The mechanism by which LiCl attenuates the impaired ability of learning and memory of APP/PS1 double transgenic mice may involve elevating the expression of $\alpha 7$ nicotinic acetylcholine receptors via regulation of the Wnt/β-catenin pathway.

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Abstract

**Background:** We examined the mechanism by which lithium chloride (LiCl) attenuates the impaired learning and memory of APP/PS1 double transgenic (APP/PS1) mice.

**Methods:** Six- or twelve-month-old APP/PS1 and wild-type (WT) mice were divided randomly into 4 groups: WT, WT+Li (100 mg LiCl/kg body weight, gavage once daily), APP/PS1 and APP/PS1+Li. Primary rat hippocampal neurons were exposed to β-amyloid peptide oligomers (AβOs), LiCl and/or XAV939 (inhibitor of Wnt/β-catenin) or transfected with small interfering RNA against the β-catenin gene. Phosphor-glycogen synthase kinase-3β (GSK3β) (ser9), total GSK3β, β-catenin, cyclin D1, and α7 nAChR protein and mRNA were quantified by Western blotting or real-time PCR, respectively; senile plaques and α7 protein by immunohistochemical or immunofluorescent staining; Aβ42 by ELISA; and cell viability by CCK8. Learning and memory were assessed utilizing the Morris water maze test.

**Results:** In the brains of APP/PS1 mice, the level of Aβ was increased and those of α7 nAChR, phosphor-GSK3β (ser9), β-catenin, and cyclin D1 (at the protein and/or mRNA level) reduced. Treatment with LiCl for 2 months at 4 or 10 months of age attenuated all of these effects. Similar changes in the levels of these proteins were observed in primary neurons exposed to AβOs and these effects were attenuated by LiCl and aggravated by XAV939. Inhibition of β-catenin expression lowered the level of α7 nAChR protein in these cells.

**Conclusion:** LiCl attenuates the impaired ability of learning and memory of APP/PS1 mice via a mechanism that might involve elevation of the level of α7 nAChR as a result of altered Wnt/β-catenin signaling.

**Keywords**
AβOs; APP/PS1 mice; LiCl; α7 nAChR; Wnt/β-catenin signaling pathway
Introduction

Alzheimer’s disease (AD), the most common form of dementia associated with aging, is characterized by progressive loss of cognitive abilities, in particular memory and learning [1, 2]. The hallmarks of this disease in the brain include intracellular neurofibrillary tangles containing highly phosphorylated tau, extracellular amyloid plaques containing β-amyloid peptide (Aβ), an excessive loss of synapses [3, 4] and deficient cholinergic transmission [5]. In this context, hydrolysis of amyloid precursor protein (APP) by β- and γ-secretases leads to accumulation of Aβ oligomers (AβOs), thereby inducing onset of neuronal toxicity [6].

The cholinergic pathway is involved directly in learning and memory [7] and the “cholinergic hypothesis” proposes that the reduction in cholinergic innervation is responsible for the cognitive decline observed in patients with AD [8,9]. Neuronal nicotinic acetylcholine receptors (nAChRs), ligand-gated ion channels composed of combinations of five different α (2-9) and three different β (2-4) subunits, participate in a wide range of functions, including cognitive processing and learning and memory. Alterations in the expression of these receptors are associated with the impaired cholinergic neurotransmission [10] and may be responsible for complex neuronal diseases such as AD and Parkinson disease [11-14].

Indeed, expression of nAChRs is lowered in the brains of the patients with AD and of animal models of this disease, as well as in isolated neuronal cells exposed to Aβ [15, 16]. α7, the main subtype of nAChR present in both neuronal and non-neuronal cells of the human brain, consists mainly of five α7 subunits and is considered to be one of the receptors most closely related to the pathogenesis of AD [17, 18]. Aβ1-40 and Aβ1-42 bind to α7 nAChR with an affinity in the nanomolar range, resulting in gradual depletion and inactivation of this receptor [19-24].
Potential treatment of AD targeting the nAChRs may not only enhance cognition, but also attenuate other symptoms of this pathology [25, 26]. Unfortunately, current pharmacotherapy only decelerates the symptoms of AD transiently. Treatment of AD patients with cholinesterase inhibitors (ChEIs) briefly activates the nAChRs, but ChEIs cannot prevent the continuous disappearance of these receptors.

In addition to the toxic consequences due to direct binding of Aβ to nAChRs, other subcellular events have been associated with AD, including down-regulation of the canonical Wnt/β-catenin pathway, which might influence the stability of these receptors. Cytoplasmic β-catenin plays a key role in signaling via this pathway. When Wnt receptors are not activated, casein kinase 1 and glycogen synthase kinase-3β (GSK3β) phosphorylate Axin-bound β-catenin sequentially at a series of regularly spaced Ser/Thr residues located in its N-terminus. As a consequence, β-catenin is ubiquitinated and targeted for rapid destruction by the proteasome, thereby depressing the expression of downstream targets such as cyclin D1 [27].

Interestingly, Wnt/β-catenin signaling is closely associated with learning and memory in mammals [28] and this signaling is impaired both in the brains of patients with AD and animal models of this disease [29]. This dysfunction plays a key role in neuron degeneration and the impairment of synapses, creating a deleterious pathway that leads to dementia [30]. Notably, nAChRs are present both on presynaptic [31,32] and postsynaptic [33,34] membranes and loss of these receptors leads inevitably to abnormalities in synapses. Furthermore, persistent activation of Wnt signaling, either by administration of ligands or by preventing the action of inhibitors, overcomes the toxic effects of Aβ and improves cognitive performance in patients with AD [35-37].

Lithium is universally accepted as the first-choice mood-stabilizer for maintenance of bipolar disorder [38]. In addition to mood-stabilization, lithium exerts
anti-suicidal, immunomodulatory and neuroprotective actions [39], during the last
decade, has come to be regarded as a neuroprotective agent and is now widely used in
studies of neurodegenerative diseases, including AD [40-42]. It is noteworthy in this
context that the incidence of AD among patients with bipolar disorder who have been
taking lithium is lower than among those not undergoing lithium therapy [38].
Lithium chloride (LiCl) inhibits GSK3β, thereby reducing this activity in the brain of
animal models of AD [43,44], while enhancing Wnt/β-catenin signaling. There are
indications that the neuroprotection demonstrated by lithium involves this action on
Wnt/β-catenin signaling [45].

