

Original Research Article

Genetic deletion of $\alpha 7$ nicotinic acetylcholine receptors induces an age-dependent Alzheimer's disease-like pathology

Maria Rosaria Tropea^a, Domenica D. Li Puma^{b,c}, Marcello Melone^{d,e}, Walter Gulisano^a, Ottavio Arancio^f, Claudio Grassi^{b,c}, Fiorenzo Conti^{d,e,g}, Daniela Puzzo^{a,h,*}

^a Dept. Biomedical and Biotechnological Sciences, University of Catania, Catania 95123, Italy

^b Department of Neuroscience, Università Cattolica del Sacro Cuore, 00168 Rome, Italy

^c Fondazione Policlinico Universitario A. Gemelli IRCCS, 00168 Rome, Italy

^d Section of Neuroscience and Cell Biology, Dept. Experimental and Clinical Medicine, Università Politecnica delle Marche, Ancona 60020, Italy

^e Center for Neurobiology of Aging, National Institute of Science and Health for Aging (INRA-IRCCS), Italy

^f Dept. Pathology and Cell Biology, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, and Dept. Medicine, Columbia University, New York, NY, United States

^g Foundation for Molecular Medicine, Università Politecnica delle Marche, Ancona 60020, Italy

^h Oasi Research Institute-IRCCS, Troina 94018, Italy

ARTICLE INFO

Keywords:

Amyloid-beta peptide
Tau protein
Alpha7 nicotinic acetylcholine receptor
Hippocampus
Alzheimer's disease
Memory
Synaptic plasticity

ABSTRACT

The accumulation of amyloid-beta peptide ($A\beta$) and the failure of cholinergic transmission are key players in Alzheimer's disease (AD). However, in the healthy brain, $A\beta$ contributes to synaptic plasticity and memory acting through $\alpha 7$ subtype nicotinic acetylcholine receptors ($\alpha 7nAChRs$). Here, we hypothesized that the $\alpha 7nAChR$ deletion blocks $A\beta$ physiological function and promotes a compensatory increase in $A\beta$ levels that, in turn, triggers an AD-like pathology.

To validate this hypothesis, we studied the age-dependent phenotype of $\alpha 7$ knock out mice. We found that $\alpha 7nAChR$ deletion caused an impairment of hippocampal synaptic plasticity and memory at 12 months of age, paralleled by an increase of Amyloid Precursor Protein expression and $A\beta$ levels. This was accompanied by other classical AD features such as a hyperphosphorylation of tau at residues Ser 199, Ser 396, Thr 205, a decrease of GSK-3 β at Ser 9, the presence of paired helical filaments and neurofibrillary tangles, neuronal loss and an increase of GFAP-positive astrocytes.

Our findings suggest that $\alpha 7nAChR$ malfunction might precede $A\beta$ and tau pathology, offering a different perspective to interpret the failure of anti- $A\beta$ therapies against AD and to find novel therapeutical approaches aimed at restoring $\alpha 7nAChRs$ -mediated $A\beta$ function at the synapse.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting the elderly, but its intricate pathophysiology has prevented the discovery of effective therapies. The Cholinergic and the Amyloid- β ($A\beta$) Hypotheses represent the two main etiopathological theories proposed to explain the onset and progression of the disease. The Cholinergic Hypothesis (Appel, 1981) has been supported by several evidences indicating that cholinergic transmission is affected in early AD. Indeed, loss of cholinergic neurons in the nucleus basalis of Meynert, decrease of choline acetyltransferase (ChAT) activity and reduction of nicotinic receptors (nAChRs) have been highly correlated with

dementia and its progression (Burghaus et al., 2000; Dickson et al., 1995; Engidawork et al., 2001; Kuhn et al., 2015; Mufson et al., 2007; Strada et al., 1992; Whitehouse et al., 1981).

On the other hand, the $A\beta$ Hypothesis (Hardy and Allsop, 1991) posits that the increase and accumulation of $A\beta$ represent the *primum movens* in AD pathophysiology, responsible of synaptic dysfunction triggering downstream events leading to dementia [reviewed in (Gulisano et al., 2018a)].

A third leading actor in this conundrum is tau, a microtubule-associated protein involved in microtubule assembly and stabilization (Wang and Mandelkow, 2015) whose activity and function is regulated by different types of post-translational modifications such as

* Corresponding author at: Dept. Biomedical and Biotechnological Sciences, University of Catania, Catania 95123, Italy.

E-mail address: danypuzzo@yahoo.it (D. Puzzo).

<https://doi.org/10.1016/j.pneurobio.2021.102154>

Received 11 January 2021; Received in revised form 29 May 2021; Accepted 18 August 2021

Available online 25 August 2021

0301-0082/© 2021 The Author(s).

Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

phosphorylation, ubiquitination or glycosylation (Martin et al., 2011). Interestingly, tau shares numerous characteristics with A β since both proteins form insoluble deposits, i.e. senile plaques and neurofibrillary tangles (NFTs), respectively (Glenner and Wong, 1984; Grundke-Iqbal et al., 1986), and can aggregate in soluble oligomers whose increase has been highly related to AD severity (Fá et al., 2016; Hölttä et al., 2013; Lasagna-Reeves, 2012; Sengupta et al., 2017). According to the classic A β Hypothesis, tau hyperphosphorylation is triggered by A β but, recently, it has been demonstrated that the two proteins might act independently or concomitantly to impair synaptic plasticity and memory (Fá et al., 2016; Puzzo et al., 2020), probably converging onto common targets such as amyloid precursor protein (APP) (Puzzo et al., 2017; Wang et al., 2017).

Although pre-clinical and clinical data continue to support both the Cholinergic and the A β hypotheses, therapeutic strategies aimed at increasing ACh transmission are not able to act as disease-modifying drugs, and approaches expected to cure the disease by decreasing A β levels have failed so far. In particular, cholinesterase inhibitors used to treat cognitive symptoms in early to moderate AD patients (Anand and Singh, 2013) do not induce a long-term improvement of cognition and the treatment is not always effective (Connelly et al., 2005; Lemstra et al., 2007). Also, a variety of nAChR agonists, although promising in preclinical studies, had a limited efficacy when experimented in clinical trials, probably for the rapid nAChRs desensitization (Picciotto, 2000). The outcome of clinical trials with anti-A β drugs is even more puzzling since the success obtained in animal models of AD has not been replicated in humans. Active and passive immunization against A β as well as the use of drugs aimed at preventing A β formation have failed, either not showing efficacy or inducing severe side effects [for a review see (Gulisano et al., 2018a)]. Notwithstanding these discouraging results, anti-A β therapies are still under investigation with the intent to treat patients in the very early asymptomatic phase of the disease, or to select A β -responders based on a personalized approach. However, the latest unsuccessful trials with the anti-A β antibody aducanumab and the BACE inhibitor elenbecestat (see www.alzforum.org) are emblematic and confirmed that the Occam's razor strategy "if A β increases in AD patients, clearing A β from the brain is the solution" might not be the right choice against AD (Gulisano et al., 2018a; Herrup, 2015; Puzzo et al., 2015a,b).

It is undeniable that AD patients present an increase of A β and hyperphosphorylated tau, as well as an impairment of cholinergic transmission (Gulisano et al., 2018a; Ferreira-Vieira et al., 2016; Selkoe and Hardy, 2016). On the other hand, the observation that in the healthy brain A β exerts a physiological function mediated by cholinergic receptors (Gulisano et al., 2019) might offer a new perspective to tackle the intricate pathophysiology of AD (Puzzo et al., 2015a,b). Indeed, a variety of studies have demonstrated that A β enhances neurotransmitter release (Gulisano et al., 2019; Koppensteiner et al., 2016; Lazarevic et al., 2017) – for a review see (Puzzo et al., 2015a,b) – and facilitates long-term potentiation (LTP) and memory formation (Garcia-Osta and Alberini, 2009; Morley et al., 2010; Palmeri et al., 2017; Puzzo et al., 2011, 2008; Ricciarelli et al., 2014) through α 7nAChRs. In fact, picomolar concentrations of A β bind α 7nAChRs with high affinity (Wang et al., 2000) exerting an agonist-like action that regulates synaptic function (Dineley et al., 2002; Gulisano et al., 2019; Lawrence et al., 2014; Lazarevic et al., 2017; Mura et al., 2012; Oz et al., 2013; Puzzo et al., 2011, 2008). Therefore, a genetic or pharmacological deletion of α 7nAChRs prevents the A β -induced enhancement of short- and long-term synaptic plasticity as well as memory (Gulisano et al., 2019; Puzzo et al., 2011, 2008).

These observations inspired this work that aimed at understanding whether the Cholinergic and A β hypotheses might be unified looking at the disease from a different perspective summarized in one question: what are the consequences of a failure of A β physiological function when its endogenous receptor, i.e. α 7nAChR, does not work properly?

2. Material and methods

2.1. Animals

We used WT (C57BL/6 J; RRID:IMSR_JAX:000664) and α 7-KO (B6.129S7-Chrna7tm1Bay/J; RRID:IMSR_JAX:003232) purchased from The Jackson Laboratory. Histology was also performed on hippocampal slices from 3 \times Tg mice (APP^{Swe}, PS1 M146 V, and tauP301 L) genetically engineered by LaFerla and colleagues at the Department of Neurobiology and Behaviour, University of California, Irvine (Oddo et al., 2003). Colonies were established in the animal facilities at University of Catania and Università Cattolica del Sacro Cuore. Housing conditions were controlled maintaining stable hygrometric and thermic conditions (50 %; 21 °C \pm 1 °C) on 12 h light/dark cycle with ad libitum access to food and water.

All the experiments were performed according to the local Institutional Animal care and Use Committee (approval #327/2013-B, #119–2017-PR, #626–2016-PR) and the European Communities Council Directives (2010/63/EU). Experiments complied with the ARRIVE guidelines and were conducted to minimize animal suffering. To reduce number of animals, we used males for electrophysiological recordings, sex-balanced animals for behavioral experiments, western blotting and ELISA, females for histology and immunohistochemistry. Animals were used at different ages according to our scientific work plan, as detailed in the specific sections.

2.2. Electrophysiological field recordings

Extracellular electrophysiological field recordings were performed on transverse hippocampal slices as previously described (Gulisano et al., 2019; Puzzo et al., 2017). After cervical dislocation, hippocampi were removed and cut (400 μ m thickness) by a manual tissue chopper. Slices were transferred to a recording chamber and perfused (1–2 mL/min) with ACSF (composition in mM: 124.0 NaCl, 4.4 KCl, 1.0 Na₂HPO₄, 25.0 NaHCO₃, 2.0 CaCl₂, 2.0 MgCl₂, 10.0 Glucose) kept at 29 °C and continuously bubbled with an O₂/CO₂ mixture at 95 % and 5%. Slices were allowed to recover for 120 min prior to recording. Field excitatory postsynaptic potentials (fEPSPs) were recorded in CA1 *stratum radiatum* by a glass capillary filled with ACSF in response to stimulation of the Schaffer collaterals by a bipolar tungsten electrode. Basal synaptic transmission (BST) was assessed by stimulating with a series of increasing voltage pulses (from 5 to 35 V) to select healthy slices to be used for electrophysiological recordings. For Paired pulse facilitation (PPF) experiments, slices were perfused with the NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid (APV; 50 μ M) for 45 min. Two pulses with a time interval of 10, 20, 30, 40, 50, 100, 200, 500, and 1000 ms were delivered and fEPSP responses were recorded. In another series of experiments, we studied LTP. Baseline was elicited every minute, by stimulating at a voltage able to evoke a response of 35 % of the maximum evoked response in BST. After 30–45 min, slices with a stable baseline (slope variation \pm 5%) were recorded for 15 min before to induce LTP by a theta-burst (TBS) stimulation, i.e. 3 TBS trains delivered with a 15 s inter-train interval with each train consisting in 10 \times 100 Hz bursts with 5 pulses per burst with a 200-ms interburst interval, at the test pulse intensity. Recordings were performed and analyzed offline in pClamp 10 (Molecular Devices, Sunnyvale, CA, USA). PPF was plotted as the percentage of the synaptic response of the second against the first delivered stimulus. LTP was plotted as fEPSP (normalized as % of baseline) vs. time (min).

2.3. Drugs

In a series of experiments, hippocampal slices were treated with α -bungarotoxin (α -BTX; Sigma-Aldrich, 10 μ M), methyllycaconitine (MLA, Sigma-Aldrich, 10 μ M), murine anti-A β antibody M3.2 (Covance, catalog #SIG-39155, 2 μ g/mL) before tetanic stimulation. MLA was

dissolved in DMSO, aliquoted, stored at -20°C . All drugs were diluted in ACSF to the desired final concentration right before electrophysiological experiments, according to previous studies (Gulisano et al., 2019; Puzzo et al., 2008).

2.4. Behavioral studies

Fear Conditioning (FC) was performed as previously described (Puzzo et al., 2017). The apparatus consisted in a conditioning chamber, connected to an interface (Kinder Scientific, USA), located in a sound-attenuating box (Campden Inst., UK) with a computer fan installed in one side to provide a background white noise. A webcam mounted on the top of the chamber allowed video recording of the experiment. The floor, made of 36-bar insulated shock grid, was cleaned after each test with 70 % ethanol and water. The protocol lasted 3 days. Mice were handled every day for about 5 min before the experiment. During the first day the animal was placed in the conditioning chamber for 2 min prior to the conditioned stimulus (CS) delivery. CS was a tone (2800 Hz and 85 dB) delivered for 30 s. In the last 2 s of the tone, the mouse received a foot shock as an unconditioned stimulus (US) through the electrified grid floor (0.7 mA for 2 s). After the CS/US pairing, the mouse was left into the chamber for 30 s before to be placed in the home cage. Twenty-four hours after training (day 2), the mouse was placed back in the conditioning chamber for 5 min to evaluate contextual fear memory. Forty-eight hours after training (day 3) animals were placed in the conditioning chamber to evaluate cued fear memory. To this end, a novel context was created by using an acrylic black box with a smooth flat floor sprayed with vanilla odorant. After 2 min (pre-CS test), the mouse was exposed to the same tone used during the training for 3 min (CS test). Freezing (absence of movement except for that needed for breathing) was manually scored during the three days by two different operators and the averaged value was used to perform the analyses.

Novel Object Recognition (NOR) was performed as previously described (Gulisano et al., 2018b). The arena was a white plastic box ($50 \times 35 \times 45$ cm) placed on a lab bench. A webcam, connected to the computer, was fixed on the wall. The NOR protocol was performed in 5 days: 3 days of habituation, 1 day of training (T1) and 1 day of testing (T2). Objects were designed by a computer aided design software (Solidworks, France) and printed in polylactic acid with a Prusa i3-inspired 3D printer of our design. After each trial, the box and the objects were cleaned with 70 % ethanol and dried with absorbent paper. During the first day (habituation to the arena), the mouse was put into the empty arena and allowed to explore it for 10 min. During the second and the third day (familiarization with objects), the mouse was put into the arena containing two different objects, randomly chosen among our object collection and changed from day to day, for 10 min. During the fourth day, NOR training session (T1) was performed. The mouse was put into the arena and allowed to explore for 10 min two identical objects placed in the central part of the box, equally distant from the perimeter and the center. During the fifth day (24 h after T1), the mouse underwent the second trial (T2) to test memory retention for 10 min. Mice were presented with two different objects, respectively a “familiar” (i.e. the one used for T1) and a “novel” object. For Novel Object Location (NOL), the mouse was put into the arena and allowed to explore for 10 min two identical objects placed in one side of the box. On the day after, the location of one object was changed and the mouse underwent the T2 for 10 min. For both NOR and NOL experiments, animal exploration - defined as the mouse pointing its nose toward the object from a distance not > 2 cm - was measured in T2. We analyzed: i) percentage exploration of familiar vs. novel object; ii) discrimination (D) index, “exploration of novel object minus exploration of familiar object/total exploration time”; and iii) total exploration time. Mice with a total exploration time < 5 s were excluded from analysis.

