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Amyloid precursor protein maintains constitutive and adaptive plasticity of dendritic spines in adult brain by regulating D-serine homeostasis

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Abstract

Dynamic synapses facilitate activity-dependent remodeling of neural circuits, thereby providing the structural substrate for adaptive behaviors. However, the mechanisms governing dynamic synapses in adult brain are still largely unknown. Here, we demonstrate that in the cortex of adult amyloid precursor protein knockout (APP-KO) mice, spine formation and elimination were both reduced while overall spine density remained unaltered. When housed under environmental enrichment, APP-KO mice failed to respond with an increase in spine density. Spine morphology was also altered in the absence of APP. The underlying mechanism of these spine abnormalities in APP-KO mice was ascribed to an impairment in D-serine homeostasis. Extracellular D-serine concentration was significantly reduced in APP-KO mice, coupled with an increase of total D-serine. Strikingly, chronic treatment with exogenous D-serine normalized D-serine homeostasis and restored the deficits of spine dynamics, adaptive plasticity, and morphology in APP-KO mice. The cognitive deficit observed in APP-KO mice was also rescued by D-serine treatment. These data suggest that APP regulates homeostasis of D-serine, thereby maintaining the constitutive and adaptive plasticity of dendritic spines in adult brain.

Keywords amyloid precursor protein; dendritic spine; D-serine microelectrode biosensor; spine plasticity; two-photon *in vivo* imaging
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Introduction

Small protrusions of dendrites, known as spines, provide primary sites for excitatory inputs in principal neurons of most brain regions. Harboring the receptive elements of glutamatergic connections, dendritic spines are of major importance for synaptic integration and plasticity, hence a prerequisite for encoding cortical representations and adaptive remodeling of neural circuits (Nimchinsky *et al*, 2002; Yuste, 2011; Sala & Segal, 2014). To ensure these functions, the morphology and distribution of dendritic spines are maintained in a highly dynamic state and are tightly regulated (Yuste & Bonhoeffer, 2001; Kasai *et al*, 2010; May, 2011). Thus, it is not surprising that the structural parameters of dendritic spines including spine density, morphology, and plasticity are affected in an array of neurodegenerative diseases (Fiala *et al*, 2002; Fuhrmann *et al*, 2007; Bittner *et al*, 2010; Lai & Ip, 2013; Fol *et al*, 2016). As such, research into mechanisms governing functions and structural plasticity of dendritic spines, which remain largely unexplored in adult brain, holds important clues not only toward understanding the basic biology of synapses with neural mechanisms of adaptive behavior but may also reveal key areas for therapeutic interventions.

Enduring interest toward amyloid precursor protein (APP), due to its key role for the pathogenesis of Alzheimer's disease, has been refueled by recent evidence indicating its multifaceted role in synaptic physiology and development (Moya *et al*, 1994; Herms *et al*, 2004; Hoe *et al*, 2012; Muller & Zheng, 2012). While the mechanistic details remain to be elucidated, increasing evidence indicates important trans-synaptic adhesive functions for trans-membrane APP and major neurotrophic roles of secreted ectodomain APPsα in neurons (Soba *et al*, 2005; Bell *et al*, 2008; Jimenez *et al*, 2011; Aydin *et al*, 2012; Caldwell *et al*, 2013; Baumkotter *et al*, 2014). The high level of APP expression in the developing nervous system with its enrichment at nascent synapses and potent synaptogenic effects of secreted APPsα have also been implied in the formation and stability of synapses during neurodevelopment (Caille *et al*, 2004; Claasen *et al*, 2009; Wang *et al*, 2009; Weyer *et al*, 2011, 2014; Hoe *et al*, 2012; Klevanski *et al*, 2014, 2015; Hick *et al*, 2015). Despite of the key relevance of these processes for integrative mechanisms of neurons and synaptic plasticity, the role of APP in governing

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dendritic spine dynamics and adaptive remodeling of neural circuits in adult brain remains poorly defined.

In this study, we used long-term *in vivo* two-photon microscopy to elucidate the role of APP in regulating constitutive and adaptive spine plasticity in adult mouse brain. Our data show that the lack of APP impairs the structural plasticity of dendritic spines and suggest its key role in regulating D-serine homeostasis, which is a prerequisite for synaptic plasticity in the adult brain.

Results

Dendritic spine dynamics are decreased in the absence of APP

APP proved critical in the formation and stabilization of synaptic connections in the developing nervous system (Priller *et al*, 2006; Weyer *et al*, 2011, 2014; Hoe *et al*, 2012; Muller & Zheng, 2012). To find out if the dynamics of dendritic spines in adult brain are also regulated by APP, we monitored and compared the density and turnover rate (TOR) of dendritic spines in cortical pyramidal neurons of 4- to 5-month-old WT and APP-KO mice *in vivo*. Apical tufts of layer V pyramidal neurons were imaged in the somatosensory cortex (Fig 1A). While the spine densities of WT and APP-KO mice remained unchanged during our imaging period (Fig 1B), both the elimination and formation of spines were significantly lower in neurons of APP-KO mice compared to controls (Fig 1D and E), resulting in reduced spine TOR (Fig 1C). Thus, the decrease in spine TOR without change in spine density indicates a key role of APP in maintaining dendritic spine dynamics.

