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14. ABSTRACT

The focus of our studies is to test the effect of genetic manipulations targeting tau phosphatase, PP2A, on behavioral impairments resulting from shockwave exposure, and to compare those results with the effects of the same manipulations on the sensitivity to Alzheimer's disease (AD) - like impairments caused by acute Aβ exposure. The principal motivation behind this approach is the observation that hyperphosphorylated tau is a common feature of multiple neurodegenerative conditions including AD and traumatic brain injury (TBI) - associated degeneration. We previously found that the two novel lines of transgenic (Tg) mice altering PP2A activity, modify sensitivity to Aβ-induced electrophysiological and behavioral impairments, and our hypothesis is that they exert similar effects on shockwave-induced impairments. This effort has required a substantial investment in developing equipment and protocols for exposing mice to a range of shockwave exposure conditions that mimic militarily relevant exposures. We found changes in phospho-tau at 1 and 24 hrs post-injury. Behavioral analysis of the blast mice revealed changes in exploratory behavior and spatial memory which were not affected by PP2A modulation. These changes were associated with eye damage interfering with interpretation of behavioral results. However, given that we also found that blast causes tau protein changes reducing memory in normal mice, when we exposed our Tg mice to tau, we demonstrated modulation of memory loss by PP2A, confirming our initial hypothesis that therapies acting onto PP2A might be beneficial in TBI and AD.

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INTRODUCTION:

Neurodegeneration resulting from both traumatic brain injury (TBI) and Alzheimer's disease (AD) is characterized by aggregates of hyperphosphorylated tau (Ballatore et al., 2007; Dekosky et al., 2013). This observation together with the involvement of tau in other neurodegenerative disorders suggests that a common neurodegenerative mechanism involving tau hyperphosphorylation may contribute to impairments associated with both TBI and AD. Tau phosphorylation is controlled by a balance between the activity of numerous kinases and the protein phosphatase, PP2A (Martin et al., 2013), and PP2A activity is in turn controlled by C-terminal methylation of its catalytic subunit (Sents et al., 2013). To examine the effects of altered PP2A activity and tau phosphorylation on TBI and AD-related impairments, we generated two lines of transgenic mice, one that expresses the PP2A methylesterase, PME-1, and one that over expresses the PP2A methyltransferase, LCMT-1. We found that PME-1 over expression increased sensitivity to electrophysiological and behavioral impairments caused by acute oligomeric Aβ exposure, and that LCMT-1 over expression protected animals from these impairments. In this project, we have used these novel transgenic animals to examine the relationships between shockwave exposure, tau phosphorylation, and behavioral impairment.

BODY:

In June of 2013 the statement of work for this project was modified to address the need for a more comprehensive assessment of the link between the shockwave characteristics and exposure conditions, and injury related increases in tau phosphorylation. The current statement of work seeks to address 4 main questions:

1) **What are the parameters and characteristics of shockwave exposure that are necessary to produce increased tau phosphorylation in mouse brain?**
2) **What are the consequences of LCMT-1 and PME-1 transgene expression on bTBI-associated behavioral impairment and tau phosphorylation at 2 weeks and 3 months post injury?**
3) **Do LCMT-1 and PME-1 transgene expression alter the acute shockwave-induced changes in tau phosphorylation at 1 hr and 24 hrs post-injury?**
4) **Do LCMT-1 and PME-1 transgene over expression affect tau hyperphosphorylation induced by acute beta-amyloid exposure?**

All these questions have been addressed during these years as detailed below.

Shockwave exposure conditions:

In the first year, we built-up our shock tube (**Figure 1**). It consists of an adjustable driver section separated by a burst diaphragm (polyethylene terephthalate) from a driven section 76mm in diameter and 1200mm long. During blast, the animal is encased in a rigid holder to protect the lungs and bowels, so that only the head/brain is exposed to the shockwave. The animal's head is placed at the center of the exit of the shock tube and 15 mm from the end of the tube. This configuration minimizes the turbulence generated by placing an animal inside the tube due to confinement effects, as well as minimizes the

dissipation of the shock wave after exiting the tube. Within ~1 tube-diameter (in this case 76 mm) of the exit, the shock wave remains planar and well-formed. Furthermore, to minimize uncontrolled motion, the animal's head is restrained to reduce head motion. Using this system, we successfully identified shockwave exposure protocol that produces acute increases in tau phosphorylation. This protocol consisted of a single shockwave exposure of 269 ± 9.8 kPa peak over pressure, 0.73 ± 0.021 ms duration, and 67 ± 2.3 kPa-ms impulse directed at the top of the head under conditions where head is supported from below and acceleration was limited (**Figure 1B**). Values of head kinematics are shown on **Figure 1D** (animals with kinematic parameters that are statistical outliers were eliminated from the study). Sham animals underwent the same procedures, except the tube was not fired. Animals were watched closely after exposure until they were ambulatory and capable of eating and drinking. Animals were allowed to recover spontaneously, noting duration of apnea (if present) and time to righting to provide a simple monitor for exposure severity and consistency. As shown on **Figure 2,** blasted mice showed an increase in righting time regardless of the genotype. Most importantly, as shown in **Figures 3-7**, this protocol elicits increases in tau phosphorylation of at least 3 epitopes that can be detected in both hippocampal and cortical homogenates at 1 and 24 hours post-injury, but not at 2 week and 3 month post-injury. Finally, the protocol did not affect Aβ40 and 42 levels (**Figure 8)** and produced no gross anatomical or cellular alterations in the brain (**Figure 9**).

These data support the contention that traumatic brain injury can cause alterations in tau phosphorylation that may contribute to neurodegeneration, and that this process can be modeled effectively in mice.

On the role of PP2A in shockwave-related tau phosphorylation:

Tau phosphorylation is controlled by the activities of kinases and phosphatases, and the principal tau phosphatase activity in cells comes from the enzyme, protein phosphatase 2A (PP2A). The ability of PP2A to dephosphorylate tau is determined by its subunit composition, which is regulated through methylation of a specific leucine residue on the c-terminus of the catalytic subunit (Rudrabhatla and Pant, 2011; Voronkov et al., 2011). PP2A methylation is controlled by the specific PP2A methylesterase, PME-1, and the specific PP2A methyltransferase, LCMT-1. We therefore tested the effect of PME over expression on shockwave-induced increases in tau phosphorylation at both 1 and 24 hrs after injury. We found that while PME over expression correlated with increased basal levels of tau phosphorylation at specific sites, it did not increase further the shockwave-induced tau phosphorylation we observed in sibling controls (**Figures 3 and 4**). We also found that that phosphorylation returns to baseline both in PME over expressing and control animals 2 week time point after shockwave exposure (**Figure 5**), These data suggest that the effect of shockwave exposure on tau phosphorylation may reach saturation at the sites where we observe increases at 1 and 24 hrs post-injury.

LCMT over expression is predicted to promote PP2A methylation and tau dephosphorylation. To explore the possibility that LCMT over expression may protect against shockwave-induced increases in tau phosphorylation, we exposed these and control animals to shockwaves or sham treatments and harvested brain tissue at 1 hr, 24 hrs after injury. We found that LCMT over expression does not reduce shockwave-induced tau phosphorylation (**Figure 6-7**). Thus, LCMT overexpression is not sufficient

to overcome the effect of shockwave exposure on tau phosphorylation, consistent with the hypothesis that tau phosphorylation may reach saturation at the sites where we observe increases at 1 and 24 hrs post-injury.

Blast-related eye damage

Since visual impairment is a common comorbidity for TBI (Jacobs and Van Stavern, 2013), and eye damage following blast exposure has been reported in several animal models (Bricker-Anthony and Rex, 2015; Hines-Beard et al., 2012; Sherwood et al., 2014; Yan et al., 2009), we decided to histologically examine eyes from our shockwave exposed animals to assess the presence of eye damage that may affect behavioral performance. We fixed and embedded eyes harvested from animals after shockwave, sectioned and stained with hematoxylin and eosin and looked for evidence of tissue damage. We found that shockwave exposure lead to vitreous detachment, photoreceptor degeneration, pigmentary changes and subretinal hemorrhage in 50% of eyes examined from shockwave-exposed mice (**Figure 10**). Thus, shockwave exposure can damage eyes.

Shockwave-related behavioral impairment:

During these years, we performed behavioral analysis of PME and LCMT over expressing and control animals at 2-week and 3 month post-injury time points. The results of the behavioral testing are described in the following paragraphs.

In the rotarod test to assess motor function (**Figures 11-12**), we did not find any significant difference both in the pretraining fall latency and testing fall latency between various groups of mice including control sham mice, blast mice, sham mutants PME and LCMT and blast mutants PME and LCMT mice both at 2-week and 3 month post-injury time points.

In the open field environment, shockwave-exposed mice spent a significantly higher proportion of time in the center of the arena at the 2 week post-injury time point in the control animals for the PME experiments (**Figure 13**). However, there was no significant difference at 3 months, nor in the controls for the LCMT experiments both at the 2 week and 3 month time points. Furthermore, the difference was not affected by over expression of PME.

In the elevated plus maze we did not find any significant difference in the time spent in the open and closed arms as well as in the center nor in the percent of time spent in the open arm vs. the closed arm between various groups of mice including control sham mice, blast mice, sham mutants PME and LCMT and blast mutants PME and LCMT mice both at 2-week and 3 month post-injury time points (**Figures 14-15**). We reached the same conclusion when we measured the ambulatory distance in the various groups of mice tested with the elevated plus maze (**Figure 16**).

In the contextual fear conditioning and sensory threshold assessment we did not find any significant difference in the percent of freezing and capability of perceiving the electric shock between various groups of mice including control sham mice, blast mice, sham mutants PME and LCMT and blast mutants PME and LCMT mice both at 2-week and 3 month post-injury time points (**Figures 17-18**).

In the 2-day radial arm water maze task, shockwave exposed animals committed significantly more errors in navigating to the hidden platform during the second day of

this task at the 2 week post-injury time point in the control animals for the PME experiments (**Figure 19**). However, there was no significant difference at 3 months, nor in the controls for the LCMT experiments both at the 2 week and 3 month time points. Furthermore, the difference was not affected by over expression of PME.

In a visual platform water maze task, we found that shockwave exposure produced a deficit in PME over expressing animals that was not observed in injured controls (**Figure 20**). The deficit could be observed not only at 2 weeks but also at 3 months after the injury and to a lesser extent in the LCMT mice. Performance of this task requires a simple non-spatial association between a visual cue and an escape platform. Neither transgene expression nor shockwave exposure affected swimming speed (**Figure 21**) suggesting comparable motivation and motor performance among these groups. This selective impairment, therefore, suggests that PME and LCMT over expressions may sensitize some portion of the visual system to the effect of shockwave exposure.

We also measured the time spent immobile in forced swim and tail suspension tasks to assess the effect of shockwave exposure and PME/LCMT over expression on affect (**Figures 22-23**). All these experiments did not show any significant difference between various groups of mice including control sham mice, blast mice, sham mutants PME and LCMT and blast mutants PME and LCMT mice both at 2-week and 3 month post-injury time points

PP2A plays a key role in shockwave-related behavioral changes induced by tau

The observed eye damage (**Figure 10**) raises the question of whether and to what extent shockwave-induced eye damage may affect behavioral performance in this and other rodent models of blast exposure. In our experiments, it is possible that eye damage may contribute to the apparent reduction in anxiety in the novel open field test, and could also contribute to the impairment we observe in the radial arm water maze, and the visible platform water maze task. Most importantly, it might affect the interpretation of the experiments with PME and LCMT mutants. To circumvent this possibility, we decided to use a different approach in which we extracted tau from the mouse brains exposed to blast. The extract was infused onto dorsal hippocampi through cannulae that were bilaterally implanted one week prior to performing the behavioral experiments (Fig. 4B). Mice were infused 180 and 20 min prior to testing on each day of a two-day radial arm water maze task (Alamed et al., 2006; Fiorito et al., 2013) with vehicle or tau purified from blast or sham mice (22.9 µg/ml in a final volume of 1 µl over 1 min). Infusion of tau from shockwave-exposed mice significantly impaired performance when compared to tau from sham-exposed animals, which was comparable to vehicle infused controls (**Figure 24A**). The effect was due to a cognitive impairment because controls with a visible platform test did not show any difference in speed and latency to reach a visible platform between the two groups. These data suggest that shockwave-exposed tau undergoes changes capable of altering short-term spatial memory. Next, we extended the findings on spatial memory to associative memory using the contextual fear conditioning model of associative learning. Shockwave-exposed tau and sham-tau were administered via the bilateral cannulas 180 and 20 min prior to the electric shock. Shockwave-exposed tau reduced freezing time when animals were exposed to the context 24 hrs after electric shock in that context, whereas sham-tau did not affect contextual memory (**Figure 24B**). The effect was not due to a difference in perception of the electric shock as sensory

threshold assessment did not show differences between different groups. Taken together, these findings indicate that shockwave-exposed tau undergoes specific changes that are responsible for its deleterious effects on memory.

LCMT over expression in these mice protected against tau-induced cognitive impairments in a two-day radial arm water maze task. LCMT-1 overexpressimg mice, or non-expressing sibling controls were bilaterally implanted with cannulae directed at the dorsal hippocampus and infused prior to testing on each day of a two-day radial arm water maze task with vehicle or human 4R/2N tau. Infusion of tau significantly impaired the performance of control animals but not LCMT over expressing animals in this task (**Figure 25**). The effect could be extended to contextual fear memory. LCMT over expression protected the mice from tau-induced cognitive impairments in a contextual fear conditioning task. Infusion of tau at a dose that impaired the performance in control animals did not impair performance of LCMT over expressing animals (**Figure 25**). The effect was really connected with memory because the same animals did not show behavioral differences in control experiments with the visible platform and sensory threshold assessment that might have unraveled defects interfering with cognitive assessment. Taken all together, these discoveries indicate that LCMT over expression protects against tau-induced cognitive impairments in this task.