2.5. Determination of $\text{A}\beta$ levels

Briefly, hippocampal tissues from 12 M $\alpha 7$ KO and WT mice were sonicated in lysis buffer (10 $\mu\text{L}/\text{mg}$ tissue) containing 5 M guanidine-HCl/50 mM Tris, pH 8.0). Sonicates were then diluted ten-fold with Dulbecco's PBS containing $1\times$ protease inhibitor cocktail (Sigma). Levels of murine $\text{A}\beta$ (1–42) were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Thermo Fisher Scientific, cat# KMB3441) following manufacturer's instructions. All assays were performed on F-bottom 96-well plates (Nunc, Wiesbaden, Germany). Tertiary antibodies were conjugated to horseradish peroxidase. Wells were developed with tetramethylbenzidine and measured at 450 nm.

2.6. Western blotting on hippocampal homogenates

Western blot (WB) analysis was performed as previously described (Gulisano et al., 2019; Li Puma et al., 2019) with minor modifications. Whole hippocampi from 9 and 12 months-old WT and $\alpha 7$ KO mice were homogenized in RIPA buffer (Thermoscientific) in the presence of phosphatase and protease inhibitors (Thermoscientific), and sonicated 3 times for 10 min on ice. Protein concentrations were determined by Bradford protein assay (Biorad) and 40 μg of total proteins were then loaded onto 4–15 % Tris-glycine polyacrylamide gels (Biorad) for electrophoretic separation and then transferred onto 0.45 or 0.22 μm nitrocellulose membranes (Amersham Biosciences, Buckinghamshire, UK). Membranes were blocked for 1 -h, at RT, in either a solution of 5% nonfat dry milk in Tris-buffered saline containing 0.1 % Tween-20 before incubation overnight at 4°C with the following primary antibodies: mouse 4G8, that recognizes residues 17–24 of $\text{A}\beta$ and the same sequence in APP full length (BioLegend San Diego, California, USA; 1:1000); mouse C-term APP Y188 (Abcam; Cambridge UK; 1:1000); mouse M3.2 (BioLegend; 1:1000), that recognizes residues 10–15 of murine $\text{A}\beta$ and the same sequence in APP full length; mouse pTau Ser199 (Thermo fisher Scientific; 1:1000); rabbit pTau Ser396 (SAB; Signalway Antibody Co., Ltd.; 1:1000); rabbit pTau Thr205 (SAB; 1:1000); rabbit pGSK-3 β Ser9 (Cell Signaling; 1:1000). Mouse anti-GAPDH (Abcam; 1:5000), rabbit total GSK-3 β (Cell signaling; 1:1000) and mouse Tau-5 (Thermo fisher Scientific; 1:1000) were used as loading controls. After incubation with HRP-conjugated secondary antibodies (Cell Signaling Technology) visualization was performed with ECL plus (Amersham Biosciences) using UVItec Cambridge Alliance. Molecular weights for immunoblot analysis were determined using Precision Plus Dual Color Standards (Biorad).

2.7. Tissue preparation for microscopy studies

For histology, NeuN and GFAP immunohistochemistry, fresh brains were removed, immersed in 10 % formalin for 72 h and then transferred in 4% PFA in phosphate buffer until use. For PHF-1 immunohistochemistry, animals were anesthetized by intraperitoneal injection of a cocktail of Zolazepam plus Tiletamine (120 mg/Kg) and Medetomidine (80 $\mu\text{g}/\text{Kg}$), and perfused through the ascending aorta with a flush of physiological saline followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% PFA in phosphate buffer for 5 days.

Sections (50 μm thickness) were sequentially cut with a Vibratome within 2.350 and 1.725 mm lateral range and processed for immunohistochemistry or histological stains.

2.8. Congo Red stain

Congo Red stain was performed as previously described (Wilcock et al., 2006). Sections were incubated for 20 min in a fresh prepared alkaline saturated NaCl solution. Briefly, NaCl was added to an 80 % ethanol solution while stirring, until the formation of an undissolved NaCl layer (about 5 mm thick) and 1% NaOH 1 M was added before use.

Sections were then incubated in 0.2 % Congo Red solution (Sigma-Aldrich) for 30 min and rinsed five times in 95 % ethanol.

2.9. Bielschowsky stain

Bielschowsky silver stain was performed as suggested by the manufacturer (Bielschowsky silver stain kit, VitroVivo Biotech, cat.#VB-3015). Briefly, sections were incubated in pre-warmed 40 °C Silver Nitrate solution for 18 min, washed in distilled water and then incubated with silver ammonium solution at 40 °C for 30 min. Subsequently, slices were placed in the developing solution for 75 s and in 1% ammonium hydroxide solution for 60 s. Slices were then washed in distilled water and incubated in 5% sodium thiosulfate solution for 5 min.

2.10. Immunohistochemistry

Antibodies. The following primary antibodies were used: mouse anti-PHF-1 (1:50) detecting tau Ser396/Ser404 phosphorylation sites (generous gift of Dr. Peter Davies); mouse anti-NeuN (1:100; Millipore, cat.#MAB377); mouse anti-GFAP (1:350; Sigma-Aldrich, cat.#G3893).

Immunofluorescence. Immunofluorescence was performed as previously described (Melone et al., 2019). Sections were incubated in 10 % NDS (1 h), followed by a solution containing PHF-1 primary antibody overnight at RT. Sections were rinsed in Tris (0.5 M buffered saline, pH 7.4) incubated first in 10 % NDS (15 min), and then in a solution containing the secondary antibody (Alexa Fluor 488, 1:230; Jackson) for 2 h at RT. Sections were washed in Tris, mounted, and coverslipped with propyl-gallate 0.1 M in glycerol-PBS solution (9:1). Omission of the primary antibodies in sections from each experimental group resulted in a lack of specific staining in the corresponding channel (negative control). To quench lipofuscin autofluorescence, at the end of immunofluorescence protocols, sections were incubated for 5 min in 0.1 % Sudan Black dissolved in 70 % alcohol. To minimize procedural variability, sections from all experimental groups, were exposed to immunofluorescence procedure in parallel.

Immunoperoxidase. Immunoperoxidase was performed as previously described (Melone et al., 2019). For both NeuN and GFAP optimal detection (i.e. immunoreactivity against the background level) was determined by testing different conditions (e.g., inclusion or exclusion of detergents, and a range of dilutions for primary antibodies) in a set of pilot trials. Sections were treated with H₂O₂ (1% in Tris for 25 min) to remove endogenous peroxidase activity, rinsed in Tris and then incubated in 10 % NGS (1 h) followed by a solution containing NeuN or GFAP primary antibodies (overnight at RT). Sections were rinsed in Tris and incubated first in 10 % NGS (15 min) and then in a solution containing biotinylated secondary antibody (1:180; Jackson) for 2 h at RT. Subsequently, they were rinsed in Tris, incubated in avidin-biotin peroxidase complex (ABC Elite PK6100, Vector), washed in Tris, and incubated in 3,3'-diaminobenzidine tetrahydrochloride (DAB; 0.05 % in 0.05 M Tris buffer, pH 7.6 with 0.03 % H₂O₂). Then, sections were washed, mounted, and coverslipped with dpx mounting medium. Method specificity was assessed by substituting NeuN and GFAP primary antibodies with Tris, resulting in the absence of immunoreactivity. To minimize procedural variability, sections from all experimental groups, were exposed to immunoperoxidase procedure in parallel.

Data collection and analysis.

For confocal microscopy, PHF-1 immunolabeled sections were scanned with a Leica SP2 TCS-SL microscope. For 20x of CA1, microscopical fields were acquired as 512 × 512 pixel images (pixel size of 750 nm) with pinhole 1.4 Airy unit, and to improve signal/noise ratio, 4 frames of each image were averaged. For quantitative microscopy of stratum oriens (so), stratum pyramidale (sp), and stratum radiatum (sr), microscopical fields were acquired as 512 × 512 pixel images (pixel size of 465 nm) with a planapo 63 × objective (numerical aperture 1.4) and pinhole 1.0 Airy unit. To improve signal/noise ratio, 4 frames of each image were averaged. CA1 microscopical fields were randomly selected

(8 microscopical fields/layer/4 sections from 2 animals for each experimental group). To avoid the influence of the acquisition parameters (i.e. photomultiplier gain and offset) on fluorescence intensity, all microscopical fields from all conditions were scanned and acquired with the same setting. As previously described (de Vivo, 2010), photomultiplier gain and offset were set so that the brightest pixel was just slightly below saturation, and the offset such that the darkest pixels were just above zero. To avoid the effects of the surface-depth gradient on immunodetection (Melone et al., 2005), all microscopical fields were acquired at a z-axis level yielding the maximum brightness of immunopositive profiles (de Vivo, 2010; Melone et al., 2005).

For quantitation of intensity, randomly selected subfields of 32 × 32 μm from the original microscopical fields (Melone et al., 2019) (32 for each experimental group/layer; with a total of 96 subfields for each condition) were used.

Optimal visualization of immunoreactivity was achieved by setting the threshold value to the mean pixel value over the field under study in WT and α7 KO groups (Melone et al., 2005). Intensity of threshold subfields was calculated by Image J (Schneider et al., 2012). Number and mean size of immunoreactive puncta of each subfield were obtained by transforming images to binary mode and calculated using Image J (Bozdagi et al., 2000; Bragina et al., 2006; Schneider et al., 2012). For PFH-1 positive neuronal-like cells, intensity of 2–4 regions of interest (ROI) within the cytoplasm, was calculated (area between 30 and 40 μm²) (de Vivo, 2010). For the intensity of PHF-1 positive dendritic-like profiles, value was extracted by plotting intensity pixel values along the major axis of profiles using Image J (Melone et al., 2019).

For light microscopy studies, hippocampus and CA1 of Congo Red and Bielschowsky stained sections were acquired at 4 × and 20 × and at 4 × and 40 × original magnifications, respectively (4–6 sections/2 animals for each experimental group). For NeuN and GFAP studies, hippocampi of immunostained sections were acquired at 4 ×, and at 40 × for quantitative studies in so, sp, sr. NeuN and GFAP positive cells were manually identified, and to estimate the density of cells, the area of microscopical fields (18 microscopical fields/layer/6 sections from 3 animals for each experimental group; with a total of 54 fields for each condition) was calculated by Image J.

2.11. Statistics

All experiments were performed by researchers blind with respect to treatment. All data were expressed as mean ± standard error mean (SEM). Statistical analysis was performed by using different tests, based on preliminary analyses of normal distribution. ANOVA for repeated measures was used to analyze PPF and LTP (120 min of recording after tetanus). One-way ANOVA with Bonferroni's post-hoc correction was used for PPF single time intervals. Two-tailed *t*-test was used for analyses of behavioral parameters, NeuN and GFAP immunohistochemistry. One sample *t*-test was used to compare D with zero in NOR and NOL. Given the non-normal distribution of data, assessed by D'Agostino & Pearson normality, we used Mann-Whitney test for ELISA and PHF-1 immunoreactivity; Mann-Whitney and Kruskal-Wallis One Way tests for WB experiments. Systat 9, Graphpad Prism 8, and Sigmaplot 14 software were used for statistical analyses. The level of significance was set at *P* < 0.05.

3. Results

3.1. Synaptic plasticity and memory are impaired in 12-month-old α7 KO mice

The role of α7nAChRs in cognitive functions (Picciotto, 2000) relies on their ability to modulate synaptic function through the regulation of glutamate release (Cheng and Yakel, 2015). Thus, we first evaluated whether their genetic deletion affected paired-pulse facilitation (PPF), a form of short-term plasticity that might reflect release probability. PPF

resulted unchanged in slices from $\alpha 7$ KO mice at 3, 6 and 9 months of age, but was increased at 12 months (M) compared to slices from age-matched WT animals (Fig. 1A). We then investigated long-term plasticity by recording LTP at hippocampal CA3-CA1 synapses. Potentiation was normal in hippocampal slices from animals at 3 M and 6 M in both genotypes (Fig. 1B). An initial deficiency of LTP was present at 9 M, even if it was less pronounced in respect to the impairment found at 12 M (Fig. 1C). Thus, we chose to conduct the rest of this study on 12 M animals.

The observation that release probability and plasticity were impaired in an age-dependent manner, prompted us to study different types of memory known to be impaired in AD (Puzzo et al., 2014a). Evaluation of contextual fear memory 24 h after training showed that the amount of freezing behavior was impaired in 12 M $\alpha 7$ KO compared to WT controls

(Fig. 1D), whereas no differences were found in amygdala-dependent cued fear memory (Fig. 1E).

We then evaluated recognition and spatial memory through Novel Object Recognition (NOR) and Novel Object Location (NOL) tasks. Analyses of Discrimination index (D = exploration of novel object minus exploration of familiar object/total exploration time) showed an impairment of memory in 12 M $\alpha 7$ KO (Fig. 1F). Comparison of D with zero confirmed that only WT mice were able to discriminate between the old and the novel object or its different spatial location. No differences were detected in total exploration time between the two groups of mice (Fig. 1G).

Open field test showed that locomotor activity and anxiety-like behavior were not affected (Fig. 1H,I), suggesting that the impairment of memory found in $\alpha 7$ KO was not due to motor or motivational defects.