Accordingly, lithium may be of value in treating AD. However, it is currently
unknown whether lithium can reverse the impairment of learning and memory in a
murine model of AD and, if so, whether this effect involves regulation of
Wnt/β-catenin signaling and consequent regulation of α7 nAChR. Here, we
investigated these questions by treating both APP/PS1 mice and primary neurons
exposed to AβOs with LiCl or XAV939 and then analyzing the cognitive ability of
these mice, as well as potential changes in Wnt/β-catenin and α7 nAChR in both of
these systems.
Materials and Methods

Materials

LiCl, Aβ₁-₄₂ and XAV939 (Sigma-Aldrich Inc., USA); mouse monoclonal anti-Aβ antibody (BioLegend Inc., USA); rabbit monoclonal anti-GSK3β, -phosphor-GSK3β (ser9), -β-catenin and -cyclin D1 antibodies and anti-rabbit IgG conjugated with horseradish peroxidase antibody (Cell signaling Inc., USA); rabbit polyclonal GAPDH antibodies (Gentex Inc., USA); rabbit polyclonal anti-nAChR α7 (Abcam Inc., USA); cell counting Kit-8 (Dojindo Inc., Japan); mouse monoclonal anti-NeuN antibody (Merck 112 Millipore Inc., Germany); rabbit monoclonal anti-glial fibrillary acidic 113 protein (GFAP) antibody (Dako Inc., Denmark); anti-mouse IgG labeled with CY-3 and anti-rabbit IgG labeled with 488 (Thermo scientific Inc., USA); kits for measuring Aβ₄₂ levels (Thermo scientific Inc., USA); Lipofectamine RNAiMAX Reagent (Invitrogen Inc., USA); Universal TaqMan 2×PCR mastermix (Applied Biosystems Inc., USA); and all other general chemicals (Sigma-Aldrich Inc., USA) were purchased from the sources indicated.

Experimental animals

Four-month-old B6.Cg-Tg (APP₅₆₁ and PSEN1ΔE9) mice with a 85Dbo/Mmjax background and wild-type (WT) mice of the same strain (all with a body weight of 20-30 g) were purchased from Shanghai Nanfang Biological Technology Development Co., Ltd. Following acclimatization for one week with a humidity of 30-55% and temperature of 22-25°C, each male mouse was allowed to mate with four females. When the resulting pups were 12-20 days old, the tips of their tails were cut off for extraction of DNA and genotyping by the polymerase chain reaction (PCR), with analysis of the products by 1.5% agarose gel electrophoresis. The PCR primers for target transcripts (Table 1) were designed on the basis of the complete cDNA
sequences deposited in GenBank.

All experiments described here were pre-approved by the Ethical Committee of Guizhou Medical University, China (No. 1702110).

At the ages of 4 or 10 months, the transgenic (APP/PS1 mutation) and WT mice were divided randomly into 4 groups (6 animals in each) for subsequent treatment for two months [46] as follows: (1) the WT group: WT animals received physiological saline (approximately 0.4 ml) by gavage once daily; (2) the WT+Li group: WT animals were treated with LiCl (100 mg/kg); (3) the transgenic group: animals with the APP/PS1 mutation were treated with physiological saline in the same manner as group (1); (4) the transgenic+Li group: transgenic animals were treated with LiCl in the same manner as group (2).

**Culturing and treatment of primary hippocampal neurons**

Primary neurons were prepared from the brains of neonatal Sprague-Dawley rats employing minor modifications of a published procedure [47]. In brief, the hippocampal regions were dissected out within 2-3 min after sacrifice and thereafter maintained in Hibernate-A medium on ice. After removing the meninges, the hippocampus was washed three times with Hank’s buffered saline solution and then digested with 0.25% trypsin for 10 min at 37°C. Subsequently, the incubation medium was discarded and DMEM containing 10% FBS added to terminate digestion. After washing twice more with Hank’s buffered saline solution, the digested tissue was resuspended in 2 ml Neurobasal/B27 complete medium (Neurobasal A medium with 2% B27, 1% GlutaMAX Supplement, 100 U/ml penicillin and 100 mg/ml streptomycin).

The resulting single-cell suspension was filtered and transferred into a new tube. Then, the cells were counted using trypan blue exclusion and placed onto 96- or
6-well poly-L-lysine-coated plates at a density of approximately $5.0 \times 10^4$/cm$^2$.
Thereafter, the neurons were maintained under a humidified atmosphere containing 5% CO$_2$ at 37° C, with replacement of half of the medium once every 3 days. After 10 days of such incubation, the neurons were subjected to various treatments. The purity of these primary neurons was evaluated by immunofluorescent double-staining with mouse anti-NeuN antibody followed by anti-mouse IgG labeled with CY-3 (red) and with rabbit anti-GFAP antibody followed by anti-rabbit IgG labeled with 488 (green).

AβOs were prepared by a procedure described earlier [48] and the suitable concentration for exposure chosen as also described previously [47]. Cells were first exposed to different concentrations of LiCl (0-100 mmol/L) and XAV939 (0-50 μmol/L) for different times. Thereafter, the cells were incubated with 10 μl CCK8 solution for 2 h, following which the absorption at 450 nm was determined. On the basis of the results of this test for viability, suitable concentrations of LiCl and XAV939 and incubation times were chosen.

Transfection with small interfering RNA (siRNA) was initiated by adding a mixture (1:1) of Lipofectamine RNAiMAX Reagent diluted in Opti-MEM Medium (15 μl:250 μl) and the siRNA (For5’-GACUACCUGUGUGGUAAAdTdT-3’; Rev
5’-UUACACCACAAAGGUAGUCdTdT-3’), also diluted in Opti-MEM Medium (1:50) for 5 min. Then, the cells were incubated with the siRNA-Lipid complex for 48 h at 37° C [49].

*The Morris water maze (MWM) test of spatial learning and memory*

Each mouse was forced to find a submerged escape platform in a circular pool filled with water (25-26° C) rendered opaque with powdered milk [50]. During the familiarization session and acquisition phase (four trials/day for four consecutive days), the mouse was given as long as 60 s to find the hidden platform and then
allowed to remain seated on this platform for 5 s, after which the animal was returned to its home cage. During the retention phase, the platform was removed from the pool and for 60 s the path taken by each mouse was video-filmed to determine the time required to swim to the original position of the platform, as well as the number of passes over and time spent at this position.

**Determination of Aβ42 by ELISA**

The level of Aβ42 in mouse brains was determined utilizing an Amyloid β42 Mouse ELISA kit in accordance with the manufacturer’s protocol [50]. In brief, after homogenization and centrifugation of the tissue, the pellets obtained were extracted with 5 M guanidine-HCl. The same amount of protein in each extract was diluted to a final volume of 100 μl and then incubated in wells on plates at room temperature (RT) for 2 h. The wells were then washed, filled with 100 μl Detection Antibody solution, and then incubated for one more h at RT. Thereafter, the wells were again washed, followed by incubation with 100 μl HRP-linked antibody solution for 30 min and four additional washes. Finally, Stop Solution was added and the optical density at 450 nm determined with a spectrophotometer (Bio-Rad Inc., USA).

