NIH Public Access
Author Manuscript
Behav Brain Res. Author manuscript; available in PMC 2012 September 12.

Published in final edited form as:
Behav Brain Res. 2011 September 12; 222(1): 265–269. doi:10.1016/j.bbr.2011.02.043.

Enhanced long-term fear memory and increased anxiety and depression-like behavior after exposure to an aversive event in mice lacking TIP39 signaling

Laurence Coutellier and **Ted B. Usdin**
Section on Fundamental Neuroscience, NIMH, National Institutes of Health (NIH), 35 Convent Drive, 20892 Bethesda, MD, USA

Abstract

Exaggerated recall for fear-provoking events leads to abnormal behaviors. We hypothesized that tuberoinfundibular-peptide-of-39-residues (TIP39) modulates fear memory by limiting long-term consequences of aversive experiences. We now show that mice lacking TIP39 signaling display enhanced fear-recall, anxiety and depression-like behavior two weeks after a traumatic event. We suggest that TIP39 modulates long-term fear recall and that mice lacking TIP39 or its receptor are tools for investigating fear-related psychopathologies.

Keywords

TIP39 signaling; fear memory; anxiety; depression-like behavior

Fear-related psychopathologies (e.g. phobia, post-traumatic stress disorder) are characterized by impaired memory extinction or exaggerated recall for a fear-provoking event, leading to strong and lasting trauma-induced changes in anxiety- and depressive-like behaviors. Patients affected by such pathologies present abnormalities in the serotonergic system, the noradrenergic system and/or the HPA-axis [1–3]. The complexity of these disorders makes it difficult to find effective treatments. To improve therapeutic efficacy, it is essential to obtain a better understanding of the neural basis of fear memory regulation.

Neuroanatomical, physiological and behavioral data suggest a possible role of the neuropeptide tuberoinfundibular-peptide-of-39-residues (TIP39) in fear memory. Firstly, TIP39 is synthesized by neurons projecting to brain areas enriched in its receptor, the parathyroid-hormone 2 receptor (PTH2-R). Prominent among these are regions implicated in emotional and stress responses and in the formation and expression of memory extinction, such as the amygdala and the prefrontal cortex [4–6]. Then, we previously showed that mice lacking the neuropeptide TIP39 show enhanced expression of fear in a classical Pavlovian fear conditioning paradigm [7] and that TIP39 signaling modulates memory performance under conditions of emotional arousal [8]. Finally, TIP39 signaling appears to modulate the HPA-axis [9] and could potentially act on central noradrenergic pathways [8]. Based on these observations, we hypothesized that TIP39 modulates pathways involved in fear

Corresponding author: Ted B. Usdin, Tel: +1-301-402-6976, Fax: +1-301-402-0245, usdint@mail.nih.gov.

The authors declare that there are no potential conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

memory and could contribute to the expression of long-term consequences of aversive experiences.

To explore this possibility, we assessed the long-term fear memory in TIP39 knockout (TIP39-KO), PTH2-R knockout (PTH2-R-KO) and wild-type (WT) mice after exposure to an aversive event. For the aversive event, we used a foot shock. In many rodent studies, foot shock has been regarded as equivalent to the traumatic event that leads to fear-related psychopathologies in susceptible people (e.g. [10–12]). We also evaluated trauma-induced anxiety- and depressive-like behaviors employing the elevated-zero maze test, the open-field test, the light-dark box test and the forced-swim test. We hypothesized that mice lacking TIP39 signaling would have changes in fear recall and that this would be associated with other signs of behavioral disturbance.

We tested a total of 100 WT, TIP39-KO and PTH2-R-KO male mice generated from HET × HET matings and aged 9–11 weeks at the beginning of the experiments. Animals were single-housed under a reverse light-dark cycle (lights off at 0800h) in polycarbonate cages (35.5 × 14 × 12.5 cm) starting at least two weeks before the beginning of the experiments. Bedding, nesting material, food and water were provided *ad libitum*.

