Impairment of Glycolysis-Derived L-Serine Production in Astrocytes Contributes to Cognitive Deficits in Alzheimer’s Disease

Highlights

- Astrocytes have impaired glycolytic flux in a mouse model of Alzheimer’s disease
- Consequently, astrocytes produce less glycolysis-derived L-serine
- Low NMDAR occupancy by D-serine leads to impairment of synaptic plasticity and memory
- Dietary supplementation of L-serine restores synaptic plasticity and memory

In Brief

Le Douce et al. show that glycolysis is impaired in astrocytes in the early stages of disease in a mouse model of Alzheimer’s. This leads to the reduction of both L- and D-serine synthesis and to the alteration of synaptic plasticity and memory. Dietary supplementation with L-serine restores both deficits, suggesting it to be a potential therapy.
Impairment of Glycolysis-Derived L-Serine Production in Astrocytes Contributes to Cognitive Deficits in Alzheimer’s Disease

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SUMMARY

Alteration of brain aerobic glycolysis is often observed early in the course of Alzheimer's disease (AD). Whether and how such metabolic dysregulation contributes to both synaptic plasticity and behavioral deficits in AD is not known. Here, we show that the astrocytic L-serine biosynthesis pathway, which branches from glycolysis, is impaired in young AD mice and in AD patients. L-serine is the precursor of ω-serine, a co-agonist of synaptic NMDA receptors (NMDARs) required for synaptic plasticity. Accordingly, AD mice display a lower occupancy of the NMDAR co-agonist site as well as synaptic and behavioral deficits. Similar deficits are observed following inactivation of the L-serine synthetic pathway in hippocampal astrocytes, supporting the key role of astrocytic L-serine. Supplementation with L-serine in the diet prevents both synaptic and behavioral deficits in AD mice. Our findings reveal that astrocytic glycolysis controls cognitive functions and suggest oral L-serine as a ready-to-use therapy for AD.

Context and Significance

A decrease in regional brain glucose consumption is observed in early Alzheimer’s disease, decades before neuronal death and clinical symptoms occur. Whether and how this hypometabolism is connected to impaired cognition is not yet known. Researchers in Paris, Bordeaux and their collaborators, have identified a mechanistic link that connects defective glycolysis in astrocytes to impaired L-serine production and reduced neuronal function. Supplementation with L-serine in the diet is able to rescue the cognitive deficits in a mouse model of Alzheimer’s disease. Understanding this metabolic pathway and its link to brain function may hold promise for the future treatment of other types of neurodegeneration characterized by early hypometabolism like Parkinson’s and Huntington’s.
INTRODUCTION

Alzheimer’s disease (AD) is a neurological disorder characterized by progressive memory decline and cognitive impairment. Functional and physical disruption of synapses is central to the disease process (Forner et al., 2017; Spires-Jones and Hyman, 2014). Dementia due to AD is also characterized by early and progressive metabolic disturbances as observed using [18F]-fluorodeoxyglucose positron emission tomography (FDG-PET). FDG-PET changes even precede brain atrophy and neuronal dysfunction (Gordon et al., 2018; Mosconi et al., 2006; Protas et al., 2013). As such, FDG-PET is included among the core biomarkers for clinical and prodromal AD (Garibotto et al., 2017). Although both amyloid-β (Aβ) and tau are central to AD pathogenesis, it remains enigmatic whether and how glucose dysregulation contributes to synaptic defects in AD. Recent observations suggest that changes in aerobic glycolysis prevail in the early phase of AD (An et al., 2018; Vlassenko and Raichle, 2015). One major function of aerobic glycolysis is to maintain high levels of glycolytic intermediates to support anaerobic reactions in cells (Lunt and Vander Heiden, 2011). L-serine, a non-essential amino acid, is generated through diversion of the glycolytic intermediate 3-phosphoglycerate (3PG) into the phosphorylated pathway. This L-serine biosynthesis pathway is a short metabolic sequence consisting of three enzymatic reactions starting with the oxidation of 3PG to 3-phosphohydroxypyruvate by 3-Phosphoglycerate Dehydrogenase (PHGDH). L-serine is a carbon donor for the biosynthesis of many macromolecules and is racemized into d-serine in the brain (Ehmsen et al., 2013). We and others have previously shown that the induction of long-term synaptic potentiation in the adult hippocampus requires the presence of d-serine, the endogenous co-agonist of synaptic NMDA receptors (NMDARs) (Henneberger et al., 2010; Panatier et al., 2006; Papouin et al., 2012; Yang et al., 2003). Therefore, a shortage of glucose may not only directly reduce the availability of ATP required to sustain synaptic transmission, but also decrease the production of both L- and d-serine. In this study, we investigated the contribution of the phosphorylated pathway to early synaptic and behavioral deficits in AD. Our findings highlight oral L-serine as a potential therapy for AD as well as other neurodegenerative diseases.

RESULTS

The serine biosynthesis pathway directly branches from glycolysis (Figure 1A). Alteration of aerobic glycolysis is an early feature of AD. In the brain, a substantial fraction of glucose is thought to be processed via aerobic glycolysis in astrocytes (Bak and Walls, 2018; Barros and Weber, 2018; Magistretti and Allaman, 2015, 2018). We used young 3xTg-AD mice to measure brain glucose metabolism in vivo, aerobic glycolysis flux in astrocytes, and serine levels in vivo.

Alteration of Glucose Metabolism in 3xTg-AD Mice

We measured energy metabolism in two groups of animals at two stages of progression of the pathology. We used 6- to 7-month-old 3xTg-AD female mice to study the early phase of AD, as only intraneuronal Aβ is present in the brain, and 11- to 12-month-old animals, which display senile plaques (Figures S1A–S1C for the anatomopathological evaluation of our colony of 3xTg-AD mice). In vivo 2-deoxyglucose autoradiography (Figure 1B) was followed by 3D voxel-wise statistical analysis to map the differences in cerebral glucose metabolism between control and 3xTg-AD mice (Barros et al., 2018). This approach allows statistically significant unbiased identification of metabolic differences between groups at the voxel level without operator-dependent delineation of regions of interest (Dubois et al., 2010). We found that 6- to 7-month-old 3xTg-AD mice displayed localized decreases in glucose uptake in the amygdala, entorhinal cortex, and hippocampus including CA1, dentate gyrus, and CA2 (Figures 1C and 1E). We also observed increases in glucose uptake in the striatum, auditory cortex, and retrosplenial cortex. Older 3xTg-AD mice (11 to 12 months old) displayed the same hypermetabolic clusters, but the size of the hypometabolic clusters was bigger, particularly in the hippocampus (Figures 1D and 1F). The magnitude of the changes in glucose uptake was similar at both ages. Very little neurodegeneration has been detected in 3xTg-AD mice, at least up to the age of 24 months (Oh et al., 2010; Rohn et al., 2008). Our current observation of an unchanged brain volume between the four groups of mice as measured on Nissl-stained brain sections for all animals used for in vivo metabolism (Figures S2A–S2C) corroborates the previous experimental evidence. It is therefore unlikely that cell death could contribute to hypometabolism in the two cohorts of 6- to 7-month-old and 11- to 12-month-old 3xTg-AD female mice. Since most energy is used at the level of excitatory synapses (Harris et al., 2012), we also probed whether the density of excitatory synapses was altered in 3xTg-AD mice. We performed serial block-face scanning electron microscopy at the level of the CA1 region of the hippocampus. We found that the density of excitatory synapses was unchanged in 6-month-old 3xTg-AD mice as compared to control animals (0.976 ± 0.036 versus 0.956 ± 0.012; Figures S2D–S2F). This result strongly suggests that the hypometabolism we observed in 3xTg-AD mice is not due to anatomical or structural changes and that 3xTg-AD mice display localized metabolic changes which start at an early stage of the disease. We next investigated whether the metabolic status of astrocytes was altered in 3xTg-AD mice.