PME over expression sensitized mice to tau-induced cognitive impairments in a two-day radial arm water maze task. Transgenic mice over PME-1, or non-expressing sibling controls were bilaterally implanted with cannulae directed at the dorsal hippocampus and infused prior to testing on each day of a two-day radial arm water maze task with vehicle or tau. Infusion of tau at a concentration that did not significantly impair the performance of control animals did cause significant impairment in PME over expressing animals in this task (**Figure 25**). PME over expression also sensitized mice to tau-induced cognitive impairments in a contextual fear conditioning task. Infusion of tau at a dose that did not impair the performance of control animals did impair performance of PME over expressing animals (**Figure 25**). The effect was really due to a cognitive impairment by tau because the same animals did not show behavioral differences in control experiments with the visible platform and sensory threshold assessment that might have unraveled defects interfering with cognitive assessment. Altogether, these findings suggests that PME over expression sensitizes animals to tau-induced cognitive impairments in this task.

In summary, these experiments demonstrate that regulation of phosphorylation by PP2A plays a key role in the cognitive damage induced by TBI.

KEY RESEARCH ACCOMPLISHMENTS:

- Identified a shockwave exposure protocol that produces acute increases in tau phosphorylation
- Completed planned measures of tau phosphorylation in PME/LCMT over expressing and control animals at the 1hr, 24 hr, 2 week and 3 month post-exposure time points. Changes in tau phosphorylation occurred at 1 hr and 24 hrs but not 2 weeks and 3 months in the absence of any change in Aβ levels.
- Conducted histological analysis of eyes from mice that revealed hemorrhage and retinal detachment after shockwave but not sham exposure.

- Conducted histological analysis of brains in PME/LCMT over expressing and control animals at the 1hr, 24 hr, 2 week and 3 month post-exposure time points. No effect was found.
- Completed behavioral assessment of PME/LCMT over expressing and control animals at the 2 week and 3 month post-exposure time points. Blast produced behavioral changes in control animals. Eye damage is likely to interfere with the interpretation of the behavioral observations.
- Identified a shockwave exposure protocol that produces tau extracts capable of producing cognitive changes.
- LCMT overexpression protects against tau induced behavioral changes.
- PME overexpression sensitizes towards tau induced behavioral changes.

REPORTABLE OUTCOMES:

We received an award from the DoD (Convergence Science Research Award Proposal Number: AZ140095; Project Title: TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains, Principal Investigator: Dr. Ottavio Arancio).

CONCLUSION:

The study of the long-term consequences of traumatic brain injury (TBI) is still an emerging field and the long-term consequences of TBI due to blast exposure is an even more recent problem due to both the nature of recent military conflicts and the fact that improvements in body armor are allowing individuals to survive blast exposures that were previously lethal. While there is a considerable and growing body of evidence that repetitive TBI produces a neurodegenerative tauopathy (Blennow et al., 2012). The data on neurodegeneration and tauopathy following a single blast exposure is much more limited. Reliable, reproducible and realistic animal models of blast exposure and other forms of TBI are urgently needed if we are to understand and ultimately treat or prevent these conditions. We have confronted the difficulties in establishing an animal model of human blast-induced TBI head-on by examining the biochemical response of wild type mice to a range of shockwave intensities and different exposure conditions. The experience we gained from this effort has contributed to the collective effort of the TBI research community to developing effective methodologies with which to study this phenomenon. This experience together with our investment in validating methods for biochemical and behavioral analysis of shockwave exposed mice has also positioned us to test our original hypotheses regarding the role of PP2A and tau phosphorylation in the behavioral and cognitive impairments that can result from blast-induced TBI. Specifically, we have found that tau extracted from brains of mice exposed to blast impairs cognition. Moreover, the toxic effect of tau onto cognition could be overcome by an increase in PP2A activity and magnified by a decrease in PP2A activity, suggesting that a therapy acting onto this phosphatase might be beneficial against the memory loss

occurring after TBI.

Methods:

Shockwave exposure:

We exposed four cohorts of control and PME-1 transgenic animals to single shockwaves of 269 ± 9.8 kPa peak over pressure, 0.73 ± 0.021 ms duration, and 67 ± 2.3 kPa-ms impulse. The same paradigm was used for LCMT mice. Pressure histories are measured with 3 pressure transducers (Endevco 8530B) arranged symmetrically around the exit of the tube in an incident configuration. This blast is quantitatively similar to a real world blast from a 100 g charge of trinitrotoluene (TNT) at a standoff distance of 1m. These animals were placed in the specially designed animal holder described above that supported the head from below, and shielded the body to prevent confounding lung/bowel injury and to reproduce the presence of body armor. In these experiments, shockwaves were directed at the head from above. These conditions produced no lethality in any of the animals tested.

Western blotting:

Phospho-tau levels were determined by western blot on brain homogenates prepared by sonication in hot 3%LDS/50 mM Tris pH 7.5/10 mM EDTA. Proteins were resolved on 4-12% Bis-Tris protein gels, blotted on PVDF membranes and probed with the indicated primary antibodies. Detection and quantification was carried out using Licor infrared-dye labeled secondary antibodies and an Odyssey imager. In all cases band intensities were normalized to a within-lane control band (either total tau or □-actin).

Behavioral testing:

The behavioral protocols were carried out according to the following schedule:
Days -4 to -1: Rotarod pretraining
Day 0: no testing (shockwave exposure day)
Battery Day 1: openfield (AM) and accelerating rotarod (PM) (for experiments on transgenic animals this day will be 2 weeks, or 3 months after shockwave exposure)
Battery Day 2: elevated plus maze (AM) and forced swim test (PM)
Battery Day 3 and 4: radial arm water maze task
Battery Day 5: tail suspension test (AM) and contextual fear conditioning task training (PM)
Battery Day 6: contextual fear conditioning task testing
Battery Day 7 and 8: visible platform water maze task
Battery Day 9: sensory threshold assessment

Accelerating rotarod task:

We assessed motor performance of mice using a rotarod apparatus (Med Associates) essentially as described previously (Clausen et al., 2011; Wang et al., 2011; Yu et al., 2012). This apparatus consists of a 32 mm diameter rotating rod suspended 16.5 cm above a pressure sensitive tray. The rod passes through large plastic discs that create 57 mm lanes along the rod in which lateral movement of the mice are constrained. Training on this task was carried out on 4 successive days. The first day of training consisted of 4 x 5 minute trials. On the first trial, animals were placed on the apparatus and the rotation speed was set at 4 rpm, on the second and third trials, the rotation speed was slowly ramped up from 4 to 10 rpm over the course of the trial, and on the third trial the rotation speed was ramped from 4 to 40 rpm. On this first day of training, animals that fell were returned to the rod and the trial continued for the specified 5 min period. On this and all subsequent days, animals were returned to their home cages for 45 min between trials. The second through fourth days of training consisted of 3 x 5 min trials per day with the rotation speed ramped from 4 to 40 rpm over the course of the trial. When animals fell from the apparatus the trial was terminated and the animal returned to its home cage. Rotarod testing was carried out in the morning of the second day of the behavioral battery. Testing consisted of 4 trials conducted in the same manner as described for pre-training days 2 -4.

Open field testing:

To assess the effects of shockwave exposure and our genetic manipulations on activity level, response to novelty, and anxiety, we assessed the behavior of our animals in a novel open field environment essentially as described in (Tweedie et al., 2007). We placed animals in a plexiglass chamber (43.2 cm long × 43.2 cm wide × 30.5 cm high) for a total of 30 min during which time their movements were tracked and analyzed using a video tracking system and behavioral analysis software (Ethovision, Noldus).

Elevated plus maze task:

To assess any possible anxiogenic or anxiolytic effects of shockwave exposure and our genetic manipulations, we examined the behavior of our animals in an elevated plus maze essentially as described in (Schwarzbold et al., 2010; Siopi et al., 2012). The apparatus consists of a plus shaped track with arms 18 cm long and 6 cm wide, elevated 60 cm above the bench top by a single central pillar. Two non-adjacent arms are surrounded by walls on 3 sides, and the remaining two arms are exposed. Animals were placed into the center of the apparatus and the number and duration of open vs. closed arm entries are used as an index of anxiety. Animal location during single 5 min exposure to this behavioral apparatus was monitored and analyzed using a video tracking system and accompanying behavioral analysis software (Ethovision, Noldus). After each trial, animals were returned to their home cages and the apparatus was thoroughly cleaned and deodorized with MB-10 and distilled water.

Forced swim test:

To assess the effects of shockwave exposure and our genetic manipulations on depressive behavior, we assessed the behavior or our animals in forced swim test essentially as described in (Milman et al., 2008; Tweedie et al., 2007). We placed control animals into a 4 liter plastic beaker filled half way with tap water (22-25C) for a total of 6

minutes. During this time, the animals' movements were recorded using a video camera, and the recordings were subsequently offline by a blinded observer for number, timing, and duration of periods of immobility. Following the forced swim trial, animals were dried using paper towels and returned to clean home cages partially illuminated by a heat lamp for a period of 10 minutes to prevent hypothermia.

Radial arm water-maze task:

To assess the effects of shockwave exposure and our genetic manipulations on cognitive performance, we tested our animals in a 2-day radial arm water-maze task as described previously (Alamed et al., 2006; Fiorito et al., 2013). The test was performed in a 120 cm diameter pool containing a 6-arm radial maze insert and opaque water maintained at 24°C. On each day of the task, animals are subjected to a total of 15 trials. During the first 11 odd-numbered trials of the first day, the location of the escape platform is indicated by a marker protruding above the surface of the water, while on all other trials, the submerged platform is not visible to the animals. In each trial, the number of errors (entries into arms that do not contain the platform) will be recorded. At the end of testing, the mice were dried off and placed in a clean cage with extra paper towels to prevent hypothermia.

Tail suspension test:

As a second test of the effects of shockwave exposure and our genetic manipulations on depressive behavior, we assessed the behavior or our animals in a tail suspension test essentially as described in (Schwarzbold et al., 2010). In our pilot experiment, animals' tails were gently taped approximately 2 cm from the end to a horizontal bar elevated 30 cm above the benchtop. The animals were then suspended in this position for 6 minutes while their movements were recorded using a digital video camera. Videos were later scored offline by a blinded observer for number, timing, and duration of periods of immobility. Immediately after testing animals were removed from the apparatus returned to their home cages.

Contextual and cued fear conditioning:

As an additional test of the effects of shockwave exposure and our genetic manipulations on cognitive performance, we tested animals on a contextual fear conditioning task as described previously (Francis et al., 2009; Puzzo et al., 2008). In this task, animals are placed into a conditioning chamber located inside a sound-attenuating box (72cm x 51cm x 48cm). A clear Plexiglas window (2cm thick, 12cm x 20cm) will allow the experimenter to record the animal's behavior with a video camera connected to a computer running Freeze Frame software (MED Associates Inc.). Background white noise (72dB), was provided by a single computer fan will installed in one of the side of the sound-attenuating chamber. The conditioning chamber (33cm x 20cm x 22cm) is made of transparent Plexiglas on two sides and metal on the other two. One of the metal sides has a speaker and the other one a 24 V light. The chamber has a 36-bar insulated shock grid floor. The floor is removable to facilitate its cleaning with MB-10 and then with distilled after each experimental subject. Animals were placed in the conditioning one animal at a time chamber once on each of two consecutive days. The first day of exposure mice was placed in the conditioning chamber for 2 minutes before the onset of a

discrete tone (CS) (a sound that will last 30s at 2800Hz and 85dB). In the last 2s of the CS, mice were given a foot shock (US) of 0.50mA for 2s through the bars of the floor. After the tone and shock exposure, the mice were left in the conditioning chamber for another 30s and then placed back in their home cages. 24 hours after their first exposure animals were returned to the conditioning chamber for a total of 5 min without foot shock or tone presentation. During each of these exposures, freezing behavior was scored using FreezeFrame software (Med Associates) and this parameter was used as a measure of the strength of the context-shock association (ie. memory on the second exposure) and the general level of anxiety (baseline pre-shock exposure).

Sensory threshold assessment:

As part of our pilot experiment, we also tested animals on the sensory threshold assessment task that we used to rule out any differences in shock perception that could interfere with our interpretation of the performance of animals in the contextual fear conditioning task. We conducted this assessment as described previously (Francis et al., 2009; Puzzo et al., 2008). Animals were placed into an apparatus similar to that used for contextual fear conditioning. A sequence of single, 1sec foot shocks was then given at 30 sec intervals and 0.1 mA increments from 0 to 0.7 mA. Each animal's behavior was evaluated to identify shock intensities that produced the first visible response to the shock (flinch), the first extreme motor response (run/jump), and the first vocalized distress.

Visible platform water maze task:

To complete the pilot testing of our behavioral battery, we subjected our cohort of control animals to a visible platform water maze task as described previously (Francis et al., 2009; Puzzo et al., 2008). We used this task in our behavioral battery both as another assessment of motor function and also to test for any performance deficits that might interfere with our analysis of the radial arm water maze task. We performed this task in the same 120 cm diameter pool used for the radial arm water maze task, except that the partitions were removed. Training for this task was carried out over 2 days with 3 morning and 3 afternoon trials on each day. Intertrial intervals were 15 to 20 min and rest periods between morning and afternoon sessions were at least 3 hrs. Each trial lasted for a maximum of 120 sec during which animals were required to swim to a visible escape platform located just above the water surface. Animals that did not reach the platform within the allotted time were guided to it and allowed to sit there for 15 sec before being returned to their home cage. The location of the platform was rotated among 4 different locations such that it was not be present in the same location on any two successive trials. Water temperature was maintained at approximately 24°C, and at the end of testing, the mice were dried off and placed in a clean cage with extra paper towels to prevent hypothermia. Measures of both time required to reach the hidden platform (latency) and swim speed were conducted using a video-tracking system and behavioral analysis software (Ethovision, Noldus).

Histology:

Eyes: Eyes were removed by blunt enucleation from mice sacrificed by cervical dislocation. Eyes were the placed in acidic methanol solution before paraffin embedding and sectioning. Sections were then processed with hematoxylin and eosin, and standard

images were captured under light microscopy for review.

Brains: Brain were fixed by transcardial perfusion with 4% paraformaldehyde, pior to paraffin embedding and sectioning. Sections were then processed for H&E staining or immunohistochemical staining on Ventana Benchmark automated IHC staining system using the following primary antibodies anti-APP, anti-phospho-tau antibody AT8, anti-phospho-neurofilament light chain (Nfl-1) antibody, anti-glial fibrillary acidic protein (GFAP), and anti-ionized calcium binding protein-1 (Iba-1).