**Quantification of the levels of phosphor-GSK3β (ser9), GSK3β, β-catenin, cyclin D1 and α7 nAChR by Western blotting**

Brain tissue or cultured cells in lysis buffer containing a mixture of protease inhibitors were disrupted in a glass homogenizer; the resulting homogenate centrifuged at 12,000 rpm at 4°C for 20 min; and the protein concentrations of the supernatants thus obtained determined with the BCA protein assay kit [47]. The proteins were subsequently separated by 10% SDS-PAGE and then blotted onto polyvinylidene difluoride (PVDF) membranes with a transfer unit (Bio-Rad Inc., USA).
For relative quantification of the proteins, these membranes were thereafter incubated with antibody against phosphor-GSK3β (ser9), GSK3β, β-catenin, cyclin D1, α7 nAChR, or GAPDH at 4°C overnight. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 60 min. Finally, the protein bands were detected utilizing an enhanced chemiluminescence system (ECL kit, Minipore Inc., USA) and the signals thus obtained visualized by exposure to hyper-performance chemiluminescence film for 30 s to 3 min. Signal intensity was quantified employing the Image J software.

**Determination of the level of α7 nAChR mRNA by reverse transcription and quantitative real-time PCR**

Total RNA extracted from brain tissue or cells using Trizol Reagent was used to obtain cDNA by reverse transcription with the Prime Script™ RT Master Mix cDNA Synthesis Kit (Takara Bio., USA) [51]. Quantitative real-time PCR was performed in the ABI PRISM 7300 Sequence Detection System (Applied Biosystems, USA) in accordance with the manufacturer’s protocol and analyzed with GeneAmp7300 SDS software. The level of α7 nAChR transcripts was estimated using $2^{-\Delta\Delta CT}$ (RQ value) and the formula: $\Delta\Delta CT=\Delta CT_{\text{target}}-\Delta CT_{\text{control}}=(CT_{\text{target}}-CT_{\text{GAPDH}})-(CT_{\text{control}}-CT_{\text{GAPDH}})$ with the error estimate being $\Delta\Delta CT$ plus and minus the standard deviation. The sequences of the PCR primers utilized are presented in Table 2. The level of GAPDH mRNA was used as the internal control.

**Examination of senile plagues and α7 nAChR by immunohistochemical or immunofluorescent staining**

Immunohistochemical staining for senile plagues and α7 nAChR in the brains of mice was performed as described previously [52]. Sections were first deparaffinized in xylene, then dehydrated through a series of aqueous solutions containing increasing
concentrations of alcohol; next, they were microwaved in 0.01 M citric acid buffer (pH 6.0) for 20 min to achieve antigen retrieval; and then placed in blocking buffer (Dako Inc., Denmark) for 30 min at RT. Subsequently, the sections were incubated with 6E10 and anti-α7 nAChR antibodies at 4°C overnight; and the next day, with biotinylated goat anti-mouse or rabbit IgG for 60 min at RT. Thereafter, they were incubated with the avidin-biotinylated enzyme complex and then placed in peroxidase reaction solution containing diaminobenzidine. The number of senile plaques and optical density (IOD) of α7 nAChR staining in randomly selected fields of vision (original magnification X 200) were quantified with the Image Pro Plus software (USA).

For immunostaining of α7 nAChR in isolated cells, the primary neurons were first seeded onto 6-well PLL-coated plates, washed three times with PBS and then fixed in 4% paraformaldehyde. These samples were then treated with goat serum for 30 min at RT; incubated with antibody against α7 nAChR overnight at 4°C [47]; and the next day incubated with fluorescein isothiocyanate-labelled goat anti-rabbit IgG labelled with 488 for one h at RT. Thereafter, the cells were rinsed three times in PBS and coverslipped with Vectashield (Vector Laboratories, USA). The IOD of immunofluorescent staining for α7 nAChR in randomly selected fields of vision (original magnification X 400) was quantified with the Image Pro Plus software (USA).

**Statistical analyses**

The values for the different groups of mice are presented as means±SD and compared by analysis of variance (ANOVA), followed by a least significant difference post-hoc test. Semi-quantitation of the Aβ plaques was carried out with the Bielschowsky procedure [53]. The correlation between the level of α7 nAChR protein
and spatial learning and memory was performed employing the Pearson correlation test. Differences with a value $p < 0.05$ were considered to be statistically significant. All analyses were performed using the SPSS 22.0 software (SPSS Inc., USA).
Results

Confirmation of the APP/PS1 murine genotype

When genomic DNA was utilized as the template for PCR amplification, products approximately 400 and 600 bp in size, consistent with the size of the APP and PS1 genes, respectively, were observed with the transgenic, but not WT mice (Fig. 1).

Spatial learning and memory in mice

As determined by the Morris water maze test (Fig. 2), the number of times the original position of the platform was crossed and the time spent at this position were both reduced, while the escape latency was increased in the 6- and 12-month-old APP/PS1 double-transgenic mice in comparison to the corresponding WT mice. Treatment of the transgenic animals with LiCl for 2 months beginning at 4 or 10 months of age clearly improved both their ability of learning and memory to the extent that these were the same as those of the WT mice treated with LiCl in a similar manner.

The number of the senile plaques and level of Aβ42 in the cortex of mouse brains

The brains of 6- (Fig. 3A-D and a-d) or 12-month-old (Fig. 3E-H and e-h) transgenic mice contained Aβ-immunoreactive senile plaques, the number of which was markedly reduced by exposure to LiCl beginning at either 6 or 12 months of age. No amyloid plaques were detected in the brains of WT mice with or without exposure LiCl (Fig. 3I).

The cortex of WT mice contained a very low level of Aβ42 that was unaffected by treatment with LiCl. In contrast, this level was much higher in untreated APP/PS1 animals, where the level was decreased even further by treatment with LiCl (Fig. 3J).

The viability of primary neurons exposed to AβOs, LiCl and/or XAV939
Immunostaining of the primary cultured neurons prepared from the hippocampal region of the brains of newborn rats with antibodies directed toward NeuN (a marker for neurons), GFAP (a marker for astrocytes), and DAPI (a marker for nucleus) revealed that approximately 90% of these cells were neurons (Fig. 4A-E). The viability of these primary neurons following exposure to AβOs, LiCl and/or XAV939 was assessed utilizing the CCK-8 test. Treatment with 10 mmol/L LiCl for 6 h or 1 μmol/L XAV939 for 24 h did not cause any significant cytotoxicity (Fig. 4F and G). Exposure of these primary neurons to 0.5 μmol/L AβOs for 48 h [conditions chosen on the basis of an earlier study; 47] reduced cell viability, a reduction that was attenuated by LiCl, but aggravated by XAV939 (Fig. 4H).