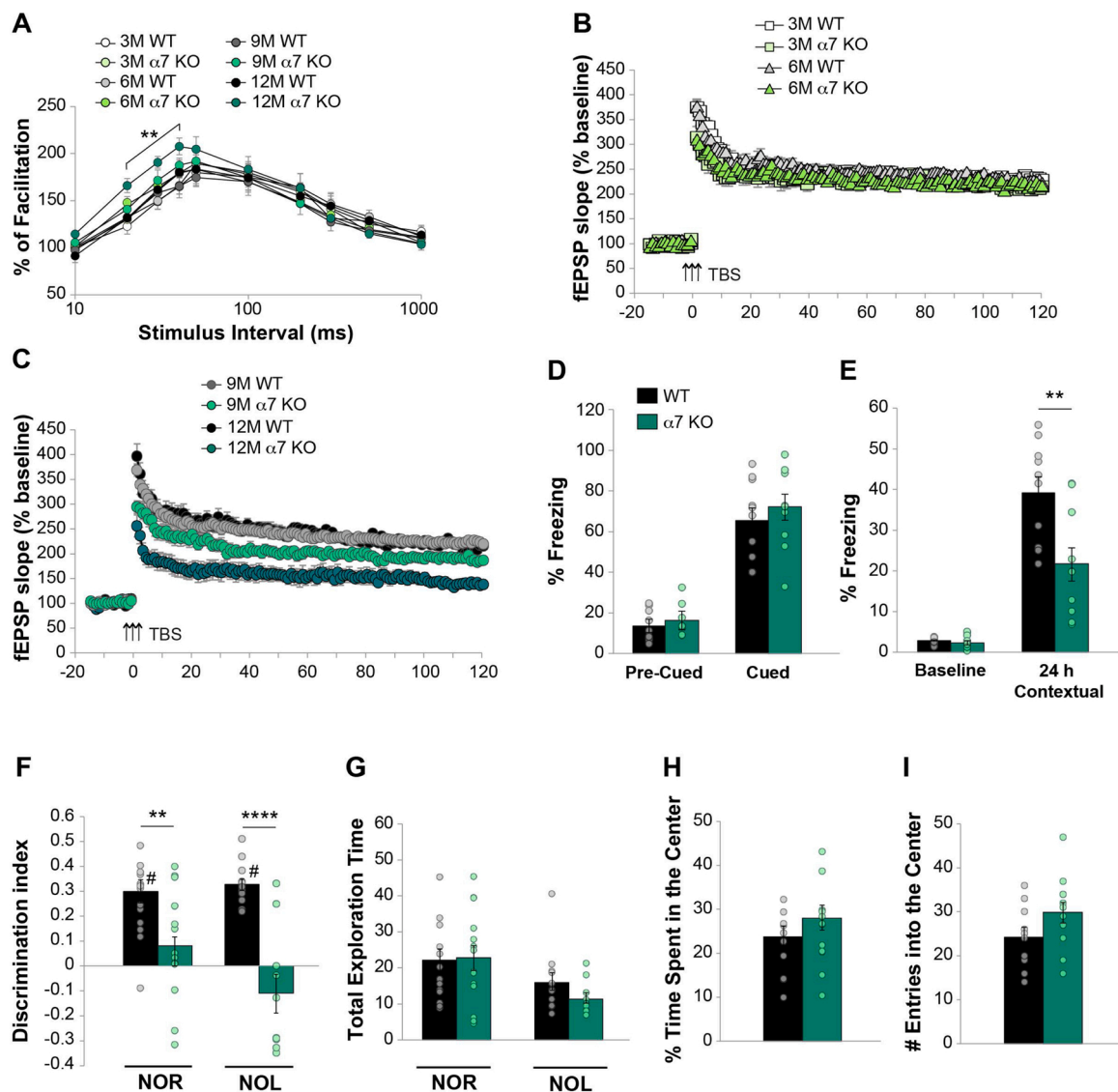


Fig. 1. Synaptic plasticity and memory are impaired in 12-month-old $\alpha 7$ KO mice. **A)** PPF changed in slices from 12 M $\alpha 7$ KO but was unaltered at other ages ($P = 0.010$ in 12 M $\alpha 7$ KO vs. WT; $N = 9$ slices from 6–7 3-month-old animals; $N = 7$ slices from 5–6 animals at other ages). **B)** LTP was normal in slices from $\alpha 7$ KO at 3 M and 6 M ($P > 0.05$ compared with age-matched WT). TBS = theta-burst stimulation. $N = 7$ slices from 5–6 animals for each condition, here and in C. **C)** LTP was impaired in slices from 12 M $\alpha 7$ KO ($F_{(1,12)} = 35.1837$, $P < 0.0001$) compared with age-matched WT. A slight impairment of LTP was present at 9 M ($F_{(1,12)} = 10.883$, $P < 0.006$ vs. 9 M WT; $F_{(1,12)} = 12.212$, $p = 0.004$ vs. 12 M $\alpha 7$ KO). **D)** Contextual fear memory was impaired in 12 M $\alpha 7$ KO mice ($t_{(18)} = 3.06$, $P = 0.007$; $N = 10/10$). **E)** No differences were detected in cued fear memory ($t_{(18)} = 0.722$, $P = 0.479$). **F)** Discrimination index (D) was impaired in 12 M $\alpha 7$ KO mice (NOR: $t_{(27)} = 2.92$, $P = 0.007$; $N = 15$ WT/12 $\alpha 7$ KO; NOL: $t_{(27)} = 5.882$, $P < 0.0001$; $N = 12$ WT/10 $\alpha 7$ KO). WT but not $\alpha 7$ KO mice were able to learn ($P < 0.0001$ D vs. zero). **G)** Total exploration time was similar among genotypes (NOR: $t_{(27)} = 0.143$, $P = 0.887$; NOL: $t_{(20)} = 0.543$, $P = 0.887$). **H–I)** No differences were present in exploratory and anxiety-like behavior tested by the open field task ($t_{(20)} = 1.081$, $P = 0.293$ for % time spent into the center; $t_{(20)} = 1.632$, $P = 0.118$ for number of entries into the center; $N = 10$ WT/12 $\alpha 7$ KO). * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, # $P \neq 0$. Data expressed as mean \pm SEM.

Thus, $\alpha 7$ KO mice showed an age-dependent impairment of hippocampal synaptic plasticity and memory.

3.2. The lack of endogenous $\alpha 7$ nAChRs induces an increase of A β production and APP expression

The impairment of synaptic plasticity and memory found in $\alpha 7$ KO mice might be due to the sole alteration of cholinergic transmission. In fact, the impairment of LTP was also present in hippocampal slices from wild type mice acutely treated with drugs inhibiting $\alpha 7$ nAChRs, such as α -BTX and MLA (Supplementary Fig. 1). However, this scenario is also compatible with the hypothesis that the cognitive phenotype is triggered by the failure of A β -mediated synaptic homeostasis. Indeed, in a situation in which A β is not able to adequately exert its physiological functions through $\alpha 7$ nAChRs, a feedback mechanism might occur, inducing a compensatory increase of A β production overtime. High levels of A β might in turn be responsible for the impairment of synaptic plasticity and memory found in $\alpha 7$ KO mice. To test this hypothesis, we evaluated whether the age-dependent damage of LTP and memory was paralleled by changes in A β production. We performed the enzyme-linked immunosorbent assay (ELISA) for mouse A β_{42} on hippocampal homogenates

from 12 M $\alpha 7$ KO and WT mice and confirmed that the deletion of $\alpha 7$ nAChRs induced a significant increase of the peptide levels (Fig. 2A). Because A β is produced by APP cleavage, we next verified whether this feedback mechanism acted through a modification of APP expression. We found an increase of APP full-length expression in 12 M $\alpha 7$ KO hippocampi (Fig. 2B,C) that was confirmed by 3 different antibodies: Y188, 4G8, and M3.2. Furthermore, 4G8 (recognizing both human and murine A β) and M3.2 (specific for murine A β) allowed detecting a 24 KDa band, presumably corresponding to soluble aggregates (i.e., pentamers), that was significantly increased in $\alpha 7$ KO hippocampi (Fig. 2D, E). However, Congo Red staining did not reveal the presence of hippocampal senile plaques (Fig. 2F).

To further confirm that the increase of A β contributed to the impairment of synaptic plasticity found in hippocampal slices from 12 M $\alpha 7$ KO mice, we investigated whether neutralization of A β by a treatment with the murine anti-A β antibody M3.2 rescued LTP. We found that M3.2 (2 μ g/mL for 20 min before tetanus) restored LTP in slices from 12 M $\alpha 7$ KO mice (Fig. 2G). Notably, the same treatment impaired LTP in slices from wild type mice (Fig. 2G).

Overall, these findings suggest that the absence of $\alpha 7$ nAChRs triggers an age-dependent increase of soluble A β and APP, which parallels the

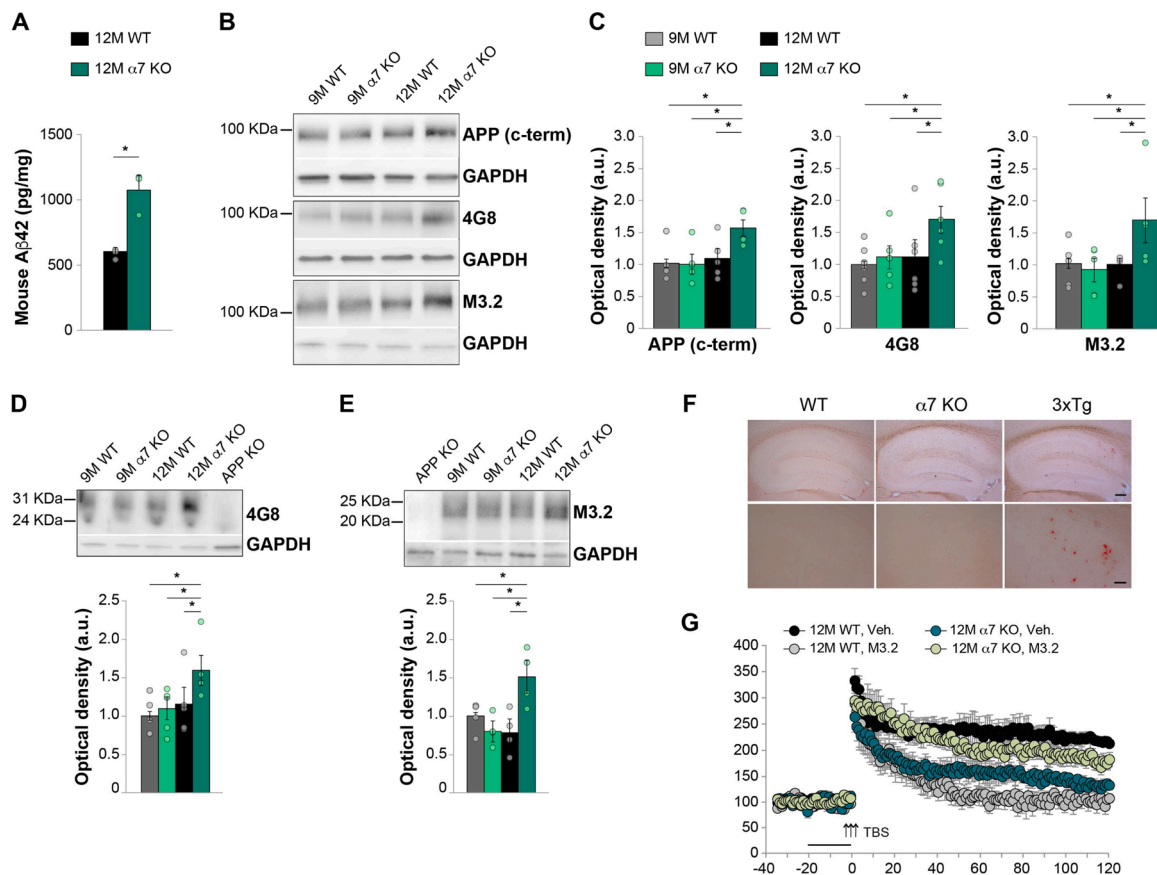


Fig. 2. A β levels and APP expression increase in 12 M $\alpha 7$ KO mice. **A)** ELISA revealed an increase of A β_{42} levels in hippocampal homogenates from $\alpha 7$ KO compared with WT mice (Mann-Whitney Rank Sum Test, $P = 0.036$, $N = 3$ and 4 animals, respectively). **B)** Western blot analysis comparing the expression of APP in hippocampi from WT and $\alpha 7$ KO animals at 9 M and 12 M assessed by 3 different antibodies, i.e. 4G8, Y188 and M3.2. **C)** Bar graphs showing the results of the densitometric analysis of the Western blots reported in (B) indicated a significant increase of APP expression in hippocampi from 12 M $\alpha 7$ KO mice compared to WT (Kruskal-Wallis One Way Analysis of Variance on Ranks, $P = 0.038$ for Y188, $N = 5$ for each condition; $P = 0.040$ for 4G8, $N = 6$ for each condition and $P = 0.046$ for M3.2; $N = 5$ for each condition). GAPDH expression level was used as loading control here, in D and E. **D)** An increase of a ≈ 25 kDa band, presumably representing A β oligomers, was detected in 12 M $\alpha 7$ KO hippocampi either when using the 4G8 (Kruskal-Wallis One Way Analysis of Variance on Ranks, $P = 0.035$, $N = 5$ for each condition) or **E)** the M3.2 antibody, specific for murine A β (Kruskal-Wallis One Way Analysis of Variance on Ranks; $P = 0.030$, $N = 4$ for each condition). APP KO mice were used as negative controls both in D and E. **F)** No plaques were detected using Congo red staining in $\alpha 7$ KO hippocampal slices. Hippocampal slices from 3xTg mice were used as a positive control. Upper panels: 4 \times magnification, scale bar 100 μ m; Lower panels: 20 \times magnification, scale bar 50 μ m. **G)** The anti-A β antibody M3.2 rescued LTP in 12 M $\alpha 7$ KO hippocampal slices ($F_{(1,12)} = 7.063$, $p = 0.021$ vs. $\alpha 7$ KO vehicle-treated slices; $N = 6/8$ from 5-6 animals), whereas it impaired LTP in WT slices ($F_{(1,11)} = 21.730$, $p = 0.001$ vs. WT vehicle-treated slices; $N = 7/6$ from 5-5 animals). TBS = theta-burst stimulation. * $P < 0.05$. Data expressed as mean \pm SEM.

impairment of synaptic plasticity and memory, with no plaque formation.

3.3. The lack of endogenous $\alpha 7$ nAChRs triggers tau hyperphosphorylation through GSK-3 β modulation

Tau hyperphosphorylation and accumulation of paired helical filaments (PHF) leading to NFTs formation represent hallmarks of AD brain lesion highly correlated with cognitive impairment (Iqbal et al., 2005). Here, we first investigated whether $\alpha 7$ nAChRs deletion modified tau phosphorylation at different residues known to be associated with neurodegeneration and AD, i.e., Ser 199, Ser 396, and Thr 205 (De Chiara et al., 2019; Neddens et al., 2018). Western blotting analysis showed a significant increase of pTau expression at Ser 199 and Ser 396 (Fig. 3A), and Thr 205 (Fig. 3B) in hippocampi from 12 M $\alpha 7$ KO mice compared to WT. In a series of complementary experiments, we found that pTau (Ser 199 and Thr 205) was not modified in hippocampi from 9 M $\alpha 7$ KO mice compared with age-matched WT (Supplementary Fig. 2).

We then turned our attention onto glycogen synthase kinase-3 β (GSK-3 β), considered a crucial molecule in AD, being a possible molecular link between A β and tau pathology (Llorens-Martín et al., 2014). In particular, we focused on GSK-3 β auto-inhibitory phosphorylation site on Ser 9 whose dysregulation induces an abnormal activation of GSK-3 β leading, in turn, to tau hyperphosphorylation (Hanger and Noble, 2011). We found a decrease in Ser 9 phosphorylation of GSK-3 β

in hippocampi from $\alpha 7$ KO mice (Fig. 3C) that paralleled the increase of tau phosphorylation.

3.4. The lack of endogenous $\alpha 7$ nAChRs causes tau accumulation and deposition in neurofibrillary tangles

We then investigated the presence of PHFs in hippocampal slices, suggestive for a more advanced stage of pathology. We used a PHF-1 antibody (a generous gift of Dr. Peter Davies) that detects Ser396/Ser404 phosphorylation sites, known to be associated with NFT formation (Götz et al., 2001).

Confocal microscopy revealed an increased PHF-1 immunoreactivity (IR) in the CA1 area of 12 M $\alpha 7$ KO hippocampi compared to WT (Fig. 4A), particularly evident in the CA1 *stratum pyramidale* and *stratum radiatum* (Fig. 4B). Analyses of PHF-1 IR revealed an increase of mean size positive puncta in the *stratum oriens*, neuronal-like cells in the *stratum pyramidale*, and dendritic-like profiles in the *stratum radiatum* (Fig. 4C-E). Consistently, Bielschowsky silver staining detected an increase of intraneuronal NFTs in the hippocampus and neocortex from $\alpha 7$ KO (Fig. 4F).

Overall, these data indicated that $\alpha 7$ KO mice present a dysregulation of tau phosphorylation resulting in an increase of PHFs and NFTs.