Tau Preparation

Tau Purification: Tau was purified from the mouse cortex. The tissue was prepared as previously described (Ivanovova et al., 2008)) with the modification that size fractionation (by filtering out all molecules below 10kDa through Sartorius Vivaspin-Turbo-15) was used in place of the immunoaffinity column for the final purification step. An advantage of this method is that it has a high yield, and preserves the natural phosphorylation state of tau and its antigenic properties.
Preparation of recombinant tau: The tau 4R/2N construct, gift of Dr. Furukawa (Yokohama U., Japan) (Furukawa et al., 2011), was transfected in Escherichia coli (Rosetta), and cells were streaked on LB agar ampicillin plates and a single colony was picked and grown overnight in LB broth with glucose and 100 mg/ml carbenicillin. Protein expression was induced with 1mM IPTG for 8 hrs when cells are pelleted at 4˚C by centrifugation at 6000g. Pellets were stored overnight at -80˚C. After a freeze-thaw cycle, cells were lysed in a 2% Triton X-100 phosphate-buffered saline and with a protease inhibitor mixture (Complete, EDTA-free; Roche). Streptomycin sulfate was added to precipitate DNA. After centrifugation, 100mM NaCl was added to the supernatant and heated at 100 °C for 15min. The precipitate was removed by centrifugation. The first step of purification for the C-terminal anionic construct used a nickel column with His-bind resin. The supernatant was loaded on His-Spin Protein Miniprep columns (Zymo Res.) and eluted with phosphate buffer containing 300mM NaCl plus 250mM imidazole. Eluted tau was then be buffer exchanged for the protein preparations into 50mM Tris-HCl pH 7.4 via Amicon Ultra Centrifugal Devices (Millipore). Protein concentration was determined by BCA assay.

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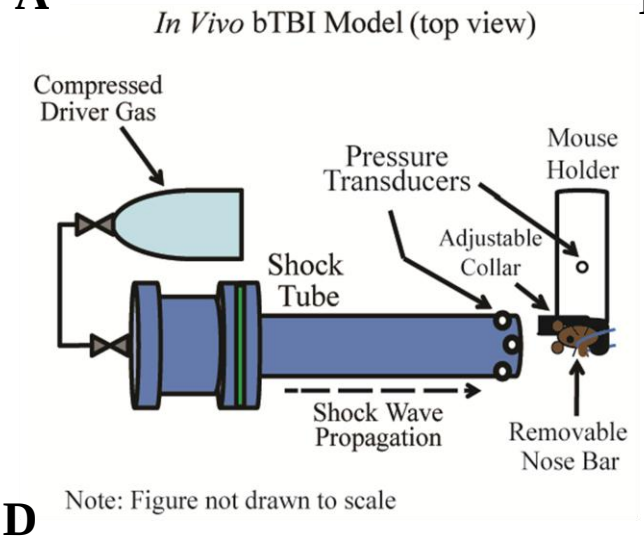
SUPPORTING DATA:

Figure 1: **Shock-tube overpressures recapitulate operationally-relevant blasts**. **A)** *In vivo* blast-TBI model (top view, figure not drawn to scale). **B)** Pressure parameters. **C)** Graph depicting the pressure vs. time history of the shock wave. Only the mouse's head is exposed to the shock wave shown in blue (In Air). The mouse's torso is protected in a rigid holder to preventing confounding injuries to the lungs and bowels (pressure in the holder in red). **D)** Mouse kinematics parameters. **E)** Picture of the mouse immobiized in the mouse holder.

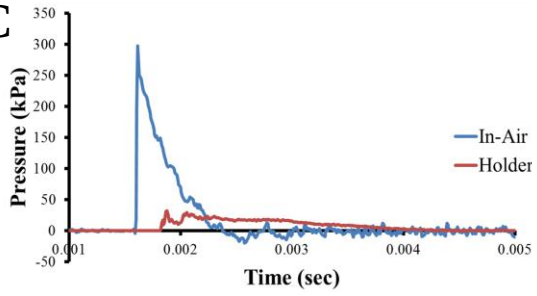
A



B Blast Parameters

Peak Pressure (kPa)	269 ± 10
Duration (ms)	0.73 ± 0.02
Impulse (kPa-ms)	67 ± 2

C



D Mouse Head Kinematics

Peak Horizontal Displacement (mm)	Peak Horizontal Acceleration (m/s^2)	Peak Vertical Displacement (mm)	Peak Vertical Acceleration (m/s^2)
3.71 ± 0.15	10372 ± 607	2.22 ± 0.21	7391 ± 466

E

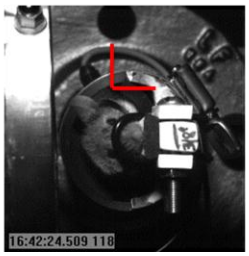


Figure 2: Righting time in PME **(A)** or LCMT **(B)** over expressing animals and their respective controls after sham or shockwave exposure.

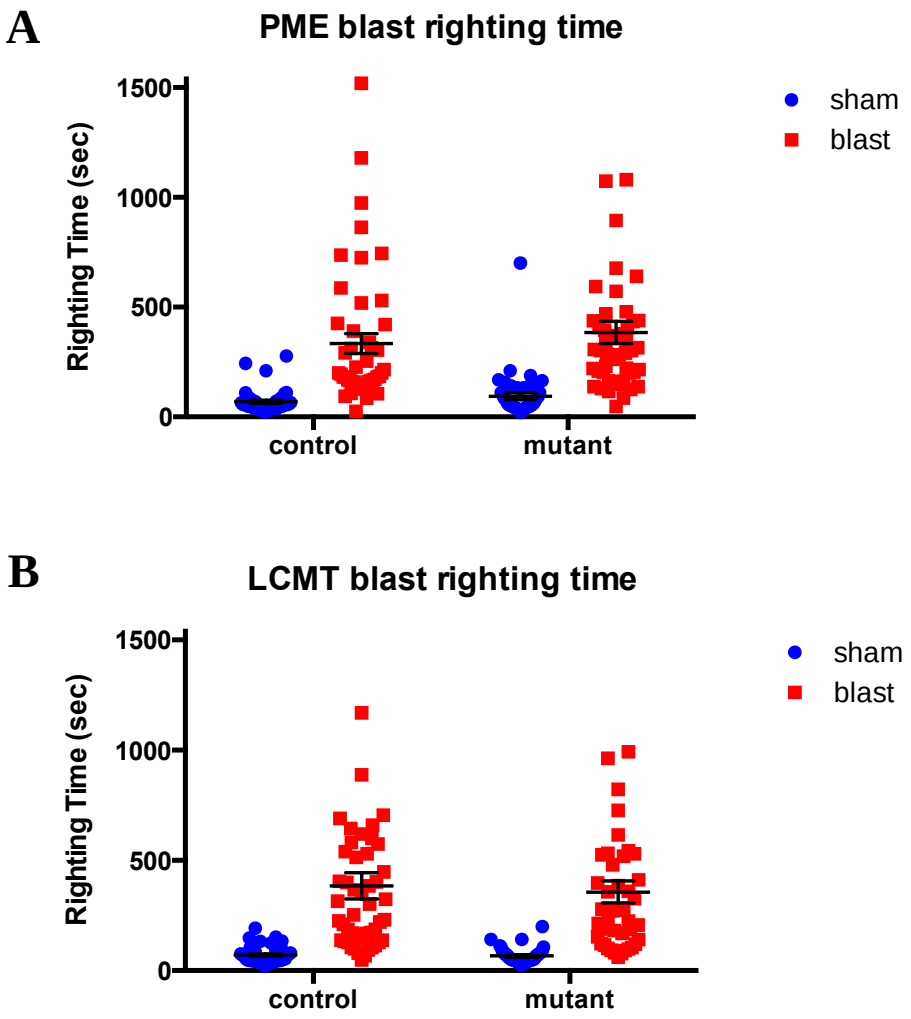


Figure 3: Tau phosphorylation in cortex and hippocampus of control or PME over expressing animals 1 hour after shockwave exposure or sham.

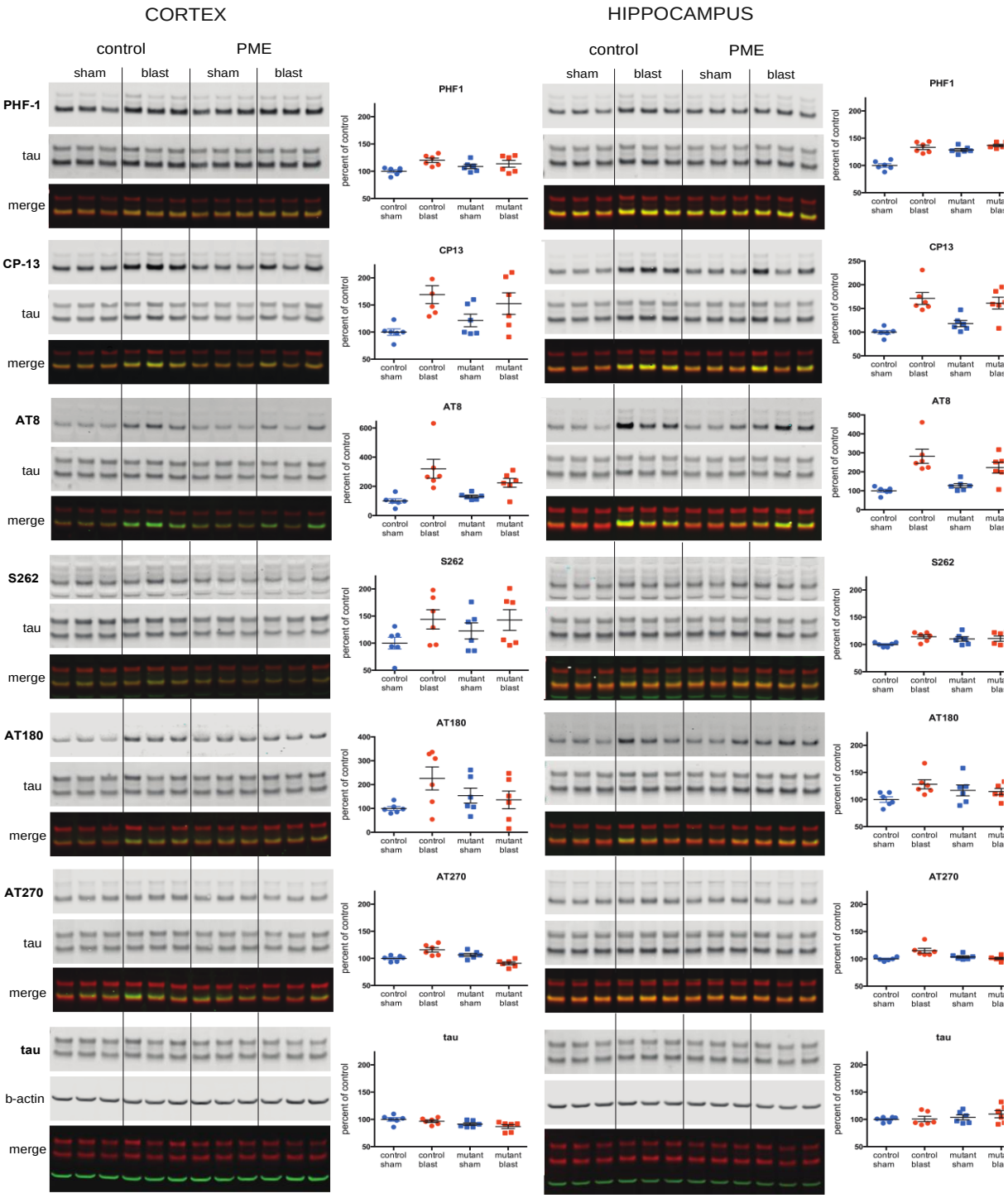


Figure 4: Tau phosphorylation in cortex and hippocampus of control or PME over expressing animals 24 hours after shockwave exposure or sham.

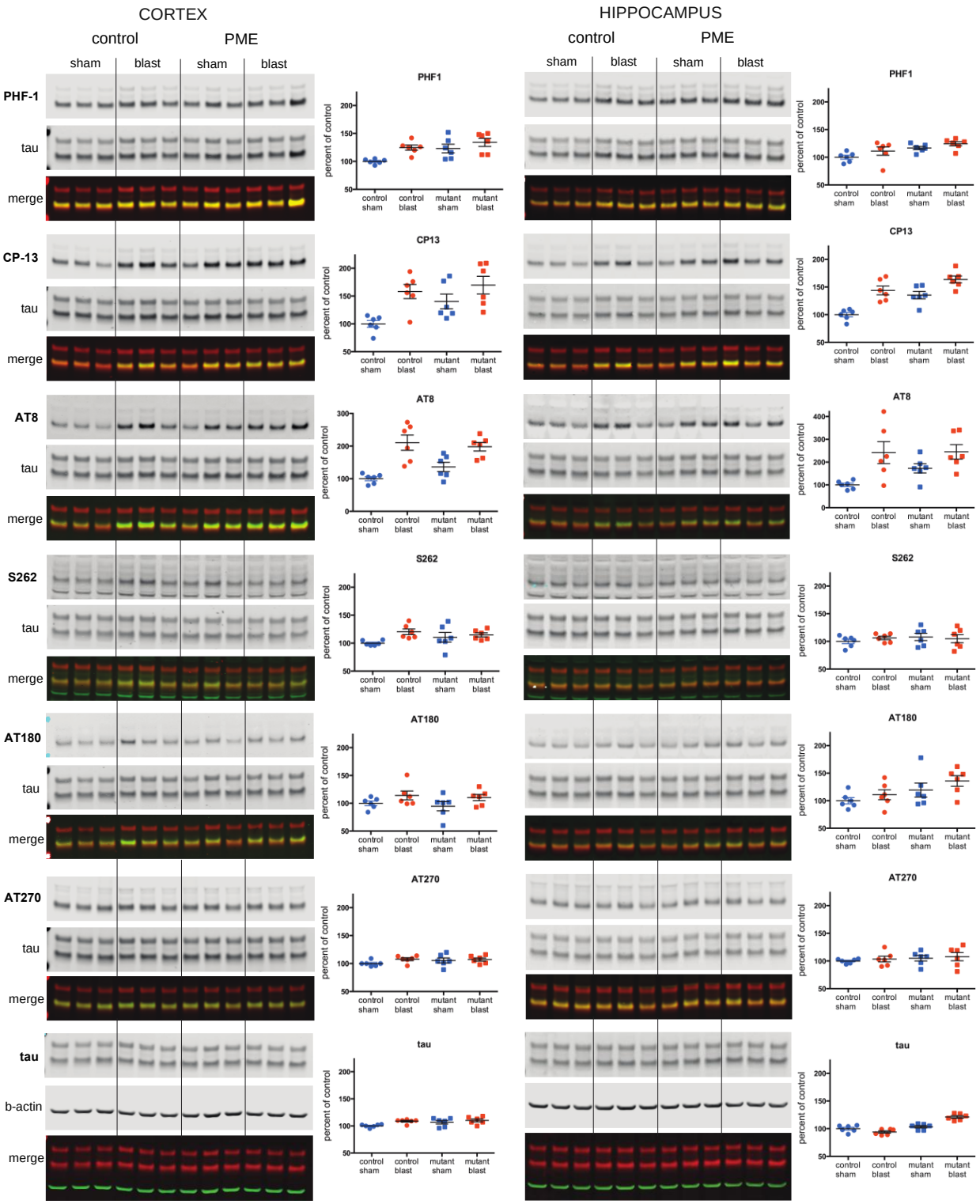


Figure 5: Average data for tau phosphorylation in hippocampus of control or PME over expressing animals 2 weeks and 3 months after shockwave exposure or sham (similar results were obtained with cortices).