Expression of proteins involved in Wnt signaling in the hippocampus and cortex of mouse brains and primary neurons

As determined by western blotting, the levels of phosphor-GSK3β (ser9) (but not total GSK3β), β-catenin and cyclin D1 in the hippocampus (Fig. 5A-C and D-F) or cortex (Fig. 5a-c and d-f) of the brains of APP/PS1 mice at 6 (Fig. 5 A-C and a-c) or 12 (Fig. 5 D-F and d-f) months of age were all lower than the corresponding levels in WT mice. Interestingly, treatment of the transgenic animals with LiCl attenuated these reductions. In addition, expression of these proteins in the hippocampus and cortex of 6- or 12-month-old WT animals was also elevated by treatment with LiCl as compared with the WT mice without the treatment of LiCl.

In the case of primary neurons, the levels of the β-catenin and cyclin D1 proteins were enhanced by exposure to LiCl alone, whereas XAV939 reduced these levels. Exposure of primary neurons to AβOs alone also reduced these levels, an effect that was attenuated by LiCl and enhanced by XAV939 (Fig. 6A and B).
In comparison with the corresponding control group, transfection of primary neurons with siRNA targeting the β-catenin gene reduced both the level of this protein and that of cyclin D1 (Fig. 6E and F).

Expression of α7 nAChR mRNA and protein in the hippocampus and cortex of mouse brains and primary neurons

In comparison with the corresponding control group, the levels of both α7 nAChR protein and mRNA were enhanced by exposure of the neurons to LiCl alone and reduced by XAV939. Moreover, these levels were reduced by exposure to AβOs, a decline that was attenuated by LiCl, but enhanced by XAV939 (Fig. 6C and D).

Moreover, transfection of neurons with siRNA targeting the β-catenin gene reduced the level of this protein, as well as that of α7 nAChR (Fig. 6G).

As assessed by western blotting and real-time PCR, the levels of α7 nAChR protein (Fig. 7A-D) and mRNA (Fig. 7E-H) were decreased in the hippocampus (Fig. 7A, C, E and G) and cortex (Fig. 7B, D, F and H) of APP/PS1 mice at 6 (Fig. 7A, B, E and F) and 12 (Fig. 6C, D, G and H) months of age. Administration of LiCl to either the WT or APP/PS1 animals elevated these levels in both of these areas of the brain.

Semiquantitative immunohistochemical analysis of mouse brains revealed localization of α7 nAChR primarily at the plasma membrane and axon (Fig. 8A-D and a-d). This immunostaining was less intense in the hippocampus (Fig. 8A, a, C and c) and cortex (Fig. 8B, b, D and d) of the APP/PS1 mice at 6 (Fig. 8A, a, B and b) and 12 (Fig. 8C, c, D and d) months of age than for the WT group. In both groups of animals, LiCl augmented the intensity of this staining.

In the case of primary neurons semiquantitative immunofluorescent analysis revealed localization of the α7 nAChR subunit mainly at the plasma membrane and axon (Fig. 9A). Exposure to LiCl alone enhanced this immunofluorescence, whereas
XAV939 reduced it. Interestingly, the reduction caused by AβOs alone was attenuated by LiCl and enhanced by XAV939 (Fig. 9D).

**Correlations between the level of the α7 nAChR and spatial learning and memory**

Correlation analysis revealed a negative correlation between the elevated level of α7 nAChR protein present in the brains of APP/PS1 mice at 6 (Fig. 10A and C) or 12 (Fig. 10B and D) months of age following exposure to LiCl and the impairment in their spatial learning and memory.
Discussion

AD is associated with a specific onset and course of cognitive and functional decline during ageing, together with aggregation of a particular Aβ with hyperphosphorylated tau protein that causes loss of synapses and neurodegeneration [54, 55]. Aβ, the level of which is associated with the extent of cognitive decline [56], is measured in the cerebrospinal fluid and brain for purposes of auxiliary diagnosis [57].

In the brain of APP/PS1 mice, one of the most common animal models of AD, the content of Aβ and number of senile plaques are elevated, while learning and memory of these animals are impaired [58]. Here, we found that in the brains of 6- and, especially, 12-month-old mice of this strain the number and size of senile plaques were markedly increased and learning and memory significantly impaired. Consistent with previous studies [47, 59], we have also confirmed that Aβ damages neurons directly, both in vitro and in vivo, leading to dysfunction and apoptosis.

The α7 and α4β2 forms are the most abundant nAChRs in the mammalian brain. The α7 nAChR subtype is widely distributed in the central nervous system, although its expression is particularly prominent in the hippocampus and prefrontal cortex, two key regions involved in neurocognition [60]. The well-defined functions of α7 nAChR in the brain include the modulation of synaptic transmission and plasticity underlying normal attention, cognition, learning and memory processes [61,62].

In this context, the level of α7 nAChR is lowered in animal models of AD and in neurons treated with AβOs and, moreover, the loss of the ability of learning and memory of APP/PS1 mice can be reversed or aggravated by activating or inhibiting the expression of α7 nAChR, respectively [63, 64]. Aβ damages nAChRs, and in particular α7 nAChR, directly, thereby altering neuronal signaling and contributing to
the synaptic dysfunction associated with AD. Computational modeling indicates that arginine-208 and glutamate-211 in α7 nAChR are involved in their interaction with Aβ [65].

Clearly, α7 nAChR plays key roles in maintaining ability of learning and memory. In our current investigation, the level of α7 nAChR protein in the hippocampus and cortex of the brains of 6- and 12-month-old APP/PS1 mice was found to be lower than in WT animals. In addition, primary neurons exposed to AβOs show a tendency towards reduced expression of α7 nAChR. Furthermore, we confirmed the damaging effect of Aβ on α7 nAChR both in vivo and in vitro, as well as the relationship between the loss of α7 nAChR and the decline in the ability of learning and memory of APP/PS1 mice. At the same time, we found that the loss of α7 nAChR is associated with a reduction in the level of the β-catenin protein.

β-catenin plays an essential role in Wnt/β-catenin signaling, which is involved in both development of the nervous system and adult synaptic plasticity, as well as in learning and memory in both normal and disease states [28, 66]. Thus, the decline of learning and memory in patients with AD appears to be related to the decrease of Wnt/β-catenin in their brain [67]. In animal models as well, there is a strong association between down-regulation of Wnt/β-catenin signaling and AD pathology [29]. In addition, GSK3β is commonly activated in connection with the pathophysiology of AD and dysregulation of this kinase influences the stability of β-catenin, as well as downregulating the expression of downstream proteins such as cyclin D1 [68, 69]. Accordingly, the Wnt/β-catenin signal pathway offers a therapeutic target for alleviating the pathological process that results in AD [70].
In the present case, we verified that Wnt/β-catenin signaling in the brains of APP/PS1 mice or in primary neurons treated with AβOs in vitro is significantly lower than in the WT animals or untreated cells.