Alteration of Glucose Metabolism in Astrocytes of 3xTg-AD Mice

Resting astrocytes produce lactate in a tonic manner via aerobic glycolysis (Sotelo-Hitschfeld et al., 2015). We monitored astrocyte lactate dynamics in real time in hippocampal slices using Laconic, a fluorescence resonance energy transfer (FRET) lactate nanosensor that was expressed in the hippocampus using an adeno-associated virus (AAV)2/9 (Figure 1G). We first confirmed the specific astrocytic tropism of the AAV2/9 expressing tdTomato under the control of the short gfaABC1D promoter (Figures S3A–S3C). Accordingly, expression of Laconic was restricted to astrocytes (Figure 1H). Lactate transport across the astrocytic plasma membrane is mediated by monocarboxylate transporters (MCTs). We therefore used AR-C155858, an MCT blocker (Ovens et al., 2010), to measure the accumulation of lactate in single astrocytes (Figure 1I) at physiological extracellular glucose concentrations (Silver and Ereciriska, 1994). The accumulation of intracellular lactate under MCT blockade observed in astrocytes of control mice was absent in 3xTg-AD astrocytes (Figure 1I). This result indicates that resting lactate...
Figure 1. 3xTg-AD Mice Display Energy Metabolism Alterations and Decreased L- and D-Serine Levels

(A) The phosphorylated pathway of L-serine biosynthesis is a short metabolic sequence consisting of three enzymatic reactions. The glycolytic intermediate 3-phosphoglycerate (3PG) is converted into phosphohydroxypyruvate (3PHP) in a reaction catalyzed by 3-phosphoglycerate dehydrogenase (PHGDH). 3PHP is metabolized to phosphoserine (3PS) by phosphohydroxypyruvate aminotransferase (PSAT1) and, finally, 3PS is converted into L-serine by phosphoserine phosphatase (PSPH). In the brain, L-serine is converted into D-serine by serine racemase (SRR). 1, 2, and 3 indicate the functional parameters we have measured in 3xTg-AD mice.

(B–D) In vivo glucose uptake was measured using 3D-reconstructed autoradiography and voxel-wise statistical analysis (B) to map the cerebral metabolic differences between (C) 6-month-old control (n = 10) and 3xTg-AD mice (n = 10), and (D) between 12-month-old control (n = 9) and 3xTg-AD mice (n = 10). Brains of 3xTg-AD mice display hypometabolic (blue) and hypermetabolic (red) clusters at both ages.
production via the aerobic glycolytic pathway is altered in 3xTg-AD astrocytes. As L-serine is generated from glucose through diversion of the glycolytic intermediate 3-phosphoglycerate into the phosphorylated pathway (Figure 1A), we next investigated whether the defective glycolytic flux in 3xTg-AD astrocytes translated into reduced L-serine production and release.

**Lower Levels of L-serine in 3xTg-AD Mice**

We first measured total L-serine level in the hippocampus and found that it was significantly lower in 3xTg-AD (880 ± 48) than in control mice (1089 ± 42 nmol/g wet tissue; Figure 1J). We then measured extracellular L-serine in the hippocampus by in vivo microdialysis and found that its concentration was also significantly lower in 3xTg-AD (7.64 ± 1.10) than in control mice (16.61 ± 1.73 μM; Figure 1K). These results strongly suggest that the production and release of L-serine is reduced in young 3xTg-AD mice. We observed these reductions in 6-month-old 3xTg-AD mice that do not exhibit Aβ plaques, overt astrocyte reactivity (GFAP staining), or activated microglia (IBA1 staining) in the CA1 region (Figure S1A and S4A–S4D). L-serine is the obligatory precursor of α-serine, the main co-agonist of synaptic NMDARs in the adult hippocampus. We next monitored the levels of α-serine in the hippocampus of 3xTg-AD mice.

**Lower Levels of α-serine in 3xTg-AD Mice**

We measured total α-serine level in the hippocampus and found that it was significantly lower in 6-month-old 3xTg-AD (244 ± 8) than in control mice (283 ± 8 nmol/g wet tissue; Figure 1J). We then measured extracellular α-serine in the hippocampus by in vivo microdialysis and found that the concentration of extracellular α-serine was also significantly lower in 6-month-old 3xTg-AD (2.90 ± 0.38) than in control mice (4.53 ± 0.72 μM; Figure 1K). These results strongly suggest that the production and release of α-serine is reduced in young 3xTg-AD mice. We did not find any difference in the level of both total and extracellular glutamate between control and 3xTg-AD mice (Figures S6D and S6E), suggesting no major impairment in glutamate synthesis, release, or uptake in young 3xTg-AD mice. We next performed electrophysiological recordings to assess the functional impact of a lower extracellular α-serine on the activity of NMDARs in 3xTg-AD mice.

**Lower Level of Occupancy of the Co-agonist Binding Site of NMDARs in 3xTg-AD Mice**

We thus recorded the NMDAR component of field excitatory post-synaptic potentials (NMDAR-fEPSPs) in acute hippocampal slices. In control mice, bath application of α-serine (50 μM) induced an increase of NMDAR-fEPSPs (126.8% ± 7.3%; Figure 2A). This is in accordance with previous work (Henneberger et al., 2010; Papouin et al., 2012), indicating that the co-agonist binding sites of synaptic NMDARs in this region are not fully saturated. Importantly, the α-serine-mediated increase in synaptic NMDAR-fEPSPs was significantly larger in 6- to 7-month-old 3xTg-AD mice (156.6% ± 8.3%; Figure 2A). This is consistent with a reduced level of occupancy of the NMDAR co-agonist binding site in 3xTg-AD mice because of a reduced level of α-serine within the cleft. Another possibility would be that the NMDAR subunit composition was switched from GluN2B to GluN2A in 3xTg-AD mice, since GluN2A-containing NMDARs have a lower affinity for α-serine than GluN2B receptors (Ferreira et al., 2017). In that case, the number of NMDARs recruited during synaptic stimulation could be reduced even if endogenous α-serine levels were unaltered. We tested this possibility by investigating the inhibitory action of a GluN2B antagonist (Ro-25-6981, 2 μM) on NMDAR-fEPSPs in both control and 3xTg-AD mice. Experiments were performed in the presence of α-serine (50 μM) to make sure that all synaptic NMDARs were available for activation and to have a better quantification of the NMDAR population present at the synapse. In wild-type (WT) animals, NMDAR-fEPSPs were slightly decreased by Ro-25-6981 (90.8% ± 4.7% of baseline; Figure 2B). Interestingly, a similar effect was observed in 3xTg-AD mice (89.8% ± 4.4% of baseline; Figure 2B). Taken together, these data indicate that the fraction of GluN2B receptors present at CA3-CA1 synapses is very limited and, most importantly, that this fraction is unchanged in 3xTg-AD mice. This result argues against the possibility of a switch of NMDAR subunits in these mice. We next monitored hippocampal excitatory synaptic transmission and plasticity in 3xTg-AD female mice, given the role played by α-serine in NMDAR activity.

**Impaired Synaptic Plasticity in 3xTg-AD Mice**

As originally reported (Oddo et al., 2003), high-frequency stimulation (HFS)-induced long-term potentiation (LTP) was impaired in 6- to 7-month-old 3xTg-AD (156.7% ± 6.7% in control versus 123.5% ± 5.4% in 3xTg-AD; Figure 2C). We found that low-frequency stimulation (LFS)-induced long-term depression (LTD) was also reduced in these animals (79.9% ± 9.5% in control versus 114.5% ± 6.6% in 3xTg-AD; Figure 2E).

**Impaired Synaptic Plasticity in 3xTg-AD Mice Is Rescued by α-Serine**

All these results support the hypothesis that the deficits in synaptic plasticity observed in 3xTg-AD mice are due to a lower concentration of α-serine in the synaptic cleft and thus to fewer NMDARs available for activation during HFS and LFS. In agreement with this hypothesis, LTP was rescued by supplying exogenous α-serine.
Figure 2. Deficits of Synaptic Plasticity at CA3-CA1 Synapses and of Spatial Memory in 3xTg-AD Mice Are Rescued by d-Serine

(A) Electrophysiological recordings were performed on acute brain slices of 6- to 7-month-old 3xTg-AD and control mice with the stimulating electrode located in CA1 and the recording electrode located in CA1 close to the subiculum. Effect of d-serine application (50 μM) on NMDAR-EPSPs in slices obtained from control

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(50 μM) to slices obtained from 3xTg-AD mice (163.6% ± 9.7% in control versus 166.1% ± 12.4% in 3xTg-AD; Figure 2D). Similarly, exogenous d-serine (50 μM) completely rescued LTD in 3xTg-AD mice (79.1% ± 11.3% in control versus 87.5% ± 4.9% in 3xTg-AD; Figure 2F). Acute application of d-serine did not affect LTP and LTD in slices obtained from control mice (Figures 2C–2F). We next assessed whether d-serine could rescue the spatial memory deficits of 3xTg-AD mice.