Development of the TIP39 line and the PTH2-R line, their general characterization and their genotyping have been previously described [7,8,13]. Animals from the TIP39 line were backcrossed for 8 generations into the C57BL/6J background while animals from the PTH2-R line were backcrossed for 4 generations into the C57BL/6J background. No general defect or gross abnormalities could be found except for a germ cell maturation defect in TIP39-KO mice. However, serum testosterone is not significantly different than WT littermates [13]. All experiments were performed between 0730h and 0930h. The shock application, the test of conditioned fear recall, the elevated-zero maze test and the open field test were performed under bright white light (150 Lux) while the light-dark box test and the forced swim test were performed under dim white light (10 Lux). All procedures were approved by the NIMH ACUC and strictly followed NIH/ILAR guidelines.

The shock application and test of conditioned fear were performed in a cubic-shaped chamber with transparent walls and a metal grid floor for shock application. Chambers were cleaned thoroughly after each trial with a 70% alcohol solution. For shock application, we modified a previously described protocol [14]: after a 198-sec acclimation period in the chamber, a single 2-sec scrambled electric foot shock of 1.5mA was administered via the metal grid. Animals remained in the chamber for another 60-sec before they were replaced in their home cages. Sessions were video-recorded and freezing behavior, defined as immobility except for respiration movements, was scored offline during the 198-sec of acclimation and the 60-sec post-shock period with a stopwatch by a trained experimenter blind to the animals' genotypes.

To test for conditioned fear recall, animals were re-exposed to the shock chamber for 3-min without further shock application and immediately returned to their homes cages. Again, all sessions were video-recorded and freezing behavior was scored offline with a stop-watch by an experimenter blind to the animals' genotypes.

Anxiety-like behaviors were assessed in the elevated-zero maze (EZM) for the TIP39 line, in the open field for the PTH2-R line and in the light-dark box for both lines. The PTH2-R line could not be tested in the EZM because a significant number of animals from this line jumped out of the maze. Instead, we used the open-field test, another test of anxiety validated for mice. The EZM consisted of a circular platform (outer diameter 46 cm, width 5.5 cm) that was elevated 40 cm above the floor and made of grey plastic. It featured two open and two enclosed (closed) segments. The closed segments were enclosed by walls

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

extending 20 cm above the surface of the maze, while there were no walls in the open segments. Each test session started by placing the mouse in one of the two open sectors facing a closed sector. The test session lasted 5-min. Performance was recorded using a video-camera placed above the EZM and TopScan video tracking software (CleverSys, Inc., VA, USA). Measurements of total path traveled and path traveled in the different sectors, percent of time spent in the open and closed segments and number of open segment entries were used for further analysis. In addition, the number of feces was counted at the end of the 5-min test session. The mouse was then returned to its home cage and the EZM was cleaned with a 70% alcohol solution.

The open-field consisted of a dark grey plastic arena (50 cm × 50 cm × 40 cm). Animals were tested four at a time, in four adjacent arenas, for 10-min. A camera was mounted above the centre of the four arenas and connected to a video-recorder and TopScan video tracking software (CleverSys, Inc., VA, USA). After the test, the animals were returned to their home cages. Measurements of the total path traveled, as well as the time spent and the path traveled near the walls and in the center of the arena were used for further analysis. The number of feces was counted at the end of each trial. The arenas were cleaned with a 70% alcohol solution between trials.

The light-dark box apparatus consisted of an illuminated (925 Lux) and a dark compartment equally sized (31 × 25 × 30 cm) and connected by a 2 cm diameter aperture at floor level. The mouse was placed in the bright compartment and allowed to explore the apparatus for 5-min before being returned to its home cage, following which the box was cleaned with a 70% alcohol solution. The time spent in the bright side and the latency to enter the dark side were scored offline by an experimenter with a stopwatch blind to the genotype. The number of transitions between the two compartments was also noted as an index of locomotor activity.