Evidence for a neuroprotective effect of lithium has accumulated over the last two decades [71] and this element has been found to be involved in the regulation of numerous genes, proteins and metabolites [72]. Most noteworthy of these is the inhibition of GSK3β by lithium, which has a wide range of beneficial effects [73,74]. Consistent with previous findings [75], we found here that LiCl enhances the ability of learning and memory of both 6- and 12-month-old APP/PS1 mice.

Moreover, both the elevated content of Aβ42 and the number or size of senile plaques in the brains of these transgenic mice were reduced by treatment with LiCl. In addition, this reduction in Aβ content was correlated to an improvement in learning and memory. Some evidence from animal models of AD indicates that LiCl can prevent neurotoxicity by reducing the production of Aβ [76], as well as that the cognitive benefits of LiCl are associated with enhanced clearance of Aβ via upregulation of brain microvascularization by LRP1 and increased bulk flow of cerebrospinal fluid [77]. In human neuronal cells, LiCl alters proteins such as the rab proteins, which have been implicated in the processing of APP in connection with the pathophysiology of AD [78]. In addition, up-regulation of Wnt/β-catenin signaling by LiCl can cause β-catenin to bind specifically to regions within the BACE1 promoter that contain putative TCF/LEF motifs and repress transcription [79]. Any or all of these mechanisms may explain the reduction in Aβ caused by lithium.

LiCl is a classic agonist of the Wnt/β-catenin pathway, which is the main reason it has been chosen for testing as a candidate drug for treating AD in numerous studies [27,42]. Here, up-regulation of the Wnt/β-catenin pathway by LiCl in 6- and
12-months-old mice with or without the APP/PS1 double mutation was verified. This effect was associated with inactivation of GSK3\(\beta\) through specific phosphorylation of Ser9 in its N-terminus [80], which elevates the levels of the \(\beta\)-catenin and cyclin D1 proteins. In addition, we found here that in primary hippocampal neurons the Wnt/\(\beta\)-catenin pathway was activated by LiCl and inactivated by XAV939 or A\(\beta\)Os. Importantly, this effect by A\(\beta\)Os could be attenuated by LiCl, but aggravated by XAV939.

In line with these findings in vitro, activation of the Wnt/\(\beta\)-catenin pathway has been reported to counteract the detrimental effects of A\(\beta\)O [81] and may thus play an essential role in the maintenance of synapses and neuronal functions [82]. Abundant evidence demonstrates that reactivation of the Wnt/\(\beta\)-catenin pathway completely restores the number of synapses and synaptic plasticity [83] and this pathway is a target for recovery of neuronal circuits following degeneration of synapses [84]. Since \(\alpha7\) nAChRs are expressed widely on both pre-synaptic and post-synaptic membranes [31,34], we hypothesize that activation of Wnt/\(\beta\)-catenin pathway by LiCl stabilizes expression of these receptors in the brain of animal models of AD.

Interestingly, the reduction in the level of \(\alpha7\) nAChR protein in the brains of APP/PS1 mice could be attenuated by treatment with LiCl. In addition, in WT animals, treatment with LiCl not only activated the Wnt/\(\beta\)-catenin pathway, but also elevated the levels of \(\alpha7\) nAChR protein and mRNA in the hippocampus or cortex. These findings indicate that LiCl may regulate expression of the \(\alpha7\) nAChR subunit during the development of AD. In this context, we found that the elevated level of \(\alpha7\) nAChR protein in the brains of APP/PS1 mice exposed to LiCl correlated negatively with their impairment in spatial ability of learning and memory. In combination with previous observations, this study further supports the important role of \(\alpha7\) nAChR in
learning and memory [61,62] and suggests that restoring normal expression of α7 nAChR may be one mechanism by which LiCl improves learning and memory in APP/PS1 mice.

Subsequently, we found that when primary neurons were exposed to LiCl, their level of the α7 nAChR subunit increased, whereas XAV939 had the opposite effect. Moreover, the decrease in the level of α7 nAChR subunit in these cells induced by AβOs was attenuated by LiCl, but aggravated by XAV939. Furthermore, transfection of primary neurons with siRNA targeting the β-catenin gene lowered not only the level of this protein and its downstream protein cyclin D1, but also reduced expression of the α7 nAChR subunit. These findings indicate that the gene encoding α7 nAChR is regulated by the Wnt/β-catenin pathway and that the reductions in α7 nAChR protein and mRNA induced by AβO may be mediated by inactivation of this pathway.

Cholinergic neurons are reliant on (α7) 5 nAChRs for maintaining the ability to learn and memory [85]. At present, relatively little is known about potential interactions between the Wnt/β-catenin pathway and the function of nAChRs. Earlier investigations revealed that activation of the canonical Wnt pathway may promote the pre-synaptic localization of α7-nAChR [86] and accelerate differentiation of stem cells in adipose tissue into cholinergic neurons [87]. Moreover, activated the canonical Wnt pathway by Wnt-7a has been reported to promote up-regulation and aggregation of α7 nAChR [88]. These observations are in line with the finding with C. elegans that mutations causing defects in the Wnt pathway lower the synaptic level of the ACR-16/α7 nAChR homolog, a change accompanied by attenuated receptor function and loss of nicotinic-related behaviors [89].

**Limitation**
In this paper, we have tentatively verified the association between $\alpha_7$ nAChR and the ability of learning and memory, and discussed the possible effect of lithium on $\alpha_7$ nAChR via the Wnt/β-catenin signaling pathway. However, not only $\alpha_7$ but also $\alpha_4\beta_2$ nAChRs are important in the learning and memory. A further investigation concerning whether $\alpha_4\beta_2$ nAChR is affected by lithium and also involved in the regulation of the Wnt/β-catenin signaling pathway on brain function in AD could be interesting.
Conclusion

The present results showed that 6- or 12-month-old APP/PS1 mice demonstrated impaired learning and memory, as well as an increase in the number of senile plagues in their brains. Interestingly, the treatment of LiCl attenuated the impaired learning and memory of these APP/PS1 mice and decreased the large number of senile plagues in their brains. Furthermore, expression of α7 nAChR, and the levels of the phosphor-GSK3β (ser9), β-catenin and cyclin D1 proteins were lowered both in the brains of these animals and in primary neurons exposed to AβOs. However, the treatment of either WT or APP/PS1 mice at 4 or 10 months of age with LiCl for 2 months increased the levels of these proteins, and the effect was also observed upon exposure of primary neurons, untreated or treated with AβOs, to LiCl. A negative correlation was observed between the elevated level of α7 nAChR protein present in the brains of APP/PS1 mice and the impairment in their spatial learning and memory following exposure to LiCl. In addition, inhibition of the Wnt/β-catenin pathway with XAV939 enhanced the neurotoxic effect of AβOs on the expressions of this pathway and the α7 nAChR subunit. Inhibition of the expression of β-catenin by siRNA in primary neurons also decreased the level of α7 nAChR. These findings suggest that LiCl can reverse the impairment in ability of learning and memory exhibited by APP/PS1 mice via a mechanism that might involve elevation of the level of α7 nAChR as a result of altered Wnt/β-catenin signaling.
Acknowledgements

Not applicable

Funding

This work was financed by grants from the Natural Science Foundation of China (U1812403), the Foundation of Guizhou Province of China (2014-06, 2014-6008, 2016-4001 and YJSCXJH 2020-149).