**Impaired Spatial Memory in 3xTg-AD Mice Is Rescued by d-Serine**

We evaluated learning and memory using the spatial reference version of the Morris water maze that is hippocampal-dependent (Morris, 1984). 3xTg-AD mice displayed a similar spatial learning performance as age-matched control mice but did not show any preference for the target quadrant during the probe trial (Figure 2G). We chronically treated mice with d-serine in the drinking water (~100mg/kg body weight), a regimen that has been previously shown to increase levels of d-serine in the brain (Otte et al., 2013). Oral supplementation of d-serine for two weeks rescued the cognitive deficits of 6- to 7-month-old female 3xTg-AD mice but did not modify the performance of control mice (Figure 2G). These results all point to defective d-serine availability at the hippocampal synapses of 3xTg-AD mice. We then asked whether the reduced production of l-serine could be responsible for the d-serine-mediated impairment of synaptic plasticity and spatial behavior. If this is the case, inactivation of the l-serine biosynthetic pathway should recapitulate the signs observed in 3xTg-AD mice. While Phgdh, the first enzyme of the l-serine synthetic pathway, was recently reported to be expressed mostly in astrocytes and oligodendrocytes, but not in neurons (see http://www.brainmaseq.org/ and http://mousebrain.org/genesearch.html), the cellular localization of PHGDH has not been thoroughly studied in the brain. We therefore performed a histological study focusing on the hippocampus.

**The Serine Synthetic Pathway Is Primarily Located in Astrocytes**

The first committed step of the l-serine biosynthetic pathway is the oxidation of 3PG to 3-phosphohydroxypropyruvate (3PHP) by PHGDH (Figure 1A). A specific antibody directed against PHGDH showed prominent staining of astrocytes not only in mouse but also in non-human primate and human brain (Figure 3A). We performed co-localization studies with confocal microscopy in a control mouse brain and confirmed that PHGDH was mainly expressed by GFAP-expressing astrocytes and not microglial cells (IBA1-positive), neurons (NeuN-positive), or oligodendrocytes (Olig2-positive) in the CA1 region of the hippocampus (Figure 3B). We next developed a conditional mouse model to interrogate the role of the astrocytic hippocampal PHGDH in synaptic plasticity and spatial behavior.

**Conditional Inactivation of Phgdh Leads to Impaired Synaptic Plasticity and Spatial Memory**

Mice conditionally lacking Phgdh in astrocytes have been previously generated by crossing Phgdh<sup>lox/lox</sup> mice with hGFAP-Cre transgenic mice (Yang et al., 2010). Since hGFAP-Cre transgene mediates gene deletions in a much wider spectrum of neural cells than just astrocytes (Zhuo et al., 2001), we locally and conditionally inactivated Phgdh by injecting an AAV expressing Cre recombinase under the control of the astrocytic gfaABC-D promoter in the hippocampus of adult Phgdh<sup>lox/lox</sup> mice (Figure 3C). At the transcriptional level, Phgdh mRNA was ~25% of its initial value found in both non-injected and AAV-GFP-injected mice (Figure 3D). At the protein level, PHGDH expression was significantly reduced in Phgdh<sup>lox/lox</sup> mice injected with AAV-Cre in comparison to mice injected with AAV-GFP (Figures 3E–3H). Astrocytes lose some of their normal functions when they become reactive (Liddelow et al., 2017). The levels of expression of mRNA for vimentin and gfa and of GFAP were not significantly different between Phgdh<sup>lox/lox</sup> mice injected with AAV-Cre or AAV-GFP (Figures S5A–S5D). We next performed a Sholl analysis on GFAP immunostained astrocytes and showed that the complexity of astrocyte processes was not altered following inactivation of Phgdh (Figures S6E and S6F). These results strongly suggest that astrocytes did not adopt a reactive phenotype following inactivation of Phgdh. The levels of expression of selected neuronal and microglial mRNAs (Neun, eaat3, cd68, and iba1) and of d-amino acid oxidase (daao), the enzyme that degrades d-serine, were not significantly different between Phgdh<sup>lox/lox</sup> mice injected with
Figure 3. Conditional Inactivation of Phgdh in Astrocytes Impairs Synaptic Plasticity and Spatial Memory

(A) Immunostaining of PHGDH in the hippocampus of a mouse, a non-human primate, and a human brain. PHGDH is mainly expressed in astrocytes.

(B) Double immunofluorescent staining of brain sections from 6-month-old control mice using PHGDH (green) and either an astrocyte marker (GFAP, red), a microglial marker (IBA1, red), a neuronal marker (NeuN, red), or an oligodendrocyte marker (Olig2, red).

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AAV-Cre or AAV-GFP (Figures S5G–SSK), indicating no marked neuronal death, no microglial activation, and no compensatory mechanisms targeting d-serine levels following local inactivation of Phgdh.

We then confirmed that the levels of L- and d-serine in the brain are determined by their de novo biosynthesis through the phosphorylated pathway in astrocytes. Indeed, the total L-serine, d-serine, and glycine content in the whole hippocampus, measured by high-performance liquid chromatography (HPLC) (Figure 3I), as well as the level of extracellular d-serine, measured in vivo via a biosensor (Figure 3J), were significantly lower in Phgdhflox/flox mice injected with AAV-Cre in comparison to mice injected with AAV-GFP. We subsequently investigated whether long-term synaptic plasticity was impaired in those mice (Figure 3K). HFS-induced LTP was significantly impaired in these animals (166.8% ± 5.8% in AAV-GFP-injected, and 133.9% ± 13.5% in AAV-Cre-injected mice; Figures 3L and 3M). Bath application of l-serine (50 μM) rescued LTP (152.2% ± 12.0%; Figures 3L and 3M), indicating that L-serine produced by the phosphorylated pathway is required for activity-dependent synaptic plasticity. We performed several behavioral tests on these mice to assess their anxiety and monitor their motor and cognitive performance. The mice with inactivated Phgdh in the hippocampus did not show any alterations in anxiety or spontaneous motor behavior, as assessed using the elevated plus-maze and the PhenoTyper (Figures 5H), as well as the level of extracellular d-serine, measured in vivo via a biosensor (Figure 5I), as opposed to mice injected with AAV-GFP. However, they did show significant impairment of spatial memory during the probe test of the Morris water maze 72 h after the location of the platform (Figure 3O), as we impaired the total L-serine, d-serine, and glycine content in the whole hippocampus as well as the extracellular levels of d-serine (Figure 3N). Accordingly, the spatial memory was restored in those animals (Figure 3O). Overall, these results show that the availability of L-serine is essential for synaptic plasticity and spatial memory in the hippocampus.

Expression of PHGDH Is Reduced in the AD Brain

We analyzed the expression of PHGDH in 6-month-old female 3xTg-AD mice that do not display Aβ plaques (Figure 4A) and found no difference as compared to control age-matched animals using immunofluorescent confocal analysis (Figure 4B) or immunoblotting (Figure 4C). This result suggests that the lower L-serine levels in 3xTg-AD mice is more likely resulting from reduced glucose uptake and less substrate available for the L-serine synthesis pathway rather than from a direct effect of Aβ on PHGDH expression, at least during the early phase of AD (i.e., astrocytes are not reactive and Aβ plaques are not formed). We then performed immunohistochemistry on human formalin-fixed paraffin-embedded sections (Figure 4D) from control individuals, intermediate AD (Braak III–IV), and advanced AD (Braak VI) patients (Table 1). Contrarily to 6-month-old female 3xTg-AD mice, advanced AD patients presented numerous Aβ plaques, hyperphosphorylated Tau, and reactive GFAP-positive astrocytes (Figure 4E). We observed a reduction of PHGDH staining (Figure 4E), which was confirmed by fluorescent immunoblotting (Figure 4F) of frozen sections from the same samples. The reduction was statistically significant in intermediate AD (~62%) and even more pronounced in advanced AD patients (~82%). These results indicate that a progressive alteration of the astrocytic serine synthetic pathway occurs in the brain of AD patients. Altogether, these data support the contribution of defective production and release of L-serine in astrocytes to the pathogenesis of AD and point to L-serine supplementation as a potential therapy. We finally asked whether supplying exogenous L-serine could also rescue both synaptic plasticity and spatial behavior deficits in 3xTg-AD mice.
Figure 4. Expression of PHGDH in 3xTg-AD Mice and in AD Human Samples
(A–C) Six-month-old female 3xTg-AD mice (A) that do not present Aβ plaques (4G8 immunostaining) (B) did not display significant reduction of PHGDH expression as observed using immunofluorescent staining or immunoblotting in the hippocampus (C). Four different concentrations of proteins were loaded per sample, and the expression of PHGDH was derived from the slopes of the regression lines (linear regression model, p = 0.79).