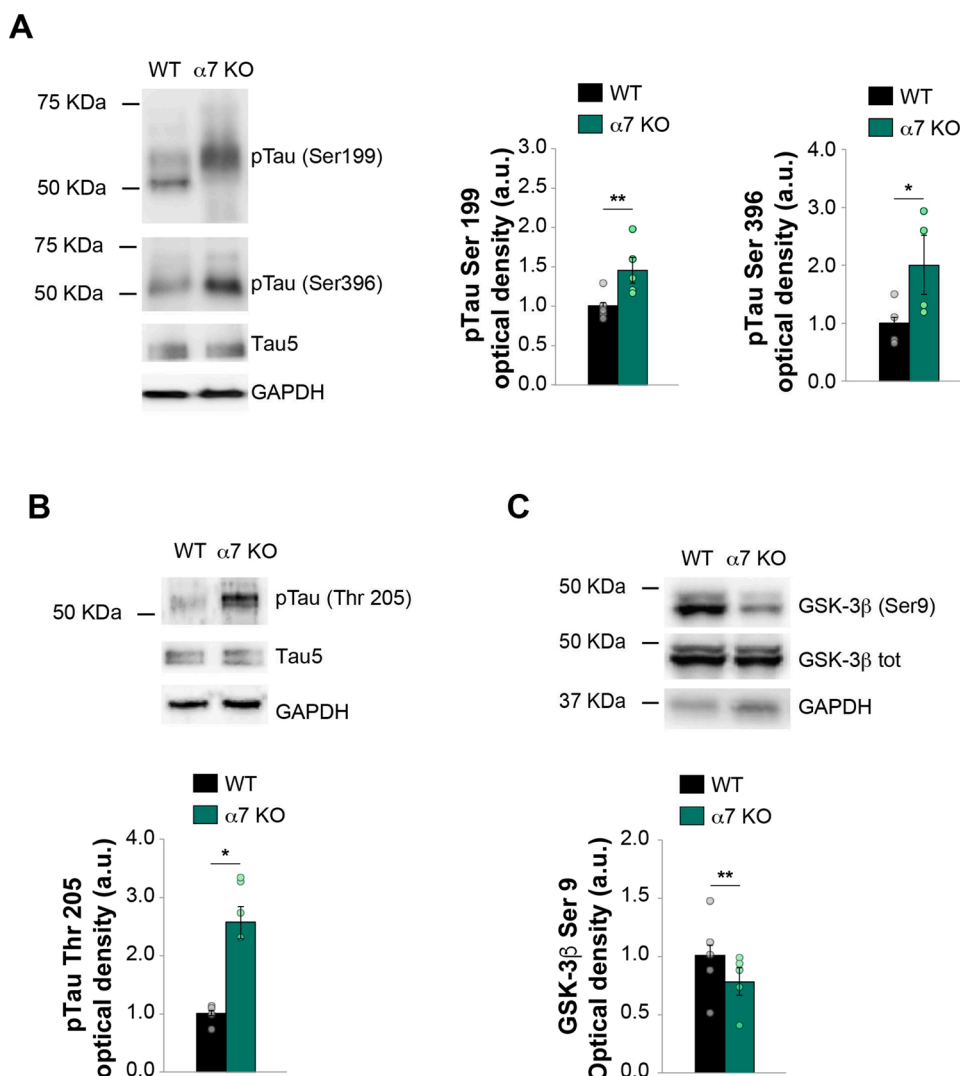


Fig. 3. Tau is hyperphosphorylated and GSK-3 β dysregulated in hippocampi from 12 M $\alpha 7$ KO mice. A) Representative images of WB assay (cropped images based on MW here and in the following panels) for the expression of phosphorylated tau (pTau) at Ser 199 and at Ser 396 performed on hippocampi from 12 M WT and $\alpha 7$ KO mice. On the right, bar graphs showing the increase of pTau Ser 199 and Ser 396 expression in hippocampi from $\alpha 7$ KO compared to WT (Mann-Whitney Rank Sum Test for pTau Ser 199: P = 0.008; N = 5/5; for pTau Ser 396: P = 0.028; N = 4/4). Tau5, specific for murine tau, was used to normalize pTau densitometric signal from WT and $\alpha 7$ KO tissues. GAPDH was used as loading control. B) WB assay for the expression of pTau at Thr 205. Lower panel, bar graph showing the increase of the expression of pTau at Thr 205 in hippocampi of $\alpha 7$ KO compared to WT (Mann-Whitney Rank Sum Test, P = 0.028; N = 4/4). C) WB images showing the expression of GSK-3 β phosphorylated at Ser 9, total GSK-3 β and GAPDH as loading control. Lower panel, bar graph showing the decrease of GSK-3 β Ser 9 in $\alpha 7$ KO hippocampi compared to WT (Mann-Whitney Rank Sum Test; P = 0.007; N = 5/5). *P < 0.05, **P < 0.01. Data expressed as mean \pm SEM.

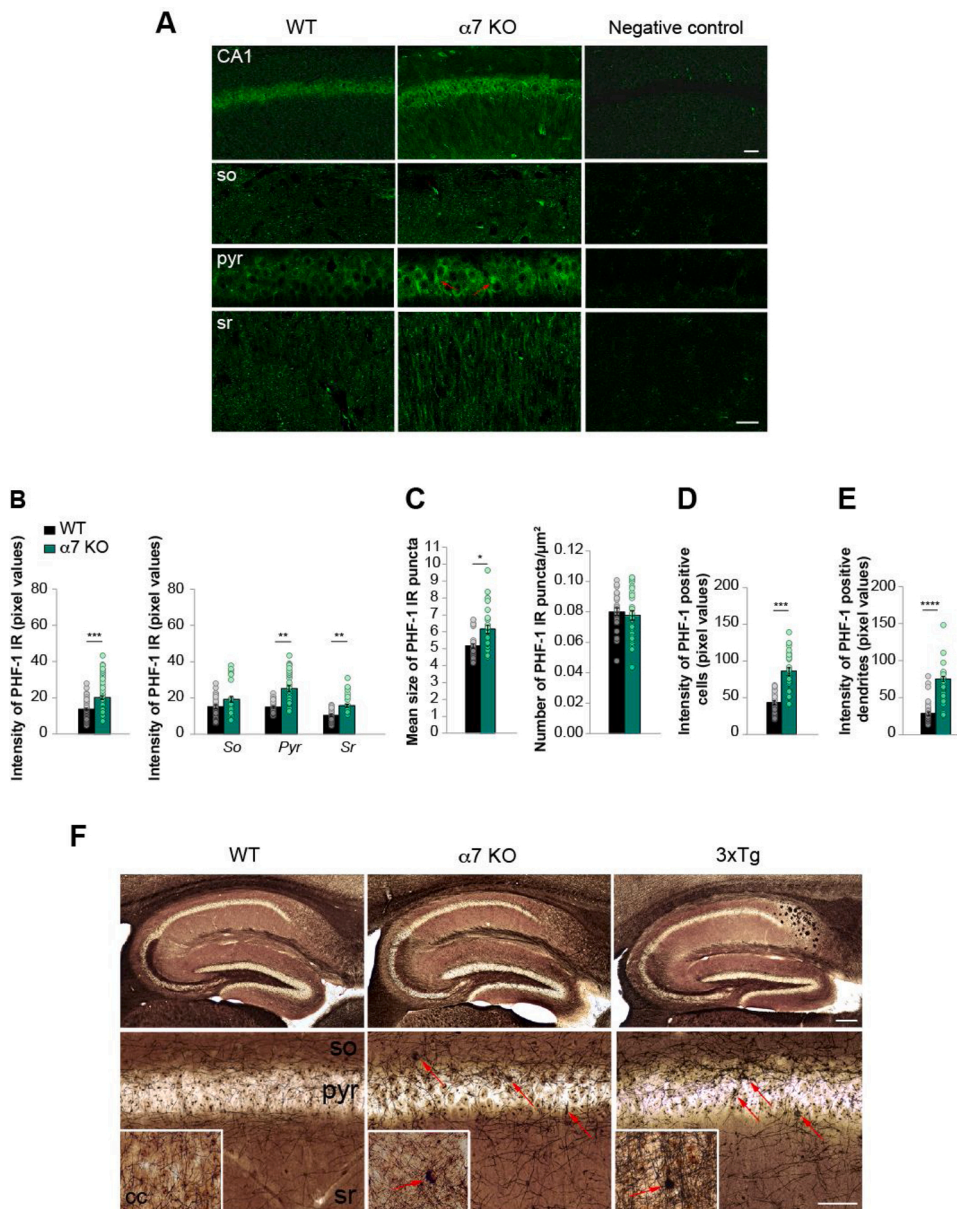


Fig. 4. PHFs immunoreactivity and neurofibrillary tangles in hippocampi from $\alpha 7$ KO mice. A) Representative confocal images of PHF-1 immunofluorescence in the hippocampus from 12–15 M WT and $\alpha 7$ KO mice. Negative control represents WT sections treated with the same immunofluorescence protocol, omitting the primary antibody. Upper panels: CA1 area, 20 \times , scale bar 50 μ m; Lower panels: stratum oriens (so), stratum pyramidale (pyr) and stratum radiatum (sr), 60 \times , scale bar 25 μ m. Red arrows indicate intraneuronal accumulations. B) Bar graphs showing the increase of PHF-1 immunoreactivity (IR) in hippocampi from $\alpha 7$ KO (Mann-Whitney test $P = 0.0002$). On the right, analyses of PHF-1 IR in so, pyr and sr. C) Bar graphs showing puncta staining analyses in so. Positive puncta mean size increased in $\alpha 7$ KO ($P = 0.0113$), whereas number of puncta was not modified ($P > 0.99$). D) Bar graphs showing analyses of cytoplasmic PHF-1 IR intensity in the pyr. Positive cells increased in $\alpha 7$ KO ($P = 0.0002$). E) Bar graphs showing an increase of PHF-1 IR intensity in apical dendrites of the sr in $\alpha 7$ KO ($P < 0.0001$). $N = 32$ subfields (4 sections from 2 animals/genotype). F) Bielschowsky silver staining showed the presence of silver-positive NFTs in $\alpha 7$ KO. Hippocampi from 3 \times Tg mice were used as positive control. Representative inserts showing a clear identifiable NFT in $\alpha 7$ KO and 3 \times Tg cerebral cortex (cc). Red arrows indicate intraneuronal accumulation. Upper panels: 4 \times , scale bar 100 μ m; Lower panels: CA1 area, 40 \times , scale bar 50 μ m; Inserts: 40 \times , scale bar 50 μ m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data expressed as mean \pm SEM.

3.5. The lack of endogenous $\alpha 7$ nAChRs induces neuronal loss and increases GFAP-positive astrocytes

Besides A β and tau pathology, because hippocampal neuronal loss highly correlates with cognitive deficits and AD progression (Donev et al., 2009), we evaluated whether $\alpha 7$ KO mice presented neuronal depletion. We found a decrease in the mean number of NeuN positive cells in hippocampal slices from $\alpha 7$ KO compared to WT controls (Fig. 5A). A statistically significant decrease was found in the stratum pyramidale and stratum radiatum, whereas no differences were detected in the stratum oriens (Fig. 5A).

Finally, because either the increase of A β and tau as well as the loss of neuronal function influence astrocytes (González-Reyes et al., 2017; Phatnani and Maniatis, 2015), we evaluated GFAP (Glial Fibrillary Acidic Protein) positive astrocytes. Quantification of GFAP-positive cells revealed an increase that was significant in all the hippocampal strata, i. e. oriens, pyramidale and radiatum, in hippocampi from $\alpha 7$ KO compared to WT mice (Fig. 5B).

Taken together, these findings suggest that $\alpha 7$ KO present a decrease of neurons and an increase of astrocytes in the hippocampus.

4. Discussion

In this work we showed that genetic deletion of $\alpha 7$ nAChRs is sufficient to induce an AD-like pathology characterized by synaptic plasticity and memory impairment, A β and tau neuropathology, neuronal loss, and increase of GFAP-positive astrocytes.

We used the $\alpha 7$ KO mouse model that does not overproduce A β due to manipulations of genes directly involved in its production (i.e., APP or presenilins). This allowed us to avoid one of the main limitations of research in the AD field, which is the use of models inspired by rare forms of inherited early onset Familial Alzheimer's disease (FAD) characterized by a genetic-driven rise of A β production (Hardy et al., 1998). Indeed, FAD only accounts for the 2–3 % of AD cases (Qiu et al., 2009), whereas the prevalent form of dementia is sporadic AD, which affects the elderly and it is not associated with genetic mutations directly leading to an increase of A β burden (Herrup, 2015).

We first focused on synaptic plasticity, whose disruption is thought to be the early pathogenetic event in AD (Selkoe, 2002). $\alpha 7$ KO mice presented an age-dependent impairment of short- and long-term plasticity, as indicated by the increase of PPF and the reduction of LTP,

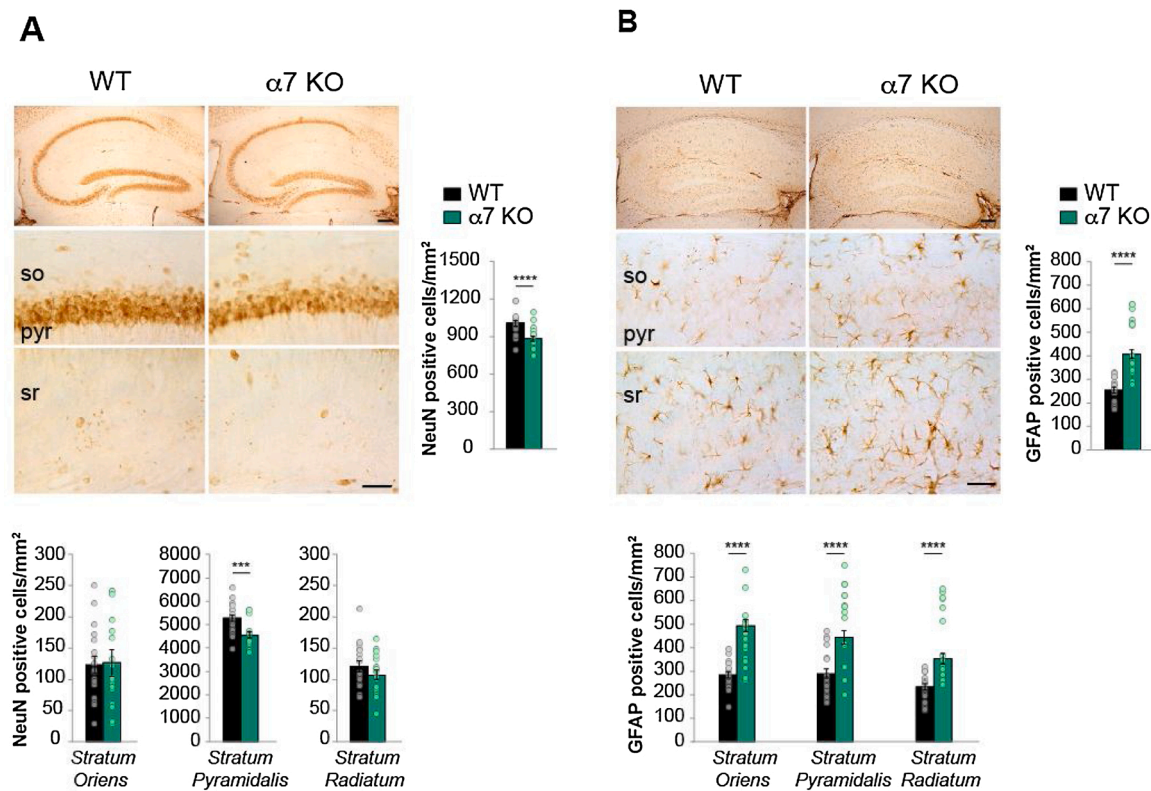


Fig. 5. Neuronal loss and increase of astrocytes in $\alpha 7$ KO hippocampi. **A)** Representative images of NeuN staining in the hippocampal formation of 12–15 M WT and $\alpha 7$ KO mice. Upper panels: 4 \times , scale bar 100 μ m; Middle panels: stratum oriens (so) and stratum pyramidalis (pyr) of CA1 area, 40 \times ; Lower panels: stratum radiatum (sr), 40 \times , scale bar 50 μ m. On the right, bar graph showing an increase of neuronal loss in hippocampi from $\alpha 7$ KO ($t_{(34)} = 3.938$; $P < 0.0001$). On the bottom, analyses of CA1 layers evidenced a significant loss of neurons in the stratum pyramidalis of $\alpha 7$ KO hippocampi ($t_{(34)} = 3.617$; $P = 0.001$). No differences were detected in stratum radiatum and stratum oriens. $N = 6$ sections from 3 animals/genotype. **B)** Representative images of GFAP staining in the hippocampus of 12–15 M WT and $\alpha 7$ KO mice. Upper panels: 4 \times , scale bar 100 μ m; Middle panels: so and pyr of CA1 area, 40 \times ; Lower panels: sr, 40 \times , scale bar 50 μ m. On the right, bar graph showing an increase of astrocytes in the three layers: stratum oriens ($t_{(34)} = 4.786$; $P < 0.0001$), stratum pyramidalis ($t_{(34)} = 5.874$; $P < 0.0001$). On the bottom, analyses of CA1 layers showed an increase of astrocytes in the three layers: stratum oriens ($t_{(34)} = 4.786$; $P < 0.0001$), stratum pyramidalis ($t_{(34)} = 4.621$; $P < 0.0001$), and stratum radiatum ($t_{(34)} = 4.321$; $P < 0.0001$). $N = 6$ sections from 3 animals/genotype. *** $P < 0.001$, **** $P < 0.0001$. Data expressed as mean \pm SEM.