Depression-like behaviors were tested in both lines in the forced-swim test. Animals were brought to the procedure room in their home cage at least one hour prior to testing. A clear Plexiglas cylinder (diameter, 20 cm) was filled with water (24–25 °C). Each mouse was tested for 6-min. The time spent immobile during the last 4-min of the test was scored manually with a stopwatch. Immobility was defined as floating motionless or using movements only necessary to keep the head above water. At the end of testing, mice were dried with a towel and placed in their home cage on a heating pad until fully dried.

The testing of the animals followed a precise experimental schedule. The animals of each line exposed to the shock were separated in two cohorts based on the incubation time following the shock. The first cohort was tested for fear recall and anxiety- and depression-like behavior one week after the exposure to the shock: 6 days after the shock, animals were tested for their conditioned fear recall. Seven, 8 and 9 days after the shock they were tested respectively in the EZM (TIP39 line) or the open field test (PTH2-R line), the light-dark box test and the forced swim test. The second cohort was tested two weeks after the exposure to the shock: 14 days after the shock, animals were tested for their conditioned fear recall. Fifteen, 16 and 17 days after the shock they were tested respectively in the EZM (TIP39 line) or the open field test (PTH2-R line), the light-dark box test and the forced swim test.

Data were analyzed using Prism 4 software (GraphPad Software Inc., SanDiego, CA, USA). All data were tested for normality with the Kolmogorov-Smirnof test. The two cohorts of WT and TIP39-KO and, WT and PTH2-R-KO, tested one week and two weeks after the shock were independent groups of animals. Thus, data from each cohort were tested independently using t-tests to assess for a genotype difference at each incubation time.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Statistical significance was set as $p \leq 0.05$ and trend as $p \leq 0.1$. All data are presented as mean ± standard error of the mean.

We first observed that exposure to a single shock induces higher fear recall in mice lacking TIP39 signaling 14 days but not 6 days after the shock. We controlled for spontaneous freezing in the shock chamber prior to the shock exposure. We observed a very low level of freezing in response to the exposure to the unfamiliar chamber in the TIP39 line (WT=0.09 ± 0.06%; TIP39-KO=0.22 ± 0.11%; $t_{48}=1.07$, p=0.290 – Figure 1a) and in the PTH2-R line (WT=0.03 ± 0.03%; PTH2-R-KO=0.18 ± 0.10%; $t_{48}=1.34$, p=0.186 – Figure 1b). We also controlled for the direct response of the animals to the shock by recording the freezing behavior for the 60 seconds following the shock. We observed no difference between WT and TIP39-KO mice (6.74 ± 1.31% and 5.03 ± 0.78%, respectively; $t_{48}=1.09$, p=0.280 – Figure 1a) or between WT and PTH2-R-KO mice (9.68 ± 1.85% and 6.95 ± 1.13%, respectively; $t_{48}=1.28$ p=0.206 – Figure 1b). Therefore, the foot shock experience did not differ between genotypes.

We assessed fear recall by analyzing the freezing response of mice 6 or 14 days after exposure to the shock. We first observed that the freezing level of all animals was low, when compared to data found in other studies testing the C57Bl/6 strain in a fear conditioning paradigm. This low level of freezing could be the results of both the genetic background of our transgenic lines and the housing/testing conditions: genetic background significantly affects the behavior of transgenic animals [15,16] and inbred mouse strains differ significantly in their contextual fear conditioning [17,18]. Our mice were produced with embryonic stem cells derived from a SV129 strain and were then crossed with another strain, C57BL/6J. Thus the behavioral testing was performed on a mixed genetic background which could have resulted in lower levels of freezing than the one observed in the C57Bl/6 inbred strain. Then, our mice were housed under a reverse light-dark cycle and testing was performed during the animal's active (dark) phase. It has been reported [19] that when comparing the performance in a fear conditioning paradigm of animals during the day and night, mice acquired the conditioning faster in the day than in the night. Furthermore, the recall for context consistently peaked during the day and mice trained at night exhibited a greater degree of extinction than mice trained during the day. It has been suggested that that the ability to learn the fear-conditioning task is modulated by the circadian system [19] and testing our mice during their dark phase might have resulted in lower level of freezing. Finally, another factor that might have contributed to the low level of freezing in our experiment is the method we used to measure freezing. Instead of using an automated system (e.g. beam break or motion detection softwares), we manually recorded the level of freezing based on a more rigorous definition of freezing than the one used by automated systems.