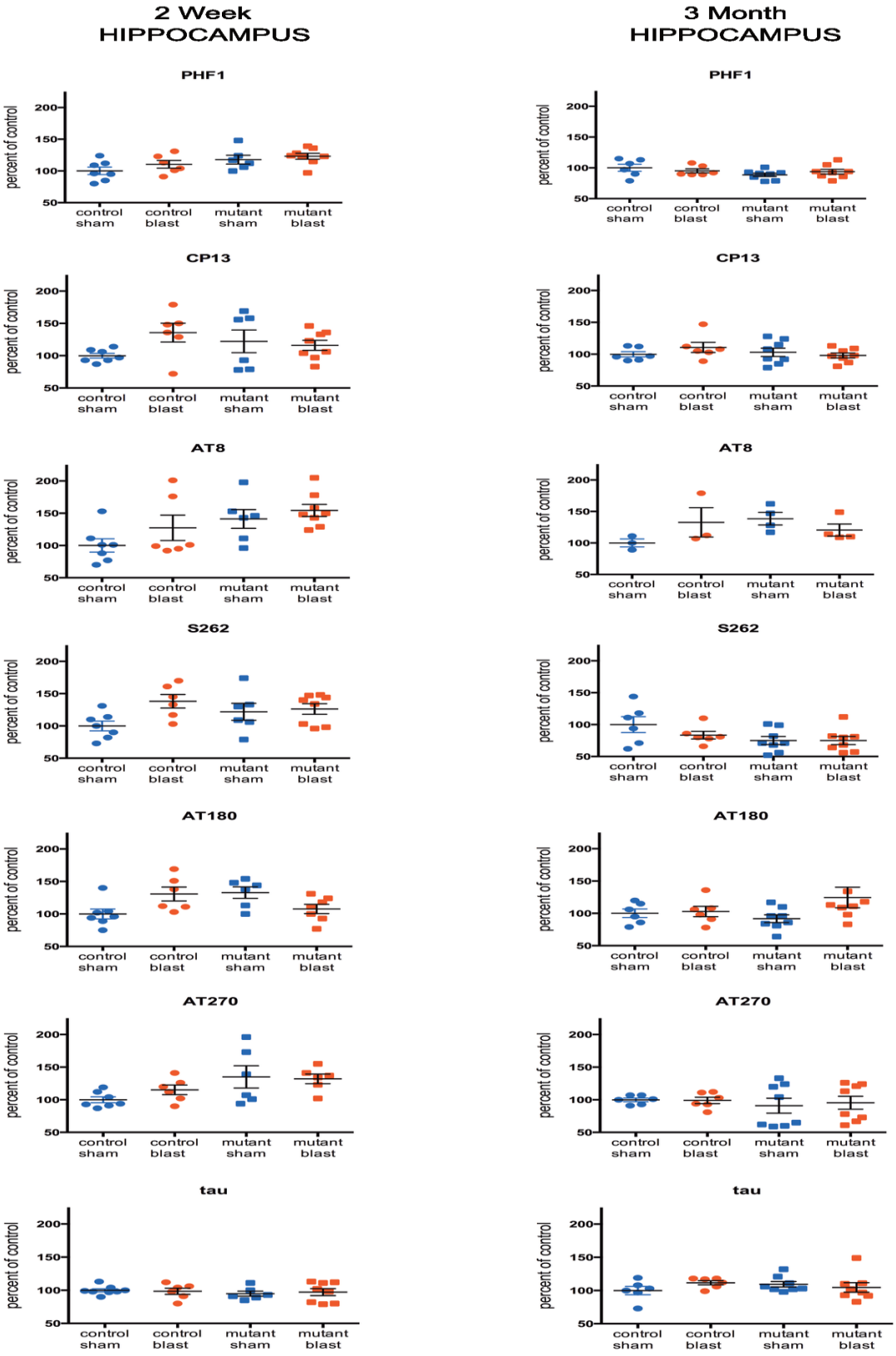


Figure 6: Tau phosphorylation in cortex and hippocampus of control or LCMT over expressing animals 1hr after shockwave exposure or sham.

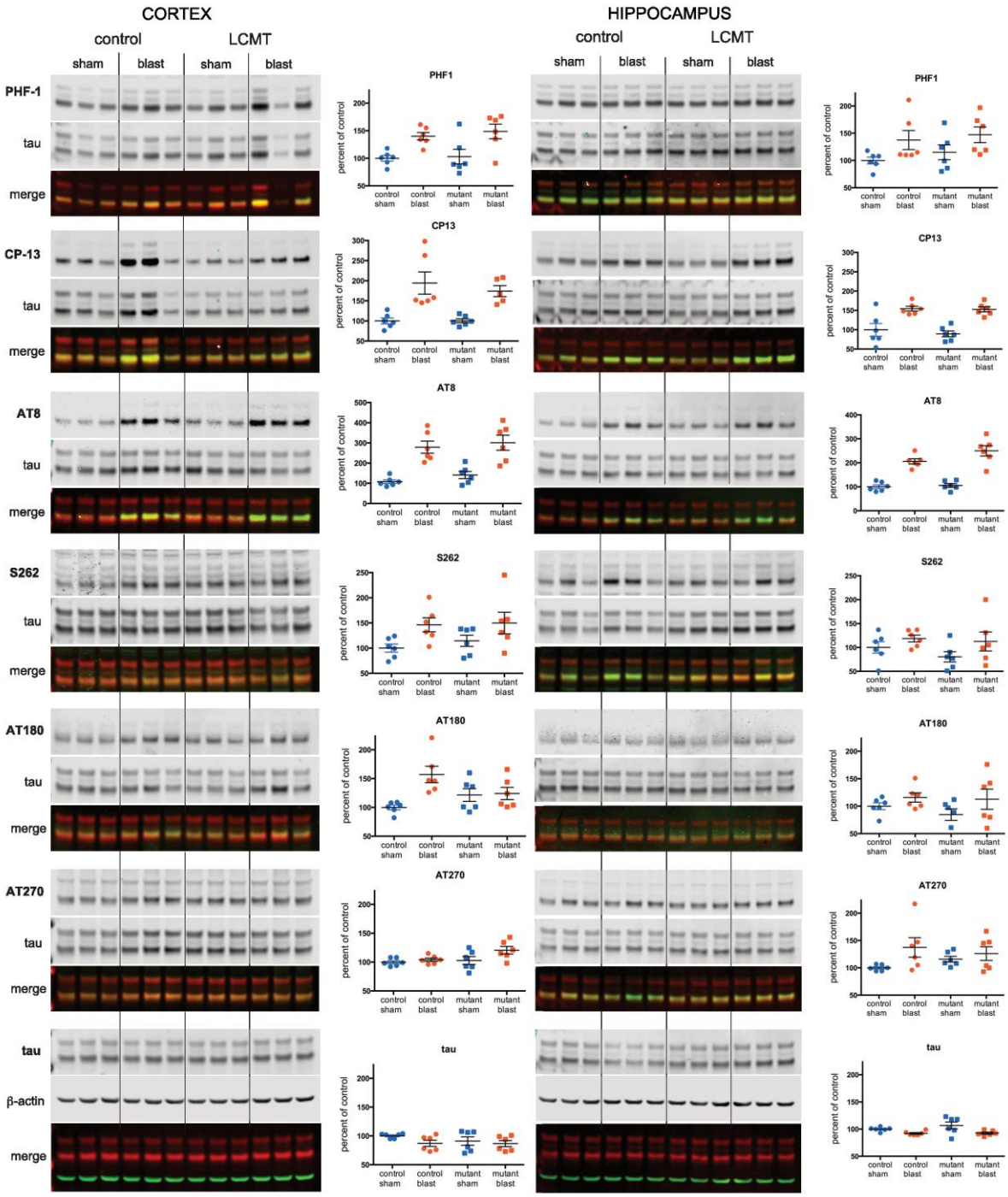


Figure 7: Tau phosphorylation in cortex and hippocampus of control or LCMT over expressing animals 24hrs after shockwave exposure or sham.

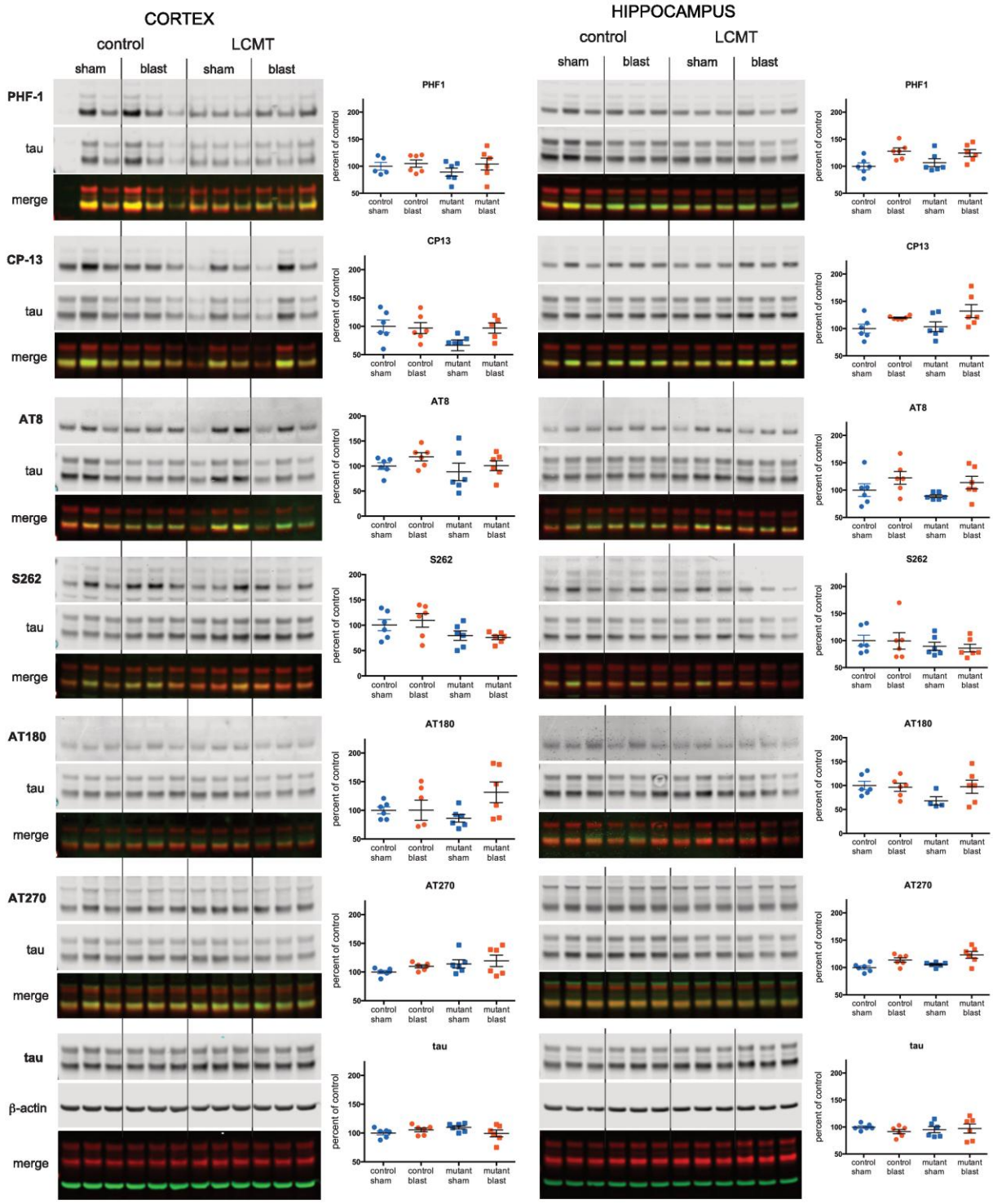


Figure 8: Acute cortical Aβ40 and 42 levels 1 hr post-blast in PME or LCMT over expressing animals and their control littermates 1 hour after shockwave exposure or sham.

