexes. In the juvenile cohort, only 10 of the 27 animals were female. Within the females, the genotype distribution was 4 and 6. The adult cohort was more evenly distributed, with females accounting for 12 of 25 animals, a distribution of 7 and 5. However, of the 13 males in the adult cohort, only 2 were WT. The most important point concerning the unequal distributions of sexes in the current study was that it did not allow for an evaluation of sex effects. At least one previous study in humans with another subset of NCL, JNCL, has shown that females suffer from a more severe form of the disease. Specifically, although females experienced the onset of symptoms approximately 1 year later than males, they died 1 year earlier and exhibited lower functional capability, earlier loss of independent function, and lower physical quality of life (Cialone et al., 2012). As such, follow-up studies would benefit from a more even distribution of genders across genotypes in order to properly evaluate if males and females of this animal model show a different onset, progression, or time-course of disease symptoms.

Future studies would also benefit from including immunohistopathology results in order to confirm, at specific ages, exactly what INCL pathology is present. This would

allow for the pairing of behavioral results at a specific time point to the matching neuropathology present in the *Ppt1-/-* brain. It may also be worth considering testing additional cohorts of *Ppt1-/-* mice on selected behavioral measures to determine the reliability of the initial positive findings. The number of tests and test sequence may have an impact on the behavioral results, and replications using independent cohorts may be necessary to evaluate this possibility. Additionally, the current study employed a behavioral battery that lasted approximately 39 days. It is established in the INCL literature that pathology in specific brain areas can progress significantly over the period of a month. The difference between a *Ppt1-/-*mouse at 1 month of age and 2 months of age may be significant, depending on the measures being employed. Likewise, after 5 months of age certain functions deteriorate rapidly in *Ppt1-/-* mice, and a shorter, more focused battery of behavioral tests would help to isolate deficits at more specific ages.

Conclusions

These findings confirm that the *Ppt1-/-* mouse is a useful animal model of INCL and provide a broad characterization of the behavioral phenotype for this mutant strain during the juvenile period and adulthood. *Ppt1-/-* mice display a complex profile of cognitive and motor disabilities, including possible deficits in sensorimotor, locomotor, and certain learning/memory functions that progressively deteriorate with age. The results of the current study establish novel behavioral markers of the *Ppt1-/-* mouse model of INCL that both clarify the phenotype of this mouse strain and also provide useful benchmarks for evaluating treatment efficacy.

References

Bolivar, V. J., Ganus, J. S., & Messer, A. (2002). The development of behavioral abnormalities in the motor neuron degeneration (mnd) mouse. *Brain Research, 937*(1-2), 74-82.

Brown, J. A., Emnett, R. J., White, C. R., Yuede, C. M., Conyers, S. B., O'Malley, K. L., . . . Gutmann, D. H. (2010). Reduced striatal dopamine underlies the attention system dysfunction in neurofibromatosis-1 mutant mice. *Hum Mol Genet, 19*(22), 4515-4528.

Cialone, J., Adams, H., Augustine, E. F., Marshall, F. J., Kwon, J. M., Newhouse, N., . . . Mink, J. W. (2012). Females experience a more severe disease course in batten disease. *J Inherit Metab Dis, 35*(3), 549-555.

Cooper, J. D. (2003). Progress towards understanding the neurobiology of Batten disease or neuronal ceroid lipofuscinosis. *Curr Opin Neurol, 16*(2), 121-128.

Fowler, Miller, B. R., Gaither, T. W., Johnson, M. A., & Rebec, G. V. (2009). Force-plate quantification of progressive behavioral deficits in the R6/2 mouse model of Huntington's disease. *Behav Brain Res, 202*(1), 130-137.

Fowler, S. C., Birkestrand, B. R., Chen, R., Moss, S. J., Vorontsova, E., Wang, G., & Zarcone, T. J. (2001). A force-plate actometer for quantitating rodent behaviors: illustrative data on locomotion, rotation, spatial patterning, stereotypies, and tremor. *J Neurosci Methods, 107*, 107-124.

Galvin, N., Vogler, C., Levy, B., Kovacs, A., Griffey, M., & Sands, M. S. (2008). A murine model of infantile neuronal ceroid lipofuscinosis-ultrastructural evaluation

of storage in the central nervous system and viscera. *Pediatr Dev Pathol, 11*(3), 185-192.

Girden, E. R. (1992). *ANOVA: Repeated measures.*: Sage Publications, Inc.

Griffey, M., Macauley, S. L., Ogilvie, J. M., & Sands, M. S. (2005). AAV2-mediated ocular gene therapy for infantile neuronal ceroid lipofuscinosis. *Mol Ther, 12*(3), 413-421.

Griffey, M., Wozniak, D., Wong, M., Bible, E., Johnson, K., Rothman, S. M., . . . Sands, M. S. (2006). CNS-directed AAV2-mediated gene therapy ameliorates functional deficits in a murine model of infantile neuronal ceroid lipofuscinosis. *Mol Ther, 13*(3), 538-547.