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Total L-serine content in the whole hippocampus was significantly higher in the two groups of animals that received the diet (see Figure 1J for comparison with untreated animals) up to a level that was not different between control and 3xTg-AD mice (496 ± 11 versus 512 ± 28 nmol/g wet tissue for control and 3xTg-AD mice, respectively; Figure 5B). Using in vivo microdialysis, we found that the levels of extracellular L- and D-serine were higher in 3xTg-AD mice receiving the diet than in untreated 3xTg-AD mice (24.91 ± 6.29 versus 7.64 ± 1.10 μM and 8.22 ± 1.79 versus 2.90 ± 0.38 μM for L- and D-serine, respectively; see Figure 1K for comparison with untreated animals). The diet did not significantly alter the total levels of other amino acids measured in the hippocampus; only a modest increase in phenylalanine level was observed (Figures S6A–S6I). We then observed that in 3xTg-AD mice fed with L-serine, bath application of D-serine (50 μM) induced a similar increase of NMDAR-fEPSPs to the one measured in control animals (126.3% ± 3.5% in control versus 124.4% ± 2.3% in 3xTg-AD; Figure 5C). This result indicates that the chronic supplementation of L-serine was able to rescue the deficit of D-serine availability observed at hippocampal synapses in 3xTg-AD mice, thereby restoring the level of occupancy of the NMDAR co-agonist binding site to its control values. Accordingly, LTP was fully rescued in those animals (152.0% ± 3.2% in control versus 154.5% ± 5.0% in 3xTg-AD; Figure 5D). We finally probed the spatial memory performance in 3xTg-AD mice fed with L-serine and found that it reached a level similar to the one measured in control mice fed with L-serine (Figure 5E). Altogether, these data support the use of oral L-serine as a ready-to-use and safe therapy for AD.

**DISCUSSION**

Glial cells have been recently implicated in AD. Astrocytes and microglia are now recognized as potential cellular mediators of synapse dysfunction and loss (Hong et al., 2016; Jo et al., 2014). Here, we have identified an astrocytic metabolic pathway that directly contributes to early synaptic deficits underlying cognitive decline in AD, well before microglia and astrocytes become reactive (Liddelow et al., 2017). We showed that the production of L-serine, a non-essential amino acid that is generated from glucose, is impaired in AD astrocytes.

The L-serine biosynthetic pathway has been well studied in cancer cells that exhibit increased glucose metabolism to lactate (the so-called Warburg effect) and in which multiple copies of Phgdh divert glycolytic flux and provide L-serine and glycine for rapid cell growth and proliferation (Locasale et al., 2011; Possemato et al., 2011). L-serine is a central node for the biosynthesis of many molecules such as sphingolipids and phospholipids. It also supplies carbon to the one-carbon pool, which is

### Table 1. Demographic Data on Studied Cases

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**AD (III–IV); Alzheimer’s disease, Braak III–IV.**

**AD (VI); Alzheimer’s disease, Braak VI.**

**PMD: postmortem delay; N/A: Not Available; # used for immunoblotting.**

**Oral L-Serine as a Potential Therapy for AD**

We first assessed the effects of acute L-serine (50 μM) on LTP. Bath application of L-serine (50 μM) fully rescued LTP in slices obtained from 3xTg-AD mice (156.8% ± 7.4% in control versus 160.5% ± 6.4% in 3xTg-AD; Figure 5A). Since long-term treatment with exogenous L-serine can compensate for its deficient biosynthesis of many molecules such as sphingolipids and phospholipids, we next assessed the effects of chronic oral L-serine supplementation upon both the long-term synaptic plasticity and the spatial memory performance of 3xTg-AD and control mice. Four-month-old mice were given a 10% L-serine-enriched diet for 2 months (Figure 5B). Total L-serine content in the whole hippocampus was significantly higher in the two groups of animals that received the diet (Figure 5B) as compared to untreated animals (Figure 1J). Two months after chronic L-serine diet, total L-serine content was no longer significantly different between control and 3xTg-AD mice (1590 ± 87 versus 1844 ± 73 nmol/g wet tissue, respectively; Figure 5B). The diet also boosted de novo production of D-serine since its content in the whole hippocampus was significantly higher in the two groups of animals that received the diet (see Figure 1J for comparison with untreated animals) up to a level that was not different between control and 3xTg-AD mice (496 ± 11 versus 512 ± 28 nmol/g wet tissue for control and 3xTg-AD mice, respectively; Figure 5B). Using in vivo microdialysis, we found that the levels of extracellular L- and D-serine were higher in 3xTg-AD mice receiving the diet than in untreated 3xTg-AD mice (24.91 ± 6.29 versus 7.64 ± 1.10 μM and 8.22 ± 1.79 versus 2.90 ± 0.38 μM for L- and D-serine, respectively; see Figure 1K for comparison with untreated animals). The diet did not significantly alter the total levels of other amino acids measured in the hippocampus; only a modest increase in phenylalanine level was observed (Figures S6A–S6I). We then observed that in 3xTg-AD mice fed with L-serine, bath application of D-serine (50 μM) induced a similar increase of NMDAR-fEPSPs to the one measured in control animals (126.3% ± 3.5% in control versus 124.4% ± 2.3% in 3xTg-AD; Figure 5C). This result indicates that the chronic supplementation of L-serine was able to rescue the deficit of D-serine availability observed at hippocampal synapses in 3xTg-AD mice, thereby restoring the level of occupancy of the NMDAR co-agonist binding site to its control values. Accordingly, LTP was fully rescued in those animals (152.0% ± 3.2% in control versus 154.5% ± 5.0% in 3xTg-AD; Figure 5D). We finally probed the spatial memory performance in 3xTg-AD mice fed with L-serine and found that it reached a level similar to the one measured in control mice fed with L-serine (Figure 5E). Altogether, these data support the use of oral L-serine as a ready-to-use and safe therapy for AD.

(D) Human post-mortem brain samples included the hippocampus, parahippocampal gyrus, and fusiform gyrus and presented numerous Aβ plaques (6F/3D immunostaining).

(E) Expression of PHGDH was reduced in an advanced AD brain (Braak VI) that presented amyloid plaques (Aβ staining using 6F/3D antibody), hyperphosphorylated Tau (AT8), and GFAP reactive astrocytes as observed on formalin-fixed paraffin-embedded sections.

(F) Fluorescent immunobLOTS of fresh brain tissues from controls (n = 5), AD Braak III–IV (n = 4), and AD Braak VI (n = 5). Four different concentrations of proteins were loaded per sample, and the expression of PHGDH was derived from the slopes of the regression lines. Expression of PHGDH was significantly lower in intermediate AD cases (–62%) and in advanced AD patients (–82%) as compared to controls (linear regression model, p < 0.001). Data are presented as mean with SEM.
Figure 5. Deficits of Synaptic Plasticity at CA3-CA1 Synapses and of Spatial Memory in 3xTg-AD Mice Are Rescued by L-Serine

(A) Bath L-serine (50 μM) application in hippocampal acute slices rescued LTP in 3xTg-AD mice to the same level as control mice (Mann-Whitney U test, p = 0.97; control: n = 8; 3xTg-AD: n = 5). Traces represent typical fEPSPs evoked before (1) and after (2) LTP induction. Bar graph summarizing experiments presented in (A).

(B) 3xTg-AD and control 4-month-old mice received chronic oral L-serine supplementation (food enriched with 10% L-serine for 2 months). The total concentration of L- and D-serine in the hippocampus was similar in both groups of mice after L-serine treatment.

(C) Chronic L-serine diet restored NMDAR co-agonist binding site occupancy measured in 3xTg-AD mice to the same value measured in control mice (Mann-Whitney U test, p = 0.63; control: n = 5; 3xTg-AD: n = 13). Traces of representative NMDAR-fEPSPs recorded before (1) and during (2) D-serine application (50 μM). Bar graph summarizing results presented in (C).

(D) Chronic oral L-serine supplementation fully restored the LTP deficits of 3xTg-AD mice (Mann-Whitney U test, p = 0.8; control: n = 7; 3xTg-AD: n = 7). Representative traces of characteristic fEPSPs evoked before (1) and after (2) LTP induction. Bar graph summarizing results presented in (D).

