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Neurobiology of Disease

Reduced Expression of the PP2A Methylesterase, PME-1, or the PP2A Methyltransferase, LCMT-1, Alters Sensitivity to Beta-Amyloid-Induced Cognitive and Electrophysiological Impairments in Mice

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Beta-amyloid (Aβ) is thought to play a critical role in Alzheimer's disease (AD), and application of soluble oligomeric forms of Aβ produces AD-like impairments in cognition and synaptic plasticity in experimental systems. We found previously that transgenic overexpression of the PP2A methylesterase, PME-1, or the PP2A methyltransferase, LCMT-1, altered the sensitivity of mice to Aβ-induced impairments, suggesting that PME-1 inhibition may be an effective approach for preventing or treating these impairments. To explore this possibility, we examined the behavioral and electrophysiological effects of acutely applied synthetic Aβ oligomers in male and female mice heterozygous for either a *PME-1* KO or an *LCMT-1* gene-trap mutation. We found that heterozygous *PME-1* KO mice were resistant to Aβ-induced impairments in cognition and synaptic plasticity, whereas *LCMT-1* gene-trap mice showed increased sensitivity to Aβ-induced impairments. The heterozygous *PME-1* KO mice produced normal levels of endogenous Aβ and exhibited normal electrophysiological responses to picomolar concentrations of Aβ, suggesting that reduced PME-1 expression in these animals protects against Aβ-induced impairments without impacting normal physiological Aβ functions. Together, these data provide additional support for roles for PME-1 and LCMT-1 in regulating sensitivity to Aβ-induced impairments, and suggest that inhibition of PME-1 may constitute a viable therapeutic approach for selectively protecting against the pathologic actions of Aβ in AD.

Key words: Alzheimer's disease; beta-amyloid; cognitive impairment; protein methylation; protein phosphatase 2A; synaptic plasticity

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Significance Statement

Elevated levels of β-amyloid (Aβ) in the brain are thought to contribute to the cognitive impairments observed in Alzheimer's disease patients. Here we show that genetically reducing endogenous levels of the PP2A methylesterase, PME-1, prevents the cognitive and electrophysiological impairments caused by acute exposure to pathologic concentrations of Aβ without impairing normal physiological Aβ function or endogenous Aβ production. Conversely, reducing endogenous levels of the PP2A methyltransferase, LCMT-1, increases sensitivity to Aβ-induced impairments. These data offer additional insights into the molecular factors that control sensitivity to Aβ-induced impairments, and suggest that inhibiting PME-1 may constitute a viable therapeutic avenue for preventing Aβ-related impairments in Alzheimer's disease.

Introduction

Multiple lines of evidence suggest a role for the serine/threonine phosphatase, PP2A, in Alzheimer's disease (AD). Among these are the observations that (1) PP2A expression and activity are reduced in brains from AD patients (Gong et al., 1993, 1995; Vogelsberg-Ragaglia et al., 2001; Sontag et al., 2004); (2) reducing PP2A activity in animal models results in AD-like pathology and cognitive deficits (Kins et al., 2001; Sun et al., 2003; Schild et al., 2006; Wang et al., 2010; Yin et al., 2010; Louis et al., 2011;

Bolognin et al., 2012); (3) PP2A is the principal phosphatase for phosphorylated forms of microtubule-associated binding protein, tau, that are linked to AD (Martin et al., 2013); and (4) pharmacological activators of PP2A have been found to reduce cognitive impairment and pathology in AD-related mouse models (Corcoran et al., 2010; van Eersel et al., 2010; Vitek et al., 2012; Basurto-Islas et al., 2014; Asam et al., 2017; Van der Jeugd et al., 2018).

PP2A is a heterotrimeric protein composed of a catalytic subunit, a structural subunit, and a regulatory subunit, and its activity is controlled, in part, through the highly regulated assembly of the multiple isoforms that exist for each of these subunits (Sents et al., 2013). One of the processes that regulates PP2A subunit assembly is C-terminal methylation of the catalytic subunit, which is catalyzed by a specific methyltransferase and methylesterase, in a process that is conserved from yeast to mammals (Sents et al., 2013). Methylation of the PP2A catalytic subunit affects its affinity for the different regulatory subunits that control the substrate specificity of the heterotrimeric holoenzyme; and in particular, methylation promotes the formation of B55α subunit-containing enzymes that exhibit the highest tau phosphatase activity (Longin et al., 2007; Xu et al., 2008). In addition, dysregulated PP2A activity resulting from impaired PP2A methylation is thought to be one of the molecular mechanisms by which hyperhomocysteinemia leads to increased AD risk (Zhuo et al., 2011).

In mice, the PP2A methylesterase is encoded by the *PME-1* gene, and the PP2A methyltransferase is encoded by the *LCMT-1* gene. Consistent with the notions that decreased PP2A methylation decreases its tau phosphatase activity (Sontag and Sontag, 2014), and tau hyperphosphorylation contributes to AD-related impairments (Wang and Mandelkow, 2016), we found previously that overexpression of PME-1 in transgenic mice increased their sensitivity for AD-related impairments resulting from acute exposure to elevated levels of β-amyloid (Aβ) (Nicholls et al., 2016). Conversely, we found that overexpression of LCMT-1 in transgenic mice protected animals from Aβ-induced impairments (Nicholls et al., 2016). To further explore the possibility that PME-1 inhibition may constitute a viable therapeutic avenue for AD treatment or prevention, we sought to determine whether reducing the expression of endogenous PME-1 or LCMT-1 might also affect Aβ sensitivity. To do this, we used two previously described lines of mice that carry either a KO mutation in the *PME-1* gene (Ortega-Gutierrez et al., 2008), or a gene-trap (GT) mutation in the *LCMT-1* gene (MacKay et al., 2013). We tested the sensitivity of animals heterozygous for these mutations to both cognitive and electrophysiological impairments produced by acute exposure to oligomeric Aβ preparations and found that reduced expression of these genes altered the sensitivity of mice to Aβ-induced impairments. These results provide further support for roles for PME-1 and LCMT-1 in regulating the pathologic response to Aβ, and suggest that PME-1 inhibition may be a viable therapeutic avenue for preventing or treating its pathogenic effects.

Materials and Methods

Animals
Subjects in this study were heterozygous *PME-1* KO mice (Ortega-Gutierrez et al., 2008), *LCMT-1* GT mice (MacKay et al., 2013) (https://www.jax.org/strain/023332), and WT control siblings. Equivalent numbers of 4- to 6-month-old male and female, heterozygous mutant and WT mice generated from crosses of heterozygous animals were used for all experiments. Animals were housed individually following surgeries to
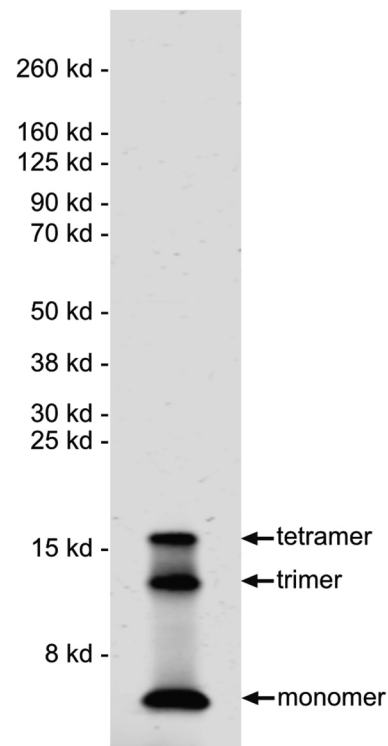

Figure 1. Representative Western blot of synthetic Aβ preparations used in behavioral and electrophysiological assays. Oligomerized synthetic human A$\beta_{1\text{-}42}$ peptide was run on a 10%–20% Tricine gel and probed with the monoclonal antibody 6E10, which recognizes an epitope contained within residues 1–17, revealing the distribution of monomeric and oligomeric species indicated. The pattern of immunoreactivity observed is consistent with that originally reported by Stine et al. (2003) and in subsequent publications (Puzzo et al., 2008; L. Lee et al., 2014; Asam et al., 2017).

implant cannulae. Surgical procedures were performed under anesthesia, and all efforts were made to minimize suffering.

Experimental design and statistical analysis
Behavioral testing was conducted during the light phase of a 12 h light/dark cycle. Animals were tested in cohorts of 10-12 animals consisting of equivalent numbers of each of the genotype $\times$ treatment groups. Behavioral testing consisted a battery of tasks conducted over a period of 2 weeks in the following order: 2 d radial arm water maze, contextual fear conditioning, visible platform water maze, open field behavior, and sensory threshold assessment. Separate groups of 3- to 4-month-old animals were used for electrophysiological experiments and Western blotting. All procedures involving animals were conducted in strict accordance with protocols approved by the Columbia University Institutional Animal Care and Use Committee (USDA Registration #21-R-0082; AAALAC Accreditation #000687; NYDOH #A141). Tests for statistical significance between groups were performed using GraphPad Prism 7. In experiments involving only two groups, Student's *t* tests were performed to test the significance of the effect of genotype. In experiments with more than two groups, regular, two-way, or two-way with repeated-measures ANOVA was performed followed by planned *post hoc* comparisons between groups. The details and results of all statistical tests are described in the corresponding figure legend for each experiment. Data are presented as means for each group $\pm$ SEM.