Availability of data and materials

Not applicable

Authors’ contributions

ZZG planed the experiment and revised the paper. JX performed the experiment, processed experimental data and wrote initial draft of the manuscript. LYR, XXZ, WWH, YX, KC, YTD and JH participated in part of the experiment work. XLQ, WFY and YX discussed the results. All authors read and approved the final manuscript.

Ethics approval

Animal use for this study was approved by the Ethical Committee of Guizhou Medical University, China (No. 1702110).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
References

1. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. Lancet. 2011; 377(9770):1019-1031.

2. Castellani RJ, Rolston RK, Smith MA. Alzheimer disease. Dis Mon. 2010; 56(9):484-546.

3. Grimm MO, Hundsdörfer B, Grösgen S, Mett J, Zimmer VC, Stahlmann CP, et al. PS dependent APP cleavage regulates glucosylceramide synthase and is affected in Alzheimer's disease. Cell Physiol Biochem. 2014; 34(1):92-110.

4. Selkoe DJ. Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. Nat Cell Biol. 2004; 6(11):1054-1061.

5. Coyle JT, Price DL, DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science. 1983; 219(4589):1184-1190.

6. Querfurth HW, LaFerla FM. Alzheimer's disease. N Engl J Med. 2010; 362(4):329-344.

7. Anagnostaras SG, Murphy GG, Hamilton SE, Mitchell SL, Rahnama NP, Nathanson NM, et al. Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat Neurosci. 2003; 6(1):51-58.

8. Bartus RT, Dean RL 3rd, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science. 1982; 217(4558):408-414.

9. Contestabile A. The history of the cholinergic hypothesis. Behav Brain Res. 2011; 221(2):334-340.

10. Mitsushima D, Sano A, Takahashi T. A cholinergic trigger drives learning-induced plasticity at hippocampal synapses. Nat Commun. 2013; 4:2760.

11. Buckingham SD, Jones AK, Brown LA, Sattelle DB. Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection.
12. Jürgensen S, Ferreira ST. Nicotinic receptors, amyloid-beta, and synaptic failure in Alzheimer's disease. J Mol Neurosci. 2010; 40(1-2):221-229.

13. Lombardo S, Maskos U. Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. Neuropharmacology. 2015; 96 (PtB):255-262.

14. Nordberg A. Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications. Biol Psychiatry. 2001; 49 (3):200-210.

15. Braak H, Rü b U, Schultz C, Del Tredici K. Vulnerability of cortical neurons to Alzheimer's and Parkinson's diseases. J Alzheimers Dis. 2006; 9(3 Suppl):35-44.

16. Guan ZZ, Miao H, Tian JY, Unger C, Nordberg A, Zhang X. Suppressed expression of nicotinic acetylcholine receptors by nanomolar beta-amyloid peptides in PC12 cells. J Neural Transm (Vienna). 2001; 108(12):1417-1433.

17. Gotti C, Moretti M, Bohr I, Ziabreva I, Vailati S, Longhi R, et al. Selective nicotinic acetylcholine receptor subunit deficits identified in Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies by immunoprecipitation. Neurobiol Dis. 2006; 23(2):481-489.

18. Pym L, Kemp M, Raymond-Delpech V, Buckingham S, Boyd CA, SattelleD. Subtype-specific actions of beta-amyloid peptides on recombinant human neuronal nicotinic acetylcholine receptors (alpha7, alpha4beta2, alpha3beta4) expressed in Xenopuslaevis oocytes. Br J Pharmacol. 2005; 146(7):964-971

19. Rajasekhar K, Chakrabarti M, Govindaraju T. Function and toxicity of amyloid beta and recent therapeutic interventions targeting amyloid beta in Alzheimer's disease. Chem Commun (Camb). 2015; 51(70):13434-13450.

20. D'Andrea MR, Nagele RG. Targeting the alpha 7 nicotinic acetylcholine receptor
to reduce amyloid accumulation in Alzheimer's disease pyramidal neurons. Curr Pharm Des. 2006; 12(6):677-684.

21. Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP, Reitz AB. beta-Amyloid (1-42) binds to alpha7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. J Biol Chem. 2000; 275(8):5626-5632.

22. Wang HY, Lee DH, Davis CB, Shank RP. Amyloid peptide Abeta (1-42) binds selectively and with picomolar affinity to alpha7 nicotinic acetylcholine receptors. J Neurochem. 2000; 75(3):1155-1161.

23. Wu J, Kuo YP, George AA, Xu L, Hu J, Lukas RJ. beta-Amyloid directly inhibits human alpha4beta2-nicotinic acetylcholine receptors heterologously expressed in human SH-EP1 cells. J Biol Chem. 2004; 279(36):37842-37851.

24. Lamb PW, Melton MA, Yakel JL. Inhibition of neuronal nicotinic acetylcholine receptor channels expressed in Xenopus oocytes by beta-amyloid1-42 peptide. J MolNeurosci. 2005; 27(1):13-21.

25. Toublet FX, Lecoutey C, Lalut J, Hatat B, Davis A, Since M, et al. Inhibiting acetylcholinesterase to activate pleiotropic prodrugs with therapeutic interest in Alzheimer's Disease. Molecules. 2019; 24(15):2786.

26. Saxena M, Dubey R. Target enzyme in Alzheimer's Disease: acetylcholinesterase inhibitors. Curr Top Med Chem. 2019; 19(4):264-275.

27. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell. 2012; 149(6):1192-1205.

28. Maguschak KA, Ressler KJ. Wnt signaling in amygdala-dependent learning and memory. J Neurosci. 2011; 31(37):13057-13067.

29. Tapia-Rojas C, Inestrosa NC. Loss of canonical Wnt signaling is involved in the
pathogenesis of Alzheimer's disease. Neural Regen Res. 2018; 13(10):1705-1710.

30. Inestrosa NC, Toledo EM. The role of Wnt signaling in neuronal dysfunction in Alzheimer's Disease. Mol Neurodegener. 2008; 3:9.

31. Chu ZG, Zhou FM, Hablitz JJ. Nicotinic acetylcholine receptor-mediated synaptic potentials in rat neocortex. Brain Res. 2000; 887(2):399-405.

32. Roerig B, Nelson DA, Katz LC. Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT3 receptors in developing visual cortex. J Neurosci. 1997; 17(21):8353-8362.