(E) 3xTg-AD mice fed with a food enriched in 10% L-serine for 2 months spent more time in the target quadrant (one tailed one sample t test against chance level [25%], p = 0.007), similarly to control mice fed with the same diet (p = 0.001), during the probe test of the Morris water maze. AQ, adjacent quadrant; OQ, opposite quadrant; TQ, target quadrant. Data are presented as mean with SEM.
involved in folate metabolism, contributing to purine synthesis (Mattaini et al., 2016). This metabolic pathway is particularly essential for the nervous system as homozygous mutations of each of the three enzymes of the serine biosynthesis pathway cause a syndrome called Neu-Laxova, a rare autosomal-recessive disorder characterized by severe peripheral and central nervous system malformations (microcephaly), leading to prenatal or early postnatal death (Acuna-Hidalgo et al., 2014). This clinical phenotype of L-serine biosynthesis is reminiscent of defects in brain serine transport induced by mutations in SLC1A4, the gene coding for the neutral amino acid transporter ASCT1 (Damseh et al., 2015), underlying the critical role of L-serine biosynthesis and shuttling. L-serine treatment during pregnancy and after birth improves fetal brain growth and prevents neurological symptoms (de Koning et al., 2004), suggesting that exogenous L-serine can compensate for its lack of de novo production in the brain.

In the present work, we focused our attention on glycolysis as a possible regulator of L-serine production in the brain as L-serine is generated from glucose. In AD, early alterations of cerebral glucose metabolism, as measured using fluoro-2-deoxy-D-glucose (FDG) and positron emission tomography (PET), are well established. Several studies have shown that abnormalities in FDG-PET can even predict progression from mild cognitive impairment (MCI) to AD (for references see Cohen and Klunk [2014]). We used 3D analysis of in vivo brain glucose metabolism to show that 3xTg-AD mice display metabolic impairment in specific brain regions, including the hippocampal formation. Vascular dysregulation (Iturria-Medina et al., 2016), as well as endothelial glucose transporter 1 (GLUT1) reductions, are mechanisms by which Ají could likely exert its deleterious metabolic effects (Winkler et al., 2015). Glucose, the almost exclusive energy substrate of the brain, is glycolytically processed to produce pyruvate and lactate in astrocytes, whereas neurons predominantly process glucose through the pentose phosphate pathway to produce NADPH and utilize pyruvate and lactate from astrocytes to feed the tricarboxylic acid (TCA) cycle and the associated oxidative phosphorylation to produce ATP (Herrero-Mendez et al., 2009). Our observation that PHGDH, whose substrate is a glycolytic intermediate, is exclusively expressed by astrocytes is consistent with the notion that astrocytes are glycolytic cells. Even if we do not provide a causal link between the defective hippocampal glycolysis and the alteration of the phosphorylated pathway, our findings suggest that astrocytic energy metabolism may control L-serine production, thereby influencing glutamatergic neurotransmission. In addition to lactate (Suzuki et al., 2011; Yang et al., 2014), we propose that another byproduct of aerobic glycolysis (L-serine) may play an important role in plasticity and learning.

In the brain, L-serine is the obligatory precursor of D-serine (Ehmsen et al., 2013), a physiological ligand of the co-agonist site of synaptic NMDARs in the hippocampus, which is required to sustain synaptic transmission (Panatier et al., 2006; Papouin et al., 2012). Our electrophysiological recordings show a reduced level of occupancy of the NMDAR glycine-binding site in 6- to 7-month-old 3xTg-AD mice. Accordingly, D-serine completely rescued both forms of synaptic plasticity (LTP and LTD) as well as spatial memory. Chronic D-serine supplementation has long been considered a therapeutic option for pathological conditions involving NMDAR malfunction such as schizophrenia (Kantrowitz et al., 2016), but its slow and weak diffusion through the blood-brain barrier and potential nephrotoxicity have limited its clinical use. L-serine represents a more favorable therapeutic option because it is considered to be safe by the Food and Drug Administration; it has been approved as a normal food additive, it is widely sold as a dietary supplement, and is well-tolerated, even at high doses. L-serine is currently prescribed for the treatment of Neu-Laxova syndrome, which results in L-serine deficiency (van der Crabben et al., 2013) and is currently in a Phase I clinical trial as a treatment for hereditary sensory autonomnic neuropathy type 1 (Garofalo et al., 2011) and amyotrophic lateral sclerosis (Levine et al., 2017). L-serine supplementation was recently shown to ameliorate motor and cognitive performance in rare NMDA-related severe encephalopathy leading to glutamatergic signaling deficiency (Soto et al., 2019). Glutamate excitotoxicity has long been implicated in AD pathophysiology, as supported by the use of the uncompetitive NMDAR partial antagonist memantine to treat moderate-severe AD patients. Although it is possible that increasing D-serine concentrations in AD patients may exacerbate glutamate excitotoxicity, this would probably not occur at an early stage of the pathology, when memantine is not yet effective (Schneider et al., 2011). It is noteworthy that people living in Okinawa, a Japanese island known for the overall longevity of its population, have a diet characterized by an L-serine content that is four times higher than that of the average American (Cox and Metcalf, 2017).

This study finally points to direct impairment of the phosphorylated pathway in AD, as shown by a marked reduction of PHGDH expression in AD patients. This is the first demonstration of the selective expression of PHGDH in human hippocampal astrocytes, as well as its decrease during the progression of AD. Since we did not observe any change in PHGDH expression in 6-month-old 3xTg-AD mice that do not display Ají plaques and whose astrocytes are not reactive, it is therefore highly conceivable that the primary event leading to less serine production would be related to altered glycolysis flux in early AD and that PHGDH expression would be affected in later AD stages. The fact that brain glucose metabolism is altered in the early phase of AD patients is compatible with such a hypothesis. Unfortunately, there is no consensus on the evolution of L- and D-serine levels in AD (Biernans et al., 2016; Lin et al., 2017; Madeira et al., 2015). Additional studies are necessary to determine whether, where, and when the levels of L- and D-serine change during disease progression. Altogether, these data raise the prospect of L-serine supplementation as a treatment option for AD and possibly other neurodegenerative diseases.

**Limitations of Study**

There are some limitations to our study, which should be acknowledged. First, we only performed this study in a single model of AD (3xTg-AD mice). It would be important to investigate whether the reduced production of L-serine by astrocytes that we uncovered is a general feature that can be found in another transgenic AD mouse models that do not present the Tau mutations. Second, we analyzed a rather limited number of
human hippocampal samples and it may be worthwhile to complete the analysis with more samples coming from other brain regions as well. Finally, an intriguing question that will be important to investigate in the future is the impact of the supplementation of L-serine in the diet on the amyloid load and the Tau hyperphosphorylation.

**STAR★ METHODS**

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  - Human Samples
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  - Postmortem Image Registration, Three-Dimensional Reconstruction and Analysis
  - Generation of AAVs and Stereotoxic Injection in Mice
  - FRET Imaging of Brain Slices
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  - Supplementation of L- or D-serine
- **QUANTIFICATION AND STATISTICAL ANALYSES**
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**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.cmet.2020.02.004.

**ACKNOWLEDGMENTS**

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**AUTHOR CONTRIBUTIONS**

J.L.D. performed the confocal analysis, carried out the biochemical, qRT-PCR and the metabolic studies and developed the AAVs with the help of M. Maugard, A.-P.B. and M.-C.G.; J.V. and M. Matos performed electrophysiology on 3xTg-AD mice with the help of A. Panatier; M. Maugard performed all experiments on Phgdh+/− mice, immunoblotting, and all behavioral tests with the help of K.C.; P.J. carried out electrophysiology on Phgdh+/− mice; P.-A.V., P.J., and M.G.-G. performed microdialysis; N.T.T., M.T., S.G., A.G., and A. Pruvost measured extracellular amino acid concentrations using CE-LIF or LC-MS/MS; E.F. performed the histological analysis of the 3xTg-AD colony and performed the in vivo metabolic study with the help of G.B. and M.G.-G.; X.T. carried out the FRET imaging experiments with the help of J.P., E.G., and B.C.; M.V., Y.B., A.-S.H., and T.D. performed the 3D image processing and analysis of the in vivo metabolic data; S.M. and M. Maugard measured extracellular α-serine using biosensors; A.K. and S.F. provided the Phgdh+/− mice and measured amino acid concentrations using HPLC; L.S., V.S., and C.D. performed and analyzed immunohistochemical staining on human samples with the help of P.-A.V.; G.K. performed EM studies; P.H. and E.B. provided materials and discussed the data; B.C., S.H.R.O., and G.B. obtained funding for the research; and A. Panatier and G.B. coordinated the study and wrote the manuscript.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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**REFERENCES**


# STAR METHODS

## KEY RESOURCES TABLE

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(Continued on next page)
**LEAD CONTACT AND MATERIALS AVAILABILITY**

Further information and requests for resources and reagents should be directed to and will be made available upon reasonable request by the Lead Contact, Gilles Bonvento (gilles.bonvento@cea.fr). This study did not generate new unique reagents.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Mouse Models**

All experimental procedures using animals were performed in strict accordance with French regulations (Code Rural R214/87 to R214/130). They conform to the ethical guidelines of both the European Directive (2010/63/EU) and the French National Charter.