resulting in memory loss. Previous studies already showed that the deletion of $\alpha 7$ nAChRs caused synaptic plasticity deficits, even if at a later age (22–24 months) with respect to our study (Ma et al., 2014). Our results are consistent with data indicating that, at physiological concentrations, A β requires $\alpha 7$ nAChRs to sustain synaptic functions (Lawrence et al., 2014; Mura et al., 2012; Puzzo et al., 2011, 2008), both at pre-synaptic level, where it enhances release probability (Gulisano et al., 2019; Koppensteiner et al., 2016; Lazarevic et al., 2017), and at post-synaptic level, where it is needed for long-lasting LTP and memory formation (Garcia-Osta and Alberini, 2009; Gulisano et al., 2019; Morley et al., 2010; Palmeri et al., 2017; Puzzo et al., 2011).

$\alpha 7$ nAChRs mediate the raise of pre-synaptic intracellular Ca^{2+} levels during neuronal activity, thus modulating glutamate release, synaptic transmission, and cognitive function (Picciotto, 2000). Hence, it was crucial to exclude that the observed $\alpha 7$ KO phenotype was exclusively due to the failure of cholinergic transmission, as it occurred after an acute pharmacological inhibition of $\alpha 7$ nAChRs [for a review see (Yakel, 2014)]. Our finding that the impairment of synaptic plasticity and memory is paralleled by the increase of APP expression and A β levels suggests that the loss of $\alpha 7$ nAChRs might trigger a chain of events through a negative feedback mechanism aimed at restoring calcium entry inside neurons by stimulating A β production. This was further confirmed by the rescue of LTP in slices from $\alpha 7$ KO mice treated with the anti-A β antibody M3.2. Notably, treatment with the same antibody impaired LTP in slices from wild type mice, as previously demonstrated (Garcia-Osta and Alberini, 2009; Morley et al., 2010; Puzzo et al., 2011), confirming that caution is needed when proposing treatments aimed at

decreasing A β levels, since the protein is physiologically needed to ensure synaptic function.

Our hypothesis that $\alpha 7$ nAChRs deletion drives A β elevation is supported by extensive literature demonstrating a tight link between $\alpha 7$ nAChRs and A β (Oz et al., 2013). A β and $\alpha 7$ nAChRs co-localized inside neurons or at extracellular level in senile plaques (Nagele et al., 2002; Wevers et al., 1999) and high A β levels induced $\alpha 7$ nAChR alterations (Dineley et al., 2002, 2001; Dougherty et al., 2003; Li et al., 2011; Liu et al., 2001). The lack of $\alpha 7$ nAChRs accelerated the pathology in the Tg2576 mouse model of AD ensuing decrease of hippocampal ChAT activity paralleled by a pronounced loss of pyramidal neurons (Hernandez et al., 2010). In 3 \times Tg mice a reduction of $\alpha 7$ nAChRs was found in the same brain regions where intraneuronal A β 42 accumulation occurred, determining cognitive deficits (Oddo et al., 2005). Interestingly, an increase of $\alpha 7$ nAChR-specific antibodies, able to induce A β accumulation and memory impairment in animal models (Lykhmus et al., 2015), has been found in plasma samples of early-onset AD patients (Koval et al., 2011).

About the mechanisms underlying $\alpha 7$ nAChR regulation of A β production, few studies focused on the ability of $\alpha 7$ nAChRs activation to reduce A β synthesis by shifting APP processing towards the non-amyloidogenic pathway (Mousavi and Hellström-Lindahl, 2009; Nie et al., 2010; Qi et al., 2007), for example by regulating γ -secretase activity and expression (Nie et al., 2010). This enhanced the production of soluble APP α (sAPP α) recognized for its neuroplasticity and neuro-protective functions able to counteract A β neurotoxicity [for reviews (Buckingham et al., 2009; Hefter et al., 2020)]. Hence, the deletion of

$\alpha 7$ nAChRs might determine an aberrant APP processing leading to A β elevation. However, we cannot exclude that the increase of APP expression found in aged $\alpha 7$ KO mice was due to different mechanisms not involving a direct interaction between $\alpha 7$ nAChRs and APP. In fact, since the lack of $\alpha 7$ nAChRs prevents A β to exert its physiological function, this might cause the unbalance of several neuroplasticity pathways (Gulisano et al., 2019) that, in turn, are known to influence APP expression and processing. See for example how cGMP levels regulate A β production during LTP by acting on BACE-1/APP approximation (Palmeri et al., 2017).

The increase of APP we found in $\alpha 7$ KO mice raises another series of considerations. Its occurrence in adult age might explain why $\alpha 7$ KO mice do not present a peculiar phenotype until the age of 12 months. Even if it is beyond the scope of this work, we can speculate that in an initial phase other nAChR subtypes, i.e., $\alpha 4\beta 2$ nAChRs, might compensate for the absence of $\alpha 7$ nAChRs, a common phenomenon in genetically modified animal models. Moreover, it is conceivable that homeostatic changes occur overtime until a critical stage, when compensation is no longer possible. This trend mirrors the course of the disease since synaptic disruption is thought to begin long before the clinical manifestation. Nevertheless, even if in $\alpha 7$ KO mice the increase of APP is aimed at restoring A β function, it eventually leads to a vicious cycle in which A β levels reach high concentrations becoming extremely neurotoxic.

In this context, APP might exert a double role as it acts as A β precursor and cell surface receptor (Deyts et al., 2016) able to bind A β and tau (Fogel et al., 2014; Lorenzo et al., 2000; Shaked et al., 2006; Takahashi et al., 2015; Van Nostrand et al., 2002). APP enhances tau phosphorylation (Greenberg et al., 1994) and serves as a common target for extracellular oligomers of A β and tau to enter neurons and impair LTP and memory (Puzzo et al., 2017; Wang et al., 2017). Hence, increased APP expression might contribute to worsening the course of the disease with different mechanisms.

As for the interplay between $\alpha 7$ nAChRs and tau in AD, results are conflicting (Rubio et al., 2006). Although some works have shown that the increase of $\alpha 7$ nAChRs stimulates tau phosphorylation (Ren et al., 2007; Wang et al., 2003), most studies showed that a reduction of $\alpha 7$ nAChRs is concomitant with tau hyperphosphorylation in brains of AD patients or animal models (Wu et al., 2010). Here, we found that $\alpha 7$ nAChRs deletion induced an increased age-dependent expression of tau phosphorylated at Thr 205, Ser 199 and Ser 396, residues known to be involved in AD onset and progression. In particular, pTau at Thr 205 seemed to be involved in tau spreading, as demonstrated in Tg/hTau mice injected with tau (Miao et al., 2019) and, together with pTau at Ser 199, has been correlated with Braak stage V/VI in patients (Neddens et al., 2018). As for pTau at Ser 396, it is considered a key marker of tau hyperphosphorylation since it increases in CA1 pyramidal neurons of AD patients and is crucial for PHFs formation (Furcila et al., 2018; Mondragón-Rodríguez et al., 2014). Consistently, here we found an increase of its expression by WB and PHF-1 immunoreactivity at hippocampal level.

These modifications of tau phosphorylation were accompanied by a concomitant decrease of GSK-3 β phosphorylated at Ser 9, a residue involved in the auto-inhibitory regulation of the kinase. In line with our results, $\alpha 7$ nAChR agonists reduced tau phosphorylation *in vitro* and *in vivo* by increasing GSK-3 β activity in mouse models of AD and hypothermia-induced tau hyperphosphorylation (Bitner et al., 2009; Hu et al., 2008), effects that are reversed by selective $\alpha 7$ nAChR antagonists (Hu et al., 2008).

Another finding of our study is that $\alpha 7$ KO mice exhibited an increase of APP and A β levels but no senile plaques. This is in line with several studies demonstrating that soluble oligomers increase in the early stages of AD and appear more toxic than insoluble aggregates [for a review see (Selkoe and Hardy, 2016)]. Consistently, in animal models, low molecular weight A β oligomers (dimers), even though unable to initiate plaque formation (Müller-Schiffmann et al., 2016), induce synaptic dysfunction and trigger the AD cascade (Kawarabayashi, 2004; Mc

Donald et al., 2015, 2010; Shankar et al., 2008). Studies performed on the arcA β mice, carrying the Swedish and the Arctic mutations, confirmed that insoluble A β deposits are not needed to initiate the cascade of events leading to AD as the impairment of synaptic plasticity and memory occurs before plaques formation (Knobloch et al., 2007). However, we cannot exclude that in our study the absence of plaques might be due to a lower propensity of murine A β to form insoluble deposits, as evidenced in previous studies (Puzzo et al., 2015a,b, 2014b).

The controversial role of A β deposition and its poor correlation with AD symptoms is also supported by several observations in humans showing that AD patients can manifest dementia without A β deposits and, conversely, plaques might be present in cognitively intact elderly subjects (Arriagada et al., 1992; Chételat et al., 2013; Delaère et al., 1990; Driscoll et al., 2006; Iacono et al., 2009; Katzman et al., 1988; Sloane et al., 1997; Zolochovska and Taglialatela, 2016).

On the contrary, tau hyperphosphorylation leading to PHFs and NFTs formation is highly related to cognitive impairment in AD (Nelson et al., 2012), as it also contributes to functional and structural alterations of pyramidal neurons (Merino-Serrais et al., 2011). In this manuscript we have shown that $\alpha 7$ KO mice presented all these tau-related pathologic signs starting at 12 months of age, concomitant to the increase of APP expression and A β levels. In this scenario, the initial impairment of LTP found in 9 M $\alpha 7$ KO animals might be interpreted as the result of the failure of A β physiological function that, when no longer compensated, would trigger the chain of events leading to the AD-like pathology at later age.

Dementia has also been strongly correlated with the degree of neuronal loss especially in the hippocampus and neocortex in humans (Donev et al., 2009). However, AD mouse models do not always mimic this aspect of the disease (Wirths and Bayer, 2010). Here, NeuN experiments have shown a reduction of neuronal number in hippocampi from $\alpha 7$ KO mice. This might be, in part, independent from the neurotoxic effect exerted by A β and/or tau but due to the lack of $\alpha 7$ nAChRs in view of their neuroprotective and trophic role. A variety of evidences reported that stimulation of $\alpha 7$ nAChRs protected against glutamate neurotoxicity, oxygen and glucose deprivation, neuronal ischemic damage, and neuronal apoptosis [for reviews see (Buckingham et al., 2009; Kume and Takada-Takatori, 2018)], suggesting a general pro-survival function. Notably, $\alpha 7$ nAChR-mediated neuroprotection might be mediated by a direct effect on neuronal cells or an indirect action through glial cells. In fact, cultured astrocytes treated with $\alpha 7$ nAChR agonists showed a significant reduction of inflammatory cytokines secretion, accompanied by a decrease in neuronal apoptosis (Patel et al., 2017).

Finally, we have documented an increase of GFAP-positive astrocytes in $\alpha 7$ KO hippocampi, in line with previous reports obtained in AD models (Ceyzériat et al., 2018; González-Reyes et al., 2017). Astrocytes have a well-recognized role in modulating synaptic function by uptaking/releasing several gliotransmitters (including glutamate) and regulating the levels of neurotransmitters released from presynaptic terminals as well as glutamate receptor activity [for a review see (Malarkey and Parpura, 2008)]. They overexpress β -secretases and stimulate A β production (Rossner et al., 2005) but also participate in A β clearance in physiological conditions (Mulder et al., 2012; Ries and Sastre, 2016). Furthermore, they are involved in the activation of intracellular signaling leading to tau hyperphosphorylation (Chiarini et al., 2017). Several reports have also highlighted a crosstalk between astrocytes and the cholinergic system (Pirttimäki et al., 2013). Septo-hippocampal lesions of cholinergic fibers induce astrocytosis and β -secretase overexpression (Hartlage-Rübsamen et al., 2003), whereas activation of $\alpha 7$ nAChRs by physiological and pathological concentrations of A β triggers Ca²⁺ elevations and glutamate release from astrocytes (Lee et al., 2014; Pirttimäki et al., 2013), thereby playing a crucial role in regulating neuronal activity and plasticity (Bazargani and Attwell, 2016). Here, the increase of GFAP-positive cells suggests an astrocyte activation/remodeling (Escartin et al., 2021) that might contribute to synaptic

dysfunction, even if further studies are needed to better investigate the intricate interplay among A β , tau and α 7nAChRs at the tripartite synapse.

In conclusion, here we have demonstrated that α 7nAChRs malfunction might be upstream the increase of A β and tau in the cascade of events leading to AD, supporting the hypothesis that if A β lacks its endogenous receptor, a negative feedback mechanism is triggered to overcome the failure of its physiological function. Even if AD is a multifactorial disease and α 7nAChRs malfunction might not be the only etiopathological factor, our findings contribute to understand why ACh-tailored therapies have a time-limited efficacy. In fact, the increase of ACh in the synaptic cleft by cholinesterase inhibitors or the use of AChR agonists might counteract the disease only for a brief period of time after which they might even be responsible for an exhaustion of the cholinergic system. Most importantly, considering the plethora of evidence supporting the importance of A β in synaptic function, anti-A β therapies might represent a paradox. In fact, they aim at decreasing the level of a physiological protein whose increase might have a compensatory significance. To summarize with a provocative but enlightening conceptual comparison, it would be as administering anti-insulin drugs in type II diabetes, where hyperinsulinemia is the mere consequence of a compensatory mechanism aimed at counterbalancing the lack of its function caused by receptors resistance.

Even if further studies are needed to better delineate A β , tau and α 7nAChRs crosstalk, our data suggest that the role of A β in AD needs to be reassessed, taking into account mechanisms underlying the transition from physiology to pathology to ensure a novel, safe and rational approach to patients. To this end, α 7 KO mice might represent an interesting model to evaluate the cascade of events leading to the increase of A β without exploiting FAD human genes.