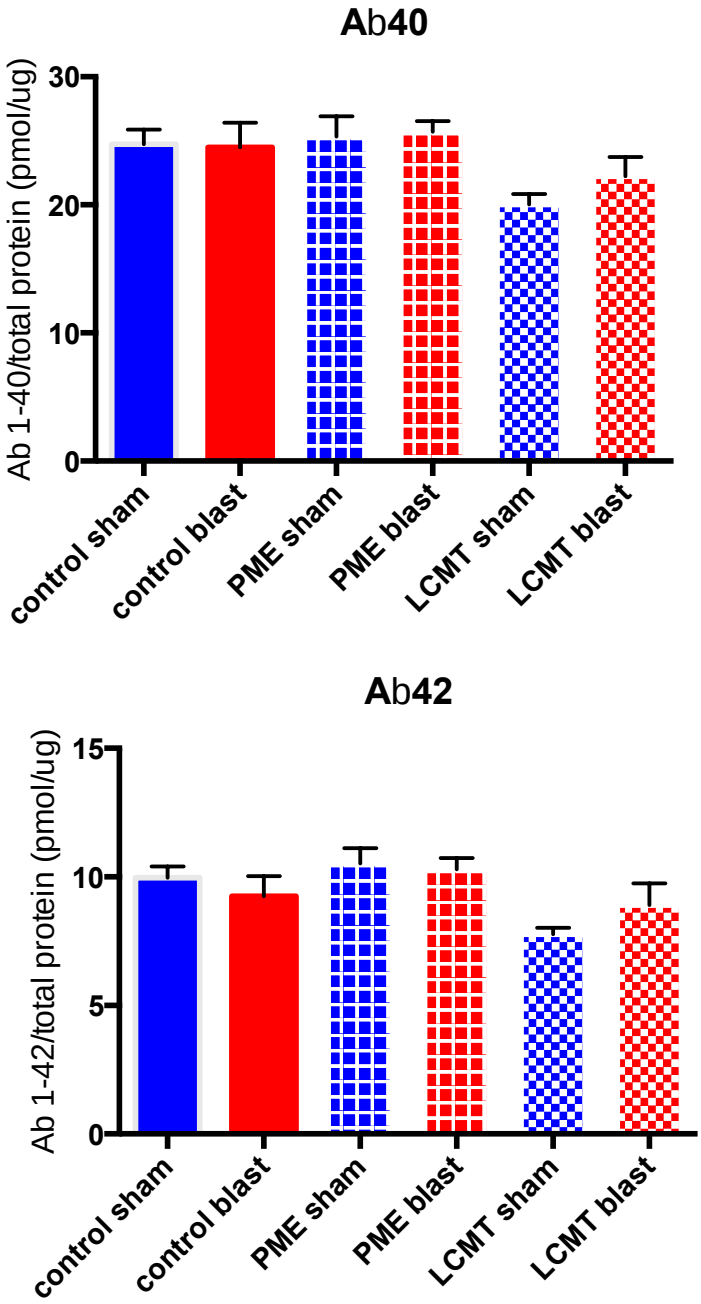


Figure 9: Shockwave exposure results in no gross anatomical or cellular alterations

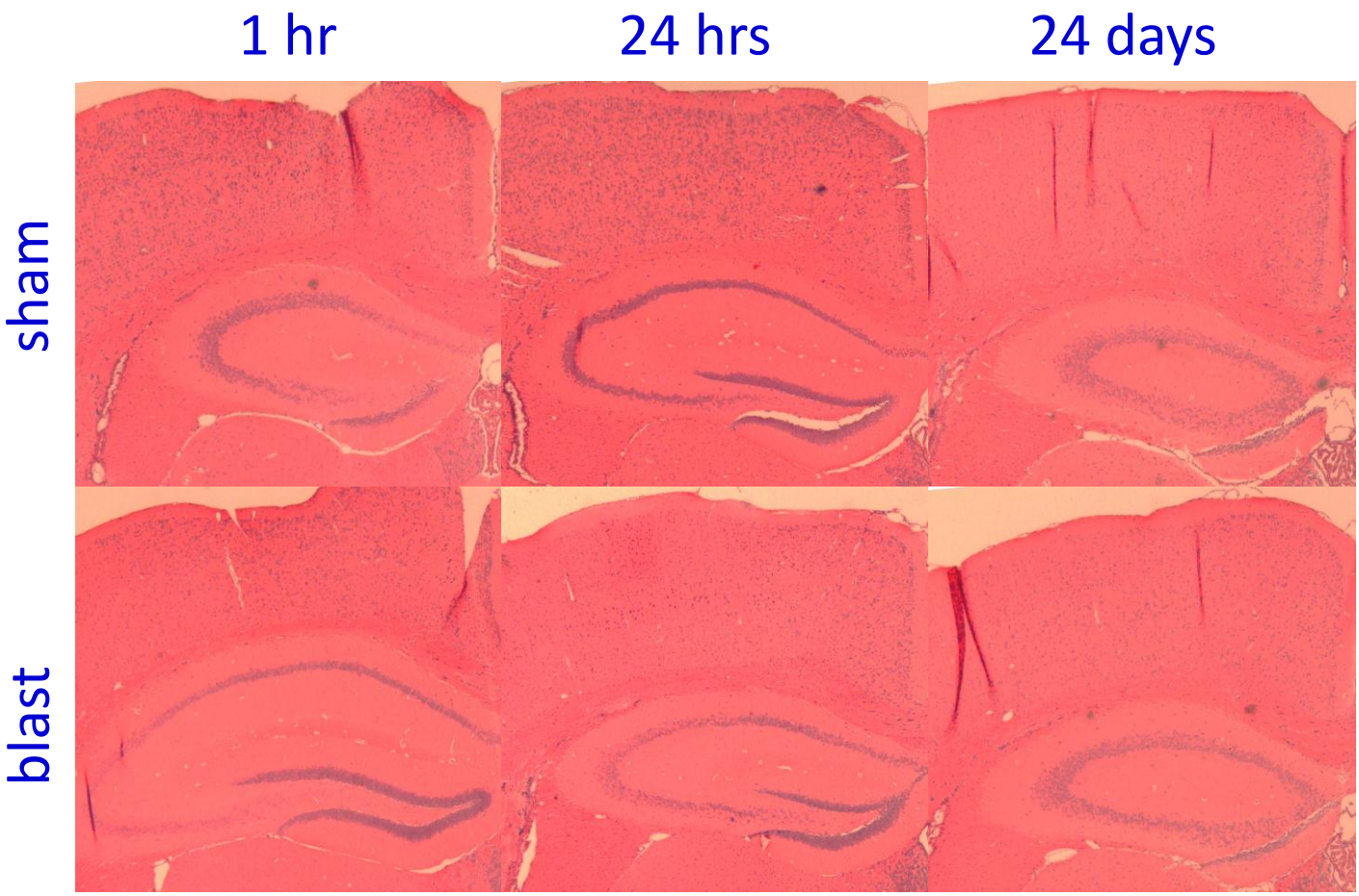


Figure 10: Hematoxylin and eosin staining of shockwave-exposed eyes. Fifty percent of the eyes harvested 24 days after injury from blast exposed animals exhibited evidence of vitreous detachment **(A)** and photoreceptor degeneration, pigmentary changes **(B)**, and subretinal hemorrhage **(C).**

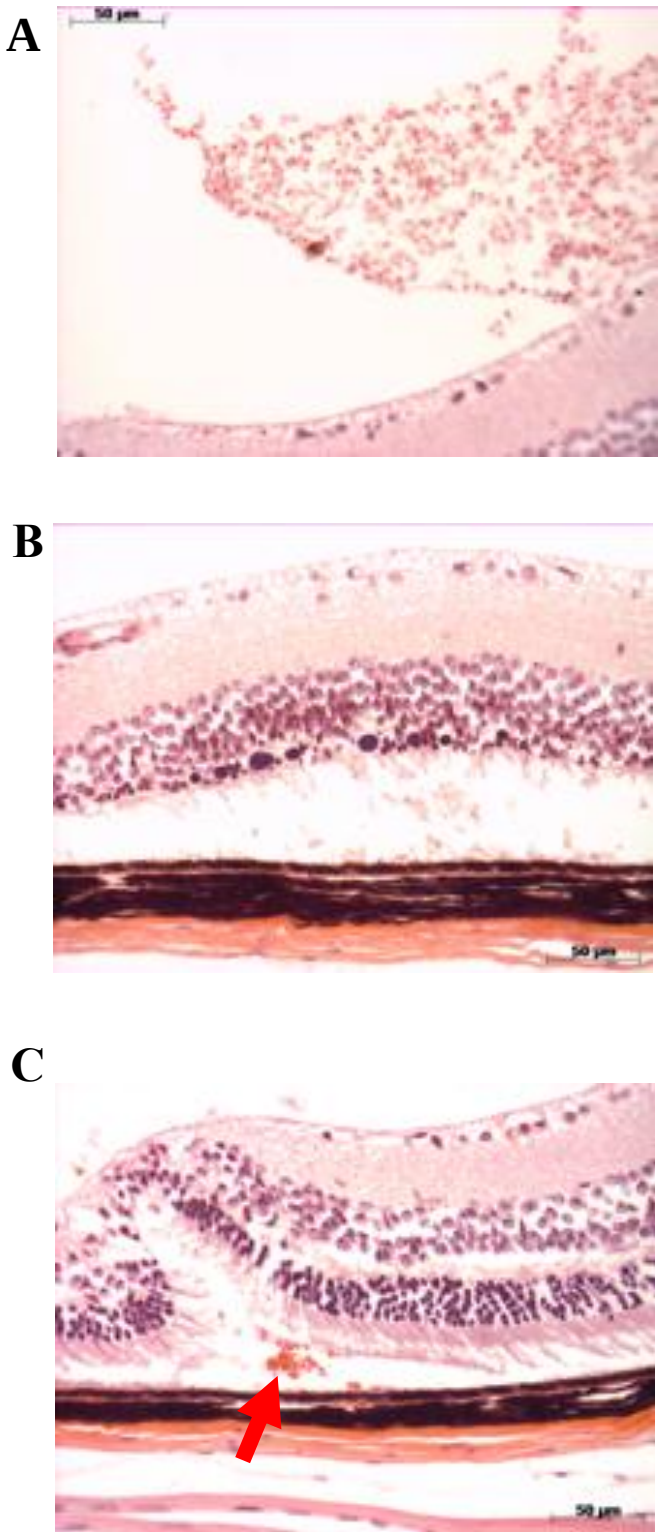


Figure 11: Pretraining fall latency during rotarod testing in PME and LCMT over expressing animals and their controls at 2 weeks and 3 months before shockwave exposure

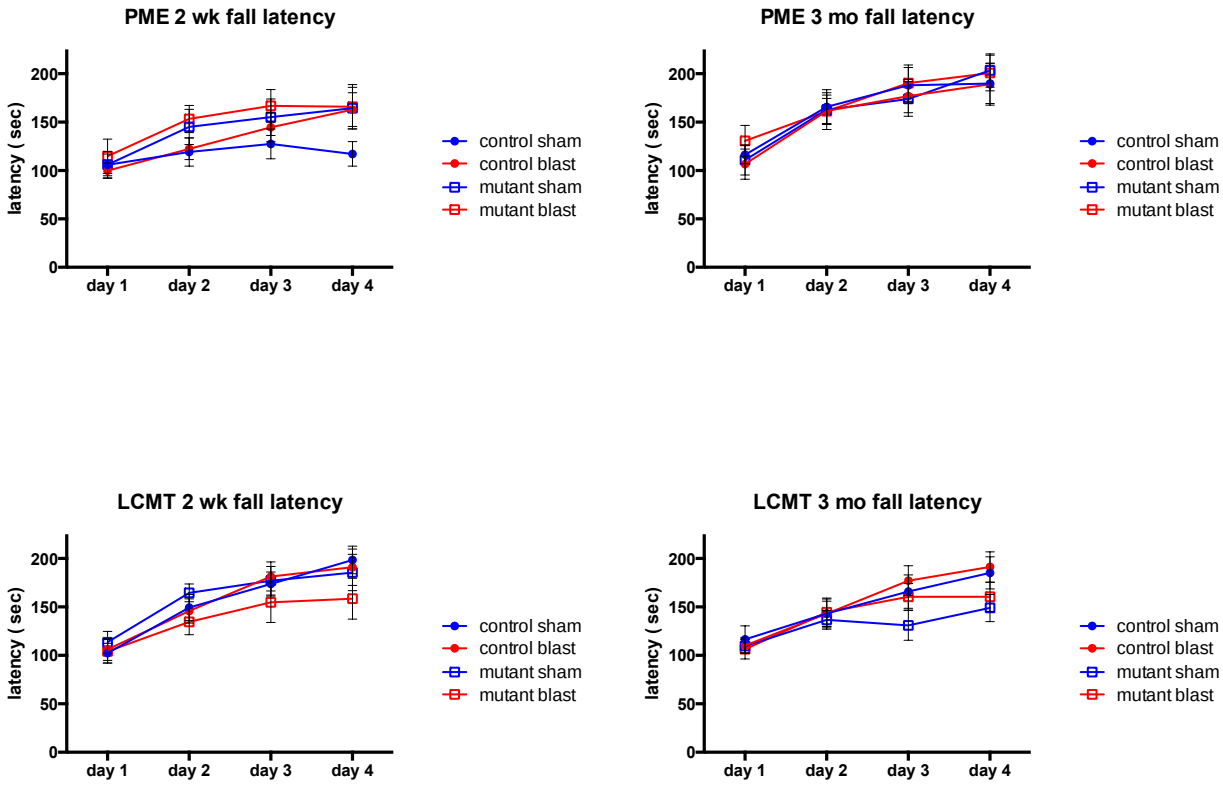


Figure 12: Testing fall latency during rotarod testing in PME and LCMT over expressing animals and their controls at 2 weeks and 3 months after shockwave exposure