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**Oligonucleotides**

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**Software and Algorithms**

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<td>Ethovision for behavioral studies</td>
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<td>32 KaratTM system 7.0 for capillary electrophoresis</td>
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<td>Fiji for confocal images</td>
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<td>BrainVisa for 3D autoradiography</td>
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used introducing two loxP sites flanking exons 4 and 5 and were backcrossed with the C57BL/6J strain for more than 10 generations. We

isosflurane (2% in O₂) and two catheters were inserted into the femoral artery and vein, respectively. Body temperature was main-

fasted for 12 h before the experiment but had free access to water. On the day of the experiment, the mice were anesthetized with

10). Experiments were performed in conscious, lightly restrained animals that were previously habituated to the constraint. Animals

month-old female control mice (n = 9), 12-month-old female 3xTg-AD mice (n = 10), and 12-month-old female control mice (n =

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Human Samples
Frozen brain tissue (hippocampus, parahippocampal gyrus and fusiform gyrus) from Controls (n = 5), AD Braak III-IV (n = 4), and AD

Braak VI (n = 5) were provided by the GIE NeuroCeb biobank in Paris. Paraffin sections of the same brain area from Controls (n = 6; age average 66.3yr, female and 73.5yr, male), AD Braak III-IV (n = 7; age average 79.4yr, female and 75.5yr, male) and AD Braak VI (n = 8; age average 81.3yr, female and 73.8yr, male) were provided by the Laboratory of Neuropathology at the Hôpital Pitié-Salpêtrière.

We did not analyze the influence of sex on our results because of the low number of samples. Previous immunostaining of Tau (AT8,

8; age average 81.3yr, female and 73.8yr, male) were provided by the Laboratory of Neuropathology at the Hoˆpital Pitie´-Salpeˆtrie` re. 73.5yr, male), AD Braak VI (n = 5) were provided by the GIE NeuroCeb biobank in Paris. Paraffin sections of the same brain area from Controls (n = 6; age average 66.3yr, female and 73.5yr, male), AD Braak III-IV (n = 7; age average 79.4yr, female and 75.5yr, male) and AD Braak VI (n = 8; age average 81.3yr, female and 73.8yr, male) were provided by the Laboratory of Neuropathology at the Hôpital Pitié-Salpêtrière. We did not analyze the influence of sex on our results because of the low number of samples. Previous immunostaining of Tau (AT8,

METHOD DETAILS

In Vivo [14C]-2-Deoxyglucose Uptake
We measured [14C]-2-Deoxyglucose (2-DG) uptake in four experimental groups: 6-month-old female 3xTg-AD mice (n = 10), 6-

month-old female control mice (n = 9), 12-month-old female 3xTg-AD mice (n = 10), and 12-month-old female control mice (n =

Experiments were performed in conscious, lightly restrained animals that were previously habituated to the constraint. Animals

fasted for 12 h before the experiment but had free access to water. On the day of the experiment, the mice were anesthetized with

isosflurane (2% in O₂) and two catheters were inserted into the femoral artery and vein, respectively. Body temperature was main-

fused for recovery from anesthesia for 1 h and intravenously injected with [14C]-2DG (16.5 μCi/100 g body weight; Perkin Elmer, Boston, MA, USA). Glycemia was measured using a DIGL-100 QuantiChrom Glucose Assay Kit. The mice were euthanized by injection of a lethal dose of sodium pentobarbital 45 min after injection. The brains were rapidly removed and immediately frozen at −40 °C in isopentane. They were cut into 20 μm-thick coronal sections with a CM3050S cryostat (Leica, Rueil-Malmaison, France). Every other section was mounted on a coverslip, rapidly heat-dried, and placed against autoradiographic film (Kodak Biomax MR) for 10 days together with radioactive [14C] standards (146C, American Radiochemical Company, St Louis, MO, USA). The sections not used for the autoradiography study were mounted on Superfrost glass slides that were then processed for Nissl staining, to obtain additional information about brain anatomy.

Postmortem Image Registration, Three-Dimensional Reconstruction and Analysis

Autoradiographic and Nissl-stained sections were digitized using a high-resolution flatbed scanner (ImageScanner III; GE Healthcare Europe, Orsay, France) at 600 dpi (pixel size 42 × 42 μm²) and 1,200 dpi (pixel size 21 × 21 μm²) in-plane resolution, respectively. Image processing was performed using our in-house software platform BrainVISA (http://brainvisa.info/). Each anatomic volume was first reconstructed in 3D, and each autoradiographic volume was reconstructed in 3D using the corresponding anatomic volume as a geometrical reference for co-registration (Dubois et al., 2008). The non-rigid registration approach is based on FFD (Free Form Deformation, polynomial model) and has been previously found to be very robust (Lebenberg et al., 2010; Rueckert et al., 1999). Autoradiographic images were converted from gray scale to activity (nCi/g) using the [14C] standards and normalized at 200 nCi/g. A brain template was created using all the anatomic brain volumes at a final resolution of 0.1 × 0.1 × 0.1 mm³. Regional differences in cerebral glucose uptake between 3xTg-AD and control mice were then assessed using a voxel-wise two-tailed unpaired t test implemented in BrainVISA (Vandenbemgehe et al., 2016).

Generation of AAVs and Stereotaxic Injection in Mice
The plasmid encoding the sensor Laconic (San Martin et al., 2013) was obtained from Addgene (www.addgene.org). The plasmid encoding Cre-ires-GFP was kindly provided by Martine Cohen-Salmon. We generated adenov-associated viral vectors (AAV2/9) expressing Laconic, td-Tomato, GFP or Cre-ires-GFP under the control of the short gfaABC₁D promoter. The design, production, and titration of AAV2/9 vectors have been described previously (Berger et al., 2015). Mice were anesthetized with a mixture of ketamine
(150 mg/kg) and xylazine (10 mg/kg). Lidocaine (5 mg/kg) was injected subcutaneously under the scalp 5 min before the beginning of surgery. The mice received bilateral stereotaxic injections of AAVs administered by a 10-μl Hamilton syringe via a 34-gauge blunt needle. The stereotaxic coordinates used were as follows: AP - 2 mm; L ± 2 mm; V – 1.2 mm with the tooth bar set at 0. Mice received a dose of 10^{10} viral genomes (VG) in a total volume of 2 μl per injection site at a rate of 0.2 μl/min. At the end of the injection, the needle was left in place for 5 min before being slowly removed. The skin was sutured, and the mice were allowed to recover for three to six weeks.

**FRET Imaging of Brain Slices**

**Injections of AAVs in Mice**

Five-month old female 3xTg-AD and control mice were injected in the dorsal hippocampus with an AAV2/9 expressing Laconic under the control of the short gfaABC_{3D} promoter and used four to six weeks later.

**Slice Preparation**

The mice were deeply anesthetized with Ketamine/Xylazine. Heparin (1000 U) was directly injected into the left heart ventricle to prevent blood coagulation, and the mice were perfused transcardially with oxygenated ice-cold sucrose artificial cerebrospinal fluid (ACSF) containing: 126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_{2}PO_{4}, 2 mM CaCl_{2}, 1 mM MgCl_{2}, 26 mM NaHCO_{3}, 2.5 mM glucose, 25 mM sucrose, and 3 mM kynurenic acid (Sigma) as previously described (Hay et al., 2016). The brains were quickly removed and sagittal slices (300 μm thick) containing the hippocampus were cut with a vibratome (VT1000S; Leica). Slices were allowed to recover 10 min at 37°C in oxygenated sucrose ACSF and then placed at room temperature for 1 h in ACSF saturated with O_{2}/CO_{2} (95%/5%), containing 2.5 mM glucose and 22.5 mM sucrose. The slices were transferred to a submerged recording chamber and perfused with oxygenated ACSF lacking kynurenic acid.