In the TIP39 line, we did not observe a genotype effect 6 days after the shock (WT: 7.77 ± 0.86%; TIP39-KO: 8.39 ± 1.38%; $t_{20}=0.38$, p=0.709). However, 14 days after the shock TIP39-KO mice showed increased freezing over WT (WT: 11.58 ± 2.68%; TIP39-KO: 19.35 ± 1.93%; $t_{26}=2.29$, p=0.031 – Figure 1a). Similarly, in the PTH2-R-KO line, WT and PTH2-R-KO mice did not present a different freezing response when re-exposed to the context 6 days after the shock (WT: 7.38 ± 1.54%; PTH2-R-KO: 11.03 ± 2.00%; $t_{17}=1.43$, p=0.172). But mice lacking a functional PTH2-R showed a higher level of freezing than the WT 14 days after the shock (WT: 11.68 ± 2.29%; PTH2-R-KO: 19.56 ± 2.99%; $t_{29}=2.07$, p=0.048 – Figure 1b).

We also observed that fear incubation leads to increased anxiety- and depression-like behavior in mice lacking TIP39 signaling. In the TIP39 line, one week after the shock, no difference between WT and TIP39-KO mice was observed in the EZM test, the light-dark

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

box test or the forced swim test (Figure 2). Two weeks after the shock, we observed that TIP39-KO mice entered the dark compartment sooner (t_{25}=2.85, p=0.009 – Figure 2a) and spent less time on the bright side (t_{25}=3.67, p=0.001 – Figure 2b) during the light-dark box test. This was not linked to difference in locomotor activity since the number of transitions from one compartment to the other did not differ between the two genotypes (WT: 7.20±1.08 vs. TIP39-KO 9.50±1.34; t_{25}=1.35, p=0.188). In the EZM test, TIP39-KO mice had a higher level of locomotion in the open section of the maze compared with WT (t_{26}=2.09, p=0.046 – Figure 2c). No other parameters from the EZM were affected by the genotype. In the forced swim test, we observed greater immobility in TIP39-KO than WT mice (t_{17}=3.18, p=0.005 – Figure 2d). In the PTH2-R line, we observed no difference between the WT and the PTH2-R-KO one week after the shock in the open field test, the light-dark box test or the forced swim test (Figure 3). Two weeks after the shock, 4 out of 10 PTH2-R-KO mice tested in the open field had to be removed from the analysis because of a high level of freezing in the arena, as demonstrated by a very low level of locomotion (total path traveled was significantly lower than the mean value – 2 SEM). Similarly, 3 PTH2-R-KO mice had to be removed from the analysis of the light-dark box because of very low locomotor activity (0 or 1 transition between the two compartments). When these animals were removed, we observed a strong trend for the PTH2-R-KO mice to travel a longer distance near the walls and to spend more time near the walls than the WT during the open field test (t_{14}=2.13, p=0.051 and t_{14}=2.11, p=0.054, respectively – Figure 3a and b). In the light-dark box test, no significant difference between WT and PTH2-R-KO mice was detected (Figure 3c). In the forced-swim test, we observed that PTH2-R-KO mice spent more time immobile than WT mice (t_{18}=2.11, p=0.049 – Figure 3d).

In the present study we tested whether the TIP39/PTH2-R system could modulate pathways mediating fear memory and contribute to the long-term consequences of an aversive experience. One week after exposure to a traumatic event in the form of a foot shock, TIP39-KO and PTH2-R-KO mice did not differ from the WT mice in their fear recall or trauma-induced anxiety- and depression-like behaviors. However, mice lacking the neuropeptide TIP39 or with an inactivating mutation in its receptor, the PTH2-R, showed enhanced contextual fear memory two weeks after the presentation of the shock. This increased fear memory was associated with an increased level of anxiety-like and with signs of depressive-like behavior. The present behavioral data suggest involvement of TIP39 signaling in the modulation of fear memory recall and in the long-term consequences of exposure to an aversive event.