Adaptive plasticity of dendritic spines is impaired in APP-KO mice

To investigate whether reduced spine dynamics has a functional consequence in neural circuit remodeling in adult brain, both WT and APP-KO mice were exposed to environmental enrichment (EE) over 5 weeks, with spine density and dynamics monitored (Fig 2A). EE is known to provide a spectrum of synaptic inputs, which activate and lead to adaptive synaptic alterations within the adult brain (Nithianantharajah & Hannan, 2006; Mora *et al*, 2007; Sale *et al*, 2014). In agreement with earlier reports (Berman *et al*, 1996; Kozorovitskiy *et al*, 2005; Jung & Herms, 2014), in WT mice environmental enrichment induced a steady increase of spine density. In sharp contrast, environmental enrichment failed to increase spine density in APP-KO mice (Fig 2B). Of note, unlike WT mice demonstrating gradual decline in dendritic spine elimination upon environmental enrichment (EE), the rate of spine elimination in APP-KO genotype remained unaltered (Fig 2C). Collectively, these data demonstrate an essential role of APP in regulating constitutive turnover of dendritic spines and their adaptive remodeling in the adult brain.

Impaired spine plasticity in APP-KO mice coincides with altered spine morphology

Structural plasticity of dendritic spines is closely correlated with spine morphology, which presents a reliable indicator of the developmental state and strength of excitatory synaptic inputs of cortical neurons (Hayashi & Majewska, 2005; Bosch & Hayashi, 2012). Classified in three major groups—stubby, mushroom, and thin spines, the relative fraction of various spine types in the brain is regulated by synaptic activity and developmental mechanisms (Benavides-Piccione *et al*, 2002; Konur *et al*, 2003). To find out whether impaired plasticity of dendritic spines in APP-KO mice correlates with aberrations in spine morphology, we assessed spine type distribution in adult WT and APP-KO mice housed under standard or enriched conditions (Fig 3). In APP-KO mice, the fraction of thin spines was reduced while the relative number of mushroom spines was enhanced irrespective of housing conditions (Fig 3A–C). Counting of stubby spines revealed no differences between two genotypes (not shown). As thin spines are more dynamic and responsive to external stimulation (Bourne & Harris, 2007), the reduction in thin spines paralleled by an increased fraction of mushroom spines support our observations on spine plasticity impairments of APP-KO genotype.

D-serine homeostasis is impaired in APP-KO mice

Synaptic plasticity depends on the glial release of D-serine, the endogenous co-agonist of N-methyl-D-aspartate (NMDA) receptors (Panatier *et al*, 2006; Henneberger *et al*, 2010). D-serine release from glial cells occurs mainly through calcium-dependent exocytosis (Martineau *et al*, 2014). Interestingly, calcium signaling in APP-KO astrocytes is dysregulated (Hamid *et al*, 2007; Linde *et al*, 2011). All these facts prompted us to measure cortical extracellular and total D-serine concentrations in APP-KO mice. We found that extracellular concentration of endogenous D-serine measured using microelectrode biosensors was dramatically decreased in the absence of APP (Fig 4A), which was in contrast to the increase of total D-serine and L-serine in APP-KO mice, quantified using HPLC (Fig 4B and C). These results suggest that the maintenance of D-serine homeostasis requires APP.

Exogenous D-serine treatment restores D-serine homeostasis and the structural plasticity of dendritic spines in APP-KO mice

As the extracellular D-serine concentration is decreased, we tested whether treatment with exogenous D-serine could rescue the impaired structural plasticity of dendritic spines in APP-KO mice. D-serine was supplemented to the drinking water of APP-KO mice housed under standard or enriched conditions, and dendritic spines were monitored over several weeks. To our surprise, exogenous D-serine treatment not only restored extracellular D-serine level (Fig 4A), but also normalized the concentrations of total D-serine and L-serine in APP-KO brain (Fig 4B and C). Also, as illustrated in Fig 5A, D-serine treatment in APP-KO mice increased constitutive spine dynamics under standard housing conditions (Fig 5B and C) and rescued the adaptive gain of spines upon environmental enrichment (Fig 5E). Likewise, treatment of APP-KO mice with D-serine enhanced the fraction of thin spines and lowered the relative number of spines with mushroom morphology (Fig 5F and G). In WT mice, spine dynamics and adaptive plasticity were not altered following D-serine administration (Fig EV1). These data suggest that constitutive and adaptive structural plasticity of dendritic spines depend on the homeostasis of D-serine, which is impaired in the absence of APP.