**Fluorescence Imaging**

Two-photon images were obtained with a custom-built 2-photon laser scanning microscope based on an Olympus BX51WI upright microscope (Olympus) with a 40X (0.8 NA) water-immersion objective and a titanium:sapphire laser (MaiTai HP; SpectraPhysics) tuned at 850 nm for mTFP excitation. Galvanometric scanners (model 6210; Cambridge Technology) were used for scanning, and a piezo-driven objective scanner (P-721 PIFOC; Physik Instrumente GmbH) was used for z stack image acquisition. A two-photon emission filter was used to reject residual excitation light (E700 SP; Chroma Technology). A fluorescence cube containing 479/40 and 542/50 emission filters and a 506-nm dichroic beam splitter (FF01-479/40, FF01-542/50 and FF506-Di02-25x36 Brightline Filters; Semrock) was used for the separation of the mTFP and Venus signals. Two imaging channels (H9305 photomultipliers; Hamamatsu) were used for simultaneous detection of the two types of fluorescence emission. An image stack of 10 to 20 images with a 1-μm interval was acquired every 30 s. Occasional x, y, and z drifts were corrected using custom macros developed from ImageJ plugins TurboReg,StackReg, MultiStackReg and Image CorrelationJ. mTFP and Venus fluorescence intensities were measured at the soma of astrocytes. Fluorescence ratios (R) were calculated by dividing the mean mTFP intensity of a given ROI by the mean Venus intensity of the same ROI. The ratios were normalized to the baseline ratio (R0) calculated in the same ROI during the 10-min prior to the application of AR-C155888 (1μM). Cells exhibiting a baseline with a slope smaller than 0.5% min^{-1} were considered to be stable and further analyzed. Effect of monocarboxylate transporter blockade was quantified by averaging the normalized ratio (R/R0) during the last five minutes of drug application.

**Electron Microscopy**

Six-month-old female mice (3 control and 3xTg-AD) were euthanized with an overdose of pentobarbital and immediately perfused, via the heart, with a buffered mix of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Brains were then removed and stored overnight in PBS at 4°C. Coronal vibratome sections were cut at 80 microns thickness, post-fixed in potassium ferrocyanide (1.5%) and osmium (2%), then stained with thiocarbohydrazide (1%) followed by osmium tetroxide (2%). They were then stained overnight in uranyl acetate (1%), washed in distilled water at 50°C, before being stained with lead aspartate at the same temperature. They were finally dehydrated in increasing concentrations of alcohol and then embedded in Spurs resin and hard-ened at 65 degrees for 24 h between glass slides. The regions containing the hippocampal region CA1 were trimmed from the rest of the section using a razor blade and glued to an aluminum stub and mounted inside scanning electron microscope (Zeiss Merlin, Zeiss NTS). Serial sections were cut from the face using an ultramicrotome mounted in the microscope (Gatan, 3View) and the block face imaged after every cut using a beam voltage of 1.7 kV and pixel size of 6 nm with a dwell time of 1 microsecond. Series of 200 – 300 sections were collected, aligned in the FIJI imaging software (www.fiji.sc) and synapses counted in the TrakEM2 plugin. Synapses were counted through discrete volumes through the series of images and classified according to their morphology. Asymmetrical synapses were presumed excitatory (glutamatergic) and symmetric, inhibitory (GABAergic). A total of 1067 synapses were counted in the 3xTg-AD mice and 1094 in the controls.

**Immunohistochemistry of Human Brain Samples**

Immunohistochemistry was performed on a VENTANA Discovery XT automated staining instrument according to the manufacturer’s instructions. All solutions were from Roche Diagnostics (Meylan, France). Briefly, slides were de-paraffinized using EZprep solution for 30 min at 75°C, followed by epitope retrieval with RiboCC solution for 20 min at 95°C. Primary rabbit polyclonal PHGDH antibody (Frontier Institute, Hokkaido, Japan) was diluted with the provided Discovery Ab Diluent at 1:300 and applied manually. Slides were developed using the DABMap detection kit, counterstained with hematoxylin II for 8 min, washed, dehydrated, and coverslipped.
After rinsing, the membrane was incubated with a fluorescent secondary antibody (1:5000, IRDye/C210 solutions as previously described (Janes, 2015; McDonough et al., 2015). The slope was determined for each sample from four dilutions.

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5'-CCTTCACCATGTCACAAACTGGA-3' (reverse); 5'-AGCAGACTATCGGCAGGGAACAT-3' (forward) and 5'-GCGGCTCTAGG GACTCGTTC-3' (reverse); 5'-TCAGAGGGTCAGCCGCCAC-3' (forward) and 5'-TGCAGGGCTCTTCGGCTTC-3' (reverse); 5'-ATGGCTATCGGCAGATTG-3' (forward) and 5'-CTCGAGACATGGCAGGACAT-3' (reverse); 5'-ATGGCAATGCTGGGACCAAA-3' (forward) and 5'-GCCCTTTTACCTCCAGCCC-3' (reverse); 5'-GTTGCAAGAGGAGTGAT-3' (forward) and 5'-GATGATGACCGAGA GTGTAGATA-3' (reverse). Relative expression was assessed using the ΔΔCt calculation.

Microdialysis
Age matched control and 3xTg-AD mice received a unilateral cannula implantation surgery. Briefly, mice were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg). Buprenorphine (0.05 mg/kg, subcutaneous) was given pre-operative as well as lidocaine (0.5% in water, 50μl subcutaneous at the level of the scalp). Mice were fixed in a stereotaxic frame, the skull was exposed, cleaned, and a bonding agent (OptiBond® FL) was applied. A guide cannula (CMA P000138) was inserted above the CA1 region of the hippocampus (coordinates: AP, -2 mm; L, 2 mm from bregma; V, 0.5 mm from dura) and sealed in place with a photopolymerizable dental cement (Tetric EvoFlow®). Mice recovered 4-7 days before sampling. Mice were habituated to the polycarbonate enclosure (STANK/WF, Instech) for 3 days. On the day of sampling (usually at 1 pm), a microdialysis probe (CMA 7, 1mm cuprophane) was inserted through the guide cannula. Probes were perfused with aCSF (147 mM NaCl, 2.7mM KCl, 1.2 mM NaHCO3, 470 mM borate buffer pH 10.25 and 12.5 mM cysteic acid, as internal standard, 2.2 mM asparagine, 1.5% sodium pentobarbital). Tissues were weighed, homogenized, and centrifuged. Proteins in the supernatant were removed by adding 100 μl of 10 mM sodium borate buffer pH 10 containing 5 mM cysteic acid, as internal standard, and 6 μl of the derivatization solution containing 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) at 2.5 mg/mL and 250 μM hydrochloric acid in methanol / water (50/50 v/v). The mixture was heated at 65°C for 30 min and the samples kept at 7 ± 2°C in the CE autosampler until analysis. Quantitative analysis of L-serine was performed using Capillary Electrophoresis coupled to Laser-induced Fluorescence detection as previously described (Esaki et al., 2011). Capillary electrophoresis experiments were carried out using a PA800 plus instrument (Sciex, formerly Beckman Coulter, Redwood City, CA, USA). The instrument was coupled with LIF detection equipped with a 3.5 mW argon-ion laser. Excitation and emission wavelengths were 488 and 520 nm, respectively. Data acquisition and instrument control were carried out using 32 Karat™ system software version 7.0 (Sciex). Electroforetic conditions were: 175 mM borate buffer pH 10.25 and 12.5 mM cysteic acid in 10% acetonitrile. The flow rate of the mobile phase was 0.15 mL/min with an isocratic mode. The samples were diluted (1/2) in 5% perchloric acid. Samples were derivatized with a fluorescent labeling reagent, 4-fluoro-7-nitro-2,1,3-benzoxadiazole. Amino acids were separated using HPLC and fluorescence of the derivatized amino acids was detected at 530 nm with excitation at 470 nm as previously described (Esaki et al., 2011).

Microelectrode Biosensor Recordings
We measured extracellular D-serine concentrations in vivo in anesthetized Phgdhfloxtflox mice using microelectrode biosensors. They were prepared at the AniRA-Neurochem Technological platform (Lyon, France) as previously described (Pernot et al., 2008; Vasylieva et al., 2011). Biosensors were calibrated with D-serine in PBS (0.01 M, pH 7.4) before and after in vivo recordings, and their selectivity was verified by the absence of detection of serotonin (20–40 μM). Mice were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg), an Ag/AgCl reference electrode was placed under the skin of the neck, and the biosensor implanted in the hippocampus (AP - 2 mm; L ± 2 mm; V – 1.2 mm from Bregma). A control biosensor coated with bovine serum albumin was inserted ~100μm away from the guide cannula. The CROWNPAK CR-I(+) column (5 μm I-CLASS and a Xevo-TQ-XS triple quadruple mass spectrometer (Waters SAS, Saint-Quentin-en-Yvelines, France) with an ESI interface was equipped with a 3.5 mW argon-ion laser. Excitation and emission wavelengths were 488 and 520 nm, respectively. Data acquisition, electrophoretic conditions were: 175 mM borate buffer pH 10 containing 5 mM cysteic acid, as internal standard, and 6 μl of the derivatization solution containing 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) at 2.5 mg/mL and 250 μM hydrochloric acid in methanol / water (50/50 v/v). The mixture was heated at 65°C for 30 min and the samples kept at 7 ± 2°C in the CE autosampler until analysis. Quantitative analysis of L-serine was performed using Capillary Electrophoresis coupled to Laser-induced Fluorescence detection as previously described (Lorenzo et al., 2013). Capillary electrophoresis experiments were carried out using a PA800 plus instrument (Sciex, formerly Beckman Coulter, Redwood City, CA, USA). The instrument was coupled with LIF detection equipped with a 3.5 mW argon-ion laser. Excitation and emission wavelengths were 488 and 520 nm, respectively. Data acquisition and instrument control were carried out using 32 Karat™ system software version 7.0 (Sciex). Electroforetic conditions were: 175 mM borate buffer pH 10.25 and 12.5 mM β-cyclodextrin for the electrolyte. Separation was performed in a 50 cm x 75 μm Id silica capillary at 21 kV and at 25°C.

LC-MS/MS
We used high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of extracellular D-serine, L-serine and L-glutamate (Sugimoto et al., 2015). The LC-MS/MS system consisted of an UPLC Acquity I-CLASS and a Xevo-TQ-XS triple quadruple mass spectrometer (Waters SAS, Saint-Quentin-en-Yvelines, France) with an ESI interface. The CROWNPAK CR-Ⅲ(+) column (5 μm, 3 mmID, 150 mm) Daicel Corporation, Tokyo, Japan) was used for the chiral chromatographic separation in a container cooled between 0 and +1°C during the analysis. The mobile phase consisted of 0.3% trifluoroacetic acid in 10% acetonitrile. The flow rate of the mobile phase was 0.15 mL/min with an isocratic mode. The samples were diluted (1/2) in the mobile phase and 10 μL was injected into the LC-MS/MS system. In these conditions, retention times were 4.60, 4.82 and 11.0 min for D-serine, L-serine and L-glutamate, respectively. Precursor-product ion pair transitions were chosen for a multiple reaction monitoring in positive ionization mode.

HPLC
Total amino acid concentrations were measured by preparing hippocampal homogenates from mice anesthetized with a lethal dose of sodium pentobarbital. Tissues were weighed, homogenized, and centrifuged. Proteins in the supernatant were removed by adding 5% perchloric acid. Samples were derivatized with a fluorescent labeling reagent, 4-fluoro-7-nitro-2,1,3-benzoxadiazole. Amino acids were separated using HPLC and fluorescence of the derivatized amino acids was detected at 530 nm with excitation at 470 nm as previously described (Esaki et al., 2011).
from the D-serine biosensor to record background current. Amperometric recordings were performed for 1 h and the current recorded by the control biosensor was subtracted from that recorded by the D-serine biosensor in order to estimate the specific D-serine current, and hence its extracellular concentration.

**Electrophysiology**

**Slice Preparation**

Experiments were carried out on adult mice (six to seven months old) in accordance with the French National Code of Ethics on Animal Experimentation and approved by the Committee of Ethics of Bordeaux (authorization no. A50120109). Briefly, mice were anesthetized with isoflurane and euthanized. The brain was quickly removed from the skull and placed in cold ACSF saturated with 95% O₂ and 5% CO₂. ACSF was used for dissection, slicing, and recording. The ACSF composition was: 126 mM NaCl, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 1.3 mM MgCl₂, 2 mM CaCl₂, 26 mM NaHCO₃, and 10 mM glucose (pH 7.3, 300-305 mOsm/kg). A block of tissue containing the hippocampus was prepared and hippocampal sagittal slices (350 μm) were cut using a vibratome (Leica, VT1200S, Germany). The slices were incubated 30 min at 32°C and then allowed to recover for at least one h at room temperature.

**Field Recordings**

For electrophysiological recordings, the slices were transferred into a recording chamber continuously perfused with ACSF (3 mL/min). A cut between CA3 and CA1 was made to avoid epileptiform activity, due to the presence of picrotoxin (100 μM) added to the ACSF just before recording. CA3-CA1 areas were identified with differential interference contrast microscopy (Olympus BX50). Field excitatory postsynaptic potentials (fEPSPs) were recorded with a Multiclamp 700B amplifier (Axon Instruments, Inc.) using pipettes (3.5-5 MΩ) filled with ACSF and placed in the stratum radiatum of the CA1 area, close to the subiculum. NMDA fEPSPs were isolated with low Mg²⁺ ACSF (0.2 mM) in the presence of picrotoxin (100 μM) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f] quinoxaline-2,3-dione (NBQX) (10 μM) to block GABAₐ and AMPA/kainate receptors, respectively. Synaptic potentials were evoked at 0.05 Hz by orthodromic stimulation (100 μs) of Schaffer collaterals using either a glass pipette filled with ACSF or a bipolar tungsten stimulating electrode placed in the stratum radiatum > 200 μm away from the recording electrodes. LTP was induced by applying a high-frequency stimulation (HFS) protocol (100 Hz train of stimuli for 1 s, repeated four times at 20 s intervals) performed in current clamp mode. LTD was induced by applying a low frequency stimulation protocol (LFS) consisting in a 1-Hz train of stimuli applied for 30 min. The change in the slope of the fEPSP was evaluated 50-60 min post-HFS or post-LFS, and normalized to the slope measured during the 10 min immediately before HFS or LFS. L-serine (50 μM) and D-serine (50 μM) were bath applied 20 min before the recording and then during the whole experiment of long-term synaptic plasticity. Average fEPSP traces presented in figures were obtained from 10 min of recordings. Signals were filtered at 2 kHz and digitized at 10 kHz via a DigiData 1322 interface (Axon Instruments, Inc.). Data were collected and analyzed offline using pClamp 9 software (Axon Instruments Inc.).

**Behavioral Analyses**

**Elevated Plus-maze**

The apparatus consisted of four arms elevated 40 cm above floor level. Two of the arms contained 15 cm-high walls (enclosed arms) and the other two none (open arms). Each mouse was placed in the middle section facing an open arm and left to explore the maze for a single 5-min session. The time spent in open and closed arms and the duration of head-dipping into open arms were analyzed using a video-camera and a video-tracking system (Ethovision, Noldus Information Technology Inc.).

**Spontaneous Motor Behavior**

We used the PhenoTyper® chamber (Noldus Information Technology Inc.) to monitor the spontaneous motor behavior of mice for 47 h. A video-tracking system was used to collect behavioral data during this period.

**Morris Water Maze**

Experiments were performed in a circular tank filled with water and equipped with a 10-cm diameter platform submerged 0.5 cm beneath the surface of the water. Visual clues were positioned around the pool, to provide the mouse with spatial landmarks, and luminosity was maintained at 400 lux. The mice were initially exposed to a learning phase, which consisted of daily sessions (three trials per session) on five consecutive days. The starting position varied between the four cardinal points for each trial. A mean interval of 30 min was left between trials. The trial was stopped when the animal reached the platform. A 60 s cutoff was used, after which the mice were gently guided to the platform. Once on the platform, the animals were allowed to rest for 30 s before being returned to their cage. Long-term spatial memory was assessed 72 h after the last training trial (fifth day) in a probe trial in which the platform was removed. The position of the animals was monitored by a video-tracking system (Ethovision, Noldus Information Technology Inc.) for 60 s.

**Supplementation of L- or D-serine**

Phgdh<sup>fox/flox</sup>, 3xTg-AD and control mice were placed on a rodent diet (Altromin 1324) enriched with 10% L-serine (w/w) for two months (Garofalo et al., 2011) including the period of the Morris water maze test. Two other groups of 3xTg-AD and control mice were given D-serine (600 mg/L, Sigma-Aldrich, St. Louis, MO, USA) in the drinking water for two weeks, including the period of the Morris water maze test. This protocol resulted in a daily dose of approximately 100 mg/kg D-serine per body weight (average weight: 30 g, average drinking volume: 5 mL/day).
QUANTIFICATION AND STATISTICAL ANALYSES

No statistical methods were used to determine sample sizes but our sample sizes (4 to 10) are similar to those generally employed in the field. Randomization was not employed. Investigators that performed electrophysiological recordings were blinded to group allocation. Other experiments were not performed blind to group allocation. For each analysis, normality of residues and homoscedasticity were assessed. Data are presented as the mean ± SEM. A p value of less than 0.05 was considered statistically significant. Statistical analyses were performed using Statistica, R or Prism software. We used the nonparametric Mann-Whitney U test for comparison between two groups and the nonparametric Kruskal-Wallis test followed by Dunn post doc test for multiple comparisons between groups. Other specific statistical tests are mentioned in the figure legends.

DATA AND CODE AVAILABILITY

All data generated or analyzed during this study are included in this published article and its supplementary information files.