Mice with or without TIP39 signaling displayed high level of freezing when placed in the context in which they received a foot shock one week earlier. The absence of TIP39 signaling does not seem to alter the ability of mice to remember the context in which they encountered an aversive event after a one-week delay. This suggests that TIP39 is not critically involved in pathways required for the acquisition, consolidation or short-term recall of an aversive event. Two weeks after the shock, WT mice still displayed high level of freezing to the context but TIP39-KO and PTH2-R-KO mice froze significantly more. This enhanced contextual fear memory of mice lacking TIP39 signaling cannot be attributed to higher spontaneous freezing since they did not show higher freezing behavior before the conditioning. This indicates that mice lacking TIP39 or the PTH2-R were not more prone to freezing when introduced to a novel environment. Furthermore, the immediate response to the shock of TIP39-KO and PTH2-R-KO mice was similar to that of the WT and we noted in a previous study that mice lacking TIP39 showed normal nociceptive response to a foot shock [7]. Therefore differences in freezing behavior two weeks after the shock are not likely to be caused by an increased nociceptive responsiveness to foot shock. The enhanced long-term fear recall in mice lacking TIP39 signaling is most likely to be explained by changes in fear memory pathways.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Associated with this enhanced fear recall two weeks after the shock, mice without TIP39 signaling showed a high level of anxiety- and depression-like behaviors. TIP39-KO mice spent less time in the bright side of the light-dark box and spent more time immobile in the forced-swim test. PTH2-R-KO mice spent more time near the walls of the open field and more time immobile in the forced swim-test. We did not detect an increase in anxiety-like behavior in the PTH2-R-KO mice in the light-dark box using the standard parameters. However, 40% and 30% of PTH2-R-KO mice displayed a very low level of locomotion (i.e. high freezing) when placed in the open-field and the light-dark box, respectively. These animals had to be removed from the analysis, significantly reducing our sample size, and thus, the power of our statistical analysis. Thus, increased anxiety-related behavior could have prevented us from finding a significant difference between the PTH2-R-KO and WT mice in the time spent in the bright side of the light-dark box. Furthermore, we cannot exclude the possibility that this disruption in locomotor activity in the PTH2-R line could be responsible for the differences between the phenotypes of the two lines. Altogether, our data indicate that not only do mice lacking TIP39 signaling have an exaggerated response to cues reminding them of the shock, but two weeks after the traumatic event, they still displayed high anxiety and depression-like behaviors in a context that is independent of the aversive event. We also noticed that TIP39-KO mice had a higher level of locomotor activity in the open segment, but not the closed segment of the elevated-zero maze. A priori, this could indicate increased exploration in the open segment, but since the time spent in the open segment and the locomotor activity in the closed segment were not affected by the genotype, this high level of locomotor activity could actually reflect increased arousal in a fearful context. This anxious and depressive phenotype in mice lacking TIP39 signaling was not observed one week after the aversive event indicating that it is not a direct effect of the lack of TIP39 signaling. Rather, it reflects abnormal long-term consequences of exposure to an aversive event in TIP39-KO and PTH2-R-KO mice. We suggest that normal TIP39 signaling lessens the long-term consequences of a traumatic event, while the absence of TIP39 signaling delays recovery or allows deleterious incubation effects. We suggest that the neuropeptide TIP39 could be involved in mechanisms that normally limit long-term behavioral changes following exposure to a traumatic event. However, more studies will be required to confirm this hypothesis. In the experiments described here separate groups of animals were tested one and two weeks after shock. It will be important to study a large cohort evaluated at several times following shock so that the effect of time can be rigorously evaluated.