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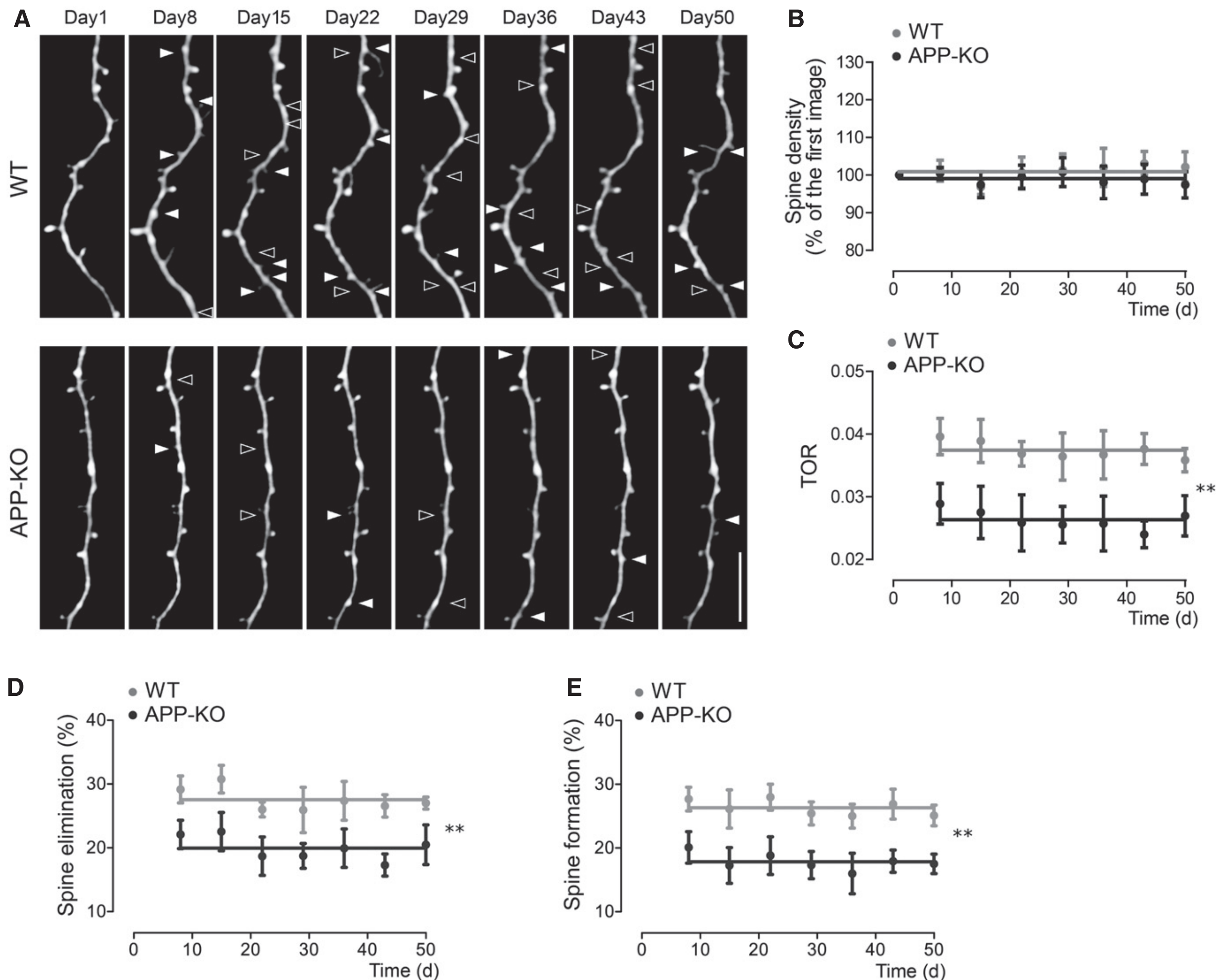

Figure 1. Constitutive plasticity of dendritic spines is decreased in the cortex of adult APP-KO mice.

A Consecutive *in vivo* imaging of the same apical dendrites from layer V pyramidal neurons in the somatosensory cortex over 7 weeks reveals formation and elimination of dendritic spines (white and empty arrowheads, respectively) in WT and APP-KO mice. All mice were housed in standard conditions. Scale bar, 10 μm.

B–E Graphical representations of the relative spine density, spine turnover rate (TOR), elimination, and formation over the imaging period. Results are presented as mean ± SEM. Nonlinear regression (*F*-test) has been used for fitting the data points and comparing the differences between groups. $N = 4$ mice per group; $^{**}P < 0.01$.

Treatment with D-serine rescues cognitive deficit of APP-KO mice

The structural plasticity of dendritic spines has important implications for cognition and memory (Kasai *et al*, 2010; Lai & Ip, 2013). To investigate whether the impaired spine plasticity in APP-KO mice is associated with perturbed cognitive performance, we subjected mice to the novel objective recognition test. As a baseline, we monitored motor performance, which was not significantly different between genotypes (Fig 6A). In contrast to WT mice that spend more time exploring a novel object, APP-KO mice displayed memory deficits, as they spent similar time to explore novel and already familiar objects (Fig 6B). Strikingly, treatment of APP-KO mice with D-serine, restored the preference for the novel object, as indicated by significantly longer exploration time (Fig 6B). These results indicate that exogenous D-serine treatment improved cognitive performance in APP-KO mice, which may relate to the restoration of spine dynamics and remodeling.

Discussion

We have shown here that in adult APP-KO mice, dendritic spine dynamics and remodeling are impaired. This finding assigns an important role to APP in governing structural plasticity of dendritic spines. Remarkably, the impaired spine plasticity and cognitive deficits could be rescued by exogenous D-serine administration, which also restores unbalanced D-serine homeostasis in APP-KO brain.

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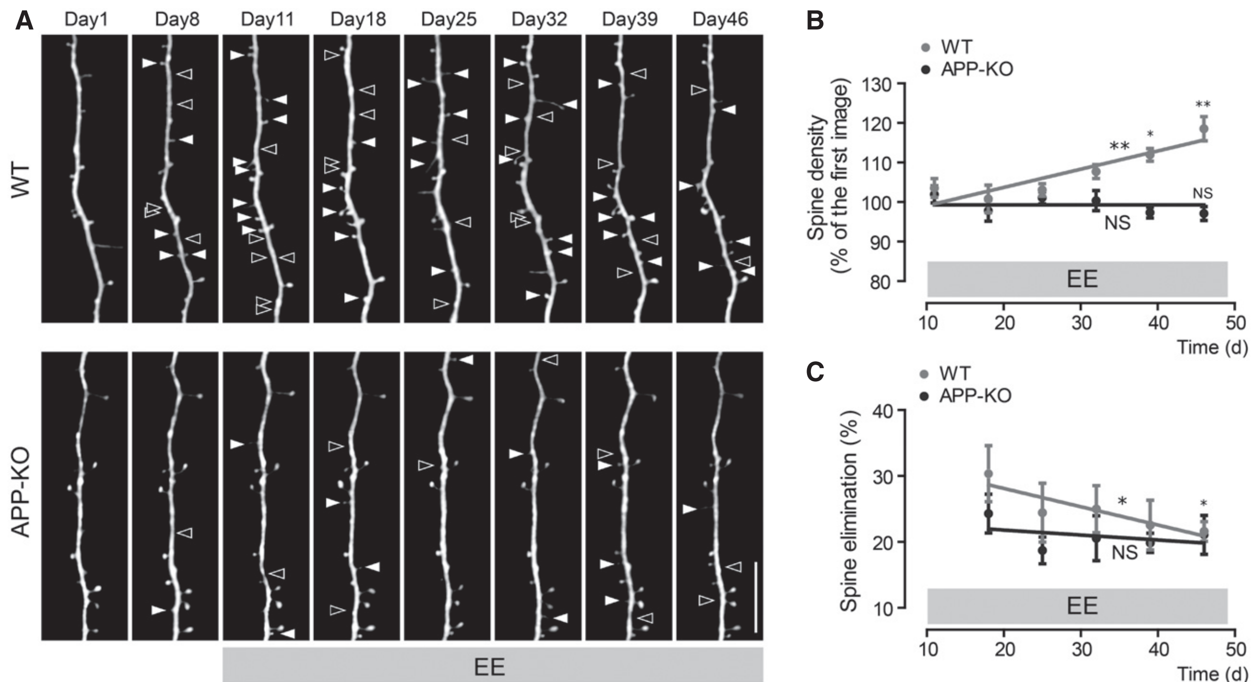