Author contributions

M.R.T. contributed to conceptualization, behavioral and imaging studies, writing of the original draft; D.D.L.P. performed western blot experiments and contributed to the writing of the manuscript; M.M. contributed to conceptualization, execution and supervision of imaging studies; W.G. contributed to conceptualization, electrophysiological experiments, writing of the original draft; O.A. contributed to the conceptualization, writing, review and editing of the manuscript; CG and FC contributed to the conceptualization, formal analysis of the data, writing, review and editing of the manuscript and provided resources; DP contributed to the conceptualization, formal analysis, visualization, writing of the original draft, supervised the whole work, administered the project. All authors discussed results and commented on the manuscript.

Funding

This work was supported by Alzheimer's Association IIRG-09-134220 and University of Catania (Project Piaceri, Linea 2) (D.P.), Università Politecnica delle Marche (PSA PJ040046_2018) (F.C.), R01-AG034248 (O.A.).

Declaration of Competing Interest

The authors declare no competing financial interests.

Acknowledgements

We thank Peter Davies (Albert Einstein College of Medicine, Bronx, NY, USA) for providing the PHF-1 antibody.

Appendix A. Supplementary data

The Peer Review Overview and Supplementary data associated with

this article can be found in the online version, at doi:<https://doi.org/10.1016/j.pneurobio.2021.102154>.

References

- Anand, P., Singh, B., 2013. A review on cholinesterase inhibitors for Alzheimer's disease. *Arch. Pharm. Res.* <https://doi.org/10.1007/s12272-013-0036-3>.
- Appel, S.H., 1981. A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer disease. *Ann. Neurol.* 10, 499–505. <https://doi.org/10.1002/ana.410100602>.
- Arriagada, P.V., Growdon, J.H., Hedleywhyte, E.T., Hyman, B.T., 1992. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimers-disease. *Neurology* 42, 631–639. <https://doi.org/10.1212/WNL.42.3.631>.
- Bazargani, N., Attwell, D., 2016. Astrocyte calcium signaling: the third wave. *Nat. Neurosci.* <https://doi.org/10.1038/nn.4201>.
- Bitner, R.S., Nikkel, A.L., Markosyan, S., Otte, S., Puttfarcken, P., Gopalakrishnan, M., 2009. Selective α 7 nicotinic acetylcholine receptor activation regulates glycogen synthase kinase3 β and decreases tau phosphorylation in vivo. *Brain Res.* <https://doi.org/10.1016/j.brainres.2009.01.069>.
- Bozdagi, O., Shan, W., Tanaka, H., Benson, D.L., Huntley, G.W., 2000. Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. *Neuron* 28, 245–259. [https://doi.org/10.1016/S0896-6273\(00\)00100-8](https://doi.org/10.1016/S0896-6273(00)00100-8).
- Bragina, L., Melone, M., Fattorini, G., Torres-Ramos, M., Vallejo-Illarramendi, A., Matute, C., Conti, F., 2006. GLT-1 down-regulation induced by clozapine in rat frontal cortex is associated with synaptophysin up-regulation. *J. Neurochem.* 99, 134–141. <https://doi.org/10.1111/j.1471-4159.2006.04030.x>.
- Buckingham, S.D., Jones, A.K., Brown, L.A., Sattelle, D.B., 2009. Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection. *Pharmacol. Rev.* 61, 39–61. <https://doi.org/10.1124/pr.108.000562>.
- Burghaus, L., Schütz, U., Krempel, U., de Vos, R.A., Jansen Steur, E.N.H., Wevers, A., Lindstrom, J., Schröder, H., 2000. Quantitative assessment of nicotinic acetylcholine receptor proteins in the cerebral cortex of Alzheimer patients. *Mol. Brain Res.* 76, 385–388. [https://doi.org/10.1016/S0169-328X\(00\)00031-0](https://doi.org/10.1016/S0169-328X(00)00031-0).
- Ceyzeriat, K., Ben Haim, L., Denizot, A., Pommier, D., Matos, M., Guillemaud, O., Palomares, M.A., Abjean, L., Petit, F., Gipechtein, P., Gaillard, M.C., Guillemier, M., Bernier, S., Gaudin, M., Aurégan, G., Joséphine, C., Déchamps, N., Veran, J., Langlais, V., Cambon, K., Bemelmans, A.P., Baijer, J., Bonvento, G., Dhenain, M., Deleuze, J.F., Olié, S.H.R., Brouillet, E., Hantraye, P., Carrillo de Sauvage, M.A., Olasso, R., Panatier, A., Escartin, C., 2018. Modulation of astrocyte reactivity improves functional deficits in mouse models of Alzheimer's disease. *Acta Neuropathol. Commun.* <https://doi.org/10.1186/s40478-018-0606-1>.
- Cheng, Q., Yakel, J.L., 2015. The effect of α 7 nicotinic receptor activation on glutamatergic transmission in the hippocampus. *Biochem. Pharmacol.* 97, 439–444. <https://doi.org/10.1016/j.bcp.2015.07.015>.
- Chételat, G., La Joie, R., Villain, N., Perrotin, A., De La Sayette, V., Eustache, F., Vandenbergh, R., 2013. Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. *Neuroimage Clin.* <https://doi.org/10.1016/j.nicl.2013.02.006>.
- Chiarini, A., Armato, U., Gardenal, E., Gui, L., Dal Prà, I., 2017. Amyloid β -exposed human astrocytes overproduce phospho-tau and overrelease it within exosomes, effects suppressed by calcilytic NPS 2143-Further implications for Alzheimer's therapy. *Front. Neurosci.* <https://doi.org/10.3389/fnins.2017.00217>.
- Connelly, P.J., Prentice, N.P., Fowler, K.G., 2005. Predicting the outcome of cholinesterase inhibitor treatment in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 76, 320–324. <https://doi.org/10.1136/jnnp.2004.043539>.
- De Chiara, G., Piacentini, R., Fabiani, M., Mastrodonato, A., Marocci, M.E., Limongi, D., Napoletani, G., Protto, V., Coluccio, P., Celestino, I., Li Puma, D.D., Grassi, C., Palamara, A.T., 2019. Recurrent herpes simplex virus-1 infection induces hallmarks of neurodegeneration and cognitive deficits in mice. *PLoS Pathog.* 15, e1007617. <https://doi.org/10.1371/journal.ppat.1007617>.
- de Vivo, L., 2010. GLT-1 promoter activity in astrocytes and neurons of mouse hippocampus and somatic sensory cortex. *Front. Neuroanat.* 3 <https://doi.org/10.3389/neuro.05.031.2009>.
- Delaère, P., Duyckaerts, C., Masters, C., Beyreuther, K., Piette, F., Hauw, J.J., 1990. Large amounts of neocortical β A4 deposits without neuritic plaques nor tangles in a psychometrically assessed, non-demented person. *Neurosci. Lett.* 116, 87–93. [https://doi.org/10.1016/0304-3940\(90\)90391-L](https://doi.org/10.1016/0304-3940(90)90391-L).
- Deys, C., Thinakaran, G., Parent, A.T., 2016. APP receptor? To Be or not to Be. *Trends Pharmacol. Sci.* 37, 390–411. <https://doi.org/10.1016/j.tips.2016.01.005>.
- Dickson, D.W., Crystal, H.A., Bevana, C., Honer, W., Vincent, I., Davies, P., 1995. Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiol. Aging* 16, 285–298. [https://doi.org/10.1016/0197-4580\(95\)00013-5](https://doi.org/10.1016/0197-4580(95)00013-5).
- Dineley, K.T., Westerman, M., Bui, D., Bell, K., Ashe, K.H., Sweatt, J.D., 2001. Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal α 7 nicotinic acetylcholine receptors: in vitro and in vivo mechanisms related to Alzheimer's disease. *In Vivo (Brooklyn)* 21, 4125–4133.
- Dineley, K.T., Bell, K.A., Bui, D., Sweatt, J.D., 2002. β -amyloid peptide activates α 7 nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *J. Biol. Chem.* 277, 25056–25061. <https://doi.org/10.1074/jbc.M200066200>.
- Donev, R., Kolev, M., Millet, B., Thome, J., 2009. Neuronal death in Alzheimer's disease and therapeutic opportunities. *J. Cell. Mol. Med.* 13, 4329–4348. <https://doi.org/10.1111/j.1582-4934.2009.00889.x>.