33. Zolles G, Wagner E, Lampert A, Sutor B. Functional expression of nicotinic acetylcholine receptors in rat neocortical layer 5 pyramidal cells. Cereb Cortex. 2009; 19(5):1079-1091.

34. Kassam SM, Herman PM, Goodfellow NM, Alves NC, Lambe EK. Developmental excitation of corticothalamic neurons by nicotinic acetylcholine receptors. J Neurosci. 2008; 28(35):8756-8764.

35. Vallée A, Vallée JN, Guillevin R, Lecarpentier Y. Riluzole: a therapeutic strategy in Alzheimer's disease by targeting the WNT/β-catenin pathway. Aging (Albany NY). 2020; 12(3):3095-3113.

36. Shruster A, Eldar-Finkelman H, Melamed E, Offen D. Wnt signaling pathway overcomes the disruption of neuronal differentiation of neural progenitor cells induced by oligomeric amyloid β-peptide. J Neurochem. 2011; 116(4):522-529.

37. Jin N, Zhu H, Liang X, Huang W, Xie Q, Xiao P, et al. Sodium selenate activated Wnt/β-catenin signaling and repressed amyloid-β formation in a triple transgenic mouse model of Alzheimer's disease. Exp Neurol. 2017; 297:36-49.

38. Nunes PV, Forlenza OV, Gattaz WF. Lithium and risk for Alzheimer's disease in elderly patients with bipolar disorder. Br J Psychiatry. 2007; 190:359-360.
39. Rybakowski JK. Challenging the negative perception of lithium and optimizing its long-term administration. Front Mol Neurosci. 2018; 11:349.

40. Van Gestel H, Franke K, Petite J, Slaney C, Garnham J, Helmick C, et al. Brain age in bipolar disorders: effects of lithium treatment. Aust N Z J Psychiatry. 2019; 53(12):1179-1188.

41. Forlenza OV, De-Paula VJ, Diniz BS. Neuroprotective effects of lithium: implications for the treatment of Alzheimer's disease and related neurodegenerative disorders. ACS Chem Neurosci. 2014; 5(6):443-450.

42. Vo TM, Perry P, Ellerby M, Bohnert K. Is lithium a neuroprotective agent? Ann Clin Psychiatry. 2015; 27(1):49-54.

43. Martinez A, Perez DI. GSK-3 inhibitors: a ray of hope for the treatment of Alzheimer's disease? J Alzheimers Dis. 2008; 15(2):181-191.

44. Hampel H, Lista S, Mango D, Nisticò R, Perry G, Avila J, et al. Lithium as a treatment for Alzheimer's Disease: the systems pharmacology perspective. J Alzheimers Dis. 2019; 69(3):615-629.

45. Cisternas P, Zolezzi JM, Martinez M, Torres VI, Wong GW, Inestrosa NC. Wnt-induced activation of glucose metabolism mediates the in vivo neuroprotective roles of Wnt signaling in Alzheimer disease. J Neurochem. 2019; 149(1):54-72.

46. Zhao L, Gong N, Liu M, Pan X, Sang S, Sun X, et al. Beneficial synergistic effects of microdose lithium with pyrroloquinoline quinone in an Alzheimer's disease mouse model. Neurobiol Aging. 2014; 35(12):2736-2745.

47. Dong YT, Cao K, Tan LC, Wang XL, Qi XL, Xiao Y, et al. Stimulation of SIRT1 attenuates the level of oxidative stress in the brains of APP/PS1 double transgenic mice and in primary neurons exposed to oligomers of the Amyloid-β peptide. J
48. Klein WL. Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets. Neurochem Int. 2002; 41(5):345-352.

49. Berardo C, Siciliano V, Di Pasqua LG, Richelmi P, Vairetti M, Ferrigno A. Comparison between Lipofectamine RNAiMAX and GenMute transfection agents in two cellular models of human hepatoma. Eur J Histochem. 2019; 63(3):3048.

50. Cao K, Xiang J, Dong YT, Xu Y, Li Y, Song H, et al. Exposure to fluoride aggravates the impairment in learning and memory and neuropathological lesions in mice carrying the APP/PS1 double-transgenic mutation. Alzheimers Res Ther. 2019; 11(1):35.

51. Duan J, Zhang Q, Hu X, Lu D, Yu W, Bai H. N\textsuperscript{4}-acetylcystidine is required for sustained NLRP3 inflammasome activation via HMGB1 pathway in microglia. Cell Signal. 2019; 58:44-52.

52. Cao K, Dong YT, Xiang J, Xu Y, Hong W, Song H, et al. Reduced expression of SIRT1 and SOD-1 and the correlation between these levels in various regions of the brains of patients with Alzheimer's disease. J Clin Pathol. 2018; 71(12):1090-1099.

53. Yamamoto T, Hirano A. A comparative study of modified Bielschowsky, Bodian and thioflavin S stains on Alzheimer's neurofibrillary tangles. Neuropathol Appl Neurobiol. 1986; 12(1):3-9.

54. Pereira JB, Janelidze S, Ossenkoppele R, Kvartsberg H, Brinkmalm A, Mattsson-Carlsgren N, et al. Untangling the association of amyloid-β and tau with synaptic and axonal loss in Alzheimer's disease. Brain. 2021; 144(1):310-324.

55. Pinto T, Lanctôt KL, Herrmann N. Revisiting the cholinergic hypothesis of behavioral and psychological symptoms in dementia of the Alzheimer's type.
56. Pietrzak RH, Laws SM, Lim YY, Bender SJ, Porter T, Doecke J, et al. Plasma cortisol, brain Amyloid-β, and cognitive decline in preclinical Alzheimer's disease: A 6-year prospective cohort study. Biol Psychiatry Cogn Neurosci Neuroimaging. 2017; 2(1):45-52.

57. Gao H, Liu M, Zhao Z, Yang C, Zhu L, Cai Y, et al. Diagnosis of mild cognitive impairment and Alzheimer's disease by the plasma and serum amyloid-beta 42 assay through highly sensitive peptoid nanosheet sensor. ACS Appl Mater Interfaces. 2020; 12(8):9693-9700.

58. Shi Q, Chowdhury S, Ma R, Le KX, Hong S, Caldarone BJ, et al. Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice. Sci Transl Med. 2017; 9(392): eaaf6295.

59. Xiang J, Cao K, Dong YT, Xu Y, Li Y, Song H, et al. Lithium chloride reduced the level of oxidative stress in brains and serums of APP/PS1 double transgenic mice via the regulation of GSK3β/Nrf2/HO-1 pathway. Int J Neurosci. 2020; 130(6):564-573.

60. Bertrand D, Lee CH, Flood D, Marger F, Donnelly-Roberts D. Therapeutic potential of α7 nicotinic acetylcholine receptors. Pharmacol Rev. 2015; 67(4):1025-1073.