Gupta, P., Soyombo, A. A., Atashband, A., Wisniewski, K. E., Shelton, J. M., Richardson, J. A., . . . Hofmann, S. L. (2001). Disruption of PPT1 or PPT2 causes neuronal ceroid lipofuscinosis in knockout mice. *Proc Natl Acad Sci U S A, 98*(24), 13566-13571.

Ho, N., Liauw, J. A., Blaeser, F., Wei, F., Hanissian, S., Muglia, L. M., . . . Chatila, T. A. (2000). Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca^{2+}/calmodulin-dependent protein kinase type IV/Gr-deficient mice. *Journal of Neuroscience, 20*(17), 6459-6472.

Kamate, M., & Hattiholi, V. (2012). Novel neuroimaging finding in palmitoyl protein thioesterase-1-related neuronal ceroid lipofuscinosis. *Pediatr Neurol, 46*(5), 325-328. doi: 10.1016/j.pediatrneurol.2012.02.021

Kielar, C., Maddox, L., Bible, E., Pontikis, C. C., Macauley, S. L., Griffey, M. A., . . . Cooper, J. D. (2007). Successive neuron loss in the thalamus and cortex in a

mouse model of infantile neuronal ceroid lipofuscinosis. *Neurobiol Dis, 25*(1), 150-162.

Kielar, C., Wishart, T. M., Palmer, A., Dihanich, S., Wong, A. M., Macauley, S. L., . . . Gillingwater, T. H. (2009). Molecular correlates of axonal and synaptic pathology in mouse models of Batten disease. *Hum Mol Genet, 18*(21), 4066-4080.

Macauley, S. L., Roberts, M. S., Wong, A. M., McSloy, F., Reddy, A. S., Cooper, J. D., & Sands, M. S. (2012). Synergistic effects of central nervous system-directed gene therapy and bone marrow transplantation in the murine model of infantile neuronal ceroid lipofuscinosis. *Ann Neurol*. doi: 10.1002/ana.23545

Macauley, S. L., Wozniak, D. F., Kielar, C., Tan, Y., Cooper, J. D., & Sands, M. S. (2009). Cerebellar pathology and motor deficits in the palmitoyl protein thioesterase 1-deficient mouse. *Exp Neurol, 217*(1), 124-135.

Maloney, S. E., Noguchi, K. K., Wozniak, D. F., Fowler, S. C., & Farber, N. B. (2011). Long-term Effects of Multiple Glucocorticoid Exposures in Neonatal Mice. *Behav Sci, 1*(1), 4-30.

Miao, N., Levin, S. W., Baker, E. H., Caruso, R. C., Zhang, Z., Gropman, A., . . . Quezado, Z. M. (2009). Children with infantile neuronal ceroid lipofuscinosis have an increased risk of hypothermia and bradycardia during anesthesia. *Anesth Analg, 109*(2), 372-378.

Mitchison, H. M., Ming, J. L., & Cooper, J. D. (2004). Selectivity and types of cell death in the neuronal ceroid lipofuscinoses (NCLs). *Brain Pathol, 14*, 86-96.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods, 11*, 47-60.

Munasinghe, J., Zhang, Z., Kong, E., Heffer, A., & Mukherjee, A. B. (2012). Evaluation of neurodegeneration in a mouse model of infantile batten disease by magnetic resonance imaging and magnetic resonance spectroscopy. *Neurodegener Dis, 9*(4), 159-169.

Prusky, G. T., Alam, N. M., Beekman, S., & Douglas, R. M. (2004). Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system. *Invest Ophthalmol Vis Sci, 45*(12), 4611-4616.

Roberts, M. S., Macauley, S. L., Wong, A. M., Yilmas, D., Hohm, S., Cooper, J. D., & Sands, M. S. (2012). Combination small molecule PPT1 mimetic and CNS-directed gene therapy as a treatment for infantile neuronal ceroid lipofuscinosis. *J Inherit Metab Dis*. doi: 10.1007/s10545-011-9446-x

Shacka, J. J. (2012). Mouse models of neuronal ceroid lipofuscinoses: useful pre-clinical tools to delineate disease pathophysiology and validate therapeutics. *Brain Res Bull, 88*(1), 43-57.

Wozniak, D. F., Hartman, R. E., Boyle, M. P., Vogt, S. K., Brooks, A. R., Tenkova, T., . . . Muglia, L. J. (2004). Apoptotic neurodegeneration induced by ethanol in neonatal mice is associated with profound learning/memory deficits in juveniles followed by progressive functional recovery in adults. *Neurobiol Dis, 17*(3), 403-414.

Wozniak, D. F., Xiao, M., Xu, L., Yamada, K. A., & Ornitz, D. M. (2007). Impaired spatial learning and defective theta burst induced LTP in mice lacking fibroblast growth factor 14. *Neurobiol Dis, 26*(1), 14-26.