- Dougherty, J.J., Wu, J.L., Nichols, R.A., 2003. β -Amyloid Regulation of Presynaptic Nicotinic Receptors in Rat Hippocampus and Neocortex. *J. Neurosci.* 23, 6740–6747. <https://doi.org/10.1523/JNEUROSCI.23-17-06740.2003>.
- Driscoll, I., Resnick, S.M., Troncoso, J.C., An, Y., O'Brien, R., Zonderman, A.B., 2006. Impact of Alzheimer's pathology on cognitive trajectories in nondemented elderly. *Ann. Neurol.* <https://doi.org/10.1002/ana.21031>.
- Engidawork, E., Gulesserian, T., Balic, N., Cairns, N., Lubec, G., 2001. Changes in nicotinic acetylcholine receptor subunits expression in brain of patients with Down syndrome and Alzheimer's disease. *J. Neural Transm. Suppl.* https://doi.org/10.1007/978-3-7091-6262-0_17.
- Escartin, C., Galea, E., Lakatos, A., O'Callaghan, J.P., Petzold, G.C., Serrano-Pozo, A., Steinhäuser, C., Volterra, A., Carmignoto, G., Agarwal, A., Allen, N.J., Araque, A., Barbeito, L., Barzilai, A., Bergles, D.E., Bonvento, G., Butt, A.M., Chen, W.-T., Cohen-Salmon, M., Cunningham, C., Deneen, B., De Strooper, B., Díaz-Castro, B., Farina, C., Freeman, M., Gallo, V., Goldman, J.E., Goldman, S.A., Götz, M., Gutiérrez, A., Haydon, P.G., Heiland, D.H., Hol, E.M., Holt, M.G., Iino, M., Kastanienka, K.V., Kettenmann, H., Khakh, B.S., Koizumi, S., Lee, C.J., Liddel, S.A., MacVicar, B.A., Magistretti, P., Messing, A., Mishra, A., Molofsky, A.V., Murai, K.K., Norris, C.M., Okada, S., Olet, S.H.R., Oliveira, J.F., Panatier, A., Parpura, V., Pekna, M., Pekny, M., Pellerin, L., Perea, G., Pérez-Nievas, B.G., Priege, F.W., Poskanzer, K.E., Quintana, F.J., Ransohoff, R.M., Riquelme-Perez, M., Robel, S., Rose, C.R., Rothstein, J.D., Rouach, N., Rowitch, D.H., Semyanov, A., Sirko, S., Sontheimer, H., Swanson, R.A., Vitorica, J., Wanner, I.-B., Wood, L.B., Wu, J., Zheng, B., Zimmer, E. R., Zorec, R., Sofroniew, M.V., Verkhratsky, A., 2021. Reactive astrocyte nomenclature, definitions, and future directions. *Nat. Neurosci.* 24, 312–325. <https://doi.org/10.1038/s41593-020-00783-4>.
- Fá, M., Puzzo, D., Piacentini, R., Staniszewski, A., Zhang, H., Baltrons, M.A., Li Puma, D. D., Chatterjee, I., Li, J., Saeed, F., Berman, H.L., Ripoli, C., Gulisano, W., Gonzalez, J., Tian, H., Costa, J.A., Lopez, P., Davidowitz, E., Yu, W.H., Haroutunian, V., Brown, L.M., Palmeri, A., Sigurdsson, E.M., Duff, K.E., Teich, A.F., Honig, L.S., Sierks, M., Moe, J.G., D'Adamio, L., Grassi, C., Kanaan, N.M., Fraser, P. E., Arancio, O., 2016. Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory. *Sci. Rep.* 6, 19393. <https://doi.org/10.1038/srep19393>.
- Ferreira-Vieira, T., Guimaraes, I.R., Silva, F., Ribeiro, F., 2016. Alzheimer's disease: targeting the cholinergic system. *Curr. Neuropharmacol.* <https://doi.org/10.2174/1570159x13666150716165726>.
- Fogel, H., Frere, S., Segev, O., Bharill, S., Shapira, I., Gazit, N., O'Malley, T., Slomowitz, E., Berdichevsky, Y., Walsh, D.M., Isacoff, E.Y., Hirsch, J.A., Slutsky, I., 2014. APP homodimers transduce an amyloid- β -mediated increase in release probability at excitatory synapses. *Cell Rep.* 7, 1560–1576. <https://doi.org/10.1016/j.celrep.2014.04.024>.
- Furcila, D., DeFelipe, J., Alonso-Nanclares, L., 2018. A study of amyloid- β and phosphotau in plaques and neurons in the Hippocampus of Alzheimer's disease patients. *J. Alzheimers Dis.* 64, 417–435. <https://doi.org/10.3233/JAD-180173>.
- Garcia-Osta, A., Alberini, C.M., 2009. Amyloid beta mediates memory formation. *Learn. Mem.* 16, 267–272. <https://doi.org/10.1101/lm.1310209>.
- Glenner, G.G., Wong, C.W., 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* 120, 885–890. [https://doi.org/10.1016/S0006-291X\(84\)80190-4](https://doi.org/10.1016/S0006-291X(84)80190-4).
- González-Reyes, R.E., Nava-Mesa, M.O., Vargas-Sánchez, K., Ariza-Salamanca, D., Mora-Muñoz, L., 2017. Involvement of astrocytes in Alzheimer's disease from a neuroinflammatory and oxidative stress perspective. *Front. Mol. Neurosci.* 10 <https://doi.org/10.3389/fnmol.2017.00427>.
- Götz, J., Chen, F., van Dorpe, J., Nitsch, R.M., 2001. Formation of neurofibrillary tangles in P3011 tau transgenic mice induced by Abeta 42 fibrils. *Science* 293, 1491–1495. <https://doi.org/10.1126/science.1062097>.
- Greenberg, S.M., Koo, E.H., Selkoe, D.J., Qiu, W.Q., Kosik, K.S., 1994. Secreted β -amyloid precursor protein stimulates mitogen-activated protein kinase and enhances τ phosphorylation. *Proc. Natl. Acad. Sci. U. S. A.* <https://doi.org/10.1073/pnas.91.15.7104>.
- Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y.C., Zaidi, M.S., Wisniewski, H.M., 1986. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J. Biol. Chem.* 261, 6084–6089. <https://doi.org/10.1074/jbc.M111.271320>.
- Gulisano, W., Melone, M., Ripoli, C., Tropea, M.R., Li Puma, D.D., Amato, A., Palmeri, A., D'Adamio, L., Grassi, C., Devanand, D.P., Honig, L.S., Puzzo, D., Arancio, O., 2018a. Role of Amyloid- β and tau proteins in Alzheimer's disease: confuting the amyloid cascade. *J. Alzheimers Dis.* 136, 1383–1398. <https://doi.org/10.3233/JAD-179935>.
- Gulisano, W., Melone, M., Li Puma, D.D., Tropea, M.R., Palmeri, A., Arancio, O., Grassi, C., Conti, F., Puzzo, D., 2018b. The effect of amyloid- β peptide on synaptic plasticity and memory is influenced by different isoforms, concentrations, and aggregation status. *Neurobiol. Aging* 71, 51–60. <https://doi.org/10.1016/j.neurobiolaging.2018.06.025>.
- Gulisano, W., Melone, M., Ripoli, C., Tropea, M.R., Li Puma, D.D., Giunta, S., Cocco, S., Marcotulli, D., Origlia, N., Palmeri, A., Arancio, O., Conti, F., Grassi, C., Puzzo, D., 2019. Neuromodulatory action of picomolar extracellular A β 42 oligomers on presynaptic and postsynaptic mechanisms underlying synaptic function and memory. *J. Neurosci.* 39, 5986–6000. <https://doi.org/10.1523/JNEUROSCI.0163-19.2019>.
- Hanger, D.P., Noble, W., 2011. Functional implications of glycogen synthase Kinase-3-Mediated tau phosphorylation. *Int. J. Alzheimers Dis.* 2011, 1–11. <https://doi.org/10.4061/2011/352805>.
- Hardy, J., Allsop, D., 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci.* [https://doi.org/10.1016/0165-6147\(91\)90609-V](https://doi.org/10.1016/0165-6147(91)90609-V).
- Hardy, J., Duff, K., Hardy, K.G., Perez-Tur, J., Hutton, M., 1998. Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nat. Neurosci.* 1, 355–358. <https://doi.org/10.1038/1565>.
- Hartlage-Rübsamen, M., Zeitschel, U., Apelt, J., Gärtner, U., Franke, H., Stahl, T., Günther, A., Schliebs, R., Penkowa, M., Bigl, V., Roßner, S., 2003. Astrocytic expression of the Alzheimer's disease β -secretase (BACE1) is stimulus-dependent. *Glia* 41, 169–179. <https://doi.org/10.1002/glia.10178>.
- Heffer, D., Ludewig, S., Draguhn, A., Korte, M., 2020. Amyloid, APP, and electrical activity of the brain. *Neuroscientist.* <https://doi.org/10.1177/1073858419882619>.
- Hernandez, C.M., Kaye, R., Zheng, H., Sweatt, J.D., Dineley, K.T., 2010. Loss of α 7 nicotinic receptors enhances beta-amyloid oligomer accumulation, exacerbating early-stage cognitive decline and septohippocampal pathology in a mouse model of Alzheimer's disease. *J. Neurosci.* 30, 2442–2453. <https://doi.org/10.1523/JNEUROSCI.5038-09.2010>.
- Herrup, K., 2015. The case for rejecting the amyloid cascade hypothesis. *Nat. Neurosci.* 18, 794–799. <https://doi.org/10.1038/nn.4017>.
- Hölttä, M., Hansson, O., Andreasson, U., Hertz, J., Minthon, L., Nägga, K., Andreason, N., Zetterberg, H., Blennow, K., 2013. Evaluating Amyloid- β oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease. *PLoS One.* <https://doi.org/10.1371/journal.pone.0066381>.
- Hu, M., Waring, J.F., Gopalakrishnan, M., Li, J., 2008. Role of GSK-3 activation and 7 nAChRs in A 142-induced tau phosphorylation in PC12 cells. *J. Neurochem.* 106, 1371–1377. <https://doi.org/10.1111/j.1471-4159.2008.05483.x>.
- Iacono, D., Markesbery, W.R., Gross, M., Pletnikova, O., Rudow, G., Zandi, P., Troncoso, J.C., 2009. The Nun Study: clinically silent AD, neuronal hypertrophy, and linguistic skills in early life. *Neurology.* <https://doi.org/10.1212/WNL.0b013e3181b01077>.
- Iqbal, K., Alonso, A., del, C., Chen, S., Chohan, M.O., El-Akkad, E., Gong, C.-X., Khatoun, S., Li, B., Liu, F., Rahman, A., Tanimukai, H., Grundke-Iqbal, I., 2005. Tau pathology in Alzheimer disease and other tauopathies. *Biochim. Biophys. Acta* 1739, 198–210. <https://doi.org/10.1016/j.bbdis.2004.09.008>.
- Katzman, R., Terry, R., DeTeresa, R., Brown, T., Davies, P., Fuld, P., Renbing, X., Peck, A., 1988. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann. Neurol.* 23, 138–144. <https://doi.org/10.1002/ana.410230206>.
- Kawarabayashi, T., 2004. Dimeric amyloid protein rapidly accumulates in lipid rafts followed by apolipoprotein e and phosphorylated tau accumulation in the Tg2576 mouse model of Alzheimer's disease. *J. Neurosci.* 24, 3801–3809. <https://doi.org/10.1523/JNEUROSCI.5543-03.2004>.
- Knobloch, M., Konietzko, U., Krebs, D.C., Nitsch, R.M., 2007. Intracellular A β and cognitive deficits precede β -amyloid deposition in transgenic arcA β mice. *Neurobiol. Aging.* <https://doi.org/10.1016/j.neurobiolaging.2006.06.019>.
- Koppens, P., Trinchese, F., Fá, M., Puzzo, D., Gulisano, W., Yan, S., Poussin, A., Liu, S., Orozco, I., Dale, E., Teich, A.F., Palmeri, A., Ninan, I., Boehm, S., Arancio, O., 2016. Time-dependent reversal of synaptic plasticity induced by physiological concentrations of oligomeric A β 42: an early index of Alzheimer's disease. *Sci. Rep.* 6, 32553. <https://doi.org/10.1038/srep32553>.
- Koval, L., Lykhmus, O., Kalashnyk, O., Bachinskaya, N., Kravtsova, G., Soldatkina, M., Zouridakis, M., Stergiou, C., Tzartos, S., Tsetlin, V., Komisarenko, S., Skok, M., 2011. The presence and origin of autoantibodies against α 4 and α 7 nicotinic acetylcholine receptors in the human blood: possible relevance to Alzheimer's pathology. *J. Alzheimers Dis.* 25, 747–761. <https://doi.org/10.3233/JAD-2011-101845>.
- Kuhn, J., Hardenacke, K., Lenartz, D., Gruendler, T., Ullsperger, M., Bartsch, C., Mai, J. K., Zilles, K., Bauer, A., Matusch, A., Schulz, R.-J., Noreik, M., Bührle, C.P., Maintz, D., Woopen, C., Häussermann, P., Hellmich, M., Klosterkötter, J., Wiltfang, J., Maarouf, M., Freund, H.-J., Sturm, V., 2015. Deep brain stimulation of the nucleus basalis of Meynert in Alzheimer's dementia. *Mol. Psychiatry* 20, 353–360. <https://doi.org/10.1038/mp.2014.32>.
- Kume, T., Takada-Takatori, Y., 2018. Nicotinic acetylcholine receptor signaling: roles in neuroprotection. *Nicotinic Acetylcholine Receptor Signaling in Neuroprotection*. Springer, Singapore, Singapore, pp. 59–71. https://doi.org/10.1007/978-981-10-8488-1_4.
- Lasagna-Reeves, C.A., 2012. Identification of oligomers at early stages of tau aggregation in Alzheimer's disease. *FASEB J.* 26, 1946–1959.
- Lawrence, J.L.M., Tong, M., Alfulaij, N., Sherrin, T., Contarino, M., White, M.M., Bellinger, P.P., Todorovic, C., Nichols, R.A., 2014. Regulation of presynaptic Ca $^{2+}$, synaptic plasticity and contextual fear conditioning by a N-terminal β -amyloid fragment. *J. Neurosci.* 34, 14210–14218. <https://doi.org/10.1523/JNEUROSCI.0326-14.2014>.
- Lazarevic, V., Fienko, S., Andres-Alonso, M., Anni, D., Ivanova, D., Montenegro-Venegas, C., Gundelfinger, E.D., Cousin, M.A., Fejtova, A., 2017. Physiological concentrations of amyloid beta regulate recycling of synaptic vesicles via α 7 acetylcholine receptor and CDK5/calceurin signaling. *Front. Mol. Neurosci.* <https://doi.org/10.3389/fnmol.2017.00221>.
- Lee, L., Kosuri, P., Arancio, O., 2014. Picomolar amyloid- β peptides enhance spontaneous astrocyte calcium transients. *J. Alzheimers Dis.* 38, 49–62. <https://doi.org/10.3233/JAD-130740>.
- Lemstra, A.W., Richard, E., van Gool, W.A., 2007. Cholinesterase inhibitors in dementia: yes, no, or maybe? *Age Ageing* 36, 625–627. <https://doi.org/10.1093/ageing/afm117>.
- Li, S.F., Wu, M.N., Wang, X.H., Yuan, L., Yang, D., Qi, J.S., 2011. Requirement of α 7 nicotinic acetylcholine receptors for amyloid beta protein-induced depression of hippocampal long-term potentiation in CA1 region of rats in vivo. *Synapse* 65, 1136–1143. <https://doi.org/10.1002/syn.20951>.
- Li Puma, D.D., Piacentini, R., Leone, L., Gironi, K., Marcocci, M.E., De Chiara, G., Palamara, A.T., Grassi, C., 2019. Herpes simplex virus Type-1 infection impairs adult

- hippocampal neurogenesis via Amyloid- β protein accumulation. *Stem Cells* 37, 1467–1480. <https://doi.org/10.1002/stem.3072>.
- Liu, Q.-S., Kawai, H., Berg, D.K., 2001. Beta-Amyloid peptide blocks the response of alpha 7-containing nicotinic receptors on hippocampal neurons. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4734–4739. <https://doi.org/10.1073/pnas.081553598>.
- Llorens-Martín, M., Jurado, J., Hernández, F., Ávila, J., 2014. GSK-3 β , a pivotal kinase in Alzheimer disease. *Front. Mol. Neurosci.* 7 <https://doi.org/10.3389/fnmol.2014.00046>.
- Lorenzo, A., Yuan, M., Zhang, Z., Paganetti, P.A., Sturchler-Pierrat, C., Staufenbiel, M., Mautino, J., Vigo, F.S., Sommer, B., Yankner, B.A., 2000. Amyloid beta interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease. *Nat. Neurosci.* 3, 460–464. <https://doi.org/10.1038/74833>.
- Lykhmus, O., Voytenko, L., Koval, L., Mykhalskiy, S., Kholin, V., Peschana, K., Zouridakis, M., Tzartos, S., Komisarenko, S., Skok, M., 2015. $\alpha 7$ Nicotinic acetylcholine receptor-specific antibody induces inflammation and amyloid $\beta 42$ accumulation in the mouse brain to impair memory. *PLoS One* 10, e0122706. <https://doi.org/10.1371/journal.pone.0122706>.
- Ma, L., Turner, D., Zhang, J., Wang, Q., Wang, M., Shen, J., Zhang, S., Wu, J., 2014. Deficits of synaptic functions in hippocampal slices prepared from aged mice null $\alpha 7$ nicotinic acetylcholine receptors. *Neurosci. Lett.* 570, 97–101. <https://doi.org/10.1016/j.neulet.2014.04.018>.
- Malarkey, E.B., Parpura, V., 2008. Mechanisms of glutamate release from astrocytes. *Neurochem. Int.* 52, 142–154. <https://doi.org/10.1016/j.neuint.2007.06.005>.
- Martin, L., Latypova, X., Terro, F., 2011. Post-translational modifications of tau protein: implications for Alzheimer's disease. *Neurochem. Int.* 58, 458–471.
- Mc Donald, J.M., Savva, G.M., Brayne, C., Welzel, A.T., Forster, G., Shankar, G.M., Selkoe, D.J., Ince, P.G., Walsh, D.M., Medical Research Council Cognitive, F., Ageing, S., 2010. The presence of sodium dodecyl sulphate-stable Abeta dimers is strongly associated with Alzheimer-type dementia. *Brain* 133, 1328–1341. <https://doi.org/10.1093/brain/awq065>.
- Mc Donald, J.M., O'Malley, T.T., Liu, W., Mably, A.J., Brinkmalm, G., Portelius, E., Wittbold, W.M., Frosch, M.P., Walsh, D.M., 2015. The aqueous phase of Alzheimer's disease brain contains assemblies built from ~4 and ~7 kDa A β species. *Alzheimer's Dement.* 11, 1286–1305. <https://doi.org/10.1016/j.jalz.2015.01.005>.
- Melone, M., Burette, A., Weinberg, R.J., 2005. Light microscopic identification and immunocytochemical characterization of glutamatergic synapses in brain sections. *J. Comp. Neurol.* <https://doi.org/10.1002/cne.20743>.
- Melone, M., Ciriachi, C., Pietrobon, D., Conti, F., 2019. Heterogeneity of astrocytic and neuronal GLT-1 at cortical excitatory synapses, as revealed by its colocalization with Na $^{+}$ /K $^{+}$ -ATPase α isoforms. *Cereb. Cortex* 29, 3331–3350. <https://doi.org/10.1093/cercor/bhy203>.
- Merino-Serrais, P., Knafo, S., Alonso-Nanclares, L., Feraud-Espinosa, I., Defelipe, J., 2011. Layer-specific alterations to CA1 dendritic spines in a mouse model of Alzheimer's disease. *Hippocampus*. <https://doi.org/10.1002/hipo.20861>.
- Miao, J., Shi, R., Li, L., Chen, F., Zhou, Y., Tung, Y.C., Hu, W., Gong, C.-X., Iqbal, K., Liu, F., 2019. Pathological tau from Alzheimer's brain induces site-specific hyperphosphorylation and SDS- and reducing agent-resistant aggregation of tau in vivo. *Front. Aging Neurosci.* 11 <https://doi.org/10.3389/fnagi.2019.00034>.
- Mondragón-Rodríguez, S., Perry, G., Luna-Muñoz, J., Acevedo-Aquino, M.C., Williams, S., 2014. Phosphorylation of tau protein at sites Ser396-404 is one of the earliest events in Alzheimer's disease and Down syndrome. *Neuropathol. Appl. Neurobiol.* <https://doi.org/10.1111/nan.12084>.
- Morley, J.E., Farr, S.A., Banks, W.A., Johnson, S.N., Yamada, K.A., Xu, L., 2010. A physiological role for amyloid-beta protein: enhancement of learning and memory. *J. Alzheimers Dis.* 19, 441–449. <https://doi.org/10.3233/JAD-2009-1230>.
- Mousavi, M., Hellström-Lindahl, E., 2009. Nicotinic receptor agonists and antagonists increase sAPP α secretion and decrease Abeta levels in vitro. *Neurochem. Int.* 54, 237–244. <https://doi.org/10.1016/j.neuint.2008.12.001>.
- Mufson, E., Counts, S., Fahnestock, M., Ginsberg, S., 2007. Cholinergic molecular substrates of mild cognitive impairment in the elderly. *Curr. Alzheimer Res.* <https://doi.org/10.2174/156720507781788855>.
- Mulder, S.D., Veerhuis, R., Blankenstein, M.A., Nielsen, H.M., 2012. The effect of amyloid associated proteins on the expression of genes involved in amyloid- β clearance by adult human astrocytes. *Exp. Neurol.* 233, 373–379. <https://doi.org/10.1016/j.expneurol.2011.11.001>.
- Müller-Schiffmann, A., Herring, A., Abdel-Hafiz, L., Chepkova, A.N., Schäble, S., Wedel, D., Horn, A.H.C., Sticht, H., De Souza Silva, M.A., Gottmann, K., Sergeeva, O. A., Huston, J.P., Keyvani, K., Korth, C., 2016. Amyloid- β dimers in the absence of plaque pathology impair learning and synaptic plasticity. *Brain* 139, 509–525. <https://doi.org/10.1093/brain/awv355>.
- Mura, E., Zappettini, S., Preda, S., Biundo, F., Lanni, C., Grilli, M., Cavallero, A., Olivero, G., Salamone, A., Govoni, S., Marchi, M., 2012. Dual effect of beta-amyloid on $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors controlling the release of glutamate, aspartate and GABA in rat hippocampus. *PLoS One* 7, e29661. <https://doi.org/10.1371/journal.pone.0029661>.
- Nagele, R.G., D'Andrea, M.R., Anderson, W.J., Wang, H.-Y., 2002. Intracellular accumulation of beta-amyloid(1-42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer's disease. *Neuroscience* 110, 199–211. [https://doi.org/10.1016/S0306-4522\(01\)00460-2](https://doi.org/10.1016/S0306-4522(01)00460-2).
- Neddens, J., Temmel, M., Flunkert, S., Kerschbaum, B., Hoeller, C., Loeffler, T., Niederkofler, V., Daum, G., Attens, J., Hutter-Paier, B., 2018. Phosphorylation of different tau sites during progression of Alzheimer's disease. *Acta Neuropathol. Commun.* 6, 52. <https://doi.org/10.1186/s40478-018-0557-6>.
- Nelson, P.T., Alafuzoff, I., Bigio, E.H., Bouras, C., Braak, H., Cairns, N.J., Castellani, R.J., Crain, B.J., Davies, P., Tredici, K.D., Duyckaerts, C., Frosch, M.P., Haroutunian, V., Hof, P.R., Hulette, C.M., Hyman, B.T., Iwatsubo, T., Jellinger, K.A., Jicha, G.A., Kövari, E., Kukull, W.A., Leverenz, J.B., Love, S., MacKenzie, I.R., Mann, D.M., Masliah, E., McKee, A.C., Montine, T.J., Morris, J.C., Schneider, J.A., Sonnen, J.A., Thal, D.R., Trojanowski, J.Q., Troncoso, J.C., Wisniewski, T., Woltjer, R.L., Beach, T. G., 2012. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *J. Neuropathol. Exp. Neurol.* <https://doi.org/10.1097/NEN.0b013e31825018f7>.
- Nie, H.Z., Shi, S., Lukas, R.J., Zhao, W.J., Sun, Y.N., Yin, M., 2010. Activation of $\alpha 7$ nicotinic receptor affects APP processing by regulating secretase activity in SH-EP1- $\alpha 7$ nAChR-hAPP695 cells. *Brain Res.* <https://doi.org/10.1016/j.brainres.2010.07.110>.
- Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kaye, R., Metherate, R., Mattson, M.P., Akbari, Y., LaFerla, F.M., 2003. Triple-transgenic model of Alzheimer's disease with plaques and tangles. *Neuron* 39, 409–421. [https://doi.org/10.1016/S0896-6273\(03\)00434-3](https://doi.org/10.1016/S0896-6273(03)00434-3).
- Oddo, S., Caccamo, A., Green, K.N., Liang, K., Tran, L., Chen, Y., Leslie, F.M., LaFerla, F. M., 2005. Chronic nicotine administration exacerbates tau pathology in a transgenic model of Alzheimer's disease. *Proc. Natl. Acad. Sci.* 102, 3046–3051. <https://doi.org/10.1073/pnas.0408500102>.
- Oz, M., Lorke, D.E., Yang, K.S., Petroianu, G., 2013. On the interaction of -amyloid peptides and 7-nicotinic acetylcholine receptors in Alzheimer's disease. *Curr. Alzheimer Res.* 10, 618–630.
- Palmeri, A., Ricciarelli, R., Gulisano, W., Rivera, D., Reboisio, C., Calcagno, E., Tropea, M. R., Conti, S., Das, U., Roy, S., Pronzato, M.A., Arancio, O., Fedele, E., Puzzo, D., 2017. Amyloid- β peptide is needed for cGMP-Induced long-term potentiation and memory. *J. Neurosci.* 37, 6926–6937. <https://doi.org/10.1523/JNEUROSCI.3607-16.2017>.
- Patel, H., McIntire, J., Ryan, S., Dunah, A., Loring, R., 2017. Anti-inflammatory effects of astroglial $\alpha 7$ nicotinic acetylcholine receptors are mediated by inhibition of the NF- κ B pathway and activation of the Nrf2 pathway. *J. Neuroinflammation.* <https://doi.org/10.1186/s12974-017-0967-6>.
- Phatnani, H., Maniatis, T., 2015. Astrocytes in neurodegenerative disease. *Cold Spring Harb. Perspect. Biol.* <https://doi.org/10.1101/cshperspect.a020628>.
- Piccio, M., 2000. Nicotinic receptors in the brain links between molecular biology and behavior. *Neuropsychopharmacology* 22, 451–465. [https://doi.org/10.1016/S0893-133X\(99\)00146-3](https://doi.org/10.1016/S0893-133X(99)00146-3).
- Pirttimäki, T.M., Codadu, N.K., Awni, A., Pratik, P., Nagel, D.A., Hill, E.J., Dineley, K.T., Parri, H.R., 2013. $\alpha 7$ Nicotinic Receptor-Mediated Astrocytic Gliotransmitter Release: A β Effects in a Preclinical Alzheimer's Mouse Model. *PLoS One* 8, e81828. <https://doi.org/10.1371/journal.pone.0081828>.
- Puzzo, D., Privitera, L., Leznik, E., Fa, M., Staniszevski, A., Palmeri, A., Arancio, O., 2008. Picomolar amyloid- β positively modulates synaptic plasticity and memory in hippocampus. *J. Neurosci.* 28 <https://doi.org/10.1523/JNEUROSCI.2692-08.2008>.
- Puzzo, D., Privitera, L., Fa, M., Staniszevski, A., Hashimoto, G., Aziz, F., Sakurai, M., Ribe, E.M., Troy, C.M., Mercken, M., Jung, S.S., Palmeri, A., Arancio, O., 2011. Endogenous amyloid- β is necessary for hippocampal synaptic plasticity and memory. *Ann. Neurol.* 69, 819–830. <https://doi.org/10.1002/ana.22313>.
- Puzzo, D., Lee, L., Palmeri, A., Calabrese, G., Arancio, O., 2014a. Behavioral assays with mouse models of Alzheimer's disease: practical considerations and guidelines. *Biochem. Pharmacol.* 88, 450–467. <https://doi.org/10.1016/j.bcp.2014.01.011>.
- Puzzo, D., Loreto, C., Giunta, S., Musumeci, G., Frasca, G., Podda, M.V., Arancio, O., Palmeri, A., 2014b. Effect of phosphodiesterase-5 inhibition on apoptosis and beta amyloid load in aged mice. *Neurobiol. Aging* 35, 520–531. <https://doi.org/10.1016/j.neurobiolaging.2013.09.002>.
- Puzzo, D., Gulisano, W., Arancio, O., Palmeri, A., 2015a. The keystone of Alzheimer pathogenesis might be sought in A β physiology. *Neuroscience* 307, 26–36. <https://doi.org/10.1016/j.neuroscience.2015.08.039>.
- Puzzo, D., Bizzoca, A., Loreto, C., Guida, C.A., Gulisano, W., Frasca, G., Bellomo, M., Castorina, S., Gennarini, G., Palmeri, A., 2015b. Role of F3/contactin expression profile in synaptic plasticity and memory in aged mice. *Neurobiol. Aging* 36, 1702–1715. <https://doi.org/10.1016/j.neurobiolaging.2015.01.004>.
- Puzzo, D., Piacentini, R., Fa, M., Gulisano, W., Li Puma, D.D., Staniszevski, A., Zhang, H., Tropea, M.R., Cocco, S., Palmeri, A., Fraser, P., D'Adamo, L., Grassi, C., Arancio, O., 2017. LTP and memory impairment caused by extracellular A β and Tau oligomers is APP-dependent. *Elife* 6, 1–21. <https://doi.org/10.7554/eLife.26991>.
- Puzzo, D., Argyrousi, E.K., Staniszevski, A., Zhang, H., Calcagno, E., Zuccarello, E., Acquarone, E., Fa, M., Li Puma, D.D., Grassi, C., D'Adamo, L., Kanaan, N.M., Fraser, P.E., Arancio, O., 2020. Tau is not necessary for amyloid- β -induced synaptic and memory impairments. *J. Clin. Invest.* 130, 4831–4844. <https://doi.org/10.1172/JCI137040>.
- Qi, X.-L., Nordberg, A., Xiu, J., Guan, Z.-Z., 2007. The consequences of reducing expression of the alpha7 nicotinic receptor by RNA interference and of stimulating its activity with an alpha7 agonist in SH-SY5Y cells indicate that this receptor plays a neuroprotective role in connection with the pathogenesis. *Neurochem. Int.* 51, 377–383. <https://doi.org/10.1016/j.neuint.2007.04.002>.
- Qiu, C., Kivipelto, M., von Strauss, E., 2009. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues Clin. Neurosci.* 11, 111–128. <https://doi.org/10.1097/ALN.0b013e318212ba87>.
- Ren, K., Thinschmidt, J., Liu, J., Ai, L., Papke, R.L., King, M.A., Hughes, J.A., Meyer, E. M., 2007. $\alpha 7$ Nicotinic receptor gene delivery into mouse hippocampal neurons leads to functional receptor expression, improved spatial memory-related performance, and tau hyperphosphorylation. *Neuroscience* 145, 314–322. <https://doi.org/10.1016/j.neuroscience.2006.11.023>.
- Ricciarelli, R., Puzzo, D., Bruno, O., Canepa, E., Gardella, E., Rivera, D., Privitera, L., Domenicotti, C., Marengo, B., Marinari, U.M., Palmeri, A., Pronzato, M.A., Arancio, O., Fedele, E., 2014. A novel mechanism for cyclic adenosine

- monophosphate-mediated memory formation: role of amyloid beta. *Ann. Neurol.* 75, 602–607. <https://doi.org/10.1002/ana.24130>.
- Ries, M., Sastre, M., 2016. Mechanisms of A β clearance and degradation by glial cells. *Front. Aging Neurosci.* <https://doi.org/10.3389/fnagi.2016.00160>.
- Rossner, S., Lange-Dohna, C., Zeitschel, U., Perez-Polo, J.R., 2005. Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme. *J. Neurochem.* 92, 226–234. <https://doi.org/10.1111/j.1471-4159.2004.02857.x>.
- Rubio, A., Pérez, M., Ávila, J., 2006. Acetylcholine receptors and tau phosphorylation. *Curr. Mol. Med.* 6, 423–428. <https://doi.org/10.2174/15665240677435444>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Selkoe, D.J., 2002. Alzheimer's disease is a synaptic failure. *Science* 298, 789–791. <https://doi.org/10.1126/science.1074069>.
- Selkoe, D.J., Hardy, J., 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* 8, 595–608. <https://doi.org/10.15252/emmm.201606210>.
- Sengupta, U., Portelius, E., Hansson, O., Farmer, K., Castillo-Carranza, D., Woltjer, R., Zetterberg, H., Galasko, D., Blennow, K., Kayed, R., 2017. Tau oligomers in cerebrospinal fluid in Alzheimer's disease. *Ann. Clin. Transl. Neurol.* 4, 226–235. <https://doi.org/10.1002/actn.3382>.
- Shaked, G.M., Kummer, M.P., Lu, D.C., Galvan, V., Bredesen, D.E., Koo, E.H., 2006. Abeta induces cell death by direct interaction with its cognate extracellular domain on APP (APP 597-624). *FASEB J.* 20, 1254–1256. <https://doi.org/10.1096/fj.05-5032fje>.
- Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., Brett, F. M., Farrell, M.A., Rowan, M.J., Lemere, C.A., Regan, C.M., Walsh, D.M., Sabatini, B. L., Selkoe, D.J., 2008. Amyloid- β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med.* 14, 837–842. <https://doi.org/10.1038/nm1782>.
- Sloane, J.A., Pietropaolo, M.F., Rosene, D.L., Moss, M.B., Peters, A., Kemper, T., Abraham, C.R., 1997. Lack of correlation between plaque burden and cognition in the aged monkey. *Acta Neuropathol.* 94, 471–478. <https://doi.org/10.1007/s004010050735>.
- Strada, O., Vyas, S., Hirsch, E.C., Ruberg, M., Brice, A., Agid, Y., Javoy-Agid, F., 1992. Decreased choline acetyltransferase mRNA expression in the nucleus basalis of Meynert in Alzheimer disease: an in situ hybridization study. *Proc. Natl. Acad. Sci.* 89, 9549–9553. <https://doi.org/10.1073/pnas.89.20.9549>.
- Takahashi, M., Miyata, H., Kametani, F., Nonaka, T., Akiyama, H., Hisanaga, Sichi, Hasegawa, M., 2015. Extracellular association of APP and tau fibrils induces intracellular aggregate formation of tau. *Acta Neuropathol.* 129, 895–907. <https://doi.org/10.1007/s00401-015-1415-2>.
- Van Nostrand, W.E., Melchor, J.P., Keane, D.M., Saporito-Irwin, S.M., Romanov, G., Davis, J., Xu, F., 2002. Localization of a fibrillar amyloid β -protein binding domain on its precursor. *J. Biol. Chem.* 277, 36392–36398. <https://doi.org/10.1074/jbc.M204676200>.
- Wang, Y., Mandelkow, E., 2015. Tau in physiology and pathology. *Nat. Rev. Neurosci.* 17, 22–35. <https://doi.org/10.1038/nrn.2015.1>.
- Wang, H.Y., Lee, D.H., Davis, C.B., Shank, R.P., 2000. Amyloid peptide Abeta(1-42) binds selectively and with picomolar affinity to alpha7 nicotinic acetylcholine receptors. *J. Neurochem.* 75, 1155–1161. <https://doi.org/10.1046/j.1471-4159.2000.0751155.x>.
- Wang, H.-Y., Li, W., Benedetti, N.J., Lee, D.H.S., 2003. A 7 nicotinic acetylcholine receptors mediate β -Amyloid peptide-induced tau protein phosphorylation. *J. Biol. Chem.* 278, 31547–31553. <https://doi.org/10.1074/jbc.M212532200>.
- Wang, Z., Jackson, R.J., Hong, W., Taylor, W.M., Corbett, G.T., Moreno, A., Liu, W., Li, S., Frosch, M.P., Slutsky, I., Young-Pearse, T.L., Spires-Jones, T.L., Walsh, D.M., 2017. Human brain-derived $\alpha\beta$ oligomers bind to synapses and disrupt synaptic activity in a manner that requires APP. *J. Neurosci.* 37, 11947–11966. <https://doi.org/10.1523/JNEUROSCI.2009-17.2017>.
- Wevers, A., Monteggia, L., Nowacki, S., Bloch, W., Schutz, U., Lindstrom, J., Pereira, E.F. R., Eisenberg, H., Giacobini, E., De Vos, R.A.I., Steur, E.N.H.J., Maelicke, A., Albuquerque, E.X., Schroder, H., 1999. Expression of nicotinic acetylcholine receptor subunits in the cerebral cortex in Alzheimer's disease: histotopographical correlation with amyloid plaques and hyperphosphorylated-tau protein. *Eur. J. Neurosci.* 11, 2551–2565. <https://doi.org/10.1046/j.1460-9568.1999.00676.x>.
- Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T., DeLong, M.R., 1981. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* 10, 122–126. <https://doi.org/10.1002/ana.410100203>.
- Wilcock, D.M., Gordon, M.N., Morgan, D., 2006. Quantification of cerebral amyloid angiopathy and parenchymal amyloid plaques with Congo red histochemical stain. *Nat. Protoc.* 1, 1591–1595. <https://doi.org/10.1038/nprot.2006.277>.
- Wirths, O., Bayer, T.A., 2010. Neuron loss in transgenic mouse models of Alzheimer's disease. *Int. J. Alzheimers Dis.* 2010, 1–6. <https://doi.org/10.4061/2010/723782>.
- Wu, J., Ishikawa, M., Zhang, J., Hashimoto, K., 2010. Brain imaging of nicotinic receptors in Alzheimer's disease. *Int. J. Alzheimers Dis.* 2010, 548913. <https://doi.org/10.4061/2010/548913>.
- Yakel, J.L., 2014. Nicotinic ACh receptors in the hippocampal circuit; functional expression and role in synaptic plasticity. *J. Physiol.* 592, 4147–4153. <https://doi.org/10.1113/jphysiol.2014.273896>.
- Zolozhevska, O., Taglialatela, G., 2016. Non-demented individuals with Alzheimer's disease neuropathology: resistance to cognitive decline may reveal new treatment strategies. *Curr. Pharm. Des.* 22, 4063–4068.