61. Kabbani N, Nichols RA. Beyond the Channel: Metabotropic signaling by nicotinic receptors. Trends Pharmacol Sci. 2018; 39(4):354-366.

62. Martín-Sánchez C, Alés E, Balseiro-Gómez S, Atienza G, Arnalich F, Bordas A, et al. The human-specific duplicated α7 gene inhibits the ancestral α7, negatively regulating nicotinic acetylcholine receptor-mediated transmitter release. J Biol Chem. 2021:100341.
63. Cao K, Dong YT, Xiang J, Xu Y, Li Y, Song H, et al. The neuroprotective effects of SIRT1 in mice carrying the APP/PS1 double-transgenic mutation and in SH-SY5Y cells over-expressing human APP670/671 may involve elevated levels of α7 nicotinic acetylcholine receptors. Aging (Albany NY). 2020; 12(2):1792-1807.

64. Zhao L, Xiao Y, Xiu J, Tan LC, Guan ZZ. Protection against the neurotoxic effects of β-Amyloid peptide on cultured neuronal cells by lovastatin involves elevated expression of α7 nicotinic acetylcholine receptors and activating phosphorylation of protein kinases. Am J Pathol. 2018; 188(4):1081-1093.

65. Roberts JP, Stokoe SA, Sathler MF, Nichols RA, Kim S. Selective co-activation of α7- and α4β2-nicotinic acetylcholine receptors reverses beta-amyloid-induced synaptic dysfunction. J Biol Chem. 2021:100402.

66. Fortress AM, Frick KM. Hippocampal Wnt signaling: memory regulation and hormone interactions. Neuroscientist. 2016; 22(3):278-294.

67. Folke Jonas, Pakkenberg Bente, Brudek Tomasz. Impaired Wnt signaling in the prefrontal cortex of Alzheimer's disease. Mol Neurobiol, 2019; 56: 873-891.

68. Lauretti E, Dincer O, Praticò D. Glycogen synthase kinase-3 signaling in Alzheimer's disease. Biochim Biophys Acta Mol Cell Res. 2020;1867(5):118664.

69. Grimes CA, Jope RS. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Prog Neurobiol. 2001; 65(4):391-426.

70. Huang M, Liang Y, Chen H, Xu B, Chai C, Xing P. The role of fluoxetine in activating Wnt/β-catenin signaling and repressing β-Amyloid production in an Alzheimer mouse model. Front Aging Neurosci. 2018; 10:164.

71. Rybakowski JK, Suwalska A, Hajek T. Clinical perspectives of lithium's neuroprotective effect. Pharmacopsychiatry. 2018; 51(5):194-199.
72. Roux M, Dosseto A. From direct to indirect lithium targets: a comprehensive review of omics data. Metallomics. 2017; 9(10):1326-1351.

73. Sofola-Adesakin O, Castillo-Quan JI, Rallis C, Tain LS, Bjedov I, Rogers I, et al. Lithium suppresses Aβ pathology by inhibiting translation in an adult Drosophila model of Alzheimer's disease. Front Aging Neurosci. 2014; 6:190.

74. Rockenstein E, Torrance M, Adame A, Mante M, Bar-on P, Rose JB, et al. Neuroprotective effects of regulators of the glycogen synthase kinase-3beta signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. J Neurosci. 2007; 27(8):1981-1991.

75. Rivera DS, Lindsay C, Codocedo JF, Morel I, Pinto C, Cisternas P, et al. Andrographolide recovers cognitive impairment in a natural model of Alzheimer's disease (Octodon degus). Neurobiol Aging. 2016; 46:204-220.

76. Phiel CJ, Wilson CA, Lee VM, Klein PS. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature. 2003; 423(6938):435-439.

77. Pan Y, Short JL, Newman SA, Choy KHC, Tiwari D, Yap C, et al. Cognitive benefits of lithium chloride in APP/PS1 mice are associated with enhanced brain clearance of β-amyloid. Brain Behav Immun. 2018; 70:36-47.

78. Thompson AJ, Williamson R, Schofield E, Stephenson J, Hanger D, Anderton B. Quantitation of glycogen synthase kinase-3 sensitive proteins in neuronal membrane rafts. Proteomics. 2009; 9(11):3022-3035.

79. Parr C, Mirzaei N, Christian M, Sastre M. Activation of the Wnt/β-catenin pathway represses the transcription of the β-amyloid precursor protein cleaving enzyme (BACE1) via binding of T-cell factor-4 to BACE1 promoter. FASEB J. 2015; 29(2):623-635.
80. Sutherland C, Leighton IA, Cohen P. Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. Biochem J. 1993; 296:15-19.

81. Huang Min, Liang Yubin, Chen Hongda, et al. The role of fluoxetine in activating Wnt/β-catenin signaling and repressing β-Amyloid production in an Alzheimer mouse model. 2018; 10:164.

82. Arráuzola MS, Silva-Alvarez C, Inestrosa NC. How the Wnt signaling pathway protects from neurodegeneration: the mitochondrial scenario. Front Cell Neurosci. 2015; 9:166.

83. Marzo A, Galli S, Lopes D, McLeod F, Podpolny M, Segovia-Roldan M, et al. Reversal of synapse degeneration by restoring Wnt signaling in the adult hippocampus. Curr Biol. 2016; 26(19):2551-2561.

84. Cisternas P, Oliva CA, Torres VI, Barrera DP, Inestrosa NC. Presymptomatic treatment with and rographolide improves brain metabolic markers and cognitive behavior in a model of early-onset Alzheimer's Disease. Front Cell Neurosci. 2019; 13:295.

85. Dineley KT. Beta-amyloid peptide--nicotinic acetylcholine receptor interaction: the two faces of health and disease. Front Biosci. 2007; 12:5030-5038.

86. Farias GG, Vallés AS, Colombres M, Godoy JA, Toledo EM, Lukas RJ, et al. Wnt-7a induces presynaptic colocalization of alpha 7-nicotinic acetylcholine receptors and adenomatous polyposis coli in hippocampal neurons. J Neurosci. 2007; 27(20):5313-5325.

87. Liu B, Deng C, Zhang Y, Zhang J. Wnt3a expression during the differentiation of adipose-derived stem cells into cholinergic neurons. Neural Regen Res. 2012; 7(19):1463-1468.
88. Inestroza NC, Godoy JA, Vargas JY, Arrazola MS, Rios JA, Carvajal FJ, et al. Nicotine prevents synaptic impairment induced by amyloid-β oligomers through α7-nicotinic acetylcholine receptor activation. Neuromolecular Med. 2013; 15(3):549-569.

89. Jensen M, Hoerndli FJ, Brockie PJ, Wang R, Johnson E, Maxfield D, et al. Wnt signaling regulates acetylcholine receptor translocation and synaptic plasticity in the adult nervous system. Cell. 2012; 149(1):173-187