The two-week delay necessary to observe a significant difference between mice with or without TIP39 signaling is not surprising. Previous studies have shown that a period of non-disturbance, or incubation, of 7 days is necessary to differentiate between strains of mice that do or do not develop strong and lasting fear responses and physiological and behavioral abnormalities following an aversive event [11,15,20]. It has also been demonstrated that contextual fear conditioning relies on the short-term acquisition of configural information, which relies on the hippocampus, and on long-term fear recall, which employs the prefrontal cortex [21]. The behavioral changes observed in TIP39-KO and PTH2-R-KO mice preferentially 2 weeks after the shock relate well with the neuroanatomical data showing that TIP39 neurons project to the medial prefrontal cortex, but not the hippocampus. This suggests that the neuropeptide TIP39 may influence fear regulatory pathways, especially those controlling long-term fear recall, possibly by acting at the prefrontal cortex level. Altogether, our findings suggest that mice lacking TIP39 signaling could be used as a tool to help shed light on how the long-term consequences of traumatic experiences develop and open a new avenue to the study of fear-related psychopathologies such as post-traumatic stress disorder.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Acknowledgments

This research was supported by the Intramural Program of the NIH, National Institute of Mental Health. We thank Milan Rusnak for mouse management.

References

1. Dai J-X, Han H-L, Tian M, Cao J, Xiu J-B, Song N-N, et al. Enhanced contextual fear memory in central serotonin-deficient mice. Proc Natl Acad Sci U S A. 2008; 105:11981–6. [PubMed: 18695238]
2. Mueller D, Cahill SP. Noradrenergic modulation of extinction learning and exposure therapy. Behav Brain Res. 2010; 208:1–11. [PubMed: 19931568]
3. Yehuda R, Southwick SM, Krystal JH, Bremner D, Charney DS, Mason JW. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. Am J Psychiatry. 1993; 150:83–6. [PubMed: 8417586]
4. Dobolyi A, Palkovits M, Bodnar I, Usdin TB. Neurons containing tuberoinfundibular peptide of 39 residues project to limbic, endocrine, auditory and spinal areas in rat. Neuroscience. 2003; 122:1093–105. [PubMed: 14643775]
5. Dobolyi A, Palkovits M, Usdin TB. The TIP39-PTH2 receptor system: unique peptidergic cell groups in the brainstem and their interactions with central regulatory mechanisms. Prog Neurobiol. 2010; 90:29–59. [PubMed: 19857544]
6. Faber CA, Dobolyi A, Sleeman M, Usdin tB. Distribution of tuberinfundibular peptide of 39 residues and its receptor, parathyroid hormone 2 receptor, in the mouse brain. J Comp Neurol. 2007; 502:563–83. [PubMed: 17394159]
7. Fegley DB, Holmes A, Riordan T, Faber CA, Weiss JR, Ma S, et al. Increased fear- and stress-related anxiety-like behavior in mice lacking tuberinfundibular peptide of 39 residues. Genes, Brain and Behavior. 2008; 7:933–42.
8. Coutellier L, Logemann A, Kuo J, Rusnak M, Usdin TB. TIP39 modulates effects of novelty-induced arousal on memory. Genes, Brain and Behavior. 2010 In press.
9. Dimitrov E, Usdin TB. Tuberoinfundibular peptide of 39 residues modulates the mouse hypothalamic-pituitary-adrenal axis via paraventricular glutamatergic neurons. J Comp Neurol. 2010; 518:4375–94. [PubMed: 20853513]
10. Maren S. Building and burying fear memories in the brain. The Neuroscientist. 2005; 11:89–99. [PubMed: 15632281]
11. Siegmund A, Wotjak CT. A mouse model of posttraumatic stress disorder that distinguished between conditioned and sensitised fear. J Psychiatr Res. 2007; 41:848–60. [PubMed: 17027033]
12. Wang W, Liu Y, Zheng H, Wang HN, Jin X, Chen YC, et al. A modified single-prolonged stress model for post-traumatic stress disorder. Neurosci Lett. 2008; 441:237–41. [PubMed: 18577419]
13. Usdin TB, Paciga M, Riordan T, Kuo J, Parmelee A, Petukova G, et al. Tuberoinfundibular peptide of 39 residues is required for germ cell development. Endocrinology. 2008; 149:4292–300. [PubMed: 18483145]
14. Kamprath K, Wotjak CT. Nonassociative learning processes determine expression and extinction of conditioned fear in mice. Learn Mem. 2004; 11:770–86. [PubMed: 15537742]
15. Cook MN, Bolivar VJ, McFadyen MP, Flaherty L. Behavioral differences among 129 substrains: implications for knockout and transgenic mice. Behav Neurosci. 2002; 116:600–11. [PubMed: 12148927]
16. Smith DR, Gallagher M, Stanton ME. Genetic background differences and nonassociative effects in mouse trace fear conditioning. Learn Mem. 2007; 14:597–605. [PubMed: 17823243]
17. Balogh SA, Wehner JM. Inbred mouse strain differences in the establishment of long-term fear memory. Behav Brain Res. 2003; 140:97–106. [PubMed: 12644283]
18. Bolivar VJ, Pooler O, Flaherty L. Inbred strain variation in contextual and cued fear conditioning behavior. Mamm Genome. 2001; 12:651–6. [PubMed: 11471061]
19. Chaudhury D, Colwell CS. Circadian modulation of learning and memory in fear-conditioned mice. Behav Brain Res. 2002; 133:95–108. [PubMed: 12048177]