Figure 2. Adaptive plasticity of dendritic spines is impaired in APP-KO mice.

A Consecutive *in vivo* imaging of the same apical dendrites from layer V pyramidal neurons in the somatosensory cortex over 46 days reveals formation and elimination of dendritic spines (white and empty arrowheads, respectively) in WT and APP-KO mice. Prior to the exposure to environmental enrichment (EE), all mice were housed in standard conditions. Scale bar, 10 μm.

B, C Graphical representations of the relative spine density and spine elimination over the period of the exposure of mice to EE. Results are presented as mean ± SEM. Nonlinear regression (*F*-test) has been used for fitting the data points. Repeated one-way ANOVA was performed followed by Dunnett's test. WT $n = 5$ mice and APP-KO $n = 6$ mice; *$P < 0.05$, **$P < 0.01$, NS = no significant difference.

These converging results pinpoint the functional role of APP in affecting the homeostasis of D-serine and thereby maintaining constitutive and adaptive plasticity of dendritic spines in the adult brain.

As a ubiquitous type I trans-membrane glycoprotein expressed in the brain, APP with its cleavage product Aβ has long been implicated in AD (Hardy & Selkoe, 2002; Selkoe, 2008). Produced by β/γ proteolysis of APP, Aβ40/42 peptides represent the main constituents of amyloid plaques in AD brain and are considered a major cause of synapse loss and neurotoxicity, leading to cognitive decline and memory deficits. At the same time, the role of APP and its fragments in synaptic physiology has been widely recognized with several studies demonstrating the essential role of APP and related APP-like proteins (APLPs) for synaptogenesis (Muller *et al*, 1994; Priller *et al*, 2006; Hoe *et al*, 2012; Muller & Zheng, 2012; Hick *et al*, 2015). In fact, recent evidence emphasizes the crucial role of protective effects mediated by full-length APP and APPsα on synapses and neurons (Ring *et al*, 2007; Yang *et al*, 2009; Hick *et al*, 2015; Klevanski *et al*, 2015; Fol *et al*, 2016). Hence, deciphering molecular mechanisms mediating APP functions is essential not only for basic research of synaptic physiology but also for translational neuroscience. Because the morphology and dynamics of dendritic spines correlate with the strength and stability of excitatory synapses, decrease in the fraction of thin spines with reduction in spine turnover in APP-KO mice are consistent with impaired structural plasticity. The lower fraction of dynamic thin spines with an increase in more stable mushroom spines suggest that the excitatory inputs of layer V pyramidal neurons of APP-KO mice are hardwired more rigidly and are less prone to contextual and behavioral remodeling.

Extracellular D-serine is involved in the modulation of plasticity at glutamatergic synapses, as endogenous co-agonist of synaptic NMDA receptors (Panatier *et al*, 2006). Indeed, long-term potentiation (LTP), a classical form of NMDA receptor-dependent synaptic plasticity, depends on extracellular D-serine, which is mainly released from astrocytes (Henneberger *et al*, 2010). The mechanism underlying astrocytic D-serine released into extracellular space is largely attributed to calcium-dependent exocytosis. Disrupting calcium signaling in astrocytes reduces D-serine release (Takata *et al*, 2011; Shigetomi *et al*, 2013). Intriguingly, *in vitro* experiments in astrocytes of APP-KO mice have previously indicated an important physiological of APP for regulating calcium signaling (Hamid *et al*, 2007; Linde *et al*, 2011). Together with our *in vivo* data, this suggests that dysregulated calcium signaling in APP-KO astrocytes might decrease their ability to release D-serine into the extracellular space. At the same time, disrupted exocytosis in astrocytes triggers D-serine accumulation in their vesicular pools, explaining why total D-serine levels did not decrease but actually increased in APP-KO compared to control mice. Note that total D-serine level measured by HPLC is more than 99% intracellular (Pernot *et al*, 2012). So far, the only D-serine storage mechanism described in literature is the pool of vesicles located in astrocytes (Martineau *et al*, 2014), while D-serine synthesized in neurons is not stored, but released through Asc1 transporters and eliminated through the blood or taken up by astrocytes.

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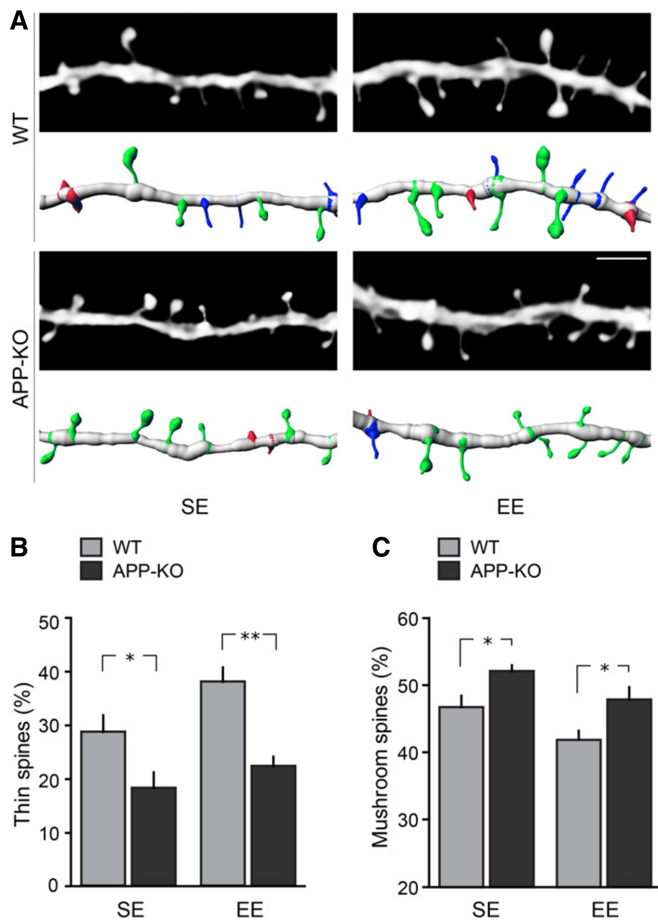

Figure 3. Dendritic spine morphology is altered in APP-KO mice.

A Typical confocal images of apical dendrites with spines (z-projections) from layer V pyramidal neurons in the somatosensory cortex of WT and APP-KO mice (top and bottom, respectively) housed in standard and enriched environments (left and right, respectively). For classification of spine types, 3D reconstructions by Imaris have been applied. Thin, mushroom and stubby spines are encoded in blue, green, and red, respectively. Scale bar, 2 μm.

B, C Summary plots of thin and mushroom spine fractions in WT and APP-KO mice exposed to standard (SE) and enriched environments (EE). Results are presented as mean ± SEM. Two-tailed Student's *t*-test was used and n = 6 mice in all experimental groups; *$P < 0.05$, **$P < 0.01$, NS = no significant difference.

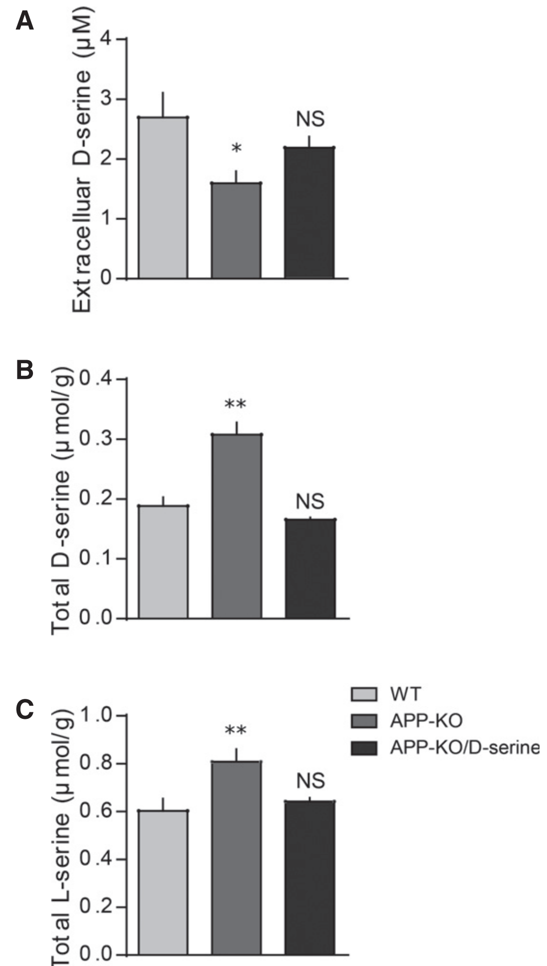

Figure 4. Quantification of D-serine concentrations in WT and APP-KO mice.

A Extracellular D-serine concentrations in cortex were detected by enzyme-based microelectrode biosensors.

B, C High-performance liquid chromatography was performed to quantify total D-serine and L-serine contents in mouse brain of two genotypes.

Data information: APP-KO mice were fed with D-serine over 1 month. Results are presented as mean ± SEM. One-way ANOVA was used for analysis followed by Bonferroni's multiple comparisons test and $n > 6$ mice per group; *$P < 0.05$, **$P < 0.01$, NS = no significant difference as compared to WT.

Therefore, D-serine accumulation in astrocytic vesicles resulted in increased D-serine amount in our HPLC measurements. In addition, APP fragments, including amyloid beta-peptide and secreted APP, have been reported to induce the release of D-serine from microglia by increasing serine racemase (SR) expression (Wu *et al*, 2004, 2007). All these findings suggest that APP might modulate extracellular D-serine release in different cell types via different mechanisms.

To restore the decreased level of extracellular D-serine, we treated APP-KO mice chronically with oral administration of D-serine. As we expected, we observed in APP-KO mice that extracellular D-serine concentration recovered, as well as deficits in behavioral and spine dynamics. Surprisingly, the total D-serine and L-serine, the precursor of D-serine, levels in APP-KO brains were also normalized to control level after exogenous D-serine treatment. This observation clearly links APP deficiency to alteration of production, maintenance and/or release of D-serine. Further investigations are needed to dissect the specific components resulting in unbalanced D-serine homeostasis, which may also involve *in vitro* studies. However, *in vitro* condition may not guarantee the physiological balance between intracellular and extracellular amino acid concentrations, important for a functional tonic and phasic D-serine release. For example, prolonged recording session in slices, as well as in cell cultures, might dilute D-serine concentration and diffusion between cells. Although our data cannot rule out the contribution of synaptic and neurotrophic functions of APP in APP-KO mice, the restorative effects of exogenous D-serine suggest that APP regulates

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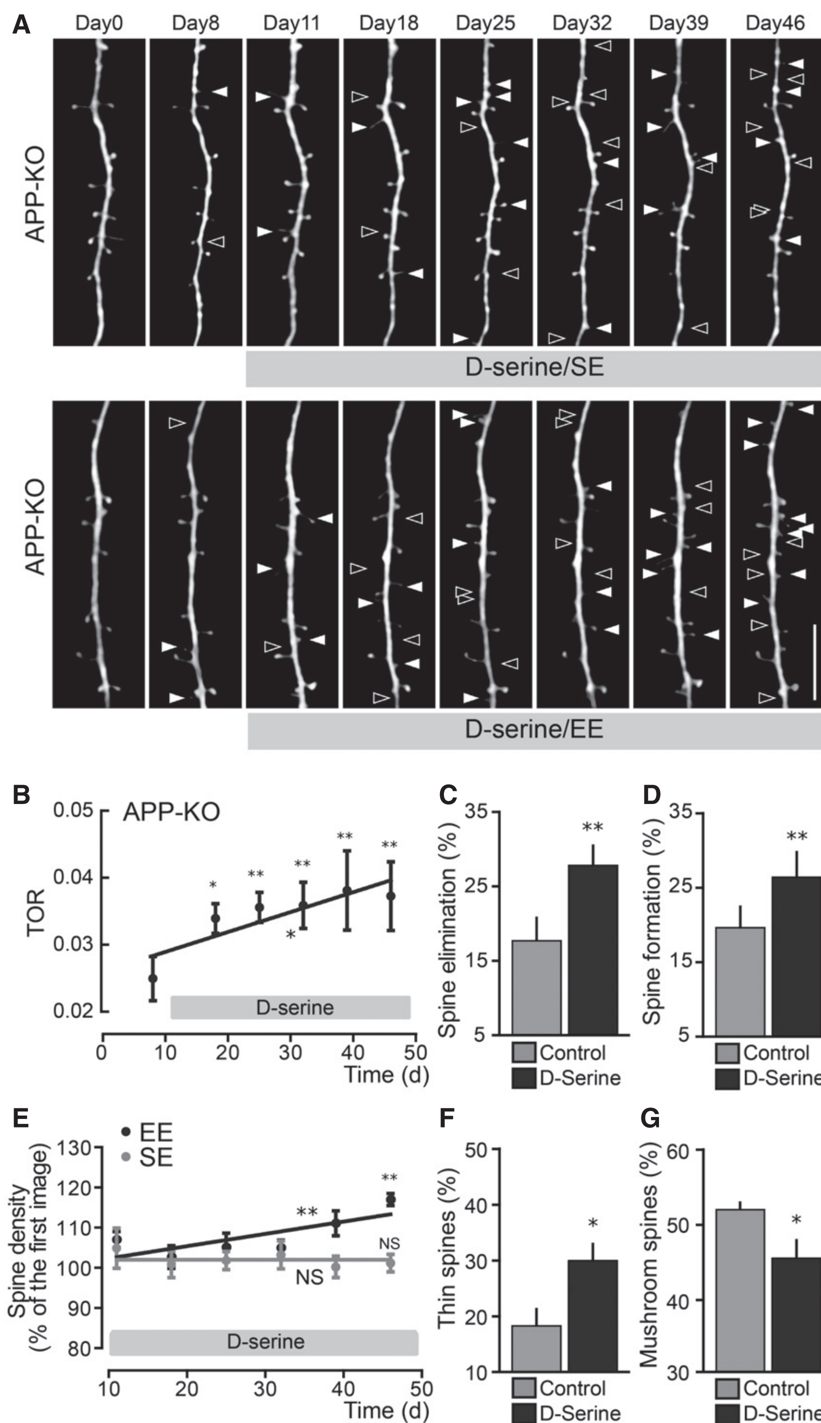

Figure 5. Treatment of APP-KO mice with exogenous D-serine restores the structural plasticity and morphology of dendrite spines.

A Consecutive *in vivo* imaging of the same apical dendrites from layer V pyramidal neurons in the somatosensory cortex of APP-KO mice housed under standard or enriched environment. Note that both groups of mice received D-serine after the second imaging time point (8 days); white and empty arrowheads point to newly formed and eliminated spines, respectively. Scale bar, 10 μm.

B Spine TOR prior and during continuous D-serine treatment.

C, D Summary plots of the fraction of spine elimination and formation in APP-KO mice before and after D-serine treatment (8 and 46 days, respectively).

E Relative spine densities in D-serine treated APP-KO mice housed under standard and enriched environments.

F, G Summary plots of the fraction of thin and mushroom spines in control and D-serine treated APP-KO mice.

Data information: For illustration purpose, the control data from Fig 3B and C are presented also here. Results are presented as mean ± SEM. Nonlinear regression (*F*-test) has been used for fitting the data points. Two-tailed Student's *t*-test was used in (C, D, F and G) and repeated one-way ANOVA was performed followed by Dunnett's test in (B and E). $N = 5$ mice in each group; $^{*}P < 0.05$, $^{**}P < 0.01$, NS = no significant difference.

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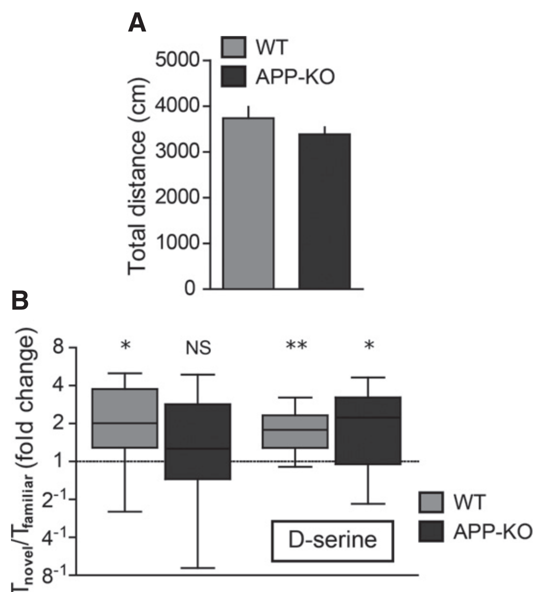

Figure 6. Exogenous D-serine treatment rescues cognitive decline in APP-KO mice.

A Quantification of total distance that mice traveled for 10 min (mean ± SEM).

B The ratios of mice explored between novel and familiar objects. Lines in boxes indicate mean levels and whiskers represent the data from minimal to maximal range. The box extends from 25 to 75% of values.

Data information: Wilcoxon signed-rank test was performed to compare data to the hypothetical value 1. $N = 9–12$ mice per group; $*P < 0.05$, $**P < 0.01$, NS = no significant difference.

the dynamics and plasticity of dendritic spines *in vivo* via controlling D-serine homeostasis.

To conclude, our data corroborate a major role of APP for structural plasticity and adaptive remodeling of cortical synapses in the adult brain. They also indicate that lack of APP holoprotein leads to impaired D-serine homeostasis, which is associated with dysfunctional synaptic plasticity. Further research of APP-mediated functions is likely to provide valuable insights into the biology of dendritic spines and may open avenues for discovery of novel therapeutic targets for AD, a scientific investment with immense beneficial potential.

Materials and Methods

Experimental animals

All protocols and procedures involving animals were approved and conducted in accordance with the regulations of LMU and the government of Upper Bavaria (Az. 55.2-1-54-2532-62-12). GFP-M mice (Feng *et al*, 2000) were purchased from Jackson Laboratory, USA. APP-KO mice were described previously (Magara *et al*, 1999; Ring *et al*, 2007). APP-KO ($APP^{-/-}$) × GFP-$M^{+/-}$ mice were generated by interbreeding. All transgenic mice were maintained on C57BL/6 background. Female transgenic mice at the age of 4 months were used for imaging, and female age-matched wild-type (WT) littermates were used as controls. Mice were housed and bred in pathogen-free environment in the animal facility at the Centre for Neuropathology and Prion Research of the Ludwig Maximilian University Munich (LMU), with food and water provided *ad libitum* (21 ± 1°C, at 12-h/12-h light/dark cycle). All mice were either housed singly in standard cages (30 × 15 × 20 cm) or in groups in an environmentally enriched (EE) cage (80 × 50 × 40 cm) equipped with platforms and variety of toys, which were relocated every 2–3 days. In experiments with D-serine treatment, every other day D-serine (Sigma-Aldrich) was prepared freshly and supplemented into drinking water (0.55 mg/ml).

Longitudinal *in vivo* two-photon imaging experiments

The surgical procedure of chronic cranial window implantation and the details of experiments have been described previously (Fuhrmann *et al*, 2007; Holtmaat *et al*, 2009). In brief, under anesthesia with ketamine/xylazine (120 and 10 mg/kg, respectively) (WDT/Bayer Health Care), cranial window (4.0 mm) was implanted above the somatosensory cortex of mice after open-skull craniotomy. After 4 weeks of recovery period, *in vivo* two-photon microscopy was carried out using LSM 7 MP microscope (Carl Zeiss) equipped with 20× objective (NA 1.0; Carl Zeiss). Mice were anesthetized with isoflurane (1% in 95% O_2 and 5% CO_2), and body temperature was kept at 37°C with the heating pad (Fine Science Tools GmbH). Apical dendrites originating from GFP-positive layer V pyramidal neurons were imaged in consecutive sessions at specified time points. GFP was excited with a femtosecond laser (Mai Tai DeepSee, Spectra Physics) at a wavelength of 880 nm. The imaging session did not exceed 60 min. Special efforts were made to keep the intensity of laser and data acquisition settings consistent throughout the experiments. Due to limitation in axial resolution, only laterally protruding spines were included into analysis. Emerging or disappearing spines over two consecutive imaging sessions over 1 week were defined as forming or eliminating spines, with their fractions normalized to the total spine number. Spine turnover rate (TOR) was defined with the following formula: $(TOR) = (N_f + N_e)/(2 \times N_t \times D)$, where N_f = formed spines, N_e = eliminated spines, N_t = total spines, D = interval days between imaging sessions. For illustration purposes only, distracting neighboring dendritic elements were removed (LSM Image examiner, Zeiss) and high-resolution (0.138 μm/pixel per frame with 1 μm/pixel z-direction) maximal projection images were deconvolved (AutoQuantX3, Media Cybernetics), with contrast and brightness adjusted.

Confocal microscopy and spine morphometry

To achieve a better resolution of spine morphologies, *ex vivo* confocal microscopy of GFP positive somatosensory neurons was used. Mice were injected with a lethal dose of ketamine/xylazine (200/14 mg/kg, i.p.), perfused transcardially with phosphate-buffered saline (0.1 M PBS, 50 ml) followed by paraformaldehyde (150 ml, 4% in PBS). Brains were extracted and postfixed in PFA at 4°C overnight and cut in coronal plane (60 μm) with the vibratome (VT 1000S, Leica). Sections containing somatosensory cortex were incubated in 0.1% Triton X-100, 5% normal goat serum (NGS) for 2 h at room temperature and exposed to rabbit anti-GFP antibody tagged

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with Alexa488 (1:200, Invitrogen) in PBS with 5% NGS for 2 h at room temperature. After three washes with PBS, slices were mounted with fluorescent media and covered for microscopic analysis. Apical dendrites of layer V pyramidal cells were imaged in slices through 40× oil immersion objective (NA 1.3; Carl Zeiss), using LSM780 confocal microscope (Carl Zeiss). Images were deconvoluted (AutoQuantX3, Media Cybernetics) with dendrites and spines reconstructed using Imaris (Bitplane) at high resolution (0.069 μm/pixel per frame with 0.395 μm/pixel z-direction). Morphological subtypes of dendritic spines were identified as follows: mushroom spine: max_width(head)/min_width(neck) > 1.4 and max_width (head) > 0.2 μm and min_width(neck) > 0 μm; stubby spine: length (spine)/mean_width(neck) ≤ 3 or min_width(neck) = 0 μm or min_width(neck) > 0.5 μm; thin spine: length(spine)/mean_width (neck) > 3. Fractions of spine subtypes (of total spine number) were assessed and compared.

Microelectrode biosensor recordings

To measure extracellular D-serine concentrations *in vivo*, microelectrode biosensors were prepared as previously described (Pernot *et al*, 2008; Vasylieva *et al*, 2011). Briefly, the biosensors consisted of a 25 μm 90% Pt/10% Ir wire with a tip that extended out of a pulled glass micropipette by 100 μm. The platinum wire was covered with an electropolymerized layer of poly-m-phenylenediamine and an enzyme layer *Rhodotorula gracilis* D-amino acid oxidase (Pollegioni *et al*, 1997). Biosensors were calibrated for D-serine and serotonin detection in PBS (0.01 M, pH 7.4) before and after *in vivo* recordings. For electrochemical biosensor recordings, mice were anesthetized by isoflurane (5% for induction, then 1–1.5%) and immobilized in a stereotactic apparatus with body temperature maintained at 37°C. An Ag/AgCl reference electrode was placed under the skin of the neck and biosensors were implanted in cortex (2.0 mm AP, ± 1.4 mm ML, 1.5 mm DV from Bregma). Simultaneously, a D-serine biosensor was inserted into the area of interest and a control biosensor coated with bovine serum albumin was inserted contralaterally. The control biosensor detects the same currents as the D-serine biosensor (i.e. endogenous oxidizable molecules, pH changes, and endogenous peroxide) with the exception of D-serine, and both control and D-serine biosensors record about 10 pA of background current in PBS. Amperometric recordings were performed for about 60 min at the end of which the difference between the current recorded by the control biosensor was subtracted from that recorded by the D-serine biosensor and divided by the sensitivity in standard solutions at 37°C.

HPLC measurements

To measure total D-serine concentrations, brain homogenates were prepared from mice anesthetized with a lethal dose of sodium pentobarbital. Homogenates were centrifuged at 10,000 *g* for 12 min to collect supernatant. HPLC measurements were performed using a Waters Alliance instrument (Waters Corporation, Guyancourt, France) with a Shiseido Capcell PAK C18 MG100 column (4.6 × 250 mm; 5 μm). The column and sample compartments were kept at 27 and 8°C, respectively, and flow rate was set at 0.9 ml/min and run time was 41 min for all analyses. Amino acid standards and brain samples were derivatized in 325-μl aliquots of a solution composed of treated with 1 mg/ml N-acetylcysteine and 2 mg/ml *o*-phthaldialdehyde in a 0.1 M borate buffer, pH 10.5. Amino acids were eluted in a gradient composed of phase A (sodium acetate 50 mM, pH 6.5) and phase B (methanol 100%) and evolving as follows: 0–7 min phase A 95% and phase B 5%, 7–19 min phase A 80% and phase B 20%, 19–27 min phase A 10% and phase B 90%, and 28–41 min phase A 95% and phase B 5%. Amino acid derivatives were detected using a Waters fluorescence detector (excitation 340 nm–emission 450 nm) and data were acquired using the Empower Pro software package (Waters Corporation, Guyancourt, France). Calibration of D-serine detection was performed using a 4-point standard curve.

Novel objective recognition test

To evaluate cognition and memory, novel objective recognition test was used as described before (Leger *et al*, 2013). Mice, with or without D-serine treatment over 5 weeks, were allowed to explore an open-field arena (40 × 40 cm) freely in the absence of objects for 10 min with motor activity recorded. One day later, mice were placed in the open-field arena with two identical sample objects with exploration period of 10 min. Mice were returned to their home cages. After 24 h, mice were put back to the arena with one of the sample objects changed into a novel one. This test phase lasted 5 min. The time spent on each object was then calculated.

Statistics

For statistical analysis and comparison, GraphPad Prism 5 was used. Comparison between two different groups was performed using two-tailed Student's *t*-test. Wilcoxon signed-rank test was used test whether the quotient of interaction time with the novel object divided by interaction time with the familial object significantly differed from a hypothetical value of 1 (equal interaction times). In the longitudinal measurements of spine analysis, repeated one-way ANOVA was performed followed by Dunnett's test. For line fittings, an extra sum-of-squares *F*-test was used to determine whether the slopes significantly differed from the hypothetical value of 0 (i.e., no dynamic change). If the null hypothesis (no dynamic change) could not be rejected with a P value < 0.05, then a slope of 0 is shown. The numbers of mice per group for *in vivo* imaging are chosen based on our previous experience and stated in figure legends. About 8–12 dendrites were imaged in each mouse; the length of each dendrite was 25–35 μm. The data are presented as the means for every mouse. Results are presented as mean ± SEM unless further specified. P-value < 0.05 was defined as statistically significant. Analysis was performed blinded with respect to mouse genotype and no explicit form of randomization was used.

Expanded View for this article is available online.

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Author contributions

CZ, MMD, UCM, and JH have designed this study. CZ, SC, SM, EM, CS, YS, SS, and KZ have collected and analyzed the data. CZ, SC, and CS have drafted the article. UCM and JH have revised the article critically. All authors have approved the version of the manuscript to be published.

Conflict of interest

The authors declare that they have no conflict of interest.

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