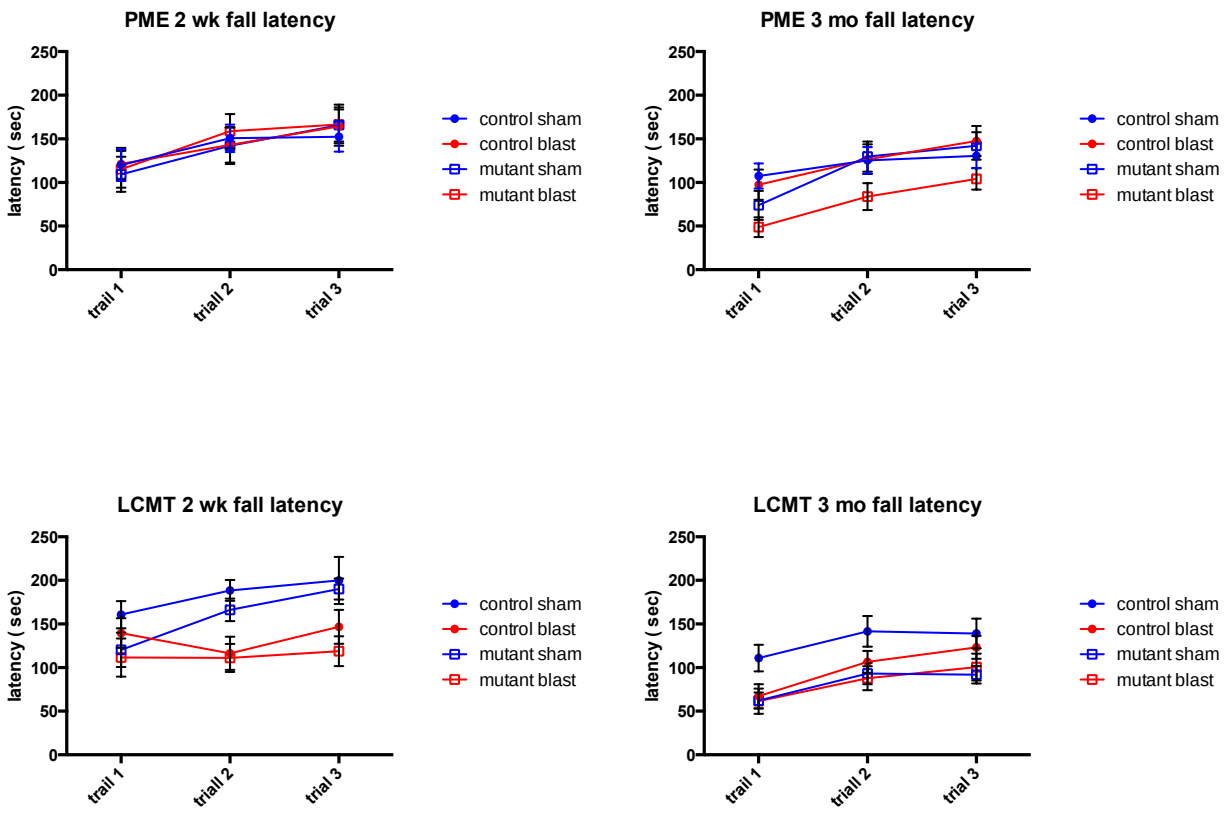
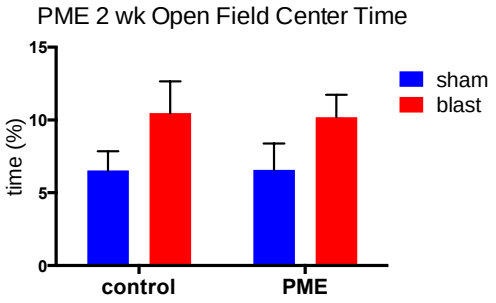
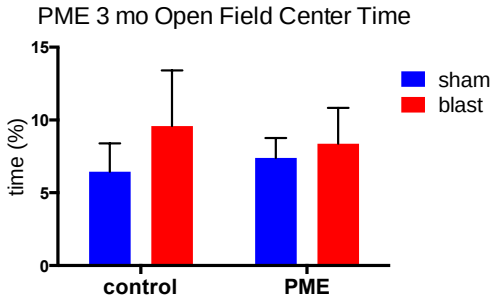


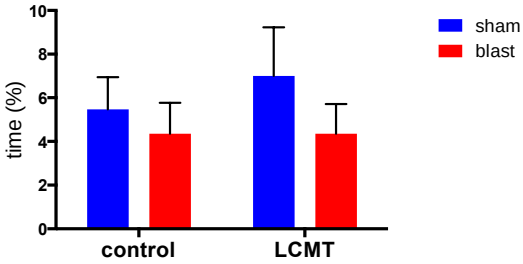
Figure 13: Percent time spent in the center of the arena during open field testing in PME or LCMT over expressing animals and their controls 2 weeks or 3 months after sham or shockwave exposure



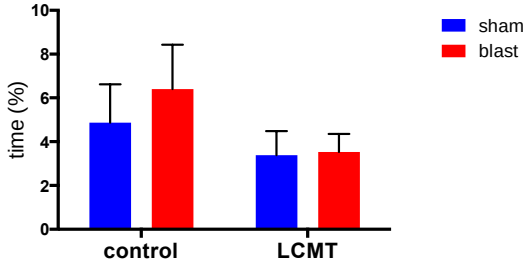
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.3821	1	0.3821	F (1, 59) = 0.007871	P = 0.9296
genotype	0.2043	1	0.2043	F (1, 59) = 0.004208	P = 0.9485
treatment	224.1	1	224.1	F (1, 59) = 4.615	P = 0.0358
Residual	2864	59	48.55		



ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	10.49	1	10.49	F (1, 32) = 0.1773	P = 0.6765
genotype	0.1613	1	0.1613	F (1, 32) = 0.002727	P = 0.9587
treatment	38.13	1	38.13	F (1, 32) = 0.6446	P = 0.4280
Residual	1893	32	59.16		



ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	8.539	1	8.539	F (1, 55) = 0.2221	P = 0.6393
genotype	8.633	1	8.633	F (1, 55) = 0.2245	P = 0.6375
treatment	51.53	1	51.53	F (1, 55) = 1.340	P = 0.2520
Residual	2115	55	38.45		



ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	6.923	1	6.923	F (1, 54) = 0.2025	P = 0.6545
genotype	68.29	1	68.29	F (1, 54) = 1.998	P = 0.1633
treatment	10.22	1	10.22	F (1, 54) = 0.2988	P = 0.5869
Residual	1846	54	34.19		

Figure 14: Time spent in the arms during elevated plus maze testing in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure

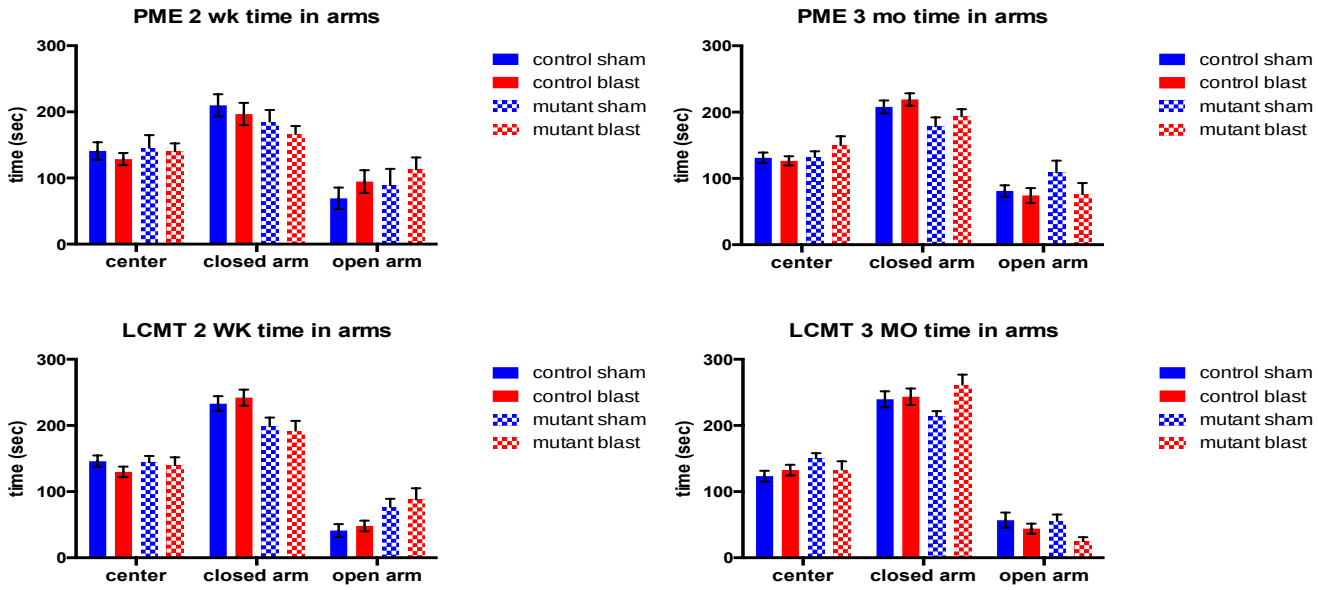


Figure 15: Percent time in open vs. closed arm during elevated plus maze testing in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure

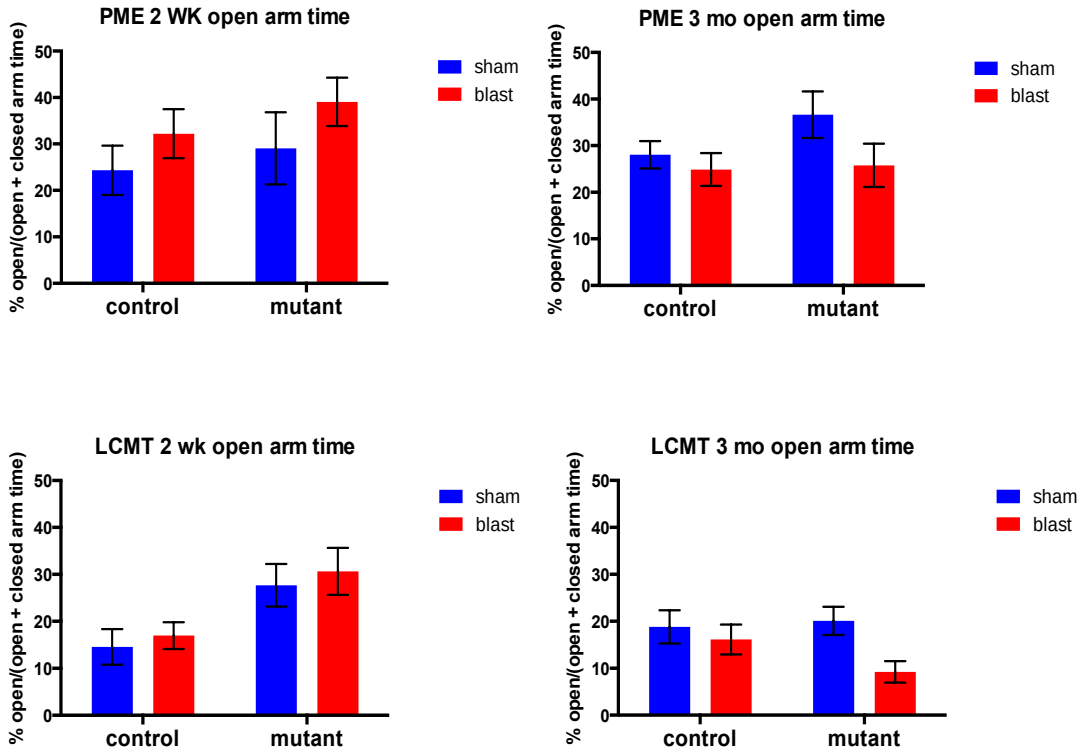


Figure 16: Ambulatory distance during elevated plus maze testing in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure

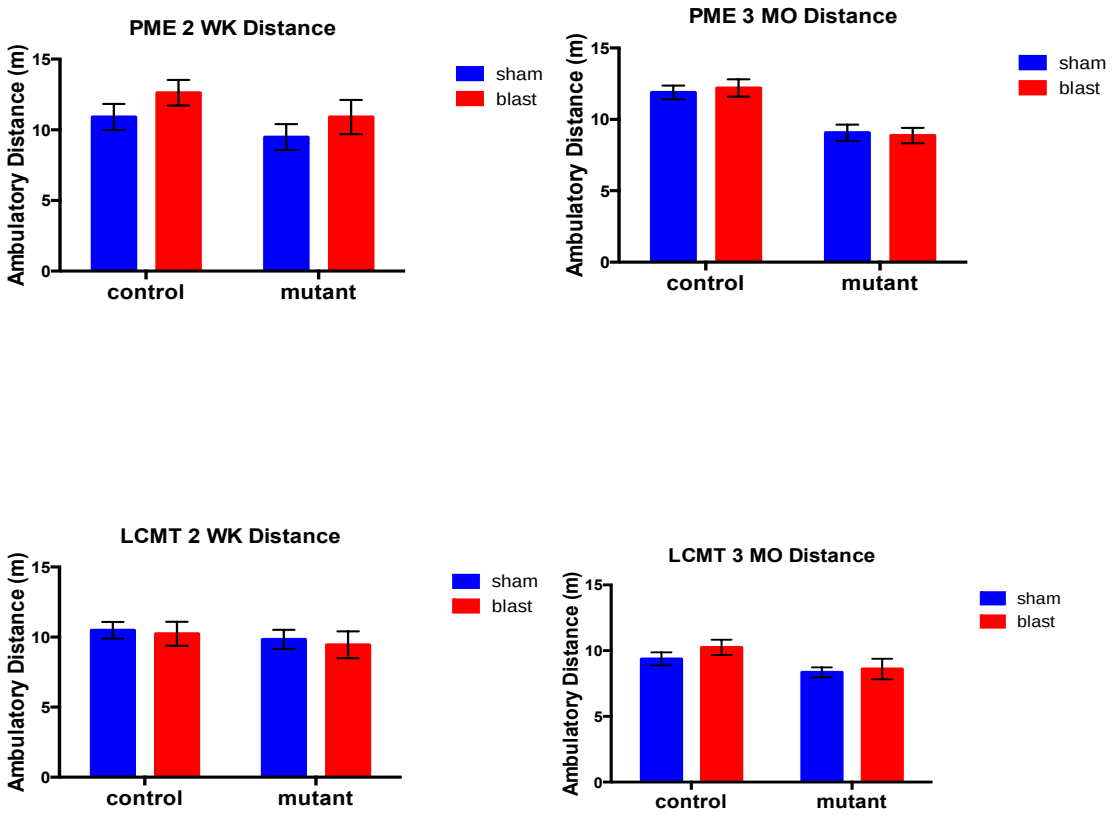


Figure 17: Percent freezing during contextual fear conditioning (CFC) testing at 24 hrs after the electric shock in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure.

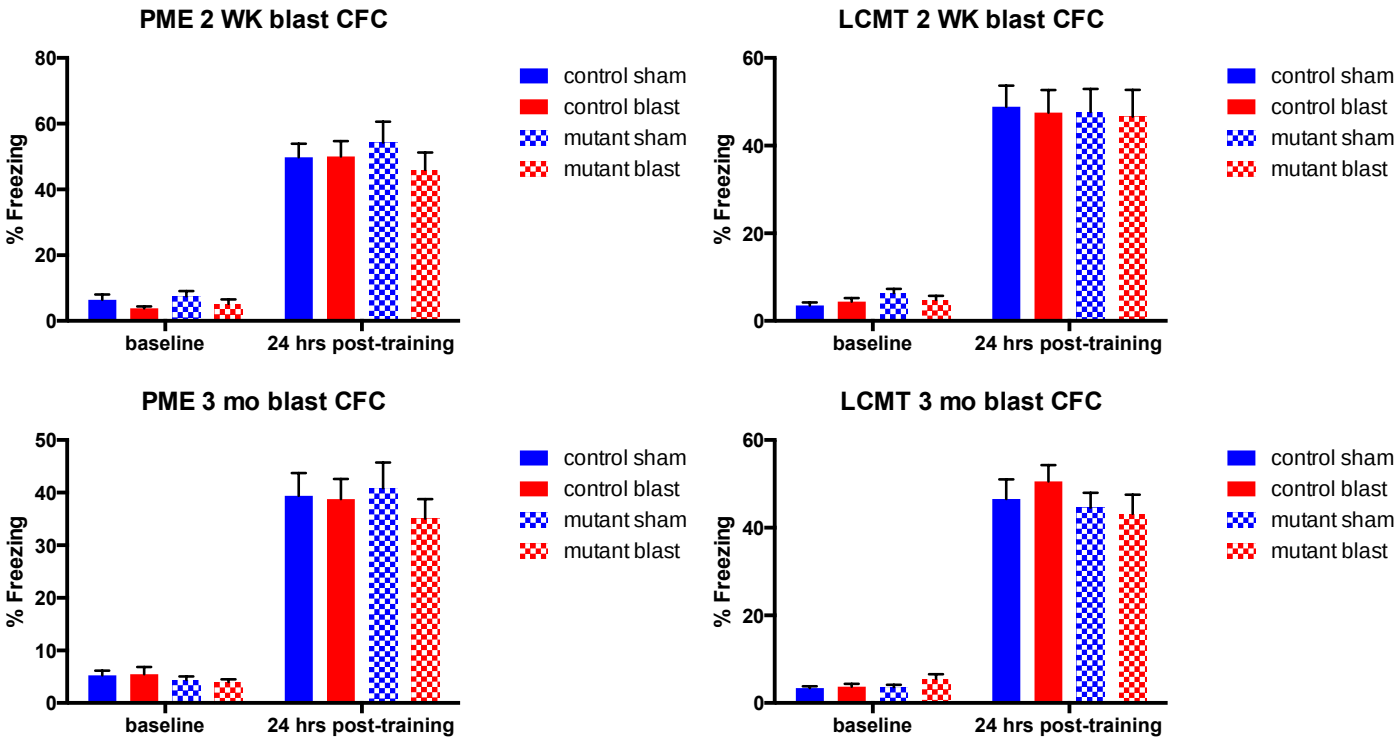


Figure 18: Sensory threshold assessment in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure.

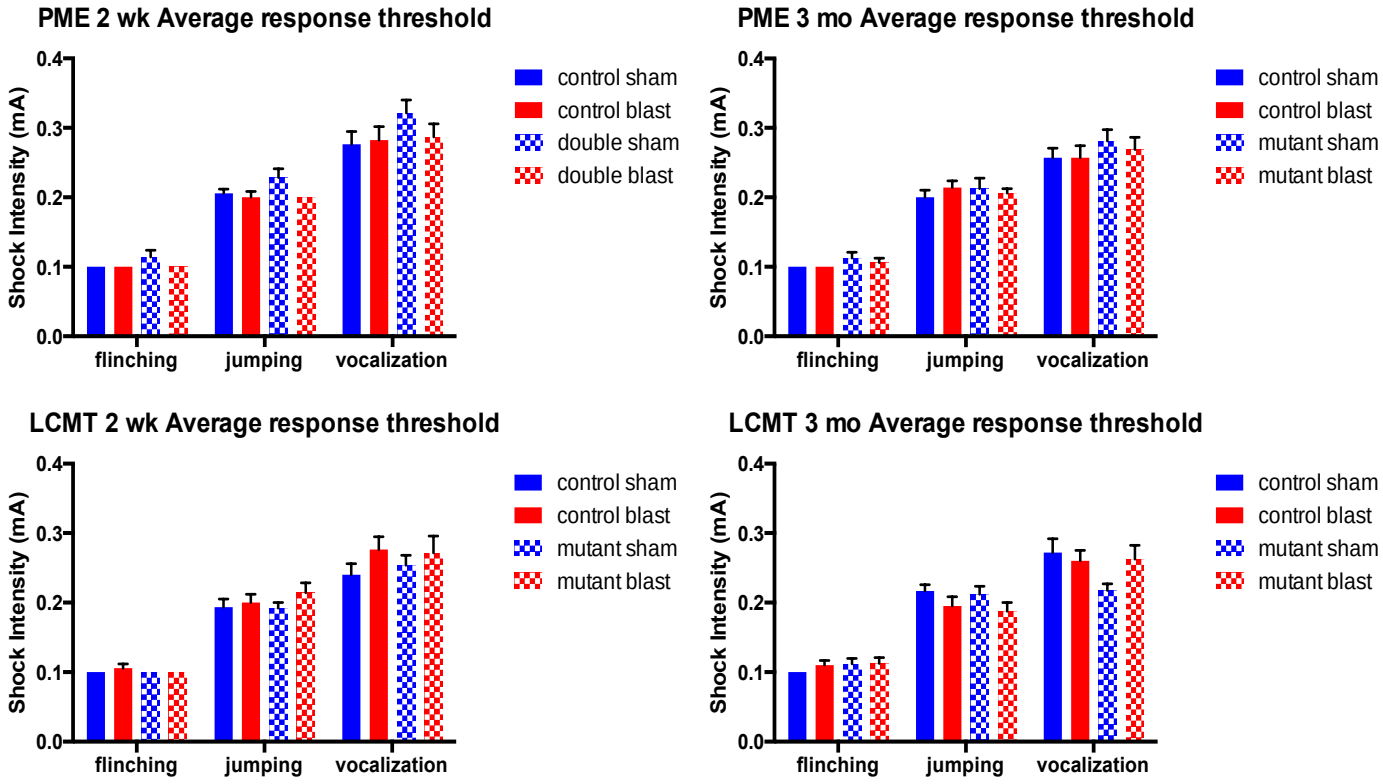
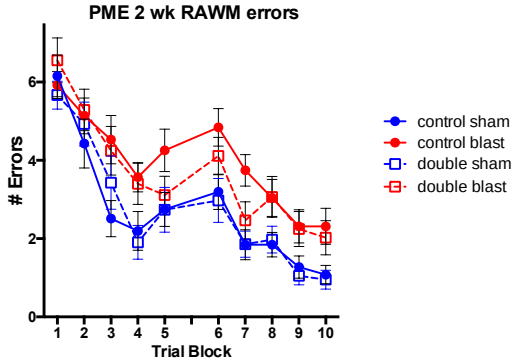
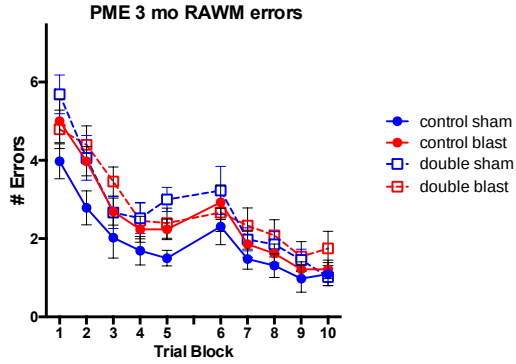


Figure 19: Number of errors during 2-day radial-arm water maze (RAWM) testing in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure



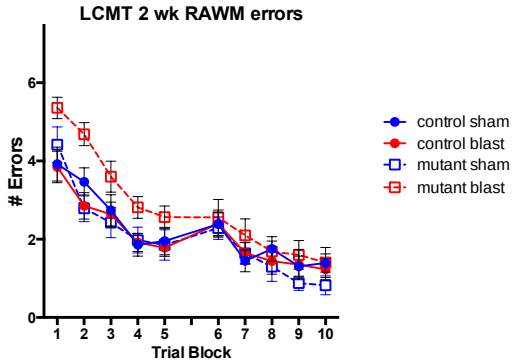
Block 6-10 ANOVA Table

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	10.97	12	0.9140	F (12, 236) = 0.7647	P = 0.6865
trial block	190.1	4	47.51	F (4, 236) = 39.75	P < 0.0001
group	126.7	3	42.23	F (3, 59) = 4.953	P = 0.0039
Subjects (matching)	503.0	59	8.525	F (59, 236) = 7.132	P < 0.0001
Residual	282.1	236	1.195		



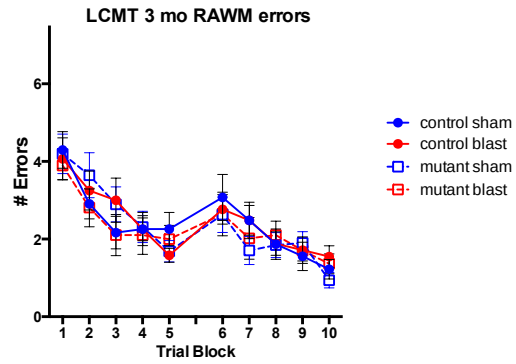
Block 6-10 ANOVA Table

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	8.911	12	0.7426	F (12, 224) = 0.6799	P = 0.7700
trial block	90.78	4	22.69	F (4, 224) = 20.78	P < 0.0001
group	16.45	3	5.485	F (3, 56) = 0.9196	P = 0.4374
Subjects (matching)	334.0	56	5.964	F (56, 224) = 5.462	P < 0.0001
Residual	244.6	224	1.092		



Block 6-10 ANOVA Table

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	4.316	12	0.3597	F (12, 220) = 0.6347	P = 0.8114
trial block	53.20	4	13.30	F (4, 220) = 23.47	P < 0.0001
group	8.015	3	2.672	F (3, 55) = 0.4953	P = 0.6870
Subjects (matching)	296.7	55	5.394	F (55, 220) = 9.517	P < 0.0001
Residual	124.7	220	0.5667		



Block 6-10 ANOVA Table

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	11.31	12	0.9423	F (12, 268) = 1.039	P = 0.4135
trial block	88.13	4	22.03	F (4, 268) = 24.28	P < 0.0001
group	3.977	3	1.326	F (3, 67) = 0.1432	P = 0.9337
Subjects (matching)	620.1	67	9.256	F (67, 268) = 10.20	P < 0.0001
Residual	243.1	268	0.9073		

Figure 20: Average latency to the platform during visible platform water maze testing in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure

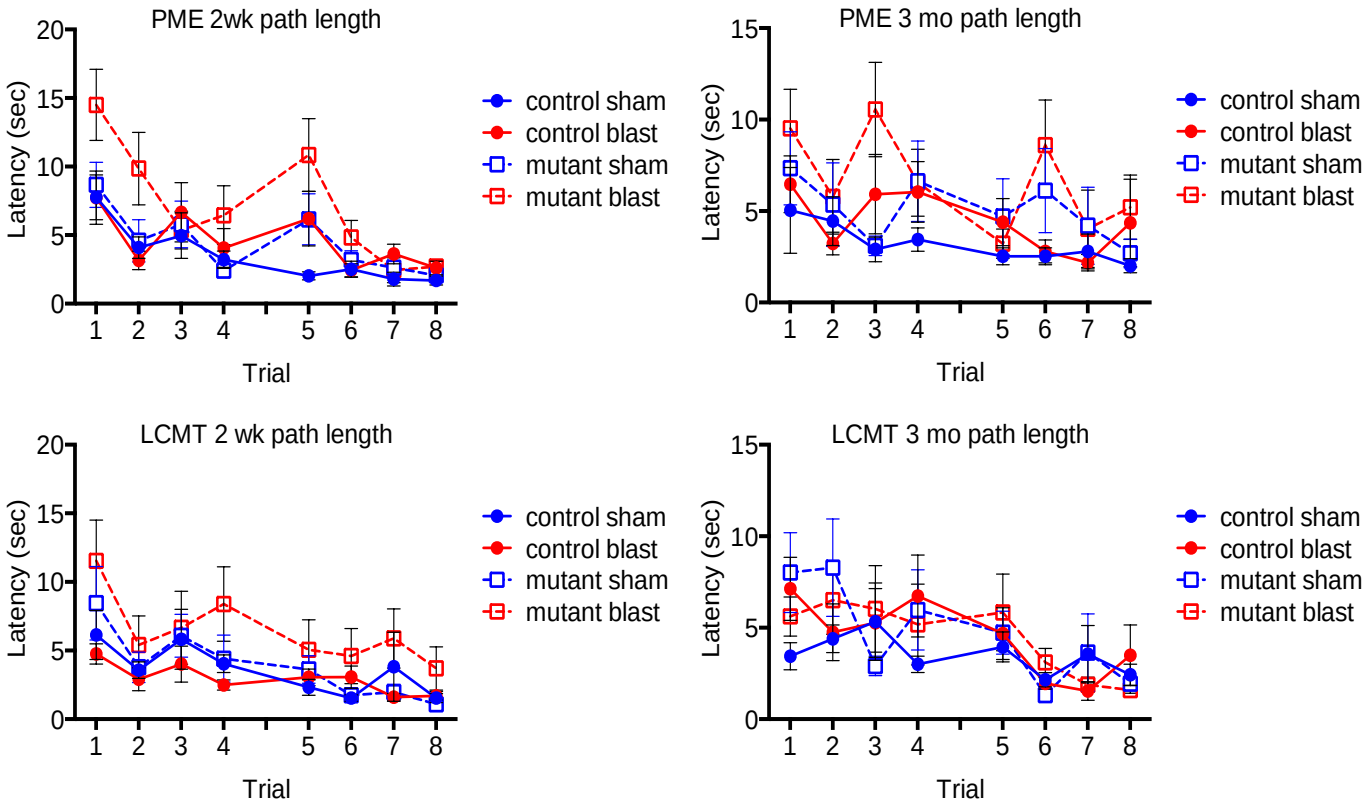


Figure 21: Average speed during visible platform water maze testing in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure

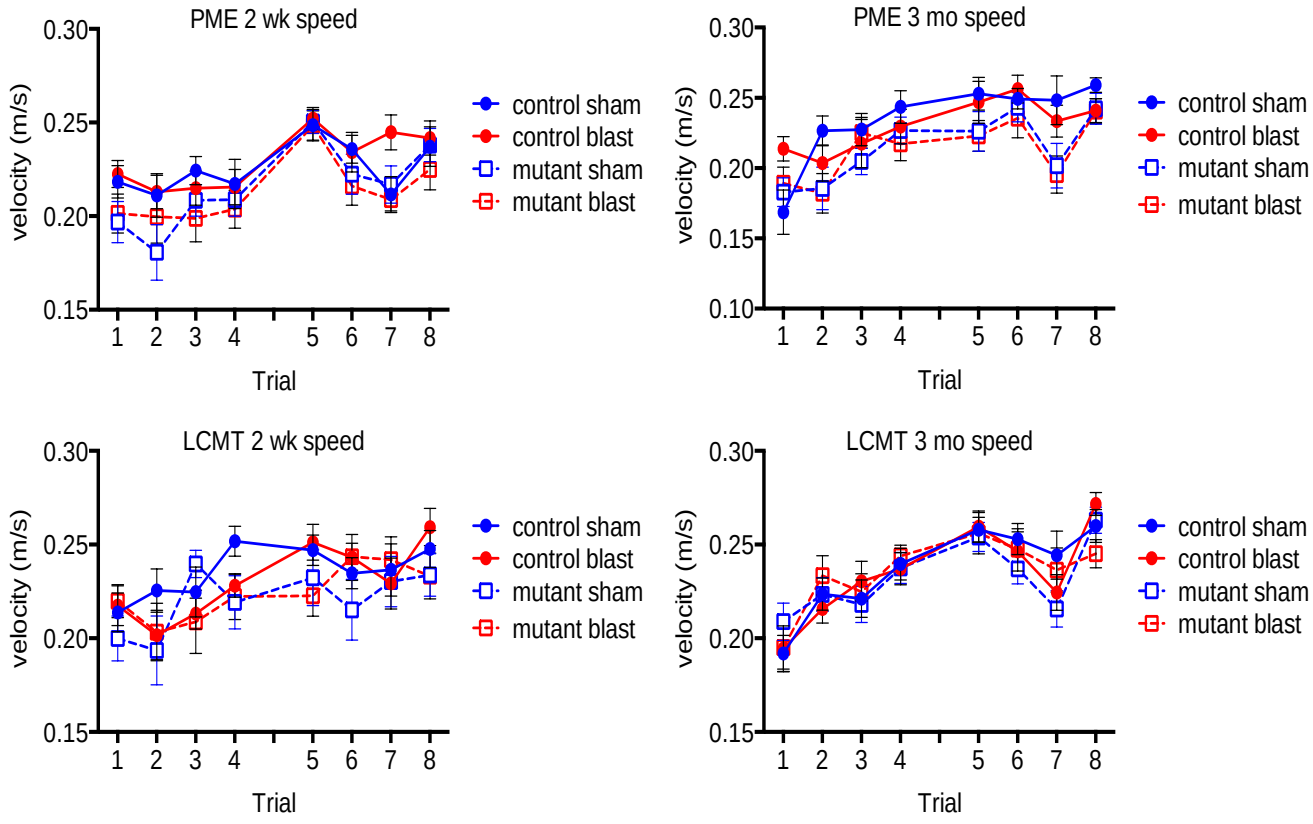


Figure 22: Time spent immobile during tail suspension test in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure

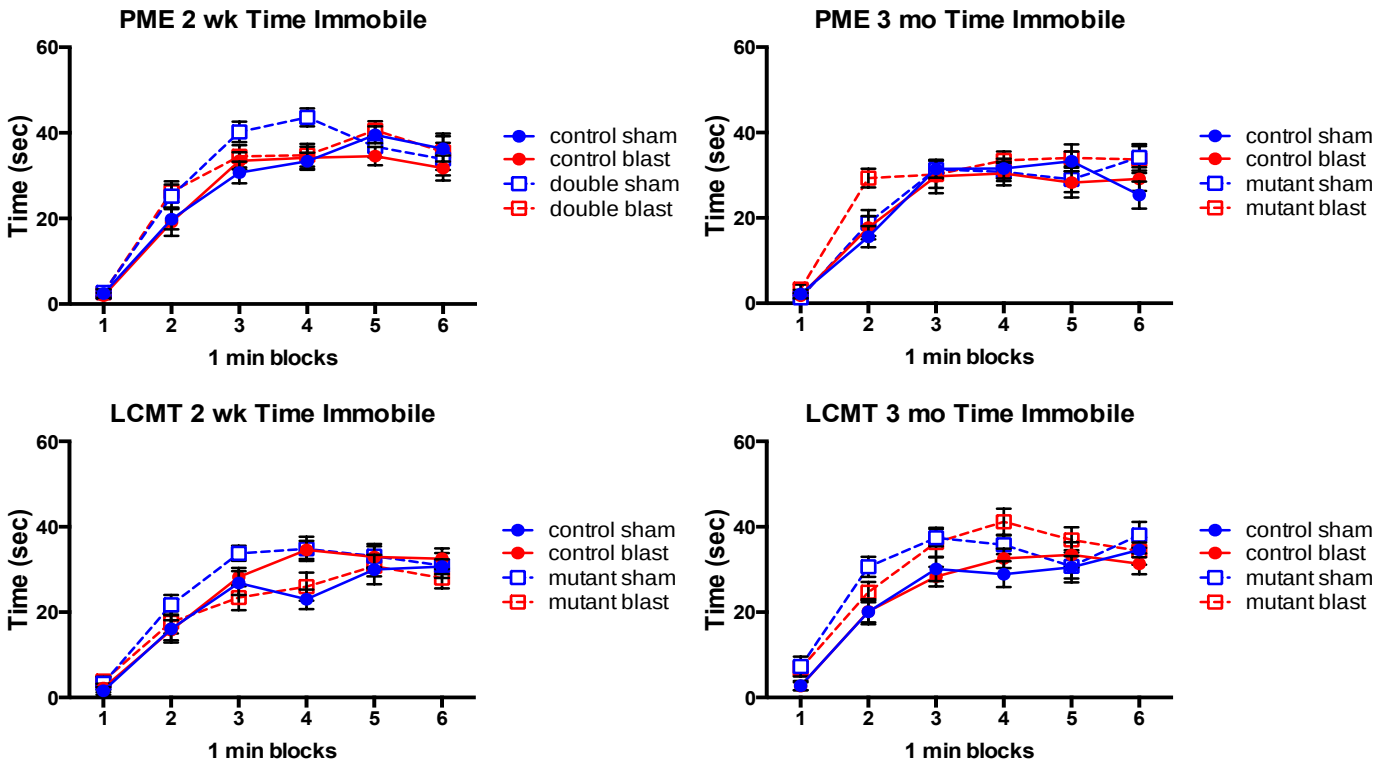


Figure 23: Time spent immobile during the forced swim test in PME or LCMT over expressing animals and their controls 2 weeks and 3 months after sham or shockwave exposure

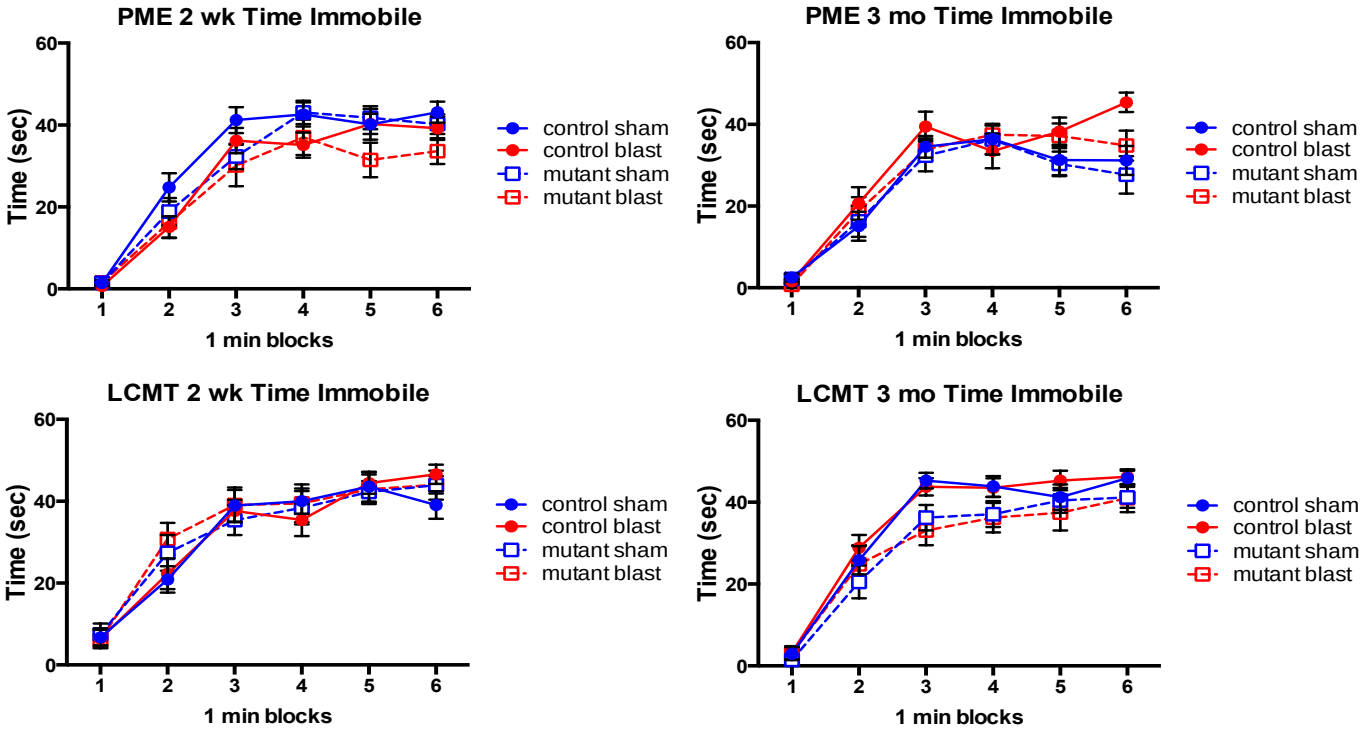


Figure 24: Blast tau Induced Behavioral Impairment A) Tau from shockwave-exposed animals impairs 2-day radial arm water maze performance when infused into wild type mice. **B)** Tau from shockwave-exposed animals impairs contextual fear conditioning when infused into wild type mice.

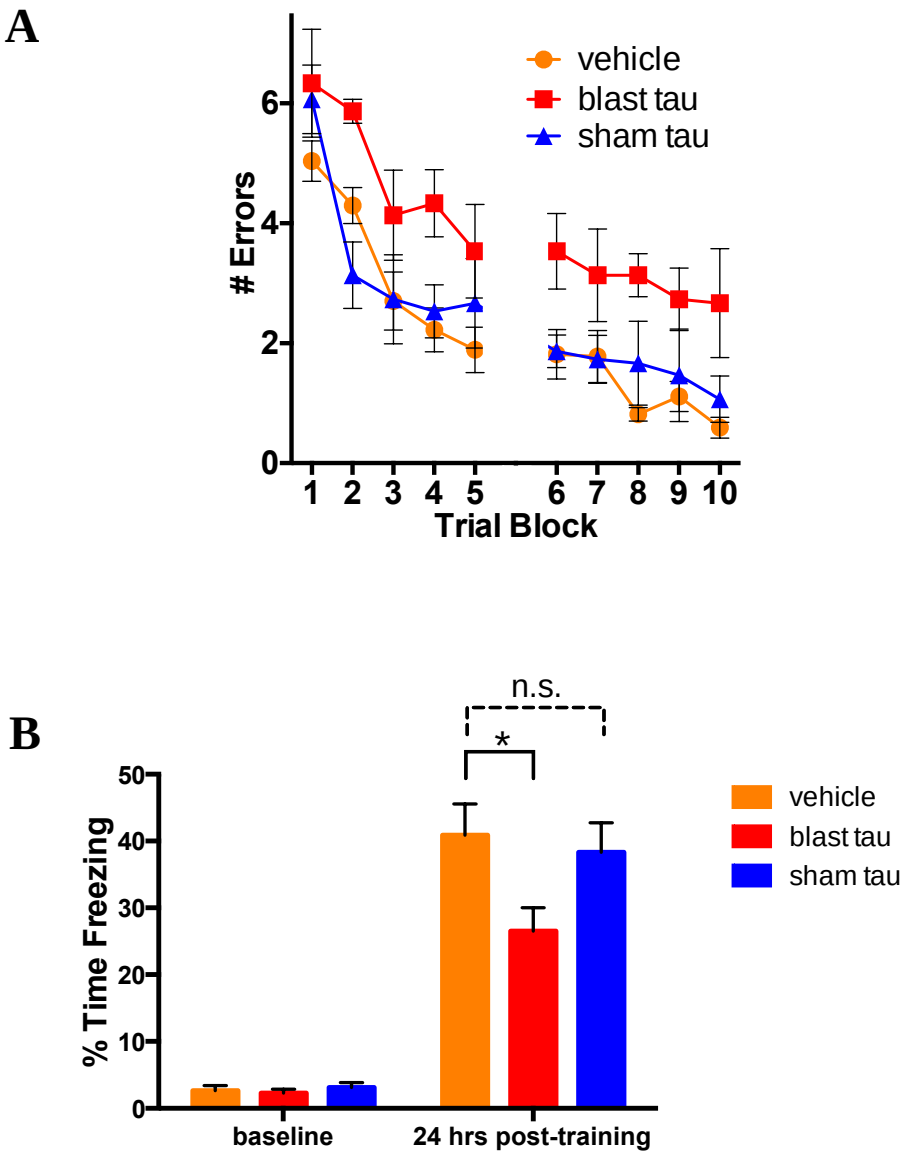


Figure 25: LCMT over expression *reduces* and PME over expression *enhances* sensitivity to recombinant oligomeric tau both in the radial arm water maze task and during contextual fear conditioning.