20. Yamamoto S, Morinobu S, Takei S, Fuchikami M, Matsuki A, Yamawaki S, et al. Single prolonged stress: toward an animal model of posttraumatic stress disorder. Depress Anxiety. 2009; 26:1110–7. [PubMed: 19918929]

21. Squire LR, Alvarez P. Retrograde amnesia and memory consolidation: a neurobiological perspective. Curr Opin Neurobiol. 1995; 5:169–77. [PubMed: 7620304]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

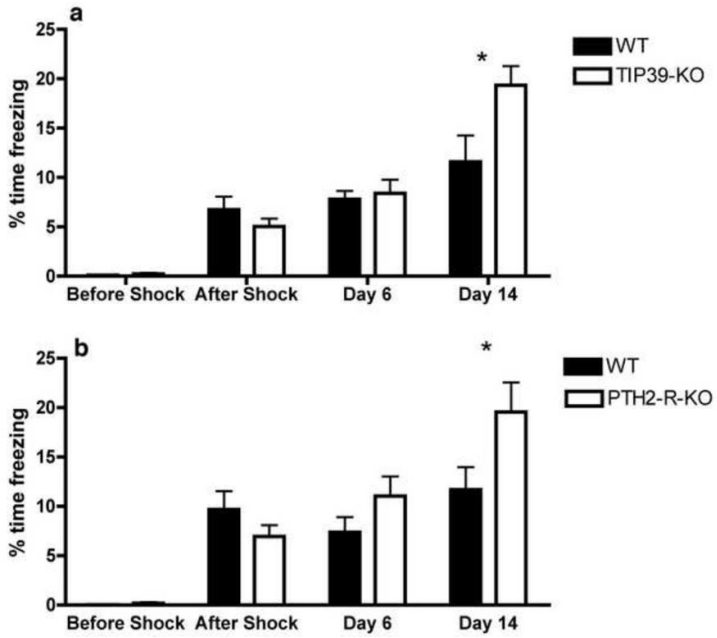

Figure 1.