Aβ treatment
Oligomeric Aβ was prepared according to previously described protocols (Stine et al., 2003; Fa et al., 2010). Synthetic Aβ peptide corresponding to amino acids 1-42 of human amyloid precursor protein was purchased from the UCLA Biopolymer Laboratory. The lyophilized peptide was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol, dried, and
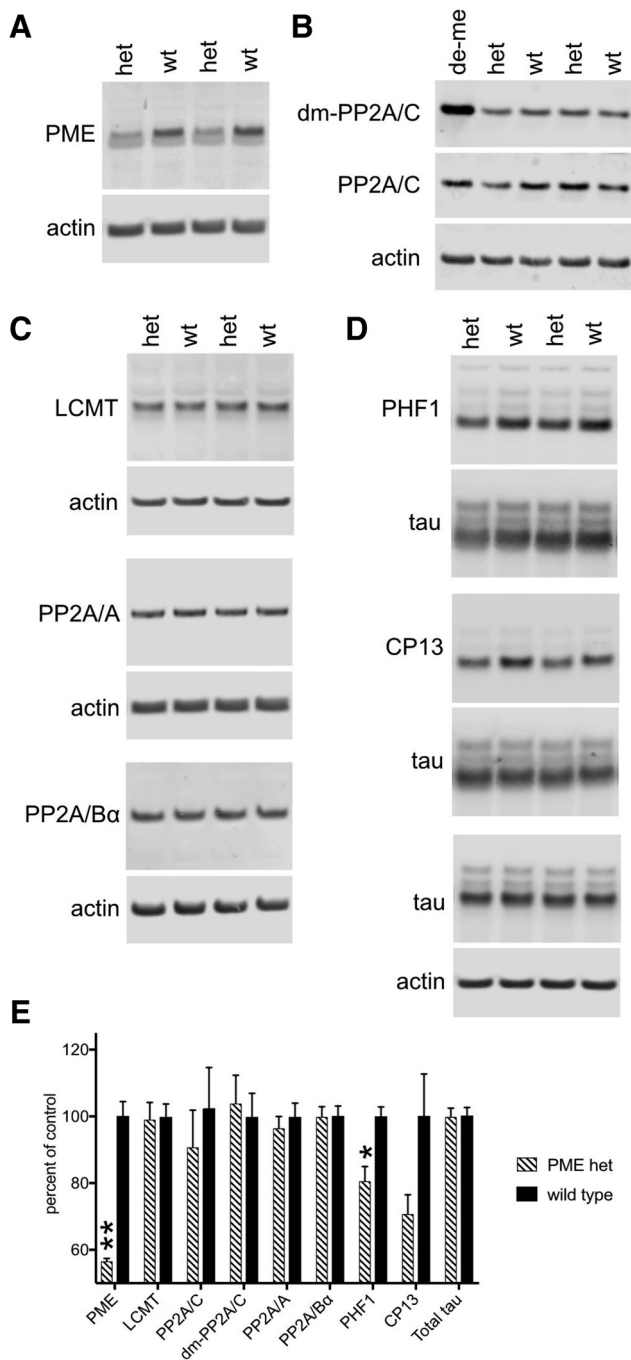
Figure 2. Heterozygous *PME-1* KO mice exhibit reduced PME-1 protein expression and increased tau phosphorylation. **A**, Representative Western blot showing anti-PME-1 immunoreactivity in hippocampal homogenates from *PME-1* KO heterozygotes (het) or WT sibling controls together with anti-β-actin immunoreactivity from the same lane as a loading control. **B**, Representative Western blot showing anti-demethyl PP2A/C immunoreactivity detected with the 1D6 monoclonal antibody in hippocampal homogenates together with the corresponding anti-β-actin immunoreactivity and total PP2A/C immunoreactivity detected after stripping and reprobing the same blot. The lane labeled "de-me" contains homogenate treated with NaOH to demethylate PP2A/C and serve as a positive control for the demethyl-specific anit-PP2A/C antibody. **C**, Representative Western blots showing anti-PP2A/A, PP2A/Bα, and LCMT-1 immunoreactivity in hippocampal homogenates from *PME-1* KO heterozygotes (het) or WT sibling controls together with corresponding same-lane anti-β-actin immunoreactivity as a loading control. **D**, Representative Western blots showing anti-tau phospho S396/404 (PHF1) and anti-tau phospho S202 (CP13) together with corresponding total tau immunoreactivity from the same lane, and anti-total tau together with corresponding anti-β-actin immunoreactivity. **E**, Histogram of mean immunoreactivity ($\pm$ SEM) for the indicated proteins normalized to within-lane anti-β-actin immunoreactivity (total PP2A/C

resuspended to 5 mM in dimethyl sulfoxide by bath sonication for 10 min. Aliquots were stored at $-20°C$. Before use, aliquots were diluted in aCSF to 100 μM and incubated for 24 h at 4°C. This procedure yielded a mixture of oligomeric Aβ species, as shown in Figure 1, that is consistent with previous reports (Stine et al., 2003; Puzzo et al., 2008; L. Lee et al., 2014; Asam et al., 2017). For experiments involving *in vivo* infusion of Aβ, animals were implanted with a 26-gauge guide cannula (Plastics One) into the dorsal part of the hippocampi (coordinates: posterior = 2.46 mm, lateral = 1.50 mm to a depth of 1.30 mm) (Paxinos and Franklin, 2013) under anesthesia with 20 mg/kg Avertin. Cannulas were fixed to the skull with acrylic dental cement (Paladur), and animals were allowed to recover for 6-8 d following surgery before behavioral testing; 1 μl of Aβ was infused into each hippocampus at a concentration of 200 or 75 nM as indicated in the text, over a period of 1 min through cannulas connected to a microsyringe by a polyethylene tubing. After infusion, the needle was kept in place for an additional minute to allow diffusion of Aβ into the tissue. For experiments involving *ex vivo* treatment, Aβ was bath-applied to hippocampal slices at a concentration of 100 or 75 nM for 20 min before theta-burst stimulation (TBS) as indicated in the text.

Radial arm water maze

Testing was performed in a 120-cm-diameter pool containing a six arm radial maze insert and filled with opaque water as described previously (Alamed et al., 2006). Mice were tested in 15 × 1 min trials on each of 2 consecutive days. The location of the escape platform was held constant during testing, but the start location was pseudorandomly varied throughout. On the first day, training alternated between visible and hidden platform trials, while on the second day only hidden platform trials were conducted. Water temperature was maintained at $\sim24°C$, and mice were dried and placed in a clean heated cage between trials to prevent hypothermia. Entries into maze arms that did not contain the escape platform were scored as errors. Aβ or vehicle was administered 20 min before, and again midway through testing on each day. Data are presented as the average number of errors committed during blocks of three training trials.

Visible platform water maze

This task was conducted in 120-cm-diameter pool filled with opaque water, but without the partitions used for the radial arm water maze task. Training for this task was conducted over 2 d with three morning and three afternoon trials on each day. Aβ or vehicle was administered 20 min before each block of three trials on each day. Intertrial intervals were 15-20 min, and rest periods between morning and afternoon sessions were 2-3 h. Each trial was a maximum of 120 s during which time the 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 s before returning to their home cage. The location of the platform was varied among four different locations such that it was not present in the same location on any two successive trials. Water temperature was maintained at $\sim24°C$, and animals were dried and placed in a clean warmed cage after each trial to prevent hypothermia. Animal movements were recorded using a video-tracking system and time required to reach the hidden platform (latency) and swim speed were determined using Ethovision behavioral analysis software (Noldus).

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immunoreactivity for demethyl-PP2A/C or total tau immunoreactivity for PHF1 and CP13 antibodies) and expressed as percent of mean control value. All blots were performed on homogenates prepared from 7 heterozygous *PME-1* KO animals and 7 WT sibling controls and compared using unpaired t tests: PME-1 expression: $t = 9.843$, **$p < 0.0001$, demethyl PP2A/C: $t = 0.3559$, $p = 0.7287$, PP2A/C expression: $t = 0.7076$, $p = 0.4927$, PP2A/A expression: $t = 0.6319$, $p = 0.5393$, PP2A/Bα expression: $t = 0.0673$, $p = 0.9474$, LCMT-1 expression: $t = 0.1324$, $p = 0.8969$, PHF1: $t = 3.691$, *$p = 0.0031$, CP13: $t = 2.124$, $p = 0.0551$, total tau: $t = 0.1204$, $p = 0.9062$).
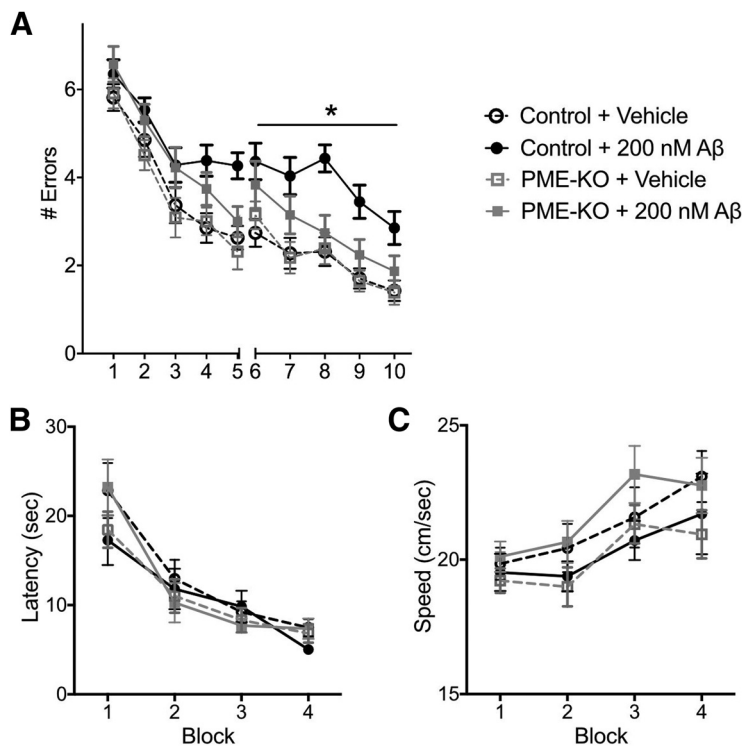
Figure 3. Heterozygous PME-KO mice are resistant to Aβ-induced impairments in a 2 d radial arm water maze task. ***A***, Plot of average number of errors committed ($\pm$SEM) during each 3-trial training block of a 2 d radial arm water maze task for heterozygous *PME-1* KO mice and WT sibling controls treated with 200 nm Aβ or vehicle as indicated. Two-way repeated-measures ANOVA for errors on day 2 (blocks 6-10) with group and block as factors shows a significant effect of group ($F_{(3,71)} = 9.028$, *$p < 0.0001$). Bonferroni *post hoc* comparisons show that Aβ-treated controls were significantly different from vehicle-treated controls ($p < 0.0001$), vehicle-treated *PME-KO* ($p < 0.0001$), and Aβ-treated *PME-KO* ($p = 0.0220$) groups. $N = 18$ control+vehicle, 20 control+Aβ, 19 PME+vehicle, and 18 PME+Aβ. ***B***, Plot of the average escape latency ($\pm$SEM) for the indicated groups during training on a visible platform Morris water maze task reveals no significant differences between groups (two-way repeated-measures ANOVA for latency with group and block as factors shows no significant effect of group: $F_{(3,68)} = 0.4588$, $p = 0.7120$). ***C***, Plot of the average swim speed ($\pm$SEM) for the indicated groups during training on the visible platform Morris water maze task described in ***B*** reveals no significant differences between groups (two-way repeated-measures ANOVA for speed with group and block as factors shows no significant effect of group $F_{(3,68)} = 1.086$, $p = 0.3612$).

Contextual fear conditioning

Animals were placed into a transparent Plexiglas conditioning chamber (33 cm × 20 cm × 22 cm) (Noldus PhenoTyper). Animal movement was recorded using an overhead video camera connected to a personal computer, and freezing behavior was scored and analyzed using Ethovision XT software (Noldus). Footshocks were administered through a removable metal grid floor, and the entire apparatus was cleaned and deodorized between animals with distilled water and 70% ethanol. Animals were placed in the conditioning chamber once on each of 2 consecutive days. On the first day of exposure, mice were placed in the conditioning chamber for 2 min before the onset of a discrete 30 s, 2800 Hz, 85 dB tone, the last 2 s of which coincided with a 0.8 mA footshock for experiments on *PME-1* KO mice, and 1.0 mA for experiments on *LCMT-1* GT mice. After the tone and shock exposure, the mice were left in the conditioning chamber for another 30 s before returning to their home cages. At 24 h after their first exposure, animals were returned to the conditioning chamber for 5 min without footshock or tone presentation. Aβ or vehicle was administered 20 min before the initial context exposure only.

Open field behavior

Animals were placed into a Plexiglas arena (27.3 cm long × 27.3 cm wide × 20.3 cm high) for 10 min on each of 2 successive days during which time their movements were tracked using arrays of infrared beams connected to a computerized tracking system, and analyzed using

behavioral analysis software (Med Associates). Aβ or vehicle was administered 20 min before testing on each day.

Sensory threshold assessment

Animals were placed into the same apparatus used for contextual fear conditioning. A sequence of single, 1 s footshocks were then administered at 30 s intervals and 0.1 mA increments from 0 up to a maximum of 0.7 mA. Each animal's behavior was monitored by the experimenter to determine their thresholds for first visible response to the shock (flinch), their first gross motor response (run/jump), and their first vocalized response. Aβ or vehicle was administered 20 min before testing.

Electrophysiological studies

Extracellular field potential recordings were performed on acute hippocampal slices prepared as described previously (Fa et al., 2010) from heterozygous PME-1 KO and, LCMT-1 GT mice and their WT control siblings. Animals were killed by cervical dislocation, a method of euthanasia approved by the Panel on Euthanasia of the American Veterinary Medical Association that yields viable anesthetic-free tissue suitable for electrophysiological recordings. Brains were then rapidly removed and cooled in ice-cold aCSF consisting of the following (in mM): 124 NaCl, 4.4 KCl, 1 Na$_2$HPO$_4$, 25 NaHCO$_3$, 2 CaCl$_2$, 2 MgCl$_2$, and 10 glucose. Hippocampi were then dissected and sliced into 400 μM sections using a tissue chopper. Slices were incubated at 29°C in an interface chamber under continuous perfusion (2 ml/min) with oxygenated aCSF and allowed to recover for a minimum of 90 min before recording responses in the CA1 region to stimulation of Schaffer collateral projections with a bipolar electrode. Input/output relationships were determined before each recording and stimulus intensities that elicited 30% of the maximal response were used. Stable baselines were obtained for a minimum of 15 min before drug or vehicle application and a TBS protocol consisting of three trains separated by 15 s intervals with each train consisting of 10 bursts at 5 Hz and each burst consisting of 5 pulses at 100 Hz was used to elicit LTP.

Western blotting

Animals were killed by cervical dislocation, an American Veterinary Medical Association-approved method that allows for rapid removal of nonhypoxic brains. Hippocampi were then rapidly dissected, snap frozen in liquid nitrogen, and stored at −80°C before homogenization for Western blot analysis. Hippocampal homogenates were prepared by sonication at 95°C in aqueous buffer containing 2% lithium dodecyl sulfate and 50 mM Tris, pH 7.5. Total protein concentrations were determined by bicinchoninic acid assay according to the manufacturer's instructions (Pierce), and 15 μg of total protein was loaded per lane on SDS-PAGE gels. Proteins were transferred to PVDF membranes, which were then blocked with Odyssey Blocking Buff (Tris-buffered saline) (LI-COR) for 1 h at room temperature. Blots were probed with the indicated primary antibodies: [rabbit anti-PME (1:1000) Millipore #07-095 RRID:AB_310373; mouse anti-LCMT-1 clone 4A4 (1:1000) Cell Signaling #5691 RRID:AB_10949100; mouse anti-demethyl PP2A/C clone 1D6, (1:1000) Millipore #05421 RRID:AB_309726; rabbit anti-PP2A/C clone 52F8 (1:1000) Cell Signaling #2259 RRID:AB_561239; rabbit anti -PP2A/A (1:1000) Millipore #07-250 RRID:AB_310467; mouse anti-PP2A/B55alpha clone 2G9(1:1000) Millipore #05-592 RRID: AB_309827; mouse anti-phospho-tau clone PHF1(1:500) Peter Davies; mouse anti-phospho-tau clone CP13 (1:500) Peter Davies; rabbit anti-
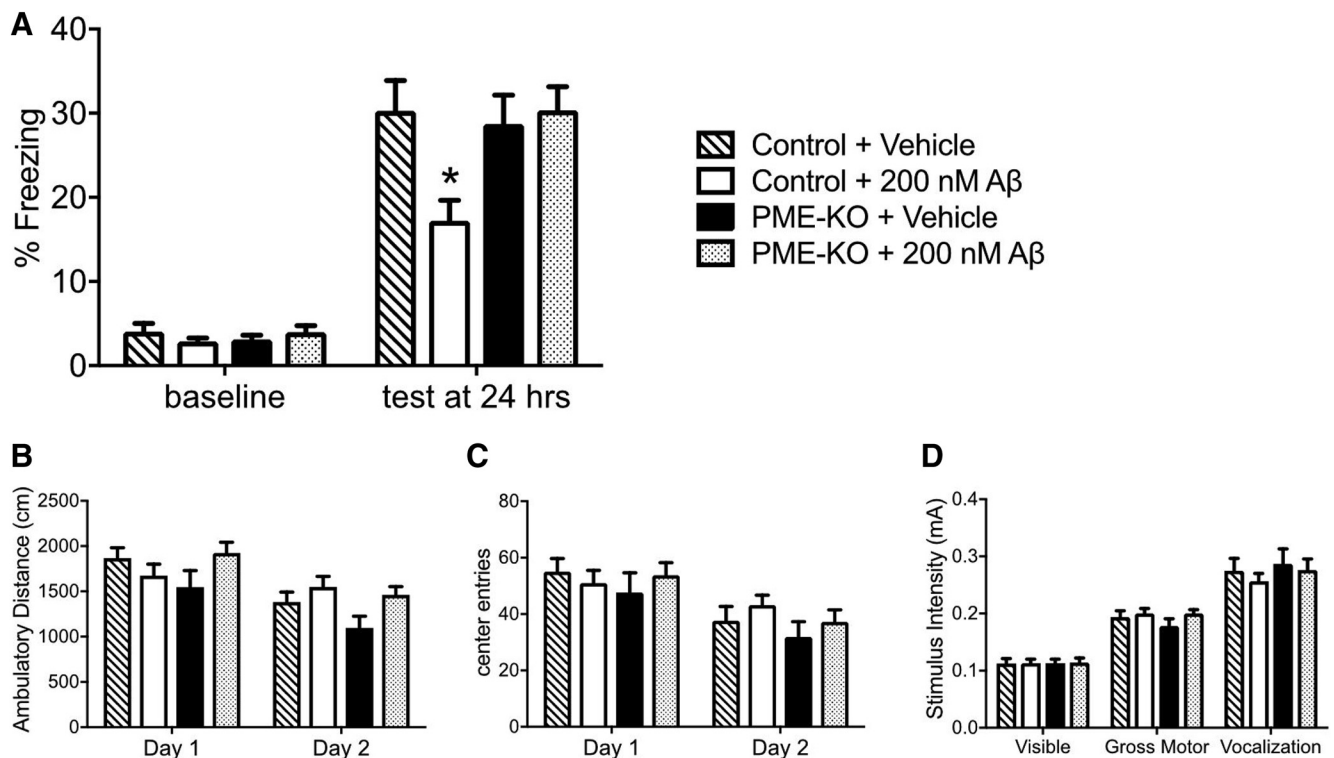
Figure 4. Heterozygous *PME-KO* mice are resistant to Aβ-induced impairments in contextual fear conditioning. ***A***, Plot of average percent of time spent freezing ($\pm$SEM) during initial exposure to the training context (baseline) and 24 h after footshock for the indicated groups. ANOVA for freezing at 24 h showed a significant difference among groups ($F_{(3,70)}$ = 4.424, p = 0.0066). Tukey's multiple comparisons show that only the control+Aβ group was significantly different from all other groups (p = 0.0218 vs control+vehicle, p = 0.0367 vs PME-KO+vehicle, and p = 0.0164 vs PME-KO+Aβ*). No differences in baseline freezing on day 1 were observed among groups (ANOVA: $F_{(3,70)}$ = 0.7894, p = 0.5039). N = 16 control+vehicle, 20 control+Aβ, 20 PME-KO+vehicle, and 18 PME-KO+Aβ. ***B***, Plot of average distance traveled ($\pm$SEM) for the indicated groups during 10 min exposures to an open field environment on subsequent days revealed no significant differences between groups. Two-way repeated-measures ANOVA for distance with day and group as factors shows a significant effect of day ($F_{(1,49)}$ = 41.31, p < 0.0001) but not group ($F_{(3,49)}$ = 2, p = 0.1262). Pairwise Bonferroni comparisons between groups within a training day revealed no significant differences with p values < 0.05. N = 10 control+vehicle, 16 control+Aβ, 15 PME+vehicle, and 12 PME+Aβ. ***C***, Plot of average number of entries into the center of an open field arena ($\pm$SEM) for the indicated groups during two 10 min exposures on subsequent days revealed no significant differences between groups. Two-way repeated-measures ANOVA for entries with day and group as factors shows a significant effect of day ($F_{(1,49)}$ = 47.28, p < 0.0001) but not group ($F_{(3,49)}$ = 0.5627, p = 0.6422). Pairwise Bonferroni comparisons between groups within a training day revealed no significant differences with p values < 0.05. N = 10 control+vehicle, 16 control+Aβ, 15 PME+vehicle, and 12 PME+Aβ. ***D***, Plot of average threshold for responses to footshocks of increasing intensity for the indicated groups revealed no significant differences in footshock perception (ANOVA for visible response: $F_{(3,79)}$ = 0.0092, p = 0.9988; for gross motor response: $F_{(3,79)}$ = 1.119, p = 0.3467; for vocalization: $F_{(3,79)}$ = 0.4069, p = 0.7484). N = 16 control+vehicle, 23 control+Aβ, 23 PME+vehicle, and 21 PME+Aβ.

tau clone EP2456Y (1:4000) Abcam #Ab76128 RRID:AB_1524475; mouse anti-β-actin (1:8000) LI-COR #926-42 212; rabbit anti-β-actin (1:8000) Licor #926-42 210 RRID:AB_1850027] overnight at 4°C, then washed and incubated with goat anti-rabbit (IRDye 800CW LI-COR) and goat anti-mouse (IRDye 680RD LI-COR) at room temperature for 2 h. For analysis of demethyl and total PP2A/C, blots were first probed with demethyl-specific antibody then stripped for 20 min at room temp in LI-COR Newblot PVDF stripping buffer (#928-40 032) plus 0.5% SDS before reprobing with the total PP2A/C antibody. Immunoreactive bands were detected using an Odyssey imager and analyzed using ImageStudio software (RRID:SCR_013715).

Alkaline demethylation
Demethylation of PP2A/C was conducted as described by Yu et al. (2001). Homogenates were incubated first for 10 min in 0.5 M NaOH at 4°C, then neutralized by addition of HCl to a final concentration of 0.5 mM and Tris, pH 6.8, to final concentration of 0.1 M.

Aβ ELISA
Levels of soluble murine Aβ 1-40 and Aβ 1-42 were measured in diethylamine-extracted hippocampal homogenates using commercially available ELISA kits (Wako) as described previously (Teich et al., 2013).

Results

Heterozygous *PME-KO* mice exhibit reduced PME-1 protein expression and decreased tau phosphorylation

To examine the effect of reduced PME-1 expression on Aβ sensitivity, we used a line of mice described previously that carry a KO mutation in the *PME-1* gene (Ortega-Gutierrez et al., 2008). Western blots performed on hippocampal homogenates from mice heterozygous for this mutation showed a 43.4 $\pm$ 0.9% reduction in PME-1 protein levels compared with WT littermates (Fig. 2A,E). Previous analysis of PP2A/C methylation levels in brains from homozygous embryonic day 18 *PME-1* KO mice showed pronounced changes in PP2A/C methylation compared with WT siblings (Ortega-Gutierrez et al., 2008); however, we observed no significant changes in demethyl-PP2A/C levels in hippocampal homogenates from heterozygous *PME-1* KO mice (Fig. 2B,E). Additional Western blot analysis of LCMT-1 protein in these animals suggested that this was not due to a compensatory decrease in LCMT-1 protein expression (Fig. 2C, E). Despite the unaltered basal levels of PP2A/C methylation in heterozygous *PME-1* KO animals, reduced PME-1 expression did result in a 20 $\pm$ 4% decrease in tau phosphorylation at
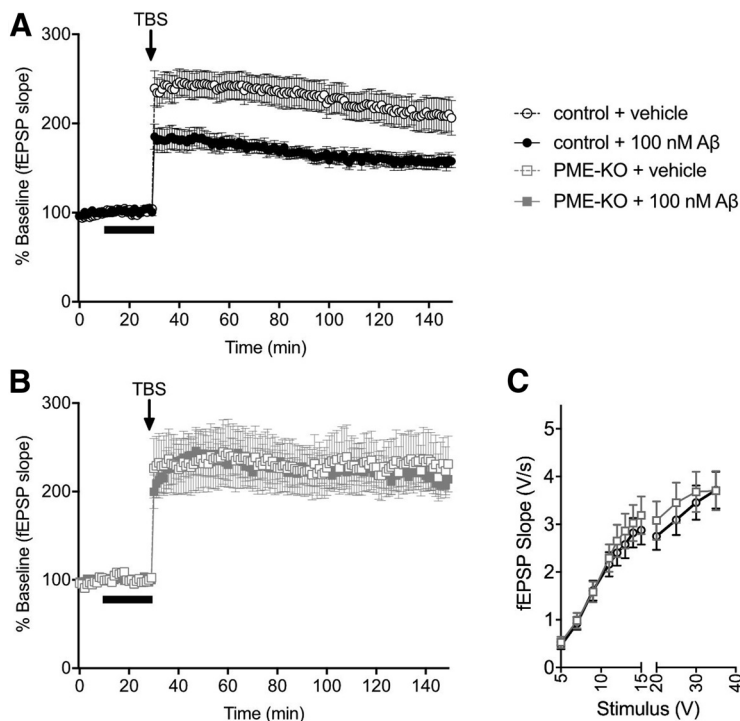
Figure 5. Heterozygous *PME-KO* mice are resistant to Aβ-induced impairments in LTP. *A*, *B*, Time course of averaged Schaffer collateral fEPSP responses (±SEM) in hippocampal slices prepared from heterozygous *PME-1* KO or WT control mice treated with vehicle or 100 nM Aβ (horizontal bar) 20 min before delivery of TBS (arrow). Aβ treatment significantly reduces potentiated responses following TBS in slices from control mice (*A*) but not slices from heterozygous PME-1 KO mice (*B*). Two-way repeated-measures ANOVA of last 20 min for group with time and group as factors shows a significant effect of group ($F_{(3,54)}$ = 3.148, p = 0.0323). Fisher's *post hoc* comparisons show significant differences between: control+Aβ versus control+vehicle (p = 0.0468), control+Aβ versus PME+Aβ (p = 0.0124) but not PME+vehicle versus PME+Aβ (p = 0.6141) or PME+vehicle versus control+vehicle (p = 0.4557). N = 13 control+vehicle, 14 control+Aβ, 11 PME+vehicle, and 20 PME+Aβ. *C*, Plot of stimulus-response relationship (±SEM) at Schaffer collateral synapses in hippocampal slices prepared from heterozygous PME-1 KO or WT control mice reveals no significant differences between groups (two-way repeated-measures ANOVA for genotype with stimulus intensity and genotype as factors: $F_{(1,89)}$ = 0.1954, p = 0.6595). Data obtained from slices before Aβ treatment and LTP recordings shown in *A* and in Figure 4*A*, *B*. N = 40 control and 51 PME-1 KO.

residues S396/404 that was statistically significant (p = 0.003), and a 29 ± 6% decrease in tau S202 phosphorylation that was not (p = 0.055) (Fig. 2*D*,*E*). These changes in tau phosphorylation were not accompanied by significant differences in total tau levels, or PP2A/C, PP2A/A, or PP2A/Bα subunit expression between heterozygous *PME-1* KO animals and controls (Fig. 2*C–E*).

Heterozygous *PME-KO* mice are resistant to Aβ-induced impairments in a 2 d radial arm water maze task
To determine whether reduced PME-1 expression affects the sensitivity of mice to Aβ-induced cognitive impairments, we tested the performance of heterozygous *PME-1* KO and WT animals infused with 200 nM synthetic oligomeric Aβ peptide or vehicle on a 2 d radial arm water maze task. This test of short-term spatial memory is both hippocampus-dependent and sensitive to acutely elevated Aβ levels (Alamed et al., 2006; Fiorito et al., 2013; Watterson et al., 2013). In this task, animals navigate to an escape platform in a fixed location at the end of one of the maze arms, and the number of errors or wrong-arm entries committed during each block of training trials is used as a measure of their ability to learn and remember its location. Previously, we found that transgenic overexpression of LMCT-1 protected animals

from Aβ-induced impairments in this task (Nicholls et al., 2016), and here we observed a similarly protective effect for reduced PME-1 expression in the *PME-1* KO heterozygous mice. While administration of 200 nM Aβ significantly impaired the performance of WT animals, the performance of heterozygous *PME-1* KO animals was comparable with that of vehicle-treated animals (Fig. 3*A*). This improved performance did not appear to result from generally enhanced performance in heterozygous *PME-1* KO animals since vehicle-treated heterozygous *PME-1* KO animals performed comparably to vehicle-treated WT controls (Fig. 3*A*). To test for potential differences in visual perception, motivation or swimming ability among these groups, we also assessed their performance in a visible platform water maze task, and found no significant differences in either escape latency (Fig. 3*B*) or swimming speed (Fig. 3*C*) during four 3-trial training blocks conducted across 2 d, suggesting that differences in these variables do not account for the differences observed among groups in the 2 d radial arm water maze task.

Heterozygous *PME-KO* mice are resistant to Aβ-induced impairments in contextual fear conditioning
As an additional test of the ability of reduced PME-1 expression to protect against Aβ-induced cognitive impairments, we tested Aβ- and vehicle-treated, heterozygous *PME-1* KO and WT animals in a contextual fear conditioning task. This task requires animals to make an association between an aversive footshock and a novel context, and has been found to be both hippocampus-dependent and sensitive to acutely elevated Aβ levels (Maren et al., 2013; Nicholls et al., 2016). In this task, animals are subjected to a footshock in a novel conditioning chamber, and the amount of time spent freezing during reintroduction to that chamber 24 h later in the absence of footshock is used to assess their memory for the context in which the aversive stimulus (footshock) was administered. Like the 2 d radial arm water maze task, this task was also used previously to reveal the protective effects of transgenic LMCT-1 overexpression against Aβ-induced cognitive impairments (Nicholls et al., 2016). Here, we found that Aβ infusion of WT animals, 20 min before their first exposure to the conditioning chamber significantly reduced freezing responses at 24 h compared with vehicle-treated controls, whereas, in contrast, Aβ infusion did not impair the performance of heterozygous *PME-1* KO animals (Fig. 4*A*). These differences were not accompanied by differences in baseline freezing responses or freezing responses between the vehicle-treated groups at 24 h, suggesting that reduced PME-1 expression in the heterozygous *PME-1* KO mice prevented the Aβ-induced cognitive impairment that was observed in Aβ-treated WT animals. There were also no significant differences between these groups in their immediate postshock freezing behavior (30 s postshock % freezing ± SEM: 24.75 ± 4.83 control+vehicle, 19.74 ± 3.63 control+Aβ, 20.99 ± 3.61 PME-1 KO+vehicle, and 18.14 ± 4.32 PME-1 KO+Aβ; ANOVA:

$F_{(3,70)} = 0.4377$, $p = 0.7267$). To test for possible differences in baseline activity levels or anxiety among these groups that might confound our interpretation of their behavior in the contextual fear condition task, we examined their behavior in an open field environment and found no significant differences in ambulatory activity (as assessed by total distance traveled; Fig. 4B) or anxiety (as assessed by entries into the center; Fig. 4C). To test for possible differences in shock perception among these groups that might affect their performance in the contextual fear condition task, we also examined their responses to a range of shock intensities and found comparable thresholds for the first visible, first gross motor, and first vocal response to footshocks of increasing intensity among these groups (Fig. 4D).

Heterozygous *PME-KO* mice are resistant to Aβ-induced impairments in LTP

Activity-dependent changes in the efficacy of synaptic transmission are thought to underlie particular forms of learning and memory, and Aβ-induced impairments in synaptic plasticity are thought to contribute to AD-associated behavioral impairments (Ondrejcak et al., 2010). Since our behavioral data suggested that reduced PME-1 expression protects against Aβ-induced cognitive impairments, we sought to determine whether reduced PME-1 expression might also protect against Aβ-induced impairments in synaptic plasticity. To do this, we performed extracellular field potential recordings of LTP at Schaffer collateral synapses in acute hippocampal slice preparations from heterozygous *PME-1* KO and WT animals. Treatment of WT slices with 100 nM Aβ 20 min before administration of a theta-burst stimulus train (TBS) significantly impaired potentiated responses relative to vehicle-treated control slices (Fig. 5A), whereas similarly treated slices from heterozygous *PME-1* KO mice showed no Aβ-induced impairment (Fig. 5B). Potentiated responses in slices from vehicle-treated heterozygous *PME-1* KO mice were comparable with those in vehicle-treated WT slices (Fig. 5A,B), and a comparison of the slope of evoked responses at increasing stimulus intensities obtained in slices from heterozygous *PME-1* KO and WT mice revealed no significant difference in the stimulus-response relationship between these animals (Fig. 5C) that might confound interpretation of these results. Congruent with our previous data on the ability of transgenic LCMT-1 overexpression to protect against Aβ-induced LTP impairment, these data suggest that reduced PME-1 expression also protects against Aβ-induced impairments in synaptic plasticity, and that this action may underlie its ability to protect against cognitive impairments caused by acute exposure to elevated Aβ levels.

Heterozygous *PME-KO* mice show normal responses to physiological levels of Aβ and produce normal levels of endogenous Aβ

One possible mechanism by which the pathologic effects of exogenously applied Aβ could be altered in *PME-1* KO mice is through changes in endogenous Aβ production. If endogenous Aβ levels
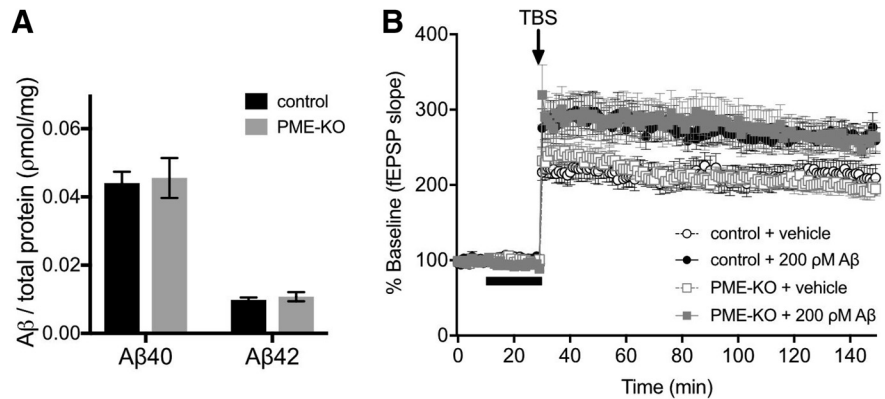
Figure 6. Heterozygous *PME-KO* mice show normal Aβ-induced increases in LTP and normal levels of endogenous Aβ. **A**, Histogram of amount of murine Aβ 40 or 42 per mg of total protein detected by ELISA in hippocampal homogenates prepared from heterozygous *PME-1* KO mice or WT controls showed no effect of *PME-1* genotype on endogenous Aβ 40 or 42 production. Results of t test comparisons for Aβ 40: $t = 0.2227$, $p = 0.8275$, and Aβ 42: $t = 0.5887$, $p = 0.5670$. $N = 7$ mice per group. **B**, Time course of averaged Schaffer collateral fEPSP responses ($\pm$SEM) in hippocampal slices prepared from heterozygous PME-1 KO or WT control mice treated with vehicle or 200 pM Aβ (horizontal bar) 20 min before delivery of TBS (arrow). Aβ treatment significantly increased potentiated responses following TBS in slices from both control and heterozygous *PME-1* KO mice. Two-way repeated-measures ANOVA of last 20 min for group with time and group as factors shows a significant effect of group: $F_{(3,48)} = 5.185$, $p = 0.0035$. Fisher's *post hoc* tests show significant differences between control+vehicle versus control+Aβ ($p = 0.0187$) and PME+vehicle versus PME+Aβ ($p = 0.0051$) but not control+vehicle versus PME+vehicle ($p = 0.4284$) or control+Aβ versus PME+Aβ ($p = 0.8770$). $N = 14$ control+vehide, 12 control+Aβ, 16 PME+vehicle, and 10 PME+Aβ.

were reduced in these mice, then this could potentially increase the threshold for impairments caused by exogenously applied Aβ. To test this possibility, we measured the levels of soluble endogenous Aβ 40 and 42 in hippocampal homogenates prepared from heterozygous *PME-1* KO and WT control mice and observed no significant differences between these groups for either peptide (Fig. 6A), suggesting that reduced endogenous Aβ production does not underlie the effects of reduced PME-1 expression on Aβ sensitivity.

In contrast to the cognitive and electrophysiological impairments caused by exposure to nanomolar concentrations of Aβ, treatment of mice or tissue with picomolar concentrations of Aβ has been found to enhance both cognitive performance and LTP (Puzzo and Arancio, 2013), and these effects are thought to reflect a normal physiological role for Aβ at nonpathologic concentrations (Puzzo et al., 2008, 2011). To determine whether reduced PME expression might also alter the response to picomolar concentrations of Aβ, we measured theta-burst–evoked LTP at Schaffer collateral synapses in acute hippocampal slices from heterozygous *PME-1* KO and WT control mice pretreated with either 200 pM oligomeric Aβ or vehicle. We found that application of 200 pM Aβ significantly enhanced LTP to a similar extent in both WT and heterozygous *PME-1* KO animals (Fig. 6B), suggesting that, while reduced PME-1 expression prevents LTP impairments caused by nanomolar Aβ concentrations, it does not affect the electrophysiological response to picomolar Aβ concentrations. This selectivity is consistent with our previous observations in PME-1- and LCMT-1-overexpressing transgenic mice (Nicholls et al., 2016) and further supports the notion that this pathway may represent a viable avenue to selectively target the pathologic effects of elevated Aβ levels without affecting Aβ's normal physiological functions. The observation that reduced PME-1 expression does not affect picomolar Aβ-induced LTP enhancement is also consistent with the normal baseline LTP and behavioral responses we observe in vehicle-treated heterozygous *PME-1* KO animals and slices (Figs. 3-5).
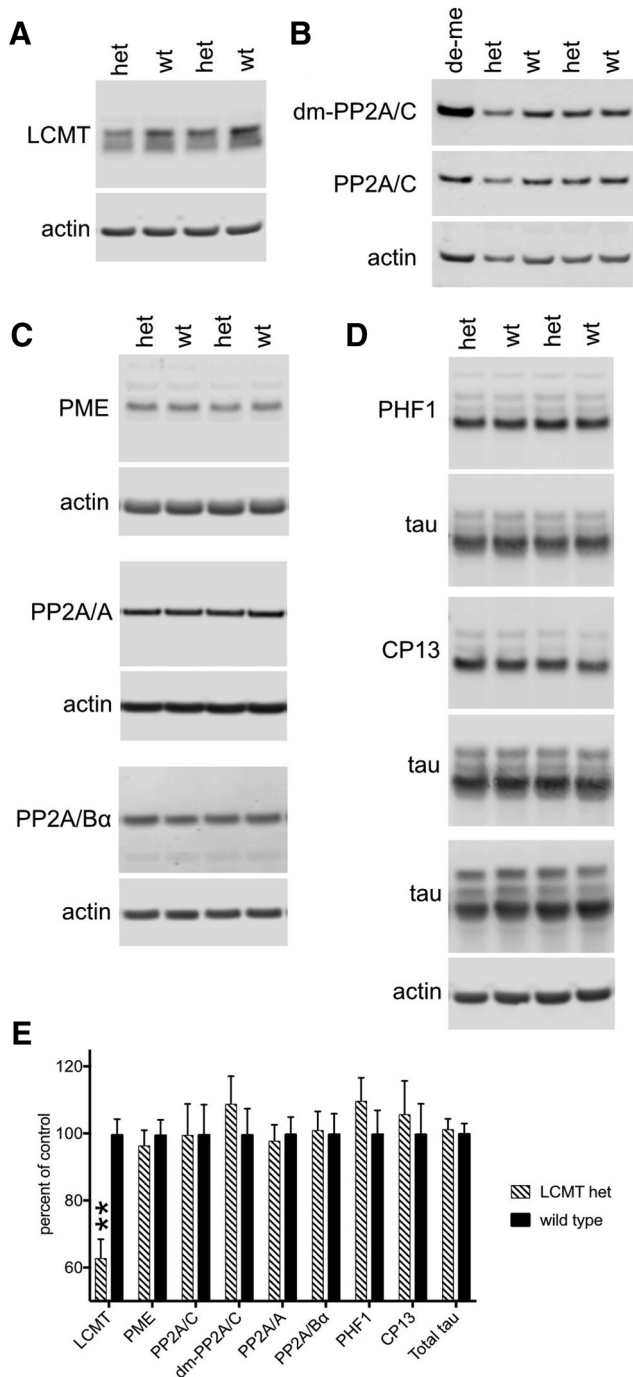
Heterozygous *LCMT-GT* mice exhibit reduced LCMT-1 protein expression

PME-1 and LCMT-1 play opposing roles in regulating PP2A/C methylation, and our previous analysis of PME-1- and LCMT-1-overexpressing transgenic mice suggests that these enzymes also have opposing effects on Aβ sensitivity (Nicholls et al., 2016). Consistent with this view, the data presented above from heterozygous *PME-1* KO mice suggest that the effects of reduced PME-1 expression are analogous to increased LCMT-1 expression, in that both manipulations decrease sensitivity to cognitive and electrophysiological impairments caused by acute exposure to elevated levels of Aβ. To test this relationship further, we used a previously described line of mice that carry a GT insertion in the *LCMT-1* gene (MacKay et al., 2013) to test the prediction that reduced LCMT-1 expression may increase sensitivity to Aβ. Western blot analysis performed on hippocampal homogenates from mice heterozygous for this mutation showed a $37.1 \pm 5.6\%$ reduction in LCMT-1 protein levels compared with WT littermates (Fig. 7A,E). We obtained no viable homozygous *LCMT-1* GT progeny from crosses of heterozygous *LCMT-1* GT animals, so heterozygous *LCMT-1* GT mice and their WT siblings were used for all experiments. A previous description of PP2A/C methylation levels in brains from homozygous *LCMT-1* GT mice reported a 15% increase in demethylated PP2A/C levels compared with WT siblings (MacKay et al., 2013); however, we observed only an $8.9 \pm 8.2\%$ increase in demethyl-PP2A/C levels in hippocampal homogenates from heterozygous *LCMT-1* GT and WT adult mice that was not statistically significant (Fig. 7B, E). The unaltered demethyl-PP2A/C levels in heterozygous *LCMT-1* GT mice did not appear to be due to a compensatory decrease in PME-1 protein expression since no differences in PME-1 protein levels were observed in Western blots of these homogenates (Fig. 7C,E). Neither were differences observed between heterozygous *LCMT-1* GT and WT siblings in total tau levels, or in PP2A/C, PP2A/A, or PP2A/Bα subunit expression (Fig. 7C–E). Western blot analysis of tau phosphorylation in heterozygous *LCMT-1* GT and WT animals using the phospho-specific PHF1 and CP-13 antibodies showed a $9.7 \pm 6.9\%$ increase in tau phosphorylation at residues S396/404 and a $5.7 \pm 9.9\%$ increase in tau phosphorylation at S202, respectively, neither of which reached statistical significance (Fig. 7D,E).

Heterozygous *LCMT-GT* mice exhibit increased sensitivity to aβ-induced impairments in a 2 d radial arm water maze task

To determine whether reduced LCMT-1 expression affects the sensitivity of mice to Aβ-induced cognitive impairments, we tested the performance of heterozygous *LCMT-1* GT mice on the 2 d radial arm water maze task as described above. However, to reveal potential increases in Aβ sensitivity in heterozygous

Figure 7. Heterozygous *LCMT-GT* mice exhibit reduced LCMT-1 protein expression. **A**, Representative Western blot showing anti-LCMT-1 immunoreactivity in hippocampal homogenates from *LCMT-1* GT heterozygotes (het) or WT sibling controls together with anti-β-actin immunoreactivity from the same lane as a loading control. **B**, Histogram of mean immunoreactivity ($\pm$SEM) for the indicated proteins normalized to within-lane anti-β-actin immunoreactivity (total PP2A/C immunoreactivity for demethyl-PP2A/C or total tau immunoreactivity for PHF1 and CP13 antibodies) and expressed as percent of mean control value. **C**, Representative Western blot showing anti-demethyl PP2A/C immunoreactivity detected with the 1D6 monoclonal antibody in hippocampal homogenates together with the corresponding anti-β-actin immunoreactivity and total PP2A/C immunoreactivity detected after stripping and reprobing the same blot. The lane labeled "de-me" contains homogenate treated with NaOH to demethylate PP2A/C and serve as a positive control for the demethyl-specific anit-PP2A/C antibody. **D**, Representative Western blots showing anti-PP2A/A, PP2A/Bα, and PME-1 immunoreactivity in hippocampal homogenates from *LCMT-1* GT heterozygotes (het) or WT sibling controls together with corresponding same-lane anti-β-actin immunoreactivity as a loading control. **E**, Representative Western blots showing anti-tau phospho S396/404

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(PHF1) and anti-tau phospho S202 (CP13) together with corresponding total tau immunoreactivity from the same lane, and anti-total tau together with corresponding anti-β-actin immunoreactivity. All blots were performed on homogenates prepared from 7 heterozygous PME-1 KO animals and 7 WT sibling controls and compared using unpaired t tests: LCMT-1 expression: $t = 5.203$, **$p = 0.0002$, demethyl PP2A/C: $t = 0.7975$, $p = 0.4420$, PP2A/C expression: $t = 0.2968$, $p = 0.7717$, PP2A/A expression: $t = 0.3691$, $p = 0.7185$, PP2A/Bα expression: $t = 0.1068$, $p = 0.9167$, PME-1 expression: $t = 0.5871$, $p = 0.5680$, PHF1: $t = 1$, $p = 0.3370$, CP13: $t = 0.431$, $p = 0.6741$, total tau: $t = 0.2733$, $p = 0.7892$).

LCMT-1 GT mice, we administered oligomeric Aβ at a subthreshold dose that did not impair the performance of WT animals. We found that, while the performance of Aβ-treated WT mice was comparable with vehicle-treated controls, Aβ treatment at this dose did significantly impair the performance of heterozygous LCMT-1 GT animals (Fig. 8A). The LCMT-1 GT mutation alone did not appear to affect performance in this task since vehicle-treated heterozygous LCMT-1 GT animals performed comparably to vehicle-treated WT controls. Neither were any differences observed among groups in either escape latency (Fig. 8B) or swimming speed (Fig. 8C) during testing in the visible platform water maze task, suggesting that behavior in the radial arm water maze task was not impacted by potential differences in visual perception, motivation, or swimming ability. Together, these data from the 2 d radial arm and visible platform water maze tasks are consistent with the prediction that reduced LCMT-1 expression increases sensitivity to Aβ-induced cognitive impairments.

Heterozygous *LCMT-GT* mice show increased sensitivity to Aβ-induced impairments in contextual fear conditioning

As an additional test of the effect of reduced LCMT-1 expression on Aβ-induced cognitive impairments, we tested Aβ and vehicle-treated, heterozygous LCMT-1 GT and WT animals in a contextual fear conditioning task. As in the 2 d radial arm water maze task above, we infused these animals with Aβ at a concentration that did not significantly impair the performance of WT control animals, and found that nevertheless this dose significantly impaired the freezing responses of heterozygous LCMT-1 GT animals when tested 24 h after training (Fig. 9A). The baseline freezing levels before footshock were comparable among these groups (Fig. 9A), and there were no significant differences in their immediate postshock freezing behavior (30 s postshock % freezing ± SEM: 9.91 ± 2.36 control + vehicle, 10.83 ± 1.97 control+Aβ, 9.66 ± 1.54 LCMT-GT+vehicle, and 9.12 ± 1.42 LCMT-GT+Aβ; ANOVA: $F_{(3,66)} = 0.1536$, $p = 0.9270$). Moreover, we found no significant differences in ambulatory activity (as assessed by total distance traveled; Fig. 9B) or anxiety (as assessed by entries into the center; Fig. 9C) when exposed to an open field environment. To test for possible differences in shock perception among WT and heterozygous LCMT-GT animals treated with vehicle or Aβ, we examined their responses to increasing footshock intensities and found comparable thresholds for the first visible, first gross motor, and first vocal response to footshocks of increasing intensity among all groups (Fig. 9D). The increased sensitivity of heterozygous LCMT-GT animals to Aβ-induced impairments in contextual fear conditioning is consistent with the increased sensitivity of these animals to Aβ-induced impairments in the 2 d radial arm water maze, and with the
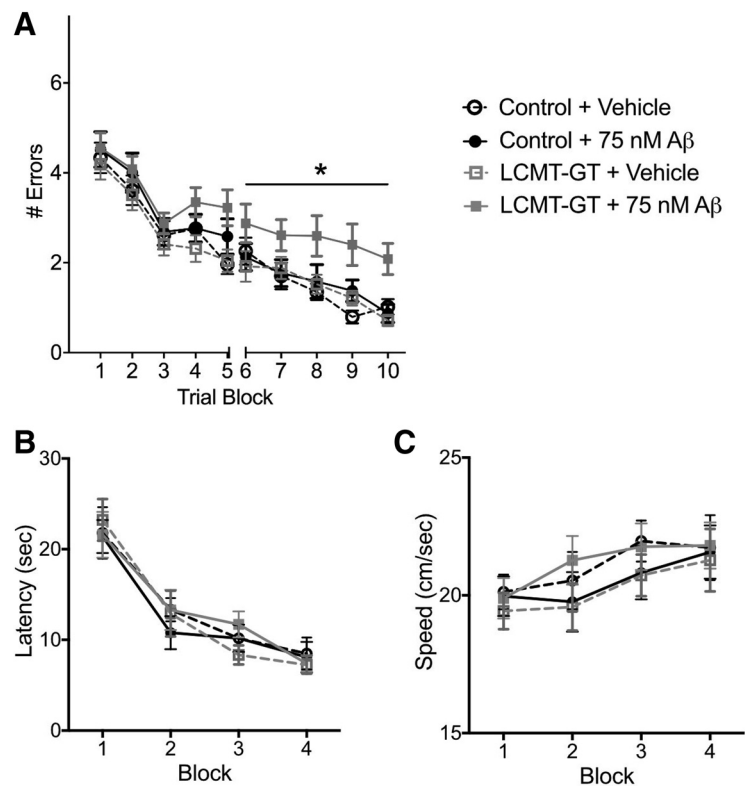

Figure 8. Heterozygous *LCMT-GT* mice are more sensitive to Aβ-induced impairments in a 2 d radial arm water maze task. *A*, Plot of average number of errors committed (±SEM) during each 3-trial training block of a 2 d radial arm water maze task for heterozygous LCMT-1 GT mice and WT sibling controls treated with 75 nM Aβ or vehicle as indicated. Two-way repeated-measures ANOVA for errors on day 2 (blocks 6-10) with group and block as factors shows a significant effect of group ($F_{(3,75)} = 4.733$, *$p = 0.0044$). Bonferroni *post hoc* comparisons show that the Aβ-treated LCMT-GT group was significantly different from vehicle-treated control ($p = 0.0089$), Aβ-treated control ($p = 0.0303$), and vehicle-treated LCMT-GT ($p = 0.0074$) groups. $N = 18$ control+vehicle, 16 control+Aβ, 21 LCMT-GT+vehicle, and 24 LCMT-GT+Aβ. *B*, Plot of the average escape latency (±SEM) for the indicated groups during training on a visible platform Morris water maze task reveals no significant differences between groups (two-way repeated-measures ANOVA for latency with group and block as factors shows no significant effect of group, $F_{(3,75)} = 0.1239$, $p = 0.9457$). *C*, Plot of the average swim speed (±SEM) for the indicated groups during training on the visible platform Morris water maze task described in *B* reveals no significant differences between groups (two-way repeated-measures ANOVA for speed with group and block as factors shows no significant effect of group, $F_{(3,75)} = 0.3459$, $p = 0.7922$).

increased Aβ sensitivity we observed previously in PME-1-overexpressing transgenic animals (Nicholls et al., 2016).

Heterozygous *LCMT-GT* mice exhibit increased sensitivity to Aβ-induced LTP impairments

To test the possibility that reduced LCMT-1 expression also increases sensitivity to Aβ-induced impairments in synaptic plasticity, we treated acute hippocampal slices from WT and heterozygous LCMT-GT animals with vehicle or with subthreshold doses of 75 nM Aβ for 20 min before TBS. Potentiated responses in WT slices treated with Aβ at this concentration were comparable with vehicle-treated controls; however, treatment of heterozygous LCMT-GT slices at this concentration resulted in significantly reduced potentiation relative to both vehicle-treated heterozygous LCMT-GT slices and controls (Fig. 10A,B). A comparison of the slope of evoked responses at increasing stimulus intensities obtained in slices from heterozygous LCMT-GT and WT mice revealed no significant difference in the stimulus-response relationship between these groups (Fig. 10C) that might confound interpretation of these results. Together, these results
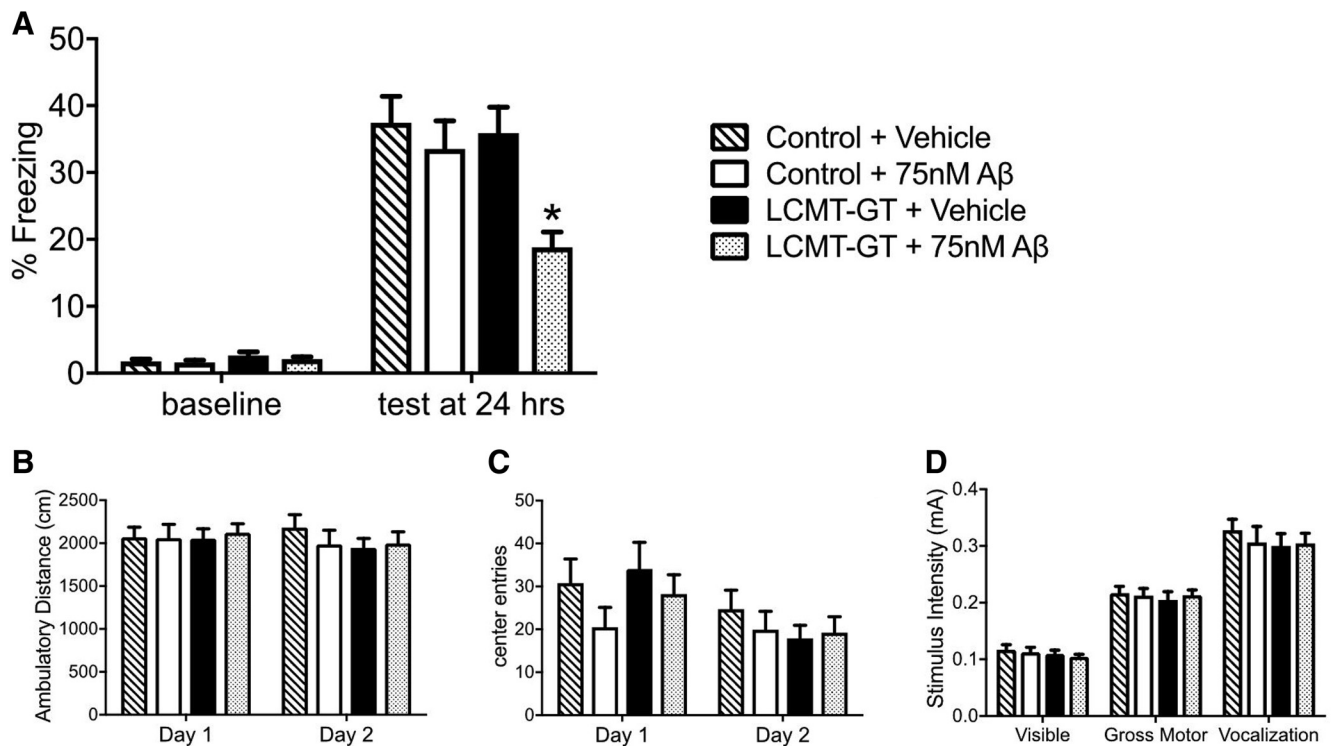
Figure 9. Reduced LCMT-1 expression increases sensitivity to Aβ-induced impairments in contextual fear conditioning. **A**, Plot of average percent of time spent freezing ($\pm$SEM) during initial exposure to the training context (baseline) and 24 h after footshock for the indicated groups. ANOVA for freezing at 24 h showed a significant difference among groups ($F_{(3,66)}$ = 5.932, p = 0.0012). Tukey's multiple comparisons show that only the LCMT+Aβ group was significantly different from vehicle-treated controls (*p = 0.0015), and that the LCMT+Aβ group was also significantly different from Aβ-treated controls (p = 0.0272). No differences in baseline freezing on day 1 were observed among groups (ANOVA: $F_{(3,66)}$ = 1.284, p = 0.2873). N = 16 control+vehicle, 16 control+Aβ, 19 LCMT-GT+vehicle, and 19 LCMT-GT+Aβ. **B**, Plot of average distance traveled ($\pm$SEM) for the indicated groups during 10 min exposures to an open field environment on subsequent days revealed no significant differences between groups. Two-way repeated-measures ANOVA for distance with day and group as factors showed no significant effect of day ($F_{(1,44)}$ = 1.284, p = 0.2634) or group ($F_{(3,44)}$ = 0.1835, p = 0.9070). Pairwise Bonferroni comparisons between groups within a training day revealed no significant differences with p values < 0.05. N = 10 control+vehicle, 9 control+Aβ, 13 LCMT-GT+vehicle, and 16 LCMT-GT+Aβ. **C**, Plot of average number of entries into the center of an open field arena ($\pm$SEM) for the indicated groups during two 10 min exposures on subsequent days revealed no significant differences between groups. Two-way repeated-measures ANOVA for entries with day and group as factors shows a significant effect of day ($F_{(1,54)}$ = 7.45, p = 0.0085) but not group ($F_{(3,54)}$ = 0.6784, p = 0.5691). Pairwise Bonferroni comparisons between groups within a training day revealed no significant differences with p values < 0.05. N = 10 control+vehicle, 9 control+Aβ, 13 LCMT-GT+vehicle, and 16 LCMT-GT+Aβ. **D**, Plot of average threshold for responses to footshocks of increasing intensity for the indicated groups revealed no significant differences in footshock perception (ANOVA for visible response: $F_{(3,74)}$ = 0.5746, p = 0.6335; for gross motor response: $F_{(3,74)}$ = 0.1716, p = 0.91523; for vocalization: $F_{(3,74)}$ = 0.3167, p = 0.8133). N = 18 control+vehicle, 16 control+Aβ, 21 LCMT-GT+vehicle, and 23 LCMT-GT+Aβ.

suggest that reduced LCMT-1 expression increases sensitivity to Aβ-induced LTP impairments, and that these impairments in synaptic plasticity may underlie the increased sensitivity of these animals to Aβ-induced cognitive impairments.

Discussion

Our data show that mice with reduced PME-1 expression as a result of a heterozygous *PME-1* KO mutation were resistant to cognitive impairments caused by acute exposure to synthetic oligomeric Aβ preparations in both a 2 d radial arm water maze and a contextual fear conditioning task. We also found that reduced PME-1 expression prevented Aβ-induced impairments of LTP in acute hippocampal slice preparations from these animals, suggesting that reduced PME-1 expression may protect against Aβ-induced cognitive impairments by preventing the impairments in synaptic plasticity that are thought to underlie them. These protective effects of reduced PME-1 expression are consistent with previously described experiments in which we found that transgenic overexpression of PME-1-sensitized mice to Aβ-induced impairments, whereas overexpression of LCMT-1 protected against Aβ-induced impairments (Nicholls

et al., 2016). In addition, we found that reduced PME-1 expression in heterozygous *PME-1* KO mice did not significantly affect endogenous Aβ levels, LTP enhancement by picomolar Aβ exposure, or baseline behavioral or electrophysiological responses. Together, these data argue that the effect of reduced PME-1 expression on Aβ sensitivity is selective for impairments caused by pathologically elevated concentrations of Aβ, but does not interfere with normal physiological Aβ functions. This interpretation is also consistent with our data from PME-1- and LCMT-1-overexpressing transgenic mice where we saw altered sensitivity to nanomolar Aβ exposure but no evidence for effects on picomolar Aβ sensitivity or endogenous Aβ production (Nicholls et al., 2016).

The antagonistic relationship that exists between the biochemical activities of PME-1 and LCMT-1, as well as our previous data on the effects of PME-1 and LCMT-1 overexpression (Nicholls et al., 2016), suggest that reduced LCMT-1 expression should increase sensitivity to Aβ-induced impairments, and this is what we observed when we treated *LCMT-1* GT mice with subthreshold doses of Aβ that did not elicit impairments in WT controls. We found that reduced LCMT-1 expression resulted in increased sensitivity to Aβ-induced cognitive impairments in

the 2 d radial arm water maze and contextual fear conditioning tasks, as well as increased sensitivity to Aβ-induced LTP impairments. These effects are consistent with the opposing biochemical functions of LCMT-1 and PME-1, the effect of reduced PME-1 expression reported here, and our previous data on the effects of transgenic PME-1 and LCMT-1 overexpression (Nicholls et al., 2016).

The fact that reduced PME-1 expression selectively protects against impairments caused by nanomolar concentrations of Aβ without interfering with normal physiological Aβ functions suggests that high concentrations of Aβ produce impairments via a pathway that is sensitive to reduced PME-1 expression, whereas low physiological concentrations of Aβ act via a pathway that is independent from, or less sensitive to, reduced PME-1 expression. Our current hypothesis for the mechanism by which reduced PME-1 expression affects Aβ sensitivity is that reduced PME-1 expression acts via PP2A to increase tau dephosphorylation and reduce the pathologic response to nanomolar concentrations of Aβ. This model is consistent with published data showing that PME-1 regulates PP2A activity and subunit assembly (Sents et al., 2013; Kaur and Westermarck, 2016), that PP2A acts as the principal tau phosphatase (Sontag and Sontag, 2014), and that tau acts downstream of Aβ to contribute to Aβ-induced impairments (Bloom, 2014; Spires-Jones and Hyman, 2014). This model is also consistent with the decreased levels of tau phosphorylation that we found in the heterozygous PME-1 KO mice. However, in our experiments, we did not observe a significant increase in basal levels of PP2A methylation in the heterozygous PME-1 KO mice that would have been consistent with reduced PME-1 expression acting through PP2A methylation to exert its protective effects. Instead, this result raises the possibility that reduced PME-1 expression may act on PP2A via a methylation-independent mechanism, such as reduced binding to PP2A or its subunits that could alter PP2A stability, assembly, activity, or substrate interactions (Kaur and Westermarck, 2016). It is also possible that other PME-1 targets, such as the related phosphatases, PP4 and PP6 (Hwang et al., 2016), may mediate the protective effects of reduced PME-1 expression.

Published data suggest that PME-1 plays a largely inhibitory role in regulating PP2A (Sents et al., 2013; Kaur and Westermarck, 2016), and so the effect of reduced PME-1 expression on Aβ sensitivity that we describe here is consistent with reports of the protective effects of PP2A activators in other AD models. These include sodium selenate, a PP2A activator that has been shown to reduce tau phosphorylation and cognitive impairments in mouse tauopathy models (Corcoran et al., 2010; van Eersel et al., 2010) and is being pursued in early clinical trials as a potential AD therapeutic (Cardoso et al., 2019); COG1410, an ApoE peptide mimetic that activates PP2A by inhibiting the PP2A inhibitor, SET/I2, and protects against impairments in a mouse AD model (Vitek et al., 2012); and FTY720/fingolimod, an
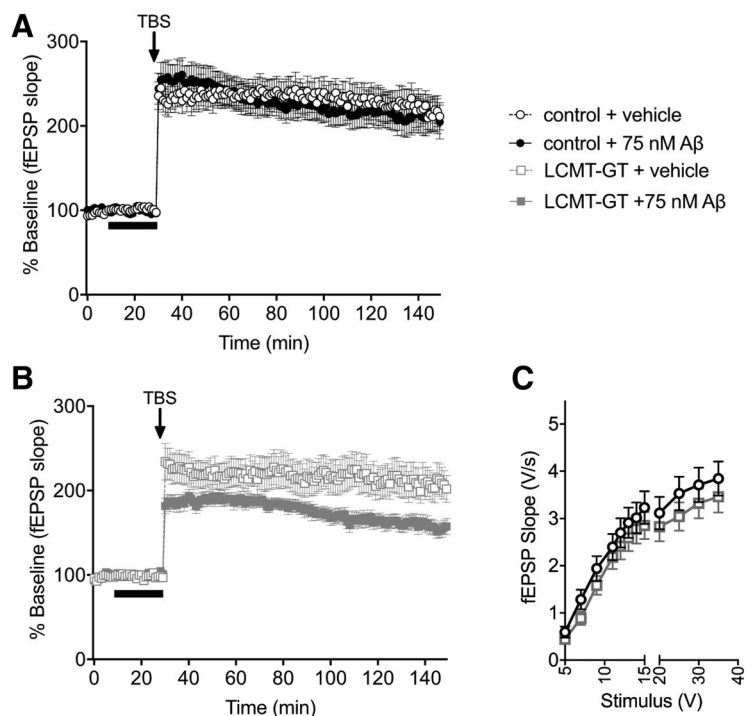

Figure 10. Reduced LCMT-1 expression increases sensitivity to Aβ-induced LTP impairments. Time course of averaged Schaffer collateral fEPSP responses (±SEM) in hippocampal slices from WT controls (*A*) or heterozygous LCMT-GT mice (*B*) treated with vehicle or 75 nM Aβ (horizontal bar) 20 min before delivery of TBS (arrow) showed significantly increased sensitivity to Aβ-induced LTP impairment in the heterozygous LCMT-GT mice. Two-way repeated-measures ANOVA of last 20 min of responses with time and group as factors shows a significant effect of group: $F_{(3,45)} = 3.095$, $p = 0.0362$. Fisher's *post hoc* tests show significant differences between LCMT+Aβ versus LCMT+vehicle ($p = 0.0421$), LCMT+Aβ versus control+vehicle ($p = 0.0132$), and LCMT+Aβ versus control+Aβ ($p = 0.0284$), but not control+Aβ versus control+vehicle ($p = 0.6560$), control+Aβ versus LCMT+vehicle ($p = 0.9229$). $N = 12$ control+vehicle, 15 control+Aβ, 13 LCMT+vehicle, and 13 LCMT+Aβ. *C*, Plot of stimulus-response relationship (±SEM) at Schaffer collateral synapses in hippocampal slices prepared from heterozygous LCMT-GT or WT control mice reveals no significant differences between groups (two-way repeated-measures ANOVA for genotype with stimulus intensity and genotype as factors: $F_{(1,93)} = 0.7503$, $p = 0.3886$). Data obtained from slices before Aβ treatment. $N = 47$ control and 48 LCMT-1 GT.

immunosuppressant compound used in the treatment of multiple sclerosis that also protects against Aβ-induced impairments (Fukumoto et al., 2014; Joshi et al., 2017) and was subsequently found to activate PP2A in addition to acting on other targets (Cristóbal et al., 2016). A potential obstacle for therapeutic approaches that target PP2A, however, stems from the large number of PP2A isoforms and substrates that exist and concern over a lack of specificity that might result from broad PP2A activation. The data we present here suggest that it may be possible to develop PP2A-based therapies for AD using PME-1 inhibitors to selectively alter the activity of PP2A isoforms that are regulated by demethylation (Longin et al., 2007; Xu et al., 2008). One candidate in this regard is eicosanoyl-5-hydroxytryptamide, which was identified in a screen for naturally occurring compounds that inhibit PP2A demethylation (K. W. Lee et al., 2011) and subsequently found to reduce AD-related impairments in rats that overexpress an endogenous PP2A inhibitor (Basurto-Islas et al., 2014), and to protect against Aβ-induced impairments in experiments similar to those described here (Asam et al., 2017). Additionally, two other small-molecule inhibitors of PME-1 have been identified that may represent viable starting points from which to pursue this approach (Bachovchin et al., 2011a,b). However, additional research will be required to explore further the efficacy of these and other PME-1 inhibitors

and realize the therapeutic potential of this pathway for the treatment of Alzheimer's disease and other disorders.

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