PP2A methylation controls sensitivity and resistance to β-amyloid–induced cognitive and electrophysiological impairments

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Elevated levels of the β-amyloid peptide (Aβ) are thought to contribute to cognitive and behavioral impairments observed in Alzheimer's disease (AD). Protein phosphatase 2A (PP2A) participates in multiple molecular pathways implicated in AD, and its expression and activity are reduced in postmortem brains of AD patients. PP2A is regulated by protein methylation, and impaired PP2A methylation is thought to contribute to increased AD risk in hyperhomocysteinemic individuals. To examine further the link between PP2A and AD, we generated transgenic mice that overexpress the PP2A methylesterase, protein phosphatase methylesterase-1 (PME-1), or the PP2A methyltransferase, leucine carboxyl methyltransferase-1 (LCMT-1), and examined the sensitivity of these animals to behavioral and electrophysiological impairments caused by exogenous Aβ exposure. We found that PME-1 overexpression enhanced these impairments, whereas LCMT-1 overexpression protected against Aβ-induced impairments. Neither transgene affected Aβ production or the electrophysiological response to low concentrations of Aβ, suggesting that these manipulations selectively affect the pathological response to elevated Aβ levels. Together these data identify a molecular mechanism linking PP2A to the development of AD-related cognitive impairments that might be therapeutically exploited to target selectively the pathological effects caused by elevated Aβ levels in AD patients.

Alzheimer's disease | protein phosphatase 2A | methylation | β-amyloid | cognitive impairment

Multiple observations suggest a role for the serine/threonine protein phosphatase 2A (PP2A) in the molecular pathways that underlie Alzheimer's disease (AD). Analyses conducted on postmortem AD brains have found reduced PP2A expression and activity, and studies conducted in animal models have found that inhibiting PP2A produces AD-like tau pathology and cognitive impairment (1–3). One of the ways in which PP2A may affect AD is through its role as the principal tau phosphatase (4–7). PP2A also interacts with a number of kinases implicated in AD including glycogen synthase kinase 3β (GSK3β), cyclin-dependent kinase 5 (CDK5), and ERK and JNK as well as amyloid precursor protein and the NMDA and metabotropic glutamate receptors (reviewed in ref. 2).

PP2A is a heterotrimeric protein composed of a catalytic, scaffolding, and regulatory subunit. Each subunit is encoded by multiple genes and splice isoforms, and the subunit composition of a particular PP2A molecule determines its subcellular distribution and substrate specificity (reviewed in ref. 2). One of the ways in which PP2A activity is regulated is through C-terminal methylation of the catalytic subunit (reviewed in refs. 8 and 9). Impaired methyl-donor metabolism is a risk factor for AD (10, 11), and PP2A dysregulation caused by impaired methylation is thought to be one of the molecular mechanisms contributing to this increased risk (12–14). Methylation promotes the formation of PP2A holoenzymes that contain Bα regulatory subunits (7, 13, 15–19), and these forms of PP2A exhibit the greatest tau phosphatase activity (6, 7).

PP2A methylation is catalyzed in vivo by the methyl transferase, leucine carboxyl methyltransferase 1 (LCMT-1) (20–22), and its demethylation is catalyzed by the methylesterase, protein phosphatase methylesterase 1 (PME-1) (23–25). To explore the role of PP2A in AD further, we generated lines of transgenic mice that overexpress these enzymes and tested their effect on the sensitivity of animals to electrophysiogical and behavioral impairments caused by β-amyloid (Aβ). We found that LCMT-1 overexpression protected animals from Aβ-induced impairments, whereas overexpression of PME-1 worsened Aβ neurotoxicity. Neither transgene affected endogenous Aβ levels, suggesting that they acted by altering the response to Aβ rather than Aβ production. We also found that PME-1 and LCMT-1 overexpression were without effect on the electrophysiological response to picomolar Aβ application, suggesting that they selectively affected the response to pathological Aβ concentrations. Together these data indicate that this pathway has potential as a therapeutic avenue for AD that acts not by targeting Aβ production but by selectively altering the response to pathological levels of Aβ.

Results

PME-1 Overexpression in Transgenic Mice. To test the effect of reduced PP2A methylation on the sensitivity to Aβ-induced

Significance

Elevated levels of the β-amyloid peptide (Aβ) are thought to contribute to the cognitive impairments associated with Alzheimer's disease (AD). We found that by genetically targeting the methylation of protein phosphatase 2A (PP2A) in transgenic mice, we could alter the sensitivity of animals to electrophysiological and cognitive impairments caused by Aβ exposure without affecting Aβ production or the electrophysiological response to low concentrations of Aβ. These data support a role for PP2A methylation in contributing to AD risk and identify a potential therapeutic pathway that might be exploited to target the pathological actions of Aβ selectively.

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impairments, we generated mice carrying a transgene encoding FLAG epitope-tagged murine PP2A methylesterase (PME-1) under the control of a synthetic *tetO* promoter. To drive expression of the *PME-1* transgene in neurons in the forebrain, we crossed these animals with mice carrying a second transgene that was under the control of a promoter region from the calcium calmodulin kinase IIα (*CaMKIIα*) gene and expressed a synthetic tetracycline-responsive transactivator (tTA) (26). In this system, *PME-1* transgene expression is activated when tTA binds to the *tetO* promoter in cells where tTA expression is driven by the *CaMKIIα* promoter (Fig. 1*A*). Although not used in the current experiments, this system also affords the ability to suppress *tetO* promoter-driven transgene expression through doxycycline administration. *PME-1* transgene-specific RNA in situ hybridization revealed expression throughout the forebrain, including the striatum, olfactory bulb, cortex, and hippocampus, in animals that carried both the *tetO-PME* and *CaMK-tTA* transgenes but not in single-transgenic siblings (Fig. 1*B*, *Left*). Immunohistochemistry on brain sections from these animals using an antibody specific to the FLAG epitope tag detected transgenic protein in cell bodies and dendrites of pyramidal cells of the hippocampal CA1 region of double-transgenic animals that was not present in single-transgenic control animals (Fig. 1*B*, *Right*).

Quantitative Western blot analysis of hippocampal extracts from *tetO-PME/CaMKtTA* double-transgenic animals revealed a significant increase in PME-1 expression compared with single-transgenic control animals (Fig. 1*C*). Transgene expression in these animals did not affect PP2A catalytic subunit expression, PP2A Bα regulatory subunit expression, LCMT-1 expression (Fig. 1*C*), or levels of the methylation metabolites S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) (Fig. S1). Quantitative Western blot analysis did reveal a significant reduction in methylated PP2A levels in animals overexpressing PME-1 as compared with controls (Fig. 1*D*). This change was accompanied by an increase in tau phosphorylation at sites reported to be targets of PP2A (5, 13), but not in total tau levels (Fig. 1*E*), as well as increased phosphorylation of amyloid precursor protein (APP) at threonine 668 (Fig. 1*F*), which also is a target of PP2A (13). ELISA measurements of $A\beta_{1–40}$ and $A\beta_{1–42}$ levels in hippocampal homogenates from animals overexpressing PME-1 and control animals revealed no significant change in basal levels of either peptide (Fig. 1*H*).

PME-1 Overexpression Increases Sensitivity to Behavioral and Physiological Impairments Caused by Exposure to Subthreshold Doses of Oligomeric Aβ but Not to Picomolar Aβ Doses. Mice overexpressing PME-1 were overtly indistinguishable from their single-transgenic control siblings. They were fertile and were recovered at the expected frequencies from crosses of double-transgenic males to wild-type females (Fig. S2). Analysis of these animals' behavior in a novel open field revealed no genotype effects (Fig. S3).

To test the effect of PME-1 overexpression on the behavioral impairments that result from acute Aβ exposure, we tested these animals in a hippocampus-dependent contextual fear-conditioning task previously shown to be sensitive to Aβ administration (27–29). Vehicle-treated animals overexpressing PME-1 exhibited a level of freezing 24 h after training in this task similar to that of vehicle-treated single-transgenic control siblings (Fig. 2*A*). Control animals infused with a subthreshold dose of oligomeric Aβ (bilateral infusions of 1 μL of 75 nM oligomeric Aβ) also exhibited levels of freezing similar to those in the vehicle-treated controls. However, animals overexpressing PME-1 that received a subthreshold dose of Aβ exhibited significantly less freezing than the other three groups, suggesting that animals that overexpress PME-1 are more sensitive to Aβ-induced cognitive impairment in this task. We observed no differences in baseline freezing, open-field ambulatory activity, or shock perception among these groups, suggesting that these differences in contextual fear conditioning did not result from differences in baseline behaviors or sensory perception (Fig. 2*A* and Fig. S4).

As a second test of the effect of PME-1 overexpression on Aβ-induced cognitive impairments, we tested these animals on a 2-d radial arm water maze task (29, 30) and found that

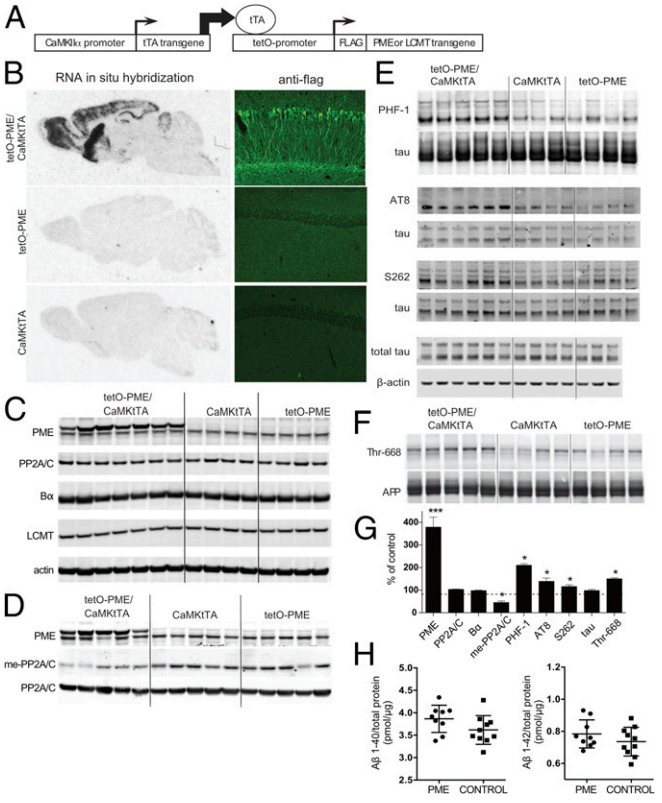

Fig. 1. *PME-1* transgene expression in the forebrain of transgenic mice. (*A*) Diagram of the tetO/tTA system in which FLAG-tagged *PME-1* transgene expression is driven by the tTA transactivator under the control of a *CaMKIIα* promoter fragment. (*B*) Representative images of transgene-specific RNA in situ hybridization of whole-brain sagittal sections (*Left*) and immunofluorescent images of anti–FLAG-tag staining in hippocampal CA1 region pyramidal cells (*Right*) from animals with the indicated genotypes. (*C*) Western blot analyses of PME-1, PP2A catalytic (PP2A/C), and regulatory (Bα) subunit and LCMT-1 protein expression in hippocampal homogenates carried out using primary antibodies directed against the proteins indicated at left and normalized to β-actin reveal a 379 ± 44% increase in PME expression in *tetO-PME/CaMKtTA* animals (upper transgenic + lower endogenous bands) vs. *tetO-PME* or *CaMKtTA* controls ($P < 0.001$) and unchanged levels of PP2A/C and Bα (103 ± 1.4 and 98 ± 2.7% of control, respectively; $P > 0.05$.) (*D*) Western blot analyses of PP2A methylation in hippocampal homogenates from transgenic animals overexpressing PME-1 carried out using a methyl-PP2A/C–specific and a methylation-insensitive total PP2A/C antibody reveal a 54.8 ± 6.6% reduction in methylated PP2A levels in *tetO-PME/CaMKtTA* animals vs. controls; $P < 0.001$. (*E*) Western blots showing increased tau phosphorylation but normal tau expression in hippocampal homogenates from transgenic animals overexpressing PME-1 were conducted using the phospho-specific tau antibodies PHF-1, S262, and AT8 or total tau and normalized to total tau or β-actin as indicated (PHF-1: Ser396/Ser-404: 210 ± 7%, $P < 0.001$; AT8: Ser202/Thr205: 139 ± 15%, $P < 0.05$; anti–phospho-S262: 116 ± 7.5%, $P < 0.05$; total tau: 98 ± 5.4%, $P > 0.05$ compared with controls). (*F*) Western blots using phospho-Thr-668–specific and total anti-APP antibodies reveal a 150 ± 5% increase in APP phosphorylation in hippocampal homogenates from transgenic animals overexpressing PME-1 compared with controls; $P < 0.001$. (*G*) Graph of the mean percent of control values (± SEM) obtained for the blots shown in *C*–*F*. (*H*) Graphs of the values and means (± SEM) obtained by ELISA for $A\beta_{1–40}$ (*Left*) or $A\beta_{1–42}$ (*Right*) conducted on hippocampal homogenates from animals overexpressing PME-1 ($n = 9$) and controls ($n = 10$) and normalized to the total amount of protein in each sample; $P > 0.05$ for $A\beta_{1–40}$ and $A\beta_{1–42}$ PME vs. control comparisons.

PME-1 overexpression also sensitized animals to Aβ-induced impairments in this task. PME-1 overexpression did not affect the performance of vehicle-infused animals, and a subthreshold dose of Aβ did not affect the performance of control animals. However, a subthreshold dose of Aβ did significantly impair the performance of animals overexpressing PME-1 (Fig. 2*B*). Tests

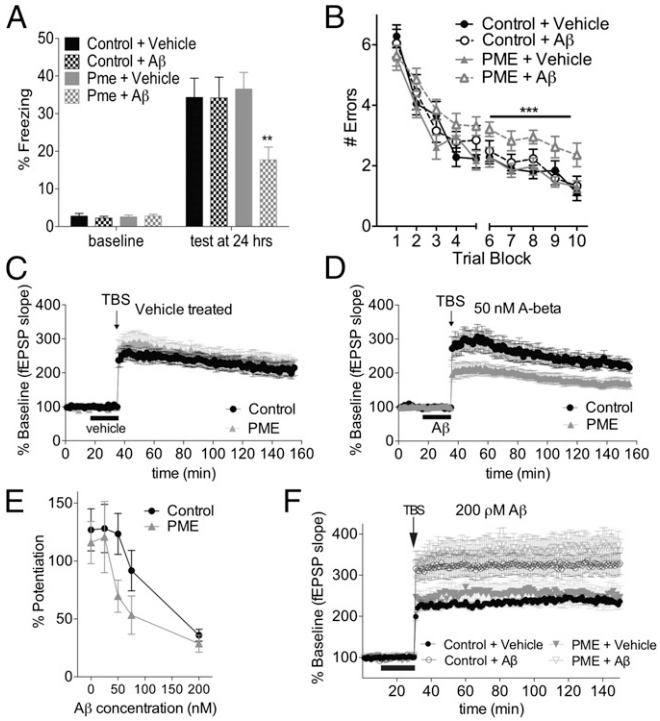

Fig. 2. PME-1 overexpression increases behavioral and electrophysiological impairments caused by subthreshold doses of Aβ without affecting picomolar Aβ responses. (*A*) Average percent of time (± SEM) spent freezing during the initial exposure to the training context (baseline) and 24 h after foot shock for the indicated genotype and treatment groups (n = 13 animals per group). Two-way ANOVA effect of training: $F(1,48) = 138.45$, $P < 0.0001$; training × group interaction: $F(3,48) = 3.43$, $P < 0.05$. Bonferroni post hoc comparisons of freezing at 24 h for treated animals overexpressing PME-1 vs. all other groups were $P < 0.01$ and $P > 0.05$ for baseline freezing responses. (*B*) Average number of errors committed (± SEM) during each three-trial training block of a 2-d radial arm water maze task for the indicated genotype and treatment groups. Two-way repeated-measures ANOVA with block and treatment as factors for PME vehicle vs. PME + Aβ on day 2 (blocks 6–10): $F(1,24) = 20.81$, $P < 0.0001$ for treatment; $P > 0.05$ for control vehicle vs. PME vehicle and for control vehicle vs. control Aβ on day 2. n = 13 animals per group. (*C* and *D*) Time course of averaged Schaffer collateral fEPSP responses in slices treated with vehicle (*C*) or 50 nM Aβ (*D*) 20 min before delivery of theta-burst stimulation (arrow). Repeated-measures ANOVA for PME + Aβ vs. control + Aβ: $F(1,22) = 5.23$, $P < 0.05$; $P > 0.05$ for control vehicle vs. PME vehicle and for control vehicle vs. control Aβ. $n > 10$ slices per group. (*E*) Dose–response curve showing increased Aβ-induced LTP inhibition in animals overexpressing PME-1 compared with controls. Plotted are average potentiated responses (± SEM) measured 105–115 min after theta-burst stimulation in slices treated with oligomeric Aβ at the indicated concentration 20 min before potentiating stimulation. Two-way ANOVA for genotype: $F(1,132) = 5.42$, $P < 0.05$. (*F*) Time course of averaged Schaffer collateral fEPSP responses (± SEM) in hippocampal slices from animals overexpressing PME-1 and control animals treated with vehicle or with 200 pM Aβ 20 min before delivery of theta-burst stimulation (arrow). Repeated measures ANOVA: PME + Aβ vs. PME + vehicle: $F(1,15) = 5.893$, $P < 0.05$; PME + Aβ vs. control + Aβ: $F(1,20) = 0.6677$, $P > 0.05$. n = 8 PME + vehicle, 9 PME + Aβ, 11 control + vehicle, and 13 control + Aβ slices.

of these animals on a visible platform version of the Morris water maze revealed no differences in escape latency or swim speed among these groups, suggesting that the combination of PME-1 overexpression and subthreshold Aβ exposure does not measurably impact motor performance, perception, or motivation in a nonspatial water maze task (Fig. S4).

Activity-dependent changes in the efficacy of synaptic transmission within the hippocampus are thought to be required for particular forms of memory, and interference with these changes, caused by elevated levels of Aβ, is thought to contribute to AD-associated memory impairments (31). Because PME-1 overexpression enhances Aβ-induced memory impairments, we sought to determine whether PME-1 overexpression also might enhance Aβ-induced impairment of activity-dependent synaptic plasticity. To do so, we tested the ability of Aβ to impair theta-burst–induced long-term potentiation (LTP) at Schaffer collateral synapses (32–34). This protocol produced robust potentiation that was comparable in animals overexpressing PME-1 and controls, suggesting that PME-1 overexpression alone did not affect LTP under these conditions (Fig. 2*C*). However, bath application of a subthreshold dose of Aβ (50 nM), which produced no significant effect on LTP in slices from control animals, significantly impaired LTP in slices from animals overexpressing PME-1 (Fig. 2 *C* and *D*). This increase in the sensitivity of slices overexpressing PME-1 to Aβ-induced LTP impairment also could be seen as a shift in the relationship between Aβ concentration and the corresponding LTP impairment (Fig. 2*E*). This enhancement of Aβ-induced LTP impairment was not accompanied by a shift in the stimulus intensity/response relationship in these animals (Fig. S4) and parallels the behavioral data we describe above, suggesting that PME-1–mediated enhancement of Aβ's effects on synaptic plasticity may lead to the enhanced Aβ-induced behavioral impairments we observed.

The impairment of LTP by Aβ is thought to reflect a pathological process that occurs at high Aβ concentrations in patients with AD. However, at low (picomolar) concentrations of Aβ, LTP is enhanced, and this effect is thought to reflect a normal physiological function of the protein (33, 35, 36). To determine whether PME overexpression also affects sensitivity to picomolar concentrations of Aβ, we recorded theta-burst–evoked LTP at Schaffer collateral synapses in the presence or absence of 200 pM oligomeric Aβ. In these experiments, picomolar Aβ application caused a significant enhancement of LTP over corresponding vehicle-treated slices that was comparable in both control animals and animals overexpressing PME-1 (Fig. 2*F*). These results are consistent with the observation that baseline LTP, behavior, and Aβ production are all normal in mice overexpressing PME-1 and suggest that PME-1 overexpression may selectively affect the response of these animals to pathological levels of Aβ without affecting Aβ's normal physiological function.

LCMT-1 Overexpression in Transgenic Mice. Because we found that PME-1 overexpression sensitized animals to Aβ-induced impairments, we sought to determine whether LCMT-1 overexpression might exert an opposite effect on Aβ sensitivity. To do so, we generated mice carrying a transgene encoding FLAG epitope-tagged murine PP2A methyltransferase (LCMT-1) under the control of a synthetic *tetO* promoter and crossed these animals with mice carrying the same *CaMK-tTA* transgene. *LCMT-1* transgene-specific RNA in situ hybridization revealed expression throughout the forebrain, including the striatum, olfactory bulb, cortex, and hippocampus, in animals that carried both the *tetO-LCMT* and *CaMK-tTA* transgenes but not in single-transgenic siblings (Fig. 3*A*, *Left*). Anti-FLAG epitope tag immunohistochemistry on brain sections from *tetO-LCMT/CaMKtTA* double-transgenic animals revealed transgenic protein in cell bodies and dendrites of pyramidal cells of the hippocampal CA1 region of double-transgenic animals that was not present in single-transgenic control animals (Fig. 3*A*, *Right*).

Quantitative Western blot analysis of hippocampal extracts from *tetO-LCMT/CaMKtTA* double-transgenic animals revealed a significant increase in *LCMT-1* expression compared with single-transgenic control animals (Fig. 3*B*). As was the case for PME-1 overexpression, *LCMT-1* transgene expression in these animals did not affect PP2A catalytic subunit expression, PP2A Bα regulatory subunit expression (Fig. 3*B*), or methylation metabolite levels (Fig. S1), nor did it affect endogenous PME-1 expression (Fig. 3*B*). Western blot analysis using methyl-PP2A/C–specific antibodies failed to reveal any significant change in PP2A methylation in *tetO-LCMT/CaMKtTA* double-transgenic animals (Fig. 3*C*). However, this result likely reflects the nearly saturating levels of PP2A methylation that exist under basal conditions (37). In addition, with the exception of a modest decrease in paired helical

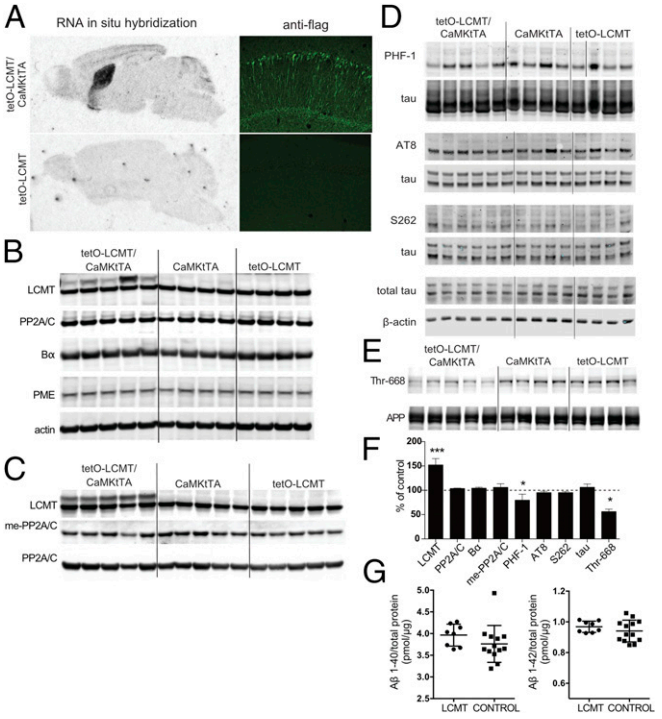

Fig. 3. *LCMT-1* transgene expression in the forebrain of transgenic mice. (*A*) Representative images of transgene-specific RNA in situ hybridization of whole-brain sagittal sections (*Left*) and immunofluorescent images of anti–FLAG-tag staining in hippocampal CA1 region pyramidal cells (*Right*) from animals with the indicated genotypes. (*B*) Western blot analyses of LCMT-1, PP2A/C, regulatory (Bα) subunit, and PME-1 protein expression in hippocampal homogenates from *tetO-LCMT/CaMKtTA* double-transgenic animals and single-transgenic siblings carried out using primary antibodies directed against the proteins indicated at left and normalized to β-actin reveal a 152 ± 13% increase in LCMT expression in *tetO-LCMT/CaMKtTA* animals (upper transgenic + lower endogenous bands) vs. *tetO-LCMT* or *CaMKtTA* controls ($P < 0.001$) and unchanged levels of PP2A/C and Bα (104 ± 1.0 and 104 ± 2.0% of control, respectively; $P > 0.05$). (*C*) Western blot analyses of PP2A methylation in hippocampal homogenates from transgenic animals overexpressing LCMT-1 carried out using a methyl-PP2A/C–specific and a methylation-insensitive total PP2A/C antibody reveal no change (105.9 ± 7.3% of control) in methylated PP2A levels in *tetO-LCMT/CaMKtTA* animals vs. controls; $P > 0.05$. (*D*) Western blots showing tau phosphorylation and total tau expression in hippocampal homogenates from transgenic animals overexpressing LCMT-1 conducted using phospho-specific tau antibodies PHF-1, S262, and AT8 or total tau and normalized to total tau or β-actin as indicated. PHF-1: 79.5 ± 12.4% of control, $P < 0.05$; AT8: 95 ± 2.9% of control, $P > 0.05$; S262: 95 ± 2.4% of control, $P > 0.05$; total tau: 106 ± 6.7% of control, $P > 0.05$. (*E*) Western blots showing reduced APP phosphorylation (56 ± 5% of control) in hippocampal homogenates from transgenic animals overexpressing LCMT-1 conducted using a phospho-Thr-668 antibody (*Upper*) and a phosphorylation-insensitive total APP antibody for normalization (*Lower*); $P < 0.001$. (*F*) Graph of the mean percent of control values (± SEM) obtained for the blots shown in *B–E*. (*G*) Graphs of the values and means (± SEM) obtained by ELISA for $A\beta_{1–40}$ (*Left*) or $A\beta_{1–42}$ (*Right*) conducted on hippocampal homogenates from animals overexpressing LCMT ($n = 8$) and controls ($n = 13$) and normalized to the total amount of protein in each sample. $P > 0.05$ for $A\beta_{1–40}$ and $A\beta_{1–42}$ LCMT vs. control comparisons.

filament 1 (PHF-1) antibody immunoreactivity, we did not observe corresponding decreases in phospho-tau immunoreactivity in animals overexpressing LCMT-1 (Fig. 3*D*). However, this result also may be caused by the relatively low levels of phosphorylation that exist at these sites under basal conditions (38). We did find that LCMT-1 overexpression led to a decrease in APP phosphorylation at the PP2A-sensitive Thr-668 site (Fig. 3*E*). Like PME-1 overexpression, LCMT-1 overexpression was without significant effect on basal hippocampal $A\beta_{1–40}$ or $A\beta_{1–42}$ levels (Fig. 3*F*).

LCMT-1 Overexpression Reduces Physiological and Behavioral Impairments Caused by Exposure to Nanomolar Concentrations of Aβ Without Affecting Responses to Picomolar Concentrations of Aβ. Like the mice overexpressing PME-1, animals overexpressing LCMT-1 were overtly indistinguishable from their single-transgenic control siblings. They were fertile and were recovered at the expected frequencies from crosses of double-transgenic males to wild-type females (Fig. S2). Analysis of these animals' behavior in a novel open field revealed no genotype effects (Fig. S5). To determine whether LCMT-1 overexpression might protect against Aβ-induced impairments, we subjected animals overexpressing LCMT-1 to the contextual fear-conditioning and radial arm water maze tasks described above. In these experiments, however, we infused animals with Aβ at a higher concentration that produced behavioral impairments in control animals (bilateral infusions of 1 μL of 200 nM oligomeric Aβ).

We found that LCMT-1 overexpression protected against Aβ-induced impairment in the contextual fear-conditioning task. Vehicle-treated control animals exhibited a robust freezing response when tested 24 h after training that was significantly reduced in Aβ-infused controls but was unaffected in similarly infused animals overexpressing LCMT-1 (Fig. 4*A*). This effect was specific to the Aβ-induced impairment in these animals, because vehicle-treated animals overexpressing LCMT-1 showed levels of freezing comparable to those of vehicle-treated controls. Moreover, we observed no differences in baseline freezing, open-field ambulatory activity, or shock perception among these groups (Fig. 4*A* and Fig. S6), suggesting that contextual fear-conditioning performance was not affected by differences in baseline behaviors or sensory perception.

LCMT-1 overexpression also protected against Aβ-induced behavioral impairment in the radial arm water maze task. Aβ significantly impaired performance in control animals but not in similarly infused animals overexpressing LCMT-1 (Fig. 4*B*). The performance of vehicle-treated animals overexpressing LCMT-1 was comparable to that of vehicle-treated controls, suggesting that this difference was specific to the Aβ response of these animals. Tests on a visible platform version of the Morris water maze revealed no differences in escape latency or swim speed among these groups, suggesting that neither transgene expression nor Aβ treatment measurably affected motor performance, perception, or motivation in a nonspatial water maze task (Fig. S6).

To test whether LCMT-1 overexpression also protected against Aβ-induced LTP impairment, we recorded theta-burst–induced LTP in Aβ-treated or vehicle-treated acute hippocampal slice preparations from these animals and controls. Bath application of Aβ at a dose that significantly impaired LTP in single-transgenic controls produced significantly less impairment in animals overexpressing LCMT-1 (Fig. 4 *C* and *D*). This effect was not accompanied by changes in the stimulus/response relationship in these animals (Fig. S6). As was the case for mice overexpressing PME-1, this correlation between LCMT's behavioral and electrophysiological effects suggests that LCMT-1 overexpression may protect against Aβ-induced behavioral impairments by reducing Aβ-induced electrophysiological impairments.

Because LCMT-1 overexpression reduced the sensitivity of animals to impairments caused by nanomolar concentrations of Aβ, we sought to determine whether it also reduced the response to low, picomolar concentrations of Aβ. To do this, we recorded theta-burst–evoked LTP at Schaffer collateral synapses in the presence or absence of 200 pM oligomeric Aβ and found that LTP enhancement was comparable in control animals and animals overexpressing LCMT-1 (Fig. 4*E*). As was the case for animals overexpressing PME-1, these results are consistent with the observation that baseline LTP, behavior, and Aβ production are all normal in mice overexpressing LCMT-1 and indicate that LCMT-1 overexpression may protect selectively against the pathological actions of Aβ without affecting Aβ's normal physiological functions.

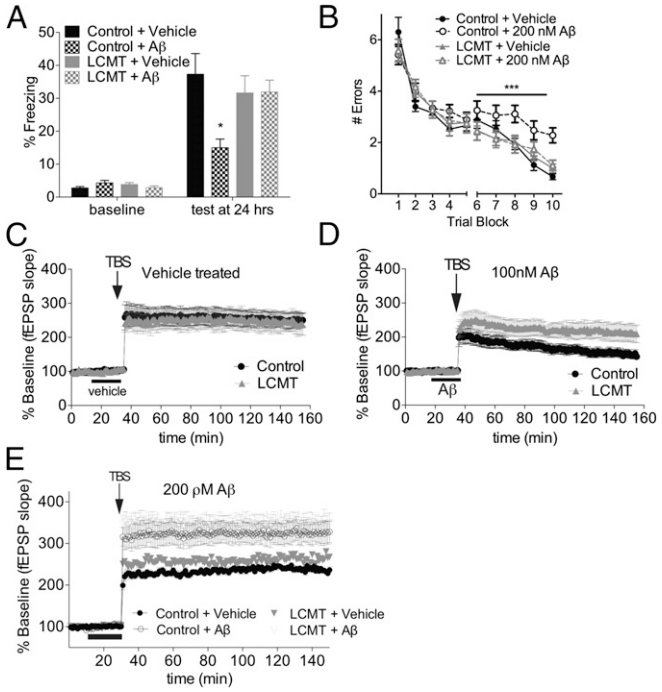

Fig. 4. LCMT-1 overexpression reduces behavioral and electrophysiological impairments caused by oligomeric Aβ. (*A*) Average percent of time spent freezing (± SEM) during initial exposure to the training context (baseline) and 24 h after foot shock for the indicated genotype and treatment groups Two-way ANOVA effect of training: $F(1,43) = 134.51$, $P < 0.0001$; training × group interaction: $F(3,43) = 5.50$, $P < 0.01$. Bonferroni post hoc comparisons of freezing at 24 h: $P < 0.01$ for Aβ-treated vs. vehicle-treated controls and $P > 0.05$ for LCMT + AB vs. vehicle-treated controls. $P > 0.05$ for all baseline freezing response comparisons ($n = 11$ or 12 animals per group). (*B*) Average number of errors committed (± SEM) during each three-trial training block of a 2-d radial arm water maze task for the indicated genotype and treatment groups ($n = 11$ or 12 animals per group). Two-way repeated-measures ANOVA with block and treatment as factors for control + vehicle vs. control + Aβ on day 2 (blocks 6–10): $F(1,21) = 27.74$; $P < 0.0001$ for treatment; $P > 0.05$ for control + vehicle vs. LCMT + Aβ and control vehicle vs. LCMT vehicle. (*C* and *D*) Time course of averaged Schaffer collateral fEPSP responses in slices treated with vehicle (*C*; $n = 9$ LCMT, 11 control slices) or 100 nM Aβ (*D*; $n = 9$ LCMT, 13 control slices) 20 min before delivery of theta-burst stimulation (arrow). Repeated-measures ANOVA for treatment on vehicle vs. Aβ-treated controls: $F(1,22) = 10.73$; $P < 0.01$. Repeated-measures ANOVA for genotype on Aβ-treated LCMT vs. control slices: $F(1,20) = 5.13$, $P < 0.05$. (*E*) Time course of averaged Schaffer collateral fEPSP responses (± SEM) in hippocampal slices from animals overexpressing LCMT-1 and control animals treated with vehicle or 200 pM Aβ 20 min before the delivery of theta-burst stimulation (arrow) ($n = 12$ LCMT + vehicle, 15 LCMT + Aβ slices). Repeated-measures ANOVA for treatment: LCMT + Aβ vs. vehicle: $F(1,25) = 8.125$, $P = 0.0086$; Repeated-measures ANOVA for genotype: LCMT + Aβ vs. control + Aβ $F(1,26) = 0.4638$, $P = 0.5019$. Pooled control data are plotted in both Fig. 2*E* and here for comparison.

Discussion

Here we show that PME-1 overexpression sensitizes mice to electrophysiological and behavioral impairments caused by acute exposure to nanomolar concentrations of synthetic, oligomeric Aβ and that LCMT-1 overexpression protects against these impairments. These complementary effects of PME-1 and LCMT-1 overexpression on Aβ sensitivity are consistent with the opposing actions of these enzymes on PP2A methylation (8, 9) and support the contention that altered PP2A methylation underlies this altered Aβ sensitivity. However, we cannot rule out the possibility that PME-1 and LCMT-1 act on PP2A via mechanisms that do not require methylesterase or methyltransferase activity, such as PME-mediated displacement of manganese ions from the PP2A catalytic subunit (25) or stabilization of inactive forms of PP2A (39, 40). LCMT-1 and PME-1 also may interact with as yet unidentified substrates. Although PP2A is the only known substrate for these enzymes, a recent study did find that the closely related phosphatase, PP4, can interact with a mutant form of PME-1 when overexpressed in cultured cells (41).

The effects of PME-1 and LCMT-1 overexpression on Aβ sensitivity exhibit two features with particular relevance for the potential of this pathway as a therapeutic target for AD treatment or prevention. First, the overexpression of PME-1 and LCMT-1 alters the response of cells and animals to Aβ. Although this altered Aβ sensitivity might have been explained by altered levels of endogenous Aβ that shift the threshold for exogenous Aβ-induced impairment, we detected no changes in basal $Aβ_{40}$ and $Aβ_{42}$ levels in brain homogenates from these animals, no changes in baseline LTP or behavioral performance, and no evidence for occlusion of the electrophysiological response of these animals to picomolar Aβ exposure. This effect on Aβ sensitivity is a clear distinction from therapeutic strategies that seek to alter Aβ production or clearance. The second notable feature of this pathway is that it appears to alter selectively the response to pathological concentrations of Aβ. PME-1 and LCMT-1 bidirectionally altered both the electrophysiological and behavioral responses to nanomolar Aβ administration, without affecting baseline LTP, behavioral performance, or the ability of the administration of 200 pM Aβ to enhance LTP. Given that normal physiological levels of Aβ play an important role in regulating neuronal activity (35, 42–44), this ability to target Aβ's pathological actions selectively could provide an approach that reduces AD-related impairments without interfering with normal nervous system function. Identifying the molecules acting downstream of PP2A in this process also will help clarify the molecular mechanisms by which Aβ exerts its pathological effects. The transgene-dependent changes in tau and APP phosphorylation that we describe mark these molecules as candidates in this regard; however, additional work is needed to determine the relevance of these changes for Aβ sensitivity.

Together these data add to the growing body of evidence suggesting that PP2A-centric therapies may be beneficial for AD and other tauopathies (reviewed in refs. 3 and 45) and specifically highlight the PP2A-regulatory enzymes PME-1 and LCMT-1 as potential therapeutic targets for this purpose.

Methods

Transgenic Animals. *tetO* promoter-driven constructs expressing either Flag-tagged murine PME-1 or Flag-tagged murine LCMT-1 were generated using standard molecular cloning techniques and were used to generate transgenic mice by pronuclear injection into C57BL6/J oocytes. Transgene-containing animals were crossed to an existing *CaMKIIα-tTA* line (26), also in a C57BL6/J background, and double-transgenic animals were outcrossed to wild-type 129SVEV/TAC mice to generate the C57BL6/J × 129SVEV/TAC F1 animals used for experiments. All experiments were carried out in a manner consistent with National Institutes of Health guidelines (46) and were approved by the Columbia University Institutional Animal Care and Use Committee. We used adult animals between 3 and 6 mo of age for all experiments. Males were used for behavioral experiments, and males and females were used in equal proportions for all other experiments. For all experiments, animals that carried the *CaMKIIα-tTA*, *tetO-PME*, or *tetO-LCMT* transgenes alone were used as controls. We observed no differences among these control groups, suggesting that the phenotypes observed in the *CaMKIIα-tTA/tetO-PME* or *CaMKIIα-tTA/tetO-LCMT* double-transgenic animals were dependent on *PME-1* or *LCMT-1* transgene expression and were not an artifact of transgene insertion.

Oligonucleotide in Situ Hybridization and Immunohistochemistry. RNA in situ hybridization was carried out as described previously (47) using a probe specific to the 3′ UTR of both the *LCMT-1* and *PME-1* transgenes. Immunohistochemistry was carried out on sections from paraformaldehyde-perfused animals using an anti-FLAG primary antibody and imaged by confocal microscopy.

Western Blots. Western blots were carried out on hippocampal homogenates prepared from microwave-fixed or snap-frozen samples and probed with the indicated antibodies as described previously (13, 48) and as described in more detail in *SI Methods*.

Aβ Preparation and Infusion. Oligomeric Aβ was prepared from synthetic Aβ 1–42 peptides (American Peptide) as described previously (33, 49). For behavioral experiments, Aβ was infused into the hippocampus via bilaterally implanted cannulae at the indicated volumes and concentrations.

Fear-Conditioning and Water Maze Tasks. Contextual fear-conditioning and radial arm water maze tasks were carried out as described previously (27, 30). In the contextual fear-conditioning task freezing behavior was assessed during re-exposure to the training context 24 h after a single foot shock. In the radial arm water maze task, entries into non–platform-containing arms were scored as errors during 10 blocks of three trials, each conducted over 2 d. A single Aβ or vehicle infusion was administered 20 min before training in the contextual fear-conditioning task and 20 min before and midway through training on both days of the radial arm water maze task.

Electrophysiological Recordings. Field excitatory postsynaptic potential (fEPSP) recordings of synaptic responses at Schaffer collateral synapses were carried out in 400-μm acute hippocampal slices maintained in an interface chamber at 29 °C as described previously (33).

Aβ ELISA Measures. $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were determined by commercially available ELISA kits (Wako) in hippocampal homogenates prepared as described previously (50).

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