Freezing behavior expressed as the percent of time spent freezing in the conditioning chamber before the shock, directly after the shock and 1 week (Day 6) and 2 weeks (Day 14) after the shock in WT and TIP39-KO mice **(a)** and in WT and PTH2-R-KO mice **(b)**. 6 days after shock: TIP39 line: WT n= 11, TIP39-KO n= 11; PTH2-R line: WT n=9, PTH2-R-KO n=10; 14 days after shock: TIP39 line: WT n= 15, TIP39-KO n= 13; PTH2-R line: WT n=15, PTH2-R-KO n=16. * $p<0.05$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

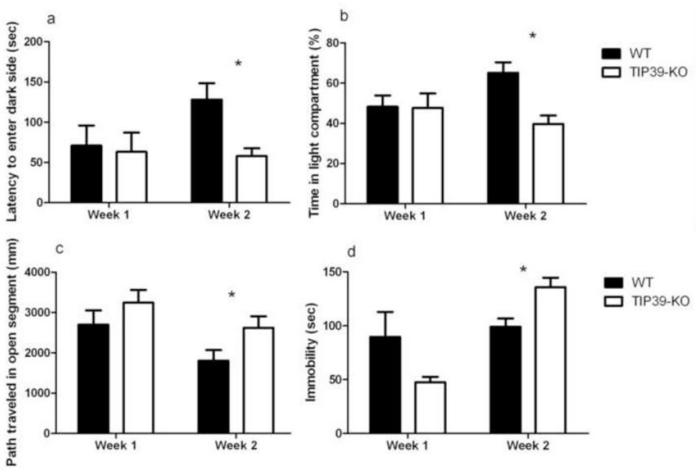

Figure 2.

Behavioral response of WT and TIP39-KO mice 1 week and 2 weeks after exposure to the foot shock. **(a)** Latency to enter the dark compartment of the light-dark box; **(b)** Percent of time spent in the light compartment of the light-dark box; **(c)** Distance traveled in the open section of the elevated-zero maze; **(d)** Time spent immobile in the forced-swim test. 1 week after shock: light-dark box WT n= 9, TIP39-KO n= 9; elevated-zero maze WT n= 11, TIP39-KO n= 11; forced-swim test WT n= 4, TIP39-KO n= 4; 2 weeks after shock: light-dark box WT n= 15, TIP39-KO n= 12; elevated-zero maze WT n= 15, TIP39-KO n= 13; forced-swim test WT n= 11, TIP39-KO n= 8. * $p<0.05$.

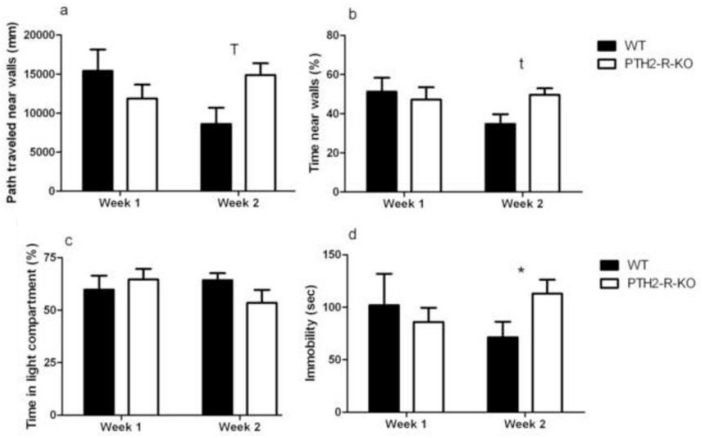

Figure 3.

Behavioral response of WT and PTH2-R-KO mice 1 week and 2 weeks after exposure to the foot shock. **(a)** Distance traveled near the walls of the open-field; **(b)** Percent of time spent near the walls of the open-field; **(c)** Percent of time spent in the light compartment of the light-dark box; **(d)** Time spent immobile in the forced-swim test. 1 week after shock: open-field WT n=9, PTH2-R-KO n=10, light-dark box WT n=9, PTH2-R-KO n=10, forced-swim test WT n=3, PTH2-R-KO n=3; 2 weeks after shock: open-field WT n=10, PTH2-R-KO n=10, light-dark box WT n=10, PTH2-R-KO n=10, forced-swim test WT n=10, PTH2-R-KO n=10; T p=0.051; t p=0.054; * $p<0.05$.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript