Aspirin binds to PPAR α to stimulate hippocampal plasticity and protect memory

Dhruv Patel^{a,1}, Avik Roy^{a,b,1}, Madhuchhanda Kundu^a, Malabendu Jana^{a,b}, Chi-Hao Luan^c, Frank J. Gonzalez^d, and Kalipada Pahan^{a,b,2}

^aDepartment of Neurological Sciences, Rush University Medical Center, Chicago, IL 60612; ^bDivision of Research and Development, Jesse Brown Veterans Affairs Medical Center, Chicago, IL 60612; ^cHigh-Throughput Analysis Laboratory and Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208; and ^dLaboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Edited by Gregory A. Petsko, Weill Cornell Medical College, New York, NY, and approved June 14, 2018 (received for review February 2, 2018)

Despite its long history, until now, no receptor has been identified for aspirin, one of the most widely used medicines worldwide. Here we report that peroxisome proliferator-activated receptor α (PPAR α), a nuclear hormone receptor involved in fatty acid metabolism, serves as a receptor of aspirin. Detailed proteomic analyses including cheminformatics, thermal shift assays, and TR-FRET revealed that aspirin, but not other structural homologs, acts as a PPAR α ligand through direct binding at the Tyr314 residue of the PPAR α ligand-binding domain. On binding to PPAR α , aspirin stimulated hippocampal plasticity via transcriptional activation of cAMP response element-binding protein (CREB). Finally, hippocampus-dependent behavioral analyses, calcium influx assays in hippocampal slices and quantification of dendritic spines demonstrated that low-dose aspirin treatment improved hippocampal plasticity and memory in FAD5X mice, but not in FAD5X/*Ppara*-null mice. These findings highlight a property of aspirin: stimulating hippocampal plasticity via direct interaction with PPAR α .

aspirin | PPAR α | plasticity | memory and learning

Alzheimer's disease (AD) is the predominantly fatal form of dementia that affects up to 1 in 10 American age 65 y and older (1). In 2017, total annual primary care payments for individuals living with AD or other dementias in the United States was estimated at approximately \$259 billion, and this is expected to rise to \$1.1 trillion by 2050 (2). Hippocampal plasticity, which has been implicated in the stimulation of learning, memory, and antidepressive response, is down-regulated during the progression of AD (3, 4). Therefore, regulation of hippocampal plasticity has long-lasting implications not only in the prevention of AD pathology, but also in the preservation of memory in healthy brains.

Acetylsalicylic acid, commonly known as aspirin, is a widely used nonsteroidal anti-inflammatory drug often consumed as an analgesic to relieve pain and fever (5, 6). The prototype target of aspirin is cyclooxygenase; by inhibiting this proinflammatory enzyme, aspirin is known to suppress the production of prostaglandins (7). Along with its extensive use as an analgesic and antipyretic, aspirin also exhibits beneficial effects in atherosclerosis, cardiovascular diseases, and several cancers (8, 9). According to Nilsson et al. (10), high-dose aspirin users also exhibit a lower prevalence of AD and better maintenance of cognitive functions. Here we demonstrate that aspirin alone is capable of stimulating hippocampal plasticity. Interestingly, while investigating the mechanism for this, we observed that aspirin binds to PPAR α at the Tyr314 residue of its ligand-binding domain (LBD). On binding to the PPAR α LBD, aspirin induces activation of PPAR α to up-regulate transcription of the *Creb* gene and associated hippocampal plasticity. Furthermore, low-dose of aspirin treatment increased the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and *N*-methyl-D-aspartate (NMDA)-mediated calcium current in hippocampal slices and improved memory and learning in the FAD5X, but not FAD5X/*Ppara*-null, mice. These studies suggest

that aspirin may have a beneficial effect in AD via PPAR α -mediated up-regulation of hippocampal plasticity.

Results

Aspirin Up-Regulates Synaptic Function. Calcium influx through NMDA- and AMPA-type glutamate receptors regulates diverse processes, including kinase and phosphatase activities, protein trafficking, structural and functional synaptic plasticity, cell growth, cell survival, and apoptosis (11–13). Therefore, we investigated whether aspirin could evoke the calcium influx in cultured hippocampal neurons. Interestingly, following aspirin treatment, both AMPA and NMDA elicited a stronger calcium influx (Fig. 1*A* and *B*). Dendritic spines are the major sites of excitatory synaptic transmission in the central nervous system, and their size and density influence the functioning of neuronal circuits (14, 15). Therefore, we examined the effect of aspirin on spine density in cultured hippocampal neurons and found that aspirin treatment significantly increased the density (Fig. 1*C* and *D*). We validated these observations by measuring spine size. Consistent with the up-regulation of spine density, aspirin treatment significantly increased spine size in the hippocampal neurons (Fig. 1*E*). Among the neurotrophins (NTs), BDNF stands out for its ability to regulate the formation of plasticity and neuronal networks in the hippocampus (16–20). Interestingly, aspirin treatment stimulated the expression of BDNF mRNA in the hippocampal neurons (Fig. 1*F* and *G*). Immunoblot (Fig. 1*H* and *I*) and immunocytochemical analyses (Fig. 1*J*) further corroborated that aspirin can up-regulate the expression of BDNF protein in E18 hippocampal neurons.

In addition to BDNF, other members of the NT family, NT3 and NT4, also participate in the regulation of synaptic plasticity (21, 22). BDNF and NT4 mediate their synaptic modulatory

Significance

Aspirin, one of the most widely used medications worldwide, binds to PPAR α ligand-binding domain at the Tyr314 residue to up-regulate hippocampal plasticity via transcription of CREB. Accordingly, low-dose aspirin also improved hippocampal function in an animal model of Alzheimer's disease via PPAR α . These results delineate a new receptor of aspirin through which it may protect memory and learning.

Author contributions: K.P. designed research; D.P., A.R., M.K., M.J., and C.-H.L. performed research; F.J.G. contributed new reagents/analytic tools; D.P., A.R., and K.P. analyzed data; and D.P., A.R., F.J.G., and K.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Published under the PNAS license.

¹D.P. and A.R. contributed equally to this work.

²To whom correspondence should be addressed. Email: Kalipada_Pahan@rush.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1802021115/-DCSupplemental.

Published online July 16, 2018.

Fig. 1. Aspirin up-regulates synaptic plasticity in hippocampal neurons. Hippocampal neurons were treated with 5 μ M aspirin for 18 h, followed by monitoring AMPA-induced (A) and NMDA-induced (B) calcium influx in a PerkinElmer VICTOR X2 luminescence spectrometer. To nullify the secondary involvement of AMPA receptor in NMDA-dependent calcium currents, hippocampal neurons were treated with NMDA together with NASPM, followed by the recording of calcium influx. Similarly, AMPA-dependent calcium influx was measured in the presence of N20C. Results are presented as the mean of three independent experiments. (C) Hippocampal neurons were treated with 5 μ M aspirin for 18 h, followed by immunostaining with neuronal marker MAP2 (white) and Alexa Fluor 647-conjugated phalloidin (red) for spines. (Scale bars: 10 μ m.) (D) Spine density was measured from phalloidin-stained hippocampal neurons in response to aspirin treatment and plotted as a function of 10- μ m-long dendrites using boxplot analysis. Results are the mean $\pm$ SD of spines measured from 13 different neurons. Significance of the mean was tested with one-way ANOVA (effector: treatment), described as $^aF_{(1, 26)} = 2.948$; $P < 0.05$ ($= 0.01113$). (E) Spine size was calculated from 13 different aspirin-treated hippocampal neurons using the strategy depicted in the cartoon. Results are the mean $\pm$ SD of spines measured from 13 different neurons. One-way ANOVA (effector: treatment) with $^aF_{(1, 26)} = 3.846$; $P < 0.05$ ($= 0.0213$) was applied to test the significance of the mean between groups. (F and G) Hippocampal neurons were treated with aspirin for 6 h, followed by monitoring the mRNA expression of *Bdnf* by (F) RT-PCR (F) and real-time PCR (G). Results are the mean $\pm$ SD of three independent experiments. $^aP < 0.01$ vs. control; $^bP < 0.001$ vs. control. (H) Following dose-dependent aspirin treatment for 18 h, the expression of BDNF protein was investigated in hippocampal neurons by immunoblot analysis. (I) Relative density of BDNF protein expression compared with actin was calculated using ImageJ software. Results are the mean $\pm$ SD of three independent experiments. $^aP < 0.001$ vs. control. (J) Hippocampal neurons were immunostained with MAP2 (green) and BDNF (red). (Scale bars: 20 μ m.)

effect through receptor tropomyosin-related kinase B (TrkB) (23). NTs also can mediate programmed cell death by activating another NT receptor, p75^{NTR} (21). Therefore, we next examined the effect of aspirin on these NTs and their receptors in cultured hippocampal neurons. Aspirin treatment increased the mRNA expression (SI Appendix, Fig. S1A and B) and protein expression (SI Appendix, Fig. S1C) of NT3 in hippocampal neurons. Similarly, aspirin also up-regulated the mRNA expression of NT4 and TrkB (SI Appendix, Fig. S1A and B). However, under similar treatment conditions, p75^{NTR} mRNA expression remained unaltered (SI Appendix, Fig. S1A and B). Taken together, these results suggest that aspirin is capable of stimulating the plasticity-associated functions in hippocampal neurons.

Aspirin Induces the Activation of PPAR α . We next investigated the mechanism by which aspirin up-regulates hippocampal plasticity. We previously showed that ligand-dependent activation of PPAR α can promote plasticity-associated function in hippocampal neurons (24). This prompted us to investigate if aspirin can activate PPAR α in hippocampal neurons. Since PPARs bind to a consensus sequence, PPRE (25), to study the activation of PPAR, we transfected mouse hippocampal neurons with tk-PPREx3-Luc, a PPRE-dependent luciferase construct, and measured luciferase activity. Aspirin treatment induced PPRE-driven luciferase activity in a dose-dependent manner, with maximum induction observed at 5 μ M (SI Appendix, Fig. S2A). Similarly, aspirin also induced PPRE-driven luciferase activity in WT

mouse astrocytes (*SI Appendix, Fig. S2B*). The inability of aspirin to induce PPARE-driven luciferase activity in *Ppara*-null astrocytes (*SI Appendix, Fig. S2B*) suggests that aspirin requires PPAR α to increase PPARE reporter activity.

Aspirin Serves as a Ligand of PPAR α . Since aspirin induced the activation of PPAR α , we examined whether aspirin can serve as a ligand of PPAR α . We first constructed a 3D structure of mouse PPAR α using homology modeling, performed with the *swissmodel* software module using human PPAR α (Protein Data

Bank ID code 1KKQ) as a template. We then used *swissdock*, a rigid body protein-ligand docking tool, to predict the interaction between aspirin and the LBD of PPAR α at the molecular level. Based on electrostatic (E_{tot}) and desolvation (E_{sol}) energies, *swissdock* in conjunction with *Chimera* software resolved and displayed the most stable docked structure. According to this analysis, we found that aspirin docked very well in the interface of the LBD of PPAR α (Fig. 2*A* and *B*). We also observed that aspirin engaged with the Tyr314 residue of the PPAR α LBD with a strong hydrogen bond interaction (3.51 Å). We further

Fig. 2. Characterization of interaction of aspirin with PPAR α at molecular level. (A) A rigid-body in silico docked pose of the PPAR α LBD with aspirin. The interaction was evaluated with free energy (ΔG) = -6.46 kcal/mol, desolvation energy (E_{sol}) = $-1,823.1$ kcal/mol, and total energy (E_{tot}) = $-1,611.88$ kcal/mol. (B) Electrostatic potential surface of interaction between the LBD and aspirin. Red indicates a negatively charged surface; blue, a positively charged surface; white, a neutral surface. Black circle highlights the docked pose of aspirin. (C) While analyzing the interaction between aspirin and PPAR α , the most stable docked structure was generated by similar rigid-body analysis with free energy (ΔG) = -5.49 kcal/mol, desolvation energy (E_{sol}) = $-2,866.05$ kcal/mol, and total energy (E_{tot}) = $-2,531.20$ kcal/mol. (D) Thermal shift assay of fPPAR α analyzed with two doses of aspirin (5 and 10 μ M). (E) Thermal shift analyses of Y314DPPAR α performed with a 5 μ M concentration of aspirin. (F) Increasing doses of aspirin altered the maximum fluorescence derived from the melting of PPAR α and Y314D PPAR α proteins, as plotted in this dose-response curve. (G) TR-FRET analysis confirming the interaction between aspirin and PPAR α . The curve was plotted as 520-nm/490-nm ratio of response with increasing doses of aspirin. Curve-fitting was done using GraphPad Prism. The analysis generated EC₅₀ (4.194 μ M) and Hill slope (9.267) values based on the sigmoidal curve-fitting equation: $Y = \text{bottom} + (X_{\text{Hill slope}} \times (\text{top} - \text{bottom}) / (X_{\text{Hill slope}} + \text{EC}_{50\text{Hill slope}}))$. Nuclear fractions of GFP-transduced (H), fPPAR α -transduced (I), and Y314DPPAR α -transduced (J) *Ppara*-null astrocytes treated with 5 μ M aspirin were extracted using a chloroform:methanol extraction procedure, followed by GC-MS analysis. Results were analyzed and confirmed after three independent experiments.

corroborated these observations by a mutation analysis, in which Tyr314 residue was mutated to aspartate (Y314DPPAR α). The docked structures of aspirin with Y314DPPAR α clearly indicated that the mutation indeed impaired the binding affinity of aspirin to PPAR α with a very weak electrostatic interaction at a distance of 7.8 Å (Fig. 2C).

Similarly, we also performed rigid body docking analyses to evaluate the affinities of aspirin-like molecules and other non-steroidal anti-inflammatory drugs (NSAIDs) with PPAR α . Five different compounds with structural similarities to aspirin, including ibuprofen, celecoxib, methyl salicylate, methyl-4-hydroxy benzoate, and naproxen, were docked with the LBD of PPAR α (SI Appendix, Fig. S3). Interestingly, our *in silico* analyses revealed that these structural homologs of aspirin displayed very weak interactions with PPAR α (SI Appendix, Fig. S3), suggesting that compared with other structural homologs, only aspirin displays a stronger affinity toward the PPAR α LBD.

To experimentally confirm the binding and activation of PPAR α by aspirin, thermal shift assays were carried out. In brief, full-length recombinant PPAR α (flPPAR α) was synthesized with lentivirus (24, 26) and its melting profile was monitored with the aid of a SYBR Green reaction at 27–94 °C. A typical sigmoidal melting curve with a melting temperature of 50.4 °C clearly indicated that the recombinant PPAR α protein is conformationally stable. Interestingly, in the melting assay revealed, 5 μ M of aspirin strongly shifted the melting curve of PPAR α to 53.6 °C (Fig. 2D). While analyzing the extent of shift of PPAR α protein in response to 5 and 10 μ M aspirin, we found that 10 μ M aspirin was unable to generate a larger shift (54.2 °C) in the thermal curve of PPAR α suggesting that 5 μ M aspirin shows optimal binding with PPAR α . In contrast, aspirin exhibited a thermal shift of only 0.98 °C with mutated Y314DPPAR α protein (Fig. 2E), indicating an interaction of aspirin with the Y314 residue of PPAR α . Consequently, our kinetic plot of maximum thermal response vs. increasing doses of aspirin ranging from 1 to 10 μ M (Fig. 2F) clearly revealed that 5 μ M aspirin displayed maximum binding affinity with flPPAR α . Again, aspirin exhibited very little binding affinity with mutated Y314DPPAR α protein (Fig. 2F).

The physiological interaction between aspirin and PPAR α can be influenced by recruitment of the coactivator PGC-1 α . Therefore, to validate the interaction of aspirin with PPAR α physiologically, we performed a time-resolved FRET coactivator assay. FRET analyses confirmed that aspirin indeed displayed a strong interaction with PPAR α (Fig. 2G). The binding curve resulted an EC₅₀ value as low as 4.19 μ M, with a Hill slope of 9.27. To further characterize the interaction between aspirin and PPAR α at the molecular level, GC-MS analysis was performed in astroglial cell lysate. In brief, *Ppara*-null astrocytes were transduced with flPPAR α or Y314D-PPAR α followed by treatment with 5 μ M aspirin for 1 h. Cells were homogenized, and nuclear chloroform extracts were prepared, passed through a GFP-affinity column, eluted, fractionated with chloroform and methanol, and then analyzed by GC-MS. Interestingly, aspirin displayed a strong interaction with PPAR α , as detected in affinity-purified nuclear extracts of GFP-flPPAR α -transduced (Fig. 2I), but not GFP-transduced (Fig. 2H), *Ppara*-null astroglial cells. The interaction was identified by the appearance of a sharp peak of acetyl salicylate at an *m/z* ratio of 180 (Fig. 2F). In contrast, Y314D mutation completely ablated the interaction of aspirin with PPAR α , as we did not observe any peak at an *m/z* ratio of 180, suggesting that Tyr314 is required for the interaction of PPAR α with aspirin (Fig. 2J).

Under physiological conditions, aspirin is known to be metabolized to other derivatives that also may exhibit an interaction with PPAR α . Accordingly, the affinity-purified chloroform fraction of GFP-flPPAR α -transduced *Ppara*-null astrocytes also identified the peak for 2-ethyl hexyl salicylate (SI Appendix, Fig. S4 A and B). However, we detected a smaller peak of 2-ethyl

hexyl salicylate in the nuclear extracts of *Ppara*-null astrocytes transduced with Y314DPPAR α (SI Appendix, Fig. S4C). Collectively, these data suggest that along with aspirin, its derivative 2-ethyl hexyl salicylate also displays a strong ligand-binding affinity with PPAR α at its Y314 residue.

Aspirin-Induced Activation of PPAR α Is Dependent on Its Interaction with the Y314 Residue of PPAR α . First, to confirm that aspirin-mediated induction of PPRE luciferase activity is due to activation of PPAR α , we overexpressed PPAR α in *Ppara*-null astrocytes. Interestingly, lentivirus-mediated insertion of flPPAR α followed by stimulation with 5 μ M aspirin significantly up-regulated PPRE luciferase activity in *Ppara*-null astrocytes (SI Appendix, Fig. S5). However, aspirin failed to induce PPRE luciferase activity in Y314DPPAR α -transduced *Ppara*-null astrocytes (SI Appendix, Fig. S5). Taken together, these results clearly demonstrate that aspirin interacts with the Y314 residue of PPAR α , and that this interaction is crucial for aspirin-induced transcriptional activation of PPAR α .

Aspirin-Induced Stimulation of Synaptic Function Is Dependent on Its Interaction with the Y314 Residue of PPAR α . We next wanted to determine whether aspirin stimulates synaptic function through PPAR α . Assessment of calcium influx through ionotropic receptors, including NMDA and AMPA receptors, has been considered the most reliable procedure for analyzing hippocampal function (11–13). Interestingly, aspirin was unable to induce calcium influx in cultured hippocampal neurons isolated from *Ppara*-null mice compared with WT mice (Fig. 3 A and B), suggesting that aspirin requires PPAR α to stimulate calcium influx in hippocampal neurons.

Next, to establish a direct role of Y314 residue of PPAR α in aspirin-induced calcium influx, we overexpressed flPPAR α and mutated Y314DPPAR α in *Ppara*-null hippocampal neurons, followed by stimulation with 5 μ M of aspirin. Interestingly, aspirin significantly stimulated both AMPA- and NMDA-mediated calcium currents in flPPAR α -transduced (Fig. 3 C and D), but not Y314DPPAR α -transduced (Fig. 3 E and F), *Ppara*-null hippocampal neurons.

To further confirm a direct role for PPAR α in the regulation of ionotropic calcium conduction in aspirin-stimulated neurons, we also overexpressed the Y314DPPAR α construct in WT hippocampal neurons, followed by stimulation with aspirin. Interestingly, infusion of Y314DPPAR α attenuated both NMDA- and AMPA-driven ionotropic calcium influxes (Fig. 3 G and H) in WT hippocampal neurons. In all cases, we also validated the effect of aspirin with gemfibrozil, a classical PPAR α agonist. Taken together, these results suggest that the interaction of aspirin with PPAR α Tyr314 is essential for aspirin-induced up-regulation of calcium influx through NMDA- and AMPA-sensitive receptors.

Along with the estimation of calcium influx, quantification of dendritic spine density is an important measure to assess hippocampal function. Therefore, we adopted a phalloidin-based quantification analysis of dendritic spines in aspirin-treated hippocampal neurons. In brief, *Ppara*-null hippocampal neurons were transduced with flPPAR α for 3–4 d and then treated overnight with aspirin. Then these cells and WT neurons were labeled with phalloidin and the dendritic marker MAP2. Accordingly, aspirin significantly increased spine density in WT, but not *Ppara*-null, hippocampal neurons. Interestingly, the introduction of flPPAR α , but not with empty vector, significantly increased spine counts in aspirin-stimulated hippocampal neurons (Fig. 3 I and J). We further confirmed these observations by measuring spine size (Fig. 3K) under the different treatment conditions. Collectively, these results suggest that aspirin up-regulates morphological plasticity in hippocampal neurons via PPAR α .

Fig. 3. Aspirin stimulates synaptic plasticity in hippocampal neurons via PPAR α . WT and *Ppara*-null E18 hippocampal neurons were treated with 5 μ M aspirin for 18 h, followed by analyzing AMPA-driven (A) and NMDA-driven (B) calcium influx. *Ppara*-null hippocampal neurons were transduced with either empty vector (blue) or flPPAR α (green) via a lentivirus strategy for 48 h, followed by stimulation with aspirin (dark green) or gemfibrozil (red). After 18 h of stimulation, neurons were analyzed for AMPA-driven (C) and NMDA-driven (D) calcium influx. Similarly, *Ppara*-null (α KO) hippocampal neurons were transduced with lenti-Y314DPPAR α (purple), followed by stimulation with aspirin (dark purple) or gemfibrozil (red). After 18 h, AMPA-stimulated (E) and NMDA-stimulated (F) calcium influx was measured. AMPA-driven (G) and NMDA-driven (H) calcium influx was measured in Y314DPPAR α -transduced WT E18 hippocampal neurons after treatment with aspirin and gemfibrozil. Calcium influx was monitored for 300 repeats in a PerkinElmer VICTOR X2 fluorimeter. Results represent three independent experiments. (I) WT and *Ppara*-null mouse hippocampal neurons transduced with lentivector containing flPPAR α were treated with aspirin for 18 h, followed by immunostaining with neuronal marker MAP2 (green) and Alexa Fluor 647-conjugated phalloidin (red) to stain dendritic spines. (Insets) Enlarged views of dendrites, boxed within white rectangles in the respective lower-magnification images. (Scale bars: 20 μ m.) (J) Spine density was measured from phalloidin-stained hippocampal neurons and plotted as a function of 10- μ m-long dendrites. Statistical analyses of spine density were performed in 13–21 dendrites per group using two-way ANOVA considering genotype [$F_{2, 105} = 135$; $P < 0.0001$ ($=0.000000$)] and treatment [$*F_{1, 105} = 3.04$; $P < 0.05$ ($=0.03107$)] as two independent variables. Interaction statistics between two independent variables were calculated as well [$F_{2, 105} = 6.14$; $P < 0.01$ ($=0.0030$)]. Tukey's HSD post hoc test was applied to assess the significance of the mean. (K) Spine size was quantified in seven to nine dendrites per group and analyzed with two-way ANOVA. The effects of genotype [$F_{2, 31} = 19.1$; $P < 0.0001$ ($=0.000000$)], treatment [$F_{1, 31} = 14.8$; $P < 0.001$ ($=0.0006$)], and their interaction [$F_{2, 31} = 2.91$; $P > 0.05$ ($=0.0692$)] were compared between groups. Tukey's HSD post hoc test was applied to measure significance.

Aspirin Stimulates the Transcription of CREB via Its Interaction with the Y314 Residue of PPAR α . CREB plays a central role in regulating hippocampal plasticity and memory (27, 28). Recently we found that PPAR α is not directly involved in the transcription of plasticity-related molecules and that PPAR α controls hippocampal plasticity via transcriptional up-regulation of CREB (24). Therefore, we investigated whether aspirin also controls *Creb* transcription via PPAR α . Accordingly, we observed that aspirin rapidly up-regulated the expression of *Creb* mRNA in primary mouse astrocytes (Fig. 4A and B). As clearly shown in Fig. 4C and D, aspirin increased the expression of CREB mRNA in a dose-dependent manner in WT and *Pparb*-null, but not *Ppara*-null, astrocytes. Immunoblot (Fig. 4E and F) and immunofluorescence analyses (Fig. 4G) again confirmed that aspirin indeed up-regulated the expression of CREB protein in the WT and *Pparb*-null, but not *Ppara*-null, astrocytes. However, overexpression of PPAR α restored the expression of *Creb* mRNA (SI Appendix, Fig. S6A and B) and CREB protein (SI Appendix, Fig. S6C–E) in *Ppara*-null astrocytes as well as hippocampal neurons (SI Appendix, Fig. S6F and G), indicating the involvement of PPAR α in aspirin-induced up-regulation of CREB. Moreover, pretreatment with GW9662, a PPAR γ antagonist, was unable to suppress the expression

of *Creb* mRNA in aspirin-treated WT astrocytes, thus nullifying the potential involvement of PPAR γ (SI Appendix, Fig. S7A and B). However, under similar treatment conditions, GW6471, a PPAR α antagonist, inhibited the aspirin-mediated expression of *Creb* mRNA (SI Appendix, Fig. S7C and D). Accordingly, aspirin also induced the recruitment of PPAR α , but not PPAR β or PPAR γ , to the *Creb* promoter (Fig. 4H–J), suggesting that aspirin stimulates the transcription of *Creb* via PPAR α .

To further verify the role PPAR α in aspirin-mediated transcription of the *Creb* gene, a PPRE containing the *Creb* promoter upstream of a luciferase reporter gene (p*Creb*-luc) construct was transfected into astrocytes, followed by stimulation with aspirin. As shown in Fig. 4K, 5 μ M of aspirin significantly stimulated *Creb* promoter-driven luciferase activity in astrocytes; however, aspirin failed to induce luciferase activity driven by mutated *Creb* promoter in which the PPRE was mutated, suggesting that aspirin-mediated transcription of *Creb* is dependent on PPAR α . To understand this specificity, we also monitored the effect of gemfibrozil, a prototype activator of PPAR α , on activation of the *Creb* promoter. Consistent with a role for PPAR α in this process, gemfibrozil also induced reporter activity driven by the *wt*-*Creb* promoter, but not by the mutated *Creb* promoter (SI Appendix, Fig. S8).

Fig. 4. Aspirin increases the transcription of CREB via PPAR α . (A and B) WT mouse astrocytes were treated with aspirin (5 μ M) for various durations, followed by measurement of the mRNA expression of CREB by RT-PCR (A) and real-time PCR (B). Results are mean $\pm$ SD of three independent experiments. ^a P < 0.05 vs. control; ^b P < 0.01 vs. control; ^c P < 0.001 vs. control. (C and D) RT-PCR (C) and real-time PCR (D) analyses were performed to analyze the mRNA expression of CREB in the WT, *Ppara*-null, and *Pparb*-null primary mice astrocytes treated with increasing doses (0, 2, and 5 μ M) of aspirin. Results are the mean $\pm$ SD of three independent experiments. ^a P < 0.001 vs. control. (E and F) WT (Left), *Ppara*-null (Middle), and *Pparb*-null (Right) mouse primary astrocytes were treated with increasing doses of aspirin and then analyzed by immunoblot analysis (E), followed by relative densitometric analysis (F) of the CREB protein. Results are mean $\pm$ SD of three independent experiments. ^a P < 0.001 vs. control. (G) Immunofluorescence analyses of GFAP (green) and CREB (red) were performed in WT, *Ppara*-null, and *Pparb*-null primary astrocytes after treatment with 5 μ M aspirin. (Scale bars: 20 μ m.) (H) Schematic representation of the *Creb* gene promoter with PPRE. (I and J) ChIP assays were performed using antibodies against PPAR α , PPAR β , and PPAR γ in aspirin-treated WT primary astrocytes. In brief, the promoter of the *Creb* gene was pulled down with PPAR α , PPAR β , and PPAR γ antibodies, followed by RT-PCR (I) and real-time PCR (J). Results are mean $\pm$ SD of three independent experiments. ^a P < 0.001 vs. control. (K) WT astrocytes were transfected with either WT-pCREB-Luc (blue) or mut-pCREB-Luc (red) constructs, followed by treatment with increasing doses of aspirin. After 2 h, luciferase activity was measured in a Promega GloMax luminometer. Results are mean $\pm$ SD of three independent experiments. ^a P < 0.05 vs. control; ^b P < 0.01 vs. control; ^c P < 0.001 vs. control.

Since aspirin binds to the Y314 residue of PPAR α , to determine whether this interaction is crucial for aspirin-mediated transcription of *Creb*, *Ppara*-null astrocytes were transduced with *lenti*-GFP, *lenti*-fl-*Ppara*, and *lenti*-Y314D*Ppara*. As expected, aspirin remained unable to induce the *Creb* promoter-driven luciferase activity in *lenti*-GFP-transduced *Ppara*-null astrocytes (SI Appendix, Fig. S9A); however, aspirin markedly induced *Creb* promoter-driven luciferase activity in *lenti*-fl-*Ppara*-transduced *Ppara*-null astrocytes (SI Appendix, Fig. S9B). In contrast, aspirin did not induce *Creb* promoter-driven reporter activity in *lenti*-Y314D*Ppara*-transduced *Ppara*-null astrocytes (SI Appendix, Fig. S9C), confirming the crucial role of PPAR α Y314 in aspirin-mediated up-regulation of *Creb* promoter activity.

To understand the functional significance of aspirin-mediated up-regulation of CREB via PPAR α , we examined whether aspirin requires PPAR α to up-regulate BDNF, one of the downstream targets of CREB. As evident from the immunofluorescence analyses, we noted that following aspirin treatment, BDNF expression was significantly higher in WT, but not *Ppara*-null, hippocampal (Fig. 5A) and cortical neurons (Fig. 5B). Similarly, aspirin was also observed to up-regulate the expression of *Bdnf* mRNA (Fig. 5C and D) in WT and *Pparb*-null, but not *Ppara*-null, astrocytes. These results were further corroborated by immunoblot (Fig. 5E and F)

and immunocytochemical (Fig. 5G) analyses demonstrating induction of BDNF protein by aspirin in WT and *Pparb*-null, but not *Ppara*-null, astrocytes.

Aspirin Enhances the Expression of Plasticity-Associated Hippocampal Proteins and Protects Memory in the FAD5X Mouse Model of AD via PPAR α .

We next analyzed whether aspirin uses PPAR α to protect hippocampal properties in vivo in mouse brain. For this, we generated *Ppara*-null mice on the FAD5X transgenic background (26). These bigenic animals are reliable tools for studying the role of PPAR α in AD-related pathologies in mice (26, 29). In this experiment, 6-mo-old FAD5X and FAD5X/*Ppara*-null mice were fed with aspirin (2 mg/kg body weight/d) for 4 wk, and then the hippocampi of these animals were tested for expression of CREB and CREB-dependent plasticity-associated proteins. Aspirin increased the expression of CREB (Fig. 6C and D) and BDNF (Fig. 6A and B) in the hippocampi of FAD5X, but not FAD5X/*Ppara*-null mice. Similarly, chronic administration of aspirin also increased the expression of other CREB-dependent proteins, including PSD95 (Fig. 6E and F and SI Appendix, Fig. S10), and NR2A (Fig. 6G and H), in the hippocampi of FAD5X mice but not FAD5X/*Ppara*-null mice, suggesting that PPAR α

Fig. 5. Aspirin increases the expression of BDNF via PPAR α . (A) WT and *Ppara*-null hippocampal neurons were treated with 5 μ M aspirin for 18 h, followed by double-labeling of MAP2 (green) and BDNF (red). (Scale bars: 20 μ m.) (B) Similar immunofluorescence analyses of BDNF were performed in WT and *Ppara*-null cortical neurons. (C and D) Primary astrocytes isolated from WT (Left), *Ppara*-null (Middle), and *Pparb*-null (Right) mice were treated with increasing doses of aspirin, and *Bdnf* mRNA was analyzed by RT-PCR (C) and real-time PCR (D). Results are mean $\pm$ SD of three independent experiments. ^a*P* < 0.05 vs. control; ^b*P* < 0.01 vs. control. (E and F) Similarly, protein expression of BDNF was determined by immunoblot analysis (E) followed by densitometric analysis (F) in WT (Left), *Ppara*-null (Middle), and *Pparb*-null (Right) mouse astrocytes treated with increasing dose of aspirin. The relative density of BDNF protein compared with actin was calculated using ImageJ software. Results are mean $\pm$ SD of three independent experiments. ^a*P* < 0.05 vs. control; ^b*P* < 0.01 vs. control. (G) Dual immunofluorescence analyses of GFAP (green) and BDNF (red) were performed in WT, *Ppara*-null, and *Pparb*-null mouse astrocytes in response to 5 μ M of aspirin. (Scale bars: 20 μ m.)

plays an essential role in aspirin-induced expression of plasticity-associated proteins in the hippocampus.

We next analyzed the effect of aspirin on the ionotropic calcium influx through NMDA and AMPA receptors in hippocampal slices of FAD5X and FAD5X/*Ppara*-null mice. Consistent with the increased expression of plasticity-associated molecules, aspirin feeding up-regulated AMPA-dependent (Fig. 6I) and NMDA-dependent (Fig. 6J) calcium influx, as measured in organotypic hippocampal slices from FAD5X animals, but not from FAD5X/*Ppara*-null bigenic mice. Taken together, these results suggest that aspirin requires PPAR α for controlling the expression of plasticity-associated molecules and their function *in vivo* in the hippocampus.

We then explored the effect of aspirin in improving hippocampus-dependent behaviors, including learning and memory, in FAD5X and FAD5X/*Ppara*-null mice. Hippocampus-dependent spatial learning and behavior can be reliably monitored using the Barnes maze test (24, 26) (Fig. 7A). As expected, and as reported in previous studies (26, 30), FAD5X mice displayed decreased spatial behaviors, as indicated by latency (Fig. 7B) and errors (Fig. 7C), compared with age-matched nontransgenic (NTG) mice. However, aspirin feeding significantly improved latency (Fig. 7B);

[$F_{1, 18} = 6.22$; $P < 0.05$ ($= 0.0226$)] and decreased errors (Fig. 7C) [$F_{1, 18} = 5.48$; $P < 0.05$ ($= 0.0310$)] in FAD5X mice. In contrast, aspirin treatment was unable to improve spatial learning in FAD5X/*Ppara*-null mice, indicating an essential role for PPAR α in aspirin-mediated improvement in hippocampus-dependent behaviors (Fig. 7B). The T-maze test assesses context-dependent hippocampal behavior, which may be a better index for testing cognition in mice. Consistently, our T-maze test also exhibited significant improvement in the performance of FAD5X mice, as demonstrated by the increased number of positive turns and reduction of errors, whereas FAD5X/*Ppara*-null mice did not show any improvement in T-maze test results following aspirin treatment (Fig. 7D and E), indicating a pivotal role of PPAR α in aspirin-mediated improvement in memory and learning in FAD5X mice.

To test the significance between groups, we performed two-way ANOVA with treatment [$F_{1, 18} = 14.68$; $P < 0.01$ ($= 0.0012$)] and genotype [$F_{1, 18} = 6.893$; $P < 0.05$ ($= 0.0172$)] as two effectors. Since the decreased latency in either the Barnes maze or T-maze test could be confounded with the increased locomotion of animals, we also monitored the speed of these animals in an open-field arena after 1 mo of aspirin feeding (SI

Fig. 6. Aspirin enhances plasticity-associated molecules and promotes calcium influx in the hippocampus of FAD5X mice via PPAR α . The 6- to 7-mo-old FAD5X and FAD5X/Ppara-null transgenic mice ($n = 5$ or 6 per group) were gavaged with aspirin (2 mg/kg body weight) for 30 d and the hippocampi were analyzed for the expression of different plasticity-associated proteins. Immunoblot analyses were performed for CREB (A), BDNF (C), PSD95 (E), and NR2A (G) in hippocampal extracts of NTG, FAD5X, FAD5X + aspirin, FAD5X/Ppara-null, and FAD5X/Ppara-null + aspirin mice. The relative densities of CREB (B), BDNF (D), PSD95 (F), and NR2A (H) proteins were measured using ImageJ. Results are presented as mean $\pm$ SEM of five or six mice per group. The significance of the mean was assessed using one-way ANOVA followed by Bonferroni's post hoc test. $^aP < 0.001$ vs. control-CREB; $^bP < 0.001$ vs. control-BDNF; $^cP < 0.001$ vs. control-PSD95; $^dP < 0.001$ vs. control-NR2A. (I and J) AMPA-dependent (I) and NMDA-dependent (J) calcium currents were measured in the hippocampal slices of NTG, FAD5X, FAD5X + aspirin, FAD5X/Ppara-null, and FAD5X/Ppara-null + aspirin mice. The arrow indicates the application of AMPA and NMDA in the assay. Results are representative of three independent experiments.

Appendix, Fig. S11A). We did not observe any significant difference in total distance moved (SI Appendix, Fig. S11B) or velocity (SI Appendix, Fig. S11C) across the different groups of animals, nullifying the possibility of interference by increased locomotion in the hippocampus-dependent behaviors.

Since hippocampal function is connected to stress and depression (31–33), we investigated the effect of aspirin on stress-related behaviors in the FAD5X and FAD5X/Ppara-null bigenic mice by analyzing their locomotive performance in the open-field arena (Fig. 7F). The FAD5X mice exhibited greater stress-related behaviors than seen in the NTG mice, as indicated by decreased center zone frequency and increased latency in corners (Fig. 7G and H). However, aspirin treatment significantly enhanced center zone frequency and reduced latency in corners (Fig. 7G and H) in the FAD5X mice. On the other hand, aspirin treatment was unable to reduce stress-related behaviors in the FAD5X/Ppara-null mice, further indicating that PPAR α is required for the amelioration of stress-related behavior on aspirin treatment. Collectively, these results demonstrate that aspirin up-regulates hippocampal plasticity, improves memory, and alleviates stress-like behaviors in a mouse model of AD via PPAR α .

Discussion

Memory loss is the earliest and most prominent symptom associated with progressive dementia in AD (34–36). To date, the Food and Drug Administration has approved very few drugs for the treatment of AD-related dementia, and these drugs provide only limited symptomatic relief but can cause unpleasant side effects, such as loss of appetite, nausea, vomiting, and diarrhea. Therefore, finding new drugs that can slow or prevent memory deficits in AD patients is an area of extensive active research. We have been endowed with a hippocampus, a key component of the medial temporal lobe memory circuit, which has the most important role in generating, organizing, and storing memory. Incidentally, the hippocampus is one of the first brain structures to exhibit neurodegenerative changes in AD. In this study, we present evidence that aspirin is capable of stimulating hippocampal plasticity and protecting memory. The World Health Organization includes aspirin in its 2017 Model List of Essential Medicines (EML) (37). The EML contains information on the most efficacious, safe, and cost-effective drugs that are needed for basic healthcare. Here we have demonstrated that aspirin evokes calcium influx, promotes spine density, and increases spine size in hippocampal neurons, ultimately leading to protection of memory and learning.

Fig. 7. Aspirin protects memory and alleviates stress-related behaviors in the FAD5X mice via PPAR α . The 6- to 7-mo-old FAD5X and FAD5X/*Ppara*-null transgenic mice were fed orally with aspirin (2 mg/kg/d) for 30 d, followed by assessment of hippocampus-dependent spatial behavior using the Barnes maze test. (A) Representative heat maps summarizing the overall activity of mice on the apparatus recorded with a Noldus camera and visualized using EthoVision XT software. (B and C) Latency (B) and number of errors made (C) by NTG, FAD5X, FAD5X + aspirin, FAD5X/*Ppara*-null, and FAD5X/*Ppara*-null + aspirin mice. Results are presented as mean $\pm$ SEM of 5–6 mice per group, and the significance of mean was assessed using two-way ANOVA followed by Bonferroni's post hoc test. Context-dependent hippocampal behavior was analyzed using the T-maze test. (D and E) Number of positive turns (D) and number of errors (E) made by NTG, control and aspirin-treated FAD5X and FAD5X/*Ppara*-null transgenic mice on an appetitive T-maze conditioning task were manually recorded. Results are presented as mean $\pm$ SEM of five or six mice per group, and the significance of the mean was monitored assessed using two-way ANOVA followed by Bonferroni's post hoc test. Stress-like behavior was investigated in an open-field apparatus. (F–H) Representative track plots (F) and graphs of center zone frequency (G) and total corner time duration (H) of NTG, FAD5X, FAD5X + aspirin, FAD5X/*Ppara*-null, and FAD5X/*Ppara*-null + aspirin mice. Results are presented as mean $\pm$ SEM of 5 or 6 mice per group, and the significance of the mean was monitored by two-way ANOVA followed by Bonferroni's post hoc test.

One of the earliest-discovered drugs, aspirin is known to inhibit the cyclooxygenase pathway. Until now, whether aspirin can directly bind to any receptor or transcription factor in cells was not clear. Here, using a combination of structural, functional, mutagenesis, mass spectrometric, and biochemical approaches, we have shown that aspirin binds to PPAR α , a nuclear hormone receptor involved in fatty acid metabolism. In silico computer-aided *swiss-dock* analyses, followed by site-directed mutagenesis and lentiviral packaging, revealed that aspirin interacts with a tyrosine residue (Y314) of the PPAR α LBD. This is interesting given our recent finding that statins, widely used cholesterol-lowering drugs, bind to

Leu331 and Tyr334 residues of PPAR α to produce a neuro-protective effect (26). However, we did not detect any interaction of aspirin with Leu331 and Tyr334 residues of PPAR α . TR-FRET analysis and a thermal shift assay also substantiated the strong interaction between aspirin and PPAR α . Lentiviral overexpression studies of flPPAR α and Y314DPPAR α , followed by GC-MS analyses, further confirmed that aspirin and its derivative, 2-ethyl hexyl salicylate, bind to PPAR α inside the cells. Interestingly, other aspirin-like molecules and NSAIDs, namely ibuprofen, celecoxib, methyl salicylate, methyl-4-hydroxy benzoate, and naproxen, exhibited much weaker interactions with the PPAR α LBD compared with aspirin, suggesting the specificity of the effect. Moreover, these results also suggest that the interaction of aspirin with PPAR α is independent of cyclooxygenase inhibition. We recently demonstrated that PPAR α is present in hippocampus, and that activation of PPAR α stimulates hippocampal plasticity (24). In the present study, we also observed that aspirin is dependent on PPAR α to evoke calcium influx and stimulate morphological plasticity in hippocampal neurons. Stimulation of both AMPA- and NMDA-mediated calcium currents in flPPAR α -transduced, but not mutated Y314DPPAR α -transduced, *Ppara*-null hippocampal neurons clearly indicates the importance of the Y314 residue of PPAR α in mediating aspirin-induced calcium oscillation in hippocampal neurons.

The mechanisms regulating hippocampal plasticity are becoming clear. Multiple studies have shown that CREB plays an important role in promoting synaptic activity, a critical signal for the formation of long-term learning and memory (38). We found that the *Creb* promoter harbors a conserved PPRE, and that PPAR α transcriptionally controls *Creb* and regulates CREB-associated plasticity genes in the hippocampus (24). Aspirin was also shown to up-regulate *Creb* specifically through PPAR α , while PPAR β and PPAR γ had no effect. ChIP analysis further showed that aspirin induces the recruitment of PPAR α , but not PPAR β or PPAR γ , to the *Creb* promoter. A lentiviral overexpression study followed by analysis of *Creb* promoter-driven luciferase activity further revealed that aspirin induces *Creb* transcription through PPAR α . These results support an essential role of PPAR α in aspirin-mediated transcriptional activation of CREB.

Owing to activation of the PPAR α -CREB pathway, aspirin increased the function of hippocampal neurons in culture as well in vivo in the brain of FAD5X mice. Oral administration of low-dose of aspirin led to up-regulation of BDNF, CREB, and other plasticity-related molecules, such as PSD95 and NR2A, along with increased calcium influx observed in hippocampal tissues of FAD5X mice. This corresponded to improved performance by FAD5X mice in the Barnes maze and T-maze tests following aspirin treatment. Interestingly, we also noted that following aspirin treatment, FAD5X animals showed significant improvement in stress-related behaviors. Consistent with the binding and activation of PPAR α , aspirin treatment remained unable to up-regulate hippocampal functions, improve memory and learning, and decrease stress-related behaviors in FAD5X/*Para*-null mice.

In summary, aspirin, a widely used analgesic, binds to the LBD domain of PPAR α and up-regulates hippocampal plasticity via PPAR α . After oral administration, aspirin improves hippocampal function and protects spatial learning and memory in an animal model of AD via PPAR α . Therefore, low-dose aspirin may find therapeutic use in AD as well as in other dementia-related illnesses.

Experimental Procedures

Cell culture, semiquantitative RT-PCR, real-time PCR, immunoblotting, ChIP protocols, luciferase assays, immunofluorescence assays, immunohistochemistry, dendritic spine quantification, and behavioral analysis are described in detail in *SI Appendix, Materials and Methods*.

Reagents and Antibodies. Antibodies and their applications, sources, and dilutions are listed in [S1 Appendix, Table S1](#). Cell culture materials (i.e., DMEM/12,

neurobasal, phenol red-free neurobasal, B27, L-glutamine, and antibiotics and antimycotics) were purchased from Life Technologies. Pharmacologic compounds, including aspirin (A5376), gemfibrozil (G9518), GW9662 (M6191), and GW6471 (G5045), were purchased from Sigma-Aldrich. All molecular biology-grade chemicals were obtained from Sigma-Aldrich or Bio-Rad. Alexa Fluor secondary antibodies used for immunocytochemistry were obtained from Jackson ImmunoResearch Laboratories, and IR dye-labeled secondary antibodies used for immunoblotting were purchased from Li-Cor Biosciences.

Animals. Mice were maintained and experiments conducted in accordance with National Institute of Health guidelines and were approved by the Rush University Medical Center Institutional Care and Use Committee. The mice used in these experiments are described in *SI Appendix, Materials and Methods*.

Thermal Shift Assay. The thermal shift assay is described in detail in *SI Appendix, Materials and Methods*.

TR-FRET Analysis. Details of the TR-FRET analysis are provided in *SI Appendix, Materials and Methods*.

DNA Constructs and Lentiviral Transductions. Generation of the pCMV6-AC-GFP lentiviral backbone expressing TurboGFP (OriGene #PS100010) and fPPAR α or Y314DPPAR α has been described elsewhere (24, 26). For biochemical experiments, 10–12 d in vitro (DIV) Ppara-null hippocampal neurons, cortical neurons, or astrocytes were transduced with lentiviral particles at a multiplicity of infection of 10 for 48 h at 37 °C. Viral integration was monitored by live GFP imaging.

GC-MS Analysis of PPAR α –Aspirin Interaction. Details of this analysis are provided in *SI Appendix, Materials and Methods*.

Calcium Influx Assay in Hippocampal Neurons. This assay is described in detail in *SI Appendix, Materials and Methods*.

Organotypic Calcium Influx Assay. Calcium influx was measured in hippocampal slices was performed as described previously (15, 26). In brief, FAD5X, 5XFAD/Ppara-null, and age-matched NTG mice were anesthetized, rapidly perfused with

ice-cold sterile PBS, and decapitated. The whole brain was carefully removed from the cranium. Dorsal slices of the hippocampus were cut at a thickness of 100 μ m using an adult mouse brain slicer matrix with 1.0-mm coronal section slice intervals. The slices were placed in the glass tray filled with cutting solution (24.56 g of sucrose, 0.9008 g of dextrose, 0.0881 g of ascorbate, 0.1650 g of sodium pyruvate, and 0.2703 g of myo-inositol in 500 mL of distilled water) and continuously bubbled with 5% CO₂ and 95% O₂ gas mixture. The glass tray was kept ice-cold during the slicing period. Slices were then carefully transferred into Fluo-4 dye containing reaction buffer. The reaction buffer was prepared before the making of brain slices using 10 mL of artificial CSF (119 mM NaCl, 26.2 mM NaHCO₃, 2.5 mM KCl, 1 mM NaH₂PO₄, 1.3 mM MgCl₂, 10 mM glucose, bubbled with 5% CO₂, and 95% O₂, followed by the addition of 2.5 mM CaCl₂) added to a bottle of Fluo-4 dye (catalog #F10471), and 250 mM probenecid. Before the transfer of slices, a flat-bottom 96-well plate (BD Falcon; catalog #323519) was loaded with 50 μ L of reaction buffer per well, covered with aluminum foil, and kept in a dark place. One individual slice was placed in each well loaded with reaction buffer, and the plate was rewrapped with aluminum foil and kept at 37 °C for 20 min. Then fluorescence excitation and emission spectra were recorded in a PerkinElmer VICTOR X2 luminescence spectrometer in the presence of NMDA (50 μ M) and AMPA (50 μ M). The recording was performed with 300 repeats at 0.1-ms intervals.

Behavioral Analysis. The open-field, Barnes maze, and T-maze tests were performed as described previously (24, 26) and in *SI Appendix, Materials and Methods*.

Statistical Analysis. Statistical analyses were performed using GraphPad Prism 7.0c software. Unless stated otherwise, one-way or two-way ANOVA was performed to determine the significance of differences between groups, followed by Tukey's honest significant difference (HSD) or Bonferroni's post hoc test for the significance of differences among multiple experimental groups. Data are expressed as mean $\pm$ SEM or mean $\pm$ SD, and $P < 0.05$ was considered to indicate statistical significance.

ACKNOWLEDGMENTS. This study was supported by Veterans Affairs Merit Award 101BX002174, National Institutes of Health Grant AG050431, and Zenith Fellows Award ZEN-17-438829 from the Alzheimer's Association.

- Karlavish J, Jack CR, Jr, Rocca WA, Snyder HM, Carrillo MC (2017) Alzheimer's disease: The next frontier. *Special Report 2017. Alzheimers Dement* 13:374–380.
- Alzheimer's Association (2017) 2017 Alzheimer's disease facts and figures. *Alzheimers Dement* 13:325–373.
- Scheff SW, Price DA, Schmitt FA, Mufson EJ (2006) Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging* 27:1372–1384.
- Scheff SW, Price DA (2001) Alzheimer's disease-related synapse loss in the cingulate cortex. *J Alzheimers Dis* 3:495–505.
- Yamamoto Y, Gaynor RB (2001) Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. *J Clin Invest* 107:135–142.
- Vane JR, Botting RM (2003) The mechanism of action of aspirin. *Thromb Res* 110:255–258.
- Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 231:232–235.
- Rothwell PM, et al. (2012) Effect of daily aspirin on risk of cancer metastasis: A study of incident cancers during randomised controlled trials. *Lancet* 379:1591–1601.
- Dai Y, Ge J (2012) Clinical use of aspirin in treatment and prevention of cardiovascular disease. *Thrombosis* 2012:245037.
- Nilsson SE, et al. (2003) Does aspirin protect against Alzheimer's dementia? A study in a Swedish population-based sample aged ≥ 80 years. *Eur J Clin Pharmacol* 59:313–319.
- Kennedy MB, Beale HC, Carlisle HJ, Washburn LR (2005) Integration of biochemical signalling in spines. *Nat Rev Neurosci* 6:423–434.
- Tada T, Sheng M (2006) Molecular mechanisms of dendritic spine morphogenesis. *Curr Opin Neurobiol* 16:95–101.
- Alvarez VA, Sabatini BL (2007) Anatomical and physiological plasticity of dendritic spines. *Annu Rev Neurosci* 30:79–97.
- Ulltair SK, et al. (2007) Regulation of spine morphology and spine density by NMDA receptor signaling in vivo. *Proc Natl Acad Sci USA* 104:19553–19558.
- Roy A, et al. (2014) Enhancement of morphological plasticity in hippocampal neurons by a physically modified saline via phosphatidylinositol-3 kinase. *PLoS One* 9:e101883.
- Autry AE, Monteggia LM (2012) Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacol Rev* 64:238–258.
- Castrén E, Antila H (2017) Neuronal plasticity and neurotrophic factors in drug responses. *Mol Psychiatry* 22:1085–1095.
- Bosch M, Hayashi Y (2012) Structural plasticity of dendritic spines. *Curr Opin Neurobiol* 22:383–388.
- Bosch M, et al. (2014) Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. *Neuron* 82:444–459.
- Meyer D, Bonhoeffer T, Scheuss V (2014) Balance and stability of synaptic structures during synaptic plasticity. *Neuron* 82:430–443.
- Chao MV (2003) Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nat Rev Neurosci* 4:299–309.
- Ceni C, Unsain N, Zeinieh MP, Barker PA (2014) Neurotrophins in the regulation of cellular survival and death. *Handb Exp Pharmacol* 220:193–221.
- Wu K, et al. (1996) Functional trkB neurotrophin receptors are intrinsic components of the adult brain postsynaptic density. *Brain Res Mol Brain Res* 43:286–290.
- Roy A, et al. (2013) Regulation of cyclic AMP response element-binding and hippocampal plasticity-related genes by peroxisome proliferator-activated receptor α . *Cell Rep* 4:724–737.
- Lemberger T, Desvergne B, Wahli W (1996) Peroxisome proliferator-activated receptors: A nuclear receptor signaling pathway in lipid physiology. *Annu Rev Cell Dev Biol* 12:335–363.
- Roy A, et al. (2015) HMG-CoA reductase inhibitors bind to PPAR α to upregulate neurotrophin expression in the brain and improve memory in mice. *Cell Metab* 22:253–265.
- Finkbeiner S, et al. (1997) CREB: A major mediator of neuronal neurotrophin responses. *Neuron* 19:1031–1047.
- Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME (1998) Ca²⁺ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* 20:709–726.
- Corbett GT, Gonzalez FJ, Pahan K (2015) Activation of peroxisome proliferator-activated receptor α stimulates ADAM10-mediated proteolysis of APP. *Proc Natl Acad Sci USA* 112:8445–8450.
- Rangasamy SB, et al. (2015) Intranasal delivery of NEMO-binding domain peptide prevents memory loss in a mouse model of Alzheimer's disease. *J Alzheimers Dis* 47:385–402.
- Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011) Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* 476:458–461.
- Kim JJ, et al. (2007) Stress-induced alterations in hippocampal plasticity, place cells, and spatial memory. *Proc Natl Acad Sci USA* 104:18297–18302.
- Lee AL, Ogle WO, Sapolsky RM (2002) Stress and depression: Possible links to neuron death in the hippocampus. *Bipolar Disord* 4:117–128.
- Shrestha P, Klann E (2016) Alzheimer's disease: Lost memories found. *Nature* 531:450–451.
- Roy DS, et al. (2016) Memory retrieval by activating engram cells in mouse models of early Alzheimer's disease. *Nature* 531:508–512.
- Selkoe DJ (2001) Alzheimer's disease: Genes, proteins, and therapy. *Physiol Rev* 81:741–766.
- World Health Organization (2017) 20th Model List of Essential Medicine. Available at www.who.int/medicines/publications/essentialmedicines/20th_EML2017_FINAL_amendedAug2017.pdf?ua=1. Accessed February 1, 2018.
- Sakamoto K, Karelina K, Obrietan K (2011) CREB: A multifaceted regulator of neuronal plasticity and protection. *J Neurochem* 116:1–9.